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Rishabha Malviya
Sonali Sundram *Editors*

Targeted Cancer Therapy in Biomedical Engineering

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Dear Healthcare Professionals,

We are dedicating this book to you. Our Love for profession shall live forever.

This book is also dedicated to friendship of editors. We work together and edited a fruitful book.

Preface

The development of targeted anticancer drug delivery and a more specialized approach to treating various cancer types have been made possible by advancements in cancer research, along with advancements in biomaterials and nanotechnology. A multidisciplinary approach integrating cancer biology, biomaterials, and nanotechnology offers the potential to improve treatment outcomes while reducing unwanted side effects. The development of a successful targeted therapy requires the optimization of therapeutic particles, cancer cell targeting, and drug release systems. BME is a growing industry in clinical application that has been continuously expanding via research in engineering medicine and biology combining different algorithms and processes by making new devices that have a significant impact on medical profession and health services.

The objective of the bioengineering technique is to deliver large doses of anticancer drugs to cancer cells, improve drug uptake by tumour cells, and reduce drug uptake in normal cells. The key strategy for targeting cancer cells is to develop a drug delivery system. Micelles and liposomes, which encapsulate drugs and are designed to target cancer cells, were developed as a result of advances in micro- and nanotechnology. This book addresses current targeted cancer treatments and focuses on the design and optimization of individual components necessary to accomplish effective cancer treatment using biomedical engineering. This book has focused on bioengineering approaches to improve targeted delivery of cancer therapeutics. This book is divided into 26 chapters covering tissue engineering in cancer research, use of biomaterials and computer-aided drug design, detection of cancer biomarkers, specific drug delivery system using nanocarriers, microfluidics and photothermal therapy in cancer treatment, role of magnetic nanoparticles, and role of vehicles for targeted cancer therapy. In a fast-evolving era, this book will help health professionals to enhance their skills to conduct health planning, make early diagnoses, and choose appropriate treatments for each patient with expected side effects and results. This book will attract audience of senior undergraduate students and graduate students

in mechanical, electrical and biomedical engineering fields and other professionals in medicine. It will be ideal for teaching and for those who are working in cancer bioengineering or interdisciplinary projects.

Greater Noida, India

Rishabha Malviya
Sonali Sundram

Acknowledgments

When the time came to dedicate my fourth book, I realized there were some specific people in this journey. Time during completing this book is one of my most challenging and experiencing journey. At first, I would like to begin by expressing our gratitude to the Almighty, whose unending blessing and supreme power enable us to accomplish all of our goals.

Then after, I am very thankful to management of Galgotias University, I want to thank you for letting me serve, for being a part of our amazing organization and for giving motivation every day.

Many thanks also go to all the authors who so kindly contributed to accomplish this book. Finally, I extend my deepest thanks to publishers for their kind and continuous support, innovative suggestions and guidance in bringing out this edition.

Greater Noida, India

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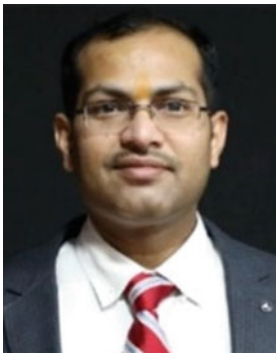
About the Editors



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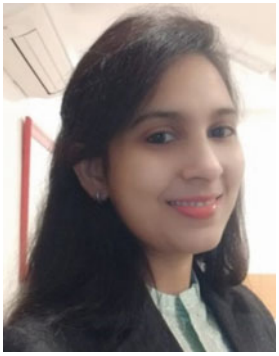

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Chapter 1
Strategies for Cancer Targeting: Novel Drug Delivery Systems Opportunities and Future Challenges

Dipak D. Gadade, Nitin Jain, Rashmi Sareen, Prabhanjan S. Giram, and Anuj Modi

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Abstract Cancer is one of the serious concerns to mankind. Conventional chemotherapy is still a primary choice of treatment for certain types of cancers. The literature reports that 27% of cancer patients' deaths occurred by terminal illnesses caused by conventional chemotherapy. They have low bioavailability, low therapeutic index, and are nonspecific. Toxicity, side effects, burst release, and unpredictable drug reactions of drugs are the major challenges in conventional cancer chemotherapy. Various approaches for targeted drug therapy for cancer to overcome biological and physicochemical barriers are discussed. Smart nanoparticulate drug delivery systems (SNDDS) are explored to overcome problems in conventional DDS. We presented an overview of nanoparticles for drug delivery, challenges in cancer therapy, cancer cell targeting mechanisms, smart strategies for cancer targeting including endogenous and exogenous stimuli-responsive systems, and the current advancement in nanoparticulate DDS.

1.1 Introduction

Cancer is amongst the leading threats worldwide and contributes to 1 in 6 deaths. As per WHO estimates about 10 million deaths were caused due to cancer in the year 2020 which will increase to 13.1 million by 2030 forecast [1, 2].

Routine tumour treatments include chemotherapy, surgery, radiation treatment, targeted treatment, and immunotherapy. In any case, constraints like the absence of specificity, cytotoxicity, and multiple drug resistance are potential problems to be tackled during cancer treatment [3]. Chemotherapy is very regularly used for cancer treatment. The chemotherapeutic agents administered through the parenteral route are non-selectively distributed in the body tissues. These drugs non-specifically kill the normal, healthy tissues along with the malignant cells. The most common side effects of chemotherapy or radiation therapy include drug induced neuropathy, bone marrow suppression, alopecia, gastrointestinal tract (GIT) and skin disorders, and generalized fatigue [3]. To avoid these side effects, design of site-specific and targeted drug delivery strategies may be adopted for cancer management [2].

Diagnosis of cancerous cells helps to prevent future consequences, but tumour suppressive microenvironment is a major challenge. The simultaneous diagnosis

and therapeutics are the recent new avenues of the nanoparticles (NPS) explored for providing better care to the affected subjects. The NPS is also reported to reduce the side effects of chemotherapy [4].

The search for innovative and novel treatment options for irradiation of cancer needs to be accelerated, to address the issues in cancer treatments. The smart nanoparticulate drug delivery techniques which made a significant contribution to improving patient survival and life expectancy are discussed in detail along with the drug delivery to a specific site by the triggered drug release.

1.2 NPS for Drug Delivery

NPS is defined as "engineered structures or particles with at least one dimension less than 100 nm or less" [5]. NPS has extensive applications in diagnostics and therapeutics. The larger surface-to-mass ratio, tiny particle size offering higher penetration in the tissues, capacity to carry both hydrophobic and hydrophilic materials, magnetism, quantum effects, high thermal and electrical conductivity, and ability to engineer the material as per requirement are the potential advantages of NPS [6]. The major goals of developments of nanomedicine are:

(a) Site-specific drug targeting
(b) Boost the therapeutic efficacy of the drugs/agents
(c) Reducing toxicity of drugs/therapeutic agents
(d) Search for safe, biocompatible, and biodegradable cargos for the drug delivery
(e) Development of single nano-units with optimal diagnostic and therapeutic capabilities
(f) Improving system stability of medicines
(g) Faster development of the newer, safer quality medicines [7]
(h) Overcomes biological barriers.

As depicted in Fig. 1.1, nanosized particles can be easily prepared from a variety of natural and synthetic materials with desired size, shape, and surface charge. It includes polymeric, metallic, lipid, or inorganic materials [8]. The surface modification of NPS is a new strategy valuable for targeting desired tissues/sites, modifying drug clearance, and plasma $t_{1/2}$ of the drugs. NPS surface properties can be tailored by modification of the surface charge, PEGylation, use of polysaccharides, and conjugation of targeting ligands through covalent or noncovalent bonding [4, 8].

There are two different synthetic methods for the development of the NPS, viz. (i) top-down and (ii) bottom-up. In the top-down approach, with larger particle size, material is converted to nanosized form. The top-down methods include (a) mechanical grinding/milling, (b) lithography technique, (c) chemical etching technique, (d) laser ablation, (e) arc discharge, (f) sputtering method, (g) electro-explosion, and (h) thermal decomposition. The bottom-up approach generally starts from the solutions, and it is converted to NPS finally. Bottom-up approaches include (a) chemical vapour decomposition, (b) biological synthesis (via bacteria, fungi, yeast, etc.), (c) spinning,

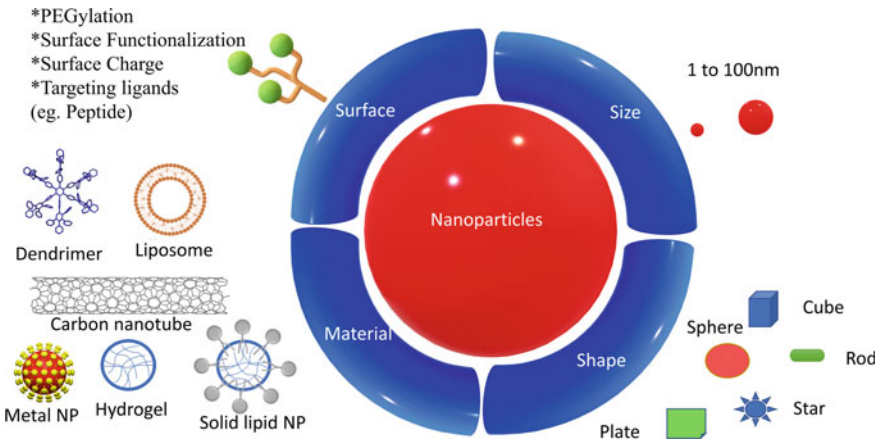

Fig. 1.1 Summary of NPS characteristics

(d) sol-gel synthesis, (e) laser pyrolysis, (f) plasma or flame spraying, and (g) reverse micelles, etc. [9].

1.3 Challenges in Cancer Therapy

Active pharmaceutical ingredients (API) used for cancer treatment have to overcome physicochemical properties for clinical translation. Although both small molecules and biologicals are used in cancer treatment, small molecules remain the mainstay for their therapeutic use. The ideal tumour treatment delivers the optimal dose of the drug at a controlled rate for a prolonged duration to the specific tumour site, preventing action on the normal tissues [10].

Current challenges to tackle in the treatment of cancer mainly include (a) the multiplicity of the drugs and complexity of the tumour treatment, (b) the varied physicochemical nature of these drugs, (c) safety concerns of the drugs used, (d) the potential problems to deliver the drug/agents to the specific target tumour avoiding their side effects, (e) biological variation leads to issues related to *in vitro in vivo* correlation, and (f) tumour suppressive microenvironment. The lower drug solubility, dissolution, and bioavailability are major factors that pose limitations in drug delivery for cancer therapy. The further challenges include improving drug distribution, multidrug resistance, reducing local toxicity, and reducing presystemic metabolism of administered drugs and targeted delivery of the drugs [11]. As depicted in Fig. 1.2, these challenges can be divided into three categories, (a) drug-related challenges, (b) drug carrier-related challenges, and (c) physiological challenges.

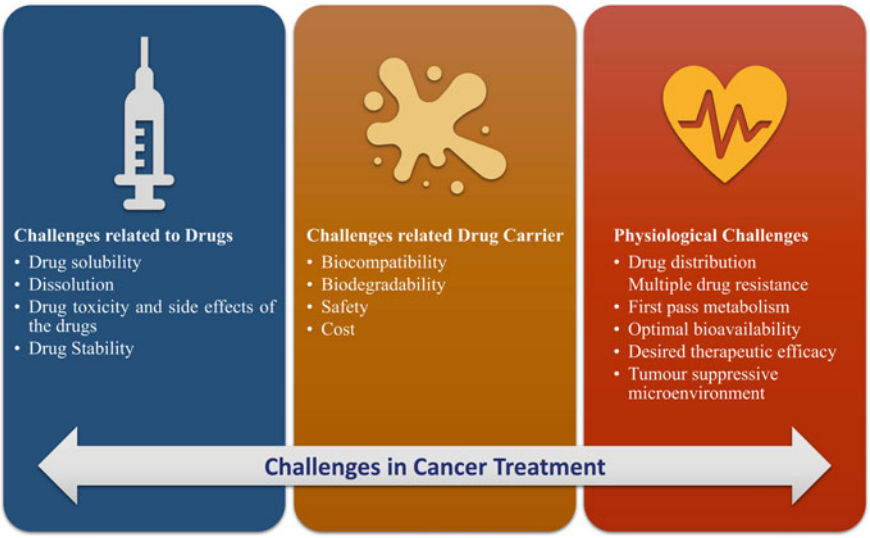

Fig. 1.2 Challenges in cancer therapy

1.4 Targeted Drug Therapy (TDT)

TDT can be defined as, the treatment in which drugs and drug delivery methods are strategically designed to "target" cancerous cells without affecting normal cells. TDT employs drugs and other substances to specifically detect and attack tumours. These therapies work in various ways including blocking inherent proteins, enzymes, and biomolecules involved in the development and spread of the tumour. Another strategy works by promoting apoptotic processes to kill cancer cells or by delivering toxic molecules that directly kill cancerous cells [12].

1.4.1 Ideal Features of Targeted Drug Therapy

The TDT shall possess the following properties for optimal and acceptable drug delivery [13]:

(a) It should be safe, physio-chemically stable, and biocompatible.
(b) The drug carrier should be inert, biodegradable, and readily eliminated from the body following drug release.
(c) It should carry the drug to the target site only without affecting normal tissues.
(d) It should provide the controlled drug release at the desired rate.
(e) The drug release rate should not alter the therapeutic effects of the drugs.
(f) There should be no or minimum drug leakage from the cargo during transportation.

Table 1.1 Benefits and drawbacks of targeted drug therapy

Benefits	Drawbacks
(a) The drug administration and delivery instructions are simplified (b) The dose of the drug can be reduced by targeted delivery (c) The drug toxicity is reduced due to specific drug targeting (d) It can avoid presystemic metabolism of the drug/therapeutic agents (e) Absorption of the drug, bioavailability, and therapeutic availability can be improved (f) The plasma drug concentration can be maintained in the therapeutic window (g) Patient compliance is improved (h) Preferential accumulation in tumour by EPR effect	(a) Toxicity from the drug carrier is possible (b) Degradation products targeted drug delivery devices may be harmful (c) Immune response from the drug carrier is possible (d) Sufficient contact time at the site of action is desired for effective drug therapy (e) Preparation, storage, and administration may require expertise in certain targeted systems (f) Targeted therapy may have a higher cost than conventional therapy

(g) The drug carrier should be inert, biodegradable, and readily eliminated from the body following drug release.
(h) It should be simple and economic.

The differentiation of normal and cancerous cells is a prime requirement for targeted cancer therapy. Hence, targeted cancer therapies have comparatively fewer undesirable effects in comparison to conventional therapy. Rapid elimination of the drug is a general issue with nonspecific and systemic administration methods of the drug. Therefore, such drug administration has higher toxicity, which can be reduced with TDT [14]. There are various benefits and drawbacks of TDT with nanomedicine, which are given below in Table 1.1.

1.4.2 Cancer Cell Targeting Mechanisms

The selective tissue penetration by circumvention of physiological barriers and biological variations is a bonus offered by nanomedicine in cancer therapy. The drug delivery with a higher dose threshold is possible with the nanoparticulate targeting approach [15]. The longer half-life and better pharmacokinetic profiles are additional advantages of NPS in oncological therapy [16, 17].

There are two basic targeting mechanisms in used targeted drug cancer therapy: (a) passive targeting and (b) active targeting.

1.4.2.1 Passive Targeting

It is referred to, as "utilizing passive diffusion for the transfer of the drug to the tumour". This targeting mechanism is based on the enhanced permeation and retention (EPR) effect. It can enable an effective localization of the NPS in tumour sites, but their uptake cannot be promoted [18, 19].

1.4.2.2 Active Targeting

Active targeting is a molecular recognition-based delivery approach. Targeting ligands are placed at the face of the NPS for active targeting. The targeting ligands bind selectively to the complimentary receptor in the target tumour site. The foremost challenge in active targeting is the identification and incorporation of selected targeting agents for delivery of NPS to the cancer site. Additionally, the targeting agent shall possess the ability to bind the tumour cell surface and need to possess a strong affinity for endocytic trigger [19].

The targeting ligands can be categorized as given below [15, 19, 20]

(A) Proteins and peptides

E.g. (i) Monoclonal antibodies (such as rituximab, trastuzumab, gemtuzumab, BR 96, vascular cell adhesion molecule-1, αvβ3 integrin, matrix metalloproteinase, HER2, epithelial growth factor (EGF) receptor, transferrin, prostate-specific membrane antigens, etc).

(b) Antibody fragments

E.g. Single chain variable antibody fragments (scFV), antigen-binding fragments (Fab protein).

(B) Nucleic acid

E.g. Aptamers (A10 RNA aptamer, anti-EGFR aptamer, anti-MUC1 protein aptamer).

(C) Carbohydrate targeting

E.g. Glucose, mannan, cellulose, etc.

1.4.2.3 Enhanced Permeation and Retention

EPR is a pathophysiological condition progressively developed in tumour where polymer drug nanosystems over 40 kDa accumulated at the cancer site allowing targeted drug delivery and retention of the drug [19]. Typically, when tumour size approaches 2 mm^3, vascular permeability is compromised, leading to the accumulation of macromolecules. Rapid uncontrolled growth of blood vessels called

Fig. 1.3 Characteristics of EPR effect

angiogenesis affects nutrition intake, waste material excretion, and oxygen transport. Abnormal tortuosity, disturbed basement membrane, and pericyte-free endothelial cells are characteristics of angiogenesis. The NPS within the size range of 20–200nm can get entry into interstitial space ensuring longer circulation time, getting concentrated in the tumour, and having low renal clearance [20]. The major pathophysiological characteristics of the EPR could be helpful in drug targeting (Fig. 1.3).

1.4.3 Approaches to Targeted Cancer Therapy

1.4.3.1 Destruction of Cancer Cells by Immunotherapy

With a better understanding of immunology and tumour pathophysiology, immunotherapy has contributed significantly to cancer treatment during last few years. Understanding basic immunology principles and tumour cell biology are the key factors in developing experimental and clinical immunotherapy. This development in immunotherapy has opened the new array of therapeutic agents for treating various types of cancer. Therapy with ipilimumab and adjuvant therapy with PEGylated interferon-a2b which were granted approval from USFDA in the treatment of melanoma are the two examples of immunotherapy. And this gives insight that understanding of immunology and tumour pathophysiology can translate the drug/therapy from laboratory to clinical use effectively. The tools utilized in developing immunotherapy may include cytokines, vaccines, cellular therapy, and antibodies [21].

To activate antitumour immunity, variety of strategies are required to overcome suppressive tumour microenvironment with therapies, systemic approach for delivery

Table 1.2 Approved immunotherapy for cancer

Approved therapy	Type of cancer	References
HPV vaccine	Cervical cancer	[23, 24]
HBV vaccine	Hepatic cancer (HCC)	[25, 26]
Antibiotics for *H. Pylori*	Gastric cancer	[27, 28]
NSAIDs (familial adenomatous polyposis, ulcerative colitis)	Colorectal cancer	[29–32]
IL-2, IFN-alpha	Melanoma, renal cell cancer (RCC)	[33, 34]
MAb of rituximab	NHL, CLL	[35, 36]

of cytokines, MAb-based passive immunization, and use of specific adjuvants for immune stimulation by refining antigen presentation to sustained immune response. Tumour prophylaxis includes vaccination and therapy for oncogenic microbial infections. For bone marrow transplantations, immune therapy is one of the important components used to treat haematologic malignancies. To generate nontoxic, safe, and effective cancer therapy, immunotherapy made a great improvement in percentage survival and played a pivotal role in cancer management. These new strategies improved systemic antitumor immunity (Innate and Adaptive) and affected the immune suppressive regulatory mechanisms (check point blockers) which are crucial for better treatment options [22]. Table 1.2 depicts approved immunotherapy for cancer.

Prophylactic Immune Response in the Development of Immune Therapy and Tumour Vaccines

The factors contributing to cancers are microbial infections associated with chronic inflammation [37, 38] and created interest in the design of therapy targeting prevent and irradiating of infections.

The infections of the liver cause chronic liver diseases like hepatitis, which progress to hepatocarcinoma. The first vaccine used for HCC was the hepatitis B virus (HBV) vaccine. Another vaccine against cervical cancer was tested recently against human papillomavirus (HPV). 250,000 deaths globally every year are attributed to HPV and contribute to 70% of cervical cancers [24, 39]. For better therapeutic outcomes, girls' vaccination against HPV prevents cervical carcinoma.

Bacterial infections also contribute to the development of tumour like *Helicobacter pylori*-related lymphomas and develop cancer of the gastric region [40]. Antibiotics are used to treat *H. pylori*, which can reduce the danger of stomach cancer. Antibiotics are also reported for treating lymphomas associated with *H. pylori* infection, and genomic alterations in the tumour cells are required for patients who are resistant to antibiotic therapy.

Immune Adjuvants and Cytokines

In response to tumour antigens, the immune system generates a systemic antitumour effect, but these responses are not able to suppress the complete development of tumours. Delivery of some immune adjuvants in the microenvironment of tumour can lead to enabling these immune responses for suppression of initial stage tumours. E.g. Use of the BCG vaccine combined with surgical removal of tumour in bladder cancer is the first treatment of choice for bladder cancer. Clinical trials suggested BCG vaccine after surgery is much more effective than chemotherapy in improving % the survival rate of cancer [41]. But treatment and the global shortage is a big challenge for BCG. Apart from adjuvants, some cytokines can also produce antitumor activity by immune modulation, e.g. cytokines IL-2 employed for the treatment of RCC and advanced melanoma. FDA-approved CpG, alum, imiquimod, and MLP to improve immune response.

Monoclonal Antibodies (MAb)

MAbs are an important class of cancer immunotherapy targeting tumour immunity cycle. Generally, as compared to conventional chemotherapy, MAb therapies are less toxic although binding to non-malignant tissue leads to significant adverse reactions [42, 43]. For cancer therapy, several MAbs are in development. About ten antibody-drug conjugates are approved by FDA, which include CD33 MAb for acute myelogenous leukaemia (AML) (gemtuzumab), CD52 MAb for chronic lymphocytic leukaemia (CLL) (alemtuzumab), and (rituximab, ibritumomab tiuxetan, and tositumomab) are anti-CD20 MAb for non-Hodgkin's lymphoma (NHL). Other, many MAb are under pipeline or clinical trials. Rituximab is extensively used as an antibody in combination with anticancer drugs like doxorubicin, cyclophosphamide, vinca alkaloid (vincristine), and prednisone as part of the typical treatment of choice for NHL [44]. This combination has proven beneficial in clinics. Table 1.3 showed some FDA-approved MAb for the treatment of cancer.

Bone Marrow Transplantation and Donor Leukocyte Infusion

Bone marrow ablative therapy is useful in bone marrow transplantation from a healthy donor to a recipient, specifically in haematological cancer. Recurrence of tumour after transplantation is the most common problem despite a high response rate. Patients who receive allogenic transplant (graft-versus-leukaemia (GVL) effect) bone marrow rejection risk are uncommon [45].

Table 1.3 FDA-approved MAb for cancer

Brand/commercial name	Generic name	Cancer target site/receptor	Approved indications
Nexaver	Sorafenib	VEGF	RCC, hepatocellular carcinoma,
Gleevec/Glivec	Imatinib mesylate	BCR-ABL	Acute lymphocytic leukaemia, chronic myeloid leukaemia, gastrointestinal stromal tumour
Iressa	Gefitinib	EGFR	Non-small cell lung (NSCL) cancer
Nolvadex	Tamoxifen	Oestrogen	Breast cancer, ductal carcinoma in situ
Tarceva	Erlotinib	EGFR	NSCL cancer, pancreatic cancer
Tykerb	Lapatinib	Her2	Her2 positive breast cancer

Immune-Modulating Antibodies

For cancer treatment, MAb with an immune-modulating effect is in various preclinical and clinical stages, e.g. mogamulizumab, ipilimumab, tremelimumab, etc. Many of these antibodies antagonize inhibitory regulatory circuits for antitumor responses. Another class of antibodies stimulates antitumor cytotoxicity, agonistic to T cell coreceptors are being developed successfully and have proven efficacy in clinics.

Novel Adjuvants

For the treatment of a number of initial stage tumours, BCG and imiquimod have proven useful; however, for systemic delivery, neither adjuvant is suitable. As a result, researchers focused on identifying adjuvants for systematic delivery that could be used for the treatment of a wider range of tumours that include natural killer T cell agonist a-galactosyl ceramide (a-galcer, KRN7000) and agonists of toll-like receptors 9 (TLR9). TLR9 agonist, a receptor, needed for the microbial CpG recognition via pathogen associated molecular pattern. DNA when delivered into the circulation can activate immune responses [46, 47].

Antagonizing T Regulatory Cells

Regulatory T cells (Tregs) are the major hurdle for dampening antitumour immunity. Tregs are a specific subpopulation of T cells (CD8, CD4, MDSC) that can suppress immune response, to control inherent homeostasis and self-tolerance. Tregs infiltrate into a variety of tumours, and large Treg infiltrates are linked with poor prognosis

in a large range of cancers [48]. Tregs are responsible for the failure of vaccination strategies in addition to inhibiting spontaneous immunity, which can produce antigen-specific Tregs. The denileukin diftitox drug is a conjugate of IL-2 and diphtheria toxin, evolved to treat T cell malignancies [49].

1.5 Inhibition of Growth of Cancerous Lesions by Interruption of Protein Synthesis Signals

The elongation process in protein synthesis has gained the attention of cancer researchers as protein synthesis is a very precisely controlled process. Elongation factor 2 (EF2) is the main inherent protein that is involved in the elongation of peptide chains. Due to this fact, the EF2 is focused on the development of new therapies. EF2 enzyme exclusively translocate the codons in ribosomes from A to P. Due to ADP ribosylation/phosphorylation as a result of EF2 inactivation, the protein synthesis is delayed, and the protein synthesis resume as EF2 gets free [50].

The most common strategy to inactivate EF2 is through the phosphorylation of Thr56 with EF2 kinase. The phosphorylation of EF2 hinders functional binding to the ribosome, resulting in the halting of the elongation of peptide chains [51]. EF2 kinase and EF2 have an opposite action. EF2 kinase and EF2 follow a definite pattern in the cell cycle (Table 1.4).

Thus, regulation in EF2 activity opens various channels for application in cancer treatment. As apparent in the above discussion, the EF2 plays an important role in the actions of some drugs. E.g. Doxorubicin treatment results in phosphorylation of EF2, due to which protein synthesis gets stalled, which results in cell arrest in the G2/M phase. A comparatively new drug "Ontak" is developed, to target EF2 directly, and is successfully employed in the treatment of several haematological cancers [52, 53].

Table 1.4 Characteristics of protein elongation activity during cell cycle [53]

Cell cycle phase	Protein elongation capability	Mechanism of control	Output of dysregulation
G1: protein synthesis	EF2 functional	Blocks EF2 kinase (EF2K) by Phosphorylation of Ser366	G1 arrest due to forced activation of EF2K
S: DNA synthesis	EF2K functional	Activation of EF2K due to increased cAMP and Ca^{+2} levels	EF2K inhibition leads to entry into S phase
G2/M: Proofreading and cell division	EF2K functional	Inactivation of EF2K by phosphorylation at Thr56	EF2K inactivation leads to G2/M arrest

1.6 Angiogenesis Inhibition

The angiogenesis process is a complex process that is regulated by some biomolecules in the body. Some endogenous systemic or local signals performs the functions of smooth muscle cells and endothelial cell in order to repair blood vessel, which is damaged. This regeneration of blood vessels involves the sprouting of endothelial cells from existing blood cells.

The process of angiogenesis consisted of the production of protease, migration, and proliferation of endothelial cells followed by vascular tube formation and synthesis of the basement membrane.

Angiogenesis is an important process during embryo development, improved organ perfusion, and wound healing. Opposite to the chemical signals that are involved in blood vessel formation, some chemical signals are angiogenesis inhibitors. (Table 1.5) Angiogenesis inhibitors tend to erupt the blood vessels and support the degradation of existing blood vessels. The balance between activators and inhibitors is crucial for vascular homeostasis [54].

1.6.1 Angiogenesis's Role in Cancer

Tumour needs oxygen supply and other nutrients for growth of blood vessels. Availability of abundant oxygen results in metabolism of endothelial cells utilizing oxygen to form vascular network in vivo or sprouts in vitro. As oxygen is a critical factor for cell growth in both cancer cell and healthy cells, the hypoxic tumourous cells will not divide. Due to the release of proteins like EGF, VEGF, oestrogen basic, IL-8, TNF, prostaglandin E1, and E2, the endothelial cells are active vigorously in growing cancers [55, 56]. Angiogenesis inhibitors can be very effective in cancer

Table 1.5 Mechanism of action of angiogenesis inhibitors

Angiogenesis inhibitors	Mechanism of action
Soluble VEGF-1	Decay receptors for VEGF-B
Angiostatin	Affects endothelial cell adhesion, migration, proliferation (ECAMP) process
Thrombospondin-1 and 2	Affects ECAMP process
Angiopoietin-2	Opposite action to Angiopoietin 1
Platelet factor-4	Prohibit VEGF binding
Endostatin	Affects ECAMP process
Antithrombin-III Fragment	Affects ECAMP process
Vasostatin	Affects ECAMP process
Maspin	Blocks proteases
Endorepellin	Affects ECA process

therapy. E.g. Combination of drug therapy of bevacizumab with paclitaxel and carboplatin resulted in improved response for % survival in advanced or recurrent NSCL cancer patients, and pulmonary haemorrhage has been seen observed as major toxicity concern [57]. The platinum-based chemotherapy, with bevacizumab in ovarian cancer, stops progression and improved the survival rate [58]. Thalidomide is used for multiple myeloma and other cancers. Revlimid® (Lenalidomide) is used for multiple myeloma and myelodysplastic syndrome [59].

Angiogenesis contributes significantly to the tumour progression. Inhibition of angiogenesis in tumour inhibits tumour progression but does not eradicate the tumour. Hence, combinational therapy with an anti-angiogenesis agent and chemotherapy available in clinical practice [56].

1.7 Specific Drug Delivery for the Destruction of Malignant Cells

Specific drug delivery to cancer cells is useful for a safe and effective regime. As compared to conventional cancer drugs, the novel/specific drug delivery approaches (N-SDDS) added new opportunities in cancer management. Some of the approaches of N-SDDS include triggered release, receptor or ligand-based targeting, intracellular drug targeting, use of stem cells, gene delivery, magnetic drug targeting, etc. Site-specific approaches lead to selective detection and eradication of cancer with minimum adverse effects. N-SDDS can also help in reducing the multiple drug resistance and the higher influx of therapeutic agents to cancer cells. Hence, it is necessary to develop N-SDDS to effectively deliver chemotherapeutic agents to malignant cells. Due to non-specificity, the conventional delivery system tends to accumulate the drug in cancer cells as well as normal cells. This problem can be sorted with site-specific delivery techniques [60]. The goal of therapy is to minimize the general toxicity of chemotherapeutic agents and to bring overall improvement in quality of life.

1.8 Induction of Apoptosis

Apoptosis is a process of a cell's natural programmed cell death [61]. The process of apoptosis gets activated in numerous conditions such as DNA damage activated by both extracellular and intracellular signals. These two apoptotic pathways correlate with signal type. Nucleic acid damage, cytokine deprivation, and growth factor deprivation are the intracellular signals, whereas death-inducing signals are the most common extracellular signals developed by cytotoxic T cells in response to the cell that is infected or damaged. Within the cell, changes take place like caspases get activated which split cellular components necessary for normal cellular function, once apoptosis is signalled. This activity leads to the shrinkage of apoptotic cells and

undergoes changes in the plasma membrane that signal the macrophage response [61–63].

The common things in cancer, which are present in all types of cancerous cells irrespective of the cause or cancer type, consists of uncontrolled cell growth, angiogenesis, and apoptosis evasion [64]. One of the main functions of apoptosis is to prevent cancer. Mainly, the intrinsic pathway is responsible for the inhibition of cancer, whereas a wide range of pathways inhibits the apoptosis process. Cancer cells tend to survive longer due to loss of apoptotic control; this gives more chances of mutations, which can lead to more invasiveness in tumour progression, activate angiogenesis, and deregulate cell proliferation with altered differentiation. There are many causes of apoptosis evasion by cancer cells, but mainly the upregulation of BCL-2 (antiapoptotic protein) and depletion of BAX or BAK are responsible for apoptosis evasion. There is the possibility to control or terminate the uncontrolled cell growth by utilizing apoptosis in cancer therapy. This is the most suitable and successful non-surgical way of cancer treatment by utilizing cell's mechanism for death. This can be used for all types of cancer. Several anticancer drugs target various steps in both intrinsic and extrinsic pathways [62, 65, 66]. For treatment, any stage in these pathways can be targeted, but there is no proof that which target is the most effective. With the designing of more apoptosis-inducing anticancer agents, the most effective targets for apoptosis can be determined.

1.9 Smart Strategies for Cancer Targeting

The smart NPS for the treatment of cancer is a topic of interest in the field as revealed by the literature search from PubMed (dated Oct 10, 2022) shown in Figure 1.4. To increase precision and specificity through drug delivery, smart nanoplatforms are designed in a temporally and spatially controlled manner. Smart nanoplatforms result in poor specificity, and high toxicity and induce multidrug resistance.

The major challenges in drug accumulation at the malignant site are insufficient drug concentration, efflux of drugs from tumour, poor and incomplete drug release inside the tumour, and leading to the drug-resistant tumour microenvironment. The suboptimal drug concentration at the tumour site could not destroy cancer cells, resulting in acquired drug resistance [67].

The stimuli-sensitive NPS (SSNPS) could tackle these challenges in drug delivery. SSNPS is designed to instantly release the drug with the intrinsic or extrinsic stimuli at the desired site of action. SSPNS identifies the signals either from internal (endogenous) or external (exogenous) stimuli or both triggers the required drug release to the specific target site of the tumour [68, 69].

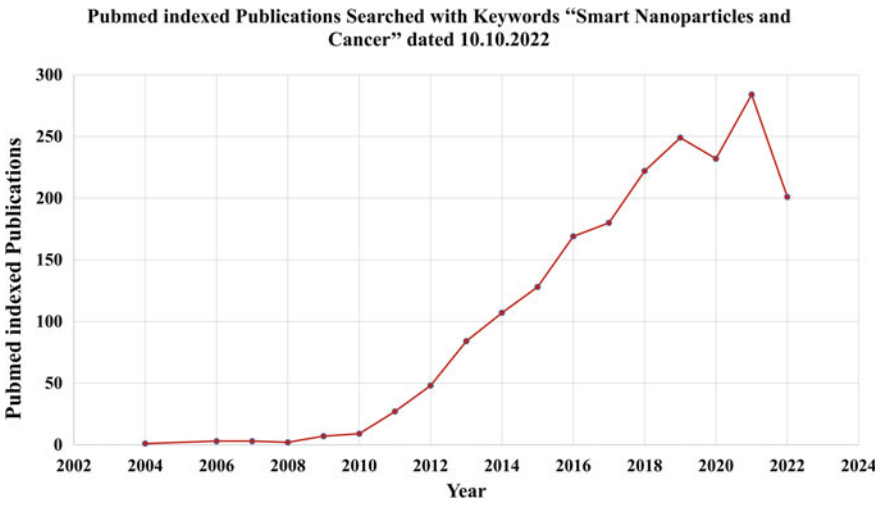

Fig. 1.4 Pubmed indexed publications searched with keywords "smart nanoparticles and cancer"

1.10 Smart Endogenous Stimulus Strategies

1.10.1 pH-Responsive NPS

The tumour cells and inflammatory cells are known to exhibit the acidic microenvironment. Therefore, the acidic pH of the tumour cells can be positively employed for the specific targeting of the tumour. Abnormally fast metabolism and uncontrolled growth, leading to higher production of lactic acid and other end products, develop an acidic environment in the tumours [70]. It provides a unique opportunity for the formulators to design the targeted NPS utilizing the exceptional acidic microenvironment of cancerous cells for reducing the toxicity associated with antineoplastic drugs. Selection of the pH-sensitive and biocompatible polymers is crucial in the formulation of the pH-responsive NPS.

pH-triggered drug release technique is commonly used for extended-release or delayed release. It is useful for drug release at the specific site of action. The pathological changes of the tumour or inflammatory responses change pH of microenvironment promoting the drug release.

The doxorubicin (DOXO) conjugated polyamidoamine (PAMAM) dendrimers were designed by the covalent conjugation of DOXO at the periphery of partially acetylated generation 5 (G5) dendrimer modified with the folic acid linked through a pH-responsive cis-aconityl linkage. It shows pH-responsive drug release at pH 5–6 which is close to acid microenvironment of tumour. The developed NPS was shown to target cancer cells over expressing folic acid receptors in KB cells [71]. In order to overcome issues in NPS, especially the leakage of the drug from self-assembled NPS and interference in recognition of the target site leading to nonspecific drug

distribution, the biocompatible and biodegradable NPS with polylactic-co-glycolic acid (PLGA) core covered by the hydrophilic bovine serum albumin (BSA-PLGA-NPS) shell were reported. These BSA-PLGA-NPS reveals increasing pH-triggered drug release of DOXO at the lower pH when tested with pH 5.0, 5.8, and 6.5. Retention of loaded cargoes increased in the presence of higher amounts of serum proteins gives better stability of the system due to the crosslinking of the albumin [72].

Cubosomes of lumefantrine (Benflumetol) with inorganic material calcium phosphate were reported by Sethuraman V. et al. for site-specific treatment of lung cancer. pH-responsive drug release at pH 4.0 was reported, revealing time dependent cellular uptake in A549 adenocarcinoma cell line [73]. Calcium phosphate is a biodegradable, biocompatible carrier for the drugs. The potential to transport a variety of biomolecules and ability to enter lysosomes by endocytosis (specific pinocytosis) makes it an ideal carrier for the drug carrier. Calcium phosphate gets easily dispersed at endosomal/lysosomal acidic pH thereby increasing osmotic pressure inside the cell leading to endosomal escape.

Eudragit S100 is employed for the delivery of 5-fluorouracil (5-FU) and leucovorin in colon cancer. The NPS was prepared by a modified double solvent evaporation technique. About 90% of the drug released at pH 7.4 shows the burst release from NPS. Significant cytotoxicity was reported with the drug-loaded Eudragit S100 NPS compared to the free drug combination [74].

Nanogels offer dual benefits of NPS and macroscopic hydrogels, where the introduction of acid-responsive chemical modification or protonatable functional groups is shown to be helpful in the cancer microenvironment. An account of pH-sensitive nanogels for cancer treatment, synthetic methods, and applications for cancer treatment is presented recently by Li Z. et al. The stability of the nanogel carriers the precision of the stimuli response, and the safety of the crosslinking agents are major issues that need to be addressed in the development of nanogels [75]. Representative examples of SSNPS with the endogenous trigger are shown in Table 1.6.

1.10.2 Enzyme-Responsive NPS

Enzymes are significant components of the biological system which offer promising capacities and ideal attributes to speed up metabolic responses. NPS which has the ability to release drug in response to an enzymatic trigger can be designed with the help of nanomaterials. The ill effects of antineoplastic agents are reduced by enzymatic catalysis of labile functional groups to give enzyme-responsive drug release when desired. There are some enzymes that are specifically overexpressed in cancerous cells, and those can be considered as a target for cancer treatment [83].

The phospholipase A2 (sPLA2) is abundantly expressed in the pancreatic, breast as well as prostate cancers. Protease enzymes matrix metalloproteinases (MMPs), cathepsins, and urokinase-type plasminogen activators are abnormally elevated in malignant cells. Especially, MMP is associated with progression, migration,

Table 1.6 Smart SSNPS for the treatment of cancer with endogenous-triggered strategies

S. No.	Drug	Trigger for the smart NPS	Responsive polymeric system/material	Target/cell line	References
1	DOXO	pH	PLGA core covalently covered with a crosslinked BSA NPS	Human breast cancer MCF-7 cells	[72]
2	Lumefantrine (Benflumetol)	pH	Calcium phosphate NPS-loaded lipidic cubosomes	A549 adenocarcinoma cell line	[73]
3	5-Fluorouracil and Leucovorin	pH	Eudragit S100	Murine colon carcinoma (CT26) cells and human adenocarcinoma colorectal cells (HT-29)	[74]
4	DOXO	pH	Polyamidoamine (PAMAM) dendrimers	Cancer cells overexpressing folic acid receptors KB cells	[71]
5	Curcumin	pH	Hyaluronic acid-based drug conjugate NPS	4T1 and MCF-7 cancer cells	[76]
6	Cytochrome C/single-stranded DNA	Thermoresponsive pH change	Gold NPS	B16 F10 melanoma cell and MDCK-GFP cell suspension	[77]
7	Paclitaxel	pH and temperature	Di(ethylene glycol) methyl ether methacrylate polymers. The polymer is modified by the hyaluronic acid	MDA-MB-231 cell suspension	[78]
8	Cisplatin	The enzyme (matrix metalloproteinase)	Gelatin NPS with concanavalin-A	Lung cancer cell line A549 cells	[79]

(continued)

Table 1.6 (continued)

S. No.	Drug	Trigger for the smart NPS	Responsive polymeric system/material	Target/cell line	References
9	Cabazitaxel	Enzyme (matrix metalloproteinase)	Block copolymer Micelles	C4–2 cells	[80]
10	DOXO, Docetaxel	Enzyme (proteinase k)	Polytyrosine polymeric NPS	RAW 264.7 Cells and HCT-116 human colorectal cancer cells	[81]
11	Paclitaxel	Redox responsive	Black phosphorus quantum dots are incorporated into cysteine-based poly-(disulphide amide)	Hepatic cell carcinoma (HepG2) human NSCL cancer (H1650) cells, mouse fibroblast (NIH 3T3) cells, and murine breast cancer (4T1) cells	[82]

and metastatic cancerous state. Cathepsins are overexpressed in the premalignant cells. Glycosidase enzymes viz. β-glucuronidase and β-mannanase, oxidoreductase enzyme viz. NAD(P) H:quinone oxidoreductase1 are also employed for smart drug release in cancer therapeutics.[83]

MMP-responsive gelatin NPS were reported, showing "on-request" controlled drug release in lung cells. MMPs are protease catalysts and profound in the tumours during various phase of cell cycle. These cells overexpress mannose receptors on the cell surface. Metallic cisplatin-loaded gelatin NPS was coated with concanavalin-A (CON-A). The surface functionalization of gelatin NPS with CON-A helps to improve the cellular uptake of the NPS. Gelatin is biocompatible, and it is a material with flexible physicochemical properties for drug delivery applications. The activity of cisplatin is greatly improved by multiple mechanisms [79].

Prostate-specific membrane antigen (PSMA) is a membrane glycoprotein present in normal as well as tumour cells, but it is overexpressed about a thousand times, especially in advanced castration-resistant prostate cancer [84, 85]. DUPA is a chemical moiety having a high affinity to the PSMA. The conjugation of DUPA or its derivatives with antineoplastic drugs can excellently target the prostate cancer cells [86]. The micelles of cabazitaxel, a taxane compound developed with DUPA, show MMP-2-responsive drug release. It is also reported that drug encapsulation in these micelles can reduce its dose and systemic toxicity [80].

Polytyrosine polymeric self-assembled NPS of DOXO revealed to have exceptionally high drug loading, higher stability, and faster enzyme-triggered intracellular drug release. About 63.1% drug loading was observed with DOXO payload, possibly assigned to strong π–π interaction between poly-tyrosine and DOXO. The results

of cell line studies reveal a better picture of in vitro antiproliferative effect when compared with the liposomal DOXO. About 17.5% drug loading was observed with docetaxel when evaluated for drug loading [81].

The higher aqueous instability of the amorphous calcium carbonate NPS has been positively converted to gain water-responsive lipase-triggered drug release of the DOXO from NPS. The NPS was coated with monostearin (MS) to ensure that the drug release will occur in the lipase-overexpressed SKOV3 cells, avoiding leakage during circulatory transport. The MS/ACC-DOXO NPS reveal a 12.45-fold plasma concentration against free drug in an animal study by EPR effect and also increases the half-life of the drug [87].

1.10.3 Redox Reaction-Responsive NPS

The utilization of redox-responsive NPS would ensure that the medication is delivered inside the target cells, and the lower number of reductive moieties in the general fails to break the redox-responsive chemical bonds. Additionally, the redox-responsive NPS overcome the main issue of uncontrolled drug release leading free drug with nonspecific distribution in the body which may be toxic to normal cells [88].

The paclitaxel-loaded black phosphorus quantum dots (P-BPQD) NPS were reported as a cancer theranostic tool hydrophobic cysteine-based poly-(disulphide amide) (Cys-PDSA) polymers which work as black phosphorus quantum dots NPS was reported which is responsive to the glutathione (GSH) via disulphide-mediated reduction. Additionally, the animal study result shows the biodegradability and biocompatibility of P-BPQD [82].

There are various approaches to redox-responsive NPS for therapeutic use. The one includes the utilization of gate men that uses the redox-sensitive bonds all through the system framework. Keratin is an inherent protein containing cysteine residue which is a potential glutathione degradable gatekeeper for the stimuli-responsive NPS, and it is nonimmunogenic [89]. Alternatively, redox-responsive linkers such as polymers and biomolecules (e.g. albumin and myglobin) [90], nanovalves like cyclodextrins [91], or metallic quantum dots can be utilized for the redox-responsive NPS approach.

1.11 Smart Exogenous Stimulus Strategies

1.11.1 Magnetic NPS

Magnetic NPS (MNPs) are widely explored for theranostic application in cancer. Superparamagnetism properties of MNP, higher magnetic moment, magnetocaloric

effect, nanosized particles, and larger specific surface area of MNP are specifically useful for biomedical and theranostic applications. MNPs can be classified as iron oxide NPS, metallic NPS with only a metallic core, shell-based ferrites, and shell-based metallic NPS. The functionalization of MNP allows the tailor-made drug delivery for the precision medicine [92–94].

The fabrication of citrate stabilized Fe_3O_4 NPS (CA-MNP) was reported. These CA-MNPs were reported to have pharmaceutical stability, desired magnetization, cytocompatibility, and better absorption rate required for cancer treatment. These CA-MNPs are loaded with positively charged DOXO HCl by electrostatic interaction [95]. The adjustment of these CA-MNPs was accounted for, where chitosan via crosslinkers to the NPS forms a cover shell like structure. Such MNPs show the higher drug release at the pH 5. The cytotoxicity studies performed in NIH/3T3, MBA-MB-231, and 4T1 cell lines show no significant cytotoxicity. The selective activity against cancerous cells against the normal cells makes them suitable treatment of breast cancer [96].

1.11.2 Thermoresponsive NPS

Hyperthermia therapy traces back to 1898, for cervical cancer. A particular impact of hyperthermia is observed at temperatures around 41.5 ± 1.5 °C. There are three zones covered by hyperthermia: focal (central), fringe (peripheral), and external (outer). The direct and immediate site of heat transfer is called central zone, where cells usually die due to necrosis. The indirect heat transfer occurs in peripheral and outer zones, and it is also associated with the apoptosis and change in microenvironment [97, 98]. It leads to cell membrane fluidity and deregulates normal functioning by the changes in inherent transport proteins, ion channels, receptors, and lipids. Hyperthermic temperatures can be achieved clinically by either ablation or mild hyperthermia [99] which are depicted in Fig. 1.5.

The synthesis of water biodegradable thermoresponsive fluorescent polymer prepared from poly(N-isopropylacrylamide) and allylamine is reported, which was conjugated with magnetic NPS (TR-MNP). These TR-MNP NPS were compatible with human dermal fibroblasts and normal prostate epithelial cells, as observed in the *in vitro* cell line studies. The cell death observed in cancerous cells when treated with empty TR-MNP was attributed to effects of hyperthermia, and the magnetic field was responsible for the targeting and localization of the drug at the target site [100].

1.11.3 Photoresponsive NPS

In the photoresponsive NPS, on photostimuli drug release is triggered from the nanosystem. This is a non-invasive method in which the target area is exposed to the

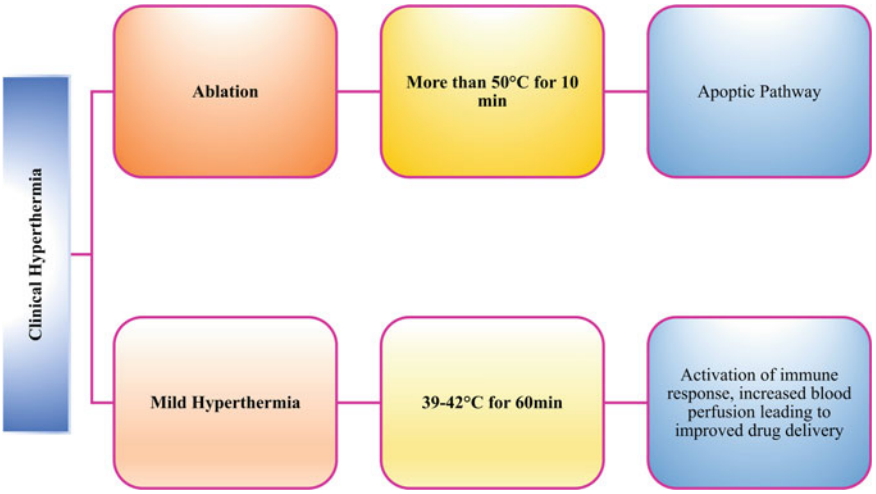

Fig. 1.5 Clinical methods to achieve hyperthermia

photons resulting in drug release, wherein light varying of wavelength is used and exposure time and intensity modulated for desired targeted drug release [101]. The photochemical reactions generally do not need additional catalysts or agents, and the by-products are usually nontoxic [102]

Phototriggered DDS are four types: (i) photooxidation, (ii)photocleavage, (iii), photopolymerization, and (iv) photoisomerization [103] as depicted in Fig. 1.6.

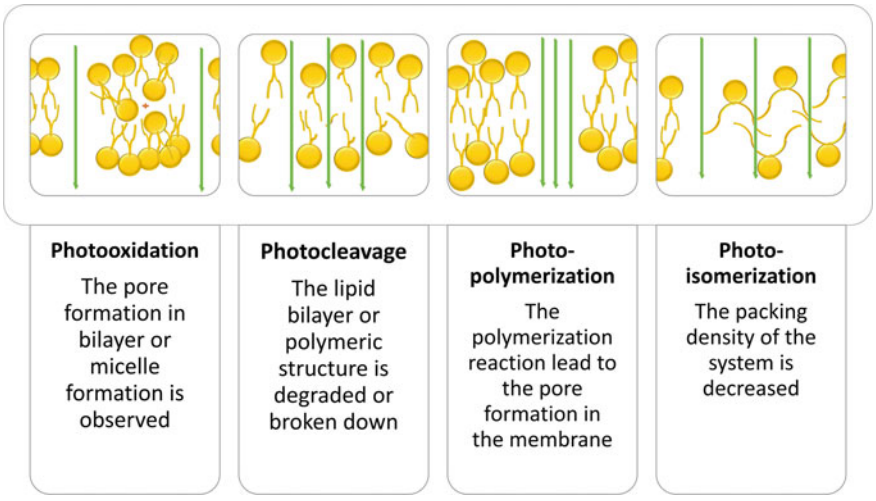

Fig. 1.6 Types of phototriggered DDS

(a) Photooxidation reactions in which radical/ROS like singlet oxygen ($^1O_2$), superoxide anions ($^{\bullet}O^{-2}$), hydroxyl radicals ($^{\bullet}OH$), or hydrogen peroxide (H_2O_2) are generated [104].
(b) Photocleavage-type drug delivery involves a linker within the polymer or lipid to form NPS which is cleaved on the trigger of appropriate light wavelength, compromising the structure of the NPS and leading to the drug release [105, 106].
(c) In the photopolymerization mechanism, in situ polymerization of monounsaturated or polyunsaturated bonds of chemical moiety occurs within the nanosystem. On exposure to the light of appropriate wavelength, the crosslinking causes a change in structural conformation (usual shrinkage of the system). This causes the drug release from the cargo [106, 107].
(d) Photoisomerization—The conformational changes occur by rotation around the double bond upon phototrigger; generally, trans to cis conversions occur.

The micelles integrating chemotherapy and phototherapy were developed using a poly(dithienyl-diketopyrrolopyrrole) (PDDP) polymer encapsulating DOXO in the hydrophobic cores of polymeric micelles which are formed by Pluronic F127. The PDDP polymer works as photothermal mediator and absorbs NIR light useful for hyperthermia. The drug release in micelles occurs by the shrinking and swelling of micelles due to the temperature variation. The enhanced cytotoxicity was observed under 808-nm laser irradiation HeLa cell lines [108].

1.11.4 Ultrasound-Responsive NPS

The ultrasound-responsive NPS is utilized for the physicochemical changes in response to exogenous ultrasound wave trigger. The ultrasound waves can work as an exogenous stimulus for drug release [109]. The schematics are shown in Fig. 1.7. The mechanistic drug release from the cargo occurs as a consequence of ultrasound trigger by thermal effects and/or mechanical changes through cavitation or radiation. These physical pressures cause structural instability and consequent drug release in sonosensitive nanocarriers. Additionally, these forces cause a temporary rise in blood artery permeability, which enhances the extravasation of nanomedicine to tumour interstitial areas and ultimately boosts the drug's cellular uptake [110, 111].

The advantages of this approach include non-invasiveness, ease of accessibility, freedom from ionizing radiation issues, manageable spatiotemporal application and effect, and higher patient compliance and affordability [114, 115]. The ultrasound-responsive NPS promotes drug release and cellular uptake, which is especially useful in cancer [116]. The benefits and drawbacks of external stimuli used for drug delivery are shown are summarized in Table 1.7

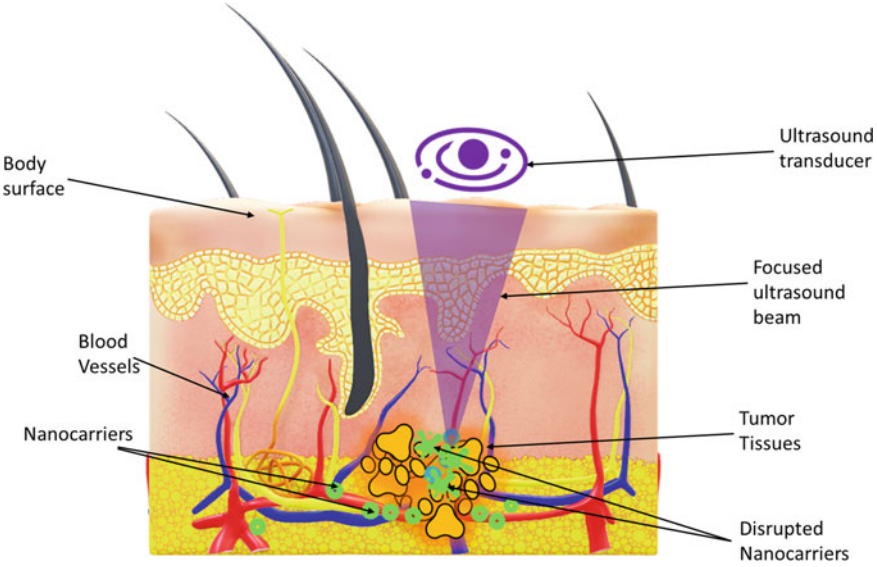

Fig. 1.7 Ultrasound radiation-responsive drug release is obtained from NPS. The controlled ultrasound radiations are concentrated at the desired tumour site. The higher amplitude ultrasound waves either disrupt or activate NPS

1.11.5 Electric Field Stimuli-Responsive NPS

Electric impulses are used for the alteration of cellular physiology, especially to increase the permeation of the cell membrane. This approach is used in cancer electrotherapy to deliver biological material or drugs [112].

1.12 Dual Stimulus-Responsive NPS

Paclitaxel-loaded pH and temperature-responsive NPS have been reported. The NPS were developed using chitosan and di(ethylene glycol) methyl ether methacrylate polymers. The polymer was modified by hyaluronic acid to actively target breast cancer cells that overexpress CD44. The NPS exhibits quick drug release in the acidic microenvironment (pH 5) than alkaline environment (pH 7.4) and at a temperature of 40 °C compared to 37 °C. NPS reported to have minimal off-target toxicity, and additionally, it has shown to induce the apoptosis in the tumour[78].

Table 1.7 Benefits and drawbacks of external stimuli in drug delivery [112, 113]

S. No.	External stimuli	Benefits	Drawbacks
1	Magnetic stimuli	Freedom from ionizing radiowaves, deeper reach is feasible, magnetism obtained via alternating magnetic field, therapeutic and diagnostic application possible	Limited to surface effect in cancer, accumulation can cause thromboembolism or higher toxicity, costly in terms of instrumentation required
2	Thermal stimuli	A non-invasive method for drug delivery, ease in the generation of heat and control, no ionizing radiations	Limited penetration power and severe hyperthermia can reduce patient compliance
3	Photo/light stimuli	Easy to use and tune, inexpensive, free from damaging radiations, higher precision possible, comparatively economic	Limited tissue penetration (near-infrared (NIR) can be used to enhance penetration)
4	Ultrasound stimuli	Non-invasive, easily accessible, better possible control, more patient compliance, comparatively economic	Limited penetration depth, difficulty in targeting moving targets like blood cells, difficulty in targeting nonhomogeneous tissues, and temperature rise can cause patient noncompliance
5	Electric stimuli	A potential alternative as it eliminates the need for chemicals increases immunogenic response and increases cellular permeation for the drug delivery	Efficiency depends on tumour size, control of the electric field is required for the safety of the patient to avoid side effects like oedema, erythema or scars, etc.

1.13 Smart Nanotechnology for Targeting of Cancer

Researcher's aim is to modify the characteristics of tumour characteristics for improved safety and obtain controlled drug release and further side effects. Explaining the tumour microenvironment has rising the treatment guidelines and diagnostic strategies such as upgrading the extracellular matrix can enhance tumour response to chemotherapeutic methods and specific access of drugs to tumour site [117]. Smart NPS is utilized to release therapeutic agents as a consequence of an exterior stimuli like heat, light, magnetic field, and ultrasound [118–120] and internal stimuli like ions, enzymes, pH, reduced oxygen levels, and proteins [121].

Recently, NPS gained a lot of interest owing to their bioimaging. The nanobiomedicine has potential as a targeting tool and could provide the optimal drug release. To overcome the poor aqueous solubility of API, they are loaded in NPS. The nature of coating on the surfaces of NPS, i.e. hydrophilic polymers, is combined with amphiphilic surfactants, permitting prompt, and uniform drug release [122,

123]. NPS prevents drug degradation, alters plasma $t_{1/2}$ and clearance, enhances the aqueous solubility of drugs, and controls the release kinetics of antineoplastic agents [124, 125]. Various smart nanocarriers are discussed below:

1.13.1 Lipid-Based Nanoparticles (LBNPS)

LBNPS presents potential carriers for the delivery of antineoplastic agents. The use of lipid-based NPS in cancer treatment has opened new opportunities by improving the anticancer activity of chemotherapeutic agents.

This delivery system has certain advantages site-specific drug release, high drug loading, low manufacturing cost, protection of active functional groups, biocompatibility, low immunogenic nature, high stability via encapsulation, ease of preparation, and large-scale production. Apart from that, LBNPS helps in the reduction of therapeutic dose and systemic side effects. This system also helps to overcome drug resistance and improve the drug concentration in tumour tissue. LBNPS showed promising results both in lab studies and clinical cancer therapy [126]. These systems have the advantage of biocompatibility and biodegradability. The main component of this system is the phospholipid, which is arranged in a bilayer structure vide their amphipathic properties. They form vesicles in aqueous media and result in higher solubility and stability of chemotherapeutic agents when they get incorporated into a bilayer structure. Liposomes entrap diverse types of drugs with varied physicochemical properties. Cholesterol is used extensively in the formulation of liposomes as it lowers the fluidity of NPS and improves the drug permeability, increasing the biological stability of NPS [127]. These are classified considering particle size and the number of lamellae. The liposomes with multiple bilayers having sizes between 0.5 and 10 nm are called multilamellar vesicles. Liposomes with a single bilayer having a size > 100 nm are known as unilamellar vesicles, when size ranges from 10 to 100 nm is known as small unilamellar vesiclular liposomes. Generally, nanocarrier vectorization through liposomes can be achieved either by passive targeting or active targeting. The influx of liposomes into tumour cells through molecular movement into cells. While active targeting involves structural modification in liposomes containing antibodies that can target malignant cells [128]. Another method can be utilized for liposomes in which stimulus-sensitive structure is created while forming liposomes. Activation modulus like temperature, magnetic field, or pH is used for site-specific delivery of anticancer agents [129].

The thermoresponsive liposomes developed from dipalmitoylphosphatidylcholine (DPPC) and cholesterol and another commercial material thermodox containing MSPC (1-myristoyl-2-stearoyl-sn-glycero-3-phosphocholine) can be used for preparation. These materials release the drug at body temperature while encapsulating the drug at a little higher temperature of 40 °C [130]. The DOXO-based thermoresponsive liposomes prepared from DPPC, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-PEG2000 (DSPE-PEG2000), and MSPC studied for

Table 1.8 USFDA-approved commercial liposomal formulations for cancer

Product name	Type	Drug	Uses
Doxil®	PEGylated liposomal formulation	DOXO	Ovary and breast cancer
DaunoXome®	Preservative free liposomal formulation	Daunorubicin	HIV-associated Kaposi's sarcoma
Lipo-Dox®	PEGylated liposomal formulation	DOXO	Multiple myeloma, ovary and breast cancer
Onivyde	PEGylated liposomal formulation	Irinotecan	Metastatic pancreatic cancer
Marqibo®	Liposomal formulation	Vincristine sulphate	Acute lymphoblastic leukaemia
Vyxeos®	Liposome	Daunorubicin and Cytarabine	Acute myeloid leukaemia
Depocyt®	Liposomal suspension	Cytarabine/Ara-C	Neoplastic meningitis
Myocet®	Nonpegylated liposomes	DOXO	Breast cancer
Mepact®	Liposomes	Mifamurtide	Non-metastatic bone tumours

targeting brain tumours using magnetic resonance directed focused ultrasound system developing local hyperthermia [131].

The pH-triggered liposomes release the drug inside the tumour around pH 5.7 and are stable at physiological pH [132]. Dioleoylphosphatidylethanolamine (DOPE), cholesteryl hemisuccinate (CHEMS), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-n-[poly(ethyleneglycol)]-hydroxy succinimide are pH-sensitive materials and can be used for liposome preparation [133].

Table 1.8 depicts USFDA approved commercial liposomal formulation for cancer therapy [134, 135].

1.13.2 Solid Lipid NPS (SLNs)

SLNs are novel colloidal DDS that contains lipids that maintain a solid state at body temperature. Solid lipid NPS is 50–1000 nm in hydrodynamic diameter. The lipids used in the formulation of SLNs are used to create a matrix for drug entrapment and these particles also consist of a mixture of glycerides and fatty acids. The lipid matrix is stabilized with the help of surfactants or polymers. SLNs are having several advantages temporal delivery of drugs, stability of the system for long period, controlled release of the drug, protection of susceptible drugs, use for delivery of lipophilic and hydrophilic drugs, ease of manufacturing, nontoxic and low cost. These advantages make SLNs, a potential drug transport carrier for chemotherapeutics agents [136]. SLNs have some disadvantages, which includes lower encapsulation efficiency and drug expulsion during storage. Due to the distinctive properties of SLNs, they have

been studied broadly as a carrier for antineoplastic agents. SLNs are utilized to improve drug absorption and bioavailability, protection of susceptible therapeutic agents and reduce the side effects of anticancer agents by site-specific delivery.

The therapeutic effect of camptothecin can be increased with pH-responsive PEGylated cationic polyoxyethylene NPS reported by Chaung et al. When tested in the human lung carcinoma (NCI-H358, CRL5802, CL1-5 cell lines), human colon cancer (HCT-116 cell line), and human HCC (HCC-36 cell line), these nanocarriers showed good activity. Additionally, it could suppress the growth of CL1-5 and HCC-36 cell lines [137].

1.13.3 Nanostructured Lipid Carriers (NLCs)

NLCs are the second-generation lipid-based nanosystems, which are derived from SLN. NLCs are composed of a combination of various liquid and solid lipids. NLCs are designed to avoid the issue of SLNs. NLCs showed higher drug entrapment capacity and minimize drug crystallization during storage. NLCs are composed of a mixture of liquid lipids and solid lipids like ethyl oleate, glyceryl dioleate, glyceryl tricaprylate, and isopropyl myristate. The average particle size of NLCs is almost similar to SLNs, commonly in the size range of 10–1000 nm. The particle size of NLC is usually affected by lipid type and manufacturing technique. The advantages of NCLs are high drug loading, can be used for both lipophilic and hydrophilic drugs, site-specific delivery that can be surface modified, controlled drug release, and lower side effects [138].

1.13.4 Metal NPS

Metal NPS is DDS, which is extensively used in biomedicine due to various characteristics like larger effective surface area, ease of preparation, good biological properties, higher efficiency, and lower systemic toxicity. Presently, the metal NPS which is mainly used in the treatment of cancer includes silver, gold, copper, lead, platinum, and other noble metals. With modification in manufacturing techniques, the shape, size, and other morphological structure can be varied. Different methods may be utilized to synthesize noble metal NPS such as laser ablation method, ion implantation method, vacuum evaporation method, liquid phase sol–gel method, and other physical method [139, 140]. On alteration of morphological characteristics, the physicochemical characteristics of noble NPS also get altered [141]. For example, strong absorption spectra have been observed by spherical gold NPS in the visible spectral band, while rod-shaped gold NPS shows strong absorption in the NIR region.

1.13.4.1 Gold NPS

In the last decade, gold NPS has became a centre of attraction for researchers due to their excellent properties and applications. Presently, the most efficient methods used to synthesize gold NPS include two phase method, electrochemical method and seed growth [142]. Also, a quite new method, i.e. biosynthesis method, is used to synthesize gold nanoparticle with smaller particle sizes and monodispersity. Gold NPS can have different morphology like gold nanoprisms, gold nanocages, or gold nanorods, etc. [143]. These particles are extensively used in biomedicine as they offer monodispersity, tiny particle size, improved cell permeability as well as higher stability. These can be used in cancer treatment by hyperthermia and inhibition of angiogenesis. Several studies show the action of gold NPS on heparin-binding proteins like basic fibroblast growth factor (bFb-GF), vascular endothelial growth factor-165 (VEGF-165), vascular endothelial growth factor receptor-2 (VEGFR-2), and epidermal growth factor receptor (EGFR) [144]. These mechanisms limit the growth and metastasis of cancer cells. While gold NPS has no inhibitory action on non-heparin-binding protein growth factors. In addition, gold NPS also has localized surface plasmon resonance effect which helps to absorb light in the NIR band, leading to hyperthermia. Hyperthermia is produced in cancer cells leading to denaturation of protein and induces cell apoptosis which results in destruction of cancer cells. Gold NPS is also reported to have photothermal therapy and can also be surface modified to get site-specific delivery or enhanced bioavailability. The limitation in the development of gold NPS is the expensive price and limited reserves that pose a hindrance to research and commercial utilization of gold NPS.

The dual-responsive theranostic Au NPS functionalized with RVRR (Arg-Val-Arg-Arg) peptides is reported by Cheng et al. which can be used for photoacoustic imaging and photothermal therapy in cancer. These Au-RVRR NPS are activated enzymatically or by the acidic environment of tumour [145].

1.13.4.2 Silver NPS

In contrast to gold NPS, the stability profile of silver NPS (AgNPs) is poor as they get gradually oxidized in an oxygen-containing liquid. But they exhibit excellent localized surface plasmon resonance (LSPR effect), good catalytic potential, and antibacterial activity; hence, these are one of the most studied metallic nanomaterials. One of the most environment friendly methods to prepare AgNPs is low-temperature nonequilibrium contact [146]. Presently, the different silver nanostructures includes silver nanocubes, silver nanowires, and silver nanospheres. The optical characteristics of silver NPS are modified by controlling the particle size and morphological characteristics of AgNPs. Silver NPS was studied for delivering cytotoxic drugs due to its controlled release, safety, and antibacterial action. The use of silver as an antibacterial agent for the treatment of cancer has gained the attention of researchers in the last few decades. Researcher observed the oxidative stress produced by AGNPs by generating singlet oxygen in cells, producing cytotoxic action, apoptosis, and

necrosis in cancerous cells. This is a new approach of treating cancer by utilizing antibacterial action of AGNPs.

The dual-responsive (pH and ROS) biocompatible, curcumin-loaded silver NPS decked in mesoporous of silica Santa Barbara Amorphous-15 which is coated with polydopamine was reported by Song et al. It reduced the chemotherapeutic effect of curcumin against Hela cells and Taxol-resistant nonsmall cell lung cell line (A549/TAX). It has the ability to control the leakage of drugs from the cargo, reducing the side effects [147].

1.13.4.3 Platinum NPS

With the discovery of cisplatin as an antitumor agent in 1969, the metal complexes gained attention from researchers for treatment of tumour. Research outcomes of cisplatin lead to the synthesis of other metal complexes such as with silver, gold, platinum, palladium, rhodium. Organoplatinum compounds act as alkylating agents in cancer therapy [148]. These compounds can result into apoptosis and necrosis of cancerous cells, as they change the structure of DNA and inhibit the cell cycle. Several researchers suggested that platinum NPS can improve anticancer activity by activating the immune responses and regulation of various signal channels [149, 150]. Platinum nanostructures are extensively used in cancer treatment they offer characteristic properties like photothermal conversion, good catalytic performance, radio-sensitization ability, and imaging ability [151, 152].

1.13.5 Ceramic NPS

Ceramic NPS generally consists of albumin, silica, or iron oxide. Nowadays, these are used as drug delivery devices if their size is less than 50 nm. Ceramic NPS is not susceptible to changes in their structure with pH changes, and they can be able to protect biomolecules against denaturation due to change in pH and temperature. Ceramic NPS has specific dielectric, optical, magnetic properties, and good stability. Roy and co-workers have studied ceramic NPS for photodynamic therapy. They used photosensitizer conjugated anticancer agent (2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide) to be entrapped in ceramic NPS. The NPS showed higher uptake of drugs by cancer cells and destruction of cancer cells by generating singlet oxygen (ROS) [153].

1.13.6 Carbon-Based Nanosystems

Carbon-based nanosystems have gained attention for their ability to attach to several drugs or ligands. They can be used for both imaging and drug delivery to cancer.

Carbon NPS has applications in fluorescent bioimaging testing due to their inborn fluorescence and biocompatibility. Amongst all the carbon nanosystem, mainly two systems carbon dots and carbon nanotubes attracted considerable attention of researchers. Carbon dots are unique delivery system which provides a platform for binding to receptor along with chemotherapeutic agents due to presence of carboxylic and amino functional group. Similarly, carbon nanotubes also emerged popularly as a potent carrier of cytotoxic agents. This system is quite nontoxic, smaller, excellent optical properties with strong ability to bind with other element for functionalization. Carbon-based nanotubes have also been explored in prostate cancer, in preclinical studies, where this system showed the induction of hyperthermia [154].

NIR light activated sgc8 aptamers functionalized single-walled carbon nanotubes and gold nanorods reported for DOXO which shows highly tumour-specific targeting [155].

1.13.7 Semiconducting Nanosystems (SCN)

Semiconducting nanomaterials (SCN) are novel nanoparticulate DDS that are utilized for photodynamic therapy (PDT), photothermal therapy (PTT), and a combination of both. These nanosystems are having great potential to use as a cargo for anti-cancer agents due to their excellent opto-electronic properties. It is very important to analyse the development and future prospective of SCN is cancer therapy [156]. The recent report with multiresponsive smart NPS prepared of zinc ferrite ($ZnFe_2O_4$) and Ox, N-rich mpg-C3N4 semiconductor, is used for the chemisonodynamic therapy and curcumin-loaded NPS tested against lung cancer cell line (A549) [157]. The semiconductor NPS releases drug at pH (5.5), ultrasound trigger, and also shows semi-enzymatic sonocatalysis.

1.13.8 Polymeric NPS

Polymeric NPS played a crucial role in advanced DDS. Drugs are encapsulated into polymeric carriers and delivered to target site of action. The controlled drug release is important for targeted therapeutic drug delivery and bioimaging of cancerous cells using polymeric cargo [158]. Polymeric nanosystems are developed to respond to a variety of internal and external stimuli for TDT in cancer. The polymers used for the stimuli-responsive SNDDS include acrylate derivatives, polypropylene, polylactic acid, polystyrene, polyethylene oxide, polycaprolactone, etc. [159, 160]. Polymeric micelles, polymeric NPS, and dendrimers are mainly reported for the smart drug delivery targeting cancer [161].

1.14 Conclusion and Future Directions

The main goals of developments in the drug delivery are to improve efficacy and reduce toxicity (improve safety) associated with the drugs. Safety and efficacy of the antineoplastic drugs are potential issues that can be addressed with the current developments in smart NPS. Nowadays, biomolecules and vaccines are also incorporated into cancer management. There is the sudden surge in the number of biologic products approved by the regulatory agencies worldwide. Moreover, the safety and efficacy of newly developed products are necessary under the legal requirements for regulatory approvals from USFDA. Efficient drug transport and minimum side effects at low cost are mainstay in the designing of the smart NPS. The drug targeting selectively to cancer tissue can reduce these associated undesirable effects of the drug. The selection of the materials (polymers, lipids, or inorganic nature) considering the nature of drug and the target site physiology are basic elements that are considered for the development of the smart NPS. There is scope to explore the avenues in a surface modification which could help to overcome toxicity of surface modified NPS. The smart NPS must possess optimal stability, safety, release higher amount of drug in the tumour for the desired time period.

Although the interest of researchers working in nanomedicine is increased in recent past, technology transfer from laboratory to industrial scale is still a challenge [162]. The properties of the NPS (size, shape, surface charge, morphology, and dispersity) are affected by the method of preparation and material used for the preparation. To maintain these properties in scale up requires efforts of the person skilled in state of the art; otherwise, it can affect the stability of formulation and efficacy of the drugs loaded in the cargo. These properties are also crucial for the in vivo release and distribution in the target site and to decide the safety of the nanocarriers. There is a need to develop standardized procedures and manufacturing equipment's for drug carrier synthesis working at an industrial scale. In order to avoid variations in formulation quality when products are transferred from lab to industry, chemistry manufacturing and controls (CMC) must be considered. The methods and procedures developed with consideration of FDA guidelines related to quality by design can help to reduce efforts and development time for the manufacturing of smart NPS. To be smarter, the NPS must possess desired safety and efficacy.

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Chapter 2
Implementation of Biomedical Engineering Tools in Targeted Cancer Therapy: Challenges and Opportunities

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Contents

Abstract Cancer is a malignant growth of cells that is unrestricted and is one of the major reasons for death. There are two essential helpful techniques in treating illness: killing past growths or tumors of harmful cells and controlling further growth

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or development of harmful cells. Different types of cancer therapy include drug delivery, immunotherapy, radiotherapy, and robotic-assisted laparoscopic surgeries. These therapies may be generally assembled into radiotherapy to destroy cancerous cells and diminish tumor size; immunotherapy activates the immune system of the body to fight against sickness, drug delivery, and medical surgeries to remove the tumors from the body. Chemotherapy and radiation play a major role in treating cancer; however, these traditional treatments of radiation and chemotherapy have few limitations. The aim of targeted cancer therapy is (1) to convey a more portion of an antitumor medication straightforwardly to the portion of growth, to increase drug grasp by malignant cells/cancer cells (2), and (3) to reduce drug grasp by noncancerous cells. Successful cancer-targeting therapies need both passive and active targeting plans and a very well understanding of physiologic hurdles for targeted drug delivery. Targeted therapy in cancer treatment is split into two different general categories: signalling and delivery. The standard chemotherapy drugs can be taken as signal interchange therapeutics since they aim and inhibit signal transduction requisite which is required for malignant growth. Passive targeting takes favor of the larger vascular permeability and poor lymphatic drainage of tumors that result in the accretion of micro- and nanoparticles in tumor tissue. Active targeting utilizes ligands to notably target receptors that are overexpressed on cancer cells. Ligands are molecules, which are namely transferrin, folate, aptamers, and epidermal growth factor (EGF), which bind to receptors on the surface of a cell. Ligands are associated with anticancer drugs or particle-encapsulated drugs to target malignant growth or tumor endothelium. This chapter covers bioengineering procedures to improve the targeted delivery of cancer therapeutics and also includes targeting particles, moieties, and stimuli-responsive drug release implementations. This chapter focuses on different target mechanisms and design criteria.

Abbreviations

CAR	Chimeric antigen receptor
CNTs	Carbon nanotubes
CSC	Cancer stem cells
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptors
EPR	Enhanced permeability and retention
IV	Intravenous
MAB	Monoclonal antibodies
MRI	Magnetic resonance imaging
NDDs	Nanosized drug delivery systems
NP	Nanoparticles
RNA	Ribonucleic acid
SMI	Small molecule inhibitors

TAA Tumor-associated antigens
TCR T-cell receptor
VEGF Vascular endothelial growth factor

2.1 Introduction

A malignant growth or cancer is a gathering of more than 100 unique illnesses. It can foster anyplace in the body. Malignant growth can happen anywhere in the body organ; it comprises cells in trillion numbers. Human body cells start to develop new cells since the body needs them regularly. When cells become old or got damaged due to any reason, it starts dividing and new cell starts to form.

Malignant growth is the one cause of death worldwide and accounted for 9.6 million deaths in 2018. Liver, colorectal, lung, prostate, and stomach cancer are the most widely recognized disease faced by men, while thyroid, lung, cervical, colorectal, and bosom tumors are the disease faced by ladies [1].

2.1.1 Start of Malignant Growth

The cell is the fundamental unit of the human body. Cells keep dividing to form new cells since it is the requirement of the body. Whenever cells get damaged in the body, it gets divided to form new cells.

Malignant growth starts when the hereditary change obstructs this precise interaction. Cells begin to wildly divide and form new cells. These wildly grown cells form a mass which is called growth or tumors. This type of tissue growth can be dangerous or may be harmless. One harmful growth is threatening, and it can extend to other organ parts of the human body. Harmless cancer means the tumor can develop but won't spread. All kinds of malignant growth are not cancer. Few incorporate leukemias and most are kinds of lymphoma and myeloma.

2.1.2 Types of Malignant Growth/Cancer

Depending on where the growth is been started, it is been divided into four fundamental types.

- **Carcinomas**: It is the growth that happens in the skin or tissue that covers the outer layer of internal organs. It is widely recognized as malignant growth. Cases of carcinomas include breast and prostate cancer, and cellular breakdown in the lungs.

- **Sarcomas**: It develops in the tissues which help to support and connect the entire body. It can foster in muscles, bones, ligaments, fat, nerves lymph vessels, veins, ligaments, and joints.
- **Leukemias**: It is a disease found in the blood. It starts when healthy platelets get modified and grow wildly. The four fundamental sorts of leukemia are as follows:
  - Acute lymphocytic leukemia
  - Acute myeloid leukemia
  - Chronic myeloid leukemia
  - Chronic lymphocytic leukemia.
- **Lymphomas**: It is a malignant growth that grows in the lymphatic system, the vessels and glands which fight diseases from the lymphatic system. Two primary sorts of lymphomas are as follows:
  - Non-Hodgkin lymphoma
  - Hodgkin lymphoma.
- **Other types of cancers which develop on organs**
  - Bladder
  - Breast
  - Colorectal
  - Kidney
  - Lung Cancer—non-small cell
  - Lymphoma—non-Hodgkin
  - Melanoma
  - Oral and Oropharyngeal
  - Pancreatic
  - Prostate Cancer
  - Thyroid
  - Uterine.

2.1.3 Spreading Out of Cancerous Cells and Its Diagnosis

As mass growth develops, the lymphatic or circulation system will spread cancer cells to different parts of the human body. The disease cells develop and may form new growths during this circulation. This process is known as metastasis. Firstly, cancer starts spreading to the lymph nodes which help the body to fight infection. Lymph nodes are present in clusters in different parts of the body, like the neck, under the arms, and groin area [2].

Cancer can spread through the blood circulatory system to different parts of the human body such as the cerebrum, lungs, liver, and bones. The name of the tumor depends on where it started in the human body. For instance, if the breast cancer expands to the other parts, it is named metastatic breast cancer; it won't be named

with other cancer names. Deep Learning [38] can be used as a powerful method in the early recognition of cancer.

When the person visits to doctor with unusual symptoms, the diagnosis begins. Medical history and symptoms will be verified by specialist doctors. To find the cause of the symptoms, more tests and diagnose will be done. Many persons with malignant growth show no symptoms. For these people, cancer will be diagnosed during another medical test for some other symptoms.

At times, a specialist doctor finds malignant growth after a medical test in a healthy individual. Screening tests may include incorporating mammography, colonoscopy, and Pap test. One person requires more screening tests to confirm or disconfirm cancer. A biopsy is the only way to diagnose cancer. A biopsy is testing of the small tissue for additional study. After the biopsy, the person will be done with the additional diagnosis.

2.1.4 Various Kinds of Cancer Treatments

2.1.4.1 Biomarker Testing for Treatment

In biomarker testing, genes, proteins, and other substances will be tested for further information on cancer. Biomarkers can be utilized to decide if an infection is available, to decide how forceful the infection is, and also to anticipate how well the body will react to a treatment.

Right now, tissue biopsies are the best way to affirm a finding of cellular breakdown in the lungs; they are likewise the standard method for recognizing driver transformations. The deoxyribonucleic acid will be released when the cancerous cell dies. This released deoxyribonucleic acid enters into plasma. In this situation, DNA is referred to as circulating tumor deoxyribonucleic acid. The vein is used to take the blood samples, and it will be sent to the clinical laboratory to check the presence of the driver transformations.

2.1.4.2 Chemotherapy

Chemotherapy uses drugs to treat cancer. This sort of disease therapy works by holding malignant growth cells back from developing, partitioning and making more cells. Chemotherapy can be utilized as a therapy for the majority of various diseases.

Chemotherapy is a fundamental drug. This implies it goes through the circulatory system and arrives at all pieces of the body. There is a wide range of sorts of chemotherapy. As a general rule, drugs utilized for chemotherapy are strong synthetic substances that treat disease by going after cells during explicit pieces of the body affected [3]. Cancer-infected cells will be influenced more compared to ordinary cells.

The DOX agent is used in this therapy. DOX is also most effectively used to treat breast cancer. Paclitaxel is also one popular agent used in treating breast cancer. The efficiency increases when targeted drug delivery is done.

Different ways of providing chemotherapy are mentioned below.

- ***IV-based Chemotherapy***: In this method, veins are used to inject the drugs. Based on the status of cancer, it may take a couple of minutes to a couple of hours to cure.
- ***Oral-based Chemotherapy***: In this method, drugs were given through the mouth. Oral therapies for malignant growth are currently more often used.
- ***Infused chemotherapy***: During this process, shot is given in chemotherapy**.** The shot might be given in a muscle or infused under the skin. You might get these shots in the abdomen, leg also in the arm.
- ***Artery-based Chemotherapy***: From the heart to the entire body, blood circulation is carried by the artery. If the artery is used during chemotherapy, then injected medicine will reach the cancerous cell.
- ***Topical-based Chemotherapy***: In this, medicine will be in the form of a cream that has to be applied to the skin.
- ***Hormone Therapy***: Specific kinds of work carried out by the cells are controlled by the protein substances called hormones**.** This therapy is used to slow down cancer which is going to occur in the breast and prostate glands which use hormones to grow. A few hormones like testosterone, progesterone also estrogen are responsible for the proper functioning of the body. Various glands will produce various hormones.

The growth of hormones depends on the type of tumor. The rapid spread of cancer can be controlled by hormones. The tumors treated with hormones are known as endocrine therapy or hormonal therapy. This therapy is used to slow down cancer which is going to occur in the breast and prostate glands which use hormones to grow [4].

2.1.4.3 Hyperthermia

This therapy is to kill the cancer tissue using excessive heat without damaging any normal body tissues. The body tissue is heated up to 113 °F which kills cancer cells. This therapy will be done with no harm to other normal tissues. Different types of hyperthermia therapy are shown in Table 2.1.

2.1.4.4 Immunotherapy

This therapy boosts the body's immune system to battle cancer. To improve the immune system of the body, it uses the components made by the body and helps to destroy the cancerous cell. Different types of immunotherapy are shown in Table 2.2.

Table 2.1 List of different types of hyperthermia therapy

Types of hyperthermia therapy	Description
Local	The ultrasound wave's electromagnetic waves or radio waves are used to generate energy to produce heat that targets the infected cell. This method is used for small growths below the skin
Regional	A wide area of our body parts is treated using this method
Whole body	This method is used if the entire body gets affected by a tumor. In this, body temperature can be increased by placing a cancer-affected person in hot water, using warming covers and hot chambers

Table 2.2 List of different types of Immunotherapy

Types of immunotherapy	Description
Checkpoint Inhibitors-based Immunotherapy	These will block the proteins which reduce the immune system by the cancerous cell attack
T-Cell-based Immunotherapy	The innate capacity of the T cells is improved to manage the attack of cancerous cells
Monoclonal antibodies-based Immunotherapy	Proteins are created which are responsible for the immune system to fight against cancerous cells
Vaccine-based Immunotherapy	These will help to recognize the immune system of the body to fight against cancerous cells

This therapy activates the immune system of the patient system. 5 classes are classified in this: (i) lymphocyte-promoting cytokines, (ii) agonistic antibodies against co-stimulatory receptors, (iii) checkpoint inhibitors, (iv) engineered T cells such as CAR T and T cell receptors (TCR) T cells, (v) cancer vaccines.

2.1.4.5 Photodynamic Therapy

In this therapy, the drugs used are activated by light. These drugs kill any abnormal or cancer tissues. It is cytotoxic to cancer tissue. The photosensitizer is spread over the tumor site. Using molecular oxygen in the existence of light at a particular wavelength, when applied, produces reactive oxygen species, which make oxidative disturbance of the elements in the cell [5]. This makes the cells get destroyed in turn the cancer cell dies. The oxygen level is decreased in this process, and the nutrient supply is disturbed in the cell. This eventually kills the tumor.

Photodynamic treatment is a two-step process. The initial step is done using the photosensitizer. The medication might be taken by mouth, given through an IV, or

spread on the skin, contingent upon where the growth is in the body. Following 24–72 h, the majority of the medication will have left typical cells yet stay in malignant growth or precancer cells. Then, light sources are used to expose the tumor.

Light is applied depending on the location of the cancer. For skin growths, the light is pointed right at the disease. The doctor will perform an endoscope for the growths in the throat, lungs, and airways. Specialists will use a small with less weight tube for checking inside the body. This endoscope is used to send the light waves using the optic cable.

2.1.4.6 Radiation Therapy

This treatment involves high radiation, to obliterate the malignant growth cells and shrink the cancerous cell.

Working of Radiation Therapy

This treatment destroys or slows down the growth of tumor when this therapy is given at high doses. The DNA of the cancer cells is damaged which prevents them from growing further or dividing further. When the cells die due to damage, it is broken and removed from the body.

Cancer cells are not killed immediately in this therapy. It takes weeks of a treatment since enough damage has to be done to the DNA of the cells to die. Once this therapy is completed, the cancer cells keep dying for weeks or months together.

Different Radiation Therapies

There are 2 types of this. They are the internal beam and the external beam. The type of radiation therapy used depends on factors which include: tumor's location, size of the growth, cancer type, how far from normal cells, medical history and health condition of the patient, age factor, and any other medical conditions.

External Beam Radiation Therapy

In this, machine radiation is aimed at the tumor site in the body. It may be noisy and large. The machine won't touch the patient but moves around, spreading radiation to the part of the body from many directions. This procedure treats a particular part of the body, so it is called local treatment. Suppose, if the cancer is present in the lung, the radiation is directed only to the chest of the patient and not to the complete body.

Internal Radiation Therapy

In this procedure, the radiation source is put inside the human body. It can be liquid or solid. Brachytherapy uses the source in the form of a solid. The capsules, seeds, or ribbons are used to place the radiation source near the tumor. It is also a local treatment like an external beam that treats a particular part of the body. The radiation source releases the radiation to the body for a particular duration.

Systemic therapy uses the source in the form of liquid. In this, the treatment travels throughout the body in blood tissues seeking and destroying the tumors. Systemic radiation therapy is given through a vein via an intravenous line or by swallowing or by injection. With this systemic radiation, the body fluids such as sweat, urine, and saliva will also give out the radiation for a while [6].

Reason for Treating the Patient Radiation Therapy

When this therapy is used, it can cure cancer and prevent it from dividing and growing and returning. This is called palliative treatment when it is used to ease symptoms of cancer. External beam procedures will shrink the tumors to treat pain and other problems caused by cancer which include breathing issues or loss of bowel and uncontrolled bladder. The pain from cancer may spread to the bones and that can be treated with systemic radiation therapy drugs known as radiopharmaceuticals.

(a) **Stem Cell Transplant**: It is used to replace the bone marrow which is not able to produce healthy blood cells which were damaged due to cancer treatment. It is usually used to assist people with lymphoma and leukemia. This method is used to improve the body's capacity of producing cells after radiation and chemotherapy treatment. Autologous transplant uses their own body to produce stem cells. In some cases, the disease is treated with a high-portion, escalated chemotherapy, or radiation treatment therapy. The immunity of the body and the stem cells will be damaged due to this procedure. Auto-transplant is the process where the immunity of the body will be improved after the chemotherapy. Donors were used to giving stem cells to the cancerous person after the chemotherapy is known as an allogenic transplant. If the umbilical cord is used to remove the stem cells, then such a transplant is known as an umbilical cord transplant.

(b) **Surgery**: In this procedure, the cancer tissue will be removed by operating on the body. During cancer, treatment surgery is performed due to several conditions.

- ***Analysis***

Different types of malignant growth can be found by using the technique called a biopsy. There are various types of biopsies. The physician will remove some tissues by making a little slice in the skin of the human body. The magnifying instrument is used by the pathologist to verify the biopsy taken for the detection of the disease.

- ***Arranging***

Arranging a medical procedure is finished to figure out how enormous the growth is, in the event that it has spread. Physicians may take lymph nodes close to the disease to learn on the off chance that it has spread. Lymphadenectomy is the procedure of removal of lymph nodes. The protection from cancerous cells is produced by the lymph nodes.

- ***Cancer evacuation***

Eliminating growth is a typical sort of medical procedure which is known as resection. Physicians may take the entire growth or the part of the solid tissue close to it. The tissue around the cancer is known as the edge.

Cancer evacuation for the most part requires a bigger opening or cut than a biopsy. Laparoscopic surgery is an example of a less intrusive choice. These utilize tiny instruments and cuts. With a less intrusive medical procedure, the cancerous patient will have reduced pain and faster recovery.

- ***Debulking***

The removal of the part of the growth using a medical procedure is known as debulking. The entire tumor can't be removed by a physician. These tumors can harm different parts of the body [7]. Chemotherapy, radiation treatment, or different therapies may be given previously or after this kind of medical procedure. This can assist with contracting the growth and treating the disease.

- ***Palliative medical procedure***

The objective of a palliative medical procedure is to ease incidental effects brought about by cancer. It can work on your satisfaction on the off chance that you have progressed disease.

- ***Reconstructive medical procedure***

Treating malignant growth can fundamentally have an impact on how we look or how our body functions. Reconstructive medical procedures can assist with the impacts of disease therapy.

2.1.4.7 Targeted Therapy

This treatment targets the changes that will happen in cancer cells which will help to divide, grow, and spread. Small atom drug medicine is sufficiently small to enter cells adequately, so they are applied to focus on inside cells. Laboratory-generated proteins named therapeutic antibodies are used to detect cancerous cells and attach them.

- ***Classes of targeted therapy***

Monoclonal antibodies (MABs) are drugs that search for a specific protein in tumor cells and bind with it outside the cell or on the cell surface. These are created at the research center. MABs attach themselves to a protein in cancer cells and block cell growth and proliferation. There are many different MABs developed at the center which are based on the antibodies available in the human body. Different MABs target different diseased cells based on the proteins available at the tumor site. The working of these depends on the protein they target. These can kill the tumor cells or stop their growth. Cancer cells have growth factor receptors, and MABs block these receptors. Some MABs carry the drug directly to the tumor sites. MABs can also affect the immune system which can kill the tumor cells in a better way. It has some limitations. The majority of the patients develop the stubborn disease within one year of treatment, and only, some patients respond to the antibodies. The efficiency of the treatment depends on how expressive the target molecule is. These target molecules are bound by the antibodies in these therapy mechanisms. Monoclonal antibodies can affect the cancer cell solely, or they can be combined with other therapy methods and drugs to have improved efficiency [8].

Small molecule inhibitors (SMIs) target proteins available inside the cells. These block the pathways of signal transduction. SMIs hamper tumor cell growth, movement, and creation of new blood vessels. These are manufactured by combining chemicals at the laboratories. SMIs are oral drugs that remain only for an hour in the body and have to be consumed daily. SMIs are nonspecific so can target more than one protein.

mAbs and small-molecule inhibitors differ in several pharmacological properties. mAb proteins are larger compared to SMIs. mAbs are injected by means of veins whereas SMIs are consumed orally. Because of the blood–brain barrier presence, the delivery of drugs to brain tissue has less efficiency when it comes to mAbs as the molecular weight of mAb is large. mAbs are less organized for tissue penetration, blood clearance, and tumor retention than small-molecule agents due to their molecular size. mAbs remain for more days in a body than SMIs allowing for weekly once dosing compared to SMIs which require daily dosing. mAbs cannot move through cellular membrane, so it can act only on molecules which are expressed on the cell surface. SMIs can target any molecule irrespective of their location. mAbs are more specific to target compared to SMIs. The unfavorable effects associated with SMIs and mAbs are mild which are rash, acne, dry skin, diarrhea, nausea, vomiting, etc.

2.1.5 Limitation/Difficulties in Cancer Therapy

1. Finding the CSCs (Cancer Stem Cells) is very difficult.
2. The drug opposition properties of CSCs make them insusceptible to anticancer medications

3. Absence of malignant growth epigenetic profiling and explicitness of existing epi-drugs
4. Issues related to malignant growth analysis make it hard to treat
   - Oesophageal Cancer
   - Prostate Cancer
   - Pancreatic Cancer
5. Inaccessibility of compelling biomarkers for malignant growth conclusion and anticipation
6. Limitations of ordinary chemotherapeutic agents.
7. Metastasis represents a tremendous issue in malignant growth treatment

The purpose of the therapy is to get a cure the treatment for cancer. This may not be completely possible due to the limitation in treatment.

2.1.6 Organization of the Chapter

The main aim of this chapter is as follows:

In Sect. 2.1, we have briefed about cancer disease, its growth, types, and treatment methods. Section 2.2 briefs the different target techniques available for cancer treatment. It also highlights the side effects of the targeted therapy. Section 2.3 describes different drug delivery strategies for the therapy. It also describes the use of nanoparticles in the diagnosis and therapy of the tumor. Challenges faced during drug delivery and therapy are mentioned in Sect. 2.4.

2.2 Different Types of Targeting Techniques

Surgery, radiotherapy, chemotherapy, and hormone therapy are the main customary antitumor therapeutic methods. These approaches are not specifically targeting the tumor areas and hence are not much active in the major patients. Nonspecific targeting of tumor sites forces them to use higher doses of drugs so that they can reach the tumor region. In nonspecific therapy approaches, the drug is distributed throughout the body, and a small portion only reaches the tumor site. There are two main hurdles to sending the drug to the tumor region. First, drug delivery to normal cells must be stopped somehow. The next is the direct target of drugs into cancerous cells.

The therapy method which takes care of these factors is known as targeted cancer therapy. Targeted cancer therapy can identify the difference between normal and cancerous cells. Targeted drug delivery accumulates the drug in the cancerous cells and declines the drug outflow into healthy cells. The targeted delivery method of drugs raises the efficiency of the treatment and reduces the adverse effects. There are

Table 2.3 Targeted therapy for treating different cancer

Cancer	Cancer types
Blood cancers	Lymphoma, leukemia, multiple myeloma
Brain cancers	Neuroblastoma, glioblastoma
Breast cancers	HER2-positive breast cancer, BRCA gene mutation breast cancer, triple-negative breast cancer, hormone receptor-positive breast cancer
Digestive system cancers	Gastrointestinal stromal tumor, pancreatic cancer, colon cancer, neuroendocrine tumors, stomach cancer, liver cancer, gallbladder cancer
Head and neck cancers	Nasopharyngeal cancer, oropharyngeal cancer, nasal cavity, and paranasal sinus cancer, oral cancer
Lung cancers	Mesothelioma
Reproductive system cancers	Cervical cancer, prostate cancer, and endometrial cancer
Skin cancers	Melanoma
Urinary system cancers	Bladder cancer, Kidney cancer

two general targeting methods, namely passive and active targeting. Different types of targeted therapy have shown in Table 2.3.

Passive targeting relies heavily on the disease. In this approach, a specific drug is loaded into a nano-delivery system and sent to the cancer site. This approach avoids the distribution of drugs into noncancerous cells. This method is entirely dependent on diffusion-mediated transport into the tumor, size, and shape. This approach can be controlled by adjusting the size, shape, and surface dimensions of the nanoparticles. Passive targeting approaches sometimes may not differentiate between normal and cancerous cells.

Picking up the most suitable targeting agent is a challenging task in the case of an active targeting approach. Successful transportation of nanoparticle systems to diseased cells is also another challenging task of an active targeting method. The capability of targeting agents or ligands to bind themselves to the tumor surface is also the factor on which active targeting methods depend. The strong bonding between the targeting agents and tumor cell surface triggers receptor endocytosis which enables the agents to be delivered to the tumor-specific regions. This approach makes use of strong interactions such as ligand receptors or other molecular recognition to design a more specific delivery system [9]. It diminishes the chances of delivering drugs to normal cells and can easily locate the tumor sites. Active targeting takes advantage of the over-expression of certain receptors on the tumor cell surface.

In the case of passive targeting, efficiency of treatment reduces since very low drug gets to reach the tumor site. A passive target approach can pose a potential toxicity problem as it can't clearly distinguish between normal and cancerous cells. Aggregating the payload within the tumor cells does not guarantee the delivery of a therapeutic agent to the tumor region in an active target approach as the components within the cell make it difficult to release at the site.

The low molecular weight of the anticancer agents is the reason for not systematically circulating it to the cancerous cell using filtration. Drug molecules get distributed in a larger volume, and they get accumulated in normal cells due to the size and hydrophobicity of the anticancer agents.

Nontargeted drug delivery has several disadvantages over its counterpart. In the case of a targeted drug delivery system, the drug can be delivered directly to the affected cells avoiding spreading toxicity to noncancerous cells. Targeted drugs use very strong therapeutic warheads. The efficiency of these warheads is nil when used in nontargeted drugs with a maximum tolerated dose. The targeting ligands can be exploited to bring about a companion diagnostic agent that can be used to select patients whose pathologic cells overexpress the directed receptor.

Nanoparticles take favor of the leaky tumor vasculature to ensure enhanced permeability and retention (EPR) responses. Nanoparticles accumulate to a significant extent in the tumor microenvironment. The effect of EPR depends on the surface properties of the nanocarriers and their size. So, care should be taken while designing nanoparticles to achieve the maximum target and therapeutic efficacy. Attaching the ligand or a monoclonal antibody with the nanocarriers for drug delivery to cancerous sites known as a molecular recognition process is preferred in the active target approach. Targeted drug delivery entails antibody-mediated targeting of the nanocarriers or ligand mediated. The therapeutic index of the drug is improved by improving the efficiency of the medicine through ligand-mediated targeting of nanocarriers which can reduce the spreading of toxicity to normal cells. Research is being conducted on many proteins, peptides, vitamins, nucleic acids, and glycoproteins to understand features make full use of them, and derive benefits so that these can be implemented to develop nanocarriers.

In the active target method, specific ligand receptors interact with each other which can be used to define the precise interactions between the carrier and the tumor cell. These exchanges are possible only if the two components are in close vicinity. The delivery system available now cannot lead themselves to the exact site, but they reach the intended part through blood circulation and extravasation followed by intratumoral retention and distribution.

The nanomedicine field has been developed by identifying disease-specific biomarkers that have opened the door toward active targeting mechanisms through which the limitations of passive target approaches are overcome. The active target approach uses at least one targeting moiety bonded with the surface of the nanoparticles. These act together with antigens or receptors which specifically affect the tumor cells more than the normal tissues. In an active targeting, approach nanoparticles are transported through a precise path to reach the exact diseased cell. To accumulate nanoparticles in the diseased site, ligands are used to target intravascular tumor cells. A successful drug carrier can be designed by combining the active target mechanism with an optimized nanoparticle design. There are numerous active targeting methods involved such as attaching an antibody to a determined cell surface protein, and the use of a synthetic targeting moiety.

The passive drug target method involves the following steps:

- Initially, the physiological conditions of the target region have to be clear, and the nature and size of the tumor need to be found.
- Delivery carriers with a definite sub-atomic weight, atomic size hydrophilicity, and neutral charge should be prepared.
- The delivery system designed should be sensitive to the pH, temperature, and charge of an enzyme. The system should be designed such that its coating should remain stable protecting the normal tissues while circulation through blood. It should melt and release the drug at the tumor site when it reaches the intended area [10]. The carrier design should be thermo-sensitive

Many factors contribute to the design of a carrier in a targeted approach in cancer therapy. The size of the particles in a carrier should be medium in terms of nanoscale so that they can easily flow through the blood vessels. They should have a hydrophilic surface so that it is easily soluble in blood at the tumor sites.

In a passive target approach, the drug circulation to the tumor site is a prolonged passive process. The size of the nanomaterial influences the rate of reaction of the same at the tumor sites. Magnetic fields and ultrasounds are used in an active target approach to make a visual representation of the tumor, act upon nanocarriers and target them, and the drug will be released at the required site. This approach provides simultaneous scanning and treatment real-time targeting and targeting of deep-seated tissues. Magnetic nanoparticles are used as imaging agents in the case of an active target method. Ultrasound focusing creates pores in the layers for the drug to enter the target tissue. Ultrasound-based target approach releases drugs by disrupting the drug-loaded carriers. Targeted hyperthermia kills tumor and opens the route so that drugs get infused into the core of the tumor site. This approach provides the image of the tumor cell and activates the release of the drug at the same time. The ultrasound approach can be combined with the magnetic field approach to provide enhanced benefits.

2.2.1 Ligand-Based Targeting

In the ligand-based cancer therapy approach, peptides, transferrin, vitamins, etc., are used as targeting moieties. These ligands can easily identify some of the characteristics of a targeted tumor cell. Because of these identifications, the drugs present in the nanocarriers can be directly delivered to those sites only. Healthy tissues won't be identified by the nanocarriers carrying drugs as these areas have different characteristics than that of tumor sites. Ligands contain molecules that can identify the antigen or receptors at the cancerous cell and bind with them. These ligands bind with the cell surface receptors. Many factors contribute to the interaction of ligands with the receptors such as high avidity and low density. The main aims of the NDDs are to reduce the degradation of drugs, prevent side effects, improve the active effect on the diseased cells, and avoid releasing drugs the normal tissues. Transferrin-based (Tf) target approach is used to deliver the drugs to the brain. Tf ligand transports

the molecules of the delivery system across the blood–brain barrier. Tf ligand has the capacity of overcoming drug resistance so that it could be easily coupled to the delivery vehicle. Tf-based nanocarriers are known to be more efficient than its counterpart. Vitamins also can be used as targeting ligands in target therapy approaches. Storage and circulation capacity, cost, nontoxic nature, and non-immunogenic characteristics of folic acid are the main reasons for its use in target therapy. Folic acid can easily be conjugated to the nanocarriers and can easily combine with the folate receptor which is the marker overexpressed in many cancer cells. In lung, prostate, and breast cancers, cell epidermal growth factor receptors (EGFRs) are specifically available and can be used for a direct target approach. To EGFR proteins, EGF binds clearly and allows the cells to divide. EGF is used as a ligand in the drug delivery system and can act as a smart vehicle for the target approach. Tumor cells overexpress some unique proteins termed tumor-associated antigens (TAAs). These TAAs give significant bits of knowledge to antibody-mediated targeting. These antibodies-coupled nanocarriers are specific to a particular cancerous cell and stable toward the biological system. These advantages make it a more attractive drug-targeting system. Many receptors are overexpressed in many different cancer cells which can be used to respond in the case of an antibody-mediated target approach. Vascular endothelial growth factor (VEGF) is the one accountable for the development of new blood vessels at tumor sites. VEGF receptors are used in the treatment of a variety of leukemia. Aptamers are used as targeting molecules in the case of nanocarrier delivery systems. These direct the nanoparticles to tumor antigens which are available on the surface of the cancer cell. These can bind themselves to small molecules, nucleic acids, proteins, cells, tissues, and organs. Features of aptamers are easy to describe and modify, can bind easily with other molecules at the target sites, and can be engineered and produced completely in a test tube by chemical synthesis. Somatostatin analog, gastrin-related peptide, vasoactive intestinal peptide, and cholecystokinin are the peptide receptors overexpressed in certain tumor cells. These are small in size, have low immunogenicity, higher stability, and are easy to manufacture [11].

2.2.2 Side Effects of Targeted Therapy

Targeted drug therapy has unexpected aftereffects in comparison to chemotherapy. These side effects vary from one drug to another drug and from person to person. The side effects go away after the treatment. Many drugs are used in therapy because skin changes usually develop slowly over time after the treatment. Some drugs can also result in an allergic reaction which starts suddenly within minutes to hours. Allergic reactions start with the symptoms like trouble breathing, dizziness, and swelling of the lips or tongue. Some drugs target the proteins in the cancerous cell during treatment. The same proteins could be present in the skin layer as well. In such situations, drugs affect these proteins of the skin layer as well causing skin changes. Skin starts to feel sunburned, dry, and itchy, becomes more sensitive to light, and

can easily be damaged by UV rays. Skin rash also can happen due to targeted drugs. Target drugs can damage the blood vessels in the hand and feet resulting in hand-foot syndrome. Some drugs can affect hair resulting in baldness; facial hair growth can also increase. Targeted drugs can change skin and hair colors, and eyelids and around the eye also can be affected by the same. Angiogenesis inhibitors can increase blood pressure. Targeted drugs can affect blood vessel growth resulting in clotting and bleeding. Because of vessel blockage wounds get healed slowly or old wounds get opened up again. Heart damage also can happen due to these drugs, and the immune system of the body may also get affected which in turn affects healthy cells of the body. Table 2.3 shows targeted therapy for treating different cancer.

2.3 Drug Delivery Strategies

Chemotherapy is a widely utilized therapy approach for the disease. The associated drugs have a few disadvantages including the development of resistance and nonspecific targeting. The disadvantages of chemotherapy have been overcome by using combination therapy. This therapy approach uses multiple anticancer drugs. The solubility and pharmacokinetic properties of drugs affect the blood circulation time, targeting the effect of the combined drug on diseased cells. The blood circulation time can be extended; drugs can be circulated in a controlled manner at the exact tumor sites, and maximum drugs can be accumulated at the tumor area, minimizing the side effects through nanosized drug delivery systems (NDDs). NDDs are created from nanocarriers stacked with anti-drugs. It provides several advantages over previous techniques like tracking the delivery process and distribution of drugs through which effects of the therapy can be predicted. pH-sensitive NDDs are active at a specific pH range and are toxic in healthy cell regions. Thermo-responsive NDDs are also developed which work based on the temperature difference available between the normal and cancerous cells. The recent delivery system includes many different features such as temperature-responsive systems, pH, and light using which it can differentiate between normal and cancerous cells [12].

2.3.1 Drug Delivery Using AU NPs

Nanomaterials are prominently utilized in drug conveyance, determination, and treatment of cancer. Among several functionalized nanomaterials, Au NPs make great medication and antitumor specialist transporters in malignant growth treatment applications.

Nanomedicine exhibits astounding clinical execution with great remedial adequacy furthermore, less sound tissue harmfulness. Due to the performance of Au, NP-based drug delivery is considered for cancer treatment. After performing the studies, pharmaceuticals have not been approved for the Au NPs-based

nanomedicines. Various gold nanoparticle-related nanomedicines along with other biomedical uses, including drug-formed gold nanoparticles arranged for disease treatment and growth focusing, are being examined [13]. Gold nanoparticles are known to be a compelling nano-transporter for different medications, for example, peptides, pDNAs, proteins, siRNAs, and chemotherapeutic agents [14].

The combination of antitumor aptamers and gold nanoparticles-altered MSL focuses on properties was contemplated. Cancers are hard to fix because their microenvironment differs from that of ordinary tissue and a hydrogen potential lesser than the ordinary tissue decrease the adequacy of the medication. Gold nanoparticles can be utilized to convey hydrogen potential-responsive medications, for example, Morin securely to the objective for great viability [15].

One of the components of the eye, i.e., retina uses gold nanoparticles to reach the deeper sections. In this research review, how gold nanoparticles can be utilized to convey medication, and biomacromolecules to the eye are explained [16].

A cell film covering methodology opened innovative doors for multifunctional medicine conveyance stages. The framework was introduced—utilizing gold nanoparticles joined using platelet film—which can be utilized in malignant growth treatment for skin cancer. Curcumin consists of coated nanostars used for coating the platelet layer and verifying whether R/P-cGNS efficiency is improved for antitumor. The outcomes demonstrate the way that it very well may be a successful framework for malignant growth treatment [17].

2.3.1.1 Delivery Using pDNAs

Deoxyribonucleic acid-functionalized gold nanoparticles are generally utilized in biosensors and also used for drug conveyance. The deoxyribonucleic acid gold nanoparticles form can influence gold nanoparticles retention and the capability of the joined protein corona on the nanoparticles [18].

Oligonucleotides are viewed as better ligands because of their biomedical pertinence, selectivity, adaptability, high explicitness, and simplicity of synthetic control. Oligonucleotide-activated gold nanoparticles have been utilized to make nanoparticle tetramers, dimers, also trimers that have flexible nanoparticle distances, and mesoscale structures that have many nanoparticles an exact example. Deoxyribonucleic acid resources can be combined with the gold nanoparticles using covalent bonds, due to which upgrades their stability under deoxyribonucleic acid denaturing environments. Manufactured oligonucleotides changes made using an alkyne bunch when brought in the vicinity utilizing a support element. Halfway equivalent to adjusted oligonucleotides bring out oligonucleotide hybridization which at last yields a ligation response. This strategy was used to make gold nanoparticle trimers and dimers with a great creation rate and was steady in deoxyribonucleic acid denaturing conditions. Multiplexed capability is shown by the dimmers in a cell climate. They may be coordinated to two mRNA targets, concurrently or freely convey a couple of

anticancer medications to the living cells [19]. Bcl-2 DNAi-conjugated gold nanoparticles in MCF-7 cells were used to examine the blocking of the gene. The outcomes show that it is a sensible decision, and it can be utilized in clinical procedures [20].

2.3.1.2 Delivery Using RNAs

The utilization of ribonucleic acids macromolecules as remedial specialists for human immunodeficiency virus, conveyed straightforwardly toward the objective site, shows capable outcomes. Lymphocytes present in the entire body are especially hard to transfer in the presence of the human immunodeficiency virus. In this manner, a ribonucleic acids conveyance framework conveyed in gold nanoparticles was intended to do the errand. Around forty-five thousand ribonucleic acids strands were taken up per lymphocyte. Antiviral movement was not identified, so it is a favorable arrangement in the human immunodeficiency virus treatment [21].

The natural obstructions of the cerebrum present a significant test to accomplish satisfactory medication fixations for glioblastoma treatment. In current strategy has been recommended that investigates the capability of a nose-to-mind quickest pathway to sidestep the blood-mind boundary. The medication has been tried on rats and shown positive results [22]. Triple-negative bosom disease (TNBC) patients show the most terrible clinical result because of its forceful clinical course, a higher pace of repeat, and a prominent absence of FDA-supported designated treatments. Another technique for treating this disease was proposed where complex nanoparticles conveying metastasis silencer micro-ribonucleic acids (miR780) are confined to the objective malignant growth region. The productive conveyance of ribonucleic acids could diminish lung metastasis [23].

2.3.2 Nanoparticles in the cancer Diagnosis and Therapy

Devices at the nanoscale size are hundred to thousand of times smaller than nanomaterials. They resemble huge biological molecules like enzymes and receptors in size. Devices with less than 50 nm scale can easily penetrate most of the cells in the human body, but objects smaller than twenty nanometers can exit blood arteries when they circulate in the body. Nanomaterials can comfortably interact with biomolecules both outside and inside cells. These nanosize materials can identify illness and administer therapy in previously unheard-of ways since they have access to so many different parts of the body. Active and passive targeting are two types of effective nanomaterial delivery to target tissues [24].

Intravenous injection is the most used method of administering anticancer medications based on nanomaterials. This method avoids the stage of intestinal absorption that must occur following oral delivery. According to the nature, position, and environment of the tumor, the distances among the endothelial particles in cancer might vary from some hundred to thousand nm. Additionally, the nanoparticles are

Table 2.4 List of nanomaterials used in cancer diagnosis and therapy

Applications	Nanoparticles
Diagnosis (biomarkers and vivo imaging)	Quantum dots
	Nanoshells
	Magnetic nanoparticles
	Polymer dots
	Gold nanoparticles
	Graphene
Therapy	Liposomes
	Carbon nanotubes
	Polymeric micelles
	Dendrimers
	Quantum Dots

not quickly removed and concentrated in the tumor interstitium because of poor lymphatic activity. Passive targeting is based on an effect known as increased permeability and retention (EPR). Unfortunately, our understanding of EPR effects is restricted by the absence of precisely recapitulated solid tumor models in the human body. A few handfuls of subcutaneous cancer xenograft models that divide quickly and exhibit high EPR effects are the foundation of the majority of our current knowledge. Accordingly, the results of investigations using these models can present an inaccurate picture of the efficacy of passive targeted nanoparticles [25].

Various nanoparticles offer various ways to trap medicinal molecules. To accomplish controlled release goals and maintain an appropriate therapeutic dosage over time, modifying the amount of drug delivery concerning an activation signal is a crucial method. According to this, the activation variables that drive drug release are divided into open-loop and closed-loop control systems. In the first system, the release of drugs is regulated by external forces like magnetic pulses, acoustic signals, thermal power, or electric signals. Contrarily, in closed-loop systems, the presence and strength of internal stimuli close to the target sites regulate the drug release rate [26]. Applications of nanomaterials in cancer treatment are shown in Table 2.4

2.3.2.1 Nanoparticle in Cancer Diagnosis and Therapy:

(A) **Nanoparticles as Biomarkers**

A malignant growth biomarker is a quantifiable biological molecule that may be identified in the body's tissues and bodily fluids, including the blood and saliva, and urine, and serves as a sign that cancer is present. When malignant growth is present, the human body or tumor cells may deliver proteins (cell surface or secreted proteins), nucleic acids, or carbohydrates, (spread tumor DNA, miRNA, etc.) as cancer biomarkers. Certain cancer biomarker levels can be measured to enable

timely recognition of cancer or tumor recurrence and to track the effectiveness of the treatment. However, a number of obstacles have prevented the widespread use of biomarkers, including low levels of biomarkers in human body fluids, patient heterogeneity in the amount and timing of biomarkers, and the challenge of conducting prospective research. High selectivity, sensitivity, and the capacity to perform several target measurements simultaneously are all provided by nanotechnology. Nanoparticles and nanomaterials can be used to enhance biosensors to allow precise targeting [27]. Additionally, the introduction of nanoparticles increases the surface-to-volume ratio, enhancing the sensitivity of biosensors to meet the needs of particular biomolecular diagnostics. The popular nanoparticle probes for the spotting of cancer are listed in Table 2.4.

(B) **Nanoparticle-Based Drug Delivery/Medicine in Cancer**

A completely new era has begun as a result of the use of nanotechnology in the diagnosis, management, and treatment of cancer. NPs increase the intracellular concentration of medications while preventing harming the healthy tissue by active or passive targeting. To initiate and control the drug release, the chosen NPs can be created and modified to be either temperature-sensitive or pH-sensitive. The acidic TME can receive medications through the pH-sensitive drug delivery mechanism. Similarly, variations in temperature brought about by sources like acoustic waves and magnetic fields cause the temperature-sensitive NPs to release the medicines in the target region. The sizes, shapes, and surface properties of the NPs used in medical treatment are often particular because the three factors have a significant effect on the effectiveness of the transport of nano-drugs and, accordingly, regulate therapeutic efficacy [28].

(C) **Nanotechnology for Cancer Imaging/Radiation**

Contrast agents at present are used routinely in the field of medical image processing to improve image contrast and make details that would otherwise be difficult to see more visible. Because of their potential as contrast agents and because they are more biocompatible and less toxic than more traditional chemical agents, nanoparticle agents continue to draw a lot of research in this sector. Gold nanoparticles for X-ray contrast enhancement, magnetic nanoparticles for MRI enhancement, and even hybrid nanoparticles with iron oxide and gold in a polymer coating that act as contrast agents for both CT and MRI are currently being developed for this function.

Iron oxide NPs for MRI-enabled cancer detection in patients and nanocolloid technetium 99 m (99 mTc) for lymphoscintigraphy are two early examples demonstrating significant promise in the development of NP-based imaging agents for human applications [29].

The various kinds of nanoparticles used in cancer imaging are as follows:

1. **Quantum Dots**: QDs can play a significant role in the tumor diagnostic process. Due to corrective therapy, phase I problems are typically detected early enough to have a 5-year endurance rate of more than 90%. Technologies that use fluorescence imaging or fluorescence naming are crucial weapons in the medical

resolution arsenal. Quantum dots are primarily utilized to enhance fluorescence imaging due to their photochemical stability and robust fluorescence emission. The study of cell transport mechanisms, functional heterogeneity, dispersal move of membrane escort proteins, nonspecific labeling for imaging and detection, the contrast of blood and lymph vessels, intracellular delivery, the diagnosis of hepatoma in vivo, internalization of QDs by live cells, probes in other bioassays, and other biomedical applications are just a few of the biomedical applications that frequently use QDs.

Even though toxicological and pharmacological issues are impeding progress in cancer diagnosis and therapy due to considerable metal and colloidal insecurity, QDs have huge implications for bioimaging and discovery. Although these issues might not prevent the development of applications in vitro, they significantly hinder the use of in vivo malignant growth imaging in humans. The development of more resilient and toxic-free nanomaterials for in vivo theranostic use is essential for future in vivo applications. The discipline is currently developing swiftly, and significant breakthroughs are anticipated shortly. However, problems like the covering shell's degradation as a result of shifting the quantum dots should be considered.

2. **Liposomes**: Due to their biological and technological advantages, nanoliposomes are now regarded as the most effective drug delivery technology. Only, the next two trials among 34 as of 2021 are mentioned combining antineoplastic medications with nanoliposomes.

   (A) Research is being done to determine the efficacy and safety of paclitaxel liposome and S-1 as first-line therapy for advanced pancreatic cancer patients between the ages of 18 and 75.
   (B) Another study is being conducted at the same time to assess the effectiveness of pegylated liposomal doxorubicin and carboplatin (the experimental study group) to paclitaxel and carboplatin (the active comparator) in treating adult women with epithelial ovarian cancer.

3. **Carbon Nanotubes**: Due to their unique features, CNTs have been extensively researched in targeted medication delivery for cancer therapy. Because of nanomaterial-based optical properties, CNTs can be used as excellent phototherapy mediators in addition to being drug carriers for a variety of anticancer drugs. The versatility of CNTs allows for a wide range of therapeutic uses in the treatment of different malignancies. Many anticancer therapy approaches are currently focused on destroying tumor cells and the environment in which they thrive.

   CNTs are biological transporters that can endocytose cytotoxic medications like doxorubicin and the prodrugs of cisplatin and paclitaxel through cellular membranes. Drugs may be delivered to cancer cells via CNTs, maybe with superior results to drug delivery without a carrier. The concentration of drug molecules in tumors may rise with targeted drug delivery, which has the potential to lower systemic toxicity. Additionally, by altering the pH, it is possible to control how quickly medications are released from CNTs, potentially increasing therapeutic

effectiveness. CNT-drug complexes may be readily released due to the acidic milieu prevalent in many solid tumors [30].

4. **Polymeric micelles**: PMs have been widely used in pre-clinical trials recently to deliver poorly soluble chemotherapeutic drugs to treat cancer. Amphiphilic polymers self-assemble in simple ways, resulting in the formation of polymeric micelles. Researchers can experiment with different polymeric combinations for optimal loading, systemic circulation, stability, and transport to the target cancer tissues because hydrophobic, and to a lesser extent, hydrophilic polymeric blocks are widely available. Furthermore, by varying the number of monomers in each polymeric chain, polymeric micelles can be manufactured to order.

   Drugs may be coupled to polymers at their distal ends to create pharmacologically potent polymeric systems that improve the solubility and stability of the conjugates and open the door to the delivery of many drugs simultaneously. Due to the enhanced permeability and retention (EPR) effect, their nanosize enables them to assemble in the tumor microenvironment. Additionally, the stimuli-sensitive breakdown gives the micelles a reliable way to deliver the therapeutic payload [31].
5. **Dendrimers**: Dendrimers can be used in chemotherapy and gene therapy for the treatment of cancer. Antitumor medications like DOX and PTX can be highly cytotoxic to both normal and tumor cells during chemotherapy, which restricts their usage in practice. Through physical or chemical interactions, the surface-modified dendrimers can combine antitumor medicines and transport them particular to the target region with less cytotoxicity and greater cell uptake. The main issues with gene therapy are the reduction of cell uptake and the blocking of nucleic acid breakdown during delivery [32–34].

   Dendrimers are available in 3 different forms:

   (i) Polyamidoamine (PAMAM): Polyamidoamine dendrimers were first introduced as a special type of medication delivery system. Their programmable molecular size, variable cationic groups on their surface, and spherical architecture make them appealing. The potential for targeted cancer therapy with this class of nanocarriers is very great. In addition, a co-delivery system performed better in cancer therapy than either a single medication delivery system or a single-gene delivery system [35].
   (ii) PPI Dendrimers: polypropylenimine (PPI): Targeting of MCF-7 breast cancer with docetaxel (DTX) is accomplished using the propylene-imine dendrimers (PPI). To improve paclitaxel (PTX) distribution to the brain, PPI dendrimers have been employed. The possibility to treat chronic lymphocytic leukemia using PPI dendrimers is another benefit.
   (iii) PLL Dendrimers: There are lysine residues in poly-L-lysine (PLL) dendrimers. Because of its superior biocompatibility, it is employed for gene delivery. Better gene transfection is found in PLL of higher generations [36].

6. **Quantum Dots**: Early tumor identification and diagnostics, targeted gene-drug delivery, phototherapy, bioimaging, and drug delivery are the main applications of quantum dots in cancer therapy. Additionally, on account of their excellent biocompatibility, minimal cytotoxicity, and high capacity for cell absorption,

quantum dots are the preferred drug delivery system. A particular method for administering and managing medications is demonstrated by quantum dots. Drugs that are transported by nano-transporters have the potential to have a greater impact at low concentrations, travel farther at lower doses, and have fewer adverse effects on patients. A variety of natural particle drug-encapsulated nanocarriers with aptamers, antibodies, folic acid, and other modifications was made to deliver medications to particular organs.

Therapy for the identification, visualization, and treatment of malignant development is increasingly possible because of the use of quantum dots-polypeptide nanogels in drug administration. Quantum dots (nanorobots) are truly the ideal warriors for target-based actions due to their capacity to overcome challenges such as medication resistance, a lack of selectivity, and solubility [37].

Technologies that use fluorescence imaging or fluorescence naming are crucial weapons in the medical resolution arsenal. Quantum dots are primarily utilized to enhance fluorescence imaging due to their photochemical stability and robust fluorescence emission.

2.4 Challenges in Nanomaterial-Based Cancer Therapy:

1. Nanomedicines are promising in oncology chemotherapy due to their effective preparation methods. In this text, weak interactions between the guest and hosts, such as physisorption or spatially constrained domains, are primarily used to load therapeutic agents into nanocarriers. The payloads in the carrier may leak out in such weak connections; generating negative side effects before the target site is reached [32].
2. The foundation for nanomedicine-enabled chemotherapy is an effective accumulation of nanomedicines to provide improved therapeutic effects. However, the majority of currently known nanomedicines accumulate in tumor tissue by the enhanced permeability and retention effect, where the accumulative efficiency of tumor-targeted accumulation is fairly poor (less than 10% via intravenous administration) [33].
3. Metal ion-mediated chemocatalytic processes are typically responsible for the transformation of intrinsic or supplied substances from non-/less hazardous to highly toxic after being endocytosed into the tumor tissue. A variety of transition metal ions (Fe, Cu, Mn, etc.) having catalytic capabilities can be added to the nanomedicine system to accomplish this purpose. The transition metal-engineered nanomedicines have a wide range of redox properties that can accelerate certain reactions, particularly the intratumoral redox reactions that produce ROS or other hazardous compounds in situ. This is due to the strong catalytic activity of transition metal ions. Even though these transition metal ions in nanomedicine can be delivered in a concentrated manner in the acidic tumor

microenvironment, the transition metals, especially copper ions, may cause some unexpected harm to the body since these nanomedicines are often difficult to use [32].

4. The other issue is the cost-effective, mass manufacture of very sensitive, highly repeatable nanoprobes with long-term storage stability. Although the majority of the current nanoprobes are produced in laboratories under highly optimized circumstances, producing these probes in batches is still a significant issue. The detection outcomes differ significantly depending on the nanoprobe's form, size, composition, charge, and surface coating. The synthesis procedures and nanoprobe functionalization must be streamlined to reduce batch-to-batch variations. Nanoprobes may also tend to group while being stored. Additionally, the affordability of creating a platform based on nanotechnology must be taken into account [25].
5. The absence of administration routes, tempered biodistribution, the passage of nanoparticles over biological barriers, their destruction, and toxicity are some biological obstacles. The nanoparticles are often administered via intravenous injections into the circulation, which removes nanoparticles and makes it difficult for nanoparticles to remain at and react with the target site. This leads to the usage of medicine with a high concentration, which could not have the desired therapeutic benefits. However, due to numerous in vivo and in vitro studies that have demonstrated the use of 3D magnetic fields to control the movement of nanoparticles against blood flow, magnetic nanoparticles can be utilized to overcome this. However, further research is required on the impact of magnetic fields used to treat the human body, as well as the interaction of many nanoparticles with magnetic fields [28].

2.4.1 Challenges Faced During Anticancer Medication Delivery

For cancer treatment, various medications are available these days, but the side effects of these medicines also how effectively they will reach the cancerous cell are the challenges with the delivery of drugs. Along with the previously mentioned difficulties, doctors should improve the medication conveyance system.

1. Oral-Based Administration

   - The oral course is the patients' favored method of conveyance because of its straightforwardness and painlessness.
   - This course faces two significant difficulties: the medication substance's low bioavailability and the designated conveyance to a characterized segment.
   - The oral course is usually utilized in numerous disease signs and is entirely suitable for gastrointestinal tumors.
   - Later modalities of treatment, for example, noncytotoxic designated specialists, and hormonal treatment, described by a more extensive remedial window, may profit from novel oral definitions.

2. Intravenous-based administration
    - The intravenous organization is the most general course for malignant growth drugs, guaranteeing more bioavailability, and low between/intra-patient inconstancy.
    - This conveyance course faces various difficulties like non-functional properties of pharmacokinetics medication substances, and side effects caused by the mediational drugs.
    - The methodologies are presently founded on the utilization of nanomedicines.
3. Subcutaneous-based administration
    - Solid efforts have been dedicated to the subcutaneous organization of anticancer medications however not all endorsements have been so far enrolled.
    - This conveyance course faces two significant difficulties: the long duration of the medication and controlling the different delivery rates.
    - The drug delivery methodologies used are more complex like implantable central processors will want to convey drugs on request through shut circle frameworks.

2.5 Conclusion

Malignant growth is the most common source of death in humans. It is a difficult task to target the exact area of the affected cell and to deliver the proper amount of drug to the intended space. This chapter is a review of some of the approaches employed for targeted cancer therapy. The chapter has a brief overview of cancer disease, the way of tumor growth, and types of cancer disease. The chapter presents kinds of treatments available for cancer. The chapter highlights the features of passive and active target approaches. It mentions the advantages and disadvantages of these target approaches. Passive methods cannot differentiate between healthy and cancerous cells which has some shortcomings. These shortcomings can be overcome by an active target method. Different drug delivery strategies using AU NPs, pDNAs, and RNAs have been highlighted in the same. The chapter also describes the use of nanoparticles in diagnosing and therapy of cancer cells.

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Chapter 3
Exploration of Tissue-Engineered Systems for Cancer Research

Ankita Panigrahi, R. Mythreyi, Kanthesh M. Basalingappa, T. S. Gopenath, and Murugesan Karthikeyan

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Abstract Cancer drugs fail in clinical trials at a rate of more than 90% due to infiltration of myofibroblast, fibroblast, and an increase in collagen, resulting in a poor prognosis. Cancer tissues have a tumor microenvironment (TME) that is made up of cancerous and noncancerous cells, growth factors, extracellular matrix (ECM), and other substances. Tumor cells' ECM is constantly remodeled to increase stiffness, survival, proliferation, and immunosuppression. Most of the studies involve cellular models in vitro, which many a times are grown as 2D cultures. Many features are lost in vitro because of continuous ECM remodeling. To overcome this, decellularized scaffolds known as decellularized tumor ECM (dt-ECM) are used, which retain composition as well as mechanical structure. They have the potential to be a very useful tool in understanding cancer progression and formation. One of the powerful approaches to understand diseases is tissue engineering, which is a culmination cell biology, developmental biology, nanobiotechnology, material science, and related fields. Tissue engineering assists researchers in producing engineered and functional cells that will give rise to tissues, which might then go on to become organs. Engineered tissues and organoids (miniature forms) are used in drug development, screening, and disease modeling. The 3D tissue model is useful in understanding various diseases. Organoids are miniature versions of organs that are used to reduce animal usage and to develop body-on-a-chip or organ-on-a-chip. They can help to support tumor heterogeneity, which will aid in personalized medicine and reduce the likelihood of drug failure. While growing cells to 3D tissues is quite interesting, finding the right type of scaffolds is equally important, which might play major role in cell adhesion, cell motility, cell differentiation, etc. Alginate with RGD and other biomaterials, for example, has been a major focus due to important properties demonstrated by alginate that aid in cancer research. Since 3D engineered organs and tissues require an environment similar to in vivo, advanced bioreactors are being developed. Tissue-engineered organs will aid in drug screening, development of more effective drugs, and, most importantly, understanding of cancer. Recently, the use of microfluidics system which is combination of microelectronics and TE looks very promising; it offers more advantages than 3D and 2D culture. It comes with various application like study of metastasis cancer which is main causative of cancer death across the world. Integration of nanotechnology in TE helps in building new biomaterials which have shown enhanced cell adhesion and various other properties. There are various 3D models which have been developed to study and understand different types of cancer.

3.1 Introduction

According to previous research, cancer drugs fail in clinical trials at a rate of more than 90% and animals who risk their lives end up in landfills? According to the World Health Organization, cancer is the leading cause of death, taking 10 million lives alone in 2020. Researchers from all over the world are working to find a cure for this deadly disease. The challenges in cancer treatment are developing better methods to predict, detect, and eradicate the spread of cancer cells to distant healthy tissues. Cancer cells constantly remodel their extracellular matrix, which influences the infiltration of immune cells, fibroblasts, and other tissues [1]. In the case of pancreatic cancer, for example, excessive deposition of ECM molecules, such as fibrillar collagen, contributes to malignancy and limits the delivery of chemotherapeutic agents to the cancer cell. Collagen-rich stroma is being normalized to improve the efficacy of immunotherapy and chemotherapy because it is unclear which factors promote or prevent carcinogenesis [2].

For many decades, animal models and 2D cell cultures have been used to study the development of diseases such as cancer, as well as the efficacy, efficiency, and toxicity of a specific drug. The issue with animal models is that they are incompatible with live imaging techniques; they increase experimental complexity, and they are ethically questionable. However, 2D cell culture is more polarizing, as cell-to-cell and cell-to-substrate interaction is lost, making it impossible to recreate physiological and biological features of human tissue or organ [3]. To address the issues, researchers have used 3D scaffolds in tissue engineering, which can be artificial, natural, or decellularized. Tissue engineering refers to a broad range of technical advancements and knowledge in developmental biology, cell biology, material sciences, and other fields. TE is made up of scaffolds, molecular cues, and cells. It has functional properties that allow for the study of cellular properties, microenvironment, and physiology, which is not possible in 2D cell culture or animal models [4].

Due to advancement in TE, there is a need to develop better scaffolds, matrices, bioreactors, and drug delivery system. Replacing the non-degradable scaffolds with multifunctional scaffolds especially with hydrogels can reduce the risk of diseases. Using naturally derived biomaterials is safer as they are multifunctional and smart along with that they respond to various physical and chemical stimuli such as light, temperature, pH, ionic strength, salt, and redox. The development of biomimetic smart materials, hydrogels, self-healing materials has revived the field of biomaterial research [5]. Inkjet three-dimensional bioprinting, electrospinning, and biofabrication technology are some of the toolboxes which are used in TE and RM [6]. Nanomaterials have evolved which is being used in cancer for detection, treatment aspects. When nanomaterials are combined with tissue engineering, smart biomaterials are created, which play a significant role in scaffold enhancements by increasing cell adhesion and also proliferation.

Microfluidics have great potential in developing our cancer research. Its root lies in microelectronics and manipulation takes at micron level. It offers various advantages over conventional 2D and 3D cell culture like low cost, low sample

and reagent required, portability, and parallelization. The platform has proved be a great asset in cancer biology. The application includes biomimicking microfluidics system, organ-on-a-chip, studying phenotype of cancers, and most importantly shed more light on metastasis cancer as this is the leading cause of cancer [7]. We will discuss in detail about the application part in the upcoming paragraphs.

In this chapter, our main focus will be on how imbalances and mechanical constraints effect extracellular matrix and cell crowding of cancer cells, changes in cell shape, their proliferation progression which lead to their metastasis. We will also discuss about how cancer ECM is continuously being remodeled which is difficult to understand animal model or 2D cell culture system. Development of smart biomaterials which are being developed in the field of tissue engineering are being discussed in this chapter. One of the very important is microfluidics system which and its application in cancer research. We are going to see how different model systems are being developed for various types of cancer such as sarcoma and bone ovarian.

Let's first understand why three-dimension cell culture is preferred over two-dimensional culture system.

3.2 Difference in 2D and 3D Cell Culture in Physiological and Structural Aspects

Cell culture was performed first time in 1907 on the origin of nerve fibers since then the method has been developed, and it is used to observe cell growth and differentiation in vivo. Experiments can be conducted using cell bank or directly isolating primary cells. The features of primary cell lines include short life span, difficult to isolate, but they mimic cancer cells closely, so it is ideal to perform experiments using cell lines. Centers like ATCC offer cell lines for various cancer research. Cell culture system helps us in understanding the cellular morphology, mechanism of disease development, drug action, protein and gene expression, and development of tissue engineering. The choice for better cell culture will help us in understand cancer biology and hence improving our treatment approaches. The culture can be carried out using Petri dishes or in suspension. Most commonly 2D cell culture is used, but recently, 3D cell culture is preferred.

3.2.1 2D Cultures

In 2D culture, cell is attached to a petri dish or flask, and cells here grow as monolayer. The major advantage of 2D cell culture is that they require low cost and maintenance, but they come with numerous disadvantages such as cell-to-cell and cell-to-ECM interaction is lost; they don't mimic the structures and tissues present inside the body; they are mostly polarizing meaning one end is attached to the substrate, and

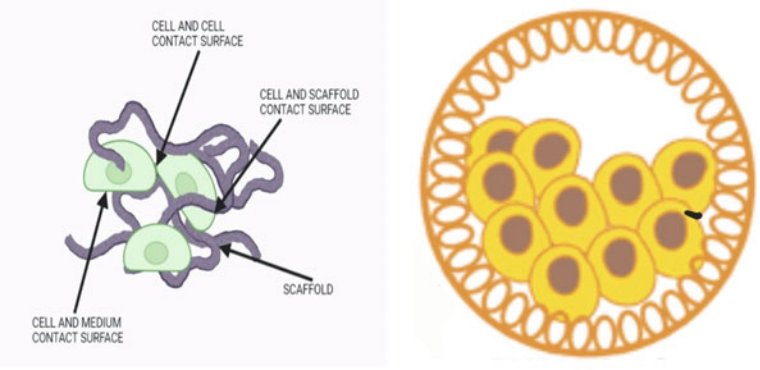

Fig. 3.1 These are types of cell culture used in biological research. **a** cells are in contact with the scaffolds, and all the cells are getting exposed to the media. **b** cells are grown in a monolayer culture, and they are nonpolar; some are attached to the surface and exposed to the media

other is exposed to cell culture media [8]. When cancer cells are cultured in lab, their proliferation, differentiation, vitality, protein and gene expression, and other cellular activities are completely different from how they naturally grow. Another drawback is that the monolayer gets access to unlimited oxygen, metabolites, nutrients, and signal molecules; furthermore, 2D also alters the topology, physiology, protein expression, splicing, and biochemistry of the cell. Because of these many disadvantages, there is a need to find better model which can replicate the natural cancer microenvironment (Fig. 3.1).

3.2.2 3D Cell Culture

For the first time, 3D cell culture was performed in soft agar solution. They are difficult to carry out and are quite expensive, but they preserve the morphology and cell-to-cell and ECM interaction along with that they receive and respond to stimuli for nearby environment, polarity of the cell is maintained. Moreover, there is similarities to in vivo growing cells in terms of topology, protein expression, signaling, and their metabolism also helpful in studying of cells initiating cancer, drug testing, response of cell to radiation, analyzing the biological and molecular feature of tumor [8].

3D culture system can be divided into three models based on preparation: -

1. Culture in concentrated medium or in gel-like substance
2. Suspension culture
3. Culture on scaffolds (Fig. 3.2; Table 3.1).

Under anchorage independent or non-scaffold based, we have hanging drop, magnetic levitation, low attachment plate and where scaffolds are used called

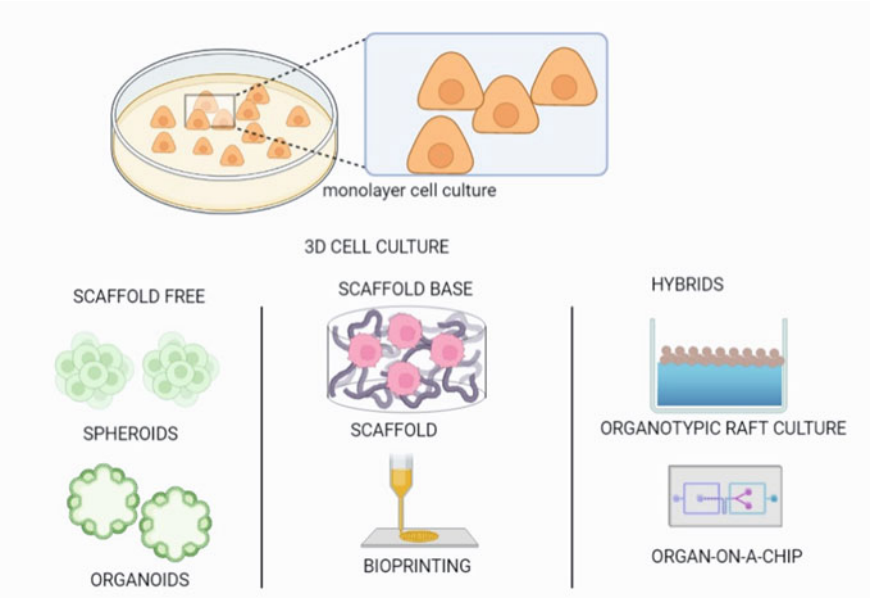

Fig. 3.2 Flowchart showing different types of 3D cell culture scaffold free, hybrid, and scaffold based. First these cells are cultured in monolayers. They grow and self-assemble to the scaffold; they resemble the extracellular matrix. These scaffolds mimic native environment of the cells. By the process of oncogenesis, cells aggregate in only non-scaffold system. Hybrids use matrix to support scaffold-free system

anchorage dependent; we can use either biological or synthetic hydrogels such animal derived have collagen, Matrigel, and gelatin and plant derived have alginate. Synthetic examples include polyethylene glycol (PEG), polylactic acid (PA), and polyglycolic acid (PGA) [13].

Specialized 3D system includes microfluidic devices and micropatterned plates such as organ-on-a-chip or lab-on-a-chip.

3D culture system is used for development of tissue engineering and are widely as a model in biological research. We will study how different types of cancer are being studied using TE later on. Let's understand the basic of tissue engineering and how it can be useful in cancer research.

3.3 Current State of Tissue Engineering

First time TE was defined in 1980s in a very broad and generalized way as understanding the structural, functional relationship in normal and pathological tissues. And the development of biodegradable as well as biological substitutes to improve, maintain, and restore function. The basic construction of TE provides models that

Table 3.1 Different types of 3D culture system along with their advantages and disadvantages

Types of 3D culture	Explanation	Advantages	Disadvantages	References
Suspension culture	Single cells are seeded on suspension medium It takes 3 days after culture to observe 3D structures	Simple, easy for conducting experiment Restricted for only some cell lines Easy to extract cells	Formation of cell aggregates Some cell lines require costly coated plate with certain materials	[9–11]
Culture in gel-like substance	Agarose is dissolved and poured in petri dish wait till it solidifies The top layer consisting of agarose and the medium with single cell is added Cells are flooded in Matrigel It takes 7 days after culture to observe 3D structures	Easy recovery Cells in Matrigel have 3D environment and allowed to interact to forms tissue Aggressiveness of the cells can be studied	Difficult in extracting and performing immunofluorescence staining of spheres Matrigel contains bioactive substances that may influence structure formation	[10, 11]
Culture on scaffold	Scaffolds can be natural or artificial such as silk, collagen, and alginate The cells can migrate and attach to the scaffolds and divide	Easy to perform immunohistochemical analysis Compatible with available functional tests	Cells get attached to scaffolds and spread like adherent culture Materials used to construct can affect cell behavior Extraction and analysis for some cell is restricted	[9, 12]

help in studying diseases such as cancer. Recently, the field of TE has extended into biomaterials, three-dimensional, bioprinting, gene editing techniques, introduction of stem cells-derived techniques as well as nanotechnology. All these have to development of smart biomaterials, production of organoids, whole organ engineering, like organ-on-a-chip or body-on-a-chip for drug testing and development [14].

Advances in biomaterials play a huge role in development of TE which can respond environment and cues. Nowadays, it is possible to develop materials with many functionalities which can mimic the natural environment. There are several engineered tissues which being used in human such as bladder, bone, skin, and cardiac tissue. The complex organs such as lungs, kidney, liver, and heart valve are being used in

animal models. For drug testing and development, miniature version of organs called organoids is being used. These organoids are being used on chip called organ-on-a-chip; they can be used to solve the problem of heterogenicity as the development of disease such as cancer is not same in all the individuals even though the cancer is same. TE is revolutionizing the field of personalized medicine. 3D tissue models resemble close to in vivo structure which is increasing our understanding of disease such as cancer and helping us to accelerate development of improved treatment for multiple diseases. This can replace the animal models which are used for biological research [4].

Use of advance bioreactors will better mimic the in vivo by providing optimized physiological conditions such as pH, temperature, oxygen consumption, and induction of cell differentiation and proliferation. Recent advancement in the field of TE is discussed below.

3.3.1 Smart Biomaterials

Biomaterials that are designed to response to physical, chemical changes with respect to nearby environment. Production of advanced polymers, fabrication technologies, and integration of nanomaterials have made the next-generation biomaterials possible. The changes in the environment such as pH, temperature, humidity, oxygen concentration, light, sound, and activities of biological substances are responded by smart biomaterials. The main driven of TE is to produce biomaterials which have the ability to replace the natural ECM in a way that they can control the stiffness, porosity, water intake, etc. Hydrogels can be build that can undergoes structural changes with response to stimuli [15]. Self-healing or shear thinning hydrogels can be injected, and they can be used to fill the defect site. Tissue glues are being developed recently which can bond the tissue and heal on its own. Specially, chondroitin based helps in tissue repair.

Affords are being given to develop ECM-based scaffolds which can revolutionize the field of TE and RM. Our body's immune system plays a very huge role when in recognizes any foreign entity. Scaffolds are being developed which can control the host immune response such as having immunomodulatory effects. Peptide such Arg-Gly-Asp (RGD) of ECM has immunomodulatory effects on both innate and adaptive immunity. They also inhibit neutrophils and phagocytic cells. Artificially, ECM is integrated with RGD peptide which shown to have increased adhesion and anti-inflammatory effects [16].

Proteases such as matrix metalloproteinases (MMPs) are being used which have immunomodulatory effects. One study shows that rate of scaffolds degradation depends on the MMPs-sensitive peptides. One more class of smart biomaterials called multidomain peptides (MDPs) hydrogels. These are injected into the ECM-mimetic materials that form a mesh at the site of target. When studied on mouse model, MDPs shown healing properties and were biocompatible [17].

These smart biomaterials are going to play a huge role in 3D printed organs. Combination of smart biomaterials and 3D printing will give rise to different architecture which can be integrated with the biological system. Also, protein or protein–protein interaction can be used to make smart biomaterials by crosslinking or by coiling. Example leucine zippers are being used to make hydrogels; its stability can be controlled by changing the temperature [4]. Level of smartness of biomaterials is classified into four levels to, namely inert, active, responsive, and autonomous.

3.3.2 Whole Organ Engineering

This field is combination of developmental biology, anatomy, and tissue engineering. The field of whole organ engineering comprises of engineering a particular organ and organ system. Biomaterials such as polylactic acid, polyglycolic acid, or combination of both are very well studied due to their biocompatibility and biodegradability, but they lack cell adhesion and cell activity. Natural polymers such as alginate are being widely used due to its hydrophobicity and similarities to natural ECM, but they limit cell proliferation and migration. The main goal of whole organ engineering is to direct cellular activity for the formation of tissues and organs. Nowadays, decellularization is gaining popularity; this is prepared by adding chemicals, physical, mechanical or combination of both. Cells form the tissues are removed and are processed into blocks or powder. They represent the ECM of a tissue. Mild detergents are used in chemical processing, and for physical, chemical processing, freeze–thaw, hydrostatic pressure is being used. There is huge rise in decellularized scaffolds in TE. Sometimes decellularization can damage important components of tissue in that case we can add synthetic materials for normal functioning [18].

The various developments that took place in the last two decade include (1) organ recellularization and decellularization, (2) bioprinting three-dimensional, (3) stem cell technologies, (4) genetic modification of tissues and cells. All these advance shows that in the next decade whole organ will be available commercially.

Let's understand the approaches for whole organ engineering:

(1) top-down approach—by this approach, scaffolds are developed to direct cells to form tissues. Whole organ or macroscopic scaffolds are seeded with a variety of cell types and remodeled to form 3D tissue or organs.
(2) bottom-up approach—by this approach, smallest element of a tissue is choose to and combined to form large construct (Fig. 3.3).

3.3.3 Spheriods and Organoids

It is well known that 2D cell culture makes it difficult to understand the microenvironment and their interaction with cell–cell and cell-ECM. To overcome this, 3D scaffolds are being developed. One the most important scaffolds is hydrogels which

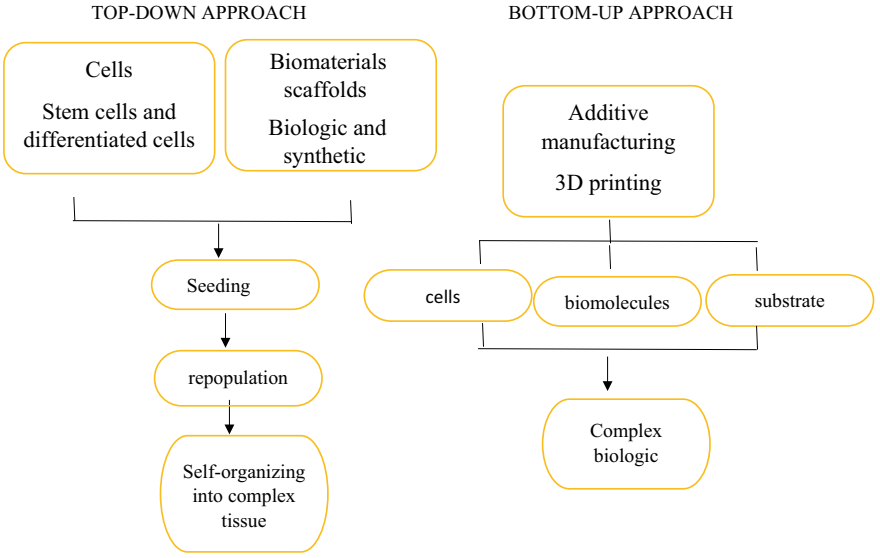

Fig. 3.3 Flowchart of top-down and bottom-up approach

has high water retention. However, hydrogels can't fully recreate the microenvironment specially tumor microenvironment. Before understanding tumor microenvironment, we need to know more about extracellular matrix. It is highly complex and dynamic composed of network of bioactive, biopolymers which are responsible for providing the tissue many structural properties. The components of ECM include elastin, collagen, proteoglycans, hyaluronic acid, fibronectin, and laminins. The most abundant collagen out of the 16 types includes types I, II, and III. They bundle to form fibrils having high tensile property, and they are interstitial collagen. Type IV collagen plays a role in connecting interstitial collagen and also helps in cell adhesion; it forms a X-shaped unit and is found in abundant number in the basement membrane. Elastin is a component of ECM, and it is highly elastic, high elastin meaning increased mechanical compliance. Proteoglycans such as glycosaminoglycans (GAGs) are highly hydrophilic, which allows them to retain water. Hyaluronic acid is one type of GAG which doesn't have hydrophilic or tissue hydrating role. Laminins have more than 15 isoforms; they are found in the basal lamina which are bound to other ECM molecules. Adhesion protein such as fibronectin contains binding domains for heparin sulfate, collagen, and integrins. All these biomolecules that we have discussed ensure that the ECM is intact and forms a composite network [19].

ECM is continuously remodeled which directs the cells to grow and interact with other cells. ECM growth and remodeling serve as microenvironment for cell differentiation into different lineages. Its remodeling plays a huge role in regeneration of tissues. As we know the structure and role of ECM, they also become the foundation of organs and whole organ engineering.

In 3D culture system, spheroids are gaining a lot of attentions; they are 3D complex composed of cells. They have advantage over 2D cell culture as they have better cell–cell and cell-ECM interactions. In tissue engineering, multiple spheroids can be combined using co-culture; they can be produced using hanging drop, microwell. Recently, phosphoproteins and glycoproteins are used to generate nanofibrils when spheroids formed in this way, they have tissue-like structure. Fragments nanofibers are injected artificially into spheroids to control their function. They are used to study response to external stimuli. For blood–brain barrier study, they are being used as multicellular spheroids are being made using pericytes, astrocytes of human. Another application of spheroids includes cancer research, disease study and provides platform for drug study. Drawback of spheroids includes their control during culturing, and if they are large, necrosis can occur in the core. However, we can improve spheroids through regenerative potential. When combined with biomaterials, they can act as scaffolds for the formation of engineered tissues.

More complex and advanced type of spheroids is organoids. They have similar physiochemical environment to that of a tissue. Their generation is a major breakthrough in the field of TE. The cell source for organoid generation can be ES, iPSCs, or autologous cells. For their generation ECM surface culture, fabrication methods are being used. Example of some organoids that have been developed includes brain, lungs, intestine, liver, and prostrate [20].

Now, let's understand the process of organoid and spheroids culture (Fig. 3.4).

Broadly, we can define spheroids as cells which are cultured on non-adherent system and have not been given any supports. They are going to revolutionize the field of personalize medicine as this aspect can't be performed on animal models as well as they are also used to study human development, disease model and their progression, and in treatment aspects.

3.4 Microfluidics and Body-on-a-Chip/Organ-on-a-Chip

Microfluidics is a branch of science which deals with the study of control, behavior, and manipulation of fluids at micron scale. The emergence of microfluidics started in 1980s has many applications such as body-on-a-chip, organ-on-a-chip technology, and inkjet printers. The advantages of microfluidics include their size ranges to few centimeters, have high resolution, low cost, high performance, require less amount of reagent, and need less analysis time.

They are made through engraved microchannels that are connected to microenvironment through small opening. With the help of these holes, fluids is injected and expelled. They are used to recapitulate the working of a human organs; we can find many models such as lung-on-a-chip, heart-on-a-chip, tumor-on-a-chip, and many others. The working mechanism of microfluidics system deals with manipulation and control of fluid at microscale level. There are various properties which are taken into consideration while building this system such as diffusion, fluidic resistance, viscous drag force, SAV ratio, inertial focusing, and surface tension. There

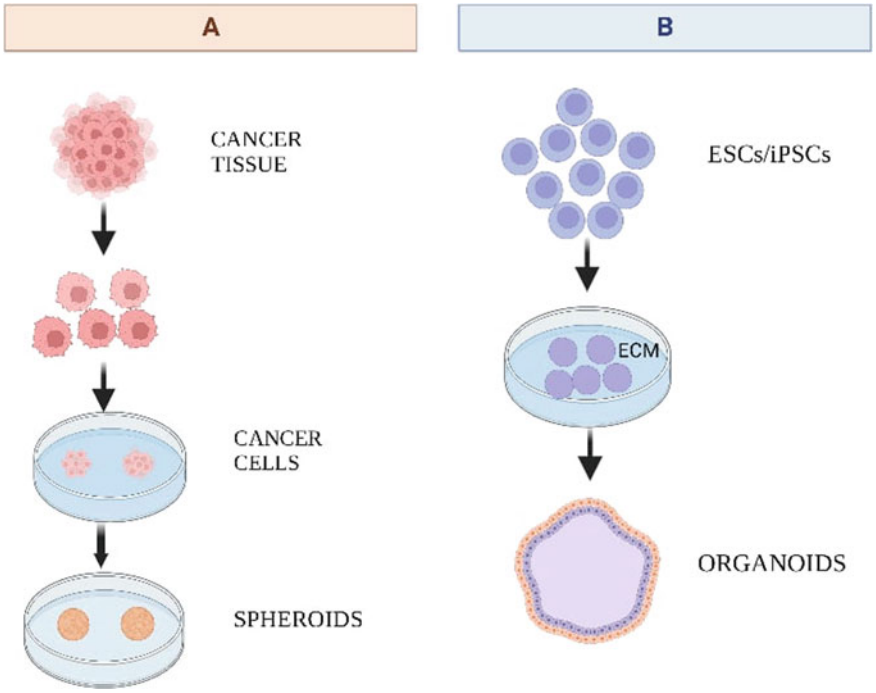

Fig. 3.4 **a** Tumor or cancer cells isolated from human and cultured to produce spheroids. **b** Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are cultured to produce organs; in presence of signaling cues and proper environmental condition, we can get spheroids. We can compare cancer organoid and human organoid by observing them under microscope

are various methods that have been employed in building microfluidics such as lithographic technique, 3D printing of channels and chambers, molding, and etching. Several materials have been used while building such as organic materials—paper, polymers—PMMA, COC, and PDMS, inorganic include silicon and glass [7].

3.4.1 Microfluidics in Cancer Research

Cells are the fundamental unit of our body; they coordinate to form tissues and then organ which makes organ system. The period of growth for the cells is defined, and formation of new cells is for replacing dying or defective cells. Cancer is defined as uncontrolled growth of cells in our body which later on spread to all the parts of our body. Tumor is formed by replication of cancerous cells which be either benign or malignant. Benign tumor does not spread whereas malignant tumor grows and spreads to others parts of the body by a process called metastasis. Cancers cells can be diagnosed by biopsy, imaging, or lab testing. Imaging tests can be performed

using MRI, PET scan, ultrasound, and X-ray. Although biopsy tissue is extracted via incision or excision. The process of metastasis is when cancer cells leave their primary site and travel to different parts of the body through circulatory or lymphatic system (Fig. 3.5).

The development of tumor has an expansive phase where invasion is absent it is encapsulated by desmoplasia. Cells of neoplastic cells escape and get detach from the primary tumor. Errors in the chromosome segregation lead to rupture of genomic DNA, and then, DNA sensing pathways are being activated and EMT, i.e., epithelial mesenchymal transdifferentiating process. EMT in the primary tumor further leads to hypoxia condition, stiffness of matrix and metabolic stressors. Nowadays, EMT is seen as transition between epithelial and mesenchymal phenotypes. Tumors cells release factors which stimulate angiogenesis. Disseminated stem cell expresses stem cells markers such as ALDH, i.e., aldehyde dehydrogenase, and some have the ability to cause metastasis. Genome wise if we compare EMT and CTCs, both have similar transcriptome [21].

From the past two decades, scientists have been using microfluidic technology for cancer research and developed many technologies with various applications. They provide great advantages such as low cost, less sample size, portability, fast processing, and highly sensitive; because of these many reasons, they have been a promising tool in cancer research.

They allow non-invasive cancer diagnosis, biomarkers analysis because cancerous cells are found in DNA, RNA, and CTCs, but they are present in low level in blood, so

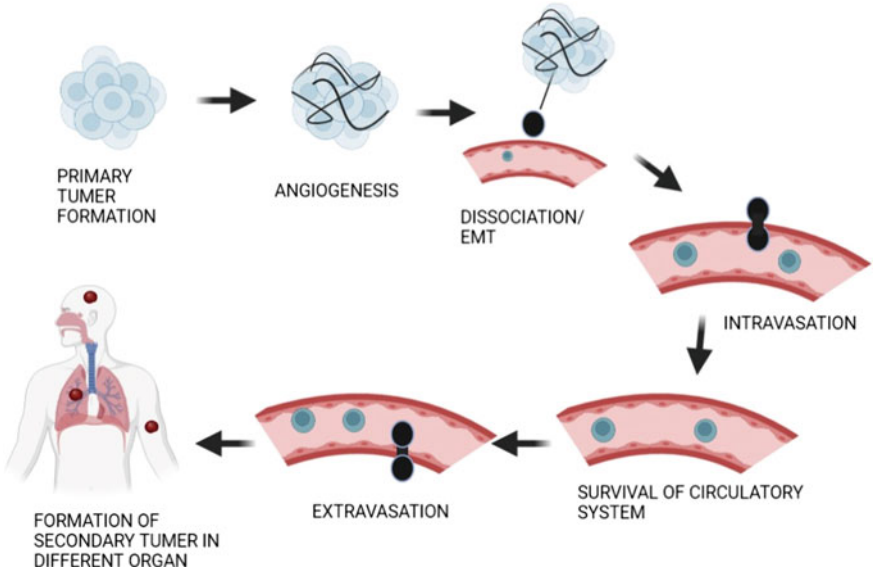

Fig. 3.5 Schematic diagram showing the process of metastasis at the primary site and moves to the secondary site forming tumors

Table 3.2 Application of microfluidics with their application

Application of microfluidics	Details	References
Organ-on-a-chip	Understanding oncology and advancing the process of drug development	[22, 23]
Studying process of metastasis	Metastasis cascade is developed by microfluidics tools which is able to replicate the physiological condition	[24]
Studying cancer cell phenotypes	Mechanical properties that influence migration of cancer cell	[25]
Replication of TME on chip	Features include tumor and ECM and their components interaction, tumor and stromal interaction	[26]
Studying shear stress	Characterizing the physiological nature of cancer cells due to shear stress	[27]
Isolation of CTCs	Label-based and label-free method for separating tumor cells from blood	[28]
Drug screening using droplet microfluidics	Allowing drug absorption confinement and release	[29]
Angiogenesis and vascularized tumor on chip	TME for oxygen and nutrient delivery to cancerous cells	[30]

highly sensitive method like microchannel-based devices can be used. Microfluidics provide platform for electrophoresis, hybridization, and PCR. We can also find the response of drugs to various stimuli. Microfluidics provides a greater picture as we can carefully analyze the concentration gradient, cell–cell analysis, and extracellular matrix components.

3.4.2 Application of Microfluidics System in the Field of Cancer Biology is Explained in the Upcoming Section

See Table 3.2.

3.4.3 Application of Microfluidics for Organ-on-a-Chip System

The phenomenon of metastasis is defined as spreading of cancer cells to different body parts through the blood or lymphatic system. The process of metastasis includes

a series of event, namely invasion, intravasation, circulation, and extravasation to the potential location. A lot of cancer research is going on across the world still the deep understanding of oncology is limited and the drug resistance mechanism remains unclear. For decades, microfluidics system is being developed to recreate and mimic the native essence of tissues and organs in vitro. They provide a more realistic model for understanding cancer and their TME. It helps researchers to understand tumor development, their vascularization, proliferation by creating cell–cell and cell-ECM interactions.

For example, Skardal et al. [31] made a device for real-time monitoring of colon cells which was moving from fabricated gut to liver in a fluidic system. It gave information regarding attachment and invasion of liver. This was the very first model using microfluidics system to understand 3D tissue to 3D target site. Another model defined by Zervantokis et al. [32], to understand the model of tumor vascular link with endothelial permeability to cytokine and paracrine signaling involving tumor cells and macrophages. Wang et al. developed a chip which shows drug response of anti-cancer medication in case of stimulating kidney cancer cells in liver.

So, the organ-on-a-chip provides a platform to better understand the metastasis cascade and further more development of personalized medicine which is specific to each patients.

3.4.4 Application of Microfluidics for Studying the Process of Metastasis

Approximately 90% of cancer deaths occur in patients with metastasis. Secondary tumor formation occurs during this process. It includes a series of sequential events like cancer cell development, growth and invasion, migration through ECM, interaction with vascular cell, invasion arrest, and extravasation at secondary site. Epithelial mesenchymal transition (EMT) is tumor associate hypoxia and stroma environment. All of the other elements, including cells in the TME signaling molecules, contribute to tumor cell intravasation. All these cells play a role in allowing tumor cells to infect the circulatory system. To implant a secondary cancer, tumor cell must overcome the shear blood flow, immune system and has to cling or attach to the capillaries at the site, extravasate, remodel the ECM and proliferation and formation of secondary tumor in the body [21].

Through conventional approaches, 2D culture system was used; then, 3D lacks the complexities, and it can't accurately remodel the TME metastasis as well as therapeutic response. Microfluidics has come a long way for research and application on cancer due to micrometer structure high sensitivity and screening to recapitulate the TME. This system helps us in understanding the process of invasion, intravasation, extravasation, and hypoxia condition like angiogenesis. Many microfluidics systems are being developed by researchers to study the process of metastasis. This is a very

powerful tool and extremely useful in oncology research because there is a sequence of events taking place in metastasis tumor formation.

In EMT, stimulators such as TGF-beta1 stimulate the epithelial cell to lose its polarity, cell–cell interaction which result in massive invasion and metastasis characteristics.

Kim et al. were able to recapture the EMT in alveolar lungs of human epithelial cell (A549) with response to stimulator like TGF-beta1. Lee et al. devised a microfluidics system to remodel the events of angiogenesis and inhibited by using growth factor like anti-vascular endothelial. To study breast cancer, Nagraju et al. [21] developed microfluidics with three cell layer laden hydrogel.

3.4.5 Application of Microfluidics to Study Cancer Phenotypes

The mechanical properties of cancer cells include constraints and stiffness which is linked to metastasis process through which cancer cell migrates in blood capillaries to plant a secondary tumor. According to study, cancer cells are more deformable than normal cells; because of their deformed shape; they are less filamentous and can squeeze through the ECM. So, there is need to know the phenotype of cancer cells to understand the metastasis progression. Microfluidics is a great tool to study cancer because it comprises of micron-level channel which can imitate the movement of tumor cells.

To assess the deformability of breast cancer, Hou et al. used microfluidics with a straight channel and two reservoirs, namely of nonmetastatic (MCF-7) and benign (MCF-10A) quantitative analysis such as cell velocity, cell deformation, and cell transit time were performed. The result was that entry time of MCF-10A was longer than MCF-7; it was assumed because of deformability of cell and due to vary in stiffness, but the transit velocity was found same.

Fluorescently labeled elastomeric contractible surface (FLECS) was developed by Pushkarsky et al. [33]; it measures the force generating behaviors by changing the tissue condition. For the phenotypic study of cancer cells, Yang et al. have developed a device called harmonic acoustics for noncontact, dynamic, selective (HANDS) by application of time-effective Fourier. Augustsson et al. [34] performed phenotyping using iso-acoustic focusing. Cell flowing in this channel starts flowing sideways because acoustic filed between cells and medium is zero.

As the metastasis potential is increased, cell–cell adhesion is decreased.

3.4.6 Application of Microfluidics for Replication of TME on Chip

In the tumor tissue, extracellular components, cancer cells, immune cells, and stromal cells are packed. The growth of tumor cells is increased or decreased by release of malignant and stromal cells. Tumor dynamics is hugely influenced by 3D nature of solid tumor. Initiation, treatment, and progression aspects are all influenced by the microenvironment of tumor cell. TME's cellular and noncellular components communicate in both directions to regulate their proliferation, function, and death.

ECM plays a critical role in cell signaling, migration, and all the other cellular activities including mechanical stabilities. Transcription factor hypoxia-inducing factor (HIF-1) is one chemical change found within tumor cells which responds to decrease in oxygen levels in the cancer cell. As tumor cell doesn't have any blood vessels, so they induce a hypoxia condition which is the driving force of angiogenesis, i.e., blood vessels formation. P53 is a tumor suppressor gene which blocks cell cycle at G1 stage, and it also induces cell death, i.e., apoptosis if the damaged DNA is not being able to repair. Mutation in p53 allows the damaged DNA to undergo cell cycle and hence starts tumor formation. Microfluidics system can be built to mimic the cellular physiology such as shear stress, nutrient delivery, and exposing to therapeutic effects. Scientist are able to mimic some physiological features like shear stress, oxygen gradients, pressure, etc.

Experiment conducted by Bhattachaya et al. [35] in-breast cancer on its role of oxygen and immune interaction. Breast cancer cells were grown in 3D scaffolds to generate physiochemical conditions.

Tumor cells on a chip are a very good platform to study tumor microenvironment (TME) and is cost-effective as well as give high-throughput drug screening. Tumor stromal interaction plays a huge role in tumor progression. Tumor stromal death could be reason of reactive oxygen species (ROS) generated in the microenvironment.

The component of ECM is embedded in the in vitro microenvironment to create framework to support structural and biochemical supports to the cell. Synthetic scaffolds are being used which are fabricated to mimic the natural ECM.

3.4.7 Application of Microfluidics to Study Shear Stress

Shear stress occurs in between stationary phase and liquid phase at the interface. It occurs inside the artery which plays a number of biological roles. In the human circulating system shear level, most of the time remains less than 70 dyne/cm^2, but in case of stenotic artery where the constriction is more than 95%, shear stress is more than 1000 dyne/cm^2. There are five portions in a microfluidics system which are generated by shear stress gradient levels include 8.38, 6.55, 4.42, 2.97, and 2.24 dyne/cm^2. Its application is also seen in umbilical vein endothelial cells. Let's understand the role of shear stress in microfluidics system.

Cancer cells are controlled by various aspects as they are present in our circulatory system; shear stress is one of the factors. It occurs when layer of fluid and viscosity of neighboring cells move together at varying speed. Deformation of CTCs can damage the fluidic force and immune cell attack. Shear stress is calculated by multiplying shear rate by fluid viscosity. Shear stress is involved in a variety of processes, including tumor cell death and metastasis tumor development.

According to research conducted, high shear rate killed more than 90% of the tumor cell in 4 h. A study conducted by Regmi et al. [36] ROS generating drug, for example, doxorubicin and cisplatin can destroy CTCs while comparing to non-ROS generating drugs, for example, Taxol and etoposide present in bloodstream.

3.4.8 Application of Microfluidics in Isolation of CTCs

Circulating tumor cells (CTCs) are cells that are shed from the primary cancer site and travel through the body and enter distance organs which give rise to secondary tumor. As we know, metastasis cancer causes 90% of the death; it is important to understand CTCs as early detection can save patient lives. Counting the CTCs number in blood gives us ideas about the stage of cancer. Understanding CTCs has huge potential for target identification in case of metastasis cancer as molecular characterization will help understand the mechanism of secondary tumor or metastatic process and will play a huge role in diagnosis and prevention. Molecular markers of CTCs include EpCAM which is a universal marker of cancer; these are being widely used in case of prostate and breast cancer. Studies have shown that EpCAM is positive in these cancers. Other marker includes human epidermal growth factor receptor 2 (HER2) for folate, estrogen, and prostate cancer [37]. Let's see the different methods to isolate CTCs based on their physical and biochemical properties (Fig. 3.6).

Label-free detection uses properties like shape, size, dielectric properties, density, and viscosity of CTCs. When compared with label-based CTC trapping, label-free has greater yield. If the CTC size is large, it is advantageous for the size-based CTCs isolation. Physical filling is done in micropores. Comparing it with label-based CTC separation is by use of specific antibodies at the target antigen.

3.4.9 Application of Microfluidics for Drug Screening Using Droplet Microfluidics

It is a new technique which gives up precise confirmation of single cell within a droplet and is used for high-throughput screening. It is compatible with biological and chemical reagents; two immiscible liquids are passed through capillary force to generate droplets. It is an excellent tool for biomedical research. Volume ranges from microliter to femtoliter. Frequency can be Hz to KHz. Many of development

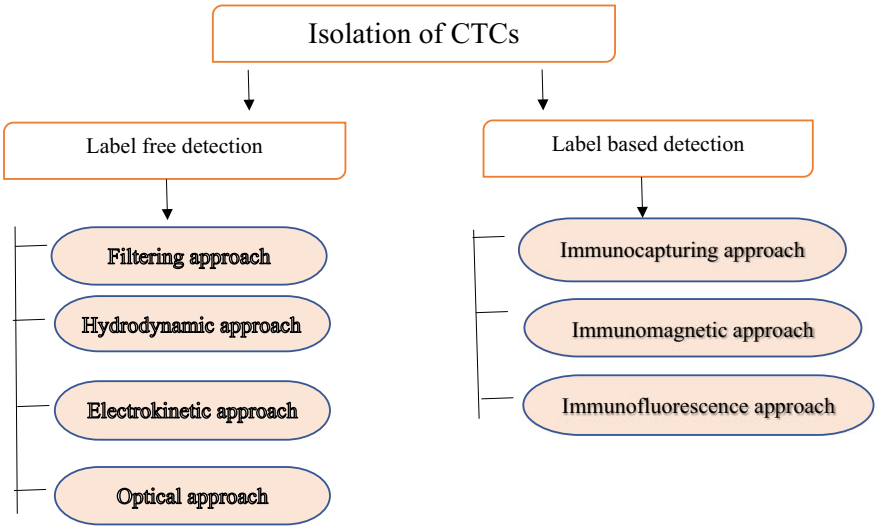

Fig. 3.6 Flowchart showing isolation of CTCs

in the field of cancer have been performed by combining with protein, nucleic acid analysis, and drug discovery [38].

In cancer research, the tiny droplets can be utilized for encapsulating anti-cancer drug as well as molecules like growth factors, antibodies, proteins, and macrophages. In an experiment conducted, droplets were combined and performed cytotoxicity against U937 cells. For optical detection, glass is considered (Fig. 3.7).

The droplet formation takes place with a T junction at the microfluidic system. The breakup of a droplet occurs from a drop in pressure as the drop is emerging.

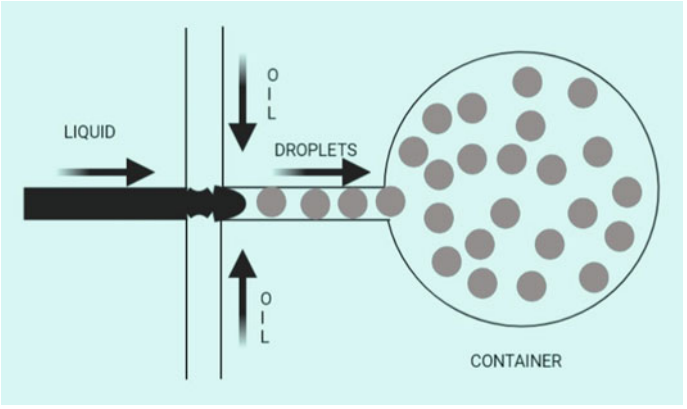

Fig. 3.7 Droplet-based microfluidics system

Commonly, T junction is used; other conformation such as Y junction is available. The flow of liquid is from top to bottom, with liquid from the left being pinched off.

3.4.9.1 Application of Microfluidics for Angiogenesis and Vascularized Tumor on Chip

The tumor vasculature has been made easier by microvascular engineering for anti-cancer drug testing, network analysis which are derived from patients. Cao et al. developed a microfluidic chip combining blood and lymph. Researchers have created lymphoma-on-a-chip to capture the interaction between cancer cells, endothelial cells, and immune cells in TME.

Vascularizing is a game changer in microfluidics for recapturing the tumor mass, interaction between cancer cells, endothelial cells, and stimulating phases of secondary tumor formation or metastasis. But, the development of biomaterials to capture the vasculature of human is a problem that is being addressed by many researchers.

3.4.9.2 Limitations of Microfluidics System

Microfluidics is very promising in cancer research, but the limitations can't be ignored like it is very much influenced by air bubbles, and there are chances of clogging. In CTCs, isolation analysis of large number of blood results in decreased throughput, but automation can solve this issue. Cancer is common in the elderly, and when analyzing their blood samples, the debris in their blood can clog the channels. Material with suitable biocompatibility and stiffness should be used. Long-term study is difficult as cell viability, functionality, and integrity is challenging.

3.5 Integration of Nanotechnology

Nanotechnology is an interdisciplinary field of research with wide application in cancer detection, and diagnosis aspects. The dimension of particle is in nanoscale. Nanotechnology in the field of TE is used to enhance the biological and physical properties of scaffolds and various other functions. Examples include titanium oxide (TiO_2), gold and silver nanoparticles, CNTs and others. TiO_2 is being used to enhance the mechanical property of tissue-engineered scaffold and increase cell proliferation. Another example of enhanced cell proliferation is use of 3D printed scaffold which is made up of PLGA used to culture ES which is derived from cardiomyocytes when TiO_2 is integrated cell proliferation is increased. Among all the discovered nanomaterials, 2D ones have considered for development of medicines. They are considered to be excellent choice for diagnosis and therapy, especially in case of cancer. The feature that makes 2D nanomaterial more unique is their shape as they have high

surface area and also belongs to carry large number of load into the cancerous tissue by passive or active targeting. More properties include their composition, configuration, functionalities, and their charge. The morphology of nanomaterial can be analyzed by scanning electron microscope (SEM), X-ray diffraction, zeta analyzer, transmission electron microscopy (TEM), Raman spectroscopy, and atomic force microscopy.

Here, we will talk about breast cancer as it is the second most diagnosed cancer worldwide. In the last five years, various nanomaterials have been studied on breast cancer such as Au NPs, quantum dots, carbon dots, magnetic NPs, mesoporous silica NPs, nanotubes, etc., have been used. In healthy cells, the human epidermal growth factor receptor 2 or HER2 is a receptor that cell regulates cell proliferation and survival. Its overexpression results in uncontrolled growth which results in breast cancer. Yang et al. used finely graphene nano-mesh and functionalized it with aptamers that would respond to HER2 protein. They used manipulated graphene in field-effect and detected single SK-BR3 in cancer cell.

A hybrid-based nanomaterial called PAA and mesoporous silica nanomaterials are used in various ways starting from drug delivery, surface modification, PAA coating, and doxorubicin hydrochloride loading. Doxorubicin is used as model guest for drug encapsulation, change in behavior at different temperature and ph. Advantages include nontoxic, large amount of drug can be loaded, and it is easy to make.

Yuan et al. developed a nanoscale drug delivery platform for anti-cancer therapy; cyclodextrin (CD) and modified poly-acrylic acid and paclitaxel are induced to a mouse which has H22 tumor, PCDAA-PTX NPs reached target site because of improved retention and permeability effect. It shows better effect than commercially available anti-cancerous drugs. Cisplatin-encapsulated nano-capsule with a cisplatin-PAA core in an amphiphilic iron oxide or polyvinyl alcohol was double emulsified to release drug. A549 is a tumor-induced mice shown anti-cancerous activity with no or little side effects.

Cancer treatment includes therapeutic functionalities and integrated multimodal imaging onto a nanoplatforms. For control drug and diagnostic process, nanocomposite system is developed which is composed of ZIF-8 and PAA, manganese oxide nanoparticles which is tumor diagnostic agent, and methotrexate which is a therapeutic and tumor biomarker agent. Wu et al. developed PAA-FeO_4 nanogel used for drug delivery and resonance imaging [39].

Nanofibers (NFs) have excellent biomaterial application in case of drug release, wound dressing, and tissue engineering. The MTT experiment shows that NFs are nontoxic, their development used as platform for cell adhesion and proliferation. Khajeh et al. reported that biocompatible nanofibers could be used for wound healing by electrospinning of PAA, poloxamer, and polyurethane.

Properties of nanomaterial provide wide varieties of research in the field of optics, biomedical sciences, and electronics. There has been progress in the field of nanomaterial that has been made, but still there are various challenges associated with tissue engineering and nanomaterials.

3.6 Tissue-Engineered Models Used in Cancer Reserch

Three-dimensional culturing is becoming promising field for cancer research. 3D models have more biomimetic properties compared to 2D cell culture. The models for in vitro analysis include spheroids, organoids, and scaffolds. In 3D tumor model, spheroids are commonly used as chemoresistance and gene expression, but they lack stressors because of culturing in static condition. The recent advancement in organoids defined as miniature versions of organs, multiple cancer studies have been performed on them as they resonate with the genetic and phenotypic epithelium, but they lack development immune cells and stromal interaction with serum. 3D culture system is gaining a lot of popularity and is being used in the cancer field to better understand the local microenvironment and tumor progression. Scaffolds have been used in TE to mimic the TME. There are various types of scaffolds, for example, polycaprolactone, PLGA, modified scaffold, and various others. We will be understanding the different 3D model systems that are being developed using TE for different types of cancer. Past decade increases in development of many 3D models by studying invasive behavior, chemoresistance, angiogenesis. Development of 3D model system for various types of cancer is hallmark of cancer research. There is scope of developing 3D model systems for increasing our understanding studying about how tumor ECM works, the whole process of tumor formation, disease progression, and its treatment.

3.6.1 Osteosarcoma

Osteosarcoma, also known as bone cancer, is characterized by high rates of metastasis and cancer relapse, and it primarily affects children and adults. There is chemotherapy available, but chances of patients with recurrent or metastatic osteosarcoma remain poor; hence, there is urgent need for effective therapy. Cancer stem-like cells (CSLCs) have infinite capability of division. A number of studies have shown that CSLC in tumor relapse as they may adopt quiescent state, overexpression of anti-apoptotic proteins. CSLC to have resistance to drugs and to regenerate in bulk and give rise to different subpopulation of cancer is called intratumor heterogeneity, and this is the main challenge faced during curing of bone cancer. The tumor microenvironment plays a huge role in drug resistant, development, and progression of osteosarcoma. Most research has been done on 2D, so only 5% of the drug is succeed in clinical trials. Bioengineered three-dimensional models are being developed which closely mimic TME. The ECM of bone is made up of calcified bone, collagen, and other ECM molecule. Hydroxyapatite has greater interest for bone repair because of its biocompatibility, cell attachment, differentiation, and proliferation.

Cold atmospheric plasmas (CAPs) are newly discovered therapy which are being used to treat cancer. It is an ionized gas at room temperature composed of high amount of ROS and NOS, ions, electromagnetic field, radiations, and electron; its

anti-cancerous activity depends mostly on ROS and NOS to kill tumor cells through oxidative stress; only, a few studies have been performed on OS by using CAP. Mostly, studies have been performed on 2D which shows promising result, but 2D doesn't mimic the ECM, so recently, studies have been performed in 3D. Three-dimensional tissue-engineered models are being developed using scaffolds made from collagen and hydroxyapatite (HA) nanoparticles. When OS cell cultured, it showed different behavior, when cold plasma-activated Ringer's solution (PAR) were applied to 3D culture survived by enhancing CSLC properties, adaption to oxidative stress, and cell proliferation. There is need to do more work on this so that we can better understand OS and treat them [40].

3.6.2 Breast Cancer

Breast cancer is the most commonly found type of cancer in found in female; it has the ability to metastasize to other parts of body such as lungs, liver, and bone, during later stages and can cause secondary cancer-related death. Traditionally, preclinical studies have been performed in 2D, but it can't recapture the tumor microenvironment, although in vivo models are extremely useful. Cancer research is expensive and requires ongoing maintenance, but it fails to demonstrate the human response to anti-cancer drugs. 3D models capture the TME; there is similarities between 3D tumor microenvironment and native structure in human body. In cancer cells, there is loss of cell adhesion which is leading to dissociation from epithelium. Stromal and cancer cells secrete growth factors which affect the cell ECM. As the tumor accumulates, tumor becomes malignant and difficult to treat. Epithelial, stromal, fibroblast, endothelial cell, adipocytes all effect the TME in tumor progression and metastasis. 3D models use biomaterials which will the cell to take their native environment. Some of the techniques to develop 3D models include fabrication, bioprinting, microfabrication. Microfluidics has a very promising role as we have discussed in earlier paragraph. Immune cells are also be added to better enhance the TME, and we can get better result. The basement membrane extract is obtained from Engelbreth-Holm-Swarm (EHS) which is composed of type IV collagen, entactin, laminin, and growth factors. Commercially, BME is also available which is used for culturing cancer cells, epithelial cell, and stem cells. Scaffolds are used for migration, metastasis, and invasion of breast cancer. Example of solid scaffold includes gelatin which is made by electrospinning, and collagen I is coated on with MCF-7 for production of breast cancer model.

When we treat it with estrogen, there is an increase or decrease in gene expression, such as cadherin expression, which is completely reversed when compared to progesterone treatment. 3D printing is another technique for breast TME engineering; through this, 3D geometric structures are produced by deposition through multiple layers. When living cells are printed, they are called bioprinting. We can control the location of cell, growth factor proteins, and create our desired 3D construct [41].

3.6.3 *Lung Cancer*

Lung cancer is one of the most commonly diagnosed cancer. Despite the use of advanced technologies, the survival rate of lung cancer is only 15%. The cost of making anti-cancerous drug is very much expensive and time-consuming. We have already discussed how ECM is remodeled because of this reason cancer models fail to capture the tumor microenvironment. 3D models are being developed to understand the cancer development and their drug optimization. Several models have been established, for example, hanging drop method (change of media is tedious), spinner flask method (takes long time), and non-adherent 3D culture system. For the development of porous substrate, poly-lactide co-gycolide is often used. Substrate or scaffolds are highly interconnected; 3D tissue model has micrometric pore which enhance cell attachment and proliferation. For lung cancer model system alginate microsphere-based pore formation, PLGA microspores (PLGAMS) are being used. Study revealed that PPMS is maintaining its structural integrity, and PLGAMS shows shrunken morphology. When collagen is coated with PPMS, it enhances the cell attachment and also their proliferation while co-culturing lung adenocarcinoma (A549) and lung fibroblast cells (MRC-5).

While comparing 2D culture and lung tumor model, tumor model has high resistance to cancer. It has been shown that PPMS has large interconnected pores, and we have got safe method of pore forming by AMS and EDTA on microspheres. PPMS has found to have high cell attachment, proliferation; models formed can be used for drug testing and also studying cancer metastasis [42] (Fig. 3.8).

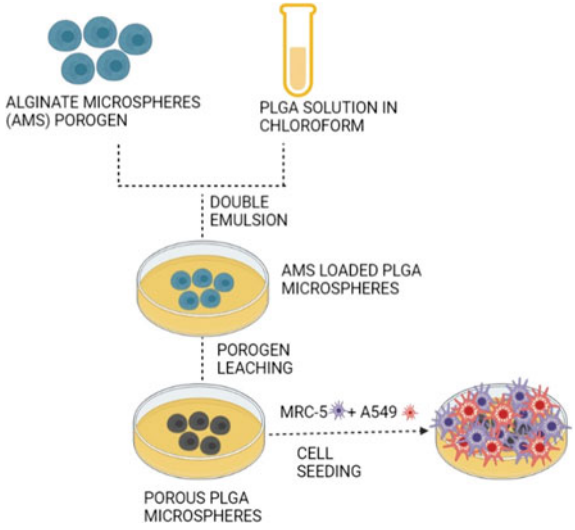

Fig. 3.8 Development of lung cancer model in 3D MRC-5 lung fibroblast and A549 lung adenocarcinoma are seeded on porous PLGA

3.6.4 Ovarian Cancer

Ovarian follicles are the functional unit of an ovary containing oocyte which is surrounded by many layers of granulosa cells and many extracellular layers. The maintenance of 3D architecture is quite challenging because of the architecture. If cancer is found in the ovary, various strategies have been used such as cryopreservation of ovarian tissue and then auto-transplantation for restoring the fertility or starting chemotherapy; but, this is not safe if there is metastasis cancer. To recapture the native environment in lab, various tissue-engineered techniques have been used such as 3D printing and ovary-on-a-chip which will help us to understand the physical structure, molecular and cellular cues, and vascularization.

Tissue engineering has developed many scaffolds which can be used in in vitro culture. They are implanted at the target site if there is a need to gain the functionalities back or repairing of any damaged tissues. In case of ovarian cancer, ovary can be fabricated in addition to enhancing the properties like biocompatibility, biodegradability, and exchange of nutrients and waste in case of biomaterials. There is proper balance between elasticity and rigidity to maintain the shape of the follicle. Conventionally, TE was using method like decellularized scaffolds, nanofibers, or hydrogels for recreating the native structure. Entirely recapturing the tissue complexities, oxygen diffusion, cellular architecture, and mass transfer is a challenge. So, 3D fabrication techniques have been developed for biomimicking the structures to pattern molecular cues and integrating required mechanical properties. Three different types of tissue construct have been developed—laser, inkjet, and extrusion-based 3D printing. Different biomaterials have been used like natural, artificial, or combination of both. The properties that the biomaterials should have include biocompatibility, suitable physical and mechanical properties, and printability. Laronda et al. developed 3D printed follicle scaffold for ovarian cancer. The interaction between scaffold and follicle has positive correlation. Microfluidics for follicle encapsulation is recapturing ovaries natural microenvironment, but it is difficult as the length of nutrient and oxygen penetration is less than 200 μm. So, we need to make sure that cells present in the core are not going under necrosis. The building of organ-on-a-chip is combination of microfluidics and tissue engineering. This is done to understand more about the tumor microenvironment and learning more about cell–cell and cell-ECM interactions. The benefits of ovary-on-a-chip include studying of diseased or healthy cells, drug testing and their development, evaluating the toxicity and safety of a particular drug. There are wide varieties of studies available for lung, liver, kidney chip, but only few studies have been performed on ovary-on-a-chip. The first study that was done on ovary is to check the ovotoxicity induced by microcystins. This approach is still in infant level; in future, there will be a lot of research focusing on this area. Innovative biomaterials are being developed like better fabrication, 3D printing as these will capture the similarities of the biomimicking materials which can be used for developed better multicellular models for follicular cancer and can innovate the field of TE and RM [43].

3.6.5 Liver Cancer

The aim of liver tissue engineering (LTE) is to build liver models which can mimic function of an in vivo as close as possible. There are two major application of LTE (i) testing drugs or pathogens and (ii) toxicity study. Due to ethical constraints, drug testing research could be hampered; on the other hand, study on primary hepatic cells can't replicate the native environment. Hydrogels are being used to study LTE as they are supportive biomaterials. There is no hydrogel that mimics the ECM of liver. Let's understand different hydrogels that are being developed for studying LTE. Comparing different hydrogels will give us a better picture as which one should be considered. The required properties include biocompatibility, biodegradability, liver cell engraftment, and long-term survival. Natural hydrogels include collagen, gelatin, fibrin, chitosan, cellulose, etc. Synthetic hydrogels for LTE include PLA, PCL, and PGA. Natural hydrogels are considered over synthetic as they have high biocompatibility, and immune response is significantly reduced [44].

3.6.6 Blood Cancer

Blood cancer, hematological malignancies, or non-solid tumors are all used for leukemia, myeloma, or lymphoma. Hamopoietic stem cells are the precursor of all blood types, and it divided into lineages. Microenvironment of bone marrow in the human body is tightly regulated which helps in maintaining balance between immune cells and blood cells. Any deformalities in BM will affect the body severely. BMN is a composed of cellular and noncellular environment and is very important factor in cancer progression. Different model systems are being used to recapture microenvironment of bone marrow. But, there is lack of complexities; the properties that should be included are the components of ECM, vascularization, differentiation, topology of material, etc. Fully building model for blood cancer is very challenging; however, continuous efforts are being put to make 3D model system which have all the desired microenvironment to make the non-solid biology much approachable [45] (Table 3.3).

3.7 Conclusion and Future Directions

2D and 3D cells provide techniques which provide necessary technique for understanding advanced research. For organs transplant, stem cell research, drug testing, 3D culture system has provided new ways of research ideas. When 3D cell culture is better understood, advanced research method will arise. For co-culturing, 3D should be considered, and its benefits are superior to that of 3D as we can apply it for studying tumor models, disease testing, and cancer models. Three-dimensional culture is able

Table 3.3 Shows the various types of cancer along with suitable type of model with their application

Type of cancer	Type of model	Material	Application	Conclusion	References
Colorectal cancer cell line is—$CaCO_2$	Spheroids, scaffold free	Methylcellulose	Tumor-related pathway	Spheroids in AKT-mTOR activity and their crosstalk	[46]
Liver cancer	Scaffolds, tumoroids	BME, Matrigel	Personalized cancer study, biomarkers	Metastasis feature is preserved	[46]
Breast cancer	Scaffold	Poly-ether urethane foam	Control metastasis of breast cancer to bone	Osteoblast mediating adhesion	[46]
Bladder cancer	Scaffold	Gelatin	Chemotherapy response	Cell–cell interaction, high proliferation	[46]
Breast and lung cancer	Hydrogel, 3D printing	Polystyrene	Adhesion of CSC to target tissue to 3D cell culture	Develop premetastatic niche	[46]
Colorectal cancer HCT-116 and HCoMECs	Microfluidics, spheroids, scaffolds	PDMS, Matrigel	3D microfluidics cell culture	Real-time analysis, co-culture	[46]

to overcome the limitation of 2D cell culture and is able to recapitulate the tumor microenvironment by integrating new technologies which are helping in creation of system that will closely mimic the in vivo condition so that we can detect, analyze, and treat cancer in a better way. The new approaches along with current tissue engineering technologies can ultimately in vitro tumor which will help advancing the field of cancer biology. The integration of multicellular, patient-specific three-dimensional interaction, chemical parameters (such as ph, oxygen, and gradient), and the composition of ECM is a great asset of TE. All the cell, tissues, and organs have specific functions, molecular cues, and have specific niches; building 3D platform with structure similar to that of native cells will useful in treatment of cancer. The use of biomimetic scaffolds having biocompatibility, biodegradability, and response to external stimuli will help in the development of tissues which will advance the field of tissue engineering. The production of organoids and spheroid has huge potential in the development of disease modeling, and it will revolutionize the field of personalized medicine because we can't perform heterogeneity test on animals, and in case of 3D models, host immune response can't be observed. The microfluidics platform has several advantages over traditional 2D and 3D culture systems. They require low concentration of reagent and sample, highly sensitive, cheap, gives high-throughput

screening, and processing time is less. Microfluidics system approaches are seen in characterization of phenotypes of cancer cells, circulating tumor cells (CTCs) and most important they helpful in studying metastasis. Microfluidics system requires to innovate in the field of metastasis target which are linked the fluid system of our body. The organ-on-a-chip is an innovative tool when if more advanced then it has the potential to fight against cancer which is caused in the distant organs. The iPSCs model system has become popular in studying cancer progression, drug development, and cancer genetics. Several studies have shown that further development of TE can be taken place by taking advantages of biomaterials. It is seen that when nanoparticles are used in scaffolds cell adhesion and proliferation of cell is increased. TE is limited to interact between cell and material if smart biomaterials having property to control the behavior and response to external stimuli of a cell, but producing such biomaterials is challenging. Use of components having magnetic or electronic properties within the tissue-engineered products when controlling the host immune response and sensing can be controlled with a remote after implantation of the product will be broadening the field of TE. Data analysis and artificial intelligence are two areas of research that have the potential to greatly popularize TE. AI will collect data and innovate TE products based on feedback.

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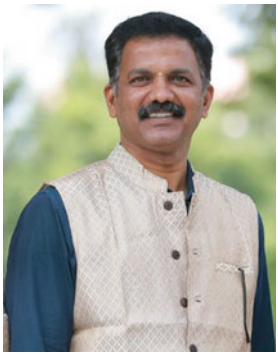

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Chapter 4
Biomaterial-Based Delivery Systems for Chemotherapeutics

Dalapathi Gugulothu, Dimple Dhawan, Alisha Sachdeva, Deepali, and Meenakshi Kanwar Chauhan

Contents

Abstract The fields of understanding the molecular basis, cell genetics, biochemical sciences, materials sciences, and technology all have significant contributions to make towards the research and fabrication of biomaterials. These materials have undergone modification in order to have interaction with biological systems in a therapeutic or diagnostic manner. In the environment where they are placed, they have a synergistic effect. Although it is a centuries-old science, it is currently evolving as a cutting-edge research platform with applications in a wide range of medical sectors, with cancer being the most intensively explored. The overview of these biomaterials-related studies, which covers the application of biomaterials as therapeutics such as immunisations and surface modulators to increase the activity of antigen-specific T cells in immunotherapy for malignancies, is included in the study. In cases of recurrent cancers, tumours that are inadequately immunogenic and tumours that are immunologically resistant, the application has been demonstrated to be reli-

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able. Biomaterials have been used to treat skin cancer and triple-negative breast cancer successfully. Patients with malignancies that lack immunogenicity respond poorly to immunotherapy treatments clinically. When taken at high concentrations, several treatments also have a chance of causing systemic toxicity, and the possibility of autoimmunity is practically always present. In order to address the drawbacks of immunotherapies, biomaterials can be used as cancer detection tools, delivery vehicles to change the pharmacokinetic properties, oral bioavailability, and regulate discharge of therapeutic drugs targeting the immune system, vaccines, and targeted nanoparticle drug delivery systems (active/passive targeting). We will examine the applications of natural, synthetic, and latest design biomaterials in cancer research in this chapter of the book.

Abbreviations

AC	Adamantane carboxylic acid
ACT	Adoptive cell therapy
ALT	Alanine aminotransferase
AMT	Absorption mediated transcytosis
APC	Antigen presenting cells
ApoA1	Apolipoprotein A1
ASGPR	Asialoglycoprotein receptor
AST	Aspartate aminotransferase
AuNP	Gold nanoparticles
BBB	Blood brain barrier
BC	Bacterial cellulose
C–Co–NPs	Cobalt nanoparticles coated with graphitic shells
–CD	Beta-cyclodextrin
CDPs	Cyclic dipeptides
CDT	Chemo dynamic therapy
CNS	Central nervous system
CpG	5′-C-phosphate-G-3
CTX	Cabazitaxel
Cur	Curcumin
DEB	Drug eluting beads
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
EGFR	Epidermal growth factor receptor
FF	Diphenylalanine
FIONs	Ferrimagnetic iron oxide nanocubes
GBM	Glioblastoma
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HAV	His-Ala-Val
HCC	Hepatocellular cancer

HDL	High density lipoprotein
HFIP	Hexafluoroisopropanol
HIV	Human immunodeficiency virus
IAP	Inhibitor of apoptosis
ICG	Indocyanine green
ICMVs	Inter bilayer-crosslinked multilamellar vesicles
IL	6 Interleukin 6
IONPs	Iron oxide nanoparticles
LDDSs	Local drug delivery systems
Lf	Lactoferrin
LFC	Lipid formulation classification
MAbs	Monoclonal antibodies
MC-38	Murine colon adenocarcinoma cells
MCTs	Multicellular tumour spheroids
MDA-MB231	M.D. Anderson—Metastatic Breast 231
MDR	Multidrug resistance
MNP	Magnetic nanoparticles
NapFF	Naphthalene-diphenylalanine
NC	Nanocrystal
NCA	N Carboxyanhydride
NDI	Naphthalene diimide
NHS	N hydroxy succinimide
NIR	Near Infrared
NPs	Nanoparticles
OLISA	Organelle co-localization-induced supramolecular self-assembly
OvCa	Ovarian cancer
PBS	Phosphate buffered saline
PDI	Perylene diimide
PDT	Photodynamic therapy
PEI	Polyethylene imine
pRNA	Package RNA
PTT	Photothermal therapy
PTX	Paclitaxel
PVA	Poly vinyl alcohol
QDs	Quantum dots
RF	Radiofrequency
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SDT	Sonodynamic therapy
siRNA	Small interfering RNA
SLN	Solid liquid Nanoparticles
SOD	Superoxide dismutase
SWNTs'	Single walled nanotubes
TCA	Tricarboxylic acid
TME	Tumor microenvironment

TNF	Tumor necrosis factor
TRAIL	Tumour necrosis factor-related apoptosis inducing ligand
WC	Watson-Crick

4.1 Introduction

With an upward trend over the past several years, cancer is known to be the foremost reasons of death globally. Abroad range of diverse disorders that are marked by unrestricted cellular proliferation are collectively referred to as cancer. It is capable of entering and spreading throughout the entire body and can start in any area of the body, including organs or tissues. Malignant cancer cells develop from normal cells as a result of genetic abnormalities, alterations in the genes for oncogenes, tumour suppressors, and DNA repair which are a few examples engaged in cellular differentiation and growth [1]. Healthy cells and cancerous cells vary from one another in a numerous ways, including the composition of their membranes, how much energy they need, and how quickly they proliferate. For instance, in typical eukaryotic cells, the leaflet located on the inside of the cellular membrane contains phosphatidylethanolamine and phosphatidylserine, whereas the outer leaflet primarily contains sphingomyelin, phosphatidyl-choline, and phospholipids that contain choline [2–5]. However, a variety of internal and environmental events that cause specific biological reactions might cause this array to vary. Cancer cell membranes also include excessive amounts of sialylated gangliosides, O-glycosylated mucins, and heparan sulphates, whereas neutral zwitterionic phospholipids and sterols [6] make up the majority of healthy cell membranes. More cationic anticancer polymers and peptides interact with cancerous tumour cells because they have a disproportionately higher surface area of microvilli than normal cells, which increases their surface area. According to Otto Warburg's theory, cancer cells consume energy differently from healthy cells [7]. Even in the absence of oxygen, cancer cells prefer the glycolytic pathway over the tricarboxylic acid (TCA) cycle to produce energy, which results in increased amounts of lactate formation and acidification [8]. Radiotherapy, chemotherapy, and pharmaceutical therapies based on biomaterials are some of the methods used to treat cancer globally [9].

Chemical science, materials science, cell biology, molecular biology, and technology all play significant roles in the research and production of biomaterials. These are compounds that have undergone modifications to connect with biological systems in order to serve as diagnostic or therapeutic tools. They have a synergistic effect on the environment in which they are positioned. Despite being an old topic, it is currently evolving into a slashing research platform with applications in various sectors of medical sciences, with cancer being the field with the most study. According to the synopsis of these biomaterials-related works, the study includes the development of biomaterials as therapeutic agents, such as immunisations and surface

modulators to boost antigen-specific T-cell response in immunotherapy for malignancies. In cases of recurrent cancers, tumours that are inadequately immunogenic and tumours that are immunologically resistant, the application has been demonstrated to be reliable. Biomaterials have been used to treat skin cancer and triple-negative breast cancer successfully. This chapter emphasises the value and range of research in the area of biomaterials in cancer treatment [10]. There have been significant advancements over the previous decade in the treatment of malignant tumours with antibodies, particularly immunomodulating monoclonal antibodies (mAbs). Despite advances in technology for antibody design and production, there are still a number of difficulties with antibody-mediated cancer therapy, including instability in vivo, poor tumour penetration, a low response rate, and unfavourable off-target cytotoxicity. It has been suggested that biomaterials-based delivery systems can increase the anticancer effectiveness of antibody medicines. Due to its remarkable therapeutic efficacy in treating a variety of tumour types, immunotherapy has somehow managed to grab attention. The evolution of local drug delivery systems (LDDSs), such as the biomaterial scaffold-based drug delivery systems, acted as a useful strategy for administering immunotherapeutic agents easily and intensively in situ by being less harmful to the body systemically because immunotherapy is not able to regulate localisation as well as sustain therapeutic concentrations at infection site. In this article, we'll go through the most recent advancements in LDDSs constructed from biomaterial scaffolds for such administration of immunotherapeutic substances such immunomodulators, immune cells, and vaccines Additionally, research is being done on co-delivery techniques for local immunotherapy, that involves chemotherapy and photothermal immunotherapy together [11–14].

Design and production of biomaterials generated from biomolecules have become viable in recent years because to developments in biomedical research. A variety of chemical, physical, mechanical, and biomimetic qualities are assimilated into biomolecules through solution processing, modification, or combining with some other natural or artificial elements to create devices and systems made of clinically responsive biomaterials. Significant innovations in the fabrication of synthetic potentially useful materials in biomedicine including medical technologies were made during the century. The ongoing emphasis on using artificial components in medical practice highlights the requirement for biomolecules to be included to increase their biomimetic properties (Fig. 4.1) [15].

4.1.1 The Generations of Biomaterials

The very first generation in biomaterials science, which began in the 1950s, was mostly composed of materials selected for therapeutic use which also guaranteed a minimum response by the host tissue and were thought to be compatible. This includes coating compounds for mechanical valves, pyrolytic carbon as well as silicone rubber [16]. It has been found that the second-generation biomaterials are related to the early or first-generation biomaterials. They are designed to have a therapeutic

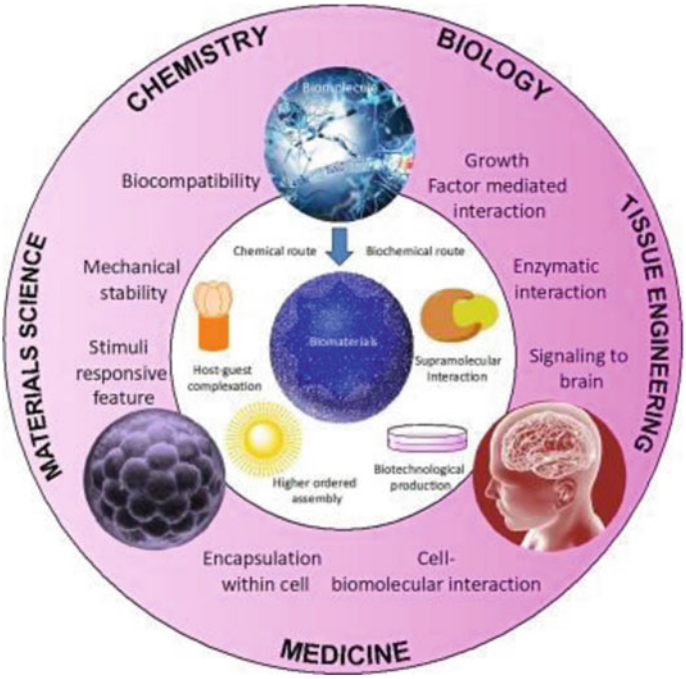

Fig. 4.1 Advancement of engineering, biology, chemistry, materials science, and medicines for biomedical applications in collaboration [15] (Copyright 2020, *Elsevier*)

effect by interacting with the tissues in which they are implanted in a controlled way. Ceramics and bioactive glasses are two examples of the clinically employed materials in dentistry and orthopaedic operations. These biomaterials also contain components with variable rates of resorption and breakdown. Because they are biodegradable, they form a contact in between implant site and also the host tissue which can be removed over time [17]. Since it offers pertinent solutions to present problems, this third generation is the one that is most frequently addressed right now. They support tissue regeneration, which is the cornerstone of the regenerative medicine and tissue engineering industries. Tissue engineering permits tissue formation and restoration with therapeutic effects using living cells. With high success rates, this has been widely employed in bladder, skin, and cartilage replacement methods [18, 19].

4.1.2 Role of Biomaterials in Cancer Therapy

However, recently the utilisation of biomaterials has grown to increasingly varied medical applications [20, 21]. Biomaterials consist of either synthetic or natural materials typically employed in medical equipments. Biomaterials were heavily used in

preclinical and clinical trials for local sustained administration and targeted therapeutic delivery because of their unique molecular, morphological, and biological characteristics [22]. Modern medical research and clinical applications have focused on biomaterials for a number of applications in medicine, such as medication administration, tissue regeneration, and angiogenesis [23–26]. Because of advances in imaging technology, a thorough knowledge of cancer biology, and the expedited delivery of therapies like chemotherapy and immunotherapy [27], the use of biomaterials has also significantly improved cancer detection and treatment [20, 28]. The use of biomaterials in numerous fields, including targeted drug delivery systems, antigen-specific T-cell boosters for the immunotherapy of cancer,implantable, injectable, and transdermal biomaterials utilising biomaterials to tailor the tumour microenvironment and 3D cell culture as well as biomaterials as an alternative to the development of cancer vaccines, will be thoroughly covered in this chapter. Adoptive cell transfer using biomaterials, intra-arterial blockage using biomedical polymers, uses for some biomaterials in the treatment of cancer [29].

4.2 Types of Biomaterials

Numerous types of biomaterials in application of cancer research were exhibited in Fig. 4.2.

4.2.1 Natural Biomaterials

Cancer treatments include a number of undesirable side effects and induce toxicity, particularly to healthy tissues, which prevents them from achieving the desired therapeutic benefits [21, 30, 31]. The creation of innovative biomaterials is among the most active fields in healthcare materials. Even though, synthetic biomaterials were employed quite extensively. The use of natural biomaterials may provide exciting options. Because they are a rich source of architectural and chemical possibilities, a natural biomaterial must be nonimmunogenic, resilient, degradable, simple to make, accessible, affordable, reusable, and sterilisable. Nature has developed a broad range of complex materials with acceptable physical, chemical, and mechanical parameters, biological processes, and also the greatest extent of biomimicry through the process of evolution [32]. There are numerous uses for natural biomaterials that emerge from the growth cycles of all living things. They include bio-inspired materials as well as bioactive and bioinert materials. Natural biomaterials have shown to be adequate to meet the requirements of the healthcare industry as they demonstrate an increasing level of sophistication. Here is an overview of the various natural healthcare biomaterials, including information about their biodiversity sources, characteristics, and possible medical applications. As other natural biomaterials are still being found and

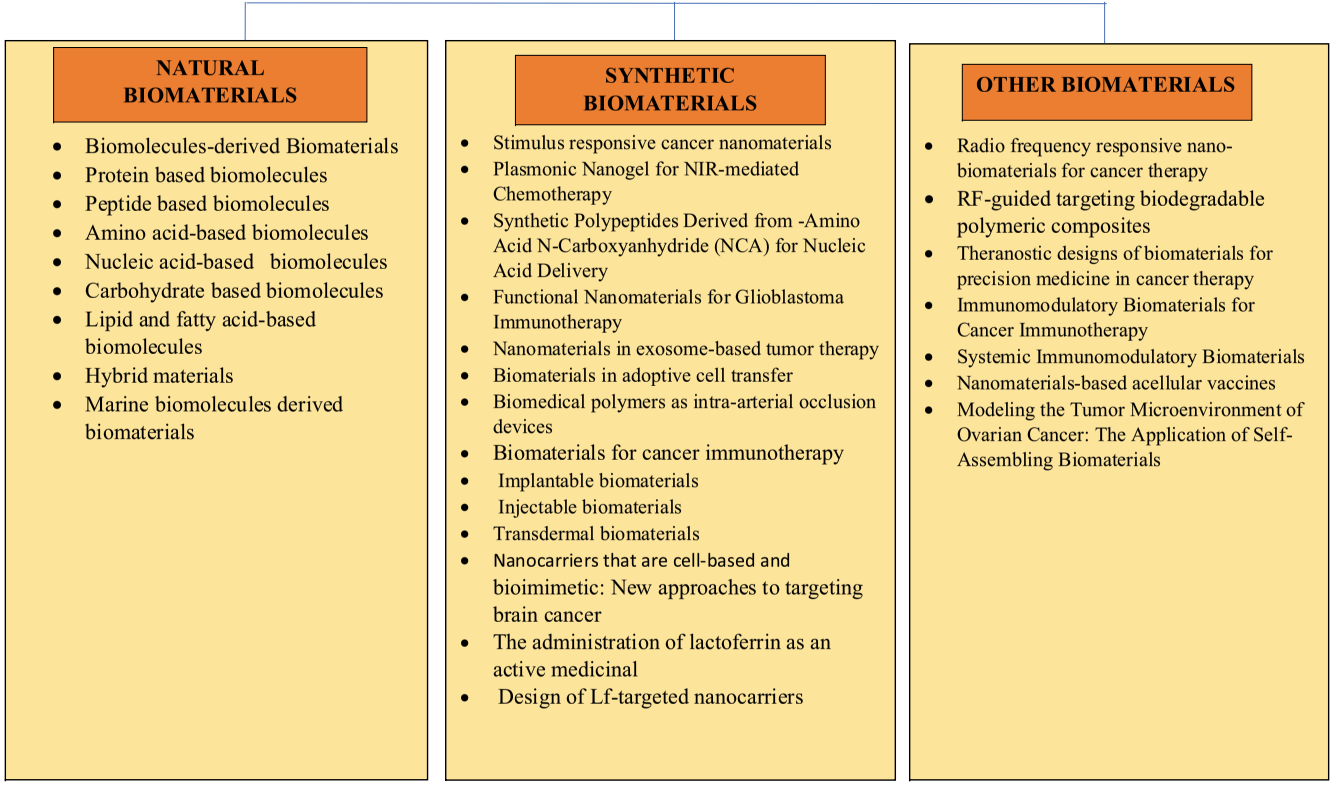

Fig. 4.2 Types of biomaterials

explored, various limits of natural biomaterials are discussed, and potential future advances are suggested [33, 34].

4.2.1.1 Biomolecules-Derived Biomaterials

Since the beginning of human civilisation, materials originating from biomolecules with animal and plant-derived sources were being used for biological purposes. In recent years, innovative biomaterials generated from proteins have been developed using precision design approaches. The development of biomaterials produced from biomolecules depends critically on the biological, chemical as well as mechanical characteristics of biomolecules and its bulk constituents. As a result, the functional characteristics and applications are significantly influenced by structural changes and there are mild noncovalent interactions within the fundamental building components. To keep life faithful, biomolecules' universal synergy and interaction are crucial. Any variation brought on by illness or injury may perturb the interaction of biomolecules within the living machinery system. Numerous human illnesses and impairments may be driven on by structural damage to the tissues and organs. The main drawback is that, if compromised under unfavourable physiological conditions, the human body is unable to regenerate most of its organs on its own. The application of materials made from biomolecules as long-term implants as well as regenerative replacements or the injured organs and tissues is currently a major focus of biomedical sciences. Biomolecule-derived biomaterials are defined as those materials used in biomedical applications that are made either from biomolecule alone or from biomolecules combined with synthetic materials [35]. The widely accessible natural raw materials keratin, silk, collagen, cellulose, and silk offer intriguing material properties and the favourable biological, chemical, and mechanical capabilities needed for the processing and manufacture of biomaterials. These materials are listed in Fig. 4.3 [15].

During the course of evolution, nature discovers biomolecules with superior molecular structures with distinctive properties that regulate numerous biological processes in living creatures. The goal of using biomolecules is the creation of durable biomaterials which imitate natural systems by exhibiting hierarchical complexity, structure, and functional significance. Tissue engineering and regenerative medicine could undergo a revolutionary change thanks to the rapidly expanding multidisciplinary field of biomaterials research and engineering. Through carefully chosen examples from the literature, we have discussed recent progress in the field of biomaterials produced from biomolecules and their uses in this chapter (Table 4.1).

4.2.1.2 Protein-Based Biomaterials

The most intriguing biomacromolecules are proteins, which have a wide range of biological roles and good material properties. All living things' phenotypic traits and

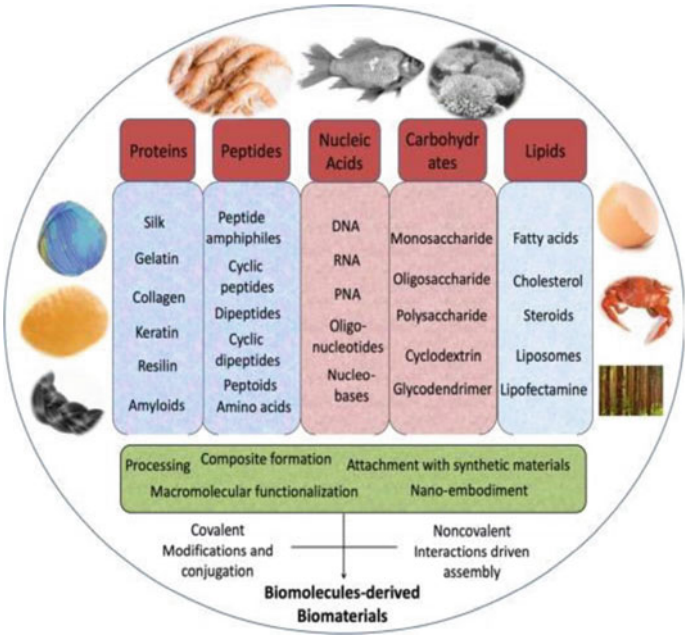

Fig. 4.3 There are several classes of biomolecules, including monomers, oligomers as well as macromolecules derived from plants and animals as well as their natural sources are employed to generate biomaterials [15] (Copyright, 2020, *Elsevier*)

functions are closely related to a complex network of proteins with clear structure–function relationships. Because of this, proteins are correctly viewed as the true workhorses of a live cell. Silk, collagen, gelatin, and keratin are the primary proteins that exhibit the superior structural and mechano-chemical characteristics of proteins [15, 37]. Figure 4.4 exhibited the various types of protein-based biomaterials [37].

Silk-Based Biomaterials

The most extraordinary protein-derived biological material is silk, in terms of mechanical strength, with exceptional toughness and stiffness [38]. The reinforced alanine- and glycine-rich crystalline (-sheet) and amorphous forms of silk, which are further changed by silkworms or spiders when they twist the fibres into threads, are the main sources of silk's mechanical toughness. Biomedical researchers are interested in reusing silk as a suitable biomaterial in addition to the widespread use of silk in the commodity textile sector. The "holy grail" of biomaterials, silk is employed for a number of things, such as drug delivery, the development of stem cell engineering, cell-specific transfection, cargo administration using biocidal coating agents, thermostabilisation of vaccinations, silk nanoparticles,implants, and artificial tissue replacement [36, 39].

Table 4.1 Different types of biomolecules, the categories that correlate to them to produce biomaterials, and their uses

Types of biomolecules	Subcategory	Applications in cancer	References
1. Proteins	Gelatin, keratin, elastic, silk, and collagens	Bioink production, neuroregeneration, food preservation, tissue engineering, and stem cell differentiation	[36–40]
2. Peptides	Dipeptides, cyclic peptides, cyclic dipeptides, foldamers, and peptidomimetics are all types of peptides	Tissue engineering, transfection, bioactive nanomaterial synthesis, photothermal therapy, anticancer medicines, anti-microbial activity, hydrogel preparation for 3D culture, and radical scavenging action	[41–64]
3. Amino acids	Polymers and tiny molecules produced from amino acids	Drug delivery, bioactive non-fouling surface modification, and anticancer therapy	[65–68]
4. Nucleic acids	RNA, DNA, nucleobases	Structural layout using DNA engineering and small molecule-driven functional design using DNA nanoarchitecture	[65, 69–89]
5. Carbohydrates	Polysaccharides, Oligosaccharides, Cyclodextrin, Monosaccharides	Coacervation, drug delivery, stem cell homing, vaccine synthesis, siRNA delivery, medication delivery using glycodendrimer-based immunomodulatory hydrogels	[90–103]
6. Fatty acids and lipids	Lipids, liposomes, steroids, bile acids,	Manufacturing of devices, delivery of drugs and genes, immunotherapy, and proteinosome assembly by microdroplet synthesis	[104–110]
7. Hybrid materials	Combining various biomolecule types with synthetic components in a single system	Integrating several types of synthetic and biomolecular components in a single system	[111]
8. Marine biomolecules-derived biomaterials	Marine proteins, marine peptides, marine lipids	Vaccine, gene and medication delivery, tissue engineering	[112–123]

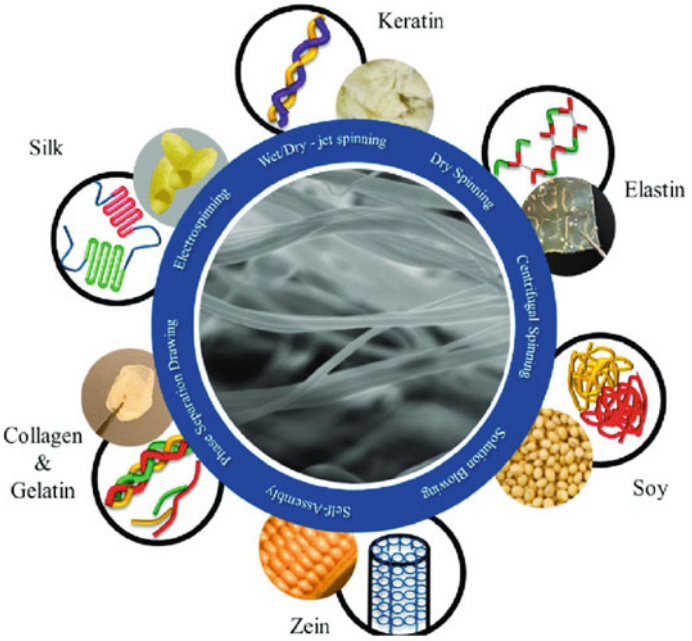

Fig. 4.4 Protein-based biomaterials (39). [This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/)]

An Anticancer Medication Delivery System Using Silkworm Silk

Since most anticancer drugs are not very soluble in water, a biomaterial carrier that can bind and release these drugs would increase their bioavailability and lead to better therapeutic outcomes. Silk fibroin has been transformed into micro- and nanoparticles, coatings, hydrogels, films, capsules, and films for drug delivery as shown in Fig. 4.5 [40].

Additional Structured Protein-Based Biomaterials

Due to their flexible mechanical characteristics, additional structural proteins including keratin, gelatin, and collagen are particularly relevant in soft tissue engineering. Collagen has the RGD sequence, a recognised integrin binding motif as well as its hydrolysed derivative gelatin, which promotes cell and tissue adhesion. Gelatin has many advantages over collagen since it is more soluble and less immunogenic [41].

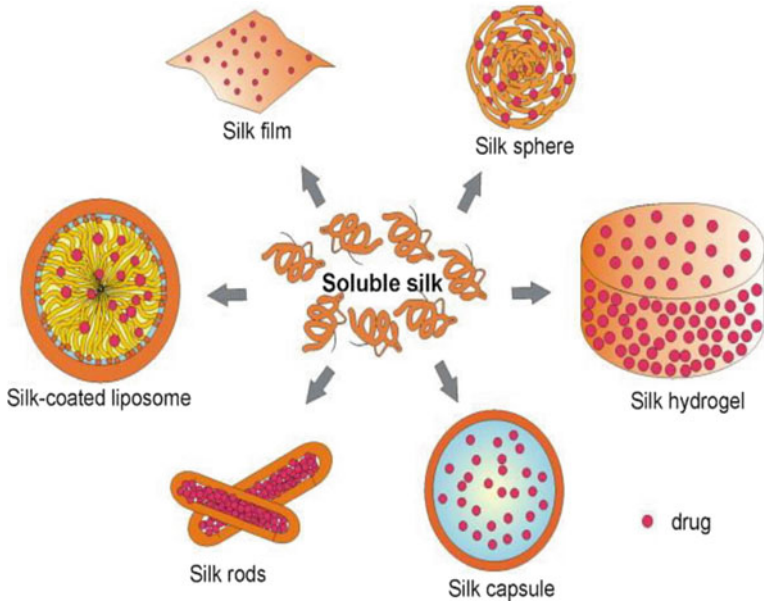

Fig. 4.5 Silk-based biomaterials for chemotherapeutic delivery [40] (Copy right 2015, *Elsevier*)

4.2.1.3 Peptide-Based Biomaterials

As a result of their tremendous potential as highly specialised drug delivery systems, self-assembled protein molecules and peptides are significant uses for such well-defined nanostructured materials in anticancer treatment. Having a substantial loading capacity for both hydrophobic and hydrophilic drugs, peptides as well as proteins also have great biocompatibility. Peptides and proteins have the ability to form nanostructures through self-assembly with different dimensions and shapes in response to shifting environmental parameters such as temperature, pH, ionic strength, and molecular interactions between the host and the guest to particularly target tumour cells, and these self-assembled nanomaterials have the ability to be embellished with useful compounds. With regard to the tumour microenvironment, stimuli-responsive elements can also be incorporated. Self-assembly allows the construction of supramolecular structures and molecular templates using nanoengineering techniques. Nanostructures including nanotubes, nanofibres, helical ribbons, fibrous scaffolds, and vesicles are created by the self-assembly of macromolecules. Due to its special qualities, comprising the ability for specific selection and targeting, minimum toxic effects, superior scalability, and simplicity of production, self-assembled proteins and peptide nanostructures seem as attractive options for upgrading traditional anticancer therapies. Nanostructures composed of

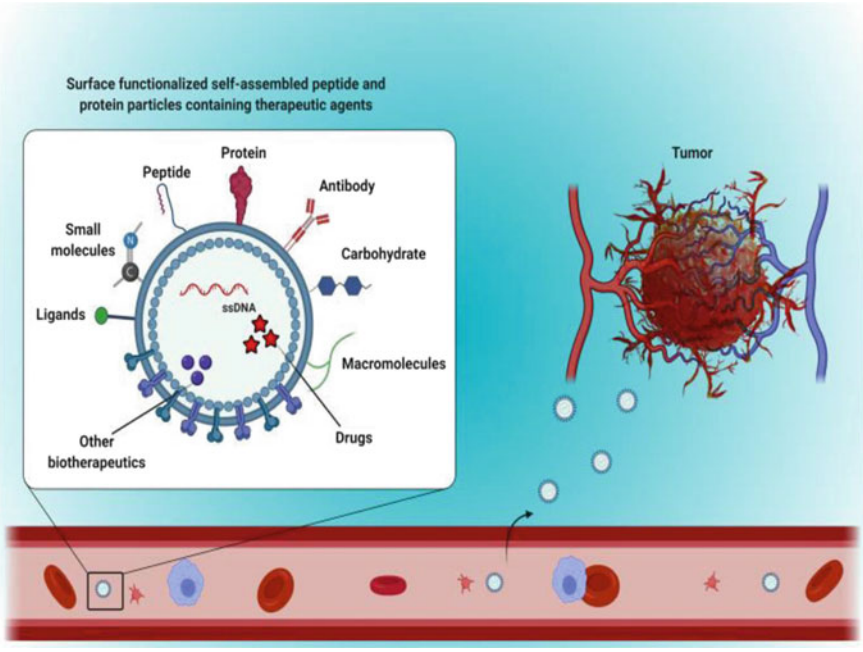

Fig. 4.6 Schematic of therapeutic agent-carrying protein and peptide particles with surface functionalisation for focusing on tumour and tumour-associated cells [42] (Copyright 2019, *Elsevier*)

self-assembled proteins and peptides may be utilised to store drugs, various bioactive substances, or imaging agents. Additionally, they could be made functional to improve targeted efficiency (Fig. 4.6) [42].

Biomaterials Based on Linear Peptides and Peptide Amphiphiles

Since they are so simple to prepare, formulation industry-level scaling up, manufacturing, and production, the peptides are being used to build functional biomaterials, which has sparked a great interest in the study of biomaterials [43, 44]. Because peptides have clearly defined chemical properties, it is advantageous to manipulate them at the molecular and structural levels. As a result, peptides both organic and synthetic are regarded as a key domain of biomolecules in the formation of biomaterials. Through the rational and scientific design of peptides, from the biological perspective, it is conceivable to imitate the functional properties of proteins while avoiding its inherently complicated structural make-up. Reverse engineering the peptide's amino acid sequence is connected to such a rational design strategy that involves reducing the structure of proteins, which in turn causes the production of higher organised structures driven by self-assembly. The molecular construction of

peptides and also how they impact pathological disorders is a crucial field of research in biomedicine [43, 44].

Dipeptide-Based Biomaterials

Dipeptides, which are peptides in their most basic form, make excellent components for designing biomaterials using a minimalist or reductionistic approach. Gazit and colleagues published the seminal paper on diphenylalanine (FF)-based nanotubes in 2003 [46]. The FF homodimer, which serves as the amyloid beta peptide's primary recognition motif, has shown to be a promising dipeptide to be used in studies of the self-assembly process for creating various material architectures for biological applications [47, 48]. Discrete silver nanowires are built using the L-form of diphenylalanine's self-assembling tubular nanostructure [45]. It's interesting to note that the diphenylalanine peptide's D- form produces nanotubes that are enzymatically stable which may be applied to the development of biosensors. Despite the fact that FF self-assembles from the bottom-up, which has been the subject of numerous investigations, its native form does not have biological relevance.

Cyclic Peptide-Based Biomaterials

Because of their unique structures and biological characteristics, one of the most preferred kinds of compounds is cyclic peptides. Cyclic peptides are relatively resistant to enzyme breakdown because of their restricted scaffold, which increases metabolic stability. In addition to providing structural stability via hydrogen bonding interactions, the stiff molecular structure also exhibits increased binding affinity for the target ligands. Cyclic peptides have the benefit of surviving hostile chemical conditions and also contribute significantly to host defence due to their excellent structural stability [49]. Cyclic peptides have peculiar receptor affinities that have been used to form agonists and antagonists [50, 51]. Histone deacetylase activity has been demonstrated to be decreased by opioid and integrin receptor antagonist cyclic tetrapeptides as well as cyclic hexapeptides [50]. The Corti group discovered that the Asn-Gly-Arg peptide which was cyclic and linear had tumour-homing properties. Both linear and cyclic systems are capable of targeting the tumour, according to a study conducted in vivo on mice. The anticancer activity of the cyclic Asn-Gly-Arg combination, however, is ten times greater than that of its linear cousin [52]. Comparing the effectiveness of linear as well as cyclic His-Ala-Val (HAV) peptides in penetrating the blood–brain barrier was the strategy used by the Siahaan group. In contrast to their linear analogues, cyclic peptides have increased in vivo activity and plasma stability because of their protease-insusceptibility property [53].

Biomaterials Based on Cyclic Dipeptides

The fundamental cyclic peptides have been known as cyclic dipeptides (CDPs), which are chemically 2,5-diketopiperazines. They possess unique properties for enzyme stability, bioactivity, self-assembly, and biomaterials [54–58]. The astonishing molecular self-assembly ability of CDPs through hydrogen-bound molecule layers and chains makes the design of new biomaterial systems alluring. In an effort to understand and analyse the molecular self-assembly enabled by noncovalent interactions towards the creation of CDP-based products, considerable research on the synthesis and structure–function correlations with various functions has been carried out [54–58]. Low molecular weight gels (LMWGs) of CDPs are being developed, and this is a promising method for creating soft materials with a variety of biomaterial uses.

Peptidomimetics-Based Biomaterials

Peptide chemistry's has the potential to create a range of structural designs, either through covalent chemistry or assembly-driven noncovalent interactions, which is a fascinating property. The researchers were motivated to develop new structural materials by the noncovalent interactions that drove peptide formation. The peptide assembly's dynamic nature enables the incorporation of many molecular interactions to form distinctive structures. The best example of peptidomimetic compounds that travel via amyloid-like assemblies to produce biomimetic materials is foldamers [59, 60]. A process known as "sheet assembly" that the foldamers frequently go through resembles the conformations of peptides and thus produces biomimetic materials having potential applications in biomedicine. The capacity of foldamers to develop and maintain secondary structure under physiological circumstances is one of their key advantages [57].

4.2.1.4 Amino Acid-Based Biomaterials

Amino acids are the fundamental components of peptides and proteins, and their use in the formation of novel biomaterials represents a prospective reductionistic approach in the field of biomaterials science. The production of peptides and proteins can be simplified by using amino acids to avoid the structural complexity, biological and chemical stability, and also the difficulties associated. Amino acids are advantageous to use in the design of biomaterials due to their excellent molecular recognition abilities, stimulus reactivity, and propensity to drive varied molecular interactions, with ability to alter chemical and physical characteristics through the side chain function (minor structural mutations). Because they may include a variety of functional and structural characteristics into such a small molecule framework, amino acids are very helpful because they could be further modified into specific materials systems or architectures using molecular architectonics [61].

Poly(Amino Acid) Nanoparticles as a Promising Tool for Anticancer Therapeutics

Amphiphilic poly (L-amino acids) (PLAAs), it is a fascinating new nanoscale platform which is widely being used to transport proteins, genes, or drugs. In order to make polymerised drug conjugates (PDCs), drugs may be chemically bonded to PLAAs or may be physically laden with them. By either guiding a drug away from normal cells or toward tumour cells, these DDCs alter the pharmacokinetics of medications, improving its clinical and pharmacological efficacy. The properties of amphiphilic block copolymers and solubilisates both significantly affect how drugs are encapsulated. Due to their biocompatability, less toxic effects, biodegradability, multiple active molecules (such as carboxyl, hydroxyl, amino, and thiol), nonimmunogenicity ease of derivatisation with adaptable physicochemical changes, and controlled release of their associated payloads, numerous studies have been done on PLAAs as a potential economic DDS. Additionally, poly(a,b-amino acid) derivatives might be degraded by the lysosome's proteolytic enzymes. Their tiny size permits passive targeting by increased permeability and retention (EPR), and the ease with which they can be surface-modified with the right targeting ligands enables active targeting by receptor-mediated endocytosis. Additionally, PLAAs are extremely important for enhancing intracellular uptake and tumour targetability. PLAAs may be produced through microbial fermentation and chemical synthesis, like solid-phase synthesis of oligopeptides as well as polycondensation for polypeptides in aqueous solutions; the hydrophilic and hydrophobic portions of amphiphilic copolymers typically self-assemble into a variety of DDSs, such as polymeric vesicles, polymeric micelles, peptide nanofibres, and polymeric solid nanoparticles. Self-assembly is principally stimulated by entropy increase from dehydration of hydrophobic areas and the formation of hydrogen bonds within molecules and water. Notably, the chemistry of the amphiphile and the equilibrium between the hydrophobic and hydrophillic segments are important factors in influencing the character of the PLAAs self-assembly product (Fig. 4.7). Amphiphile molecules prefer to form polymeric micelles when their hydrophilicity is higher, polymeric vesicles when their hydrophobicity is intermediate, and amorphous polymeric nanoparticles (polymeric solids) when their hydrophobicity is higher [62].

4.2.1.5 Nucleic Acid-Based Biomaterials

The best and most adaptable biomolecule for developing biomaterial scaffolds with a variety of uses is nucleic acids [62, 63]. Because of their extremely specific and programmable molecular recognition, nucleic acid-derived biomaterials are appealing because of their straightforward structural design, biological and chemical stability, and also ease of engineering [64, 65]. Numerous nanomaterial architectures are produced by the nucleobase-sequence dependent programmed assembly on nucleic acids and can be investigated for diverse biomedical uses [66]. RNA, DNA, nucleobases, as well as its derivatives are frequently used to construct nucleic

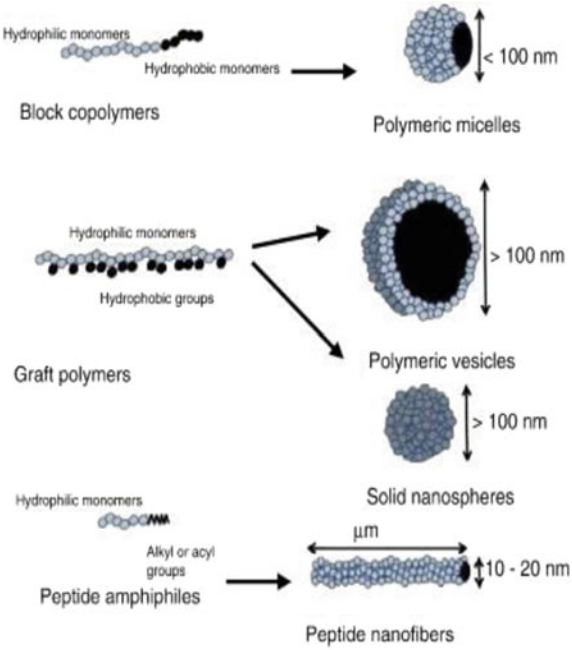

Fig. 4.7 The self-assembly of PLAA amphiphiles into peptide nanofibers, polymeric solid nanospheres, polymeric micelles, and polymeric vesicles [62] (Copyright 2019, Elsevier)

acid-based biomaterials, and sequence-specific assembly is often used to speed up the manufacturing process.

Nanoplatforms

The development of suitable nanoplatforms for drug delivery is another approach to NP design. By facilitating the concurrent utilisation materials with hydrophobic-hydrophilic as well as other physical attributes, as well as varied functions, this could also optimise the distribution and effects of some substances. Thanks to a wide variety of efficient carriers, gene therapy which specifically targets either RNA or DNA has grown to be among the most effective cancer treatment alternatives during the previous ten years.. Therapies based on DNA and siRNA have assisted in overcoming difficult obstacles like tumour microenvironment heterogeneity, drug resistance, genetic changes, tumour relapse, and metastasis. However, there are still a number of difficulties that DNA and siRNA-based drug must overcome, including permeability, stability, inadequate target selection, solubility, and unpredictable kinetics of drug release. Nanocarriers have been used to overcome various a significant negative charge and inadequate blood stability, and to lessen cytotoxicity to healthy tissues in order to solve these difficulties (Fig. 4.8). NA's anionic hydrophilic characteristics,

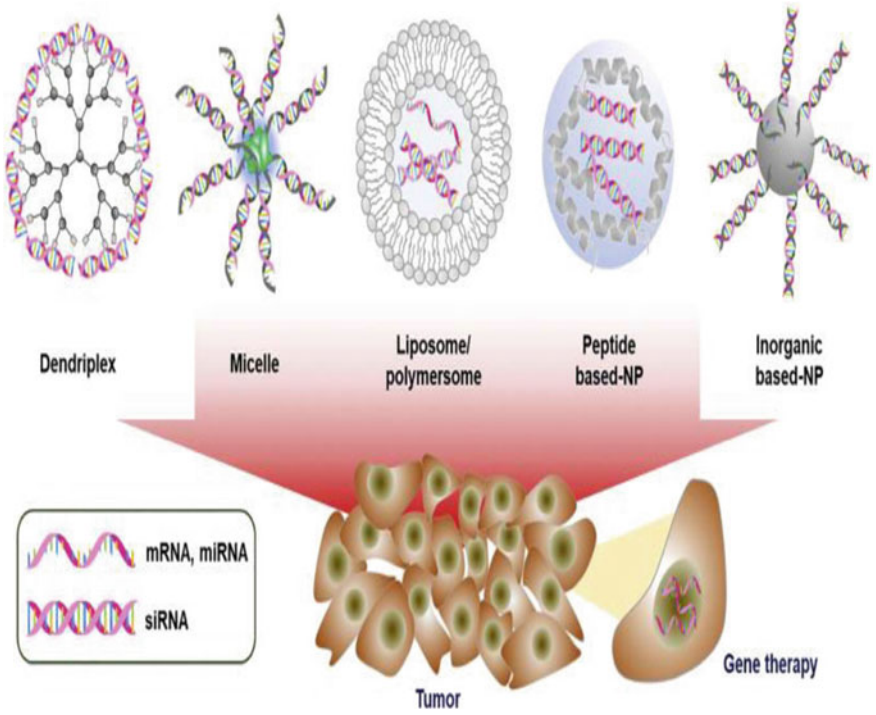

Fig. 4.8 Nanoplatforms for gene therapy [67] (Copyright 2020, Elsevier)

accessibility of enzymatic digestion, or renal excretion limits passive siRNA transport through the cell membrane in addition to its retention in blood vessels. Lipid-based nanoparticles (NP), prodrug-based nanocarriers,, polymer-lipid hybrid NP, carbon nanotubes, nanosponges, polymer-drug conjugates, supramolecular nanocarriers, inorganic gold, silver, mesoporous silica-based, and liposomes are some of the newer, more efficient delivery technologies. Due to the fact that gene therapy attacks cancer in vivo, delivery methods should also be biodegradable, biocompatible, and nonimmunogenic to minimise risk [67].

DNA-Based Biomaterials

In most cases, a helical DNA duplex structure results from Watson–Crick (WC) base pairing (canonical hydroxyl groups interactions) among two complementary single-stranded DNA molecules. Along with WC base pairing, noncanonical hydrogen bonding interactions within base pairs also participate in the creation of DNA triplexes, quadruplexes, quadruplexes branches, and assembly. Complementary and non-complementary interactions mediated by hydrogen bonds have made it possible

to create a variety of DNA structures or materials using a bottom-up construction method.

Classical DNA Nanotechnology

Because nucleic acids may be programmed, particularly DNA, it is possible to manipulate their materialistic properties for structural reformation and the creation of multidimensional nanoarchitectures. DNA nanotechnology began with the idea that DNA can serve as a material building block to construct multidimensional molecular platform by joining them as junctional structures through sequence-specific nucleobase pairing. Long DNA sequences were used as the construction blocks in the original DNA nanotechnology to make three-dimensional (3D) nanoarchitectures, also with hybridisation (base pairing) process acting as the molecular adhesive [68].

Biomaterial System Based on DNA Nanoarchitecture and Functional Small Molecules

The production of intriguing DNA nanoarchitectures has been made possible by conventional DNA nanotechnology and origami. However, some of the key problems that limit the implementation of DNA nanotechnology from its original form are the usage of expensive cost programming, cost, DNA sequences, and reproducibility. However, recently developed functional DNA nanoarchitectonics or templated DNA nanotechnology uses short oligonucleotides (RNA or DNA sequences) that are easily obtained commercially or are less expensive to synthesise, and it entails tiling these sequences centred on the assemblage of useful molecules [65, 67, 69–72]. New DNA nanoarchitectures with useful features and useable applications are expected to be produced by the functional molecule templated DNA nanotechnology. The growing field of DNA nanoarchitectonics may be expanded by using this idea of templated DNA nanoarchitectonics to develop hybrid DNA ensembles using small molecules and DNA. Because it utilises molecular architectonics to produce small molecule templated genetic material architectures and equipment for promising usage in the realms of biosensors and biomedicine, DNA nanoarchitectonics is very alluring for overcoming the challenges of classical DNA nanoscience [73–78]. Short oligonucleotides and artificial organic molecules are designed to using the hydrogen bonding capability of noncanonical and canonical nucleobases [79].

RNA-Based Biomaterials

Using RNA as the skeleton of the structure is a new development in the realm of nanotechnology based on nucleic acids with significant potential for the study of biomaterials [80–82]. Compared to its DNA twin, RNA exhibits greater structural complexity and diversity. As a result, nanotechnology based on RNA is developing

quickly and has a high potential to make a variety of functional nanoarchitectures [83]. RNA nanotechnology is concerned with the study of nanoscale RNA structures based on nucleobase pairing interactions between DNA and RNA. However, due to its predominant single-stranded structure, RNA exhibits a substantial structural and functional diversity that is comparable to that of proteins. Architectural diversity is increased by the potential for noncanonical base pairing and tertiary structural (3D) configurations of RNA structures. The Guo group has significantly advanced the field of RNA nanotechnology. By reconstructing the mutant package RNA (pRNA), a novel strategy for restoring the viral capsid's capacity to package genomic DNA was described in 1998.The authors decided on the bacteriophage 29 because pRNA is necessary for the packaging of DNA in viral procapsids. The scientists confirmed that an alteration in a pRNA loop renders the pRNA inactive and unable to support packaging. The development of a hexameric ring by the assembly of two, three, or six mutant pRNA is followed by the restoration of genomic DNA packaging. This procedure implies that the development of a hexameric ring via intermolecular interactions in the altered region of pRNA is crucial for the recovery of packing activity [84].

Nucleobase-Based Biomaterials

In the realm of materials science, the fundamental building blocks of nucleobases, nucleotides, and nucleic acids are of great interest since they display each one of the useful characteristics of their parent biomacromolecules. The storage and transfer of genetic information are made possible by these essential nucleic acid key components, which are essential biological components of life. The development of beautiful materials using nucleobases and nucleotides to extract cutting-edge functional capabilities in a compact and minimalistic design is quite alluring. Researchers have set up NDI to function properly, a very well organic semiconductor comprising thymine and adenine units that establish complementary base pairing of peptide nucleic acid (PNA) dimers, in order to take use of the intriguing characteristics of nucleobases [85]. While 2D nanoarchitectures are produced as a result of the PNA-thymine dimer directed assembly, nanoribbons were formed through the solution processing of the adenine-NDI adenine conjugate. The molecular recognition-driven formation of porous spheres with petal-like 2D sheets is the result of the complementary contact with thymine-NDI-thymine and PNA-adenine dimer. This especially bioinspired nucleobase-mediated assembly for nano- as well as micro-architectures promotes the design of advanced supramolecular material systems including applications in the optical or bioelectronics-related biomedical domains [86].

4.2.1.6 Carbohydrate-Based Biomaterials

Carbohydrates are utilised by humans for thousands of years as a source of food, fuel, textiles, and chemicals [87]. Monosaccharides, oligosaccharides, and polysaccharides are the three main types into which carbohydrates are traditionally split. Carbohydrate-based materials are today considered to be historically crucial for the transmission of human knowledge and culture thanks to the invention of Egyptian papyri. Advances in the research of the characteristics of materials of carbohydrates in addition to their innate biological qualities have made it easier to make biomaterials based on carbohydrates with a wide range of functional features as well as purposes.

Polysaccharide-Based Biomaterials

A potential biomaterial at the nanoscale interface is bacterial cellulose (BC), which is generated from the bacterium Gluconacetobacter xylinus. It has significant potential uses for tissue engineering due to its exceptional mechanical property, water retention ability, and outstanding cytocompatible characteristic. In a fascinating study, tubular implants with an inner diameter of about 6 mm were created to substitute the carotid arteries in a pig model [88]. A year of in vivo monitoring revealed the implantation of BC nanotubes at the bloodstream's inside surface by endothelisation. By forming durable vascular conduits in vivo, the implanted BC nanotube demonstrated its effectiveness as a biomaterial for tissue engineering applications [89].

Oligosaccharides-Based Biomaterials

Chemically, oligosaccharides are unique and more complex than linear peptides and oligonucleotides because they have branching structures. They are extremely complicated in terms of their chemical and physical make-up due to the structural variations and functions. The oligosaccharide-based microarray is an effective tool for studying biomolecular interactions in glycomics. Using neoglycolipid technology, Feizi and colleagues have developed oligosaccharide probes that can be used to test infections [90]. Natural pseudo-oligosaccharides, like aminoglycosides have inherent antibacterial characteristics [91]. It has been demonstrated that the hexasaccharideglobo vaccination is effective in the treatment of prostate cancer. N-acetylgalactosamine-based oligosaccharides make up the majority of tumour-targeted carbohydrate vaccines [92]. Glycodendrimers are a significant approach with potential biomedical applications in the realm of oligosaccharides, and their synthesis and usage as biomaterials is a promising method. In one study, the surface of the SWNTs was adsorbed with branched glycodendrimers to improve their water dispersibility. It has been demonstrated that the bioactive glycol coating considerably reduces the cytotoxicity of SWNTs. The multivalent glycodendrimers are a useful toolkit for examining

the interactions between proteins and carbohydrates [93]. In this regard, the Schengrund group has made and synthesised several glycodendrimers to examine how they interact with cells that are HIV-infected [94].

Cyclodextrin Based Biomaterial System

Cyclodextrins, which are made of macrocyclic rings with five or more subunits of glucose joined by -1,4 glycosidic linkages, are a significant type of oligosaccharides. Hydrophobic cargo molecules and pharmaceuticals can be trapped inside the inner core of all, and -cyclodextrins thanks to their hydrophilic outside and hydrophobic inner core, respectively. The potential of cyclodextrin to make inclusion complexes through the host–guest assembly has drawn significant attention, recently in the context of drug delivery [95]. It is extremely desirable to alter cyclodextrins using polyethylene imine (PEI) to lessen PEI's cytotoxicity. An efficient gene delivery vehicle was prepared by conjugating the PEI with -cyclodextrin, thanks to Wang and colleagues. The PEI-grafted cyclodextrin polymer exhibits better biocompatibility and can thus transfect genes in cultured neurons and the central nervous system (CNS) upon intrathecal injection. They developed a host–guest assembly cyclodextrin-based co-delivery system for use in both drug delivery and gene delivery applications in a separate investigation. The adamantane-conjugated anticancer prodrug was able to be included in the PEI-cyclodextrin system because of the host–guest interaction between the cyclodextrin and adamantane moieties [96]. It has been demonstrated that the cyclodextrin-prodrug combination can interact with anionic nucleotides like those found in DNA and siRNA. In contrast to PEI, the nucleotide-driven condensation produces spherical nanoparticles with a high transfection efficiency. The in vitro and in vivo experiments depicted that paclitaxel and the human breast cancer cell line MDA-MB-231 co-delivered considerable anticancer activity. Another illustration of a co-delivery system is the doxorubicin and tumour necrosis factor-related apoptosis inducing ligand (TRAIL)-plasmid delivery system exploiting cyclodextrin-based vector technology. The rate of tumour proliferation in vivo decreases when the co-treatment with TRAIL triggers apoptosis [97].

Monosaccharide-Based Biomaterials

Numerous varieties of monomers and polymers are included in the family of carbohydrates. Monosaccharides are said to be the fundamental units of oligo- and polysaccharides, both of which can be linear or branched. Monosaccharides have a significant deal of promise for developing innovative materials because they are the fundamental building block with all structural and functional features. In this section, we go over the published studies on the development of biomaterials for potential biomedical uses using fundamental monosaccharides. The Baker group developed a crosslinking agent based on glucopyranose to add carbohydrate functionality to a synthetic polymer system [98]. The utilisation of a monosaccharide derivative as a

crosslinking agent to produce a synthetic biomacromolecular system that resembled carbohydrates was demonstrated for the first time in this study. Recently, the biological relevance of G quadruplexes and its pathophysiological connection to cancer have been discovered [99]. The Derache group developed a number of carbohydrate-NDI conjugates as ligands for the particular G-quadruplex binding as well as cellular uptake. The experimental findings showed that, in contrast to double helix DNA, the monosaccharide linkers increased the selectivity for G-quadruplex. It was discovered that the G-quadruplex was preferentially taken up by cells by monosaccharides by a factor of 2 or 3 compared to control DNA. The compounds of NDI with monosaccharides efficiently kill malignant cell lines by translocating inside of them via the glucose transporter [99].

4.2.1.7 Lipid Based Biomaterials

Natural Lipid and Fatty Acid-Derived Biomaterials

Nature is regarded as the foremost specialist in the production of resources that are useful to everyone. Lipids and fatty acids are used to produce many unique structural formulations with distinct qualities or purposes, resulting in one of the most elegant and beautiful molecular structures ever seen in nature. It has been shown that by separating these molecules in water, it is feasible to develop compartmentalised formations from the existence of amphiphilic nonpolar and polar portions within of a single molecular system. The most magnificent lipid molecule subclass are phospholipids, which work as a protector of life in the form of cells by encasing and protecting the components of cells from the outside environment. Drugs that are taken orally must have drug delivery method based on lipid and fatty acids, known as "lipid formulations" [100]. To enhance the ability of poorly water soluble drugs to circulate and the serum stability across the gastro-intestinal (GI) tract, lipid formulations must be used when administering them. Type I (oil without surfactant system), Type II (oils with water-insoluble surfactant system), Type III (oil, surfactants, and co-solvents), and Type IV (oil, surfactants, and other co-solvents) are the four distinct sub-systems that make up the lipid formulation classification (LFC) system (water soluble surfactant and co-solvent). By using hydrophilic polymers, which prevent the accumulation of medication within the LFC system, the sustainability of the LFC systems can also be increased. It has been demonstrated in numerous novel methods that LFC therapy increases the plasma stability and solubility of the Fenofibrate [101, 102]. An intriguing method to increase the immunogenicity and retention power of antigens is the use of lipids in immunisation. Ovalbumin antigen-packed inter bilayer-crosslinked multilamellar vesicles (ICMVs), which have been developed using a novel strategy, can enhance endogenous T-cell and antibody responses [103]. In direct gene transfer applications, liposomes are essential because they can mimic the structure of viruses through non-specific interactions with anionic DNA. It is especially advantageous to mimic the viral transfection pathway in order to avoid negative immunogenic stimulations and to efficiently transfer relatively bigger DNA.

In addition to being used as a means of delivering genes, liposomes have also been investigated for potential utility in other contexts, such as protein sensing [104].

Steroid and Bile Acid-Based Biomaterials

Important categories of natural lipids include steroids and bile acids. The most basic and well-known member of the steroid family, cholesterol, is essential for maintaining the fluidity and structure of cell membranes. Recently, the utilisation of cholesterol and bile acids as attractive molecular scaffolds for synthetic organic as well as material chemistry to make practical goods has grown in favour. These steroids are helpful biomolecules to develop biomaterials for therapeutic uses due to their amphiphilic nature and simplicity of chemical modification [105, 106].

4.2.1.8 Hybrid Biomolecule-Based Biomaterials

Immunotherapy is described as a very effective method for treating illness problems by altering the immune system. Due to its clear therapeutic advantages, particularly for the treatment of cancer, immunotherapy has drawn a significant amount of interest. Immunology, material science, pharmaceutical chemistry, and biotechnology have actively collaborated to improve the field of immunotherapy. Bioengineering of the cells, cargo delivery of immunomodulatory agents, and functionalisation of immunomodulatory scaffolds are all components of the therapeutic approach. A RNA-lipoplex nanoparticle was created by Kranz et al. for use in cancer treatment. The RNA-lipid hybrid nanoparticle induces systematic transport and manifestation of tumour antigens inside the dendritic cells while shielding RNA from enzymatic destruction. Participants in this study successfully displayed de novo T-cell responses, which were followed by therapeutic trials that reduced the expansion of malignant cells. Another study described a nanodisk neoantigen vaccination method in order to treat cancer that artificially resembles high density lipoprotein (HDL).The apolipoprotein A1 (ApoA1)-mimetic peptides and phospholipid-based nanodisk enabled the simultaneous distribution of adjuvants and antigen to immune cells. As adjuvant and neoantigen, the mutant Adpgk protein from MC-38 colon cancer and the 5′-C-phosphate-G-3′ (CpG) motif attached to cholesterol moiety, respectively, were chosen. The adjuvant was positioned inside the 10.5-nm-diameter disc section of modified Adpgk that was linked to a nanodisk by a cysteine-serine-serine (CSS) linker. It was demonstrated that the adjuvant-antigen loaded nanodisk elicited in vivo tumour-specific T-cell responses. More than 85% of animal models with MC-38 tumours showed therapeutic efficacy in treating them [107].

4.2.1.9 Marine Biomolecules-Derived Biomaterials

Indeed, marine biomolecules play a crucial role in the formation of biomaterials. The reality is that the distinct structural characteristics and low weight of corals, molluscs, and sponges have also influenced the creation of useful materials with a wide range of uses. The earliest nations to utilise substances from the sea for medical purposes were ancient Asian and Egyptian civilisations. The best prospects for creating useful biomaterials from various marine biomolecular resources are the collagen and chitin found in higher and lower life forms of marine animals [108]. In a study, curcumin anticancer medication is packaged and delivered using krill lipid-based liposomes or marinosomes. On the A549 lung cancer cell line, the curcumin-trapped marinosomes demonstrated potential anticancer and antioxidant activities. Overall, marine biomolecules and the biomaterials gave rise to have exceptional cellular viability, bioactivity, and biocompatibility, making them appropriate for several biomedical applications. With more over 70% of the planet's surface covered by the seas, they are the planet's greatest home. There are many different marine species with unique chemical and biological characteristics that cannot be employed commercially in the various oceanic zones and conditions. About 75 per cent of total of the waste material from marine-origin organisms generated yearly by the shellfish production can be further investigated and treated to create new chemicals and materials [108]. The substances may possess a variety of physicochemical properties, such as low toxicity and inexpensive production, which may result in intriguing biological aspects that should be researched in the biomedical field [109, 110]. Marine organisms can be split into three classes, each of which can produce a variety of biopolymers: nucleic acids, polysaccharides, and proteins [107–115]. This chapter of the book will discuss the anticancer properties of the polysaccharides fucoidan, chitosan, carrageenan, and alginate as given in (Table 4.2). Their minimal cytotoxicity and physical resemblance to substances found in native human tissues are intriguing characteristics. However, they have certain drawbacks, such as batch-to-batch inconsistency, since the time of year and location of the harvest may have an impact on the polysaccharide characteristics, and the choice of extraction technique may also have an impact on the biological performance. In this regard, controlled and repeatable substances must be obtained using standardised, sustainable techniques [116].

4.2.2 Synthetic Biomaterials

Cancer is a group of diseases that share one feature: dysregulation of cell proliferation. Traditional cancer therapy options have limitations in clinical settings for treating distinct tumours. Examples include the inability to overcome chemotherapeutic resistance including innate resistance. Nanotheranostics combines therapeutic and bioimaging capabilities at the same time [121]. Nanomedicines are fast emerging

Table 4.2 Drug delivery system of various marine derived biomaterials and its application in cancer

	References
Fucoidan-based systems Fucoidan has been incorporated into nanosystems for drug delivery to treat cancer, including nanoparticles, micelles as well as liposomes. In comparison to free DOX, fucoidan/polyethylenimine nanoparticles with doxorubicin (DOX) added showed stronger cytotoxic 556 and more cell cycle arrest in G1-S phase in the MDA-MB-231 cancer cell line	[117]
Chitosan-based systems Paclitaxel (PTX)-loaded chitosan nanoparticles were created. In vitro cytotoxicity was analysed over MDA-MB231 breast cancer cells, with results revealing higher toxicity when compared to free drug. In the initial 24 h, about 60% of the medication was released	[118]
Carrageenan-based systems Curcumin has demonstrated antiproliferative effects in a variety of malignancies, and Sathuvan et al. created curcumin-laden with -carrageenan beads (-Car Cur) for drug delivery against lung cancer cells to overcome its poor solubility (A549). A higher level of cytotoxicity against cancer cells was produced by -Car-Cur composites than by free curcumin, according to in vitro cell-based experiments. Additionally, ROS generation rose and mitochondrial membrane potential decreased when A549 cancer cells were treated with -Car-Cur, suggesting that this approach induced cellular death	[119]
Alginate-based systems As a means of delivering DOX, Prabha and Raj created Fe_3O_4-NPs coated in polyvinyl alcohol, bovine serum albumin, and sodium alginate. These particles were found toxic to HepG2 liver cancerous cells but harmless to healthy cells of liver (L02)	[120]

and have already begun to play a significant part in a number of cancer treatment techniques. Initially, the nanomedicine field concentrated on optimising an agent's pharmacokinetics, toxicity, and/or biodistribution via nanoparticle formulation. When applied more strongly, nanomedicine can also refer to the development of innovative pharmaceuticals that make use of nanoscale material properties to enhance therapy effectiveness through molecular targeting, distinct mode of action, etc., or by carefully regulated drug administration.The goal of current and upcoming nanomedicines is to enhance both diagnostic and therapeutic outcomes, landscapes by utilising, electrical, optical, mechanical, magnetic as well as biological nanomaterial properties in novel ways [122].

4.2.2.1 Cancer Nanoparticles with Stimuli-Responsive Properties

The majority of the time, nanoparticles are created to be "on," and when they are administered to animal models, their payloads may be detected and released. The number of medicines that have been authorised, on the other hand, can be examined personally. Designing nanocarriers allows drugs to be administered to cancer sites

with "on-and-off" theranostic functions. As a result, this rationale has propelled the growth of stimulus-responsive nanomaterials, opening up new avenues for improving and controlling the environment [123, 124]. Developing functional molecules, or moieties and chemical-sensitive linkers is a common mechanism of various stimulus-responsive nanomaterials [125]. Incorporating either a moiety or structure directly into TME-responsive nanomaterials is another design strategy. Karimi M. et al., developed the use of chemical, biological, or physical stimuli in nanotheranostics and nanomaterials. These include shifts in the pH of the intracellular TME, adjustments to the protein expression levels in tumour cells, elevations in oxidative stress there, and other conditions brought on by extrinsic stimuli including, magnetic modification, near infrared stimulation, and ultrasonic manipulation. It is also feasible to combine two or more stimuli, which broadens the range of sophisticated stimulus-responsive nanomaterials including nanotheranostics [126].

4.2.2.2 Acid-Responsive Nanomaterials

The pH of the TME is thought to be an important characteristic for differentiating between cancerous and healthy tissues. Because cancer cells possess high rate of glucose absorption, their metabolism differs from that of healthy cells. This causes lactic acid fermentation in the cytosol instead of glycolysis and pyruvate oxidation inside the mitochondria in most healthy cells. The Warburg effect refers to this altered cellular metabolism.Additionally, the endocytosis of nanomaterials within tumour cells frequently results into a cascade of trafficking into pH-5.2 endo lysosomal compartments. Thus, integrating such organelles with TME pH might be a strategy that might be specifically targeted for cancer theranostic uses [122, 127].

4.2.2.3 pH-Sensitive Linkers

One method enabling acid-responsive nanotheranostics is to employ pH-sensitive linkers, like acotinyl group. By functionalising dendrimers with folic acid, Zhu et al. developed a dendrimer-coupled nanotheranostic platform for computed tomography imaging for active targeting which is pH sensitive [128], importantly when chemotherapeutic doxorubicin (Dox) molecules were conjugated to dendrimers with cis-aconitic anhydride. In the acidic TME, they were released more quickly than under physiological conditions. Kulhariand co-workers developed micelles encapsulating CTX medication to treat prostate cancer using Pluronic F68-cis-acotinyl linked-cabazitaxel (CTX) and F68- succinoyl linkedCTX. A more powerful apoptotic effect was produced by the F68-cis-acotinyl linked CTX due to an increase in pH sensitivity and drug distribution control. For pH-stimulated drug release, several chemical connections that were pH-sensitive were reported. These are some examples: b-thiopropionate, citraconic amide, and other functional groups, orthoester, acetal, cyclic acetal, carbamate, hydrazone, imine, ethyl acetate, ketal as well as

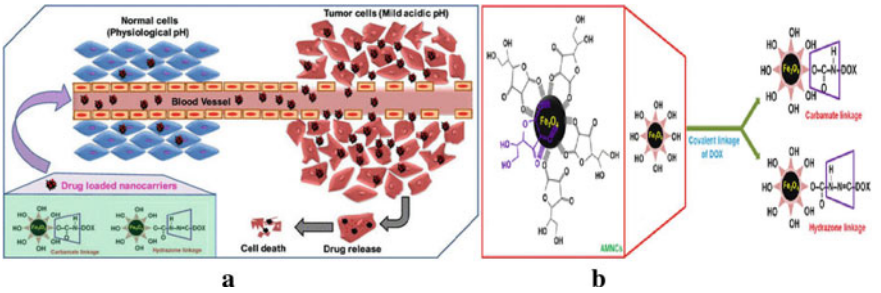

Fig. 4.9 Acid-sensitive nanomaterials with pH-sensitive acidic linkers **a** diagram of the acidic pH-responsive nanotheranostics that are only found in cancer cells. **b** Dox conjugation via acidic-sensitive carbamate and hydrazone connections to the nanobuild [131] (This is an open access paper published under an ACS AuthorChoice License, which allows for non-commercial copying and redistribution of the work or any changes)

benzoic-imines Gawali et al., for example, identified the use of Dox to encapsulate pH-labile, ascorbic acid-coated magnetic nanostructures to treat mouse cutaneous fibrosarcoma. breast cancer MCF-7 and lung cancer A549 [129, 130]. They are notable for use of carbamate and hydrazone linkages to covalently conjugate Dox to the nanoconstructs. These nanoconstructs displayed gradual and prolonged chemotherapeutic release within tumour cell lines under acidic circumstances but not below physiological pH values (Fig. 4.9a, b) [131].

4.2.2.4 Nanomaterials with pH Sensitivity

Another technique to producing pH-responsive nanotheranostics is to inject nanomaterials into tumours that breakdown quickly in acidic environments. Because tumour organelles, such as lysosome and endosomes, have intrinsically low physiological pH values (4–6), This strategy has been employed in several tumour models. To differentiate between healthy and tumour tissues, a TME with a pH of 6.5–6.8 can also be used. Therefore, these pH-sensitive stimuli may also be employed to regulate release profile of a drug or prodrug cargo activation. Calcium carbonate ($CaCO_3$) is an important class that has been used in a variety of nanotheranostic applications [132]. In general, $CaCO_3$ nanoparticles are stable, but in the presence of an acidic TME, they rapidly degrade into carbon dioxide and calcium ions, allowing cargo payloads to be discharged in a regualted manner. For example, Liu and his colleagues developed a pegylated $CaCO_3$ nanotheranostic platform encapsulating a Ce6(Mn) photosensitiser-coupled Dox medication. The controlled release of Ce6 (Mn) allowed for pH-dependent T1 signal amplification for magnetic resonance imaging and real-time drug release monitoring. Additionally, by improving cellular absorption in the TME, these nanocarriers made chemo-photodynamic therapy possible [133].

4.2.2.5 Nanomaterials that Respond to Reactive Oxygen Species (ROS)

It has been discovered that the intrinsic environment of cancer, which is characterised by elevated metabolic activity and cell proliferation, increases intracellular ROS levels. The TME can therefore be utilised in ROS-responsive nanotheranostics as a biological stimulation. In a hypoxic environment, nanoparticles can directly stimulate ROS generation in tumours. Sun et al. developed a nanotheranostic system combining ROS sensitive pegylated hyperbranched polyphosphates and Dox carrying thioketal linkers. Singlet oxygen species were produced after 660 nm irradiation, producing enhanced drug release and tumour apoptosis. For bimodal tumour imaging, photoacoustic signals from Ce6 and magnetic resonance signals from Gd3+ can also be generated by these nanomaterials. This method reduced Dox resistance inside a cell line of breast cancer with relatively high levels (MCF-7/ADR) in vitro. This nanotheranostic device showed considerable tumour accumulation due to its enhanced retention and permeability. This work was significant because it allowed for regulated spatiotemporal therapy with little harm to the kidney and liver. Yang et al. used probe IR790 to produce perylene diimide-IR790-Fe/Pt nanoparticles in a parallel development. They measured ROS levels using in vivo ratio metric photoacoustic imaging of these nanoparticles. Because IR790 can be cleaved by ROS, ratio metric photoacoustic imaging enabled real-time monitoring of ROS formation when irradiated at both 680 and 790 nm. Cisplatin drug molecules attached to nanoparticle surfaces facilitated ROS production. The reductive environment controlled cisplatin release after endocytosis into U87-MG tumour cells. After the NOXs (nicotinamide adenine dinucleotide phosphate oxidase) enzymes are activated, intrinsic O_2 was catalysed to hydrogen peroxide H_2O_2 and superoxide. The Fenton reaction then catalysed H_2O_2 into extremely cytotoxic OH, resulting in increased oxidation and decreased tumour cell proliferation [134].

The three major kinds of ROS are hydrogen peroxide (H_2O_2),superoxide anion (O_2), and hydroxyl radicals (OH), and one ROS species can change into some other by a number of reaction mechanisms. Also endogenous ROS production from the NADPH enzyme or mitochondrial metabolism and exogenous ROS production via photodynamic or non-photodynamic actions are both possible. Superoxide (O_2) produced inside of cells is mostly produced by mitochondrial aerobic metabolism or by NADPH oxidation with the help of NAPH oxidase enzymes (NOXs). Meanwhile, superoxide dismutases can quickly convert O_2 into H_2O_2 (SODs).A particular high concentration of H_2O_2 causes the formation of OH through interactions with metal cations (Fe^{2+}/Cu+), which causes irreparable harm to cellular DNA, proteins, or lipids. H_2O_2 can be converted to H_2O via glutathione peroxidase (GPx), peroxiredoxins (PRx), and catalase (CAT) [135]. In cells, ROS perform a dual-edged role. Low amounts of ROS act as cellular signalling molecules and support cellular life cycles by reversibly oxidising the thiol groups in proteins, altering the structure and function of the resulting proteins. To maintain a modest level of control over ROS, cells have a range of methods to manage the balance between their production and removal. Apoptosis or necrosis, oxidative damage to cellular components (such as lipids, DNA, and proteins), oxidative stress with excess levels of ROS, and

likely the activation of cancer-causing mutations could all occur from the imbalance. Numerous pieces of evidence point to the fact that many cancer cell types produce more ROS than healthy body cells. According to research, cancer cells have a ROS content that can exceed 100 M, or about 100 times that of normal cells. Due to their naturally fluctuating redox status, many cancer cells appear to be extremely adapted to oxidative stress. Researchers have used the increased levels of ROS to develop target-specific DDSs as a result of this abnormal metabolic change in disease tissues. There have been many ROS-responsive DDSs made and investigated for use in treating ROS-sensitive materials. Photosensitisers (PSs) as well as other chemical agents may be employed in conjunction to endogenous ROS as representative exogenous ROS sources that facilitate drug release. For usage in smart DDS applications, a wide variety of ROS-sensitive carriers or prodrugs are being studied, including mesoporous silicon, arylboronic ester, aminoacrylate, selenide/telluride, diselenide, thioketal, and arylboronicester. Depending on how these linkers were built, the principal drug release mechanism can be attributed to ROS-induced carrier solubility change, ROS-induced carrier cleavage, or ROS-induced prodrug linker cleavage [135].

4.2.2.6 NIR-Responsive Nanomaterials

The intricacies of biological tumour settings in both humans and animals need careful attention when selecting light wavelengths. Deep tissue penetration is necessary to get through skin barriers. The wavelength should have low autofluorescence, low endogenous substrate absorption, and minimal photon scattering. As a consequence, two tissue optical windows have been developed for practical usage: NIR-I (700–900 nm) and NIR-II (700–900 nm) (1000–1700 nm). UV and visible light excitation has a limited ability to penetrate tissue and can cause photodamage to biological tissues. As a result, recent research has focused on producing NIR-responsive nanomaterials [136]. Wang et al. provided a recent example of NIR- and TME-responsive nanocapsules comprised of a polymer matrix and Fe/FeO nanocrystals, and a polymer matrix. Chemotherapy, photothermal therapy magnetic resonance imaging, and fluorescence imaging are all examples of multimodal photothermal therapy, and these nanocapsules were filled with indocyanine green (ICG) and Dox. After 5 min of 808-nm irradiation at pH 6.5, the nanocapsule size decreased from 220 to 54 nm over 48 h and subsequently disintegrated without causing cytotoxicity. Furthermore, the researchers showed that managed ROS emission can enhance synergistic in vitro and in vivo treatment. Pu and colleagues discovered polymer-functionalised ceria nanoparticles Another outstanding study accomplished self-regulated NIR photodynamic treatment. For fluorescence imaging, the NIR-absorbing semiconducting polymer was utilised; the nanoceria, on the other hand, had been a regulator for controlling ROS generation under physiological settings or an acidic TME [137–139].

Zhang and colleagues offered another notable example, reporting the use of photoactivatable up conversion superballs at 808 and 980 nm to increase therapeutic

effectiveness in HeLa cells (Fig. 4.10) [140]. PDT photosensitiser zinc phthalocyanine (ZnPc) and human superoxide dismutase 1 (hSOD) siRNA were put into this NIR-responsive nanotheranostic device. NIR stimulation at two wavelengths enabled dual modal activation. First, the 980-nm excitation allowed nanoparticles to escape cellular endosomes by photochemical internalisation, increasing cellular absorption by blocking recycling endosomes. Second, 808-nm photoactivation resulted in hSOD gene knockdown, leaving HeLa cells sensitive to ROS. Finally, with 980-nm excitation, a synergistic therapeutic efficacy was demonstrated, indicating that upconverted red emission might photoactivate ZnPc for PDT [141].

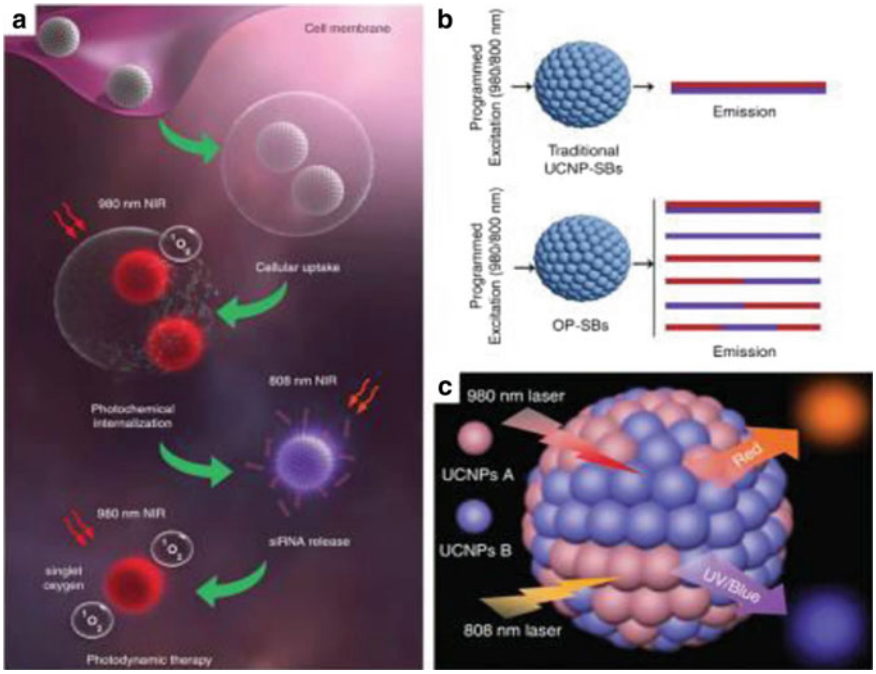

Fig. 4.10 Ananotheranostic that responds to NIR light. **a** Orthogonal photoexcitation for siRNA release, PDT, and endosomal escape with up-converting superballs [140]. **b** A comparison of the emission spectra of orthogonal photoactivatable-superballs and upconversion nanoparticle-superballs (UCNP-SBs) (OP-SBs). **c** A photograph of the emission around UV/Blue and red wavelengths following stimulation by 808 as well as 980 nm lasers. Adapted with permission from [140]. Open Access, this article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. (To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/)

4.2.2.7 Magnetic, Thermal, and Ultrasound-Responsive Nanomaterials

Magnetic-responsive nanotheranostics has two advantages: magnetic fields for tumour targeting can be developed by them which also develops significant hyperthermia in tumours exposed to various magnetic fields. According to Yan et al., for example, researchers generated a nanoconstruct by combining photothermal therapy with magnetic hyperthermia. Magnetic nanoparticles (MNPs), poly(4-styrenesulfonate, cyanine 7poly(3,4-ethylenedioxythiophene), and 2-deoxyglucose-polyethylene glycol were the main components of the nanoconstruct. Tumour cell growth was suppressed both in vitro and in vivo when photomagnetic hyperthermia and a constant trimodal imaging platform were used. Significantly, this nanoconstruct has also extended second-phase systemic circulation, with a half-life of roughly 20 h [142]. Thermally responsive nanotheranostic instruments for chemotherapeutic drug delivery have also been developed. They are typically used in conjunction with other external stimuli like infrared energy or ultrasound. These thermally sensitive cancer micro-constructions keep their structures at or under 37 °C but change conformation at 40 °C. Tang et al. presented on a new thermal-responsive nanotheranostic example. They developed organic semiconducting photoacoustic nanodroplets, which they then stabilised with perylene diimide and perfluorocarbon. Perylene diimide functions as both a photoacoustic molecular agent and a photoabsorber. This nanoconstruct was then enclosed in ZnF16Pc molecules, which act as photosensitisers. Perylenediimide, following 671-nm excitation, converted energy into heat, vaporised perfluorocarbon, and produced hypothermia, enabling the application of photothermal treatment, ultrasonic imaging, and photothermal treatment all at the same time. The $ZnF_{16}Pc$ molecule serves as an energy acceptor, transforming energy into molecular oxygen, which then produces lethal ROS for PDT. In contrast, the perfluorocarbon also provide molecular oxygen to help the TME overcome hypoxia [143]. Ultrasound responsive cancer nanotheranostics, on the other hand, have been utilised to treat a range of cancers. Following introduction of the nanocarrier into the TME, drug release was typically controlled by high-frequency sound waves. Low-frequency ultrasound imaging uses frequencies below 20 kHz, while higher frequencies can be utilised to interfere with ultrasonic nanocarriers and regulate the discharge of specific cargoes. A significant development is the recent commercialisation of a number of ultrasound-responsive cancer theranostics, including Definity, Optison, Levovist, and Senazoid [144].

4.2.2.8 Plasmonic Nanogel for NIR-Mediated Chemotherapy

Temperature-trigger chemotherapy is a cutting-edge anti-tumoural strategy in nanomedicine. This technique, however, is closely related to the effect of temperature in tumour tissue. The ablation of tumour tissue at high temperatures can obstruct a proper chemotherapy approximation. When the temperature is moderate, consequently, a negative vascularisation is formed, which promotes tumour growth and competes with the chemotherapeutic effects. Thermoresponsive polymer is

crosslinked using plasmonic gold nanoparticles to develop a nanogel system for temperature-trigger chemotherapy (Au-NPs). Doxorubicin is expelled from the porous interior of the nanogel when the thermoresponsive network collapses due to the heat produced by the Au-NPs during the application of near infrared light. Both in vivo and in vitro studies of the hybrid nanogel system were conducted and there was indication that the temperatures attained during in vivo NIR irradiation have a negative impact on tumour growth suppression, but drug-loaded systems effectively decreased tumour sizes. This work emphasises the significance of structure in temperature-triggered antitumour frameworks, where lower temperatures, which have been frequently reached in real-world settings because of the light attenuation caused by tissue, may be used to increase the antitumour effectiveness of infused pharmaceuticals in the system [145].

4.2.2.9 Synthetic Polypeptides Derived From—Amino Acid N-Carboxyanhydride (NCA) for Nucleic Acid Delivery

Gene therapy is the introduction of therapeutic nucleic acids into target cells to modify the genetic make-up of the host cells. It has been shown to be an effective method for curing or preventing a variety of diseases. Examples include ribozymes, antisense/antigene oligonucleotides (ODNs), small interfering RNA (siRNA),messenger RNA (mRNA), small interfering RNA (siRNA),microRNA (miRNA), plasmid DNA (pDNA), and other therapeutic nucleic acids. These include the tools used to silence specific genes, such as siRNA, miRNA, and ASOs. In contrast, related genes or proteins are much more likely to be stimulated by pDNA and mRNAs. In contrast to pDNA, which must be carried into cell nuclei for transcription, mRNA operates in the cytoplasm after translation. As a result, mRNA transfection does not need nuclear membrane disruption and hence provides a minimal risk of insertional mutagenesis. Numerous cancer medicines can be made more effective against cancer by using synthetic oligodeoxynucleotides with unmethylated CG dinucleotides (CpG ODN) [146–150].

4.2.2.10 Functional Nanomaterials for Glioblastoma Immunotherapy

The most dangerous type of brain tumour is glioblastoma (GBM). Despite multimodal therapies, standard treatments are not particularly effective. Immunotherapy, as opposed to traditional therapies that target tumour cells directly, employs the immune system of the body to attack cancer cells. GBM, on the other hand, has a very poor response to immunotherapy due to its severe immunosuppressive microenvironment. Furthermore, the existence of the blood–brain barrier (BBB) reduces the effectiveness of immunotherapeutics. As a result, successful GBM immunotherapy requires therapeutic medicines that not only cross the BBB quickly but also reduce the tumour microenvironment's extreme immunosuppression [151].

During animal studies and early clinical phases, immunotherapies being used to treat GBM have produced encouraging results, but they have been unable to maintain their positive effects over time. This is brought on by a variety of factors, such as the GBM's high heterogeneity and plasticity, which make it susceptible to immunotherapy resistance; the GME's strong immunosuppressive effects, low mutation load, and poor antigen presentation; and the presence of the BBB, which prevents the majority of drugs from entering and penetrating tumour tissue. The drugs short blood circulation time may also contribute to systemic toxicity including autoimmune diseases. Nanomaterials that can traverse the blood–brain barrier (BBB), enhance permeation of drugs and delivery to cancer cells, and have high stability and particular surface functional changes are needed for effective GBM therapy. Several nanomaterials were employed in clinical research and even commercialisation as nanomedicines. Polymer materials, extracellular vesicles, metal nanostructures, cell membranes, liposomes, and so on are examples. Furthermore, novel administration techniques, such as intratumouralinjection and nasal administration, are developed to maximise pharmaceutical utilisation while minimising drug loss [151].

4.2.2.11 Exosome-Based Tumour Treatment with Nanomaterials

Targeted therapeutic delivery has shown to be an important issue in tumour treatment. Endogenous carriers have showed considerable promise in therapeutic nanoparticle administration due to their outstanding biocompatibility and minimal toxicity. Because of their exceptional biocompatibility, low cytotoxicity and immunogenicity, strong capacity for both loading and protecting cargo, targeting particular cell types more favourably, able to successfully bypass physiological boundaries, and longer period of in vivo circulation, exosomes are thought of as all-natural, exogenous cargo-carrying targeted delivery methods. Nanotherapy is a significant application of nanotechnology. This discipline develops novel remedies for diseases by utilising the unique properties of nanomaterials. Although the objectives can be met, significant flaws remain. The lack of knowledge about in vivo interactions and toxicity caused by nanomaterials, as well as possible immunogenicity and systemic toxicity, impedes clinical translation of nanomaterial-based therapy approaches. As a result, exosomes and nanoparticles together have so become a popular form of nanotherapy [152–158].

4.2.2.12 Adoptive Cell Transfer Biomaterials

A significant advancement in cancer immunotherapy is the invasion of tumours by chimeric antigen receptor T cells, lymphocytes, and T cell-based ACT. It does, however, deal with the issue of T-cell proliferation and the inability to maintain effector function both during the ex vivo cell-production procedure and after transplantation. Additionally, a significant quantity of autologous cancer T lymphocytes are needed for these treatments. T cells that have been extracted straight from a

patient's blood may not respond well. In this case, "artificial APCs" have been developed using microparticles coated using anti-CD3 and anti-CD28 antibodies to provide the major and co-stimulatory signals. In order to remove magnetic fields from cells, they even use superparamagnetic iron oxide. This is now a standard practise. PLG microparticles, particularly in CD8+T cells, expressed extra signalling for the T cellular growth factor IL-2, promoting T-cell proliferation. They also facilitate the best cytokine presentation with in cellular environment, allowing transplanted cells to live and function in ACT [159–161].

4.2.2.13 Biomedical Polymers as Intra-Arterial Occlusion Devices

Lewis [163] described sulfonate-modified poly (vinyl alcohol) (PVA) hydrogel beads (DC Bead®) as degradable decorative devices which can be useful in hypervascular benign or malignant tumours, specifically arterio-venous dysfunction. The practise of aiding and treating persons who have bleeding problems is known as embolisation of blood vessels. DEBs (drug eluting beads) are PVA hydrogel beads treated with sulfonates that have been found to deliver chemotherapy directly to liver tumours' blood vessels. According to Lewis [163], they are effective in the treatment of liver cancer because they can release positively charged anticancer drugs through an ion-exchange process [162].

4.2.2.14 Biomaterials for Cancer Immunotherapy

Cancer immunotherapy, which has been around for a while, offers patients hope for the treatment of their tumours. But because it has some restrictions that must be overcome, a new upgrade was necessary. Unwanted unfavourable and side effects, localised distribution of immunomodulators, and excessive systemic immune system activation are some of the limitations related. Using biomaterials in the form of injectables, implants or transdermal drug delivery devices can be helpful to localise cancer immunotherapy and so give a solution to the problem. The following discusses a few of them [163].

4.2.2.15 Implantable Biomaterials

.A straightforward procedure is used to implant scaffolds beneath the skin in the place of lost tissue. Before being implanted, the biomaterials are loaded with cells, anticancer drugs, factors, or anything else needed for the treatment before being released carefully from enormous scaffolds created just for them. These porous scaffolds are prepared to attract immune cells to the site of implantation. Examples include poly(lactide-coglycolide), porcine gelatin, alginate, polyglyconate, collagen, and hyaluronic acid [164, 165].

4.2.2.16 Biomaterials for Injectable

They include injectable biomaterial scaffolds, which eliminates the need for surgery and implants. They work effectively in combination with cancer treatments like radioisotope therapy or chemotherapeutics. The injectables, which are liquid at ambient temperature and gel at temperature of body, are biocompatible. Cryogel, hydrogels, and inorganic scaffolds are examples of materials that allow for the controlled and localised release of cancer-fighting drugs. The use of injectable scaffolds has shown numerous encouraging results in tumours and other malignancies, according to numerous studies [166].

4.2.2.17 Transdermal Biomaterials

They consist of transdermal injections or surgically implanted devices. Through this method, melanoma, the most prevalent kind of skin cancer, has been attempted to be treated. A specific rate of drug delivery occurs from the patch via the skin and into the bloodstream. Low permeability should be one of the properties of transdermal biomaterials. They include a variety of issues, including electric fields (iontophoresis and electroporation), chemical enhancers, lipid enhancers, and pressure waves produced by ultrasound or photoacoustic effects. Microneedles are a method for penetrating the skin. Hyaluronic acid, trimethylene carbonate, poly (lactic-co-glycolic acid), polycaprolactone, poly, and granulocyte–macrophage colony-stimulating factor are few examples [167].

4.2.2.18 Uses of Some Biomaterials for Cancer Therapeutics

Polyamine analogues have the ability to destroy cancer cells and inhibit cell division. The bioactive polymers have an indirect and direct impact on cancer cells, either by killing cancer cells and reducing their development or by erasing multi-drug resistance by inhibiting P-glycoprotein activity (Pgp). Each class serves a particular purpose in accordance with its relationship between structure and activity and its cytotoxicity process. Gold particles can be used as biomaterials in a variety of fields, including biomedical sciences. Only a few applications include drug transporters, photothermal compounds used as sensitisers for radiation therapy and in biomedical imaging. Similar to this, Zhang et al. presented their research in 2020 that involved using Cu like a biomaterial to cure cancer by inducing hypoxia. Cu-MOF NPs were employed in chemodynamic treatment and sonodynamic therapy (CDT/SDT) as a novel hypoxia-responsive copper metal–organic framework nanoparticle. The nanoparticles utilised in deep tumour drug delivery had a considerable influence on cancer cell killing. The hypoxic tumour microenvironment caused the nanoparticles to exhibit excellent stability beneath normal partial pressure of oxygen and enhanced tumour accumulation that broke down and produced, intratumourally, copper ions increased penetration [168, 169].

4.2.2.19 Nanocarriers that are Cell-Based and Bioimimetic: New Approaches to Targeting Brain Cancer

Over the past 20 years, there haven't been any notable developments in the therapy of the most common and harmful forms of brain tumours. The difficulty to get drugs into the brain and spinal cord in therapeutic doses and the resulting serious side effects are the fundamental barriers to progress. To achieve successful therapy, medication must be able to cross the required biological barriers without inactivating prior approaching the proper therapeutic target. Although there has not yet been any practical translation, there has been substantial study into the production of synthetic nanocarriers to treat brain tumours for overcoming these kind of challenges. Recent research has shown focus on cell-derived or biomimetic nanocarriers because of their inherent bio-interfacing potential. A stronger selectivity for brain malignancies and the capacity to transfer therapeutic molecules would raise new expectations for the creation of secure and efficient treatments [170].

4.2.2.20 Nanocarriers for Brain Tumours

To get beyond such biological barrier and enhance dug delivery, while minimising unwanted side effects, drug-loaded nanocarriers can be created. The capacity of nanocarriers to make hydrophobic drugs more soluble and shield them from the surroundings helps overcome common problems such drug stability and poor aqueous dissolution. The characteristics of the target tissue and chemotherapeutic agents significantly influences the design and functionalisation of nanocarriers. Polymeric nanoparticles (NPs), nanostructured lipid carriers, metal-based carriers micelles, solid lipid nanoparticles (SLN), and dendrimers are a few of the nanocarriers created for brain distribution. Due of the unique characteristicsof the brain, an ideal nanocarrier system should be biodegradable, but not harmful and biocompatible. Nanocarriers having diameters in the range between 30 and 100 nm and then either positively charged for more cell absorption or neutral/negatively charged for prolonged circulation time have been developed with the goal of targeting brain tumours. These nanocarriers aim to penetrate biological barriers and enter the tumour using absorption-mediated transcytosis (AMT) without any particular functionalisation [171–173].

4.2.2.21 Lactoferrin: Drug Nanocarrier

The potential of Lf nanoparticles in cancer therapy particularly, as well as Lf's prospective use as a drug nanocarrier, attracted the researchers interest. by the expanding developments in the nanomedicine sector by 2005 [174]. Since Lf is a naturally occurring protein found in milk, there is very little risk that it will cause an unfavourable immunological response. Primarily, the Lf protein-based particles are created using gentle techniques devoid of any chemical reactions [175]. The liver and

haematological biochemical parameters are retained even after systemically administering Lf NPs at high levels, according to study groups on the substance. According to many reports, Lf exhibits anticancer activity. Numerous investigations have shown that bLf specifically blocks the plasma membrane V-ATPase, lowering the acidity of the cancer microenvironment and preventing tumour development and metastasis. This was supported by the finding that the highly malignant cancerous cells Mg-63, PC-3, Hs 578T, as well as MDA-MB-231, which had higher levels of V-ATPase over non-cancer cells, were preferentially destroyed by bLf [176, 177]. Another way that bLf differs from other usual V-ATPase inhibitors in considerations of selectivity is by inhibition of the lysosomal V-ATPase.

Additionally, colon CSCs and tumours were demonstrated to be sensitive to nano-formulated bLf's anticancer apoptotic effect, which was proven to have an indirect connection to the suppression of apoptosis inhibitors (Fig. 4.11) [174]. Lf up-regulates the antagonists of IA, HTRA2, IAP, and SMAC in addition to activating the caspases, notably caspases 8, 9, or 3.As a result, it was determined that the key function of Lf in the induction of apoptosisin cancer cells was to block IAPs. To battle chemoresistance, Lf also swiftly internalises within cancer cells and makes resistant tumours more susceptible to the impacts of chemotherapeutic drugs like doxorubicin (DOX). Additionally, TNF and IFN, two cytokines with anticancer properties, may express themselves more frequently in response to Lf nanocarriers. Iron-saturated holo-Lf exhibits more anti-tumour efficiency than apo-Lf and native Lf, suggesting that Lf's iron saturation may be related to its anticancer action; however, the underlying mechanism is yet unknown [178–180].

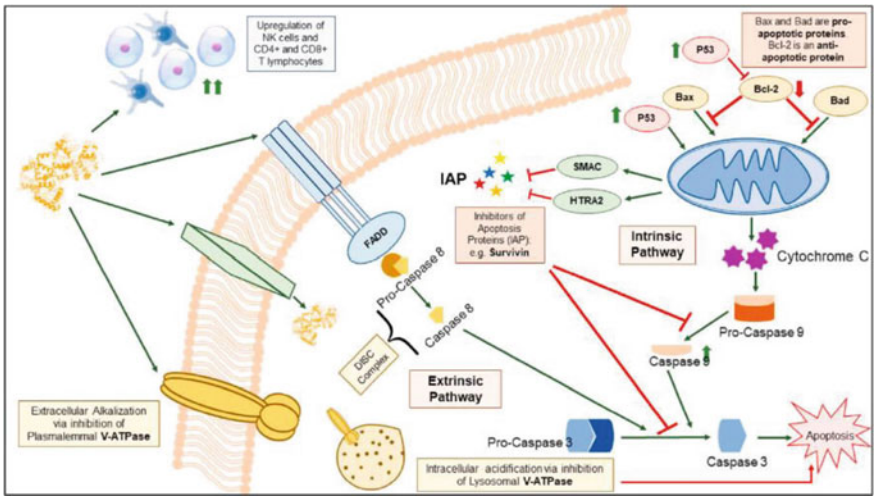

Fig. 4.11 Schematic illustration of Lf's primary anticancer mechanisms (182) (Copyright 2020, Elsevier)

4.2.2.22 The Administration of Lactoferrin as an Active Medicinal

Microparticles

For the regulated release of Lf, chitosan microparticles with a mean size of 4.9 m were created by emulsification-solvent evaporation The microparticles showed a substantial Lf loading (16.7%, w/w) and a regulated, progressive release of Lf over the course of 24 h. Oral administration was not advised due to the fact that chitosan dissolves in acidic pH and has the potential to disintegrate in acidic conditions in the stomach. This led to the encapsulation of Lf inside calcium alginate microparticles that had been electrostatically complexed and subsequently covered in chitosan. For the intestinal transport of Lf, the microparticles in the GIT at pH 1.2 and pH 6.8 retained their integrity [181–183].

Nanoparticles

Capacite phosphate nanocrystals (NCs) were electrostatically coated with cationic Fe-bLf to improve oral absorption. Chitosan was then lightly applied to the NCs. For additional protection against GIT degradation, the chitosan-coated NCs have been encased in alginate gel [184]. In acidic environment of the stomach, alginate is unionised, and creating a matrix surrounds the inner core to safeguard the trapped Fe-bLf. When the gut pH shifts to an alkaline state and the Lf content is released, the ionic sodium alginate is produced. Enhanced internalisation of nanocarriers within intestinal tissue layer was made possible by the mucoadhesive properties of Fe-b Lf NCs that were regulated by the interactions between the positively charged iron, negatively charged mucus, and chitosan. Additionally, intestinal cells' LPR and DMT1 receptors aid in the absorption of the nanocarriers. The Lf NCs with the alginate/chitosan coating showed encouraging action against many types of solid tumours [185].

Liposomes

It was demonstrated that adding bLf to liposomes strengthened their resistance to stomach digestion, thus elevated the intestinal absorption, and boosted their anti-inflammatory properties after being delivered orally or intravenously. LiposomalLf increased intracellular accumulation and also prevented proteasomal and lysosomal breakdown, which has increased its cytotoxicity against cancer cells [186, 187].

PEGylation

It is possible to create PEGylated Lf by directly conjugating and both the branching PEG N-hydroxy succinimide (NHS) esters and free amino moieties of bLf. As an

alternative, two linear PEG pnitrophenyl esters, 5 and 30 kDa, might be used to link bLf. In comparison to PEG-NHS, PEG-p-nitrophenyl active esters permitted for delayed reaction rates but considerably stronger degradation stabilities. PEGylation of Lf increased its oral bioavailability and the proteolytic half-lives of 20 kDa and 40 kDa-PEG-bLf by 2- and sixfold, respectively, in contrast to unmodified Lf. At a dosage of 30 mg/kg, 20k-PEG-bLf demonstrated a 5.4-fold longer plasma half-life and a tenfold higher rate of absorption over unmodified Lf in rats. PEGylated bLf increased its hepatoprotective effect against CCl4-induced liver diseases, which lowered AST and ALT activity, via enhancing activities of SOD and ROS scavenging in mitochondria. In contrast to both original bLf and 20k-PEG-bLf conjugates, the 40k-PEG-bLf showed superior pharmacokinetics and hepatoprotection [188–190].

4.2.2.23 Design of Lf-Targeted Nanocarriers

Due to the overexpression of Lf's receptors in malignant cells and some other tissues, such as the brain, Lf was used more frequently to alter the surface of drug nanocarriers. ForLf surface-modified nanocarriers, electrostatic complexation and covalent conjugation have been the key design considerations. Chemical conjugation utilised a variety of coupling processes, including maleimide and carbodiimide thiol coupling techniques. When Lf was conjugated to SLNs and PEGylated liposomes using maleimide and carbodiimide thiol coupling techniques, high yield and effective conjugation were generated, with conjugation efficiencies of 71.02% and 74%, respectively [191, 192]. Different types of of Lactoferrin based Nanocarriers were reported in Table 4.3.

4.2.3 Others

4.2.3.1 Radio Frequency Responsive Nanobiomaterials for Cancer Therapy

Since it is not hazardous and may enter tissues, radio frequency (RF)-aided cancer therapy is familiar in the medical community and allows for a comprehensive cancer treatment. Patients find it challenging to endure the RF ablation process because of the non-particular and intrusive property of the current treatment plan. Metal nanoparticle use has received a lot of attention lately(NPs) like gold and iron oxide in place of RF probes, which allow RF current to enter tumours. These metallic NPs have potential to be used in conjunction with stimuli-responsive polymers to deliver drugs to tumours while also improving thermal ablation [200].

Table 4.3 Pharmaceutical applications of lactoferrin based nanocarriers

S. No.	Diseases	Applications	References
1	Brain tumours	Lf-conjugated PEG-PCL polymersomes (POS) containing 40 or more Lf molecules each were co-loaded with tetrandrine and DOX (Tet) Tet, an MDR inhibitor, improved total cellular uptake by decreasing cellular resistance, which led to the smallest tumour volume and longest survival duration. Notably, Lf alteration increased POS concentration in the right side of the brain more than in the unmodified ones	[193]
2	Parkinson disease	Lf is highly expressed in microglial cells in Parkinson's disease, where it has a significant neuroprotective impact. Both holo- (iron-saturated) Lf and served to illustrate this. The hypothesised Lf neuroprotective mechanisms may be mediated by an increase in mitochondrial membrane potentials, which shows better mitochondrial activity	[194, 195]
3	Alzheimer's disease	Oxidative stress, reactive oxygen species, and the production of nitric oxide synthase are associated with elevated iron levels in Alzheimer's disease. Because Lf has antioxidant properties and works like an iron chelator within brain to restore iron haemostasis, lower amounts of TNF- and IL-6 are produced as a result of its anti-inflammatory effects	[196]
4	Colon cancer targeting	Lf has been promoted as a secure substitute with potent anticancer properties, especially against colon cancer. First, numerous studies have shown that Lf has an anti-inflammatory effect on the colon cancer. As evidenced by elevated levels of nuclear p65 protein expression, p65 + cell counts, and IKK/, NF-B was activated in Lf knockout mice, which led to an exacerbated inflammatory response. Pro-inflammatory cytokines (TNF- IL-1, and IL-6) were also found to be increased which can result in dysregulation of AKT and mTOR and the formation of tumours	[197]

(continued)

Table 4.3 (continued)

S. No.	Diseases	Applications	References
5	Liver cancer targeting	Lf enters hepatocellular carcinoma via selectively binding to LRP and asialoglycoprotein receptors (ASGPR) (HCC). In ASGPR-positive cells, such as BEL7402, HepG2, and SMMC7721, Lf-modified PEGylated liposomes improved absorption, but not in ASGPR-negative cells. In HepG2 mice, DOX-loaded Lf-PEG liposomes were superior to the placebo in their ability to treat cancer	[198]
6	Breast cancer targeting	Following oral therapy, the micro Fe-bLf NCs (80 nm) produced mitochondrial depolarisation and activation of caspase-3 in the claudin-low, triple-negative MDA-MB-231 breast cancer cells. They also induced downregulation of the apoptosis inhibitory proteins survivin and livin, along with PI3K. Fe-bLf NCs can also be used as a tumour nanotheranostic material for magnetically guided treatment of cancer and MRI contrast imaging due to various their superparamagnetic characteristics	[199]

RF Responsive Gold Based Nanobiomaterials

The surplus ionic contents formed during the Au-NPs production time are fundamental cause of background heating from Au-NPs. Most published synthesises of Au-NPs use NaOH to regulate pH, which causes the Au-NPs to be heated under RF energy unnecessarily. One must take into account this issue. To prevent this, it is preferable to use tris-buffer to modify the pH; thus, the resultant Au-NPs do not experience any additional heating from ionic counterparts [201].

John S. Kanzius, an American inventor and amateur radio operator of Erie, Pennsylvania, was the first to propose the utilisation of Au-NPs for RF aided heating [202]. NIR has some difficulties for treating the deeply embedded tumours, despite the large number of work using Au-NPs for NIR aided tumour therapy at the in vitro and in vivo levels. It has been observed that Au-NPs in spheres, cages, clusters, and other altered forms have the ability to perform NIR imaging both in vivo and in vitro [202].

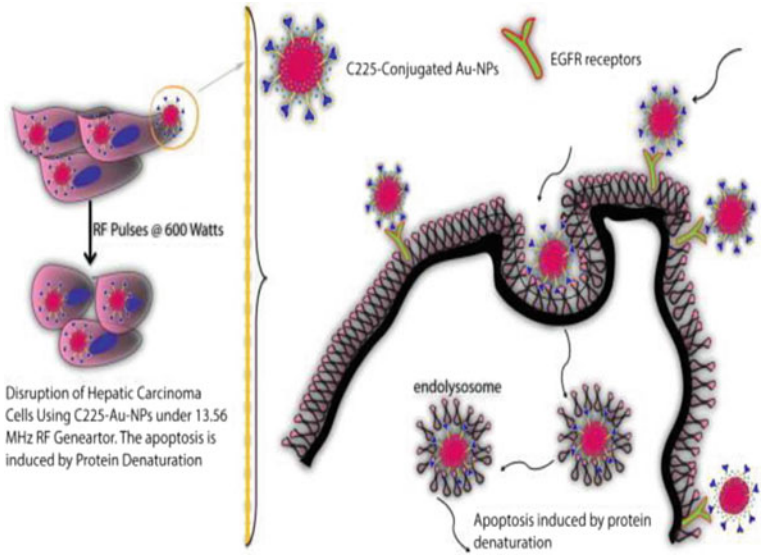

Fig. 4.12 RF aided thermal destruction of hepatic carcinoma cells using C225 conjugated Au-NPs. The apoptosis is preceded by protein denaturation under RF exposure of 600 W (211) (Copyright 2015, Elsevier)

Applications of RF responsive gold based nanobiomaterials

Several research have suggested the utilisation of Au-NPs for in vitro and in vivo RF aided tumour therapy in recent years. When monoclonal antibodies are added to Au-NPs, targeted hyperemia can be achieved, which allows for the total eradication of cancer cells [203–205]. Improvedtarget ability and RF-induced heating in pancreatic cancer cells have been seen using cetuximab (C225) attached Au-NPs (Fig. 4.12) [203]. The RF-aided death of pancreatic tumour cells (Panc-1) was clearly influenced by the Au-NPs, and additional surface modification would help with better targeting [206–208]. The RF-aided heating in cancer cells is described by Raoof et al. [209], offering fresh perspectives on the creation of hyperthermic heat in cancerous cells. The anti-epidermal growth factor receptor (EGFR) antibody (C225) linked Au-NPs may improve the stability and selectively kill hepatic cancer cell lines in vitro by reducing endolysosomal Au-NPs aggregation by raising intracellular pH. By causing protein denaturation, the C225-conjugated Au-NPs might cause apoptosis in liver cancer cells. Although antibody-coated Au-NPs demonstrated improved biomedical uses, there isn't much evidence to support their usage in RF-aided drug delivery for treatment of tumour [209].

Iron Oxide Nanoparticles (IONPs)

The utilisation of IONPs as a good candidate for RF-assisted tumour therapy is being emphasised [210]. They have received FDA approval as contrast agents [211]. They are said to be greatly bioresorbable [212], adaptable, and biocompatible [213]. The medium-sized IONPs were discovered to be an effective tumour killer at low-frequency RF irradiation among the varied sized IONPs. The largest proportion of tumour cell death was produced by small-sized IONPs at the greater exposure of radio frequency. It was discovered that the tumour-killing impact depended on concentration. The enhanced cancer killing was observed at greater IONP concentrations. After hypethermia, IONP-containing MCF-7 cells were discovered to be apoptotic and to have an elevated level of reactive oxygen species (ROS). SEM and TEM are used to see how tumour cells are changing during apoptosis [214]. According to Bae et al. [219], ferrimagnetic iron oxide nanocubes can treat magnetic hyperthermic agents. The synthetic Chito-FIONs, also known as ferrimagnetic iron oxide nanocubes, have a great potential for heating up in an RF field. Chito-FIONs are composed of numerous tiny (30-nm) FIONs that are enclosed within the chitosan shell. The numerous FIONS included into the chitosan shell can increase overall magnetic moments and cause localised heat accumulation when a magnetic field is applied [215].

Quantum Dots (QDs)

According to a 2010 study by Evan S. Glazer et al., the QDs have been used as RF-responsive nanobiomaterials for in vitro ablation of pancreatic tumour cells. By using a particular antibody to target the tumour cells, the heating of these QDs can be effectively contained within the tumour cells. The EGFR, a cell-surface receptor that is a member of the ErbB family of tyrosine kinases, exhibits a crucial function in regulation of cell proliferation, survival, and differentiation. According to reports, the majority of malignant tissues have excessive EGFR expression, making them suitable targets for anticancer therapy [216]. Excellent cytocompatible luminous superparamagnetic nanocomposites were the subject of research by Yang et al. in 2011. These were made of coated QDs with iron oxide and silica (IQ). To create Fe_3O_4/SiO_2, silica shell was first applied to the Fe_3O_4-NPs (IQs). These were further altered by assembling with thiol conjugated CdSe-ZnS QDs, which are very water soluble. These multilayered nanocomposites are designed to effectively improve RF heating and imaging of cancer cells.

The superparamagnetic and luminous properties of the IQ nanocomposites were remarkable. Within a brief period of incubation, the extremely luminous IQ NPs were discovered to be effectively absorbed into the Panc-1. In vitro tests showed that the IQ nanocomposites were not harmful to the tested cell lines. Even at higher concentrations (0.2 mg/mL), they shown great compatibility with $Panc^{-1}$ cells. Fluorescent magnetic nanocomposites at 1.7 g/mL significantly increased RF-induced cytotoxicity toward Panc-1 cells (0.8% viable cells) after only 2 min of RF exposure.

Due to their distinctive optical characteristics, the IQ composites were useful for tracking apoptotic phenomenon. Additionally, it was demonstrated that the created IQ composites have strong magnetic characteristics that may be used for in vivo MRI imaging and magnetic hypethermia. Due to its multifunctional qualities, which are very demanding in terms of current cancer treatments, the extremely biocompatible IQ compositmemay be effective as possible theragnostic agent. Since the utilised QDs had ZnS surface passivation, cadmium ion leaching might be substantially reduced, thus improving the in vivo applicability. Since ZnS-based nanocrystals are more cyto-friendly than CdSe, they would ideally be a superior solution for reducing the toxicity of CdSe core. Silica toxicity, however, may limit the in vivo potential of IQ composites, leading to organ damage. Therefore, a thorough examination of histopathology and organ distribution would be essential to comprehending its application as an effective RF sensitive nano-biomaterial [217].

4.2.3.2 Carbon Based Nanobiomaterials for RF Aided Cancer Therapy

Cobalt Nanoparticles Coated Graphitic Shells (C-Co-NPs)

C-Co-NPs, which are ferromagnetic cobalt nanoparticles with cubic crystalline graphitic carbon decorations and have a diameter of 7 nm, are good at producing RF-induced heat for nanohyperthermic applications. X-ray photoelectron spectroscopy research proved that the cobalt NPs were kept as metal inside the carbon shell. Images obtained using fluorescence microscopy and Raman spectroscopy demonstrate that they can successfully pass by the cellular and subcellular components of HeLa cells. These C-Co-NPs efficiently cooked the cancer cells by inducing localised heat in the metallic NPs at low RF pulse rates of 350 kHz. Both RF power and concentration were discovered to be reliant on this process. In comparison to SWNTs, the carbon-shelled cobalt NPs demonstrated better selectivity for RF absorption and heating. The DNA fragmentation, nucleus rupture, and membrane disintegration that the RF exposed C-Co-NPs caused in HeLa cells indicated that the cancer cells had been completely destroyed. Overall, C-Co-NPs may be a great choice for the in vivo RF-induced ablation of cancer cells as well as other medical uses including the eradication of microorganisms. One of the biggest problems with these novel RF ablating systems is the absence of preclinical research like pharmacokinetics and pharmacodynamics, which makes it challenging for translational purposes [218].

Epidermal Growth Factor (EGF)-Functionalised Carbon-Coated Magnetic Nanoparticles

It has been claimed that RF sensitive nanobiomaterials called carbon-shelled iron magnetic nanoparticles (C/Fe MNPs) can kill tumour cells. The improved cell binding potential of EGF functionalised-MNPs on breast tumour cells was demonstrated by the surface charge analysis of internalised tumour cells. The increased negative

surface charge of the EGF-MNPs that are localised within cells suggests that they are also internalised more deeply. After being treated with EGF altered NPs, a 10-min RF excitation treatment eliminated the majority of the MCF-7 breast tumour cells with less than 3% cell viability. The unaltered NPs, however, exhibited decreased tumour cell mortality and were still 63.7% viable under same experimental circumstances. Comparing the bio-conjugate to the native C/Fe MNP bio-conjugates, the bio-conjugation demonstrated superior bio-compatibility and greater targetability on MCF-7 cells. The caspase-3 defective route was used to mediate the RF-induced apoptosis [219].

Transferrin Functionalised Graphene

According to Sasidharan et al., transferrin altered carboxylated graphene is the best material for locating and destroying chronic myelogenous leukemic tumour cells with shorter RF exposures for five minutes. Their work demonstrated the capability of graphene as an RF-sensitive biomaterial that was capable of eliminating CML without the need of chemotherapeutic drugs, opening up new opportunities for RF aided cancer therapy. According to many biological tests, the RF ablation was extremely targeted at clinically important K562 cells, shedding insight on the potential for graphene-based biomaterials. Although the precise phenomenon for RFA employing transferrin linked graphene is unmentioned, it is claimed that they are more effective hyperthermic agents than SWNTs and Au-NPs. Since brain malignancies like gliomas overexpress transferrin receptors, employing transferrin attached graphene in combination with RF therapy to target glioma cells would be an excellent strategy. Comprehensive in vivo toxicity studies are perfect for the translational component of these transferrin-linked carboxylated graphene materials [220].

4.2.3.3 RF-Guided Targeting Biodegradable Polymeric Composites

Due to their ability to react to intracellular and extracellular stimuli including pH, ionic concentrations, and GSH levels [221], stimulus sensitive polymers are essential for drug delivery. Incorporating stimuli-sensitive polymers as a shell for an Au [222] and Fe_3O_4-NP based [223]. RF responsive core has received a lot of interest recently. The primary goal of adding stimuli-sensitive shells to RF responsive cores is to increase the effectiveness of drug loading in the shell while managing the release in line with the alteration of the RF sensitive base material. ACM-TNGs, an effective RF responsive nanomedicine, were recently created by combining chitin, a commonly used biopolymer, with RF responsive Au-NPs and MnO_2 nanorods. The improved RF heating under RF exposure ranging from 20 to 100 W is the result of the optimised concentrations of 0.5 mg Au, 1 mg chitin, and 0.27 mg/mL MnO_2 nanorods of ACM-TNGs. The addition of MnO_2 nanorods was found to boost the heating. The ACM-TNGs treated human ductal carcinoma cells (T47D) were discovered to be fully lifeless after being exposed to 100 W of RF for two minutes. An accurate

understanding of ACM-in TNG's vivo behaviour would have been possible with the help of animal research employing the T47D tumour model to better comprehend the toxicity profiles of the compound [224]. By encasing curcumin in chitosan-graft-Poly(N-Vinyl caprolactam) NPs with 10 nm-sized Au-NPs, which are thermal, pH, and RF responsible, it can be supplied to breast cancer cells in the most favourable RF settings. The study was commended for its RF and pH-responsive delivery from Au-NPs and thermoresponsive coating, respectively. The curcumin was released under ideal RF settings of 40 W for around 5 min, effectively increasing the rate of apoptosis in 4T1 mammary ductal carcinoma cells. Similar outcomes were seen with Fe_3O_4-NPs coated with chitosan-graft-Poly (N-Vinyl caprolactam) NPs at a little greater RF exposure of 80 W for 2 min, indicating that stimuli-sensitive Fe_3O_4-NP coating could boost RF aided curcumin's delivery in a pH induced way. Under RF conditions, curcumin might maintain its anticancer efficacy, indicating that a small amount of RF irradiation could increase drug permeation to tumour cells without destabilising curcumin. Despite the fact that 4T1 tumour models have demonstrated great retention of these NPs, it may be preferable to analyse the in vivo RF-induced release of curcumin from those models through anticancer research. If such Au- and Fe_3O_4-incorporated chitosan-graft-Poly(N-Vinyl caprolactam) NPs were actively targeted, the toxicity towards normal intestinal epithelial cells (IEC-6) might be reduced to a certain degree [225, 226].

4.2.3.4 Designs of Theranosticbiomaterials for Cancer Precision Medicine Therapy

Theranostic biomaterials have opened up a wealth of new possibilities for the advancement of precision medicine in the treatment of cancer. Additionally, when theranostic biomaterials are able to precisely film a biological process at the particular tumour locations, there is the potential to discover a new paradigm shift for the treatment of tumour. Theranostic biomaterials can visualise targets, track the effectiveness of delivery vehicles, and track therapeutic actions at the tumour location with the help of molecular imaging technology. Contributing target specificity to biomaterials for increasing therapeutic efficacy and visibility in cancer treatment may provide significant challenges in theranostic biomaterial design, which can help us discover strategies to decrease the negative impact of cancer treatment on healthy tissues.

4.2.3.5 Stimulus-Activatedtheranostic Biomaterials

Acidic pH-Responsive Theranostic Biomaterials

The Warburg effect states that the enhanced performance of the proton pump on the plasma membrane of tumour cells results in the creation of huge amount of lactic

acid in the extracellular milieu and significant amounts of energy through glycolysis in cancer cells [227]. Because of this, the tumour site's pH conditions are more acidic (pH 6.5–7.2) than those of the adjacent normal tissue (pH 7.4) [228]. Several theranostic biomaterials, that easily produce optical signals and improve therapeutic actions under mildly acidic settings but reduced activity under neutral conditions, have thus been documented employing pH variations. These pH-responsive theranostic biomaterials can offer therapeutic options for more accurate medication administration and tumour-specific diagnostics. The general methodologies for the creation of pH-responsive theranostics can be divided into two categories. Including ionisable chemical groups, such amines, which can collect or donate protons and undergo pH-dependent alterations, releasing medicines, is one tactic [229]. For pH-responsive theranostics, another tactic is of adding an acid-labile linker between the therapeutic substance and the nanocarrier [230]. When the pH is neutral, they are stable, but when the pH is acidic, they breakdown or hydrolyse. Liu et al. created self-assembled pH-responsive near infrared (NIR) emission micelles loaded with doxorubicin (Dox) to imagine fluorescence and chemotherapeutic applications as an illustration of the first technique [231]. Lim's team has introduced a pH-responsive poly (-glutamic acid)-graft-cetylester (-PGA-g-cetylester) nanoparticle made of a targeting moiety, pH-switchable fluorophores, and the anti-tumour drug paclitaxel. The construction of acid-labile systems for pH-responsive theranostics has frequently exploited hydrazone linkages with aldehyde or ketone moieties. Using hydrazone linkage formation, Lee et al. reported DOX-conjugated [G-3]-(PEO5k)8-[G-4]-(OH)16 bow-tie dendrimers in 2006 [232].

Enzyme-Responsive Theranostic Biomaterials

As possible endogenous inducers for activatable theranostics, many overexpressed enzymes engaged in intricate biochemical phenomenon originating from tumour growth, angiogenesis, and metastasis are investigated. It has potential to sustain activation of theranostic and successfully increase the signal with just a few enzyme molecules. Additionally, selective cancer imaging and therapies are made possible by the enzyme's substrate selectivity [233]. It is recognised that some proteases, such as matrix metalloproteinase (MMP) and cathepsin B, are crucial in the biochemical processes involved in the growth and spread of cancer. As it is generally known, MMPs alter the extracellular matrix's development and modify the microenvironment of malignant tumours [234]. In the starting phases of tumour metastasis, the cathepsin family is overexpressed [235]. MMPs are Ca^{2+}- and Zn^{2+}-dependent proteases that participate in the breakdown of extracellular matrix [236]. The 24 members of the MMP family are categorised according to their functions, like collagenase, gelatinase, matrilysin, or membrane-type MMPs. Ansari et al. reported multifunctional theranostic nanoparticles that can both transport medications in vivo and release pharmaceuticals by enzyme cleavage [237]. They gave the iron oxide nanoparticles attached to the MMP-14 reactive peptide sequence that also contains the angiotensin azademethylcolchicine (ICT), a fluorescent dye called fluorescein isothiocyanate

(FITC), the name CLIO-ICTs. In vitro, no damage to MMP 14 negative fibroblasts was seen; however, MMP-14-positive breast tumour cells showed cell death. After intravenously injecting CLIO-ICT into a mouse model of cancer, a remarkable tumour specific accumulation anti-tumour impact was seen.

Redox-Responsive Theranostic Biomaterials

Due to oncogenic activation, metabolic changes, etc., tumour cells experience oxidative stress from overexpressed reactive oxygen species (ROSs), such as superoxide ($[O_2]\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($[OH]\cdot$). Tumour cells overexpress redox species including glutathione (GSH) and superoxide dismutase (SOD) to combat these oxidative stressors [238]. Because of this, the TME maintains considerably higher oxidation/reduction potentials than a healthy normal cell environment. Scientists have developed a spectrum of redox-responsive theranostic biomaterials which show stablity in blood circulation yet competent of quickly disintegrating and effectively releasing medicines in tumour cells by taking advantage of these physiological variations. The GSH regulates numerous biological processes, including cell development, metabolism, and antioxidant defence, acting as a cellular protective agent [239]. GSH is reported to be four times overexpressed in tumour cells compared to normal ones to combat the oxidative microenvironment around the tumour [240]. Disulfide bond (–S–S–), diselenide (–Se–Se–), and carbon-selenium (–C–Se–), that are simply diminished by GSH, have all been used to develop redox-responsive nanoparticles using this physiological difference.

Hypoxia-Responsive Theranostic Biomaterials

Greatmagnitude of nutrients and oxygen are consumed by tumours for disproportionate proliferations. Despite the formation of numerous neovasculatures, the amount of nutrients and oxygen available is still insufficient to satisfy the demands. As a result, tumour cells experience hypoxia, a low oxygen partial pressure state. The oxygen content is typically less than 4% in many solid tumours and occasionally non-measurable in TME [241]. In activatable theranostic designs, a variety of organic functional groups with hypoxia-sensitive behaviour, like aromatic nitro or quinone groups, are employed. Hypoxia-activated theranostic agents with a diazo motif, fluorescent rhodamine 123/B with a triphenylphosphonium group, and nitrogen mustard as an anti-tumour medication were developed by Verwilst et al. [242]. By intra- and inter-strand DNA strand coupling, the alkylating chemical nitrogen mustard causes persistent DNA damage.

External Stimuli-Responsive Theranostic Biomaterials

Numerous studies have been conducted on multifunctional theranostic agents to enhance treatment efficacy and lessen unfavourable side effects. These compounds react to environmental stimuli like light, ultrasound, and radiation [243]. Due to its simplicity of usage and capacity to precisely manage wavelength and intensity, light is regarded as a beneficial external stimulus. Unfortunately, water and haemoglobin in the human body absorb the majority of visible light and obstruct light's ability to penetrate tissue. Therefore, the imaging and treatment of cancers that are deeply embedded in tissues mostly utilise the NIR area (650–900 nm) [244]. Heat and oxygen are two of the most important effectors in light-triggered therapy. It is categorised into photothermal therapy (PTT) and photodynamic therapy (PDT). PTT typically comprises of a photosensitiser that when exposed to light transforms light energy into heat, elevating surrounding temperature and burning tumour [245]. PDT, on the other hand, employs substances that cause ROS or O_2 to harm cancer cells [246]. For some solid tumours, PDT is a proven therapeutically effective ablative technique, although photosensitiser-mediated PTT is still in the research phase.

4.2.3.6 Immunomodulatory Biomaterials for Cancer Immunotherapy

Local Immunomodulatory Biomaterials

The patient may experience systemic toxicity and other unintended side effects when immunotherapy medicines are given systemically. To increase the safety of immunotherapy medications for systemic administration, researchers began to alter them or package them in nanoparticles; yet, these therapies frequently fail to accumulate the payload in tumours sufficiently to trigger an anticancer immune response. Researchers are looking at a more localised method of immunomodulation to solve these problems. Biomaterials and macroscale drug delivery systems have been created as promising therapeutic approaches to achieve this. Immunomodulation of the tumour microenvironment (TME) using a local strategy enables targeted delivery of the tumour treatment that specifically targets the tumour and immune cells that infiltrate the tumour. Current local immunomodulation biomaterials offer numerous advantages over systemic immunomodulation [247]. First, because local immunomodulatory biomaterials only require small amounts of immunomodulatory medications or agents, they do not have to worry about systemic toxicity or other negative side effects like cytokine release syndrome and vascular leak syndrome. Due to the close proximity to the treatment site, local administration reduces the dosage of medications required and frequently includes a targeting mechanism. These targeting techniques can either target circulating immune cells or aid in the recruitment of immune cells (e.g. antibodies conjugated to the payloads within the local immunomodulatory biomaterials). Local biomaterials spatiotemporally regulate medication release to maximise the immune response in addition to the low dose need.

Hydrogels

Hydrogels are injectable biomaterials which can be created from different polymers, such as natural or synthetic polymers, or a composite consisting of both, and which crosslink to create a three-dimensional (3D) network. The biocompatibility, biodegradability, and customisability of hydrogels are significantly higher than those of the other local immunomodulatory biomaterials. Immune cell maturation and proliferation are facilitated by the hydrophilic characteristics of hydrogels and their high swelling ratio. Depending on the polymer's characteristics and hydrogel's porosity, a variety of immunomodulatory drugs can be held in hydrogels. Additionally, it is possible to make hydrogels sensitive to outside stimuli. Depending on the stimuli, the hydrogel's sol–gel transition or the drug release mechanism may be caused. A number of polymers that fall under the categories of natural, synthetic, or a combination of the two can be used to make hydrogels [248]. Natural polymers are highly biodegradable and break down into natural metabolites that the body may easily eliminate. Synthetic polymers frequently do not interact with the biological environment and are nonimmunogenic in nature. However, these polymers typically produce hydrogels that are structurally weaker than synthetic equivalents. Collagen and gelatin have intrinsic chemical characteristics that make them highly bioreactive and rapidly transition to a gel. Hybrid polymers, which mix the finest qualities of natural and synthetic polymers, are being researched as a way to solve the drawbacks of hydrogels made entirely of natural or synthetic polymers. In one work, scientists created a hybrid hydrogel of hyaluronic acid that was functionalised with poly(-caprolactone-co-lactide) ester and levodopa, a stabilising agent to prevent biodegradation (HA-PCLA) [249]. A natural polysaccharide and part of the ECM in connective tissue, hyaluronic acid influences biological processes like cell migration and proliferation. Hyaluronic acid hydrogels are frequently contaminated, have a significant batch-to-batch variability, and deteriorate quickly when used alone [250]. The researchers were able to produce a hydrogel that retains the desired features of HA, primarily its high biocompatibility, while changing to a gel state when the solution reaches body temperature by mixing HA with PCLA. Granulocyte–macrophage colony-stimulating factor (GM-CSF), which enhances immune cell recruitment to hydrogel, and a nanopolyplex-based DNA vaccination were both delivered via these hydrogels.

Scaffolds

Scaffolds are 3D polymeric networks used in spatiotemporal drug release and host cell recruitment. There are many ways to use scaffolds to improve local immunomodulation. The maturation of immune cells can be aided by exposing the immune cells to cancer vaccines, anticancer antigens, or adjuvants through the use of polymeric scaffolds loaded with recruitment factors, such as GM-CSF. When the anti-tumour immune response is underway, those freshly trained immune cells might exit the scaffold. In one intriguing work, for instance, Ali et al. created a macroporous poly-lactide-coglycolide (PLG) scaffold containing GM-CSF, danger

signals (unmethylated cytosinephosphate-guanine oligonucleotide, or CpG-ODN), and tumour antigens to attract and reprogram DCs to elicit an anticancer response [251].

Microparticles

Microparticles are typically utilised to encapsulate several immunomodulatory substances, including immunotherapy medicines and cancer vaccines. They are significantly smaller than hydrogels and scaffolds. Microparticles typically have diameters between 1 and 50 m. The contact and particle diffusion with host cells are impacted by the enormous size of microparticles. For instance, immune cells, specifically APCs, can phagocytose microparticles, while smaller delivery methods, like nanoparticles, can be ingested through endocytosis and micropinocytosis [165]. These particles can be marked with antibodies to target immune cells and loaded with cancer antigens and/or immunomodulatory medicines. Microparticles are an effective platform for synergistic combination therapies to improve cancer treatment because they shield the antigen from degradation generally associated with bolus injections when utilised as a delivery mechanism for a cancer vaccination. To treat triple negative breast cancer, Davoodi et al. formulated a novel localised delivery system utilising a core–shell polymeric microparticle enclosing cisplatin and paclitaxel integrated in an injectable hydrogel [252].

Systemic Immunomodulatory Biomaterials

Immunomodulatory medicines administered systemically are a potential strategy for treating both primary tumours and malignancies that have migrated to distant locations of the body. As a result, the U.S. Food and Drug Administration has licenced numerous immunotherapy medications to treat metastatic cancer, including immune checkpoint inhibitors, toll-like receptor agonists, interleukins, and interferons [253].

Nanoparticles

One of the most adaptable biomaterials used in research is nanoparticles. They can be made from a variety of polymers and biological substances, comprising lipids, natural polymers such as hyaluronic acid, synthetic polymers like PEG, and inorganic metals like gold. Nanoparticles have the capacity to target important immune cells and promote cellular absorption thanks to the conjugation of ligands. In a work by Zhang et al., IL-2 agonists and anti-CD137 ligands were added to the surface of PEGylated liposomes to promote the growth of cytotoxic T lymphocytes and natural killer cells also as serve as a co-stimulatory signal that activates T cell [254]. The efficiency of a combined therapy using anti-CD137 and IL-2-Fc, which fuses the Fc domain to IL-2 to prolong half-life, was initially evaluated by the researchers [255].

Drug Conjugates

In cancer immunotherapy, monoclonal antibodies can detect particular antigens on or near the tumour site to trigger a cytotoxic response, but the therapeutic benefits are only partially achieved without conjugation of the monoclonal antibodies [256]. Monoclonal antibody-drug conjugates take advantage of the cytotoxic/immunotherapeutic attributes of the conjugated medication and the targeting abilities of monoclonal antibodies. These conjugations' fundamental structure consists of the drug, a linker, and an antibody. The release mechanism can be changed to concentrate the medicine at the desired spot by adjusting the linker chemistry. This is crucial for preventing drug release that is off-target when it is in circulation. These linkers may be pH-responsive, susceptible to lysosomal enzymes or enzymes present in high concentrations in tumour locations. As an illustration, Lv et al. created a polymer-PTX drug conjugate (P(L-SS-PTX))by conjugating paclitaxel (PTX), a cancer treatment medication, to 3,3'-dithiodipropionic acid functionalised methoxy poly(ethylene glycol)-b-poly(L-lysine) (mPEG-b-P(LL-DTPA)) [257]. The disulfide bond between the carboxyl groups of mPEG-b-P(LL-DTPA) and PTX in this instance serves as the conjugate linker and is unstable in reductive and acidic conditions. As soon as the drug conjugate enters the cell's endolysosomal route, this process enables drug release. Due to the lower pH and high glutathione concentrations in the extracellular matrix of the tumour, this polymer-drug combination can also be released there (GSH).

4.2.3.7 Nanomaterials-Based Acellular Vaccines

The production of homologous entire cell-based vaccinations is labour consuming, despite its potential, and there exist questions about production and bioactivity. Acellular immunisation exhibits a number of benefits as a result of these limitations, comprising ease of manufacture and consistency of batch [258].

Prefabricated Nanovaccines

Materials such as polymers (hydrogels, dendrimers, and nanofibers) [259], lipids (micelles, liposomes, and solid lipid NPs) [260], metals (gold, silver, zinc, titanium, and quantum dots) [261], carbon structures (nanotubes, nano-diamonds, and graphene) [262], and inorganic material [263] have all been used in NP delivery systems (silica). In contrast to typical adjuvants paired with antigens, Gao et al. produced PEG-b polymethacrylate polymers with pH-hypersensitive characteristic that transport antigens to DCs, that induced robust immunisation via STING pathway [264].

In Situ Assembled Nanovaccines

Recently, multiple groups have reported on a novel approach with enhanced therapeutic efficacy and DC-targeting capacity. The method uses fatty acids and other high-affinity albumin binders [265] to in situ connect endogenous serum albuminto create nano-based vaccinations. Amphiphile-based in situ assembled vaccines (amph-vaccines) were created by Irvine and colleagues and consist of a chain of an albumin-binding polymer coupled with an adjuvant or antigen. They verified using fluorescence imaging that vaccinations with fluorescein labels displayed significant accumulation in LNs [266]. Evans blue (EB) derivative (MEB), another albumin binder, and a nanovaccine derived on MEB were recently created by Chen and colleagues (AlbiVax). Two components made up this nanovaccine were MEB with a CpG link and an antigen conjugated MEB. To effectively distribute the vaccine to LNs and have an anti-tumour impact, the vaccine attaches to albumin molecules to create albumin/AlbiVax [267].

4.2.3.8 Application of Self-Assembling Biomaterials in Ovarian Cancer

Spheroids

These comprise of clusters of cell which form a necrotic core at diameters more than 500 m and preserve constant chemical gradients (such as oxygen, nutrients, and metabolites) and architectureat sizes between 200 and 500 m [268]. Spheroids are produced utilising different types of methods, comprising matrix encapsulation, suspension culture, non-adherent culture, hanging drop culture, and microfluidics [269]. Same nutrient transport, growth dynamics, and cell–cell interactions between cells cultivated as spheroids and solid tumours are seen [270]. Multicellular tumour spheroids (MCTs) are employed in particular for OvCa to simulate the tumour cell aggregates present in ascites [271, 272]. When compared to their single cell counterparts, spheroids generated from the OV-90 and OVCAR-3 clusters demonstrated better resilience to anoikis [273]. In contrast to the loose aggregates produced by OVCAR-3 cells, OVCAR-8 cells generated compact spheroids having significant migratingability. Additionally, spheroids showed size-independent resistance to anti-tumour medications [274]. Nectin-4 peptides prevented OVCAR-5 and CAOV3 spheroids from forming, which may boost the effectiveness of chemotherapeutic drugs in keeping cells as single cells or tiny aggregates [275].

Organoids

The term “Organoids” refers to 3D objects made from either pluripotent or adult stem cells [276]. Primary and metastatic tumours, pleural effusion drainage, ascites, healthy fallopian tubes, and ovarian surface epithelium are used to generate tumour-derived organoids [277]. They are being used as a transitional model between

xenografts and tumour cell lines [278]. The characteristics of malignant ascites were retained in organoids made from MCTs [279]. Instead of looking at the genetic changes that cause treatment resistance, cancer research has used tumour-derived organoids for pharmaceutical analysis [280]. DNA repair deficiencies in the organoids were connected to drug susceptibility, according to genomic analyses [281].

Microfluidic Devices

These gadgets are made up of micro-sized wells joined by channels of various shapes which can be used for perfusion, shear stress, nutrient delivery, and waste disposal [282]. The advantage of using microfluidic devices for drug testing is that different medications can be mixed and their concentrations can be changed [283]. To study tumour growth, metastasis, and medication responses, tumour-on-a-chip models can be made using cells expanded in microfluidic devices, also referred to as organ-on-a-chip devices [284]. Microfluidic devices, as opposed to static cultures, replicate crucial TME features such vascularisation, invasion, and migration of tumour cells while retaining cell viability [285]. For instance, Onal et al. developed an actuator-integrated microfluidic device to apply compression to SKOV-3 cells [286]. According to reports, cells stimulated by compression exhibited invasive morphology, enhanced proliferation, and chemoresistance [287]. OVCAR-5 cells were used in a microfluidic model that Rizvi et al. constructed utilising continuous laminar flow [288]. OvCa spheroids were brought into touch with human mesothelial cells in a microfluidic system created by Li et al. to mimic the metastatic process in peritoneal cavity [289–291]. The technology did not, however, guarantee that shear stress and individual nutrient replenishment were under control.

4.3 Conclusion and Future Perspectives

Biomolecules with ideal structural and distinguishing characteristics which control every biological processes in living beings are chosen by nature as a result of evolution. The goal of using biomolecules is to create durable biomaterials that imitate natural systems by exhibiting hierarchical complexity, structure, and functional significance. The interdisciplinary discipline of biomaterials science and engineering, which is rapidly expanding, can transform the fields of tissue engineering with the use of materials produced from biomolecules. Through carefully chosen examples from the literature, we have discussed current advancements in field of biomolecules-based biomaterials and their utility in this article. Sections devoted to proteins, nucleic acids, carbohydrates, and lipids contain thorough discussions about the biomaterials formed from several groups of biomolecules. In order to stress the top-down approaches of molecular complexity which results in reductionistic tactics, biomacromolecules, oligomers, and monomers are discussed as sub divisions

in each of these sections. A variety of molecular systems, including peptidomimetics (foldamers), cyclic peptides, peptide amphiphiles, cyclic dipeptides, and amino acids, have been thoroughly examined in the protein section. In a separate part, we discussed hybrid biomaterials that were created by combining more than two biomolecules in molecular systems that imitate the dynamic, intricate, diverse cellular, and tissue milieu. It has been emphasised that integrating biomolecules and it's components with synthetic materials can increase their chemical, mechanical, and biocompatibility qualities. The production of innovative biomaterials that can increase desired clinical benefit and enhance human life well-being is the main goal of biomaterials research and engineering in particular. Multi-disciplinary attempts toward the development of precision materials design approaches for creation of next-generation biomaterials structures and materials can benefit tremendously from the utility of biomolecules-derived materials or biomolecules-integrated synthetic materials with remarkable chemical, physical, mechanical, and biological features along with augmented biocompatibility and immunogenic response. The human body's in vivo environment differs from its in vitro environment. Therefore, it is a monumental effort to translate the in vitro results into the in vivo milieu of the living human body. New-generation radio frequency-sensitive nanobiomaterials have the potential to replace conventional oncological procedures and provide great patient compliance with efficient thermal ablation of cancerous cells. It would be advantageous to combine these substances with stimuli-responsive polymers for the regulated, continuous distribution of chemotherapy drugs to the sick location in an efficient manner. The RF-aided heating phenomenon of the QDs and carbon nanotubes is one area where generalisation of scientific discussion clarity is still needed for better knowledge. The heating method of RF-responsive nanobiomaterials needs to be understood on a broad scale. The QDs-based nanobiomaterials could be used for simultaneous imaging and drug administration using RF-induced thermal energy. The biocompatibility of semiconductor nanoparticles, such as zinc-based nanocrystals and QDs, is their most notable characteristic. These NPs might make for more effective hyperthermic agents. It would be perfect to combine a good thermoresponsive polymer with them to transport drugs, image tumours, and thermally destroy them all at the same time. If one were to consider actively going after certain cancers, one may have better results.

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Chapter 5
Immunotherapy: Targeting Cancer Cells

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Contents

Abstract Cancer has been a complex disease, and for many decades, research has been going on for designing a novel strategy for the cure and successful treatment; promising results and efforts are required. Although remarkable progress has been made in cancer medicine research concerning more efficient, specific, and less invasive modalities of cancer treatments recently, currently targeted therapy is one of the approaches aimed at targeting a particular location linked to cancer, such as tumor microenvironment or intracellular organelles, without affecting its normal surrounding and therefore benefits by increasing the specificity of the treatment. Targeted therapies are now one of the most promising therapies and have

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been embarking on their importance in oncological research and clinical oncology due to the revolution in the treatment of cancer in terms of diagnosis and the use of sophisticated diagnostic and molecular characterization technologies due to the advent of targeted therapy and immunotherapy. The advantage of targeted therapies is that it promotes effective dendritic cell (DC) maturation, T cell priming, activation, and differentiation into memory T cells that are long-lived, and thereby suggest combining cancer vaccines along with targeted therapies for boosted immune response and functioning of effector T cell. In this chapter, aspects of immunotherapy as a strategy for cancer therapy will be reviewed by analyzing the different approaches of immunotherapy and the upcoming promising therapies for cancer generally as well as for specific cancers while attempting to understand the latest developments in using immunotherapy as a novel strategy for cancer cure.

Abbreviations

ACT	Adoptive cell transfer
APC	Antigen presenting cell
CAR	Chimeric antigen receptor
CD	Cluster of differentiation
CDR	Complementarity determining regions
CGA	Cancer germline antigens
cHL	Classical Hodgkin lymphoma
CRC	Colorectal cancer
CTLA-4	Cytotoxic T-lymphocyte-associated antigen 4
FDA	Food and Drug administration
HLA	Human leukocyte antigen
HNSCC	Head and neck squamous cell carcinoma
HSCT	Hematopoietic stem cell transplants
ICI	Immune checkpoint Inhibitors
IL	Interleukin
MCC	Merkel cell carcinoma
MHC	Major histocompatibility complex
NIH	National Institute of Health
NK	Natural killer cells
PBL	Peripheral blood lymphocytes
PD-1	Programmed death 1 receptor
PD-L1	Programmed death receptor ligand 1
PD-L2	Programmed death receptor ligand 2
Ras	Rat adenosarcoma
RCC	Renal cell carcinoma
TAA	Tumor associated antigen
TCR	T cell receptor
TIL	Tumour infiltrating lymphocytes

TME	Tumour microenvironment
UC	Urothelial cancer
VAV	Vaccinia viru

5.1 Introduction to Immunotherapy

Cancer is a complex and devastating disease characterized by genomic instability which results in tumor progression as a result of the accumulation of numerous point mutations and structural alterations [1, 2]. Recent biotechnological advances are remarkably transforming the paradigm of cancer treatment using proteomic and RNA analysis, as well as tumor and cell-free DNA profiling using next-generation sequencing as well as a better understanding of immune mechanisms leading to optimizing the treatment option [3]. Targeting aberrations associated with cancers driven by oncogenes and immuno-oncology have revolutionized cancer treatment in the recent decade. Therefore, targeting cells by considering the individual abnormalities of the disease or the overall cancer pathway have yielded good clinical responses that could demonstrate severe effects for the survival of the tumor cells in solid tumors as well as malignancies [4]. Targeted therapy can be considered as a type of cancer treatment modality that involves targeting the proteins that regulate the growth of cancer cells, divisions, and differentiation. According to the National Institute of Health, National Institute of Cancer (NIC), 2022, targeted therapy is the basis of precision medicine. Targeting the cancer cells by enhancing the immune response is a type of targeted therapy. Cancer immunotherapy, one of the recent advancements of targeted therapies is also known as immuno-oncology, employs the strategy of using the defense mechanism of the body's own immune system to inhibit, limit, and destroy cancer. In oncology, using immune checkpoint inhibitors or adoptive cell transfer as a strategy has emerged as the latest foundation of anticancer immunotherapies. These treatments act by surpassing or reducing tumor-induced immunosuppression, thereby enforcing immune-mediated tumor clearance [5]. In cancer immunotherapy, the immune system is activated or the activation is boosted to attack the cancer cells naturally because the immune response is evaded during disease progression with the use of various agents and strategies. Thus, immunotherapy has been acknowledged as a promising strategy in the recent decade to treat, and even cure, especially certain types of cancer. The era of clinically marketing immunotherapy began with the Food and Drug Administration (FDA) of the United States' approval of recombinant variants of the cytokine interferon-α (IFNα), in 1986 for hairy cell leukemia as the first immunotherapy treatment for cancer [6–8]. Immunotherapy has now taken to revolutionize the field of cancer treatment and revitalized the concept of tumor immunology; many types of immunotherapies, including adoptive cell transfer (ACT) and immune checkpoint inhibitors (ICIs), have shown promise since their introduction response at clinical trials [9]. The five major approaches of immunotherapy include checkpoint inhibitors, lymphocyte-enhancing cytokines, adoptive transfer of engineered

T cells using chimeric antigen receptor (CAR) or T cell receptor (TCR), antibodies specific to costimulatory receptors, and vaccines [10]. Clinically, the breakthrough of immunotherapy for cancer came in the year 2013 with the advent of ipilimumab, CAR T cell therapies, and immune checkpoint inhibitors which became an important milestone in cancer immunotherapy, as emphasized by *The Science* journal. Following this. Several immunotherapies for cancer treatment have been approved in the last decade upon the success of clinical trials while many more are in clinical trials and many promising results are anticipated. The major immunotherapy approaches are considered to be classified under many classes, such as immune checkpoint blockade, Cytokines, CAR T cells, genetically engineered T cells with T cell receptors, cancer vaccines, oncolytic viruses, and bispecific antibodies [11]. Therefore, according to the reviews, it can be considered as a significant breakthrough in the era of cancer treatment as it has revisited the field of oncology considering the fact that, immunotherapy, aims at boosting the natural defenses to eliminate malignant cells but historically idea of targeting the immune system of the host to destroy cancer dates back to more than a century ago [12, 13].

In this chapter, addressing immunotherapy, we will discuss all the current approaches including immune checkpoint inhibitors mainly focusing on CTLA-4 and PD-1\PD-L1, CAR T cell therapy, adoptive cell transfer, and other approaches (Fig. 5.1) while also reviewing the conducted as well as ongoing clinical trials. Overall, this chapter will summarize the immunotherapy concept at the clinical trial level as well as the concept for future directions.

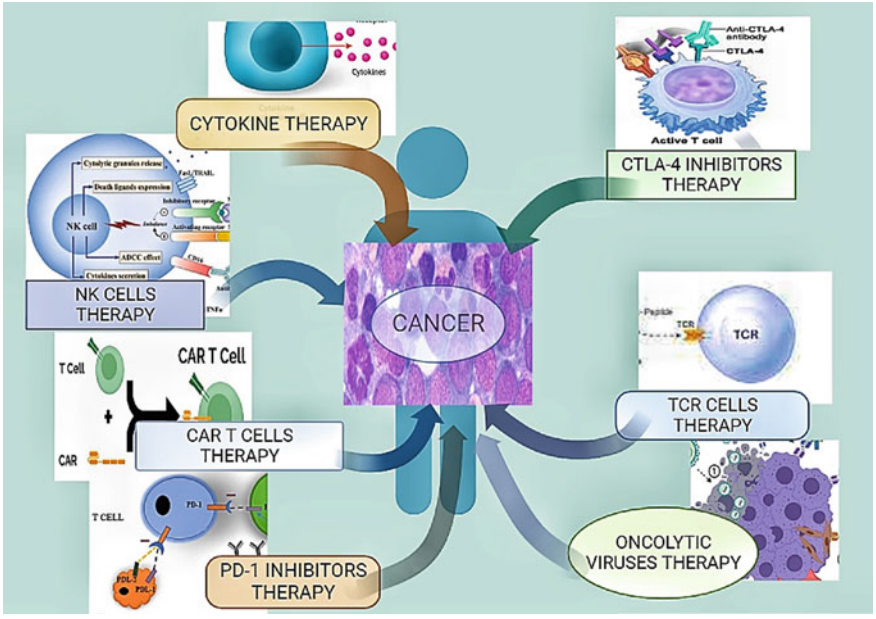

Fig. 5.1 Current approaches of immunotherapy for cancer

5.2 Approaches of Immunotherapy

In recent years, strategies therapeutically targeting the tumor microenvironment (TME) have appealed as a potential approach for the treatment of cancer due to the latest advances in understanding the essential functions of the tumor microenvironment in monitoring tumor progression and amending response to established standardized therapies [14]. Cancer therapies based on immune response are already revolutionizing the management of several types of cancer, conferring hopes to cancer patients about the emerging therapies that it would take off the emotional, financial, and physical burden of personal suffering over the coming decades [15]. Potential anticancer immune response stimulatory therapies channeling already prevalent immune responses by targeting the immune checkpoint pathways have exhibited potential clinical response among multiple types of tumors. Currently, the novel approaches being explored are inhibitors for immune checkpoint, oncolytic viruses, engineered cytokines, CD3-bispecific antibodies, vaccine platforms, and adoptive cell therapy are just a few examples [3, 16].

5.2.1 Immune Checkpoint Inhibitors

The immune system acts two roles in fighting against cancer which are preventing the tumor cell outgrowth as well as shaping the immunogenicity of tumor cells through cell engineering cancer cells are powerful enough to evade the immune system by inhibiting T lymphocyte activation [17]. Cancer cells' genetic instability contributes to the uncontrolled proliferation of cancer cells and expresses antigens which will be identified by the cells of the immune system. These antigens expressed by cancer cells are the normal proteins that are overly expressed in cancer cells and the novel proteins created due to genetic rearrangement or chromosomal aberrations. Cytotoxic $CD8^+$ T immune cells render the anticipated anti-tumor response which is specifically effective by identifying the antigens specific to the tumor presenting to the class i molecules of the major histocompatibility complex (MHC), which will lead to the killing of the targeted tumor cell. Upon required stimulation by the antigen-presenting cells after recognizing the tumor antigens, $CD8^+$ T cells become effector cells which will further initiate an immune response against the tumor through these antigen-presenting cells (APCs). Costimulatory signals are provided through receptors on the cell surface receptors (such as CD28) and cytokine molecules such as interleukin (IL-12) for embedding by antigen-presenting cells the peptides of the antigen on the MHC molecules as well as effective stimulation of T cells [18, 19].

There are two types of immune responses that is provided by the immune system which works as a defense system against foreign organism or to maintain homeostasis and the removal of damaged cells while also providing immunosurveillance through innate or non-specific immunity and adaptive or specific immunity which has several other functions in the body.

In cancers, there has been observed a huge amount of infiltrating innate immune system cells such as macrophages, mast cells, and neutrophils which attributes to enhanced angiogenesis and/or decreased prognosis. Prolonged chronic inflammatory conditions could probably lead to chances for the development of cancer for which the risk could be reduced by anti-inflammatory drugs. Immune balance, the immune status, genetic deletion or diminution of immune cells activation of anti-tumor adaptive immune responses are regulated by the genetic polymorphisms which have a suppressive effect on cancer risk and tumor growth according to human clinical trials and animal model studies [20]. Therefore, in the immunotherapeutic approach, the different components of the immune system of the patient are used to target the cancer cells only, thereby eliminating the adverse side effects coupled with the current conventional treatment options that are currently being used clinically. The cancer cells could be detected by the immune system in either of the two ways of recognizing either the molecules which are only expressed specifically in cancer cells (antigens specific to tumors) or by the molecules that are variedly expressed in cancer cells in comparison to normal cells (antigens associated with tumor) [21]. Therefore, immunotherapy can be considered a potential and promising option for the treatment of cancer because of its specificity and long-term effects which have demonstrated enhanced survival and overall tolerance [22, 23].

According to NIH, immune checkpoint inhibitors can be defined as a type of drug that blocks proteins called checkpoints which are produced by immune cells such as T lymphocytes and cancer cells. On effectively blocking the immune checkpoints, the T lymphocytes can be made to kill the cancer cells potentially which otherwise would have refrained to do so as the checkpoints control the strong immune responses.

Immune checkpoints have been known as key regulators of the responses of the immune system of T cells against the invasion by pathogens or any dysregulation in the expression of the self-antigens by coordinating the balance of inhibitory and costimulatory signals [24, 25]. The action of immune checkpoints is through the costimulatory and inhibitory pathways that control T cell immune response. However, there is a variation in the mode and level of activity of these immune checkpoints at different levels and immune responses based upon their activation [26]. The receptors which serve as immune checkpoints are essential molecules for regulating responses of the immune system. Signaling of these checkpoints depletes activation of T cells to prevent autoimmune response as well as the negative effects of the over-enhanced inflammatory response [27]. T cells become exhausted particularly during chronic inflammation which causes an upregulation of unique diversified inhibitory receptors that control the effectiveness of T cells through T cell immunoglobulin and mucin-domain containing-3, PD-1, CTLA-4, lymphocyte-activation gene 3 (LAG-3), or T cell immunoglobulin and mucin-domain containing-3 (TIM-3) [28].

Inhibitors for the immune checkpoint which target PD-1\PD-L1 or CTLA-4 signaling have demonstrated remarkable clinical responses for many types of cancers and are reforming the pragmatic medicinal approach for cancer treatment [29]. CTLA-4 and PD-1 are the two most clinically relevant immune checkpoints at the moment that have been studied for their potential role in immunotherapy for cancer. Because the costimulatory and co-inhibitory pathways have membrane-bound and

soluble receptor–ligand pairs, they are promising drug targets in immune checkpoint inhibition therapy [30].

This chapter will go over the roles of CTLA-4 and PD-1 as potential immune checkpoints for immunotherapy targeting immune checkpoints for cancer treatment.

5.2.1.1 Immunotherapy Using CTLA-4 Immune Checkpoint Inhibitors

The cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a CD28-related cell surface receptor that binds to the ligands CD80 and CD86 [31] to deliver a negative signal in the activation of T cells by poorly availing the ligands to CD28 [32] and functions in preventing the stimulation signal of T cell proliferation which is rendered by binding of CD28 with the ligands (CD80 and CD86) during the priming phase [33]. CTLA-4 has been primarily found to be localized in the intracellular compartments of the naïve T cells [34] while only a trace of CTLA-4 was found on the cell's surface which summarizes the mechanism of transportation of CTLA-4 to the surface of the cell as very critical in understanding immune response pathway of CCTLA-4. The expression of CTLA-4 in the intracellular compartments is regulated by the binding of clathrin adaptor protein 1 (AP-1) which is a plasma membrane-associated protein to one of its motifs (GVYVKM) [35]. CTLA-4 binds to B7 proteins, which compete with CD28 costimulatory signals, eventually inhibiting the excessive immune response [36]. Drs. James Allison and Tasuku Honjo were awarded the 2018 Nobel Prize in Physiology or Medicine for discovering Programmed Cell Death Protein-1 (PD-1) and Cytotoxic T Lymphocyte-associated Antigen-4 (CTLA-4), to honor their exceptional efforts toward developing the field of immunotherapy for cancer which now has received the clinical application from laboratory preclinical studies [37]. Regulatory T cells extensively express CTLA-4 while regulating the functions of effector T cells which could be attributed to their immune suppressive functions contributing to their important role in balancing peripheral tolerance. The absence of CTLA-4 genetically has demonstrated impairment in immune suppressive functions of T cells in animal models revealing that CTLA-4 is critical in immune suppressive response. According to studies, this immune suppressive effect of regulatory T cells could be the result of B7 ligands being downregulated on APCs, resulting in decreased CD28 co-stimulation, which thereby controls the effector T cells. CTLA-4 through trans endocytosis confiscates CD80 and CD86 ligands from the surfaces of cells that present antigens to reduce the accessibility of these stimulatory ligands to other T cells expressing CD28 which induces a bystander effect of immune suppression on bystander cells through regulatory T cells [38].

CTLA-4 is a well-studied immune checkpoint that plays an important role in the immune response process. CTLA-4 has been identified as an important negative regulator in T cell activation, influencing the functions and costimulatory signals of a cluster of differentiation (CD) 28 binding to B7[39]. As the CTLA-4 has an increased affinity (10- to 20-times) to its ligands B7–1 (CD80) and B7–2 (CD86) when compared to its costimulatory molecule CD28 [40], it is, thus, possible to induce the anti-tumor response by inhibiting the interaction between the ligands on

antigen-presenting cells and CTLA-4, which can suppress the immune system [41, 42]. In an animal model study using mice to understand the major role of CTLA-4 plays in immunological tolerance in which the mice which lacked the CTLA-4 gene centrally or particularly in the Forkhead box P3 (FoxP3) $^{+}$ compartment of regulatory T cells developed lymphoproliferative disorders and died at a young age confirmed the theoretical understanding of CTLA-4 as an immune checkpoint to use an approach for immunotherapy [43]. Therefore, the current studies imply that inhibiting the CTLA-4 checkpoint using anti-CTLA-4 could induce an anti-tumor response which would be supported by the proliferation and activation of effector T cells to render a greater immune response against tumor regardless of specificity. However, the exact mechanism underlying the process is not clear and requires a much deeper investigation for an effective outcome.

Ipilimumab known as a CTLA-4 inhibiting antibody was the initial and foremost inhibitor of the immune checkpoint that was validated and received approval for treating cancer patients. The anti-CTLA-4 antibodies have been approved for human use and have yielded significant results in significantly controlling the disease progression of many cancers and especially melanoma [44]. Ipilimumab is a monoclonal antibody that, based on the findings of a large clinical trial, was approved by the FDA in March 2011 for the treatment of skin cancer (advanced melanoma) including around 676 patients which confirmed that ipilimumab enhanced the overall survival (OS) of patients with melanoma for which other standard therapies were not potent enough to elicit any response in the immune system [45, 46]. The theoretical understanding behind this approach is that Ipilimumab a human monoclonal antibody (mAb) can inhibit the immune suppressive potential of CTLA-4 by allowing CD28 to bind to CD80/CD86 which leads to the activation of T cells by targeting CTLA-4 for blocking of CTLA-4's interaction with CD86 and CD80 on T cells or antigen-presenting cells [47]. Tremelimumab (human IgG2 mAb) is another CTLA-4 inhibitor, which acts similarly the way of ipilimumab, by blocking the interaction between CD28 and CTLA-4, to inhibit the immune suppressive effect of CTLA-4 due to the inactivation of immune cells [48]. Tremelimumab improved the proliferation of effector T cells by nullifying the immune suppressive potential of regulatory T cells, implicating that blocking CTLA-4 using antibodies would be a potential approach of immunotherapy; however, more detailed understanding in the underlying concept is needed to get the exact benefit from this method [49]. In conclusion, most of the clinical and animal model studies have demonstrated the inhibitory action of anti-CTLA-4 antibodies majorly against regulatory T cells in cancers but considerably some studies have implied an observation of no change in the number of regulatory T cells as a result of various parameters of different studies which could lead to a requirement of a whole new understanding of the approach. Therefore, the review of this approach suggests that antibodies blocking the CTLA-4, with or without altering the levels of CTLA-4 in the regulatory T cells, could be a new, revolutionary, and ultimately more potent immunotherapeutic strategy against the cancer cells because incomparable magnificent responses have been reported against various cancers including when treated with anti-CTLA-4, PD-1/L1 antibodies, advanced stage cancers such as lung cancer, melanoma, bladder cancer, and Hodgkin's disease

immunotherapies while benefitting not a major percentage of patients post-treatment suggesting a necessity of further extended studies [50, 51].

5.2.1.2 Immunotherapy Using PD-1\PD-L1 Immune Checkpoint Inhibitors

Programmed death 1 (PD-1) a receptor is a protein found on cells of the immune system in the body (especially T cells) that regulates the immune response of the body. The binding of PD-1 to PD-L1 another protein controls the immune reaction of T cells against cancer cells, and other types of cells according to the definition of NIH, National Institute of Cancer. Therefore, blocking PD-1 or its interaction with PD-L1 negatively regulates its control over T cells and the immune system which enhances the ability of T cells to kill cancer cells which is the anticipated immune response through this approach. The ligands of the PD-1 receptor which include PD-L1, PD-L2 along with members of B7 and CD25 families have a very important function in co-inhibiting add exhausting T cells [52]. The interaction PD-1/PD-L1 prevents the proliferation of T lymphocytes, their survival and other effective cytotoxic functions, initiates apoptosis of T cells specific to the tumor, stimulates the differentiation of T cells with $CD4^+$ into $Foxp3^+$ regulatory T cells, along with defiance of tumor cells to cytotoxic T lymphocyte attack [53]. Peripheral and central tolerance is regulated by the inhibitory signals of the PD-1/PD-L1 pathway. PD-L1 is expressed in the thymus gland which is involved in both positive as well as negative responses of the gland [54]. Another function of T cells such as reactivating and expanding is also controlled through the PD-1 pathway [55]. PD-1\PD-L1 pathway also has the role of regulating the formation of memory B cells by interacting with $PD\text{-}1^+$ follicular T helper cells in germinal centers, in the B lymphocyte cells expressing PD-L1 and PD-L1. Studies also have shown that the absence of PD-1 signaling remarkably reduces its potential to generate long-lived plasma cells [56]. The two potential ligands of PD-1 receptor PD-L1 and PD-L2 are crucial in activating T cells, promoting T cell proliferation, and enhancing the secretion of cytotoxins against cancer to the downregulated anti-tumor immune response. According to the reports, PD-L1 is found to be expressed in the hematopoietic cells and some parenchymal cells, whereas expression of PD-L2 is limited to dendritic cells and macrophages. To account for the expression of PD-L1 ligand in tumors, studies have described its extensive expression in various types such as melanoma, gynecological cancers, lung cancers, kidney tumors, bladder cancers, hematopoietic malignancies as well as in pancreatic and esophagus adenocarcinoma [53]. PD-1 is an immune checkpoint which initiate immune response of T cells in normal cells, contrarily in tumor cells, it elicits negative immune response and prevaricate the immune surveillance [57]. Therefore, the immune checkpoint negative regulation can be controlled using external therapeutic agents which can be a potential strategy in treating cancer [58–60].

The mechanism of action of PD-1 inhibitors varies depending on the treatment. Peptides/polysaccharides and small molecules target, nonpeptidic PD-1/PD-L1 small-molecule inhibitors, aptamer-drug conjugates (APDCs), and antibody-based PD-1/PD-L1 inhibitors are some of the inhibitors' mechanisms of action, as well as in combination with other inhibitory molecules [61]. Currently, PD-1/PD-L1 immune checkpoint inhibition therapy has been considered the prevailing therapy for treating many malignant cancers, including melanoma, both types of hepatocellular carcinoma, renal cell carcinoma (RCC), head and neck squamous cell carcinoma (HNSCC), classical Hodgkin lymphoma (cHL), and colorectal cancer (CRC), bladder cancer, Merkel cell carcinoma (MCC), as well as adult and pediatric solid tumors, and therefore, it is thoroughly studied through clinical trials for other malignant carcinomas [62]. In 2002, the role of the PD-1 signaling pathway responsible for rendering tumor immunity was observed for the first time, by implicating that the overexpression of the ligand of PD-1 (PD-L1) would damage the cytolytic activity of T cells and remarkably upregulate the progression of tumorigenesis and tumor invasiveness [63–65]. Potential effects of anti-PD-1 monotherapy have been seen in cancers caused by carcinogens or furthered to malignant cancers due to viral infections and desmoplastic melanoma, eventually elevating response rates of 50 to 80%.

Pembrolizumab has been approved in 2014 by FDA for treating advanced melanoma as a PD-1 signal inhibitor [66]. Other FDA-approved PD-1 inhibitors have also been commercially used for immunotherapy. They are, Nivolumab, which works by binding to the PD-1 receptor and inhibiting its interaction with both ligands PD-L1 and PD-L2, conferring the release of negative control of PD-1 pathway-mediated immune response to the tumor cells. Nivolumab was approved by later that year; the FDA approved it as a reliable treatment for unresectable or metastatic melanoma [67]. The combination of ipilimumab (CTLA-4 inhibitor) with nivolumab was primarily approved as an effective immediate treatment for specific mutations in melanoma in 2015 based on the results of a study [68]. The same combination therapy was later approved for unresectable or metastatic melanoma, regardless of mutation, the following year [69]. Nivolumab has also been approved for the treatment of metastatic squamous non-small cell lung cancer [70], metastatic renal cancer [71], classical Hodgkin lymphoma (cHL) [72], recurrent or metastatic head and neck squamous cell carcinoma [73], and locally advanced or metastatic urothelial carcinoma [74], colorectal cancer [75] as well as for hepatocellular carcinoma [76]. Another PD-1 inhibitory drug is Pembrolizumab which is a mode of action similar to that of Nivolumab and has been approved by FDA, while Avelumab has been approved for metastatic Merkel cell carcinoma (MCC) and locally advanced or metastatic urothelial carcinoma treatment, whereas Durvalumab has received approval for treating various cancers including lung cancers and urothelial cancers which has effects on PD-L1 mRNA [62, 77] Cemiplimab works by binding to PD-1 and inhibits its interaction with PD-L1 and PD-L2 by acting as a human programmed death receptor-1 monoclonal antibody [78]. Successful immune responses against tumors through the approach of inhibiting PD-1/PD-L1 needs activation and proliferation

of T cells specific to tumors found in the tumor microenvironment (TME). Therefore, the effectivity and the variations in the outcome of cancer immunotherapy have been accepted to be moderately accredited to the heterogeneity of the TME [79]. This encourages more researchers to come up with effective PD-1\PD-L1 inhibitors for immunotherapy targeting cancers. To sum up, the rising acknowledgment of the potential influence of effector cells of innate immunity to render the anti-tumor immune response and amalgamating a plethora of ways to target the adaptive immune system into PD-1/PD-L1 inhibition-based therapies reveals the importance of anti-PD 1 inhibition approach to the future immunotherapy [80].

5.2.1.3 Combining CTLA4 and PD1 Inhibitors for Immune Checkpoint Blockade

CTLA-4 and PD-1 immune are well known now as the most reliable targets among other immune checkpoints, and therefore, compounds targeting CTLA-4 and PD-1 have led to drastic development in designing treatment for advanced cancers. The primary benefits of CTLA-4 and PD-1 inhibitors are that they show remarkable durable response rates, significantly increase the survival time of responding patients and give an adaptable secure profile [81]. According to the review's recommendations, combining CTLA-4 and PD-1 inhibitory molecules would have a synergistic effect on stimulating an anti-tumor immune response, eventually leading to increased response rates in cancer patients [82]. Theoretically, a combination of inhibitors of both CTLA-4 and PD-1 or PD-L1 has the potency to induce active high number proliferation of T cells in an immune response while restoring exhausted and reduced immune responses of T cells which were targeted by regulatory T cells mediated immunosuppression. Preclinical studies and initial clinical trials demonstrated elevated anti-tumor responses using combined inhibition in comparison with immune checkpoint monotherapy. This synergistic approach effectively assesses the multiple roles played by the inhibitory molecules in regulating the response of the immune system. However, with combination therapy, disease progression was reduced and overall survival was enhanced when ipilimumab was administered after giving nivolumab to address the issues of adverse effects of the suggested combined immunotherapy [83–87]. Additional investigations could be desired to lessen the appearance and intensity of the antagonistic adversarial side effects while retaining the efficiency of the combination. Therefore, more clinical trials and investigations must be carried out to ineffectively design a combination of inhibitors of both CTLA-4 and PD-1 for an enhanced benefit of the therapies rather than giving monotherapies. Table 5.1 summarizes the CTLA-4 and PD-1 PD-L1 immune checkpoint inhibitors that have been approved by the FDA.

Table 5.1 FDA-approved immune checkpoint inhibitors

S. No.	Immune checkpoint inhibitor	Targeted immune checkpoint	Targeted type of cancer	Source of references
1	Ipilimumab	CTLA-4	Melanoma	[45, 46]
2	Nivolumab	PD-1	Metastatic squamous NSCLC, metastatic RC, cHL, urothelial carcinoma, HNSCC, CRC, HCC	[70–76]
3	Pembrolizumab	PD-1	NSCLC, HNSCC, Hodgkin lymphoma, gastric cancer, cervical cancer, PMBCL, HCC	[62, 77]
4	Cemiplimab	PD-1	Metastatic cutaneous squamous cell carcinoma	[78]
5	Avelumab	PD-L1	Metastatic MCC and locally advanced or metastatic urothelial carcinoma	[62, 77]
6	Druvelumab	PD-L1	NSCLC and advanced UC well as JQ1	[77]
7	Atezolizumab	PD-L1	Stage III-B or IV nonsquamous and squamous NSCLC	[62]

5.2.2 Adoptive Cell Therapy (ACT)

Adoptive transfer of naturally occurring or genetically engineered T cells with the ability to mediate tumor recession in patients with metastatic cancer by infusing back the genetically engineered cells into a patient following ex vivo expansion can attack the tumor and arbitrate the eradication of T cells target to regions where their targeted antigens are expressed and can secrete cytotoxic molecules that alter tumor growth. Considering the efficacy of ACT for cancer, naive T cells have been presented to be more potent than memory T cells in various model organisms such as mice, non-human primates as well as humanized mouse models. ACT has evolved as a promising advancement in cancer immunotherapy following lymphodepletion. The use of adoptive T cell-based immunotherapies to eliminate cancer is an approach that is a mere combination of basic immunology, and clinical implication proving its meaningfulness as a therapy, however, has its particular serious limitations as it has not been officially approved by FDA and requires a high budget [88]. Though many studies with adoptive immunotherapy for solid tumors have been done against skin cancers, there is advancement in understanding ACT's role in the treatment of

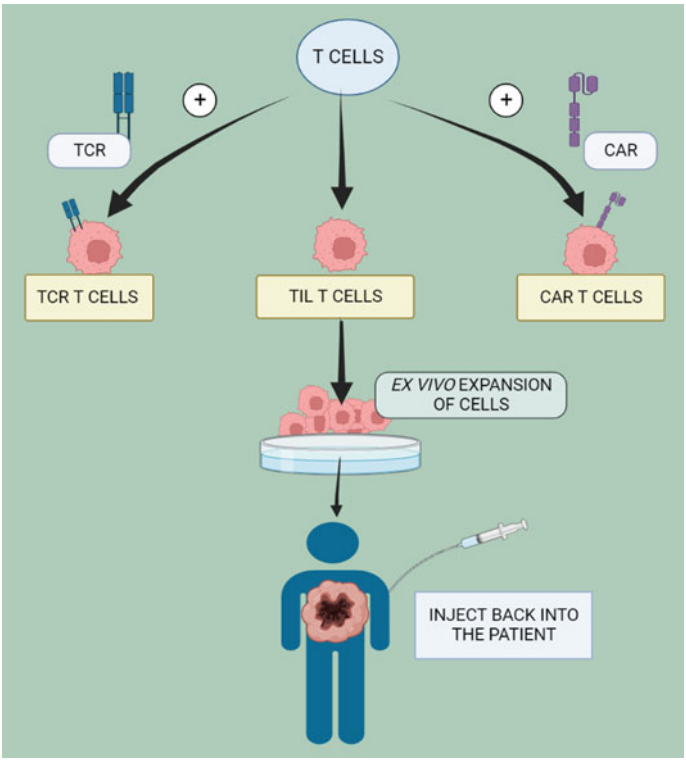

Fig. 5.2 Adoptive T cell transfer approach for immunotherapy using TILSs, CAR T cells, and TCR T cells

other types of tumors. A remarkable new 121 clinical trials, including those for non-solid tumors, were described in a recent summary by Fournier et al. of initial trials conducted using ACT since May 2015 [89].

Adoptive T cell transfer immunotherapy can be categorized into three classes based on its mechanism as ACT with tumor-infiltrating lymphocytes (TIL), ACT with TCR gene therapy, and ACT with T cells modified with chimeric antigen receptor (CAR) (Fig. 5.2) [90].

5.2.2.1 ACT with Tumor-Infiltrating Lymphocytes (TIL)

According to the National Cancer Institute, tumor-infiltrating lymphocytes are a type of immune cell that has moved from the blood into a tumor and can recognize and kill cancer cells. Rosenberg and colleagues at the National Institutes of Health's Surgery Branch (SB, NIH, Bethesda, Maryland, USA) studied and demonstrated preliminary studies using TILs, where TILs were cultured from various murine tumors that expressed anti-tumor activity in vivo [91]. TILs were first shown to be clinically

beneficial in 1994 when T cells were isolated from tumors and expanded ex vivo to target the MHC peptide complex [92]. The present approach of TIL therapy includes the ex vivo expansion of TIL from the recovered part of the tumor of a patient and transferring back into the patient following a depletion of lymphocytes preparative regimen with additional backing up using interleukin-2 (IL-2) (a cytokine) which can achieve a magnificent anticipated tumor response of at least 50% in patients with metastatic melanoma which has been already investigated in several phases I/II clinical trials which has been furthered to other types of solid tumors. The TILs have been produced from non-melanoma tumor types such as cervical cancer, RCC, breast and lung cancer (especially NSCLC) with erratic rates of tumor reactivity which have been studied currently [93]. The mechanism of producing TILs is selecting the T cells dissociated from immunosuppressive cell populations (from a tumor) and exposing them to suppress the levels of immunosuppressive cytokines in the early stages of culturing for ex vivo expansion of TIL cells to increase cell numbers after infusion of those cells back into the patient to elicit death and absolute destruction of the tumor, resulting in prolonged total diminution as well as possibly the cure of the profound cancers [94]. One strategic approach for the treatment of pancreatic cancer includes the use of TIL expanded from the tumors of the pancreas which are functional and have the ability to respond to pancreatic tumor-associated antigens which supports the approach of developing adaptive cell transfer therapy using the tumor-infiltrating lymphocytes [95]. Tumor-infiltrating lymphocytes (TIL) play a crucial function in facilitating response to chemotherapy and advancing clinical outcomes which may have an increased PD-L1 expression, especially in breast cancer including all its subtypes [96]. According to preliminary studies on antigen cloning, self-antigens that were not mutated were proposed to be potent candidates for targeting with tumor-infiltrating lymphocytes based immunotherapy [97]. TILs were frequently the source of antigens cloned from T cells derived from patients of melanoma [98–101].

5.2.2.2 ACT Using T Cell Receptor (TCR) Gene Therapy

T cell receptors (TCRs) are expressed by all types of T cells to recognize antigens. TCR recognizes and binds to specific antigens (proteins) found on abnormal, cancer, foreign, and infected cells. TCR a heterodimer consists majorly of an α and β chain which possess three major regions: (1) a variable region for antigen binding, (2) a constant extracellular region, and (3) a transmembrane domain [102]. Each of the variable domains in TCR chains comprises basically of a germline-encoded sequence recombined variable (V) and diversity (D) alleles, as well as a series of three non-consecutive hypervariable loops known as complementarity determining regions (CDRs), which are found in the chain, which also contains a junctional (J) allele [103, 104]. TCRs render recognition to epitopes derived from proteins localized within any compartment of the cell [105]. This makes the TCRs identify broad-spectrum neoantigens, cancer germline antigens, and viral oncoproteins which are examples of targets. This interaction triggers the T cells to attack the cells which express the antigens identified by TCR and empower the body to defend against

infection, cancer, or other diseases. Genetically engineering T cells and using this approach in immunotherapy have shown remarkable effects in controlling as well as curing cancers in patients in some cases which in recent years has gained a lot of attention due to superior benefits over CAR T cell therapies [106, 107]. Genetically engineered TCR which can identify tumor-associated antigens cloned into the PBL of HLA-appropriate patients followed by the expansion of these cells is one strategy to use in the adoptive cell transfer technique [108]. TCRs are expressed by the $CD8^+$ T cells, in which the ligand is most commonly a linear peptide sequence of 8-11 amino acids presented in complex with the MHC class I molecules complements of the patient which is a well coordinated process that occurs in most of the nucleate cells as well as cancer cells [109, 110]. The potency of TCR gene therapy was demonstrated for the first time to validate the feasibility and clinical applicability by targeting the melanoma differentiation antigen (MART-1) [111]. A successive investigation tumor regression was reported in 30% of the total patients with higher-affinity TCR. In addition, another study using a manipulated high-avidity TCR recognizing NY-ESO-1 antigen revealed a potential clinical response in 60% of synovial cell sarcoma patients and 45% of all skin cancer patients [112–114]. The most influential understanding is with TCR-based immunotherapies for solid malignancies currently with the adoptive transfer of TIL, in a review, currently, TCR-based therapies have been focused on tissue differentiation antigens, in which trials are going but have shown remarkable on-target/off-tumor toxicities. The cancer germline antigens (CGAs) are also becoming TCR therapeutic targets while first-phase human clinical trials are going on for viral-derived oncoprotein HLA-A2 restricted TCR therapy. There has been a new paradigm of targeting cancer neoantigens using targeted TCR therapy which has also shown promising results. The findings from these investigations and understanding implicate that when compared to linked signaling conformations accompanied by CARs, the natural, noncovalently linked signaling complex of a TCR is inherently very effective [115].

Nevertheless, the significance of resistance to TCR-based immunotherapies is progressively being acknowledged recently. Currently, the TCR-based immunotherapeutic approach is being conjugated with other current immunotherapies, such as combining with immune checkpoint inhibitors and cytokines that stimulate an immune response to overcome various paths coupled with TCR resistance. Therefore, strategies to effectively surpass the obstacle of resistance to TCR-based immunotherapies concerning the specific category of resistance are needed and will come up in the near future according to the review [115].

5.2.2.3 ACT Using CAR T Cell

Chimeric antigen receptor (CAR) T cell therapy is another approach of immunotherapy that has been considered a radical approach recently as it has shown surprisingly potent and long-lasting clinical responses [116]. Through this approach, engineered synthetic receptors CARs which act to transmit lymphocytes, to predominantly T cells, recognize and eliminate cells expressing a specific target antigen,

primarily on cancer cells CAR binds to target antigens expressed on the cell surface independently of the MHC receptor, resulting in T cell activation and intense anti-tumor responses. [117] These CARs are actually hybrid receptors that have been genetically engineered to include an antigen-binding extracellular domain, a TCR-signaling intracellular CD3 chain domain, and an additional domain for co-signaling, in order to confer co-stimulation. [118] Using gene transfer, recombinant technology T cells are engineered to facilitate the capacity of especially recognizing their target antigen through their scFv antigen-binding domain, consequentially yielding T cell activation in a totally MHC-independent manner unlike TCR gene therapy [119]. The most common method to transfer the engineered CAR T cells by retroviral infection [120]. The recombinant retrovirus will transduce T cells imbued with CARs that have antibody-like specificity, allowing the CARs to recognize MHC nonrestricted structures on the surfaces of target cells and destroy them (cancer cells specifically) [121, 122].

The first clinical success of CAR T cell therapy was approved by the Food and Drug Administration (FDA) in the USA in 2017, following the unexpected success of CD19 CAR T cell therapy against B cell malignancies [123, 124]. Second-generation CAR T cells have shown intensive clinical therapeutic responses for various hematological malignant cancers, such as B cell acute lymphoblastic leukemia, chronic lymphocytic leukemia, diffuse large B cell lymphoma, and multiple myeloma, and the efficacy of those second-generation CAR T cells is currently being studied for solid tumors such as liver metastases, glioblastoma, advanced sarcoma, mesothelioma, ovarian, and pancreatic cancers [125]. The MHC-independent recognition of its specific antigens by CAR genetically engineered T cells was first demonstrated in the first-generation CAR T cells back then in the late 1980s by the Kuwana and Eshhar groups. The manipulation of T cells with CARs has been advanced over a period of time with four generations of CAR-engineered molecules. First-generation CAR mediated the production of IL-2 through an scFv antigen-binding domain and intracellular CD3ζ in murine models; activation causes domain and non-MHC-restricted cell lysis. Costimulatory domains are present in second- and third-generation CARs additionally to elevate T cell activation, survival, and expansion, while the fourth-generation CAR T cells additionally proliferative cytokines like IL-12 or costimulatory ligands like 4-1BBL according to a couple of clinical studies [126–132]. There are some significant limitations to CAR T cell therapy, such as tumor resistance to one antigen targeting CAR construct, on-target and off-target effects, CAR T cell trafficking and tumor infiltration, and CAR T cell-associated toxicities. However, CAR T cells have shifted the paradigm of the current and future treatment scenario of a few particular hematological malignancies as they represent one of the potent examples of genetic engineering of T cells in confronting the human race with splendid ideas and hopefully marching toward developing a major cancer therapy for B cell malignancies in the following couple of years [133, 134].

Overall, to sum up, adoptive cell transfer therapy using genetically engineered T cells with chimeric antigen receptor or T cell receptor or using tumor-infiltrating lymphocytes has revolutionized the concept of immunotherapy in the past decade showing rays of hope to cancer patients, especially during the advanced stage. T cell

adoptive immunotherapy has demonstrated the keen potential to be considered as one of the most suited effective treatment cancer patients unlike the current strategies of the therapies. The future challenges associated with T cell adoptive therapy are the prediction and minimization of adverse events, and the identification of new possible targets for T cell therapy, while also utilizing allogeneic T cells as well as considering its effective role in clinical administration. In contrast, there are significant issues that must be addressed, such as the prediction and management of potential adverse events associated with efficiency, the search for new antigen targets, including neoantigens, and the use of allogeneic lymphocytes. To summarize, T cell adoptive immunotherapy will ensure an effective and safe clinical treatment for patients with various malignancies in the near future by addressing these issues of concern. Only deeper investigations, clinical trials, and a greater understanding of these approaches to cure cancer [107].

5.2.3 NK Cell Therapy

Natural killer (NK) cell is a special type of immune cell that plays an important role in immune activation against abnormal cells; it can induce targeted cell death by producing cytotoxic granules which contain granzymes and perforin as well as pathways mediated by death receptors such as FasL/Fas. NK cells can be recognized as they lack the CD3 while expressing CD56 on their surface in humans. NK cells are found in blood at levels ranging from 5 to 15% of circulating lymphocytes, as well as lymphoid and non-lymphoid organs such as the spleen, lung, and liver. NK cells secrete cytotoxic granules containing perforin and enzymes similar to granzymes if stimulated which direct the lysis of tumor cells similar to the action of cytotoxic T cells [135, 136]. These cells confer the preliminary defense against the development of cancer. Their major histocompatibility complex (MHC)-antigen stimulating independent cytotoxicity, better safety, and high feasibility for "off-the-shelf" manufacturing add an advantage to it over other approaches of immunotherapy. Their presence in higher levels lowers the cancer incidence, demonstrating NK cells' potent immunosurveillance ability making them crucial for using natural killer cell therapy as immunotherapy for treating cancers [137, 138].

According to the review, the development of preclinical natural killer cell-based immunotherapy for cancer was the result of investigations by The Ruggeri Group and Miller et. Al. on the understanding of the activation of NK cells which pioneered the clinical setting of hematopoietic stem cell transplants (HSCTs) in which NK cells showed the capacity to exert a graft versus leukemia effect. Further investigations revealed that the transfer of NK cells to host organisms from twins (HLA-haploidentical donors) in combination with a cytokine IL-2 administered following a pre-conditioning treatment with high-dose cyclophosphamide and fludarabine resulted in the successful in vivo expansion of donor NK cells with the induction of complete cancer eradication in approximately 26% of patients with poor progression of acute myeloid leukemia (AML), paving the way for the first successes of

adoptive NK cell transfer for treating hematological cancers. [139–143] NK cells have shown in vitro to be capable of killing freshly isolated human tumor cells from AML, ALL, multiple myeloma, neuroblastoma, ovarian, colon, renal cell, and gastric carcinomas [144, 145]. Autologous TILs and tumor-specific cytotoxic T cells infusion following lymphoid-depleting therapy resulted in lymphocyte-activated killer cells while in three of the clinical studies using ex vivo autologous NK cells activated by IL-2 infusions were given to patients with a variety of cancers, including non-lymphoma Hodgkin's and renal cell carcinoma, but no effective results were seen at the end [146]. One of the "off-shelf" NK cell therapeutic products oNKord,, has been designated as an orphan drug by FDA for treating acute myeloid leukemia patients. These successful milestones have stimulated a lot of ongoing investigations of NK cell-based cancer monotherapy or combining along with other disciplines of immunotherapeutic approaches for various cancers such as hematological malignancies, and solid tumors [139, 147]. Interestingly, the effective anti-leukemic responses driven through allogeneic donor-derived NK cells were not associated with graft versus host disease (GVHD) suggesting that haploidentical mature NK cells individually without transplanting stem cells stand a chance to present as an assuring tool to observe the anticipated anti-tumor responses [148]. Clinical trials involve NK cell therapy alone or in combination with other approaches such as autologous NK cell adoption [149] in which NK cells can be generated, expanded in vivo, and enhanced function for a better response. Another approach using cytokines to enhance the activity of NK cells based on IL-2 or IL-15 has also shown potential results in clinical trials [150, 151]. Combining with potential immune checkpoint inhibitors IPH2101 enhances the activity of NK cell therapy, or the use of chimeric antigen receptor engineered NK cells, which has been developed and showed promising result for future investigations to develop a most suited approach of immunotherapy using NK cells [152, 153].

With more than 100 ongoing clinical trials for NK cell therapy, targeting various solid tumors and malignancies, NK cell therapy suggests being a potent immunotherapy to cure cancer. Considering the present, the triumph of the NK cell expansion approach remains impulsive, especially for solid tumors. However, new technologies of iPSC-NK and genetic engineering approaches, as well as current NK cell development understanding subject is required to overcome the difficulties of large-scale NK cell acquirement. Nevertheless, future clinical trials will lead in the right direction of NK cell immunotherapy to result in a highly efficient anti-tumor response using NK cells by overcoming the limitations [138, 146, 153].

5.2.4 Immunotherapy Using Oncolytic Viruses

Oncolytic viruses are viral organisms that can infect and cause lysis of the tumor cells other than stimulating the response of the immune system to eradicate the disease as they can either present tropism to cancerous cells naturally or be genetically engineered to recognize specific targets or antigens [154]. The oncolytic viruses have

shown a positive response in the treatment of a few types of cancers, attributed to tumor amelioration. Viruses have already been shown to infect normal cells, which can then be used to infect cancer cells, presenting tumor-associated antigens, creating a less immune-tolerant TME, and using vehicles to express the inflammatory and immunomodulatory cytokines for an elevated immune response [155]. Consequently, the employment of oncolytic viruses clinically emerges as a substitute to modify the TME from an immunosuppressed state due to the evasion leading the tumor progression, to an inflamed condition, so that the immune system will be able to kill the cancer cells [156]. The general mechanism by which oncolytic viruses can be used as therapy has been reviewed by Santos et al. The oncolytic viruses have the ability to infect the abnormal cells (cancer cells) through the specific targets which are produced by the cancer cells such as a prostate-specific antigen, cyclooxygenase-2, and osteocalcin. Further, the pathogenic genes can be deleted and engineered to increase selectiveness toward the tumor cells as well reduce their pathogenicity toward normal cells. This genetically engineered product can be administered intraperitoneal, intrathecal, subcutaneous, intratumoral, or intravenous depending on the type of tumor. The viral infection of the tumor cells will elicit an immune response and thus show the anticipated anti-tumor effect of the oncolytic virus therapy [157]. Further studies using a combination of oncolytic viruses with immune checkpoint inhibitors or stimulating cytotoxic lymphocytes or other strategies will improve the efficiency of oncolytic virus therapy. The oncolytic viruses that can be considered for oncolytic virus therapy are adenoviruses, protoparvoviruses, vaccinia viruses (VACVs), respiratory enteric orphan virus (Reovirus), and the herpes simplex virus-1 (HSV-1) (Fig. 5.3).

Adenoviruses have shown chemical and thermal stability even outside the cell, has many mechanisms to infect a host cell and enter as well as a good depth of knowledge of their biology, attribute to their contribution to the development of immune therapy using them [158]. The most commonly used adenovirus is the fifth type of adenovirus for oncolytic viral therapy, as the TLRs in the cellular membrane or inside the cell can detect them, stimulating various mechanisms to produce a Th1 profile inflammatory response [159]. Protoparvoviruses, which have the ability to infect mammalian cells, including human cells can stimulate the secretion of protease enzyme from the lysosome to the cytoplasm, resulting in cellular necrosis of tumor cells, as well as trigger an anti-tumor inflammatory response to liberate cytokines with a Th1 profile such as IL-2 and TNF-alpha [160–162]. The vaccinia viruses (VACVs) are enveloped viruses that have been administered in the tumor microenvironment which have positively influenced the apoptotic pathways and the induction of an anti-tumor response while inhibiting tumor metastasis formation [163]. The respiratory enteric orphan virus (Reovirus), due to its inability to cause any known human disease, is being used for oncolytic virus therapy [164], as the Ras pathway can be activated because the virus is dependent on mutations of Ras, implying that reoviruses can be used to deliver viral oncolytic therapy to up to 80% of cancer patients, thereby contributing to its essential role in oncolytic virus therapy for treating various cancers [165]. The herpes simplex virus-1 (HSV-1) is another virus, with double-stranded stable DNA and a pathogen to humans that can produce infection of the mucosa or skin CNS which can also be used for oncolytic virus therapy with genetic manipulation of transgenes to

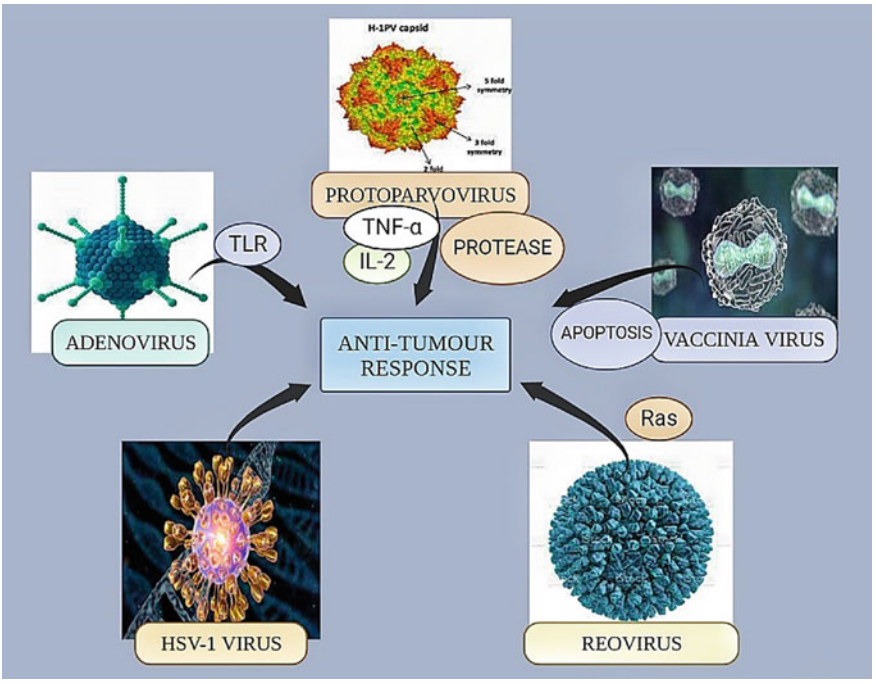

Fig. 5.3 Immunotherapy using various oncolytic viruses for initiating anti-tumor response against cancer

harness its pathogenicity [166]. The first idea here is to induce direct killing of tumor cells in which viruses can enter the TME, typically by injecting locally, and then replicate, resulting in lysis of the infected tumor cell, the release of tumor antigens, and induction of the local immune response [167]. Clinically, the oncolytic viruses have been used in oncolytic virus therapy for pancreatic cancer using reovirus [168], melanoma using prototypical poxvirus [169], breast cancer using multiple oncolytic viruses [170], hepatocellular carcinoma [171], glioblastoma [172], prostate cancer [173], and colorectal cancer [174]. The strategy of using oncolytic viruses appears aimed to clinically improve the conditions of cancer patients through the stimulation of the host immune system as a way of overcoming tumor immune evasion. With many ongoing oncolytic viral therapy, choosing the optimal oncolytic virus, selecting the effective delivery method, expected immune response, and methods to overcome physical barriers while concerning the biosafety, oncolytic virus therapy can be potentially considered as an emerging immunotherapy approach to target cancer cells while combining with other immunotherapies, immune checkpoint inhibitors or other treatment like chemotherapy or radiation therapy and effectively be used to cure cancer in patients which require further investigations and a deeper understanding of the virology.

5.2.5 *Immunotherapy Using Cancer Vaccines*

With all the recent advances in different approaches of immunotherapy including using engineered CAR T cell therapy or checkpoint inhibitors or oncolytic virus therapy, some of which have received FDA approval, unlike the most recent advances, cancer vaccines. The main purpose of vaccines is to deliver the in vivo acquired response of the immune system in defense to a specific antigen or a group of antigens which indicates giving particular functions to the antigen-presenting cells to cause triggering the responses of T helper cell to initiate the secretion of antibodies along with inducing effector T cells [175].

Cancer vaccines are an appropriate replacement for the current approaches of immunotherapy; by providing both prophylactic and therapeutic properties, cancer vaccines have the potential to recognize tumor-associated or tumor-specific antigens (TAAs or TSAs), allowing them to specifically raid and eradicate malignant cells that overexpress these antigens, resulting in a remarkable chronic therapeutic response due to memory associated with the immune response [176]. One approach of immunotherapy to guarantee a sufficient level and function of immune cells like effector T cells for efficient treatment of cancer is through therapeutic cancer vaccination [177]. The strategic approach is of vaccines to target T cells specific to tumor circulating which can be used to eradicate the tumors. Vaccine therapy has superior potential which can reduce the tumor volume as well as strongly lower the immunosuppressive effect [178]. Many types of vaccines for cancer treatment have been developed that vary depending on the targeted antigen used in the making of the vaccine. The four major types of vaccines for cancer include vaccines based on tumor or immune cells, vaccines based on peptides, vaccines based on viral delivery systems, and vaccines based on nucleic acids like mRNA [179]. Cancer vaccines based on tumor or immune cells target the tumor antigens through irradiated autologous tumor cells or allogeneic tumor cell lines, autologous tumor lysates, whole tumor-derived mRNA, or allogeneic tumor cell lines approaches [180]. The tumor antigens to target for cancer vaccines could be either differentiation antigens, cancer-testis antigens, or virus-derived antigens, and the most recently considered tumor antigen for cancer vaccines-mutanome-derived epitopes [181, 182]. The concept of using TAAs which can elicit specific cytotoxic T cell stimulus to cancer cells is the idea behind using vaccines in immunotherapy which can cause the elimination of tumors without having any adverse effects on the normal cells [183]. In recent times, cancer vaccines using nanoparticles have increased an optimally designed delivery system that can achieve cellular uptake and increased antigen interaction with immune cells [184]. Cancer antigens targeted through nanoparticle-based cancer vaccines can have approaches of virus-like nanoparticles, caged protein nanoparticles, or using adjuvants using a diverse set of delivery systems [185]. Nucleic acid (DNA- or RNA-) based vaccine is one of the upcoming promising vaccine strategies because it allows successive transport of several antigens involving a number of TAAs or somatic tumor mutations inducing both the adoptive types of immune

response which functions to enhance the possibilities of overcoming vaccine resistance, encoding the complete sequence of tumor antigens while allowing APCs to present or cross-present multiple epitopes with both classes I and II HLA, which are less restricted by human HLA types in eliciting a diverse T cell response [186]. Messenger RNA (mRNA) vaccine is one of the currently emerging revolutionary approaches as an effective alternative to DNA vaccine for the prevention of infectious diseases and anticancer treatments using suitable delivery vehicles like lipid nanoparticles (LNPs), polymers, and peptides. To date, only two FDA-approved mRNA-based vaccines are limited to prevent virus-induced malignancies [187]. Initially, cell-based vaccines were one of the preliminary types of vaccines to therapeutically treat cancer validated [188]. Virus vector vaccine monotherapy embodies a promising platform for vaccines because the viral genetic material might activate dendritic cells by stimulating pattern recognition receptors [189]. Another strategic approach of cancer vaccine utilizes the concept of manipulating tumor cells by intratumorally administering the oncolytic viruses [190]. Neoantigens are aberrant peptides that result due to genetic and epigenetic aberrations such as deletions or inversions or any other type of modification of the cancer cells and are another considerable candidate for cancer vaccines. Many clinical studies of early stage cancer patients with solid tumors have demonstrated that personalized neoantigen vaccines are acceptable, and convenient and will also be able to alter neoantigen-specific responses of T cells [191].

The feasibility, safety, and immunotherapeutic activity of targeting individual tumor mutation signatures have been demonstrated in first-in-human clinical trials of personalized cancer vaccines [192]. Cancer vaccines though are showing promising results for the future of cancer treatment has limitations, and to overcome those limitations, adjuvants have been used to improve their specificity and efficiency while reducing their toxicity. These adjuvants are compounds used in conjugation with a specific antigen that elicits more intense immunity than the response of individual antigens and therefore beneficially improve the effect of cancer vaccines [193]. Immunostimulatory adjuvants like TLR4 ligands, Saponins, STING (STimulator of INterferon Genes), Immunostimulatory cytokines like IL-2, IFN-γ, IL-12 and granulocyte-macrophage colony-stimulating factor (GM-CSF), heat shock proteins (HSPs), aluminum salts, virosomes, and virus-like particles [175]. Clinical trials of the recombinant vaccines for NSCLC with GSK biological's recombinant MAGE-A3 vaccine [194], TG4010, another Muc-1-targeting vaccine evaluated in NSCLC [195]. Prophylactic, therapeutic, and personalized cancer vaccines have significant promise as the advanced generations of therapeutic vaccines for cancer, using a combination of multiple approaches that can focus on diversified classes of the immune response [196]. The previous milestones in using cancer vaccines for treatment along with the current advances in the understanding of the basic immune biology of cancer have led to a pathway for the future of vaccine development. This suggests that cancer vaccines could be one of the most promising approaches of immunotherapy for curing as well as preventing cancer with multiple approaches to overcome all the barriers and limitations prone to other approaches of immunotherapy.

5.2.6 Cytokine Therapies

Immune cells including lymphocytes and macrophages contributing in eliciting the defense system of the body secrete proteins called cytokines. Immunotherapy based on cytokines is evolving as a potential strategy for the treatment of cancer because these cytokines are proteins that naturally originate in the host immune system and can regulate the immune response of the host cell against cancer as well as can cause the death of the tumor cell [197]. Cytokines are important immune response modulators and could be used to treat a variety of cancers. Engineering cytokines with improved therapeutic properties has emerged as a promising immunotherapy strategy for cancer cells [198]. Cytokines are membrane-bound proteins secreted by innate and adaptive immune cells in response to microbes and tumor antigens. Animal tumor model studies have shown that cytokines have anti-tumor potential because they can directly stimulate immune effector cells and stromal cells at the tumor site and improve tumor cell recognition by cytotoxic effector cells, leading to the idea of using cytokine-based immunotherapy for cancer treatment [199]. The immune system has a remarkable ability in recognizing and destroying cancer cells, which has become a profound reason of interest over the recent years in reining cytokines for cancer treatment [200]. Cytokine anti-tumor properties have resulted in an exponential increase in the number of clinical trials of drugs based on cytokines, not only as a monotherapy but also combining along with other drugs that modulate the immune response. Cytokines restrain tumor cell growth either through direct anti-proliferative or pro-apoptotic activity, or indirect stimulation of the cytotoxic T cells activity against tumor cells which was first discovered in 1957, in interferon-alpha (IFN-α) [201].

Cytokine-based approaches for cancer therapy include GM-CSF, IL-7, IL-12, IL-15, IL-18, and IL-21 have entered into clinical trials as potential candidates for immunotherapy [202]. Interferon-α (IFN-α) belonging to the type I IFN family has significant immunomodulatory effects and is FDA adjuvant treatment for high-risk melanoma; first-line treatment for patients with metastatic RCC, AIDS-related Kaposi's Sarcoma, follicular lymphoma, hairy cell leukemia, chronic myelogenous leukemia, condyloma acuminata, and cervical intraepithelial neoplasms [203]. IFN- has direct anti-tumor activity by upregulating MHC class I surface molecules, which increases caspase-dependent apoptosis in certain cancers and has anti-angiogenic effects on tumor vasculature. IFN- is the only adjuvant therapy currently approved for patients with high-risk Stage II or Stage III melanoma [204]. IFNα and Peginterferon alpha 2b or in combination with bevacizumab is the current FDA-approved IFN-α-based cytokine therapy for cancer [200, 202, 205]. Interleukin-2 (IL-2) is a cytokine produced by antigen-stimulated CD4+ T helper cells, as well as CD8+ T cells, NK–NKT cells, and activated dendritic cells to a lesser extent [206]. IL-2 triggers the proliferation of NK cells improving their ability to cause cytolysis, guiding the proliferation and activation of CD8+ T cells, as well as B cell proliferation and secretion

of antibodies fulfilling carry out its role as a potent immune regulator [207]. IL-two-based second-generation therapies are being developed by improving their pharmacokinetic and pharmacodynamic profiles [208]. Rather than cytokine monotherapy, cytokine-based combined immunotherapies using immune checkpoint inhibitors like atezolizumab, nivolumab, and a combination of nivolumab plus ipilimumab has now become promising candidates under clinical trials [209–212]. Human clinical trials using recombinant IL-15 in various studies have shown some adverse effects and therefore require a lot of understanding to develop a suitable, stable IL-15-mediated cytokine immunotherapy in the near future. Interleukin-21 (IL-21) is a pleiotropic cytokine secreted by activated CD4+ T cells that have emerged as a potential immune modulator that acts to link innate and adaptive immunity. IL-21 has been studied as a therapeutic target for the treatment of autoimmune diseases and as an immune modulator for the treatment of cancer patients [213]. Some ongoing clinical studies have been combining the IL-21-based cytokine therapy with other approaches of immunotherapy to increase the efficacy of the cytokines to induce its anti-tumor potential along with adoptive cell transfer and NK cells approach. IL-10 is a cytokine secreted by the immune cells. The immune system's innate and adaptive response to direct the activity of pro-inflammatory cytokines has been considered an immunosuppressive cytokine because it can downregulate dendritic cell antigen-presenting activity and inhibit the cytotoxic effect as well as cytokine-release functions via T lymphocytes and NK cells [214, 215]. IL-10 conjugated with polyethylene glycol (termed pegilodecakin) is being administered for clinical trials to increase IL-10 half-life to see improved effects [216, 217]. In preclinical studies using animal tumor models, combining IL-12 with immune checkpoint inhibitors has shown excellent synergistic results by eradicating the tumor [218]. All the current cytokines in the immunotherapy have been summarized in Table 5.2.

Cytokines have shown potential therapeutic anti-tumor effects in murine models as well as in the treatment of a few human cancers. Furthermore, IFN and IL-2 cytokines are approved for the treatment of certain cancers. Despite the fact that multiple novel strategies are being used to elicit the activity of cytokines due to their adverse effects, cytotoxicity, or limitations, it is hoped that by considering the understanding of the regulatory mechanisms that control the immune response validated in animal models, cytokines will eventually play a critical role in cancer immunotherapy.

Table 5.2 List of current cytokines in immunotherapy along with their status of FDA approval

S. No.	Cytokine	FDA approval status	References
1	IFN-α	Approved	[203]
2	IFN-α and Peginterferon alpha 2b	Approved	[205]
3	IL-2	Approved	[208]
4	IL-15	Clinical trials	[213]
5	IL-10	Ongoing clinical trials	[216]
6	IL-12	Preclinical studies	[217]

5.3 Future Directions

Cancer is a complex, devastating disease, and from many decades, a suitable treatment approach is being researched and yet not been satisfactory due to complications involved with the disease. Despite recent remarkable advances in the field of molecular biology and surgical adjuvant therapy, cancers are yet a dreadful and potentially dangerous disease to human life. However, there has been a paradigmatic change in the approach to treatment for cancers considering immunotherapy [219]. The most promising and emerging therapy for cancer in the current scenario is the rise of the immunotherapy approach for the treatment of cancer. A deeper understanding of the mechanisms of immunoregulation and advanced techniques for manipulating immune cells as well as engineering the immune cells will proceed to new approaches of immunotherapy and cell-based strategies to treat cancer. The advancement in technologies has been allowing new strategies to be developed for modulating the strength and specificity of the immune system to benefit the immunotherapeutic approach which has the potential to crucially contribute to cancer treatment modalities [220]. Influencing the immune system by using inhibitors of the immune checkpoints, and cancer vaccines, ACT provides a remarkable chance at revolutionizing the treatment for cancer [221]. Immunotherapy is now considered the fifth pillar of cancer treatment and management, alongside surgery, chemotherapy, radiotherapy, and targeted therapy. Recently, the success of genetically modified T cells that express chimeric antigen receptors (CAR T cells) has given cancer patients renewed hope. CAR T cell therapy research and development has resulted in a vast array of studies and clinical trials that use lymphokine-activated killer cells, tumor-infiltrating lymphocytes, and allogeneic hemopoietic stem cell transplantation to treat cancer [222]. Currently, with the FDA approval for use of CAR T cell therapy, T cell receptors, engineered T cells, immune checkpoint inhibitors, oncolytic viruses, natural killer cells, and cytokine-based monotherapies have emerged as a promising treatment modality in the very near future. The monotherapies have also shown toxicities, adverse effects, and not many efficient results in a few clinical trials which have led the paradigm toward the concept of a combination of immunotherapies for a better efficient cure for cancer. These novel combinations of two or more approaches of immunotherapy at targeting the cancer cells have become the potential strategy in developing the most suitable cancer treatment that is the need of the hour.

5.4 Conclusion

In this chapter, the different approaches of cancer immunotherapy considering the most recent advancements in the field have been discussed. Ongoing clinical trials, completed clinical trials, results of preclinical studies, animal model studies as well as a review of the most promising upcoming advancements in immunotherapy has been reviewed and discussed to highlight the development of immunotherapy against

various cancers over the past decade. The concepts discussed here suggest that there is a great scope for the approaches of immunotherapy in the near future that needs to be advanced with a deeper understanding of the immune system and all the technologies that can be employed for immunotherapy to target cancer cells.

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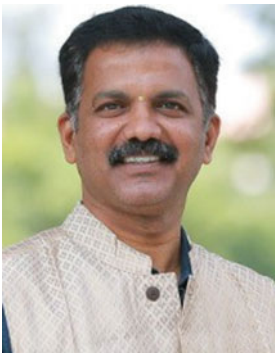

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Chapter 6
Bioinformatics Tools to Discover and Validate Cancer Biomarkers

S. Bhumika, G. O. Chandan Gowda, Kanthesh M. Basalingappa, T. S. Gopenath, and K. Gobianand

Contents

Abstract Cancer is the most deadly disease that causes death. Most likely, cancer is a problem in developed nations, with liver cancer coming in third in terms of mortality rates globally. Other than breast cancer, which is ranked fourth in the world, ovarian cancer is the most dangerous cancer in terms of maternal cancer. Ovarian cancer which is epithelial ovarian cancer (EOC) accounts for 90% of cases. Hox genes are typically expressed during organogenesis, but HOXA9 and HOXA11 are responsible for and involved in the molecular pathway of ovarian cancer. Ovarian cancer has many genes involved in the molecular pathway, and it can validate ovarian cancer treatment and therapies. In this current study, we examined bioinformatics resources for ovarian cancer biomarker validation for ovarian cancer therapies. In ovarian cancer, the genes CREB1, CD38, SIRTs, TP53, WT1, many miRNAs, etc., play novel biomarker roles and can be validated for ovarian cancer treatment. Many ovarian cancer biomarkers

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are reviewed and validated through bioinformatics tools. Different genes were discovered and validated using GEO and common DEGs through cBioPortal, KEGG, functional enrichment analysis, PPI, and oncomine along with immunohistology. Many hub genes were found and reviewed using common DEGs and GEO.

Abbreviations

AS	Advanced Stage
BC	Breast Cancer
cBioPortal	CBio Cancer Genomics Portal
CC	Cellular components
CDC42EP3	Cell division cycle 42 effector protein 3
CNVs	Copy number variants
CREB	CAMP response element binding protein
DAVID	Database for Annotation, Visualization, and Integrated Discovery
DEGs	Differentially expressed genes
EMS	Endometriosis
EOC	Epithelial Cancer
EPCAM	Epithelial cell adhesion molecule
FBXW7	F-box and WD repeat domain containing 7
FGF18	Fibroblast growth factor 18
GEO	Gene Expression Omnibus
GEPIA	Gene Expression Profiling Interactive Analysis
GO	Gene Ontology
GSEA	Gene set enrichment analysis
HGSCs	High-grade serous carcinomas
Hox gene	Homoebox gene
INAVA	Innate immunity activator
KM-Plotter	Kalpan-Meier Plotter
LGSC	Low-grade serious carcinomas
LMP	Low malignant potential
LncRNA	Long noncoding RNA
LOH	Loss of heterozygozity
m6A	N6-methyladenosine
MAL	Myelin and Lymphocyte
MCODE	Molecular complex identification
MD	Moderately Differentiated
NK	Natural killer cells
NSCLC	Non-small cell lung cancer
OC	Ovarian Cancer
PD	Poorly Differentiated
PFS	Progression-free survival
POTEE	POTE ankyrin domain family member E

PPI	Protein–Protein Interaction
PSAT1	Phosphoserine Aminotransferase 1
SBOT	Serous ovarian borderline tumours
SDC	Syndecan-3
SOC	Serous ovarian carcinoma
SOX17	SRY (sex determining region Y)-box transcription factor 17
Tcm	T central memory cells
TF	Tanscription Factor
Tgd	T gamma delta cells
TIMER	The Tumor Immune Estimation Resource
WD	Weekly differentiated
WT1	Wilms tumour gene

6.1 Introduction

A complex and scary disease or combination of disorders is cancer. Cancer has affected multicellular organisms for more than 200 million years, and there is evidence that it affected our ancestors more than a million years ago. Unlike infectious illnesses, parasites, and many environmental issues, cancer is not primarily brought on by an outside source. Human cells that have been recruited, in some ways, lost control, and to some extent undergone a metamorphosis into tumour nuclei or pathogenic organisms are its destructive agents [1]. According to Anya et al. [2], "Cancer may be understood as being analogous to a car breaking down, a process of natural selection, an infectious disease… a process of development, and even the establishment of an ecological community." The estimated incidence of cancer worldwide in 2012 was 14.1 million, with 7.4 million males and 6.7 million women affected. Therefore, 14% of all malignancies in Indian women are breast cancers (BC). An Indian lady receives a breast cancer diagnosis every four minutes. Both rural and urban India is experiencing an increase in breast cancer cases. 162,468 new instances of breast cancer were detected in 2018, and 87,090 people passed away from it.

However, ovarian cancer (OC) is the deadliest of all female reproductive system tumours. It is a silent assassin [3]. Because of the often nonspecific symptoms of ovarian cancer, it is frequently not discovered until it has progressed, making it difficult to treat curatively [4, 5]. 70% of ovarian cancer patients in stages three or four are not discovered until the disease has progressed. According to recent research, there is a 47.4% chance of surviving ovarian cancer after 5 years [6]. In women over the age of 40, the second most common cancer after breast cancer is OC, particularly in developed countries [7]. The absence of viable treatment options for advanced stage III/IV (AS). The survival rate of early-stage detection (ESD) is 90% with a five-year history, whereas the advanced stage has a rate of 20–25%. However, 70% of cases are diagnosed in the advanced stages. Inadequate diagnosis and treatment

failure are exacerbated by ineffective screening, chemoresistance, late-stage cancer with significant disease spread, and mild symptoms.

Most ovarian cancer is epithelial ovarian cancer (EOC), (approximately 90%), which can have many different tumour forms. The most prominent histological subtype of ovarian cancers (SC) is poorly and moderately differentiated (PD and MD), respectively, as opposed to well and weakly differentiated (WD) [8, 9]. According to widespread consensus [10], PD and MD SC are distinguished from SC, WD, and serous ovarian borderline tumours (SBOT) by their origin, pathogenesis, molecular profile, and clinical prognosis. Serous ovarian carcinoma (SOC) has two sub-classes, namely low-grade serous carcinomas (LGSCs) (Fig. 6.1) which make up less than 5% of all EOC, whilst high-grade serous carcinomas (HGSCs) make up 70% to 80% of all subtypes. 10%, 3%, and 10%, respectively, of cell subtypes are endometrioid, mucinous, and clear [11].

The biology and behaviour of ovarian cancer vary at the medical, cell, molecular, and cellular levels, making it a complex illness. The typical ovarian tissue is composed of a variety of distinctive elements. Additionally, endometriosis deposits, the fallopian tube lining, and the surface of the peritoneal cavity can all develop cancers with similar histology [12]. The endometrium (endometrioid), fallopian tube (serous), endocervix (mucinous), and vagina all exhibit healthy cell differentiation, as do the four major histotypes of epithelial ovarian cancer (clear cell) [13].

The improper translation of homeobox (Hox) genes, which are typically translated during gynaecological organogenesis, is closely related to histotypes. According to this, the uterus and SOC both have HOXA9, uterus and endometrioid cancer both contain HOXA10, and lower uterine segment, cervix, and mucinous malignancies

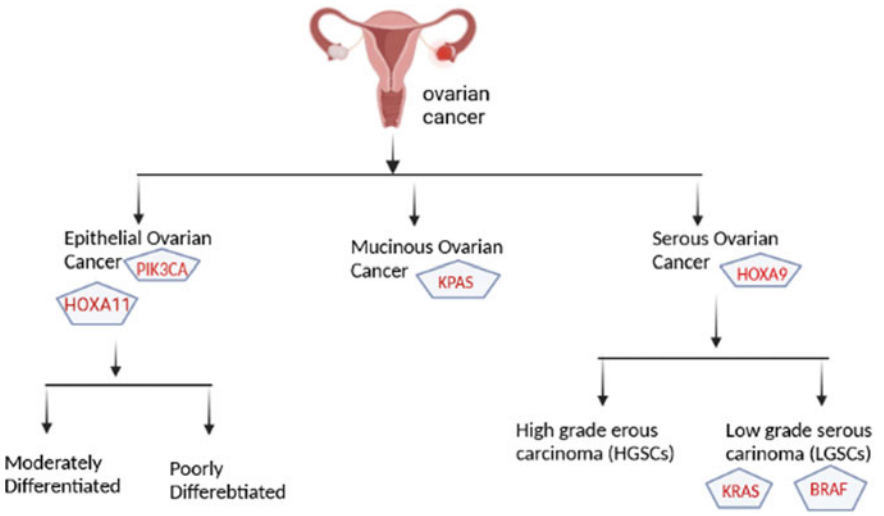

Fig. 6.1 Schematic diagram depicting the type of ovarian cancer along with the genes involved in types of ovarian cancer

all contain HOXA11. This has lately led to a re-examination of the traditional viewpoint on the According to a unique theory, clear cell, endometrioid, and serous carcinomas—all of which need ectopic endometrium—directly support the growth of the distal fallopian tube [14].

It is critical to inform women and healthcare experts about the dangers of ovarian cancer. Historically, the warning signs and symptoms of OC have been ambiguous. Symptoms like belly bloating, abdominal discomfort, frequent urination, early satiety or feeling full, or changes in bowel habits are generally not out of the ordinary for women [15, 16]. Due to this, women could put off seeking medical attention, which could delay diagnosis. In reality, the majority of women recollect experiencing these symptoms before being diagnosed, and some women who did come with these symptoms were treated without their doctors delving into the underlying source of their problems [15]. As medical professionals, it is crucial to take these vague symptoms into account when assessing patients with ovarian cancer risk factors.

Any woman, even those without obvious risk factors, can get ovarian cancer on occasion.

A summary of ovarian cancer risk variables and their relative risk probabilities is clear cell carcinomas and endometrioid carcinomas [16].

Various studies have found a substantial correlation between genetic changes and the clinical manifestation of malignancies, which suggests that the gene's location may be used as a target for prognostic and therapeutic measures [17]. Most commonly, epithelial ovarian cancer is linked to mutations in the TP53, PIK3CA, BRCA1/2, and KRAS genes. These mutations occur at varying rates in various subtypes of epithelial ovarian cancer. The P53 mutation's expression is the most frequent mutation in HGSOC. In HGSOC, 54.5% is the P53 mutation rate. In relation to endometriosis, OCCC and epithelial ovarian cancer have a high prevalence of PIK3CA mutations. LGSOC and mucinous ovarian cancer are both affected by the KRAS mutation. The functional loss of genes regulating tumour suppression, DNA repair gene defects, apoptotic death of the cell, the development of oncogenes, and epigenomic inactivation are a few of the hypothesised associations between mutations and ovarian cancer [18].

But early detection is unquestionably important because the circumstances that lead to advanced stages are poorly understood and, at times, contradictory [19, 20]. Early diagnosis biomarkers that are extremely specific to ovarian cancer must be identified and validated in order to develop non-invasive screening approaches for early disease diagnosis. Biomarkers of ovarian cancer are studied in this article using various bioinformatics tools (Fig. 6.1) [21].

Whilst ovarian cancer is brought on by genetic changes that happen at random, certain ethnic individuals get the disease as a result of congenital genetic flaws in their reproductive systems [22, 23]. Figure 6.2 depicts assets of bioinformatics tools in ovarian cancer.

The CA125 serum assay remains a key component of early detection strategies for ovarian cancer despite significant efforts to identify substitute techniques [24]. Protein [25, 26] and gene-based biomarkers [27] are only a couple of the numerous possibilities that have been made public (Table 6.1).

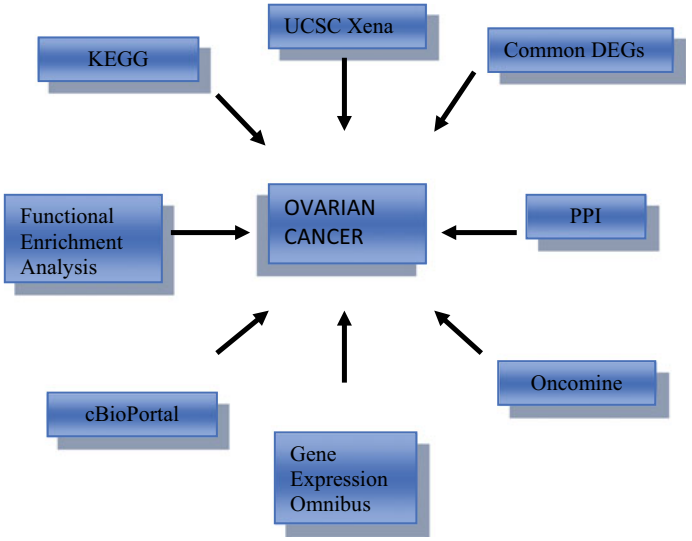

Fig. 6.2 Assets of bioinformatics tools in ovarian cancer

Table 6.1 Source of biological samples and their biomarkers in ovarian cancer

Biomarkers	Protein based	References
CA125	Serum and plasma based	[28]
Apolipoprotein A1		[29]
Osteopontin		[30, 31]
Claudine 4		[32]
Angiostanin	Urine	[33]
FGA, FGB, NT, and COL3A1 fragments		[34]
Hsp 70	Exosomes	[35]

Many early genes are expressed by the cAMP response element binding protein (CREB), a significant expression factor responsible for controlling specific gene expression and cell proliferation during embryogenesis [28]. Both benign and healthy prostates lack detectable CREB, according to an immunohistochemistry examination of original and bone metastatic prostate cancer patient samples [29]. Non-small cell lung cancer (NSCLC) is more aggressive than nearby normal tissues tumour tissues express CREB at much greater levels [17]. CREB overexpression is linked to a shorter event-free survival and a quicker time to relapse, according to the Kaplan–Meier analysis [30].

According to Li et al. [32], they identified CREB1 gene as one of the possible OC biomarkers using three different bioinformatics tools (UALCAN database analysis, functional enrichment analysis via metascape, and MEXPRESS analysis) which are associated with genes such as TP53, AKT2, AKT1, AKT3, BCL2, CCND1,

MMP9, and CCND3. Sun et al. [33] and Zhu et al. [34] examined the variation in oncomine which was used to study the expression and prognostic value of SIRTs (1-7) in ovarian cancer. GEPIA, TISIDB, and KM-Plotter, as well as CD38 (EOC), resulting in it being a novel biomarker. Sun et al. [33] and Zhu et al. [34] discovered that epithelial ovarian cancer (EOC) had higher levels of CD38 expression than normal ovarian tissue and that this higher expression is associated with a better prognosis. TIMER also discovered a link between CD38 and tumour infiltrating lymphocytes (TILs), specifically activated CD8C T cells. As a result, a new prognostic biomarker and potential immunotherapy target, CD38, are proposed to play an important immunoregulatory function in the EOC microenvironment.

Elgaaen et al. (2016) messenger RNA microarray datasets such as GSE36668, GSE14407, GSE18520, and microRNA dataset GSE47841 that offer some novel biomarkers obtained from the gene expression omnibus (GEO) database. SMC4 is a crucial biomarker that may be examined utilising differentially expressed genes (DEGs), KM plotter, the database cBio cancer genomics portal (cBioPortal), the UCSC Xena browser, and the UALCAN tools, according to Huijun Zhu et al. SMC4 plays a significant part in the biological process of OC. Liu et al. [37] demonstrated 116 DEGs that were carefully evaluated using GEPA, Oncomine, mini gene ontology (GO), KEGG, PPI, and other databases utilising 4 gene expression omnibus (GEO) datasets, such as GSE40595, GSE38666, GSE27651, and GSE66957. Additionally, they identified the core genes CDCA5, FOXM1, KIFI5, MCM2, and ZWINT as being connected to epithelial ovarian cancer (EOC). They also identified potential drugs for the treatment of OC depending on the DEGs.

Prognostic genes in OC were subjected to a thorough bioinformatics analysis by Huijun Zhu et al. to look into any potential underlying biological pathways. In their investigation, which made use of three GEO OC datasets, DEGs were eliminated (GSE26712, GSE18520, and GSE14407). 879 common DEGs were found after integrating the three datasets. The SMC4 was shown to have high expression in the analysis using the Kaplan–Meier (KM) plotter, and both the messenger RNA and protein levels were significant for the prognosis of OC. According to the results from cBioPortal, copy number amplifications were responsible for the great majority of mutations in the genome in OC, and SMC4 mutations were responsible for 7–18% of those alterations. In addition, samples overexpressing SMC4 were primarily enriched in adherens junctions, the cell cycle, spliceosomes, and according to GSEA data, ubiquitin-mediated proteolysis. High SMC4 expression in OC patients is therefore linked to risky decisions and may serve as a biomarker for the disease.

It appears that epigenetic pathways are significant in ovarian cancer. In the most recent identification, POTEE (POTE ankyrin domain family member E), which is hypomethylated in ovarian cancer, was used by Qazi and Raza [38]. They carried out an enrichment analysis on the POTEE paralog mRNA sequence and used it to pinpoint key motifs. Only three patterns were most likely to be present in the POTEE nucleotide sequence out of the six motifs we discovered, which varied in length. By analysing enrichment and occurrences, we were able to correct the best match motif to CTTCCAGCAGATGT. Since the structure of the POTEE paralog has not been empirically proven, utilising an automated template-based model to anticipate the

POTEE structure modelling procedure powered by a deep neural network. Furthermore, they utilised AlphaFold's projected POTEE structure to confirm the validity of our model, and we found that the residual stretch beginning at residues 237–958 had a significant level of confidence for each residue. A 50 ns time step replica exchange molecular dynamic simulation was also performed to evaluate the stability of the planned POTEE model. There are just 10 extremely significant, direct, and physical POTEE associators found in this network-based epigenetic research. They will learn more about the POTEE paralog thanks to their discoveries.

Qin et al. [39] employed bioinformatics analysis in the current study to identify potential genes implicated in the development and progression of epithelial OC. Using 3 microarray datasets (GSE29450, GSE14407, and GSE54388) which are retrieved from the GEO database, they selected and evaluated potential genes associated with carcinogenesis and the formation of epithelial ovarian cancer. Their bioinformatics research discovered more than 400–500 differentially expressed genes (DEGs) and nine potential hub genes (CDK1, CCNB1, CDC20, BUB1, BUB1B, CCNA2, RRM2, TIK, and AURKA). A Kaplan–Meier survival analysis revealed that seven potential high-expression genes (BUB1, CCNB1, RRM2, CCNA2, BUB1B, AURKA, and CDK1) had low overall survival (OS). The results of interactive analysis of gene expression profiling (GEPIA) show that the seven candidate genes are expressed greater in ovarian cancer samples than in healthy ovarian samples. According to immunostaining data from the human protein atlas (HPA) database, ovarian cancer tissues expressed more of the proteins CCNB1, CCNA2, AURKA, and CDK1. The level of RRM2 protein expression was identified in healthy ovarian tissue and ovarian cancer samples.

An oncological investigation found a link between clinicopathological data from patients, and the pattern of expression is BUB1B, CDK1, CCNA2, AURKA, CCNB1, and BUB1. A transcription factor (TF) gene-controlling network was developed following the identification of six genes as CCNB1, BUB1B, CCNA2, AURKA, CDK1, and BUB1 which are hub genes. This network helped identify TFs, ZBTB11, POLR2A, KLF9, and ELF1 a few examples, which were thought to be involved in controlling these hub genes.

Bioinformatics techniques were used by Kulbe et al. [40] to identify and validate epithelial ovarian cancer (EOC) biomarkers for detection. Three separate stromal or epithelial compartments from GEO were made available to them, and they utilised them (GSE29156, GSE40595, GSE14407). Utilising the broad functional database and the CSIOVDB, the findings were confirmed. When their potential as biomarkers was examined, 12 of the top 25% of the candidate based on transcript levels, genes were able to distinguish between benign and malignant tumours. Versican, Aurora kinase A, Myelin and Lymphocyte (MAL), and Syndecan-3 (SDC) are T cell differentiation proteins that discriminate between malignant and benign situations substantially superior to the previously identified biomarker fibroblast growth factor 18. (FGF18). Additionally, there was a substantial correlation between a worse prognosis with increased Myelin and Lymphocyte and AURKA expression levels. They as a result found a few potential novel biomarkers and learned that the stroma of the

Table 6.2 Ovarian cancer biomarkers and their validation through bioinformatics tools

Biomarkers	Bioinformatics tools	References
CREB1	UALCAN, metascape, and MEXXPRESS analysis	[28]
SIRTs (1-7)	GEPIA, TISIDB	[29]
CD 38	Oncomine, GEPIA, TISIDB, and KM plotter	[29, 30]
GSE36668, GSE18520, GSE14407, and GSE47841	Oncomine, KEGG	[31]
SMC4	DEGs, KM plotter, cBioPortal, UCSC Xena browser, and UALCAN tools	[32]
GSE27651, GSE40595, GSE38666, and GSE66957	GEPA, oncomine, KEGG, and PPI	[32]
CDCA5, FOXM1, KIF15, MCM2, and ZWINT	DEGs	[33]
GSE26712, GSE18520, and GSE14407	GEO, KM plotter, cBioPortal, GSEA	[32]
GSE14407, GSE29450, and GSE54388	DEGs, HPA, GEPIA, and KM plotter	[34]
GSE29156, GSE40595, and GSE14407	CSIOVDB, GEO, and KM plotter	[35]
FGF18	DEGs	[35]

EOC is a functional biomarker. Table 6.2 represents the ovarian cancer biomarkers and their validation through bioinformatics tools.

Finding the hub and core genes as well as any pertinent biochemical pathways that could be linked to the genesis of OC was the main goal of the study. They searched for DEGs in OC cells and ovarian SINE-resistant cancer cells using the GEO2R tool and a microarray dataset (GSE126519) from the GEO database. A detailed bioinformatics analysis revealed that DEGs could be involved in the incidence, prognosis, development, and expansion of OC. They examined 2708 DEGs and 10 hub genes using GO (gene ontology), KEGG, Geno Go Metacore, and PPI and discovered that the core genes FZD8, FZD6, CDK2, and RBBP8 were involved in OC and SINE-resistant OC.

Meng et al. [51] investigated the molecular pathways underlying WT1 in OC using WT1 (Wilms tumour gene). WT1 is a prognostic and diagnostic marker for OC. They discovered important molecular mechanisms and hub genes in WT1-affected ovarian cancer using different bioinformatics and other techniques. According to studies, both lifespan and rates of progression-free survival (PFS) in ovarian cancer have been correlated with widespread WT1 expression. In the ovarian cancer cell line SKOV3, WT1 downregulation increased mRNA expression of 638 genes whilst decreasing messenger RNA levels of 512 genes, the majority of which were involved in the AMPK, FoxO, and Hippo signalling pathways. Using the Cytoscape software and the STRING online tool, a protein–protein interaction network was created using 18 differentially expressed genes (DEGs).

Sixteen of the eighteen genes were connected to prognosis, according to a Kaplan–Meier plotter study. Seven of the 16 genes had distinct expression patterns in normal tissues compared to ovarian cancer tissues, according to an analysis of GEPIA datasets. IGFBP1 and FBN1 gene expression increased significantly, whilst SERPINA1 gene expression significantly decreased after WT1 interference. When compared to both healthy and ovarian cancer tissues, the correlation between WT1 expression and those of these three genes was strong. SERPINA1, IGFBP1, WT1, FBN1, and 20 other genes interacted extensively, according to studies on the Gene MANIA website.

They discovered major signalling pathways with WT1 which affect ovarian cancer, as well as 3 differentially expressed genes regulated by WT1 that are important for ovarian cancer prognosis. As a result of shedding light on the mechanisms underlying WT1 gene expression in ovarian cancer, their findings highlight the need for novel anti-ovarian cancer therapies.

To enhance OC diagnosis, prognosis, and treatment, Zhang et al. [52] employed microarray gene expression to identify gene markers, create and analyse systems, and conduct drug interaction studies and survival analysis. The DEGs, DisGeNET, and NCBI-Gene databases were used, as well as GeneMANIA, DAVID 6.8 for GO enrichment analysis, Cytoscape 3.8, for patient survival data, the UALCAN; for gene-drug correlations, PubChem, DrugBank, and DGIdb. Eight genes were found, and investigations of drug-gene interactions were conducted on them. Results determined that the OC genes HMGA1 and PSAT1 are promising medical biomarkers for early detection due to their overexpression in all four phases of the disease (stage I). According to our investigation, the seed genes share 11 medications in common. The medications cisplatin, cyclosporin, bisphenol A, progesterone, and sunitinib also exhibited a substantial interaction affinity with the ovarian cancer-promoting methylation status genes HMGA1 and PSAT1.

Yang et al. [53], work demonstrated four gene expression profiles from the GEO and DEGs in normal tissues and OC tissue (GSE54388, GSE69428, GSE36668, and GSE40595). As a result, 117 DEGs in all were discovered. In this study, 114 DEGs that were upregulated were mostly involved in the nucleus, cell division, and binding of protein, but 57 DEGs that were downregulated were mainly involved in the inhibition of transcription from the RNA polymerase II promoter, protein complex, etc. The enhanced DEGs were mostly associated with metabolic activities, antibiotic and amino acid biosynthesis, and HTLV-I infection for the KEGG pathway. KIF4A, BUB1B, FOXM1 CDC20, etc., were also identified as hub genes. Patients with OC have the levels of expression of the genes in increasing order CCNB2, KIF4A, BIRC5, BUB1B, CDC20, etc., which were statistically have poor progression-free survival, according to survival analysis of these hub genes. The hub genes' expression levels coincided at the same time, according to GEPIA2 and GEO. Finally, 62 small molecules that might be utilised to treat OC were found using the DGIdb database.

In their most recent studies, Li et al. [54] used GEO2R to screen the DEGs from three microarray datasets (GSE38666, GSE40595, and GSE66957). The hub genes were examined using survival analysis, GO, KEGG, and protein expression data. Thus, a total of 199 DEGs were found. It was discovered through KEGG pathway

analysis that DEGs are associated with the AGE-RAGE signalling pathway, carbon metabolism, and HPV infection. They also examined four highly expressive hub genes (COL4A1, TOP2ASDC1, and CDKN2A) and found a relationship between their expression and the longevity of OC patients, which was supported by the GEPIA and HPA databases.

It is critical to understand the molecular processes that regulate cancer stem cells (CSCs) if novel therapeutic strategies for epithelial ovarian cancer are to be developed (EOC). In 2020, however, Abhijeet Behara and his colleagues discovered numerous molecular markers and signalling pathways in ovarian CSCs. During the screening phase, 117 upregulated genes and 83 downregulated genes were discovered out of a total of 200 DEGs. According to GO analysis, the majority of the frequently upregulated DEGs were found to be more abundant in biological processes such as cell proliferation, tissue development, and reaction to lipids, chemicals, and organic substances in addition to cell ECM, whereas the downregulated DEGs were found to be more abundant in biological processes in the regulation of molecular function. DEGs in CSLCs that are frequently upregulated play critical roles in several cell signalling pathways.

Genes involved in interferon-alpha/beta signalling (IFIT3, IFITM1, IFIT2, etc.), fibre elasticity (BMP4, LTBP2, FBLN1, and TGF-1), and bile acid and bile salt production (BMP4, FBLN1, LTBP2, and TGF-1) were amongst the most frequently upregulated DEGs (AKR1C3, AKR1C2, and AKR1C1). A small number of downregulated DEGs in CSLCs were primarily responsible for EGFR activation via the gastrin pathway (EGFR and PRKCA).

The top 13 DEGs that were most frequently up and downregulated were ADM, AKR1C1, CXCR4, CCDC80, RIMS2 TPM1, etc. These DEGs could be identified as specific therapeutic targets and used as a diagnostic indicator as well as for long-term OC management.

Using bioinformatics, Ni et al. [56] discover potential key genes and pathways underlying the conceivably shared biological processes between ovarian cancer and endometriosis (EMS) (OC). Between EMS and OC, a total of 571 DEGs overlapped. 36 gene ontology keywords and 7 KEGG pathways were linked to enriching DEGs, and the p53 signalling pathway was the pathway that was most frequently connected with deactivation. The newly found hub genes' expression was validated using the KM plotter platform, and survival analyses revealed that CCNB1, KIF2C, CCNB2, etc., are linked to lower rates of OC survival and disease-free survival.

Song et al. [57] discovered genes involved in OC and significance in molecular pathways to investigate relevant clinicopathological significance in OC. The 14 differentially expressed genes (DEGs) discovered included nine genes (BIRC5, VEGFA, CDCA3, NCAPG, etc.) that were strongly elevated in gene ontology analysis frequently highlighting cell cycle control. These DEGs contained 7 hub genes, according to further PPI research. Expression levels of CDCA3 and UBE2C were also linked to lower overall patient survival, independent of tumour stage, or histologic grade, according to KM survival analysis. UBE2C and CDCA3 may therefore be helpful indicators for estimating the time until death in individuals with advanced serous OC.

Dogan et al. [58] used pathfinder to successfully carry out a functional study of previously confirmed target genes of circulating miRNAs in their most recent research. The GTEx and the cancer genome atlas datasets were also utilised to evaluate survival rates and disease phases using miRNA target genes. In the previous research, we used high-throughput techniques to confirm three downregulated miRNAs (hsa-miR-885-5p, etc.) with diagnostic relevance in OC patients' serum. The miRDB database was searched for these miRNAs' predicted target genes (v6.0). PathfindR used the target genes to perform an active-sub network-oriented, KEGG pathway enrichment analysis. According to the study of pathfinders enriched KEGG pathways, 24 pathways were linked to the target genes. Ubiquitin-mediated proteolysis, the spliceosome, and the Notch signalling system had the lowest p-values. It was discovered that 93 common genes had differential expression in the datasets. In the study of survival rates, no significant genes were discovered, whereas 24 significant genes were discovered in the study of sick stages. The outcomes of this work include in-silico analyses that confirmed the target genes for circulating miRNAs and the enriched pathways that are connected to OC and may be useful in novel theranostics applications.

Zheng et al. [59] discover the main malignant genes, expose the significant molecular pathways of ovarian cancer using a bioinformatic technique, and validate them using immunohistochemistry. GSE14407, GSE26712, GSE18520, and the gene expression omnibus (GEO) database were used to obtain gene expression profiles for GSE54388. The transition between G2/M was determined after analysing the four gene expression profiles, a total of 226 of the mitotic cell cycle, death, cell proliferation, and positive modulation of the canonical Wnt signalling pathway were amongst the biological processes where DEGs were found in abundance, according to the GO analysis. The largest DEG concentrations were found p53 signalling pathway during the cell cycle, the Wnt signalling pathway, the Ras signalling system, Rap1 signalling pathway, and tyrosine metabolism, according to the KEGG study. 147 nodes and 655 edges were analysed using PPI network and from which we selected 50 hub genes, revealing a relationship between 30 hub genes and the prognosis of ovarian cancer. Immunohistochemical analysis of phosphoserine aminotransferase 1 was performed (PSAT1). High levels of PSAT1 were discovered in the tumour tissues of ovarian cancer patients, and the expression level was correlated with clinical stage and tissue differentiation. Elevated PSAT1 transcription and an advanced clinical stage are separate risk factors for the lifespan and prognosis of ovarian cancer patients, according to a Cox proportional risk model.

It is still unclear exactly what molecular processes underlie the disease's development. In the Zhang et al. [60] study, a detailed bioinformatics study was conducted to identify the main genes causing severe epithelial ovarian cancer. A total of three expression datasets, which included 30 samples of ovarian surface epithelium and 46 samples of serous epithelial ovarian cancer, were obtained from the gene expression omnibus database. Batch normalisation was done after merging the three datasets. The pooled normalised data were then scrutinised for differentially expressed genes (DEGs). 1300 upregulated and 912 downregulated genes were amongst the 2,212 DEGs that were found. According to a gene ontology study, the main functions

of these DEGs were "control of the cell cycle," "DNA packing," "mitosis," and "nucleosome assembly." The key cellular components were the "extracellular area portion," "chromosome," etc., whereas the molecular activities were the "calcium ion binding," "polysaccharide binding," "Wnt receptor activity," etc. These DEGs were mostly engaged in the "Wnt signalling pathway," "pathways in cancer," "PI3KAkt signalling pathway," and "focal adhesion," according to KEGG pathway analysis.

The 20 most important DEGs were found using the PPI network, and an oncogene study of these essential genes showed that 13 of them were elevated, and two were downregulated in serous epithelial ovarian cancer. The outcomes of serous epithelial ovarian cancer may be affected by cyclin B1, polo-like kinase 1, G protein subunit transducin 1, and G protein subunit 12, according to survival research. Although further study is required to confirm the revealed potential genes, their findings have significant therapeutic ramifications for ovarian cancer early detection, prognosis, and the creation of more targeted molecular therapies.

Through the use of bioinformatics, Chengzhang et al. [61], assessed the pathogenesis of OC 332 DEGs were examined using the GEOs datasets; 94 of them showed upregulation, whereas 340 showed downregulation. A functional enrichment databases study findings revealed that the DEGs were significantly enriched in a variety of biological functions, molecular mechanisms, and cellular elements connected to tumours. KEGG's pathway enrichment analysis led to the discovery of five new cancer-related pathways. Ten hub genes in all were discovered using the PPI network's topological analysis. A survival analysis found a correlation between 7 of the hub genes and dramatically decreased patient survival durations ($P = 0.05$). The aforementioned hub genes may serve as potential therapeutic targets for the treatment of ovarian cancer.

Yang et al. [62] finding of substantial representative biomarkers or cluster modules of differential genes associated with the phases of ovarian cancer (OC) are significant since they may help with understanding the mechanisms behind OC development and determining the prognosis of OC patients. From the TCGA database, he extracted gene expression information and related clinical data for 379 patients with ovarian cancer. The genes in OC patients whose expression fluctuates across phases were found using *R*. They ran GO and KEGG pathway analyses using the database for annotation, integrated discovery, and visualisation. There were 731 distinct genes between stages II and III of ovarian cancer (DEGs 2–3) and 563 distinct genes between stages III and IV of ovarian cancer (DEGs 3–4). (DEGs 3 and 4) (DAVID). Furthermore, Cyto-Hubba was used to identify the twenty hub genes in DEGs 2–3 and 3–4, followed by Cytoscape's search tool for retrieving interacting genes (STRING), and the MCODE plug-in was used to generate protein–protein interaction (PPI) modules for these genes. Using the molecular complex identification (MCODE) method, three significant DEGs 2–3 co-expression modules and three more DEGs III-IV relevant modules were discovered in the PPI network. Furthermore, five hub genes—COL3A1, KRAS, COL1A1, COL1A2, and NRAS—these stage-related DEGs modules were chosen that had lower overall survival rates. This bioinformatics study discovered that DEGs linked to stage-related prognoses, such

as COL3A1, KRAS, and NRAS, may be harmful to the growth and spread of ovarian cancer.

Zhu et al. [63] used bioinformatics analysis to recently identify and investigate the key genes for SOC. For the purpose of performing GSEA and screening for DEGs, the GSE14407 and GSE18520 datasets from the GEO database were obtained. To find the key genes, a PPI network was built, and DEGs connected to prognosis were found using the TCGA database.

Clinical samples were also gathered to help confirm the KIF11 gene, a member of the kinesin family. When SOC as compared to normal tissue, 198 DEGs were discovered, including 81 upregulated and 117 downregulated genes. According to GSEA, 16 gene sets, including those involved in the cell cycle and DNA replication, were associated with SOC across the two datasets. The PPI network of the DEGs had 130 nodes and 387 edges. Then, using submodule analysis, 20 important genes linked to a single top-ranked module were eliminated. KIF11, CLDN3, and FGF13 were found to be significant predictors of SOC prognosis in a survival study. KIF11 was discovered to be a fundamental and predictive gene, and this discovery was later supported by clinical evidence. KIF11 was found to be associated with aggressive traits such as tumour grade, TNM stage, and lymph node invasion, and it was found to be higher in tumour tissues than in surrounding normal tissues. As a result of the current study's discovery of the critical genes and gene clusters, our understanding of the genesis and prevalence of SOC has improved. KIF11 was also identified as a possible target for SOC diagnosis and therapy and as a prospective tumour-promoting gene.

Low malignant potential (LMP) ovarian cancers have a good prognosis and act like a disease between benign and malignant tumours. Authors know very little about the genes and mechanisms that connect benign-like LMP and aggressive OEC. To identify the important potential genes and pathways that may be connected to OEC, Zhou et al. [64] merged two cohorts of profile datasets. 327 OECs and 48 LMP tumours from two datasets (GSE9891 and GSE12172) were included for the analysis of gene expression. There were 559 differentially expressed genes overlapping, with 251 upregulated and 308 downregulated. Following that, researchers looked into gene ontology, signalling pathway enrichment, and the protein–protein interaction (PPI) network. Based on significant enrichment, functional enrichment analysis classified the upregulated and downregulated genes. The DEGs PPI network complex contained 282 nodes, differentially expressed genes (DEGs), and two of the most significant k-clique modules. Finally, they were able to find biomarkers using integrated bioinformatics analysis that may be crucial in the pathogenesis of OEC. This may help us better understand the underlying aetiology and molecular processes. These putative genes and pathways might be studied further, leading to more accurate illness diagnosis and therapy.

Yang et al. [65] studied the underlying pathway and possible target genes of sanguinarine in OC. In ovarian cancer cells, he employed the Affymetrix gene expression profiling chip to analyse the alterations in the transcription of downstream target genes and the biological processes that the control and sanguinarine groups regulate. DEGs were identified using an Agilent mRNA array from Affymetrix.

All the DEGs were then analysed through GO and KEGG pathway analysis using the DAVID database. As a consequence, 1185 DEGs total—835 upregulated and 350 downregulated—were found in the sanguinarine and control groups. The GO analysis found that the majority of cellular mechanisms, including the metabolism of nitrogen compounds, transcription from the promoter of RNA polymerase II, and the negative regulation of gene expression, were concentrated in the DEGs. The cytoskeleton and endoplasmic reticulum appeared to be particularly affected by changes in cellular components (CC). The changes in molecular function appeared to be focussed mostly on protein dimerization, nucleic acid-binding transcription factor activity, and enzyme binding. The "systemic lupus erythematosus," "MAPK signalling route," "pathways in cancer," and "metabolic pathways" were the regions where the DEGs were most abundant, according to the results of the KEGG pathway analysis. His work provides insight into the mechanism regulating sanguinarine target genes in ovarian cancer cells, which may be helpful for OC diagnosis and treatment.

The majority of high-grade serous ovarian tumours have TP53 mutations, which can change the way its protein product, p53, participates in oncogenesis. The effect of these kinds on prognosis has been the subject of contradictory findings. This association in the patient group is explained by Mandilaras et al. [66]. Through a focussed next-generation sequencing approach used in an institutional molecular screening programme, 229 individuals with high-grade serous ovarian cancer had their tumours profiled. Using techniques that have been previously discussed in the literature, TP53 mutations were categorised. TP53 mutation was evaluated by immunohistochemistry on formalin-fixed, paraffin-embedded tissue. To study variations in outcomes, they created patient groupings with similar clinicopathologic features using divisive hierarchical clustering. Six distinct TP53 mutation identification methods were subsequently examined. Both the initial platinum-free period and overall survival did not demonstrate a connection with these. In 80% of cases, next-generation sequencing accurately predicted a mutation, which is comparable to the percentage of immunohistochemistry identified. Dividend-based hierarchical clustering resulted in the formation of four primary groups, with cluster 3 having a considerably poorer prognosis (p 0.0001; log-rank test). Gains of function mutations were more prevalent in this cluster, and the likelihood that these patients had received the best debulking surgery was lower.

To enhance patient outcomes, many obstacles related to OC metastases must be discussed.

Recent investigations by Liu et al. [67] have shown copy number variants (CNVs) commonly provided to changes in oncogenic drivers. He used a CytoScan HD Array to look for CNVs and loss of heterozygosity (LOH) in the whole genomes of six OC patients and human OC cell lines to identify the gene target events responsible for OC's various invasive abilities. According to the findings, there are increases at 8q21.13, and LOH at Xq11.1 and Xp21.1.were unique, particularly CNVs. After that, bioinformatics analysis was used to check for CNVs connected to ovarian cancer. Additionally, using data from the PASTAA study, interactions between transcription factors and their target genes were anticipated. By combining the above-mentioned data on gene expression and clinical outcome, six genes such as GAB2,

UGT1A1, AKT1, EGFR, COL6A3, and UGT1A8 were shown to be good targets. Six CNV-driven genes interacted with four known transcription factors (TFs) associated with cancer in the transcriptional regulatory network. Three of these four TFs were identified as probable candidate genes-related transcription factors in OC by the protein/DNA arrays. Finally, they showed that these six genes can function as possible OC indicators.

It has been established that N6-methyladenosine (m6A) RNA alteration is essential for the development and spread of tumours. The functions of m6A target genes in ovarian cancer, however, have not been elucidated. A thorough bioinformatics and in vitro research were provided by Yan et al. [68] to assess the functions of the m6A target genes. Cell division cycle 42 effector protein 3 (CDC42EP3), a possible m6A target gene, has been regulated in ovarian cancer tissues and cells. Molecular techniques were used to confirm the regulated CDC42EP3 in A2780 and TOV112D ovarian cancer cells. Using other algorithms like PrognoScan, KM plotter, LinkedOmics, and TISIDB, the biological significance of CDC42EP3 in ovarian cancer was established. These results showed that lower CDC42EP3 expression was related to a worse outcome in ovarian cancer patients. Several immune cells identified in tumours, including natural killer cells (NK), T central memory cells (Tcm), T gamma delta cells (Tgd), and others, have also been substantially associated with CDC42EP3 expression. The m6A target gene CDC42EP3 was discovered as a novel prognostic target in these researches, which also revealed possible functions for CDC42EP3 in the regulation of the immune microenvironment in ovarian cancer.

A key piece of information for identifying functional genes in ovarian cancer development and progression comes from microarray-based gene expression investigations. Zhao et al. [69] investigation, which used GEO data on OC gene expression, included 16 papers. A study into the gene expression was done using meta-analysis to find the genes that were differently expressed (DEGs). Our meta-most analysis's differentially expressed genes were chosen to confirm gene expression and gene function. In conclusion, 541 upregulated genes and 431 downregulated genes were discovered to be linked with ovarian cancer, totalling 972 DEGs with a *P*-value of 0.001 or higher, and 92 additional DEGs were discovered to be gained DEGs.

To validate gene expression profiling, the top five genes that were up- and downregulated were chosen. Several of these genes, including upregulated CD24 molecule (CD24), SRY (sex determining region Y)-box transcription factor 17 (SOX17), WFDC2, epithelial cell adhesion molecule (EPCAM), innate immunity activator (INAVA), and downregulated aldehyde oxidase 1, were found to have recurrent expression patterns in clinical patient samples from OC. Whilst upregulation of WFDC2 and INAVA promoted ovarian cancer cell migration, upregulation of WFDC2 boosted cell proliferation, and downregulation of AOX1 enhanced cell proliferation, downregulation of AOX1 was successful at inducing ovarian cancer cell death.

KIF1A expression level was used in Lu et al. [70] to study ovarian cancer and its clinical importance in the emergence of OC, as well as its possible regulatory network, examined. The differences between the expression of OC and normal tissue were investigated, as well as the relationship with the tumour stage, using the

TCGA OC data. The oncomine and Kaplan–Meier plotter programmes were utilised to investigate the relationship between KIF1A expression and prognosis. KIF1A may be involved in a variety of biological processes, including expression, DNA-templated cytolysis, and many other molecular pathways. Wnt signalling pathway, and some other molecular pathways, according to GO and KEGG analysis. Since KIF1A was shown to be highly expressed and associated with poor survival and immune infiltration in OC, it may be used as a diagnostic biomarker.

Long noncoding RNA (lncRNA) is now understood to play important roles in controlling a variety of biological activities in cancer. Although it has been suggested that titin-antisense RNA1 (TTN-AS1) plays important roles in malignancies, and its function in ovarian cancer is yet unclear. Using quantitative RT-PCR, the levels of TTN-AS1, miR-15b-5p, and F-box and WD repeat domain containing 7 (FBXW7) in ovarian cancer cells were identified. Bioinformatic techniques were used to identify the targets for TTN-AS1 and miR-15b-5p, and luciferase activity reporter assays were used to confirm these targets. Cell growth, colony formation, and cell death were investigated using the cell counting chamber, colony formation assay, and flow cytometry. During the interactive analysis of gene expression profiling, the correlation between TTN-AS1 and FBXW7 was examined. TTN-AS1 expression was shown to be diminished in the tissues and cells of ovarian cancer. Dual luciferase activity revealed that TTN-AS1 and FBXW7 share a binding site with miR-15b-5p. A functional investigation showed that overexpression of TTN-AS1 promotes apoptosis whilst inhibiting the proliferation and colony formation of ovarian cancer cells. Rescue tests indicated that FBXW7 knockdown may partially counterbalance the impact of TTN-AS1 overexpression on the behaviour of ovarian cancer cells. They concluded that the TTN-AS1/miR-15b-5p/FBXW7 axis identified in this work may help find treatment biomarkers for ovarian cancer [71].

Three microarray datasets from the GEO public database (GSE14407, GSE36668, and GSE18520) were used to examine ovarian cancer. In total, samples of 26 normal and 72 malignant ovarian tissues were compared. DEGs were obtained using GEO2R tools and Venn diagrams. Then, GO and KEGG analyses were carried out using the database for annotation, visualisation, and integrated discovery (DAVID). Then, using Cytoscape, the protein–protein interaction (PPI) system was established and visualised. A total of 232 DEGs were shared amongst the three datasets under study. The raised 108 genes had high levels of extracellular matrix/region, anchoring membrane component, cell junction and Golgi membrane, transcription factor activity, sequence-specific DNA binding, RNA polymerase II regulatory area, and DNA binding all examples of transcription factor activity. Other prominently enriched regions included cellular response and cell adhesion to interleukin-1, positive control of transcription from RNA/DNA, and transcription. Using the MCODE plug-PPI tool, network analysis revealed 14 increased genes. Nine genes were connected to considerably decreased survival amongst OC patients, whereas 4 genes had no discernible effect, according to a Kaplan–Meier plotter study. 13 DEGs had substantially higher expression in ovarian cancer tissues than in healthy tissues, according to the gene expression profiling interactive analysis (GEPIA). 11 genes, including CDC6, CDC20, PTTG1, CCNB2, etc., were largely linked to the

cell cycle, according to KEGG analyses, whilst two genes, such as SFN and RRM2, were linked to the p53 signalling pathway. Therefore, potential upregulated DEGs in OC patients might help diagnose. This will make it simpler to understand the underlying mechanisms of OC and to apply focussed therapeutic techniques [72].

A class of transmembrane proteins called the claudins is connected to tight junctions. Spite of significant research on their role in cancer, little is known about how they interact with the tumour immune microenvironment. This study examined the link between genes associated with the prognosis of ovarian cancer and the tumour immune microenvironment. The claudin family's genetic variation pattern in ovarian cancer was discovered using the cBioPortal for cancer genomics database. The mRNA expression of claudins in malignancies was investigated using the ONCOMINE and gene expression profiling interactive analysis (GEPIA) databases. The Kaplan–Meier plotter was used to investigate the prognostic potential of these genes. Gene set enrichment analysis (GSEA) was used to ascertain the enrichment of immunological markers. The tumour immune estimation resource (TIMER) was used to look into the relationships between claudins and the tumour immune microenvironment in ovarian cancer. As a consequence, 363 (62%) of the patients/samples analysed had changed claudin genes. Various malignancies were shown to express claudins abnormally. Amongst these, there was a strong correlation between overall survival and CLDN3, CLDN4, CLDN6, CLDN10, CLDN15, and CLDN16 in ovarian cancer patients. According to GSEA, CLDN6 and CLDN10 were considerably enriched in the B cell, CD4 T cell, and CD8 T cell immunological signatures. Furthermore, immune cell infiltration in ovarian cancer was found to be negatively and positively associated with CLDN6 and CLDN10, respectively.

Additionally, there were both positive and negative correlations between the expression levels of CLDN6 and CLDN10 and several immune cell gene markers in ovarian cancer.

Therefore, immune cell infiltration may be facilitated by CLDN6 and CLDN10 in ovarian cancer, and these mechanisms may be responsible for the poor prognosis. The immunological microenvironment and the predictive biomarkers CLDN6 and CLDN10 in ovarian cancer were therefore linked, according to the study team. As a result of these findings, CLDN6 (as seen in Fig. 6.3) and CLDN10 [73] have been identified as potential therapeutic targets for the treatment of ovarian cancer. As OC contains numerous biomarkers, many of which were examined by Elgaaen et al. [35] and may be a novel biomarker for ovarian cancer, more study on OC biomarkers utilising the bioinformatics methods more study on this is necessary.

Utilisation of bioinformatics methods and certain diagnostic biomarkers for ovarian cancer have been identified and validated as shown in Fig. 6.3

6.2 Conclusion

Cancer continues to be a significant societal burden all over the world. Molecular biomarkers are essential for human cancer therapy, diagnosis, and detection. The fourth-most fatal maternal illness is ovarian cancer, the most deadly kind of cancer

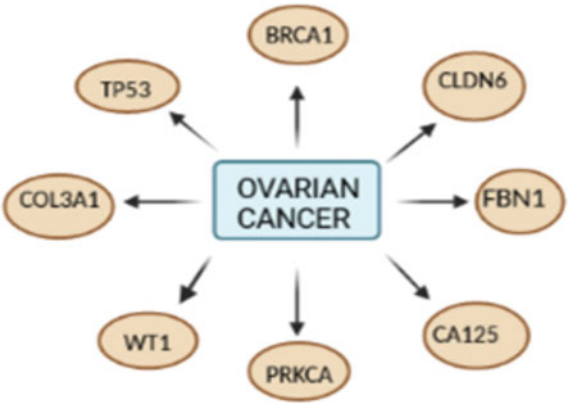

Fig. 6.3 Utilising bioinformatics methods and certain diagnostic biomarkers for ovarian cancer have been identified and validated

in women. Epithelial ovarian cancer (EOC) accounts for 90% of ovarian cancer cases, with severe ovarian carcinoma instances accounting for the remaining 10%. Organogenesis is normally when HOX genes are expressed. However, severe ovarian cancer was discovered to have HOXA9 and HOXA11. It caused the conventional understanding of the role played by the ovarian surface epithelium in ovarian cancer to be re-examined. Finding the precise gene and mechanism of ovarian cancer is essential. The most often occurring genes associated with epithelial ovarian cancer through multiple pathways are TP53, BRCA1/2, PIK3CA, and others. Finding the ovarian cancer gene is crucial, but early detection is also important because the advanced stages of the disease are poorly understood. It is crucial to identify and validate early detection biomarkers that are highly specific to OC in order to develop minimally invasive screening methods for the disease. In-depth bioinformatics sources for ovarian cancer (OC) biomarkers are examined in our work. Significant ovarian cancer biomarkers include CREB1, CD38, CA125, and SIRTs (1–7). Some novel biomarks were extracted from gene expression omnibus (GEO) databases using mRNA microarray datasets.

SMC4, RRM2, MAL, WT1, HMGA1, PSAT1, and others, such as GSE36668, GSE18520, GSE14407, GSE447841, and GSE26712, amongst others, all play a crucial role in biomarker for the validation of ovarian cancer. Using GEO and DEGs, several mRNA microarray datasets are examined. Using many bioinformatics tools, all the ovarian cancer biomarkers were discovered and validated. For example, POTEE (POTE ankyrin domain family member E), GEO2R programme, functional enrichment databases like ONCOMINE and GEPIA databases, gene set enrichment analysis (GSEA), tumour immune estimation resource (TIMER), Kaplan–Meier plotter, etc., were used for the validations. Using DEGs, a large number of hub genes for ovarian cancer were discovered, and these genes were also discovered as novel biomarkers. More investigation is necessary to pinpoint the precise molecular pathways governing OC development using these bioinformatics approaches because ovarian cancer has a large number of molecular biomarkers.

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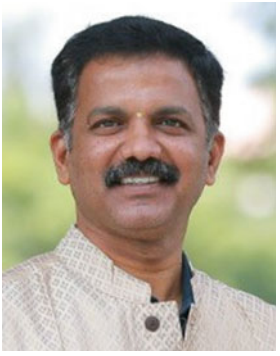

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Chapter 7
Application of Biomaterials in Cancer Research

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Contents

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Abstract Biomaterials can function efficiently once it has been implanted and is designed to work with biological systems to provide therapeutic response and aid in disease detection. Because they are a fusion of science in the research and devotement fields, biomaterials can be recognized. In many fields, including molecular biology, cellular biology, material sciences chemical sciences, and engineering and biomaterials are crucial. The competent and reliable individuality of the biomaterials makes their use in a physiological system possible. These biomaterials are meant to be used specifically with a variety of material types, including polymers, metals, composites, and ceramics. Cancer is the most common cause of serious illness and death globally. There are many distinct types of cancer research being conducted worldwide due to the unpredictable nature of the disease. Cancer treatment is still difficult because cancer cells have endogenous mechanisms for cell division and proliferation that differ from person to person. The utilization of biomaterials in the management of cancer is a vast topic for research because it involves both conventional and other new treatment modalities. The chapter that discusses the utilization of biomaterials as healing substances, with their use as vaccines and surface modulators to improve the antigen, has evaluated such studies carried out in the field of biomaterials. In this study, the novel biomaterial consisting of formulation for systemic delivery and implanted instruments issummarized. This chapter also focuses on the current advancements and utilization of biomaterials in cancer/tumor diagnosis and treatment.

Abbreviations

APCs	Antigen-presenting cells
BCRP	Breast Cancer Resistance Protein
CMC	Critical Micelle temperature
CpG-ODN	Cytosine-phosphate-guanosine-oligodeoxynucleotides
CRT	Calreticulin
CTLA-4	Cytotoxic T-lymphocyte–associated antigen 4
DCs	Dendritic cells
DEX	Dextran
DNA	Deoxyribo-neuclic acid
DOX	Doxorubicin
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HSPs	Heat shock proteins
ICD	Immunogenic cell death
ICG	*Indocyanine green*
IDO	Indoleamine 2,3-dioxygenase-1

ISPI	In situ photo immunotherapy
IV	Intravenous
LCP	Lipid Calcium Phosphate
LNPs	Lipid Nanoparticles
MN	Microneedle
MRI	Magnetic Resonance Infrared
MTO	Mitoxantrone
NIR	Near Infrared Radiation
NLCCs	Novel nanostructured lipid-carrageenan hybrid carriers
NPs	Nano Particles
PCL	Poly-epsiloncaprolactone
PD-L1	Programmed death-ligand 1
PEG	Poly Ethylene Glycol
PLA	Poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
PTX	Paclitaxel
ROS	Radiative Oxygen Spices
TAAs	Tumor-associated antigens
TEM	Tumor Microenvironment
TLR	Toll-like receptor

7.1 Introduction

Biomaterials are a substance that is utilized to substitute a live structure or becomes a component of a biomaterial device so that biomaterials can interact with the human biological system. To put it another way, it is a non-viable substance that can heal, substitute, and improve organs, tissue, and bodily processes [1]. The applications of biomaterials can be prolonged to include tissue, blood serum, prosthetics, biological fluids, diagnostics, and treatments, as well as energy storage. The live creature and its constituent parts should not be adversely affected, though, which is a crucial necessity. The fields of cellular and "molecular biology, chemistry, materials sciences, and engineering" all perform a supporting character in the research and improvement of biomaterials. These topics control the entire area, which has made a significant contribution to the study of how materials interact with the relevant physiological environment, or "bio interface," as it is known. The expansion of biomaterial in the context of medical research has been a recent topic of study, with various anticipated positive outcomes. Researchers from the fields of biotechnology, microbiology, and oncology have reported using biomaterials with a high success rate [2, 3].

Cancer prognosis, diagnosis, and therapy have advanced therapeutically over the past 50 years, refining the results and excellence of lifespan for "millions" of patients every year. Though, several ongoing issues continue to restrict our capacity to halt cancer as soon as it is discovered and to stop relapse after a successful course of

treatment. Drug toxicity and poor selectivity are just two of the obstacles preventing these expansive aims from being achieved. One of the major contributions of bioengineering is biomaterials, which are used in many cutting-edge methods for the treatment of cancer. Biomaterials are found in different natural or synthetic sources and are utilized in a variety of medical applications for supporting, nourishing improving, or replacing broken tissue or physiological function and a range of fields of medical science like biology, medicine, material science tissue engineering physics, and chemistry, and are also mixed-up with the modern field of biomaterials.

7.2 Classification of Biomaterials

7.2.1 First-Generation Biomaterial

The first generation of biomaterials, which started in the 1950s, consisted heavily of materials chosen for medical use that also provided the least amount of impact on the host tissue and were recognized as biocompatible. This is made up of "pyrolytic carbon" and silicon rubber, which is used to coat mechanical valves.

7.2.2 Second-Generation Biomaterial

The biomaterials from the second generation are those that have been linked to the original or first generation. They are designed to have a regulated reaction along the tissues where they are implanted to produce therapeutic effects. Examples of clinical applications of biomaterials include bone disease, dental surgery, and the usage of bioactive glasses and ceramics. These biomaterials also contain substances that are resorbable and have variable breakdown rates. Because of their biodegradable nature, biomaterials form a terminal between the place of implantation and the host tissue that can be removed over time [4].

7.2.3 Third-Generation Biomaterial

The third generation of biomaterial is the subject of the majority of current analyses since it suggests contemporary defenses against dangers. Biomaterials encourage the renewal of physiological tissues, which serves as the foundation for "tissue engineering" and "regenerative medicine". Utilizing living cells, "tissue engineering" aids in tissue development and regeneration to produce therapeutic effects. With high success rates for high-quality outcomes, tissue engineering has been widely used in bladder, cartilage, and skin replacements [5] (Fig. 7.1).

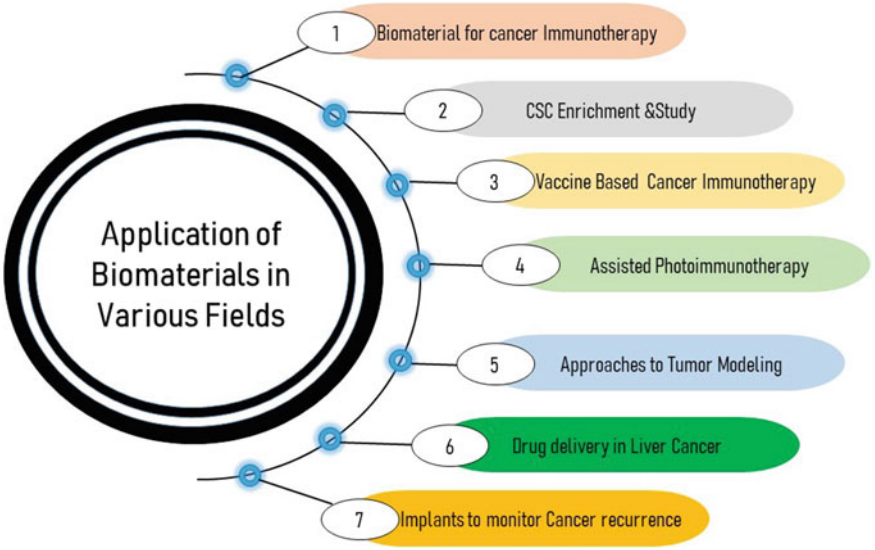

Fig. 7.1 Biomaterials are used in cancer research

7.3 Biomaterial for Cancer Immunotherapy

Cancer immunotherapy, which has been around for a while, offers hope for the recognition and treatment of cancer in cancer survivors. But because it has some restrictions that need to be made clear, a new upgrade was necessary. Several restrictions related to adverse reactions, localized delivery on and over activation of the immune system, and immunomodulatory [6]. This cancer treatment has the potential to provide patients with care that is both more precise and safe than current treatments [7]. The materials are intended to induce a potent primary and secondary anticancer immunological impact, which will inhibit metastasis and tumor growth by strengthening or mending biological processes that have been weakened or destroyed along the course of the disease [8]. Delivering cancer immunotherapy locally, using biomaterials in the form of implants, transdermal patches, or injections can help with the issue. The following are explanations of various biomaterials.

7.3.1 Implantable Biomaterials

With the use of a quick surgical operation, "implantable biomaterials", which comprise biomaterials scaffold, are inserted subcutaneously stead of the removed tissue. Since the implantation, the biomaterials have been preloaded with components, anticancer agents, cells, tissue, or other elements necessary for the procedure, and are subsequently released in a regulated way from sizable scaffolds that have

been made just for them. These manufactured porous scaffolds stimulate immune cells to the implantation site. Alginate, polyglyconate, collagen, hyaluronic acid, porcine gelatin, and poly(lactide-coglycolide) are among examples [9, 10].

7.3.2 Injectable Biomaterials

Injectable biomaterials include injectable biomaterial scaffolds, and thus, issues associated with operations and implants may be eliminated. Combination therapy for malignancies, such as radioisotope or chemotherapeutic therapy, is ideally suited for injectable biomaterials. At room temperature, the injectable has an impact on a liquid's properties such as its condition the liquid can convert into in situ gelling system and shows biocompatibility properties. Inorganic scaffolds showed the regulated and localized release of anti-cancerous chemicals by the use of cryogel and hydrogel [11]. Numerous studies have shown promising results in malignancies and tumors after the application of injectable scaffolds. For the management of breast and ovarian cancer, Wang et. al. designed the usage of fibrin hydrogel in cargos cyclophosphamide and anti-neoplastic drug in mouse models and showed suppress the recurrence of the tumor following surgical therapy to estimate drug delivery [12, 13].

7.3.3 Transdermal Biomaterials

They consists of surgically implanted or injected devices in the transdermal area. This method has proved successful in treating melanoma, and this technique also treats effectively severe skin cancer. At a specific rate, the active moiety leaves the patch and travels through the skin and into the bloodstream. Both distinctiveness and low permeability should be present in transdermal biomaterials. They mainly deal with chemical enhancers, lipid enhancers, pressure waves generated via photoacoustic or ultrasound response, electric fields (electroporation iontophoresis), and lipid enhancers. Though the skin is punctured, the method is to employ microneedles. Examples include poly(lactic-coglycolic acid), polycaprolactone, polygranulocyte-macrophage, colony-stimulating factor, and trimethylene carbonate [14, 15].

7.3.4 Novel Class of Biomaterials is Utilized for Cancer Immunotherapy

(a) **Lipid-containing Biomaterials**

APCs are the utmost effective professional DCs and have the capacity to combine both adaptive and innate immunity. APCs, especially DCs, should seize on antigens to produce cytotoxic CD8 + T cells (CTL) that can manage malignancies [14], while cellular DC vaccines, which deliver adjuvants and antigens directly to DCs in vivo, need a time-consuming and expensive research approach, lipid-based nanobiomaterials like liposomes have been studied [16] (Table 7.1).

(b) **Polymer-related biomaterials**

(i) ***Micelles***

Amphiphilic polymers self-assembled into micelles, which are nanoparticles, when the CMC was attained [17]. In the emptying lymph nodes, smaller neutrally charged nanosized particles may have more systemic accessibility. To create an amphiphilic molecule (PSA) and PSA micelles that were specifically stored in draining lymph nodes as well as systemic regions to produce systemic toxicity and speed up DC maturation, Zeng et al. added stearic acid to polyethyleneimine (PEI)-2k. Additionally, polysialic acid micelles made through tyrosinase-related protein-2 produced beneficial anticancer action and promoted the secretion of chemokine receptor-7, the expression of cluster of differentiation 86, and the manifestation of main "histocompatibility complex" category-draining lymph nodes [18]. By inducing macrophages and increasing NO production in the tumor insertion location, poly(L-arginine)-based micelles blocked by the poly(ethylene glycol), delivered to a tumor region can inhibit tumor growth [19].

(ii) ***Hydrogels***

Because of their ability to gel, hydrogels can serve as caverns for storing antigens, and they have been employed as carriers of DNA, cytokines, and proteins [20]. Approximately ten years ago, alginate microparticles containing injectable gels were studied and employed to co-deliver developed, dendritic cells, chemokines CCL21, and CCL19. According to the research, this hydrogel formulation can draw host dendritic cells to the injected area and cause them to move to nearby lymph nodes simultaneously, indicating that an ongoing process to trigger the immune response was possible.

(iii) ***Nanoparticles made from iron oxide***

Since Fe_2O_3 NPs have been accepted for employs in humans as a "magnetic resonance imaging" (MRI) contrast substance and because the byproducts of their disintegration are beneficial for the body's iron storage, they have gradually been used for both cancer imaging and cancer immunotherapy. The anticancer immunological impact of Fe_2O_3 nanoparticles can be boosted by adding different cargos, like heat shock

Table 7.1 Different biomaterials containing formulations

Example of Biomaterial	Made by	Potential vector of immunotherapy e.g.	Composed by	Mechanism of action
(a) *Liposomes*				
Lipid-based nanobiomaterials	Phospholipid bilayer	Vaccines Hepatitis A (Epaxal®), Malaria (Mosquirix), influenza (Inflexal®)	Melanoma-specific TRP2180-188 peptide and customized through the immune adjuvant CpG-ODN and DC-targeting mannose A cationic and mRNA (R), Polymer (P), cationic liposomes fabricated dramatic mRNA delivery system	Liposomes improved the tumor antigen-specific CD8 + (Called as cytotoxic T lymphocyte cells), and this indicates the tumor cell proliferation and prevention of tumor angiogenesis DC-targeting and showed their cogent anti-tumor effect
(b) *Lipid/calcium/phosphate (LCP) nanoparticles*				
Lipid-based nanobiomaterials	Lipid-coated cap		Added an anti-Checkpoints T-lymphocyte-associated protein 4monoclonal antibody to the Lipid Calcium Phosphate-based m-Ribonucleic Acid vaccine that was given to dendritic cells to contain the tumor antigen MUC1	Extended the T cell immunological impact and demonstrated the effectiveness of LCP as a carrier for tumor-associated RNA. Additionally, a vaccine (HSP70pCM-CaP) based on LCP that mimics cancer cells was described to prevent tumor invasion and eradicate tumor cells
Polymer-based biomaterials				

(continued)

protein and poly(I: C), CpG-ODN, ICG, and 70 (Hsp70). Iron oxide nanoparticles can distinguish between imaging and therapy.

(iv) ***Gold nanoparticles***

An effective therapy that combines laser photophysical properties with immunoregulation is called photothermal immunotherapy [21]. In recent years, the use of gold nanorods, NIR photosensitizers, Prussian blue, and other photothermal biomaterials has been helpful for cancer immunotherapy. The possibility that melanoma-specific

Table 7.1 (continued)

Example of Biomaterial	Made by	Potential vector of immunotherapy e.g.	Composed by	Mechanism of action
Polymer-based biomaterials	PLGA		Cancer cytomembranes	DCs' surface biomarker expression may be induced by NPs. Additionally, this strategy is advantageous for the administration of antineoplastic drugs since it may be utilized to trigger the origin of neoplastic cells through an "isotypic" combining process
			United by an "anti-PD-1 monoclonal antibody"	By increasing cytotoxic T lymphocyte cells and overcoming the tumor immunosuppressive TEM suppression, synergistic effects on tumor immunotherapy can be achieved
Inorganic biomaterials				
Siliceous nanoparticles	Organo-silane Precursors method Participate in a hydrolysis condensation reaction			A Th1 anticancer immune impact was demonstrated by silica NPs modified with amino acids, silica nanospheres doped with metals, and cytokine generation with Mg, Ca, and ZN (MS-Mg, MS-Ca, and MS-Zn)

T cells may be noninvasively visualized using traditional X-ray computed tomography increases when they have been gold NP tagged, making this technique useful for identifying path immune cells in immunotherapy. Another intriguing finding is that gold nanoparticles can predict the therapeutic impact of immune checkpoint blockage following alteration with PD-L1 antibody [22]. To strengthen anticancer immunological effects and advance cancer immunotherapy, adjuvants may also be used in conjunction with gold-based NPs.

7.4 Engineered Biomaterials for Cancer Immunotherapy

There have been various discussions of the benefits of synthetic biomaterials for cancer immunotherapy. In this study, we also demonstrated the localized delivery of the biomaterials that support this cancer treatment therapy, including hydrogels and immunotherapy [23], and microneedles. The incorporation of cancer immunotherapy and engineered biomaterials, it results in a novel approach to chemotherapy; it is hoped that this study can assist curious nonexperts in understanding the future forecast, recent problems, and developments (Table 7.2).

7.5 Biomaterials for Vaccine-Based Cancer

7.5.1 Integrating Cancer Vaccines and Biomaterials

As was shown in the prior section, a variety of biomaterials are utilized in the treatment and diagnostics of cancer, with variable results. Since there are not any efficient delivery methods, the results of the numerous vaccine experiments we covered have combined [32]. A key illustration is provided by peptide cancer vaccines, which have a clinical response rate of roughly 3% when administered to cancer patients. When cancer patients' dendritic cells are separated, reacted with peptides ex vivo, and then pumped return into them, a better clinical treatment response rate is seen. The diversity in individual responses and their poor performance without a DC delivery system show that bare peptides have difficulty penetrating dendritic cells in vivo [33]. Consequently, biomaterials are a prerequisite to reducing the biological barriers to vaccination administration in vivo [34]. As cancer vaccination methods vary, collective biomaterials are desirable to minimize the several drug delivery barriers. Because of this, biomaterials for cancer vaccines range in size from the nano-scale (such as polymeric and liposomal nanoparticles) to superior injectable or implantable synthetic scaffolds (Table 7.3).

Table 7.2 Biomaterial used in cancer immunotherapy

Delivery system	Biomaterial used	Loaded with	Types/cell lines	References
Implantable scaffold	Poly(lactide-co-glycolide)	Granulocyte-mcrophage colony-stimulating factor/CpG	Skin cancer/B16F10 melanoma	[24]
	Mesoporous silica	GM-CSF	Black 6	[25]
Injectables/spreadable hydrogel	Hyaluronic acid/polycaprolactone	GM-CSF/OVA	Murine B16-OVA melanoma cell	[26]
	HA/TCM/γ-PGA	OVA	C57 Black 6 Mice	[27]
	Factor Ia	CTX/anti-Pd-L1	Triple negative breast cancer 4T1, ID8 ovarian cancer	[11, 28]
	Alginate	Cat/CpG/^{131}I	4T1 murine breast cancer tumors; PDX grew in mice: rabbit VX2 liver tumors	[29]
	Factor Ia	CaCO3/anti-CD47	Skin cancer/B16F10 melanoma	[30]
Tansdermal MN	Hyaluronic acid	Anti-PD-1/GOx	Skin Cancer/B16F10 melanoma	[31]
	Polyvinyl alcohol	Anti-PD-L1/1-MT	Skin cancer//B16 melanoma	[32]

Table 7.3 Different delivery systems based on biomaterials

Delivery system	Carriers	Targeted organ and tissue	Reason	Assessment	References
Nanoparticle-based delivery systems					
Lipid nanoparticles liposome polymeric nanoparticles, self-assembled nanoparticles	Nanoparticle	Tissues such as solid tumors or the spleen or lymph nodes	For enhance delivery of vaccine	Self-assembled nanoparticles have been demonstrated to effectively deliver nucleic acid- and peptide-based vaccinations because they usually have high loading capacities	[35]
				Due to their success in delivering siRNA in the past, liposome nanoparticles have been extensively employed to deliver mRNA vaccines	[36]
				While both LNPs and self-assembled nanoparticles are insufficient to target specific antigen types, both rely heavily on charge-based complexation in their nanoparticle compositions	[37]

(continued)

Table 7.3 (continued)

Delivery system	Carriers	Targeted organ and tissue	Reason	Assessment	References
				Antigens with easily changeable sequences may consequently be better suited for self-assembled nanoparticles. Such as specified charges (such as DNA or mRNA) or (such as a peptide), but polymeric particles or liposomes may be more effective for other antigen classes (i.e., tumor lysate, tumor cell, or protein)	[37]
Scaffold-based delivery systems					
Polymeric and hydrogel-based scaffolds system	**Used scaffolds** Mesoporous silica micro (MSRs, alginate-based hydrogels PLGA		To induct and amended immune cells to evoke an anti-tumor response [112]	Cryogel and PLGA scaffolds have made significant progress in encasing tumor cell antigen acquisition. While PLGA is typically used to encapsulate tumor lysate antigen, cryogel is used to encapsulate and deliver irradiation to entire tumor cells	

(continued)

Table 7.3 (continued)

Delivery system	Carriers	Targeted organ and tissue	Reason	Assessment	References
Biomaterial vaccines for delivery to lymph nodes (LN)					
		Lymph node (LNs) targeting		Additional surface modifications, such as the inclusion of a cell-penetrating peptide or a DC legend targeting pattern, are meriting attention to improve vaccination uptake in significant cells like "dendritic cells"	
		Dendritic cells targeting		Due to the ability to attach a dendritic cells targeting peptide to the peptide epitope during the duration of vaccine manufacturing, this method has proven to be particularly effective for peptide-based vaccines	

7.5.2 Biomaterials for Tumor Targeting and Alteration

(a) ***Immunomodulators transform the tumor into an antigen repository***

A surgeon by the name of William Coley discovered in the nineteenth century that repetitive intratumoral injection of bacterial lysate minimizes the expansion of cancer [38]. But it was not till about a century later that scientists realized that the immunomodulator CpG was the vital substance of the lysate that stimulate tumor regression. Subsequently, this finding, directly administering immunomodulators to

tumors, has gained popularity as a method of cancer immunotherapy. Immunomodulators do not exhibit antigens, but by triggering tumor cell death and producing tumor antigens locally [39] it can convert tumor areas into antigen stores. DCs that are either already present in the tumor stromal region or are drawn to their afterward take up tumor antigens. Upon reaching adulthood, DCs move to LNs where they produce systemic anti-tumor immunity.

(b) ***Intra-tumoral injection***

The intratumoral method is the first and most accurate approach for administering immunomodulators to the tumor site which is an intratumoral injection [40]. Intratumoral injections have utilized several kinds of immunomodulators, including cytokines, antibodies stimulators of interferon genes (STING), TLR agonists, and chemotherapeutics. Even though these immunomodulators are tiny and can easily escape the tumor and enter the bloodstream in a matter of minutes, they exhibit systemic toxicity [41]. To improve the holding period of immunomodulators at the tumor site, many kinds of biomaterials have been created. The retaining of immune modulators in the tumor microenvironment has improved thanks to the utilization of particle-based drug delivery systems like polymeric NPs, inorganic nanoparticles [42], and liposomes. These systems have also reduced biological toxicity. Formulation-related hydrogels are also a helpful platform for enhancing medicine retention at tumor sites. Therapeutics can be released gradually with precisely calibrated kinetics thanks to their various degradation profiles [34, 43].

(c) ***Systemic injection and tumor targeting***

Although intratumoral injections have the potential to be beneficial, one obstacle to their widespread success is that less treatable and widely distributed metastatic cancer cannot be treated with them. New methods have been developed that make use of vaccinations that can be delivered in an organized manner and reach the tumor location to lower this barrier [44]. To enhance the efficacy that the medicine will reach the tumor area when triggering solid tumors with injectables, drug distribution periods in the circulation must be extended.

7.6 Biomaterial Implants to Monitor Cancer Recurrence

A foreign body reaction caused by synthetic biomaterials introduced into the body is distinguished by fibrosis and persistent inflammation. To create a “metastasis sensor” for the challenge of cancer chemotherapy research, “Oakes and colleagues” exploited biomaterial implants and their corresponding immunogenic features. A scoring system was created using “computational analysis” of the patterns of gene expression found in implant biopsies to forecast the availability of malignancy. This unexpected utilization of biomaterial for the initial diagnosis of neoplastic cell creates a more precise systemic sample associated to blood or liquid biopsies and reduces the

requirement for inefficient imaging by reducing the necessity for biopsy specimens from likely metastatic target tissues [45].

Synthetic biomaterial represents novel chances to increase the few available tools for detecting metastasis of tumors and determining tumor recurrence. While more invasive than blood sample collection, a biopsy of a subcutaneous implant is probably less harmful than one of an internal organ with a major risk of metastasis, like the lung. Additionally, this procedure escapes issues with tissue samples because a biomaterial implant has dimensions that are well-established for exact biopsy insertion. Only a portion of the equation involved a fresh diagnostic tool to observe the biomaterial immune impact and predict disease development. To convert the implant impact into usable and predictive information, accessible computational tools and multiplex gene expression were crucial. These prove helpful in reorganizing our conception of how we collaborate via biomaterials and their benefits for cancer research.

7.7 Biomaterial Strategies to Modulate Cancer

7.7.1 Cancer Molecular Markers

Biomaterials have lately gained prominence in cancer diagnosis investigation. Via changing the capabilities of conventional diagnostic agents, biomaterials are frequently adept in successful current diagnostic potentiality for cancer. The prognosis for cancer is considerably improved by early diagnosis. Different methods for diagnosing cancer are made easier by biomaterials.

Some e.g. of biomaterials that have been useful for enhanced cancer diagnosis

(a) ***Quantum dots***

Diagnostic procedures must be sensitive, repeatable, and reliable. This practice is used to demonstrate the utilization of fluorescent biomaterial in detection to identify specific bimolecular interactions. Fluorophores offer a chronic way to obtain precise pictures and sensitivity through enough structural resolution [46] (Table 7.4).

This chapter uses SPIONs that are 30 nm in size to demonstrate how adding folate to a tumor model of the human nasopharyngeal epidermal carcinoma, which upregulation of the folate receptor, significantly enhances magnetic resonance contrast. After being delivered to the tumor with folate, these particles produces enhanced contrast for more intensified recognition of cancerous cells, potraying them as a potential option for MR imaging of malignancies.

Table 7.4 Nanocarriers for contrast agents

Nanocarriers	Produced by	Size range	Result	Biomaterials used
Polymeric nanoparticles	Natural or artificial polymers	10–1000 nm	Polymerization of monomers	PLGA PLA PCL chitosan human serum
Liposomes	Hydrophobic contact causes the impulsive attachment of amphiphilic phospholipids in an aqueous environment	50–200 nm	Sonication or Extruction for in vivo application	Synthetic phosphocholine or Natural (egg or soy)
and micelles	Hydrophobic and hydrophilic segments	10–100 nm	Hydrophobic interactions, which cause the non-polar copolymer segments to form a micellar core, are the development's driving force	Superparamagnetic
Superparamagnetic iron oxide nanoparticles (SPIONs)				
Nanoparticle	Iron oxide core surrounded by a water-loving polymer coating	5–300 nm in diameter	Iron oxide's paramagnetic properties make it possible to accelerate MR relaxation durations and create picture contrast at the site	Starch, Dextran, PLGA, Alginate, and PEG

7.7.2 Biomaterials for Cancer Therapy

Prospective advancements in the treatment of cancer are also provided by advances in biomaterial engineering. The only available cancer treatment choices historically have been surgical removal of the prime tumor mass followed by supportive fractioned radiotherapy and chemotherapy to damage any residual malignant tissues and maybe prevent a recurrence. In addition to enhancing the effectiveness of this traditional treatment, research in the field of biomaterials has also been able to suggest new ways of therapy.

(a) ***Biomaterials for local and systemic drug delivery***

One of the main goals of the development of the biomaterial system has been to enable more efficacious cancer therapy and, generally, proper formulation. Much of what was previously discussed about the distribution of diagnostic elements are accurate is in the formulation, in that the utilization of biomaterials substrate

allows for the adjustment of the pharmacokinetics of the payload medicament. Cancer chemotherapy is characteristically administered IV, which results in minimal circulation times, restricted triggering, and limited permeation of cancerous tissues. Excluding hydrophilic drugs from the equation and using high doses can result in harmful effects on healthy tissues, mainly blood-filtering organs. Currently, it is the potential to administer IV treatment while simultaneously avoiding some of these discussed through the use of biomaterial-based drug delivery methods.

The primary substrate material is a component of biomaterial drug delivery systems. The materials that are available range from synthetic to the natural polymer.

(b) ***Alternative and novel biomaterial-based cancer treatment methods***

There are numerous alternative methods for cancer treatment using biomaterials that do not characterize conventional formulation patterns, even though we have shown with considerable strength how traditional chemotherapy can be improved by biomaterials.

(i) *Enhancing surgical intervention*

These technologies have also been used to define tumor limits before or at the time of the surgical procedure (through intraoperative magnetic resonance imaging) to improve the doctor's visual observation of once almost enough tumor has been eliminated or to explain once the resection starts to affect the nearby probably delicate, healthy tissues. The expansion of tissue engineering methods for the re-establishment of bone, tissue defects, and in some cases, entire organs have also been aided by biomaterial [47]. The availability of these technologies is mainly significant when taking into account circumstances where tumor resection reveals a significant shortage in healthy tissue or where, in the absence of substitution tissue, the survival threat of the cancer patient would make surgical treatment an impractical option.

(ii) *Enhancing radiotherapy*

Typically, radiotherapy involves either the native implantation of a radioactive substance in cancer tissue or the external application of a radiation beam or brachytherapy, both of which can be enhanced utilizing biomaterials. It is possible to use radiofrequency responsive elements, like gold, ceramic microspheres, or iron oxide nanoparticles, to increase the response of externally applied radioactive or to enable the use of minimal efficacy radiation doses; it is crucial for eradicating malignancies that have spread deeply. Azab et al. have shown that radioactive source elimination, which generally necessitates a separate surgical procedure, can be avoided by using implantable biodegradable CHI-hydrogel to manage the separation of radioactive chemicals away from the healing area into the bloodstream [48].

(iii) *Hyperthermia*

Using ferromagnetic or NIR-responsive nanoparticles directly to thermally ablate tumors is an alternative usage for these materials. For instance, the NIR-responsive

nanoparticle Auroshell, which has a silica core and a gold shell, thermally kills tumor tissue when exposed to NIR laser light from the outside and is presently undergoing clinical trials for neck and head cancer.

(iv) *Immunotherapy*

The challenge of cancer immunotherapy is to stimulate the immunological system in a direction to prevent tumor progression, in contrast to traditional immunotherapy, which has been employed to prevent an undesirable immunological impact. To confirm that the effect is restricted to the tissues of the tumor and that an adequate tumor-preventive immune action is produced, the goal is to give a focused, prolonged, and localized activation of the TME. In an animal model of metastasis melanoma, Park et al. delivered immunostimulatory chemicals, like "transforming growth factor beta" (TGF-β) and interleukin-2 inhibitors, through nanoscale liposome through a biodegradable polymer core to expressly increase animal survival and delay tumor progression [49]. Regulatory T cell suppression through biomaterial-related "danger" signal-mimicking adjuvants, implantable three-dimensional immunoregulatory niches, and nanoparticle-mediated cancer vaccination are other new methods for employing biomaterial in cancer immunotherapy [50].

7.8 Biomaterials Approaches Tumor Modeling

This method clarified several molecular abnormalities that are currently the subject of clinical investigation. However, tumor cells do not exist in a vacuum, and it is now well-accepted that the microenvironment shows a significant role in controlling the progression of cancer. The success of immune-oncology today is predicated on the idea that preventing tumor growth is more effective to treat the microenvironment than the tumor cells themselves in preventing recurrence. The tumor microenvironment, on the other hand, is compound and contains changed, extracellular matrix (ECM) deposition, mechanical indications, and cellular composition, all of which we have yet to fully understand. Model methods are needed to summarize the tumor-microenvironment interaction in vivo to further our understanding of how these many biophysical and biochemical components regulate tumorigenesis.

To summarize, 3D tissue-engineered tumor models promise to advance our novel understanding of cancer by enabling computers to analyze and summarize significant possessions of tumor microenvironment interaction. A deeper understanding of the causes of tumor start, development, progression, metabolic modification, and immune evasion is necessary. While targeting characteristics of tumors except for the tumor cells directly has revealed efficacy in the clinic, healthier, further complicated models are required. Tissue engineering has enormous potential for the acquisition of an additional thorough understanding of the fundamental biology and physical mechanisms, finally boosting the treatment of cancer patients. The contributions that are available in this particular matter serve as a model for improvement in this last section.

7.9 Biomaterials Used in Liver Cancer Treatment

Cancer, especially hepatocellular carcinoma (HCC), is a unique and complex illness, making it difficult to diagnose and treat. Biomaterial's adaptability and multifaceted nature offer potential solutions to some of these problems. By modifying their various properties, such biocompatible substances can be easily created and tailored for utilization in the investigation, recognition, and treatment of hepatic cancer. After that, we provide the many biomaterials that have been created to increase the delivery of drugs in applications for hepatocellular carcinoma, which are included in Table 7.5.

7.10 Biomaterial-Assisted Photoimmunotherapy for Cancer

Under the proper light irradiation, phototherapy, a collection of non-invasive important approaches often uses phototherapeutic chemicals to specifically kill tumor cells. "Photodynamic therapy" and "Photothermal therapy" are two common categories. To effectively convert photoenergy into heat and destroy cancer cells, PTT typically uses light-absorbing materials like gold nanostructures, various carbon nanomaterial, morphologies, sulfides, and transition metal oxides, in addition to several other organic and inorganic nanoparticles.

A type of light-triggering indigenous treatment known as phototherapy, which includes PTT and PDT, has numerous distinct advantages, such as increased selectivity and controllability as well as reduced biological harmfulness. Photothermal therapy (PTT) focuses on "light-absorbing" materials to efficiently transform light energy into heat, raising the tumor's normal temperature to destroy cancer cells. Contrarily, PDT relies on photosensitizers, which can transmit light energy to the oxygen molecules in the area, producing cytotoxic $^1O_2$ that can harm cancer cells. For the two different forms of light-triggered therapies, the therapeutic efficacy of diverse biomaterials has recently been studied.

7.10.1 Biomaterial-Assisted Photothermal Immunotherapy

(a) ***Conventional phototherapy that activates the immunological system to fight cancer***

Numerous biomaterials with high NIR absorbance have been studied in modern years for "photothermal therapy", which is useful for treating local tumors since neoplastic cells are sensitive to high temperatures. However, the main obstacle to photothermal therapy is the spread and recurrence of malignancies [69]. The optimal PTT should be capable of managing tumor spread and recurrence in addition to removing the primary

Table 7.5 Few Biomaterials that have been looked into for liver cancer treatment

Drug delivery system	Biomaterials	API	Targeting Agent	Types of therapy	References
Nano-diamonds					
Nano-diamond	Carbon	Epirubicin		Chemotherapy	[51]
PLGA particles					
Nanoparticles	Charge reversible pullulan-based (CAPL) shell and poly(β-amino ester) (PBAE)/poly(lactic-co-glycolic acid) (PLGA) core	Combretastat in A4 (CA4) and paclitaxel (PTX)	Polysaccharide pullulan backbone	Anti-angiogenesis and chemotherapy	[52]
Nanoparticles	Poly-D,L (lactide-coglycolide) (PLA)	5-FU	Anti-SM5-1	Cancer therapy	[53]
Microspheres	Poly(lactic-co-glycolic acid) (PLGA) core surrounded by a poly(L-lactic acid) (PLLA) shell layer	Chitosan-DNA NPs (chi-p53) and/or Doxorubicin (DOX)		Gene therapy and cancer therapy	[54]
Nanoparticles	Poly(gamma-glutamic acid)-poly(lactide)	Paclitaxel	Galactosamine	Cancer therapy	[55]
Nanoparticles	Biotin-/lactobionic acid modified poly(ethylene glycol)-PLGA-poly(ethylene glycol) (BLPP)	Curcumin (CUR) and 5-flurouracil	Biotin/lactiobiomic acid	Natural therapy/cancer therapy	[56]
Natural polymer-based particles					
Nanoparticles	Gelatin	Doxorubicin-lactose	Lactose	Cancer therapy	[57]
Microspheres	Gelatin and chondroitin-6-sulfate	IL-2		Immune therapy	[58]
Nanoparticles	Chitosan	None		Anticancer	[59]

(continued)

Table 7.5 (continued)

Drug delivery system	Biomaterials	API	Targeting Agent	Types of therapy	References
Nanoparticles	Chitosan	None		Chemotherapy and anti-angiogenesis	[60]
Nanoparticles	Chitosan	Trans-resveratrol	Biotin and avidin	Phytoconstituents	[61]
Nanoparticles	Chitosan	Plasmid DNA with granulocyte–macrophage colony exciting factor, interleukin 21, internal ribosome entry site, and retinoic acid early transcription factor-1	Biotin	Gene therapy	[62]
Metallic particles					
Nanoparticles	Galactosylated-carboxymethyl chitosan-magnetic iron oxide (Gal-CMCS-Fe_3O_4)	RAS association domain family 1A (RASSF1A) Gene	Galactose	Cancer therapy and gene therapy	[63]
Nanoparticles	Ultra small SPIONs	SM5-1	Anti-SM51	Immunotherapy	[64]
Nanoparticles	Gold	mIR-375	Au	Gene therapy	[65]
Nanoparticles	Au conjugated with sodium citrate or polyamidoamine dendrimers (PAMAM)	None	Au	Cancer therapy	[66]
Nanoparticles	Gold	SM5-1	Au	Immune therapy	[67]
Nanoparticles	Au with a monolayer of L-aspartate	Cisplatin, capecitabine doxorubicin	Au	Cancer therapy	[68]

tumors that have been treated. In situ photoimmunotherapy (ISPI) was first proposed in 1997, combining photothermal therapy and showing immunological stimulation with an immune adjuvant. It was proven that following PTT dying cancer cells might release TAAs, thermally stimulate HSPs, and collaborate with their antigen. These antigens would be captured by APCs, specifically DCs, and accessible to T cells to promote the adaptive immunological system.

Even though photothermal therapy can attract various immune cells and excite the immunological system, these actions might not be sufficient to produce anti-tumor immune action. As a result, many studies have been conducted on a method that combines PTT and immune adjuvants to provide an immunological effect [70]. R837, a TLR7 (toll-like receptor7) agonist permitted via the "Food and Drug Administration" (FDA), was proven to further promote the immunological impact activated by photothermal therapy on cancer patients with older melanoma as a successful paradigm in a pitot clinical research [71]. Exciting, 11 patients with numerous cutaneous metastasis underwent ISPI in one or more 6-week therapy cycles by a local injection of "ICG and R837" and local radioactivity with a near-infrared laser. This was done to demonstrate robust therapeutic action to suppress tumor development and also metastasis.

(b) ***Immune adjuvants and phototherapy agents delivered simultaneously***

With the progress of biotechnology, there is an increased focus on utilizing biomaterials, which are capable of delivering immunostimulatory components to improve the anti-tumor action stimulated by photothermal therapy as well as serving as valuable photothermal therapy substances (Table 7.6).

7.10.2 Biomaterial-Assisted Photodynamic Immunotherapy

A different form of phototherapy, PDT, is capable of producing a significant extent of cytotoxic $^1O_2$ to promote the apoptosis and cell death of neoplastic cells. It is interesting to record note that "immunogenic cell death" (ICD), also acknowledged as the necrosis and apoptosis of cancer cells after PDT, can activate the immune system by promoting CRT exposure, increasing HSP expression, and releasing TAAs. Furthermore, the $^1O_2$ generated by PDT may trigger acute local inflammation, which is capable of drawing a range of immune cells into the tumor and providing several "pro-inflammatory" cytokines, like "interleukin IL-6, IL-8, (IL)-1", and "Tumor necrosis factor"-219. These cytokines may also activate the adaptive and innate immune system to target any residual neoplastic cells.

Even though photodynamic therapy-induced inflammation may boost the immunological system, it might not be adequate to effectively target anti-tumor immunological response. In general, TAAs released by dying cancer cells following PDT could trigger the immunological system activation, but inhibitory indications are also present to block the anti-tumor immune action. To enhance the immune response,

Table 7.6 Immunoadjuant based drug delivery system

Drug delivery system	PTT agents or immuno-adjuvants	Therapy	Mechanism	Result	Used in types of cancer	References
Chitosan-coated hallow CuS nanoparticles	CpG oligodeoxynucleotides/	Photothermal and immunotherapy	The temperature of tumors may increase during NIR laser irradiation, "burning" cancer cells and releasing TAAs into the environment. In the meantime, when the temperature rose, CuS's structure might deteriorate, mend, and change into polymer complexes to discharge CpG and enhance the tumor's plasmacytoid DCs' uptake of antigens and CpG	Prevent the expansion of equally primary and distant tumors	Tumors	[72]
Iron oxide nanoparticles	CpG	Immuno-therapy	These nanoparticles might more effectively collect in the tumor area in the presence of external magnetic fields	Potent photo-the immune result	Tumors	[73]
Bovine serum albumin bioinspired gold nanorods (GNRs)	R837	Mixed PTT-induced immunotherapy			Melanoma	[74]

(continued)

Table 7.6 (continued)

Drug delivery system	PTT agents or immuno-adjuvants	Therapy	Mechanism	Result	Used in types of cancer	References
Graphene oxide	CpG	Immuno-stimulation	Localized heating via graphene under NIR light irradiation may hasten nanoparticle transport within cells	Enhancing the immunostimulatory effects and CpG-influenced proinflammatory cytokines Outstanding PTT and immunological therapeutic effectiveness were attained in animal test		[75]
Nanoparticle (PC@gcpd(Gd))	Gadolinium ion (Gd3+), CpG oligodeoxynucleotides, and graphene quantum dots (GQD) stabilized by polydopamine for fluorescence/magn	Photo-immuno therapy	Enhancing the local temperature when exposed to laser light. More significantly, the concurrently administered CpG was able to activate tumor-specific T lymphocytes by further promoting the release of IL-1βα (pro-inflammatory) cytokines and supporting the growth of dendritic cells	Demonstrated a potent anti-tumor immunological response by preventing EMT6 mouse mammary cancers from spreading	Tumor	[76]

(continued)

Table 7.6 (continued)

Drug delivery system	PTT agents or immuno-adjuvants	Therapy	Mechanism	Result	Used in types of cancer	References
Liposomes	Small compounds with strong Near infrared absorbance, IR-7, and the immunoadjuvant HA-linked CpG (Ha-CpG) are examples of inorganic photothermal agents	Photothermal and immune therapy	Interesting, IR-7-induced photothermal reaction could enhance the release of TAAs and necrosis of cancer cells	The immunoadjuvant CpG's presence proved effective in promoting DC maturation, realizing beneficial antigen presentation, and stimulating T lymphocytes to identify and eradicate cancer cells		[77]
Transdermal microneedle containing (MN) patch delivery system	Melanin-containing B16F10 tumor lysate was put into the MN	Photothermal immune therapy	MN-loaded	The polymeric MN-loaded B16F10 tumor lysate demonstrated that photothermal immunotherapy may enhance T cell infiltration and cytokine release, clearly enhancing the survival of mice after tumor challenge and evoking recognized primary and metastatic tumors	Tumor	[78]

(continued)

Table 7.6 (continued)

Drug delivery system	PTT agents or immuno-adjuvants	Therapy	Mechanism	Result	Used in types of cancer	References
Self-assembled nanoparticles	Including R848, ICG, and CpG), and hydrogel biomaterials	Immuno-stimulatory	Getting immune compounds to release after being NIR triggered and working with the TAAs produced after photothermal therapy	Successfully trigger the immune system to stop cancer spread and recurrence		[79]
DNA hydrogel	CpG-containing hexapod-DNA with gold nanoparticles	Immuno-stimulatory	By being exposed to a NIR laser, the hydrogels could break down and release the hexapod-DNA that contains CpG sequences. and release the hexapod-DNA that	Getting fantastic anti-tumor results	Tumor	[42][80]

numerous efforts have been made to combine photodynamic therapy with a variety of immune system stimulators, checkpoint-blocking antibodies, small-molecule inhibitors, and adjuvants (Table 7.7).

7.10.3 Silk as Innovative Biomaterial for Cancer Therapy

Many spiders and insects produce silk, which is a fibrous protein. In the creation of cocoons, Web sites, nests, and egg coating as lifelines, they provide structural properties. The silks that can be most clearly distinguished are from domesticated Bombyx mori silkworms, as well as from Nephila clavipes, spiders, and Araneus diadematus.

The heavy chain (390 kDa) and light chain (26 kDa) proteins of fibroin make up the two most significant components of the silkworm silk derived through the "cocoon" of B. Mori. The sericin proteins that coat these core chains join the fibroin fibers together to form the intricate strands that make up the cocoon case. Large internal repetitive patterns bound through smaller terminal domains (C- and N-terminals) comprise the modular structure of silk proteins [88]. The pattern of the six amino acid residues like (Gly-Ala-Gly-Ala-Gly-Ser)n makes up the majority of the repeated design. It has crystalline domains that produce a beta-sheet secondary structure. As well as hydrophilic, hydrophobic, amphiphilic, and amorphous areas. The alanine-rich areas of the biopolymer are responsible for silks' self-assembly capabilities and mechanical stability. Since "sericins" are the cause of the immunogenic reaction, they must be kept apart from the silk fibroin throughout the "de-gumming" procedure, which involves boiling "silk cocoons" in a basic vehicle. Several In-vitro investigations have given that fibroin supports many cell types' adhesion and proliferation following sericin extraction. A flexible biomaterial, silk fibroin may be manufactured into a variety of shapes, sizes, and structures [89].

7.10.4 An Anticancer Medication Delivery System Using Silkworm Silk

The immense majority of anticancer medications are not well dissolved in an aqueous solution; as a result, a biomaterial carrier that can mix and discharge these medications would increase active moiety systemic availability and improve treatment effectiveness. "Silk fibroin" has been produced into films, coating, hydrogel, micro-nanoparticles, and capsules [90] for drug delivery purposes. Intratumoral medication delivery techniques based on silk, synthetic delivery, and local application solvent for IV injection can be divided into two classes (Table 7.8).

Table 7.7 Biomaterial-assisted photodynamic immunotherapy

Formulation	Consist of	Result	References
Novel nanoscale metal–organic framework (MOF) based on chlorine derivatives (TBC)	Cargos the small molecular indoleamine-2,3-dioxygenase (IDO) inhibitor	After the immune system was activated by combining ICD produced by Photodynamic and IDO inhibition with the small-molecule medicines, B cells, NK cells, and T cells were found in the tumor The synergistic immune effects promoted via Photodynamic and IDO inhibitors could efficiently prevent the expansion of equally primary and distant tumors	[81]
Nanoscale cationic MOF based on the dinuclear WVI secondary building units	5, 10, 15, 20-tetra(p-benzoate)porphyrin (TBP) (photosensitizer)	Anionic CPg is utilized to increase DC maturation and antigen presentation following PDT	[82]
Multitasking UCNP nanoparticles	Ce6 and R837 (UCNP-Ce6-R837)	When exposed to a NIR laser, UCNP-Ce6-R837 NPs could promote the tumor's effective PDT destruction and release a significant quantity of TAAs, which when combined with the immune adjuvant R837 might target potent anti-tumor immune response. More intriguingly, CTLA-4 inhibition has the potential to enhance such anti-tumor immunological action to stop tumor spread and recurrence	[83]
Cu-porphyrin (TBP)-based MOF	Cu-porphyrin	Anti-PH-L1 can be used to improve the immune system and systemic anti-tumor immunity	[84]

(continued)

Table 7.7 (continued)

Formulation	Consist of	Result	References
Acid-responsive micelleplex	PD-L1 knockdown with small interfering RNAs (siRNAs)	Significantly prevent the growth and spread of tumors	[85]
Liposome containing nanoplatforms	Sulfated catalases, NIR photosensitizers, and DOX	Produced $^1O_2$ may further modify immune cytokines, stimulating the immune system to activate and enhancing in vivo tumor prevention	[86]
DEX-based nanoparticle	Biocompatible polymer, dextran (DEX)	In addition to increasing the therapeutic activity of PDT, elevated oxygen levels also reserve the immunosuppressive tumor microenvironment to promote the anti-tumor immunological response following photothermal therapy, resulting in noticeably increased synergistic effects between PD-L1 and PDT checkpoint inhibition	[87]

7.10.5 Marine-Derived Biomaterials for Cancer Treatment

This book chapter will present evidence for the anticancer properties of the polysaccharides chitosan, carrageenan, alginate, and fucoidan. Their minimal cytotoxicity and physical resemblance to substances found in native human tissues are intriguing characteristics. They do have certain limitations, such as batch-to-batch variability, because the time of year and location of the harvest may change the characteristics of the polysaccharides, and the use of a variety of extraction methods may change the therapeutic action in the end. To create controlled and repeatable chemicals, it is necessary to use standardized methodologies and sustainable thinking [101] (Fig. 7.2).

7.10.6 Bioactive Agents Made of Marine Biopolymers

(a) ***Fucoidan***

A sulfated polysaccharide called "fucoidan" can be extracted from many types of "brown algae". Despite the possibility of additional monosaccharides, it primarily consists of fucose and sulfate residues [102] (Table 7.9).

Table 7.8 Systems for administering drugs to treat cancer based on silk

Biomaterial	Forms	Active moieties/types of administration	Types of cancer	References
B. Mori silk	Film	DOX/Intratumoral	Breast cancer	[91]
B. Mori silk	Hydrogel	DOX/Intratumoral	Breast cancer	[92]
B. Mori silk	Particle	Paclitaxol/intratumoral	Gastric cancer	[93]
B. Mori silk	Particle	Doxorubicin	Breast cancer	[94]
B. Mori silk-albumin	Particle	Methotrexate	Breast cancer	[95]
B. Mori silk fibroin-chitosan fibroin-chitosan	Particle	Curcumin	Breast cancer, melanoma	[96]
B. Mori silk	Film-coated liposome	Emodin	Breast cancer, melanoma	[97]
Bioengineered silk fibroin-elastin	Particle	Doxorubicin	Cervical cancer	[98]
Bioengineered functionalized spider silk	Sphere	DOX	Breast cancer and ovarian breast	[99]
"Bioengineered spider silk and DNA"	Complexes	Plasmid deoxyribo nucleic acid/IV	Breast tumor	[100]

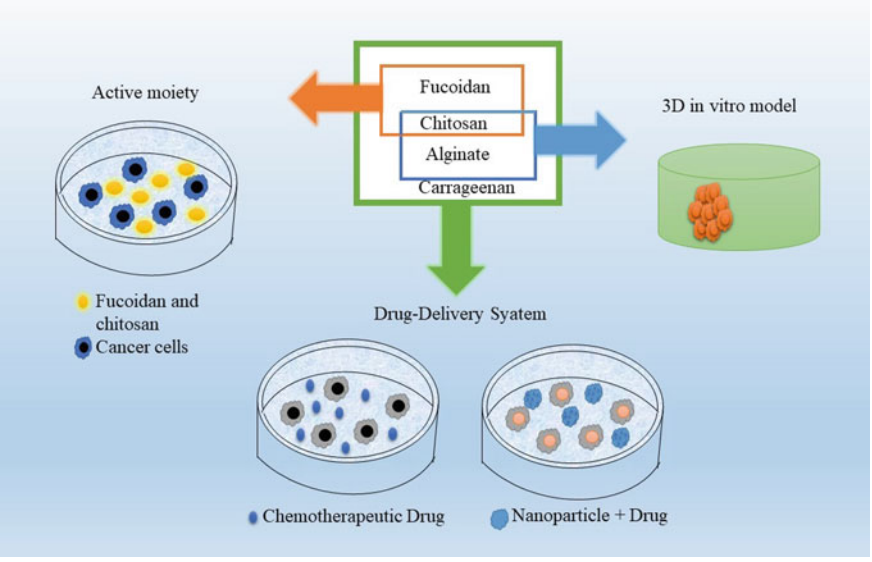

Fig. 7.2 Principal illustration of the application of several marine polysaccharides in various anticancer techniques

Table 7.9 Chitosan-, carrageenan-, alginate-, and fucoidan-based drug delivery system

Formulation	Drug	Cancer	Mechanism	References
Fucoidan-based formulation				
DOX-cargos-NPs	Doxorubicin	Breast cancer	Compared to free DOX, higher toxicity and higher cell cycle arrest in GI-Sphase	[103]
Protamine/fucoidan NPs	Protamine	Breast cancer	Protamine/fucoidan NPs displayed charge and pH responsiveness as well as selective internalization mediated by P-selection	[104]
Fucoidan and methotrexate nanoparticles	Methotrexate	MCF-7 cell line hela cell line	Both cell lines endocytose nanoparticles, with Hela cells demonstrating a higher rate of internalization	[105]
Complexed with fucoidan (Ru-Fu)	Rutin	Cervical cancer	Higher toxicity compared to normal cells and Hela cancer cells	[106]
$CaCO_3$ micro-particles coated with fucoidan and poly-L-ornithine	Doxorubicin		Induction of cell apoptosis	[107]
Gold nanoparticles coated with fucoidan	DOX	Breast cancer, Eye cancer	Loss of membrane integrity, suppression of cell development, and cytoplasmic condensation Dose-dependently promoted equally early and late apoptosis	[108]
MnO_2 nanoparticles coated with fucoidan	manganese dioxide	Tumor	Improved number of apoptotic cells	[109]
Cu_2S nanoparticles were coated with fucoidan	Copper sulfide	K562 (multidrug-resistant cells), hela (colon), and A549 (lung)	Cells apoptosis	[110]

(continued)

Table 7.9 (continued)

Formulation	Drug	Cancer	Mechanism	References
Silver nanoparticles coated with chitosan/fucoidan	Silver nanoparticles	Hela cells	Increased apoptosis	[111]
Chitosan-based system				
DOX-loaded NPs	DOX	Liver cancer	Increased p53, induced cell apoptosis, stopped the cell cycle in the G2/M phase, and decreased cell survival through this pathway	[112]
Chitosan-capped gold nanoparticles	DOX	Breast tumor cell line	Increase the effectiveness of chemo-radiotherapy on cancer cells' capability and produce cell death on the very less radiation dose (0.5 Gy)	[113]
DOX-encapsulated polymeric nanoparticle surface-capped with chitosan	DOX	tumor	Removing CD44++ cancer stem-like cells	[114]
Chitosan nanoparticles cargos with paclitaxel (PTX)	Paclitaxel	"MDA-MB231 breast cancer cells"	Apoptosis	[115]
Estrone-modified glycol chitosan nanoparticles cargos with paclitaxel	PTX	Breast tumor	Exhibited pH-responsiveness Estrone receptors revealed a greater Internalization index Cells apoptotic Tumor shrinking is caused by greater NP accumulation at the tumor location	[116]
Integrin-PLGA-chitosan Nanoparticles cargos with Paclitaxel	Paclitaxel	Lung cancer	Enhancement of apoptosis included cell cycle arrest at the G2/M phase	[117]
Chitosan nanospheres cargos with paclitaxel	PTX	Pulmonary cancer cell	Multiplying of lung cancer cells and increased apoptosis	[118]

(continued)

Table 7.9 (continued)

Formulation	Drug	Cancer	Mechanism	References
5-Fluorouracil was cargos into amino-functionalized mesoporous silica nanoparticle (MSN-NH2)-based GCs	5-FU	Colon cancer cells	Cells apoptosis and cellular uptake	[119]
Chitosan nanospheres cargos with 5-flurouracil	5-Fu	Endothelial cells	Preventing the growth of cancerous cells	[120]
PTX-loaded chitosan nanoparticles	PTX	Ovarian cancer	Cellular uptake effectiveness, and apoptotic cells	[121]
Chitosan-covered nanorods comprising "gemcitabine" and further conjugated with a "pH-sensitive peptide"	Gemcitabine	Pancreatic cancer		[122]
Chitosan iron oxide nanoparticles loading gemcitabine	Gemcitabine	Breast cancer cells	pH-associated release profile and enhanced "cytotoxicity and cellular uptake"	[123]

(b) ***Carrageenan-Based System***

A linear sulfated polysaccharide known as carrageenan is taken out of the extracellular matrix (ECM) of red sea algae belonging to the "Rhodophyceae" family. The first two of them are thickening agents that are most frequently employed in the biomedical industry, while the third is a gelling agent. Carrageenans have recently been shown to be useful in medication delivery systems for the chemotherapy of cancer. A carrageenan oligosaccharide-gold nanoparticle (Car-AuNPs) was produced by Chen et al. and utilized as a delivery mechanism for the chemotherapeutic medication epirubicin. Using common "human umbilical vein endothelial cells" and hepatic cancerous cells, the cytotoxicity of carrageenan–gold nanoparticles, epirubicin (EPI), and epirubicin-loaded Carrageenan-gold nanoparticles (EPI-Car-AuNPs) was assessed (HepG2). Carrageenan-gold nanoparticles did not influence the cellular feasibility of healthier cells, authorizing that these nanoparticles consist of excellent biocompatibility. Compared to free epirubicin, epirubicin-carrageenan-gold nanoparticles showed less cytotoxicity against healthy cells. When used against HepG2 cells, both Epirubicin and epirubicin-carrageenan-gold nanoparticles showed a dosage-dependent reduction in cell capability, with the inhibition epirubicin-carrageenan-gold nanoparticles being further pronounced than that of free epirubicin at the same dose. Additionally, the epirubicin-carrageenan-gold nanoparticles

caused cellular death in HepG2 cells by showing a cell cycle arrest at the G2\M phase. In a different research, DOX was delivered using fused nanoparticles related to $CaCO_3$ and carrageenan (-Car) derivatives of folic acid. The MG-63 osteoblastic human osteosarcoma resulting cell line was more sensitive to the cytotoxic properties of folic acid-carrageenan hybrid nanoparticles with or without doxorubicin. MG-63 cell viability was greatly reduced by folic acid-carrageenan–doxorubicin hybrid nanoparticles but not by folic acid-carrageenan. Additionally, FA-Car-DOX exhibited cytotoxicity that was comparable to that produced by folic acid-carrageenan-doxorubicin hybrid nanoparticles supernatant. To enhance DOX distribution, a GO-grafted-carrageenan nano-carrier coupled through biotin (GO-Car-biotin) was recently created. HeLa cell line vitality was severely reduced by doxorubicin-loaded GO-carrageenan-biotin nano-carrier in a concentration-dependent manner; however, human dermal fibroblast cell line viability was only marginally affected. Cells reacted with GO-Carrageenan-biotin nanocarriers loaded with DOX displayed nuclei with aberrant shapes. Additionally, FA-Car-DOX exhibited cytotoxicity that was comparable to that produced by folic acid-carrageenan-doxorubicin hybrid nanoparticles supernatant. To enhance DOX distribution, a GO-grafted-carrageenan nano-carrier coupled with biotin (GO-Carrageenan-biotin) was recently created [106]. "Human cervical cancer cell line" (HeLa) vitality was severely reduced by doxorubicin-loaded GO-Carrageenan-biotin nano-carrier in a concentration-dependent manner; however, human dermal fibroblast cell line viability was only marginally affected. Cells reacted with GO-carrageenan-biotin nanocarriers loaded with DOX exhibited nuclear fragmentation, chromatin shrinkage, and an irregularly shaped nucleus. By doxorubicin-loaded GO-carrageenan-biotin, proliferation was dramatically lowered and lactate dehydrogenase leakage was therapeutically enhanced in a concentration-dependent manner. NLCCs, which are nano-structured lipid-carrageenan hybrid carriers, were used to deliver the chemotherapeutic drug mitoxantrone hydrochloride under regulated conditions (MTO). When MCF-7MX cells overexpressing the breast cancer resistance protein were compared to the MTO solution (MTO-Sol), MTO-NLCCs considerably outperformed the MTO solution in terms of relative cellular absorption (BCRP). As a result, when compared to the MTO solution (MTO-sol), the cytotoxicity of mitoxantrone was dramatically increased in mitixantrone-NLCCs. However, after incubation with the blank NLCCs, no discernible growth inhibition was discovered, proving the NLCCs were safe to use as a solvent. Rats' in vivo pharmacokinetics of mitoxantrone-sol and mitoxantrone-NLCCs showed that the mitoxantrone-NLCCs cluster's apparent bioavailability was substantially more than that of the mitoxantrone-solution group. To overcome its limited solubility, curcumin has demonstrated an antiproliferative impact in several malignancies, according to Sathuvan et al. produced carrageenan beads with curcumin (-CarCur) for medication delivery to pulmonary cancer cells (A549). According to in-vivo cell-related experiments, free curcumin did not provide the same level of cytotoxicity against cancer cells as -Car-Cur composites. Additionally, during treatment with -Car-Cur, ROS generation enhanced and mitochondrial membrane efficacy reduced, suggesting that this system induced cell death in lung A549 cancer cells.

(c) ***Alginate containing drug delivery system***

Alginate is a "linear polysaccharide" comprised of 1,4-glycosidic linkages between the filtrates of L-glucoronic acid (G) and –D-mannuronic acid that is derived from brown sea algae (M). It has attracted significant investigation and uses in biological therapeutic effects because of its low toxicity, accessibility, biocompatibility, and moderate gelation. This study will concentrate on alginate-containing nanoparticles employed as drug delivery solvents for cancer therapy drugs like Doxorubicin and 5-fluorouracil, even though many alginate-based delivery methods have been created recently. Numerous alginate-containing nanoparticles have appeared to address the significant tissue toxicities related to DOX's high anticancer action, including cardiotoxicity. As a means of delivering DOX, Prabha and Raj created Fe_3O_4 NPs coated in polyvinyl alcohol, bovine serum albumin, and sodium alginate. These particles were poisonous to HepG2 liver cancer cells but harmless to healthy hepatic cells (L02). Peng et al. developed magnetically targetable "superparamagnetic iron oxide nanoparticles" (SPIONs) for the pH-responsive release of doxorubicin in tumor cell microenvironments. Although superparamagnetic iron oxide was cytocompatible with both normal and liver cancer cells, DOX-loaded SPIONs were more harmful to HepG2 cells than to L02 cells. Under the external magnetic field, doxorubicin-loaded nanocarriers in vivo may raise the concentration of the drug in tumor tissues, resulting in a notable suppression of tumor development and a marked decrease in the negative effects of free DOX. Additionally, oxidized sodium alginate was covalently coupled with DOX to generate an "amphiphilic macromolecular" prodrug. After that, the prodrug self-assembled onto doxorubicin nanoparticles in a hydrophilic solvent that acknowledged the acidic condition in cancer cells. The Doxorubicin-containing nanoparticles had a suppressive action on the proliferation of MCF-7 (breast cancer cells) but not on non-cancerous breast cells, according to in vitro cytotoxicity experiments (MCF-10A). Studies conducted in vivo on zebrafish further supported the DOX-NPs' effective absorption. Additionally, as compared to free DOX, DOX-NPs showed a better cardiotoxicity profile. In a different study, alginate (ALG) natural polyelectrolyte multilayers and chitosan (CHI) were interchangeably placed on poly(lactic-co-glycolic acid) (PLGA) NPs that were doxorubicin-loaded. In difference, doxorubicin-PLGA nanoparticles showed 61.35% and doxorubicin in vehicles indicated 23.77% lower anti-tumor efficiency, DOX-PLGA (CHI/ALG) nanoparticles give the highest anti-tumor effect 83.18%. Alginate consisting micelles was made utilizing alginate-g-poly(*N*-isopropylacrylamide) (PNIPAAm) and utilized to encapsulate doxorubicin in a different method. Because of the increased penetrability and retention action, these micelles gathered near the tumor area in an animal model with tumors. Interestingly, in a mouse model of cancer, DOX-loaded alginate-g-PNIPAAm micelles demonstrated outstanding anticancer treatment activity without causing any discernible adverse effects.

7.11 Conclusion

Biomaterials approaches provide versatile application in cancer research and various kinds of cancer with the use of several kinds of biomaterials like natural, synthetic, and organic–inorganic marine sources like alginate, chitosan, and carrageenan which are obtained from various sources; these biomaterial shows less toxicity in a biological system. Biomaterials show outstanding therapeutic responses to treat cancer. These biomaterials are biodegradable in nature which are easily accepted by the biological system, less toxic, and cost-effective that is why it established various types of drug delivery systems to identify and treat all types of cancer. In this chapter, we discussed the number of treatments and therapy that is utilized in cancer treatment like implantable, transdermal, and injectable scaffolds which are very useful in the cancer patient. This chapter also summarized vaccine-based cancer therapy, biomaterials used in liver cancer, phototherapy technique, silk-based biomaterial, marine biomaterials used in various cancer, and different types of biomaterial-based cancer immunotherapy. All approaches and therapies are very useful to manage cancer or tumor in survival patients. In this study, we summarized various cell line study in related to the lungs, breast, skin, and liver.

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Chapter 8
Engineered Tissue in Cancer Research: Techniques, Challenges, and Current Status

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Abstract By developing functional alternatives in the laboratory, tissue engineering (TE) has given rise to great prospects for the treatment of organ failure and tissue loss. Along with other advancements in the field of regenerative medicine (RM), TE also offered emerging technological platforms for the investigation of tumor cell development processes and potential tumor cell spreading in the field of cancer research. Recent developments in stem cell technology, such as those involving adult, embryonic, and induced pluripotent stem cells, perfectly illustrate the necessity to better understand the pertinent processes of cell growth regulation before using such procedures on patients. With the use of these TE-Cancer research models, we may explore the interactions that arise while simulating physiological and pathological situations during the early stages of replication, morphogenesis, differentiation, and growth under various provided settings. This article will provide an overview of some of the most recent breakthroughs and potential uses of TE in cancer research using TE platforms.

Abbreviations

2D	Two dimensional
3D	Three dimensional
ADAM12	ADAM metallopeptidase domain 12
BG	Bioactive glass
CNCs	Cellulose nanocrystals
C-PC	C-phycocyanin
DOX	Doxorubicin
DPV	Differential pulse voltammetry
ECM	Extracellular matrix
GBM	Glioblastoma
GO	Graphene oxide
MDR	Multidrug resistance
MMP3	Matrix metallopeptidase 3
MSN	Mesoporous silica nanoparticles
NPS	Nanoparticles
OHA	Oxidized hyaluronate
OSCC	Oral squamous cell carcinoma
PDT	Photodynamic therapy
PEO	Polyethylene oxide
PLA	Polylactic acid
PLLA	Poly-L-lactic acid
PVA	Polyvinyl alcohol
RES	Resveratrol
RNA-seq	Single-cell RNA sequencing
TAM	Tumor-associated macrophages
ZEO	Essential oil of Zataria multiflora

8.1 Origin of Tissue Engineering

The conquest of cancer persists in posing many conflicts in biomedical science. Every tissue in the body has been notably affected by this complex disease. It emerges from normal cells due to alterations affecting many genes. However, because of its widespread and lethal nature, a metamorphosis of disease has been observed where tumors are not mere an aggregates of similar cancer cells, but an intricated non-diseased organ system. Thus, cancer therapy holds the most extensive challenge of diagnosing, eliminating, and stopping tumors from outlying tissues. Back in history, surgical removal of cancer has opted for treatment. However, the most dramatic impact on cancer surgery was documented in 1894, when William Halsted developed radical mastectomy for breast cancer [1]. However, radical mastectomy was limited in treating metastasized large and small tumors. Later, Fisher suggested combining radical surgery and chemotherapy or radiation therapy to reduce cancer research morbidity. Likewise, cancer treatment using radiation was promoted after the X-ray's invention in 1895 by Roentgen. It was a turning point in this discipline when head and neck malignancies were treated with fractionated radiation in 1928. Chemotherapy was promoted to treat and cure cancer in the twentieth century by Paul Ehrlich. Early in the twentieth century, researchers created animal models of transplantable malignancies, but they were unable to create screening methods to forecast human anti-cancer activity [2–4]. Since that day, chemotherapy has evolved into targeted therapy, and the literature has been driven by the quest for agents that inhibit specific molecular targets, with the current advancement in treating some of the most difficult-to-treat malignancies, such as lung cancer and melanoma. Currently, immunotherapy has been a widely suggested treatment for cancers. However, a significant concern in cancer treatment is to prevent them. Discoveries about cancer's genetic determinants have had only minor clinical effects in treating cancers. Thus, recent advances have been focused on treating, monitoring, detecting, and classifying cancer molecularly. Perhaps, a profound knowledge of tumor etiology with specific cell types has been necessitated [5]. To comprehend the varied dimensions of tumor genesis, therapy, and advancements, the in vivo tumor environment factors have been considered in tissue engineering. Thus, in the 1980s, the concept of tissue engineering in cancer research emerged from a broad and general perspective. It is described as "implementing life science and engineering principles and approaches to the basic knowledge of model–functionaries interaction in mammalian tissues to generate biological alternatives for improvement, maintenance, and restoration of functionalities. For the first time in 1993, Langer and Vacanti published Research on the term TE. Tissue engineering is more than the sum of its ingredients [6, 7]. TE's current approaches have outlined the design, structure, and chemical architecture of native tissues, including angiogenesis. Compared to 2D methods, 3D methods are more accurate in mimicking in vivo growth and chemotherapy resistance of cancer cells. The significance of interactions with various non-malignant cell types has been identified through a number of animal studies and co-culture experiments. Biomimetic techniques have been used for years to advance tissue engineering by giving cells the components of their natural

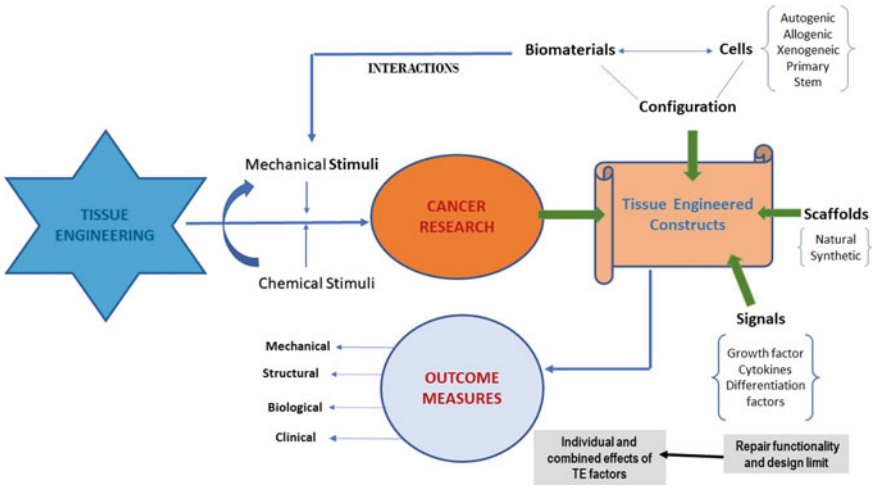

Fig. 8.1 Interactive process of tissue engineering in creating tissue engineered constructs

microenvironment they require to recreate both structure and process for a specific tissue [8, 9]. Tissue engineering has made an impact on areas other than regenerative intents. A tumor biology study can reflect the conduct of cancer-living organisms. The development of matrix- and scaffold-based culture methods that more accurately reflect the chemistry, transmitting environment and geometry of the natural ECM is specifically a result of advancements in TE. However, 3D culture systems have presented significant challenges in studying cancers [10] (Fig. 8.1).

8.2 Current Status of Tissue Engineering

The most fascinating and quickly developing topic of biomedical engineering is tissue engineering (TE). It has shown continuous evolution over the past two decades. It assimilates knowledge of technological advancements in various divisions of biology, including medicine, material science, cell biology, chemistry, and molecular biology. The phrase "tissue engineering" was first used in 1993 by two scientists, Langer and Vacanti [11]. In order to address the issue of tissue damage, it is the field that deals with biological substitutes that enhance, repair, or enhance tissue functions. Much of the growth in this area to date has been connected to the formation of model systems, which have recommended various strategies. Additionally, a few concepts in tissue development and cell biology have been defined [12]. Over the past three decades, the understanding of complex biological systems has been expanding at an accelerating rate. The engineering of novel tissues can benefit from the information acquired in the disciplines of molecular biology, biochemistry, stem cell, and cell biology. Mechanical devices, organ transplants, or surgical reconstruction are frequently used as a

substitute for tissue damage in the current situation. By generating in vitro tissues to heal in vivo damage, TE has evolved as a solution to the issue of tissue damage. Additionally, given one subject depends on advancements in the others, and the areas of genetic engineering, tissue engineering, stem cell biology, and cloning may ultimately grow together for the treatment of human disease. Understanding cells and their needs for mass transfer as well as the production of materials to serve as scaffolding and templates represents the scientific challenge in tissue engineering. This field has provided the way for the advancement of novel regenerative medicine techniques and disease simulation models [13]. The application of 3D printing technology, OOC, and iPSC is among the most important progresses. Development in tissue engineering will continue to provide goods and treatments that are both useful commercially and effective in treating diseases. The goal is to develop useful tissue constructs for the regeneration of missing or injured tissues and organs, including the spinal cord, skin, and other tissues. The generation of models to investigate function, ailment, and test and produce medications has also been a side benefit [14]. Ex vivo or in situ engineering studies can be conducted by utilizing a variety of chemicals, components, or cells to promote local tissue regeneration. The paramount significance is to transform how tissue loss is treated. The work required a "system" approach using engineering concepts rather than the conventional reductionist testing methodology. Beyond understanding a basic process, the ultimate objective was to create a product that would lessen or treat a disease condition [15–17]. Table 8.1 shows the current and recent development of tissue engineering in cancer research.

8.3 Challenges in Tumor Engineering

Cancer cells' dynamic interactions with their microenvironment have been considered for cell progression and metastasis. The interaction has involved the stromal cell's extracellular matrix components (non-cellular parts of the cells responsible for the clonal evolution, increased multidrug resistance, and heterogeneity of cancer cells) and other cellular parts [31, 32]. A multifaceted network of chemokines, cytokines, chemokines, inflammatory mediator's matrix remodeling enzymes, and growth factors maintains intercellular communication. In contrast, distance target cancer cells exchange the information through exosomes, novel tumor cells (CTCs), apoptotic bodies, and cfDNA. It is also noted that conditionally the tumor vasculature became tortuous and leaky, providing access to other tumor cells. Consequently, air and food delivery got delayed in the tumor. In some cases, stem cell production led to more differentiated precursors and migrated orderly. However, survival of one cell in a million has progressively led to new tumor cell formation because tumor cells can change their surrounding niche into a hospitable home to meet their growth needs and dissemination and recruit the neighboring non-malignant cells for their advantage [33]. Thus, cancer progression has been affected by tumor and stromal cells. When formulating treatment plans, a deeper comprehension of the tumor microenvironment and its relationship to cancer growth becomes essential. Each specific event

and different microenvironment characterized by the development of tumor [34]. Thus, the following factors must be considered that affect tumor progression.

8.3.1 Hypoxic Tumor Environment

The clinical and experimental studies have pointed out the fundamental role of hypoxia metastatic progression associated with solid tumors. Various studies reported

Table 8.1 Recent developoment of current tissue engineered contrusts and their advantages and disadvantages

Developments	Utility purpose	Advantages	Disadvantages	References
1. *3D Bioprinting*				
Cardiac patch	– Cardiac therapy	– Alternative of a heart transplant – Included patient-derived stem cells in creating personalized tissue constructs – A delivered drug to the epicardium for tissue regeneration	– Limited clinical studies have been reported – Required additional information on clinical trials	[18]
Novel κ-Carrageenan bioinks	– 3D manufacturing of Scaffold	– Novel algae-based biomaterial – Does not require supporting sacrificial solution – During 3D printing easily shaped into different structures	– Other natural materials are required for the fabrication of composites – Ethical, cultural, and regulatory concerns are required for animal materials	[19]
3D printed meniscal devices	– Meniscus reconstruction	– Biocompatible with tissue replacement – Balanced shape, and biomechanical function – Intriguing and promising method	– Native tissue complexity required integrating anatomy	[20]

(continued)

Table 8.1 (continued)

Developments	Utility purpose	Advantages	Disadvantages	References
2. *Organ on chip*				
Neural tissue vascularization on chip	– Neural tissue engineering	– Integrated the numerous cell types to recap the physiological environment of the tissue barrier – Resembled the same anatomical micro-architecture – Promoted neural cell growth and axonal regeneration	– Complexity and controllability are required to be balanced to mimic in vivo counterparts It-not suitable to produce on a large scale	[21]
Organ chips for keratoconjunctivitis sicca	– Tear gland engineering	– Promoted in situ regeneration – Helps in the healing process	– Culturing required tissue-specific natural growth factors. Culturing period is governed by the sustainable exchange of nutrients and metabolites	[22]
Cancer on chip	– Mimicking of Xenograft model	– Mimicked human cancer response directly – Experimental variations can be minimized – Utilized as alternative personalized medicine	– Clinical data can be hampered due to the complexity and variability of human tumors – Systemic comparison between experimental cancer on-chip model and clinical data becomes challenging. Extremely – Expensive clinical study	[23]

(continued)

Table 8.1 (continued)

Developments	Utility purpose	Advantages	Disadvantages	References
Vascularized lung chip	– In-vitro screening of lung tumor	– Created a natural lung cancer tissue-like structure – Given accurate drug screening – Supports dose specific action of the antitumor drug over 2D screening systems	– In-depth analysis is required to understand immunotherapy and metastasis extensively	[24]
3. *iPSC*				
Functional cardiomyocytes and cardiac tissue	– Regenerative therapy for end-stage heart failure	– Robust replacement approach for heart trans-plantation and regenerative therapies	– Complicated large-scale production	[25]
iPSC derived hepatocytes	– Wilson's disease	– Improved liver function by decreasing liver fibrosis	– Exhibited the immunosuppression	[26]
iPSC-RBCs	– Investigation of iPSC erythropoietic properties in immudeficent mice	– Expand and differentiate into RBCs – Capability to differentiate into enucleated erythrocytes	– Unavailability of appropriate and sufficient donor cells – Lack of crucial homing signals	[27, 28]
iPSC progenitor cells	– Synthetic cancer cell alternative	– Reflected responses to curative interventions	– The required high diagnosis platform of specific tissue – Limited use to repeated drug testing	[29]
TRAIL-iMSCs	– Targeted Cancer Therapy	– Provide suitable site – No effect on normal cells	– Clinical application hampered	[30]

increases in genomic instability and heterogenicity due to prolonged hypoxia are responsible for altering the gene expressions. Hypoxic tumors often resist malignancy and conventional therapies. It was also reported that hypoxia exhibited tumor cell differentiation by blocking mesenchymal MSNs and maintaining the stem cells. Several research has demonstrated that stromal and malignant cells influenced by hypoxia are also responsible for creating the diverse phenotypes of cancer cells via point mutations and chromosomal abnormalities that result in clones for distinct hypoxic conditions. As a result, HIFs aid in neovascularization, glucose metabolism, and tumor cell metastasis in order to adapt to these hypoxic settings [35, 36]. Concept by Ru Want et al., on cancer invasion and metastasis by ADAM12 is an expression hypoxia-inducible factor in breast cancer. Hypoxia activated the HIF-dependent production of metalloproteinase 12 and disintegrin, which cleaves the transmembrane domains of the HB-EGF. In vitro, inhibiting ADAM12 expression reduced hypoxia-induced movement and influx [37]. Similarly, another study by Yong Xi et al. reported esophageal cancer tumorigenesis and metastasis increased hypoxia-induced circBCAR3 expression. It was observed that hypoxia induced the upregulation of E2F7, which activated the QKI. circBCAR3 has increased by prioritizing the circularized exons [38].

8.3.2 Angiogenesis

An emerging tumor from dormancy has induced the current blood vessels through angiogenesis/neovascularization. Progression to this state showed the transition of tumors from dormant to angiogenic through several proangiogenic and antiangiogenic factors. However, host cells produced these factors in response to tumors or are either present in normal tissues [39]. This angiogenic shift thus improved cells' nutrient and oxygen supply for rapid growth. The more malignant the tumor, the greater its angiogenic potential [40]. Yadi li Aao et al. reported the angiocarpic factors contributed to HCC proliferation. A miR-130b-3p was upregulated in HCC and promoted angiogenesis and resulted in a poor prognosis. At the same time, HOXA5 was downregulated and provoked the HCC cells to induce the capillary tube formation with large tumor size, higher recurrence probability, relocation, and expansion of endothelial cells [41]. Wulong Wang et al. conveyed that tumor exosome miRNA-141 had progressed lung cancer due to GAX. The study confirmed that the upregulation of miRNA-141 promoted migration, invasion, and cell proliferation of A549 [42].

8.3.3 Acidification of TME

The extracellular pH of tumors in healthy tissues is acidic, while intracellular pH is slightly alkaline. The acidic condition of tumor microenvironmental impacts tumor

immune surveillance components, resulting in immune escape and cancer progression. This is known as tumor acidosis. However, the low pH and gradient imbalance between intra- and extracellular pH assist tumor cells to generate higher resilient phenotypes with much more invasive and chemo-resistant variants and elude immune modulation [42–44]. The disorganized tumor vasculature also prevented H+ ions release extracellularly and accumulated in the tumor environment. Despite antitumor effectors, T and natural killer cells follow apoptosis in the low pH environment. The immunosuppressive components like T-cells and regulatory myeloid cells sustain the tumor growth by blocking the antitumor immune response. In such ways, acidosis-driven tumor progression is promoted [45]. For instance, Cyril Corbet et al. the study concluded that autocrine TGF-β2 signaling was promoted by acidic microenvironment pH that favored the formation of lipid droplets as energy stores and supported anoikic resistance and cancer cell invasiveness. Besides, partial epithelial-to-mesenchymal transition (EMT) and fatty acid metabolism supported Smad2 acetylation induced by acidosis-induced TGF-β2. However, TGF-β2 stimulation and PKC-zeta-mediated translocation of CD36 facilitated the uptake of fatty acids [46].

8.3.4 Epithelial–Mesenchymal Transition

The EMT has occurred due to the addition of mesenchymal features from epithelial cells. This has raised cancer's initiating and metastatic potential and therapeutic regimen resistance. Consequently, mesenchymal fate has been activated by a transcription program [47, 48]. The transforming growth factor beta (TGF-β)/Smads pathway, a potent EMT inducer, had a significant role in the upregulation of EMT-related transcription factors [49]. Katsuno, Yoko et al. reported the role of EMT in cancer proliferation. TGF-β, as a potent discharge factor, had driven cancer proliferation in immunosuppressive and proangiogenic ways. It has induced epithelial plasticity leading to EMT. However, genetic mutations accelerate cancer progression [50]. Ayano Kabashima-Niibe et al. showed that cancer has advanced by EMT due to fibroblast cells. According to the data, myofibroblast-like cells that were positive for α-smooth muscle actin were derived from MSCs and helped to trigger EMT in populated cells. According to mice xenograft models, MSC-derived myofibroblasts continue to exhibit stem cell traits, such as increasing the expression of several genes linked to stemness, improving sphere-forming activity, and encouraging the growth of tumors. MSCs have also contributed to regulating tumor-initiating stem cell (TISC)-like characteristics. Jagged-1 siRNA and ϒ-secretase inhibitors had reduced the inhibition of E-cadherin and sphere formation [51].

8.3.5 Tumor Endothelial Heterogenicity

TECs are involved in cancer maturation. The increased expression of TECs to VEGF allowed cancer cells to enter the bloodstream. Upregulated adhesion molecules provide scaffolding for tumor cells to extravasate between TECs, which fuels the spread of metastatic disease. Angiocarpic factors, which are inductive substances provided by TECs, encourage the development and migration of tumor cells [52]. IL-8, IL-6, endothelin-1, TGF-β, and bFGF released TECs. Other endocrine agents also encouraged leukemic cell proliferation. Jag1, a protein generated from TECs, also stimulates Notch 2 in lymphoma cells to increase tumor invasiveness [53]. On TECs, CXCR7 has a role in angiogenesis and tumor formation. TECs stimulate tumor cell intravasation and metastasis. TECs constitute a heterogeneous folk induced by tumor microenvironmental factors [54]. For instance. Zhou et al. explained intratumoral heterogeneity, high therapeutic resistance, and tumor recurrence. Using scRNA-seq sequence, the transcriptome profile of tracked Prom1 + cells was examined. Prom1 identified CSC-like proliferative tumor-propagating cells in HCC tumors. These cells exhibit in situ clonal growth in initial tumors, as shown by lineage tracing. In contrast, labeled Prom1 + cells in 3D culture and allotransplantation showed higher tumorigenicity. Although Prom1 + HCC cells followed dedifferentiated status with high proliferation and stem cell features. The assortness and vitality of HCC cells were recorded. The function of malignant cells in the development of carcinoma cells was demonstrated [55].

8.3.6 Experimental Design

ECM proteins come in a huge variety, and each one has unique biochemical and biophysical characteristics that affect the phenotypic of cells. To ensure tissue homeostasis, the ECM undergoes continuous deposition, remodeling, and degradation from early development until maturity. To control cell behavior and differentiation, the ECM's composition and structure are comprehensively regulated. However, disturbed ECM results in the emergence of illnesses like cancer [56]. It has been recognized that the chemical cues the ECM provides are important catalysts for both cancer formation and progression. Biochemical microenvironment cues and physical signals have altered cellular conduct. The uncontrolled proliferation of cells in cancer remodels the extracellular matrix (ECM). The ECM is altered when mutant fibroblast cells release growth factors into the ECM, which either directly influences the ECM or changes the epithelial cells, which subsequently alters the ECM [57, 58]. Cancer cell metabolism is altered by the physical features of the ECM within the tumor. Cell substrates are accountable for the phenotypic variance of tumor cells. Compared to monolayers grown on a plastic plate, separated cells have different glutamine and redox metabolism [59]. Another illustration is the dependence of breast cancer cells on proline catabolism in spheroid models, which is not shown in conventional 2D

cultivation. These studies support the notion that metabolism can be impacted by connection to a physical substrate. A transgenic mouse model that overexpressed the ECM-degrading enzyme MMP3 in the mammary stroma eventually developed breast cancer, demonstrating the significance of cell behavior and function. A tumor microenvironment (ECM) in a tumor is made up of stromal cells and tumor cells working together [60].

8.3.7 Microenvironmental Conditions

Critical determinants of tumor angiogenesis, fibrosis, and heterogeneity include microenvironmental factors such oxygen tension and tissue dimensionality. It is interesting to note that numerous angiogenic factors and proteases, like MMPs and lysyl oxidase, that break down or rebuild the ECM, are among the genes with hypoxia-responsive regions in their promoter [61]. It is therefore necessary to better simulate the complicated biophysical properties of the TME and the tumor heterogeneity using pathologically relevant 3D tumor invasion models, such as tumor spheroids with a hypoxic core embedded in the self-assembled stroma. For example, it was supposed that tumor cells take benefits from the microhabitat that support tumors. The abundant desmoplastic tissue rich in collagen, fibronectin, and periostin that has overtaken the normal parenchyma is thought to be a contributing factor to PDAC's aggressive aggressiveness [62]. Therefore, on the triggered stroma's intrusive facade, PSCc activates continuously, and the ensuing fibrosis of the periacinar areas caused tissue hypoxia and parenchymal loss. The basal membrane, which isolates epithelial cells from the interstitial matrix, inhibits the growth of stellate cells and tumor cells. Breaking via basement membrane is an important stage in the growth of many cancers, including PDAC. Malignant cells come into intimate touch with fibrillary proteins in the ECM during this step [63].

8.4 Paradigm Shift from 2 to 3D Techniques in Cancer Research

Since the early 1900s, cells have been cultured using 2D cell culture technique. However, 2D models misrepresented tissue cells in vitro due to numerous limitations. Most cells are presently cultured using two-dimensional (2D) techniques. In contrast, cell growing in 3D cultures has shown compelling evidence for being utilized for sophisticated experiments3D cultures are better for altering the cell environment in vivo [64]. In contrast, 3D in vitro tissue models, particularly those produced by tissue engineering studies, are useful experimental tools for cancer research. Researchers can now simulate tumors and organs to use in drug testing thanks to 3D culturing techniques [65]. Furthermore, some studies have shown that these models

are getting better and are being used more frequently. Older 2D cell culture techniques are starting to lose ground to 3D cell culture techniques. Although, based on the desired experiment, each 3D culturing technique has a different set of benefits that can be used. The cell culture methods in cancer research must comprehend tumor biology, amend chemotherapy, and develop treatment strategies [66]. Qingxi Liu et al. explained cellular response of Cisplatin in 3D model and compared to traditional cell cultures. Cell behavior to drugs for cells growing differed significantly to 2D cultured cells. The activity of compounds in 3D cultures was assessed by estimating the spheroid proportions. Within the perfused 3D collagen scaffold, cells immediately take on the shape of a sphere. In contrast, at the stage just before the spherical phenotype formed, 3D collagen scaffolds appeared weaker than in 2D-cultured cells. Thus, the compact spherical structure has protected the microenvironment akin to the cells in vivo. Enhanced ROS levels in cultivated cells, however, indicated that the metabolism of the cells was more active [67]. Barros Andriea et al. had compared the therapeutic effect and the synergistic potential of two anti-cancer drugs. The synergistic effect of drug combination has been differed cell cultures. These differential effects of drug combinations have highlighted the utility of using 3D spheroids in screening drug combinations [68]. Similarly, Alexandra Arranja et al. reported use of cell culture models to understand Pluronic carriers' interactions in human cancer cell. These three-dimensional tumor spheroids were utilized to evaluate the potential toxicity and penetration of the nanocarriers. According to the findings, 2D cell models showed copolymers were the main determinants of the Pluronic nanocarriers' internalization paths and final cellular localization. F127 micelles that had been cross-linked, however, were more effectively distributed throughout the tumor spheroids. The transcellular transport of the carrier was governed by penetration depth [69]. Ala'a Al Hrout et al. studied in vivo simulation conditions of liver tumor. Liver cancer cells and fibroblasts were co-cultured. An ex vivo tumor microenvironment model that simulates tumor–stroma interactions was reported. It is straightforward but reproducible. In comparison, the expression patterns of genes and proteins were different. The study found that, in comparison with conventional 2D culture, 3D monoculture boosted gene expression [70]. Table 8.2 is showing the 3D culture technique with their characteristic parameters considered for cancer research (Fig. 8.2).

8.5 Applications of Tissue Engineering with Particular Emphasis on Cancer

The creation of functioning 3D cells is the goal of the interdisciplinary field. The ultimate objective of TE is to create biological replacements that preserve, enhance, or restore tissue function. TE could therefore avoid the issues related to tissue destruction that are currently managed by transplants, mechanical devices, or surgical reconstruction [77]. As a result, TE has provided clinics with excellent alternatives for

Table 8.2 3D culture technique with their characteristic parameters considered for cancer research

Cancer research using 3D culture	Characteristic parameters of 3D culture					
	Cell culture condition	Morphological characteristics	In vivo imitation	Drug resistance	Tumor stimulation	References
Breast Cancer model	– Dense multicellular spheroids have been developed in one day on 3D culture of breast cancer	– Loose 3D spheroids with 200–300 μm maximal size	– Showed better in vivo cellular dormancy features with no difference of caspase-8 expression	– Exhibited more excellent resistance to paclitaxel and doxorubicin	– Stimulated characteristic Hypoxia dormancy, anti-apoptotic features in vivo	[65]
Oral cancer model	Dropwise Scaffold in approximately 17 days	– A three-layer structure as replicated oral epithelium	– 3DP, mimicking human bone tissues	– Needs future assessment of novel diagnostic for managing OSCC	– Simulated closely	[71]
Cervical Cancer model	– Spheroid was prepared using liquid overlay and hanging drop method in 4 days, whereas small spheroids were prepared within three days	– Uniform spheroid with 5000 397 cells/spheroids maximum possible volume	– 4T1 and TC1 spheroids inhibited ZEO at different doses	– Resisted toxic effects of ZEO	– Dose-dependent inhibition in 4T1 399 and TC1	[72]

(continued)

Table 8.2 (continued)

Cancer research using 3D culture	Characteristic parameters of 3D culture					
	Cell culture condition	Morphological characteristics	In vivo imitation	Drug resistance	Tumor stimulation	References
Colorectal cancer model	– By seeding technique in ultra-low attachment microplates, spheroids were generated in 7 days	– Multicellular cancer spheroids	Cell viability enhanced	– Enhanced intrinsic apoptosis of LCFS	– Stimulated proliferating and quiescent tumor factors	[73]
Thyroid Cancer model	– Using the manufacturer's kit of a collagen type I solution mixed with minimal cell suspension	– Uniform polymerized spheroids	– Decreased invasion in 3D SW1736	– Resisted BRAF and MEK inhibition	– Sensitized tumor cells to MAPK inhibitors	[74]
Liver Cancer model	– By Liquid overlay technique	– Multicellular spheroid	– Enhanced survival of HepG2 due to increased expression of HIF-2 expression in vivo	– Resisted antiproliferative and proapoptotic properties	– Stimulated cell proliferation in differentiated p53 HCC cells	[75]

(continued)

Table 8.2 (continued)

Cancer research using 3D culture	Characteristic parameters of 3D culture					
	Cell culture condition	Morphological characteristics	In vivo imitation	Drug resistance	Tumor stimulation	References
Ovarian cancer model	– Suspending method by suspending the trypsinized cell in the growth medium	– Aggregated, elongated and cohesive Spheroids	– No effect of gene knockout – Decreased BCRP and COL3A1 expression	– Resisted PAC cell lines	– No sensitivity	[76]

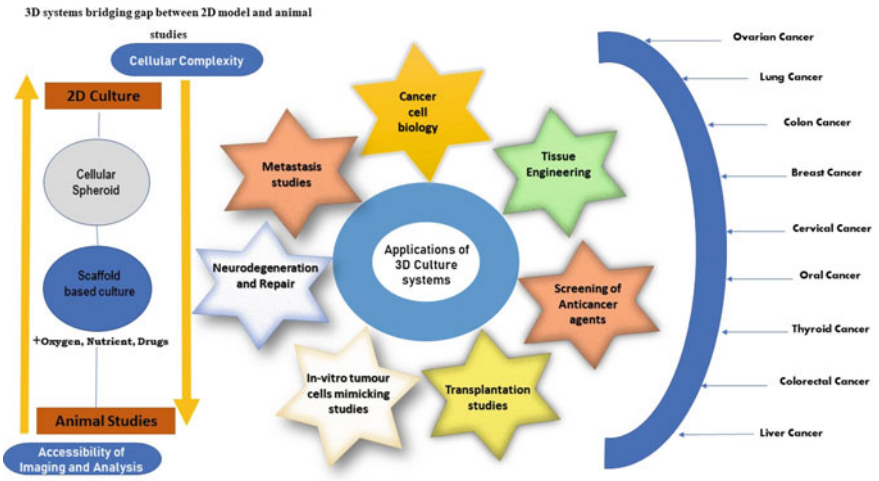

Fig. 8.2 Dynamic applications of 3D culture systems in Cancer research

tissue restoration. They can also accomplish this goal by developing in-vitro tissue-repair technologies [78]. TE also produces modified tissues, which might enable us to do in vitro research on human physiology. Research on cytology, however, is frequently conducted in 2D cells, where cells develop in non-physiological conditions. TE applications involved three essential components, i.e., framework, cellule, and biomolecules. To execute, 3D cultures, which offer the microenvironmental conditions that regulate carcinogenesis, have been produced. These 3D cultures are built using a combination of biomolecules, scaffolds, and cells. However, from last twenty years, a wide numerous hybrid biomaterials are meticulously created to guide and support tumor progression. The creation of synthetic scaffolds that incorporate the molecular makeup of the ECM and a thorough examination of their particular function in the disease are important advancements in the study of cancer [79]. However, by using microchips and microfluidic methods in cancer, TE applications have grown increasingly complicated. Complex applications of TE in cancer research are described below.

8.5.1 Three-Dimensional (3D) Cell Cultures Models

In order to imitate in vivo tumors and their surroundings, research in cancer cell biology has recently turned to three-dimensional (3D) cell culture technology as its main focus. They extended prospects to two-dimensional in vitro studies and in vivo studies models. A 3D cell prototype closely mimics tumors natural cell features and configurations. Hence, 3D cell cultures are suggested for disease modeling and drug identification [80].

8.5.1.1 Multicellular Tumor Spheroids as 3D Models

The employment of cancer spheroids has been raised recently. As reported in many studies, these models are best to understand three-dimensional structure of tumors in vitro. For instance, the curative ability of spheroid was investigated. Results manifested that cytotoxic effects T212Pb-NG001 have been induced in the human prostate C4-2 spheroids [81]. Pancreatic spheroids have mimicked in vivo characteristics of PDAC, such as microenvironmental factors and drug responses in the 3D model. In consecutive reported studies, multicellular tumor spheroid had been involved in screening the small oligonucleotides. The screening of small molecules was confirmed which was increased substantially with drug concentration [82]. Hui li ma et al. revealed that multicellular spheroids were utilized in-vitro to quantify nanoparticle penetration effects. These 3D HeLa spheroids were used as a screening tool because of intrinsic dimensions and aquaphobicity [83].

8.5.2 In Vitro Synthesis of Tissues and Organs

Biologically active scaffold analogs generate organs and tissues. Tissue-engineered scaffolds are significant for neural tissues, vascular tissues, skeletal tissue, skin, bone, and ligament engineering. They have been generally used as a carrier to control protein and drug deliveries. The scaffolds with high strength and low degradation are being developed with suitable sizes [84].

8.5.2.1 Polymeric Scaffolds Using Natural Polymers

For instance, ceramic porous scaffold is widely used in bone tumors. These scaffolds are made from nanocomposite materials that have been lightly coated with a natural polymer. Due to its resemblance to the bone's porous structure and gentamicin–gelatin coating, composite scaffolds are involved bone cancers. By combining MNPs to the basic matrix, scaffold porosity was improved [85].

The utility of multifunctional polymeric drug transporters for cancer has been revolutionized recently. They have amphiphilic properties and are capable of conforming. Pluronic polymers have surfactant-like properties, which are considered drug carriers. Studies have depicted the utility of pH-sensitive Pluronic micelle for oxidative therapy in MDR that hinders cancer treatment [86].

8.5.2.2 Synthetic Polymeric Scaffolds

Synthetic polymeric scaffolds are highly recommended in the biomedical field for specific applications. These are applicable in the field [87]. Thus, research performed by Sanjeeb K. Sahoo et al. has prepared the biodegradable porous polymeric

microparticles scaffold to assess cell maturation**.** Hydrophilic polymers were used as an internal matrix in microparticles. The 3D-like structure had been attempted by grown microparticle engulfed cells. Optimized microparticles showed better growth and cell adhesion. Thus, microparticles with tissue-like structures in cancer cells are robust systems testing anti-cancer drugs [88]. The fibroin scaffold was investigated for hepatic cancer models. Hepatic cancerous cells were grown in vitro cells. Cells were linked to the mixed sponges and developed cell–cell contact. Hence, the cell growth and their attachment to the composite scaffold were successfully improved [89].

8.5.2.3 Porous Scaffolds to Study Cancer Metastasis

Scaffolds have significantly mimicked tumor heterogeneity, metastasis, and drug uptake processes. A highly linked, porous polyester-based scaffold with pore sizes of more than 100 m was created by Mei Zhang et al. The growth pattern of the cancerous cell was equivalent to the in vivo method due to the extended doubling rate. Along the surface of the scaffold, the associated cells created clumps up to 500 m in size. The high-density random seeding technique has allowed enhanced loading (30–40%) for further testing [90]. By supplying the hollow conduit between the cell clusters, this scaffold has offered possibilities to evaluate solubilized components of culture [91].

8.5.2.4 Hydrogel Scaffolds for Tissue Repairing

Hydrogel scaffolds have shown potential in the repair and regrowth process of damaged biological tissues. They have excellent supportive cell proliferation, migration, and nutrient transport properties, which are made to mimic the native soft tissues [92]. An injectable hydrogel had been reported. This in situ injectable hydrogel has water high water retention and associated structures. Combined CNCs and CS-Au hydrogel has improved mechanical strength and resistive nature. Enhanced cell growth was reported in viability assay. As the prepared had provided the tissue-specific networks hence suggested for tissue engineering applications [93]. Hydrogel scaffolds have recently been drawn in tissue engineering applications. Hydrogel has presented excellent stability and biocompatibility. However, the 95% retaining of encapsulated cells has proved a remarkable candidate for TE [94]. The biomaterials controlled the O_2 and reduced the ROS-free radical. A polymeric cryogel scaffold (PUAO-CPO) was developed. Cryogel PUAO-CPO scaffold had been prepared by incorporating calcium peroxide (CPO) using the cryo-gelation technique in the antioxidant scaffolds. Results showed cryogels had sustained H9C2 cardio myoblast cells under hypoxic conditions. Results were standard and comparable to typical PU scaffolds [95].

8.5.3 In Vivo Engineering of Tissue and Organ

8.5.3.1 In Vivo Bone Engineering

PLLA/PEG scaffolds with pores help with bone tissue engineering. The histological and immunohistochemical analyses were performed. polyethylene glycol was used as excellent modifier. Structured PEG5 foam, which has a well-organized structure, strong mechanical characteristics, and outstanding cellular biocompatibility, may offer improved in vivo regeneration. The scaffolds that were produced were used for in vivo bone healing. This PEG5 foam with structure was able to mend bone in vivo [96].

D-printed bioceramic scaffolds are employed for regeneration and tumor treatment. These active bioceramic scaffolds offer a huge possibility for treating bone tumors by mending surgically caused bone flaws and eliminating any potential remaining tumor cells. Due to their resemblance to bioactivity, osteoconductivity, inorganic bone components, and potential osteoinductivity, these are extensively researched [97, 98]. For instance, ceramic glass for regeneration has been described in reported investigations. Similar to this, a study looked into how microduct networks affected biological characteristics. A 3D printed scaffold loaded gelatin–methacryloyl hydrogel and interconnected microchannel networks. Next, bioanalysis was carried out using these scaffolds, which were either with or without microchannels. Rat femur bone defects of crucial size were implanted with these scaffolds. In comparison with groups with no scaffolds, bone healing was improved by both solid scaffolds and scaffolds containing microchannel networks [98].

8.5.3.2 Tissue-Engineered Human Tumor Models

Tumor biology has been widely studied by three-dimensional tumor models in recreating in vivo tumor phenotypes. Several advanced 3D culture techniques have been adopted by researchers for producing clinically precise ex vivo models. Therefore, in order to provide predictive models of tumors, a new generation of bioengineered tumors has arisen that has the potential to revolutionise drug screening [99]. A biocompatible hyaluronic acid hydrogel containing the IKVAV peptide sequence, which promotes neurite growth, has been published. A hydrogel matrix displayed the conduction characteristics of neurons. By encouraging the creation of a tissue matrix and axonal growth in order to replace the lost tissue, tissue abnormalities in central nervous systems have also been healed [100]. Thus, a bioengineered hydrogel has provided a practical cancer model. However, developing these helps in explain the effects of matrix stiffness. The study's findings suggested that matrix stiffness cell proliferation within hydrogels [101].

8.5.4 Biomaterials in Tissue Engineering

To give cancer cells that had been cultivated in vitro a physiological setting, 3D models made use of a variety of polymeric biomimetic materials. These biomaterials have overcome several intrinsic drawbacks of 2D cell culture methods. They are further classified as natural, synthetic, and hybrid [102].

8.5.4.1 Development of Scaffold Using Natural Biomaterials for Drug Screening

Due to their prevalence in the natural tumor microenvironment, natural biomaterials like collagen and basement membrane extracts (like Matrigel) have been widely used in cancer research. They have provided helpful clues that encourage a cytoarchitecture akin to the in vivo arrangement. The physical design of natural matrices is well-defined and difficult to modify in order to explore the effects of biophysical signals on tumor growth. In order to create 3D scaffolds, a variety of biomaterials can be used [103]. Forrest M. Kievit et al. had created (a) chitosan-alginate 3D scaffold to encourage carcinoma cells in culture to change into a more malignant in vivo-like phenotype. These had offered glioma cells that represented the in vivo tumor a 3D microenvironment, making them a useful platform for creating and researching anti-cancer therapies [104].

NSCLC, the most common type of tumor of lung epithelial cells, was studied in 3D in vitro models. Matrigel™ and Pura Matrix™ had very low storage moduli, indicated as soft gels. Cancer cells assumed a spheroidal shape, while cells cultured on softer substrates (Matrigel™ and Pura Matrix™) displayed cortical F-actin. When cultivated in soft matrices (Matrigel™ and Pura Matrix™), such cytoskeleton reorganization resulted in alterations in the mechanical characteristics of A549 cells, which revealed a reduced young's modulus. As a result, cells cultivated in 3D soft matrices showed reduced stiffness [105].

8.5.4.2 Synthetic Biomaterials

To emulate a natural extracellular matrix, synthetic biomaterials are materialized [106]. ECM-like proteins developed during the development of biomaterial scaffolds. These molecules, which resemble complete proteins, can be produced synthetically. However, it was discovered that delivering immobilized bioactive compounds was appealing for supporting tissue regeneration. This method, which used sirolimus hybrid coatings to transmit the dextran drug release mechanism, was found to provide good drug release and enhance the biocompatibility of coated cardiovascular stents [107], whereas chitosan has gained popularity in tissue engineering. However, most research has focused on developing scaffolds due to their functional character. For

example, porous chitosan scaffolds were obtained using porogenic materials. These porous pores have improved cell proliferation in vitro and in vivo [108].

8.5.4.3 Hybrid Biomaterials in Cancer PDT Applications

Cancer therapy has been advanced lastly where biological macromolecules from hybrid nanomaterials contributed as therapeutics. They have also shown remarkable offerings for PDT applications [109]. As a result, the photoactivity biomaterial was investigated [110]. Some biomaterials for the clinical treatment of oral cancers are reviewed greatly. A new class peritumoral NIR injectable hydrogel is used for tongue tumor [111]. Besides, hybrid gel are encouraged to treat cancers [112].

8.5.4.4 2D Nanobiomaterials as a Drug Delivery System

Unique nanosheet shapes, sizable surface areas, and exceptional physicochemical features of 2D nanomaterials have generated a great deal of interest. These 2D nanomaterials are exceptionally effective nanoplatforms for drug delivery [113]. Doughty ACV et al. quoted the experimental study of Liu et al., that is, the use of GO in PTT. PTT has also been used with graphene oxide (GO) nanosheets for the treatment of cancer. It has been shown that GO nanosheets work well as photothermal absorbers. For in vivo monitoring, O-PEG nanosheets were also coupled with the NIR fluorescent dye Cy7, which demonstrated that the majority of the nanosheets localized within the kidney and tumor. Local ablation of 4T1 murine breast cancer after intravenous injection was accomplished with modest power densities (0.5 W/cm^2) in comparison with similar doses of other nanoparticles [114]. Gold nanostars (AuNSs), comprising dendrimers/AuNSs, metal compound/AuNSs, SiO_2/AuNSs, and polymer/AuNSs have been shown to have potential as a photothermal absorber in a latest report. Incident light was absorbed and scattered as a result of these AuNSs' localized surface plasmon resonance (LSPR) property. As an illustration, AuNS-based nanohybrids were created and produced. For the PTT of tumors, high photothermal conversion efficiency (50.5%) AuNSs were used in their design. Concanavalin A (ConA) can be additionally conjugated with a sensitive thermoresponsive glycopolymer composed of glucose by specific identification, and galactose can be targeted specifically to hepatocytes. Con A exerted its therapeutic activity by inducing autophagy and mitochondrial degradation, which resulted in cell death, as a result of the galactose's targeting effect. The nanohybrids demonstrated a significant collective therapeutic effect [115].

8.5.5 Drug Testing by Using Microtissues

Research reported that 3D circumstances could authentically mimic specific organ functions. These organoids are used as a model for drug screening [116]. Some examples are discussed below.

8.5.5.1 Organoid on Chip for Toxicological Screening of Anti-cancer Drugs

Organoid-on-A-chip platforms have significantly advanced drug and toxicology screening. At the same time, chip systems are used for generic drug development screening and dose testing. A cervical cancer chip has been developed. The plasmonic chip contain Au nanoparticles have been utilized for clinical metabolic fingerprints. The on-chip serum fingerprints differentiated between cancer and control subjects. The chip was sensitive and fast in detecting the small molecules [117, 118].

8.5.5.2 Microfluidic Device for Cancer Detection

Electrochemical biomarkers are valued in tumor detection. These are emerging MEMS as multidisciplinary research [119]. Thus, in a recent study performance of microfluidic systems, a RUBYchipTM™ for CTC capture was studied, whereas RUBYchip™ was ten times more efficient. Thus, it was suggested that Liquid biopsy using the RUBYchipTM helped to overcome the histological testing limitations of HER2 [120]. Likewise, isolation and enumeration of circulating tumor cells) for ovarian cancer was performed. Blood samples were taken at various times from 13 ovarian cancer patients, the connections between CTC level and time course of the disease. Because the FAST disk method was label-free, it was simple to extract the collected CTCs for use in subsequent CTC count analyses. Targeted therapy is now possible thanks to single-cell and genetic analyses. It has been demonstrated that CTCs may be useful as an indicator of treatment responses [121].

8.5.6 Tissue Engineering and Drug Delivery Applications in Cancer Treatment

Photothermal therapy has recently been used in cancer therapy. However, numerous nanomaterials employed for tumors. For example, PEGylated gold nanoparticle conjugates were developed for photodynamic therapy drug delivery of hydrophobic drugs. It was observed that Au conjugates had reduced the drug delivery time compared to free drugs. The drug delivery process was efficient and passive targeting

at the tumor site was achieved [122]. Anjum S. et al. had prepared ZnO NPs of taxifolin. Apoptotic signals were observed. Lesser dose of 27 MCF-7 cells was inhibited in lower doses [123, 124]. Polymer-coated nanosized fibers of natural origin are versatile materials. For novel biomedical applications gelatin has been thoroughly considered. For example, in situ DOX-loaded nanofibers have been developed. Cross-linked PNIPAM/gelatin was used for cross-linking. These nanofibers have rereleased DOX on each temperature rise which in turn has lowered the cells viability [125].

8.5.6.1 Electrospun Fibers as Controlled Drug Delivery Systems

The electrospinning approach has been introduced to generate ultrafine polymer fibers. Ultrafine polymers fibers have had been introduced to generate ultrafine polymer fibers. Ultrafine polymer fibers have symmetry-like ECM with natural properties, high automated strength, and absorbent mesh with significant interconnectivity. However, their applications have expanded as implantable drug delivery systems for treating localized tumors [126].

Chemotherapeutics were released under regulated conditions using electrospun fibrous films. Titanocene dichloride was delivered to lung tumor cells via PLLA electrospun nanofibers. Human lung tumor cell growth was inhibited by 11.2, 22.1, 44.2, and 68.2%, while Doxorubicin-loaded PLLA electrospun nanofibers were studied for their ability to suppress untreatable liver cancer and stop post-surgery tumor recurrence [127].

8.5.7 Novel Applications

8.5.7.1 Novel Biomaterials for Cancer Treatment

Ceramic glass nanomaterials have shown revolutionary prospects in cancer therapy. Due to their magnetic effectivity and high bioactivity, as bone fillers, bone tumors have been treated. Thus, synthesis and in vitro tests of novel magnetic biomaterials for cancer treatment were carried out. For instance, Palamed® containing ferrimagnetic particles was developed. They exhibited high bioactive and magnetic properties. The bioactivity and cytocompatibility of ATCC CRL-1427, Mg63 osteosarcoma cell, was tested and displayed good probability, form, adhesion, and density [128]. Glass nanomaterials exist in connection with rigid and gentle tissues. Bone and skin regeneration had been possible due to their proangiogenic and germicidal potential. Cu-doped bioactive nanomaterial showed promising potential for tumor treatment, especially in photothermal therapy [129].

8.5.7.2 2D Nanomaterials for Neural Tissue Regeneration and Engineering

Lately, 2D nanomaterials as regenerative medicine have been explored extensively. The creation of a 2D nanomaterials scaffold has presented a newer perspective in tissue engineering. Achieving the high drug loading capacity is simple. Graphene family nanomaterials, transition metal dichalcogenides, layered double hydroxides, MXenes, and black phosphorus are examples of 2D nanomaterials. As a result, 100–300 m Scaffold 3D graphene foams were created for NSCs. Compared to 2D graphene film, NSCs proliferated and differentiated more readily. Peripheral nerve repair has been demonstrated in experiments. Using multi-layered porous scaffolds made of PCL-polydopamine coated, arginyl glycyl aspartic acid, single- or multi-layered graphene (SG), or both [130].

8.5.7.3 Carbon Nanotubes in Cancer Therapy

Carbon nanotubes as polyvalent tools for cancer treatment are progressing swiftly. Carbon nanotubes are a unique and novel class of nanomaterials with multiple properties for cancer therapy. Yadong Zhou et al. summarized the applicability of creditable drug carrier carboxylated polyethylene multi-layered carbon nanotubes (MWCNT-COOH) in liver chemotherapy. The prepared nanocarrier delivered the anti-cancer drug Doxorubicin at a specific site of the cancer cells. The HepG2 cells and HEK293 kidney cells were used for i*n*-cytotoxicity and anti-cancer features of the nanocarrier consisting DOX. However, upgraded cytotoxicity and cell apoptosis of HepG2 liver cells were observed, which has shown potential in liver cancer therapy [131]. **Similarly**, in reported studies, **an** electrochemical smartphone system with differential pulse voltammetry measurement and a screen-printed immunosensor customized with multi-layering of MWCNTs, thionine, and gold-plated nanoparticles has made tumor detection possible. It was possible to detect CA125 cancer antigen with a high degree of accuracy. The smartphone's android application (APP) managed the detection procedure. According to the experimental findings, the log CA125 concentrations and DPV peak currents exhibited an excellent linear connection. The proposed approach has therefore provided information about tumor marker detection [132].

8.5.7.4 Smart Biomaterials in Stem Cell Repairing and Engineering

The novel MXenes biomaterials gained prospect as therapy. The multiple biologic features of tissue repairing and engineering are their exceptional features. For instance, in the given study, post-injury tissue repairing for immunomodulation has been achieved by zero-dimensional (0D) Ti3C2 MXenes-based quantum dots (QDs). This was evident by reduced human lymphocyte activation and stimulated the expansion of immunosuppressive regulatory T-cells in the stimulated lymph cell

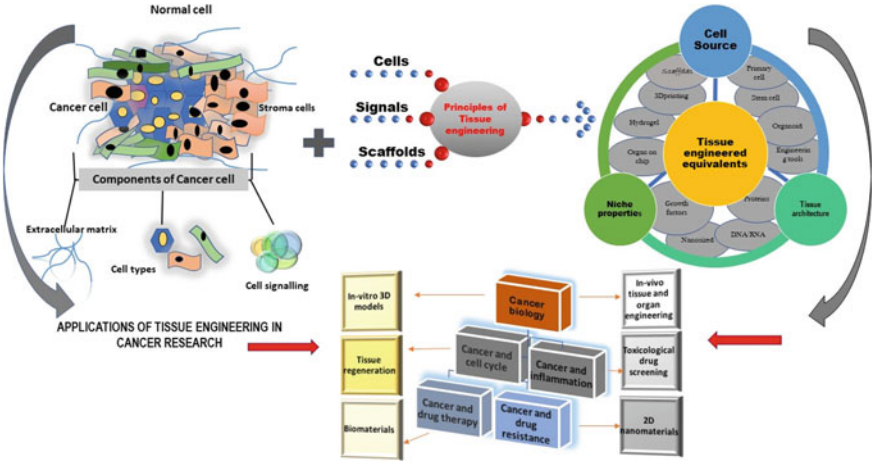

Fig. 8.3 Briefing of applications of tissue engineering in cancer research

population. Thus, two-dimensional MXenes with BMSC biocompatibility emanated from pluripotent cells was evoked by fibroblasts [133] (Fig. 8.3).

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Chapter 9
CADD for Cancer Therapy: Current and Future Perspective

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Abstract Due to the complexity of cancer treatment, there is a need to embrace more advanced approaches in cancer therapy. Targeted therapies are cancer treatment that targets proteins and enzymes that affect how cancer cells grow, divide, and disseminate. The specificity of targeted therapies is essential in understanding the most effective way to treat cancer. Previous proteomics and enzymatic studies have revealed some essential proteins and enzymes actively involved in cancer cells' growth, division, or dissemination. Some of these proteins and enzymes include; glucose transporter 1, which undertakes the function of supplying glucose to cells; hexokinase 2, which enhances glycolytic rate; phosphoglucose isomerase and glyceraldehyde 3-phosphate dehydrogenase, which performs supplementary actions in boosting the growth of tumor in an unglycolytic manner; Tyrosine kinases, which play an essential role in transduction steps mediation, causing proliferation of the cell, differentiation, migration, metabolism, and programmed cell death. Computer-aided drug design and advancements in this area have made it possible to have these essential proteins and enzymes involved in cancer in a 3D format present in databases that can be used in in silico drug design that target specific proteins and enzymes. With the computer-aided drug design, databases can be utilized in novel drug design for cancer treatments through computer-assisted structure–activity relationship studies, in silicodrug-likeness prediction, in silicoADMET studies, molecular docking simulation, molecular dynamics studies, and other in silico studies targeted drug development. These methods can hasten the course of new drug development and repurposing, thereby minimizing the likelihood of drug candidates failing in the phases of drug development.

9.1 Introduction

Targeted cancer therapy describes a cancer therapeutic approach that targets proteins involved in the regulation growth, division, and spread of cancer cells. It serves as the basis for precision medicine [1]. It involves using drugs that specifically target the proteins and genes which support the growth and development of cancer cells. Targeted therapy either concentrate on cells like blood vessel cells that are linked to the progression of cancer or it can affect the tissue environment in which cancer cells thrive [2]. Many different forms of cancer can be treated using targeted therapy.

Additionally, targeted therapies can be utilized in conjunction with other cancer therapeutic alternatives like chemotherapy. Although not every cancer type may be treated with targeted therapies, with the advent of computational approaches and their advancements, developing new targeted therapies for cancers can be made more precise, robust, time efficient, and economical. The field of targeted therapy is expanding quickly, and numerous new targeted cancer therapies are currently in different phases of clinical trials [2].

The global health issue of cancer causes one in every six fatalities worldwide. Globally, in the year 2020, there were about 10 million fatalities attributed to cancer and 19.3 million recent cancer cases. Cancer is a highly complex series of medical disorders that generally develop slowly and lose growth control [3–5]. For many years, patients had only a limited number of treatment options, which comprised of surgery, the use of radiation, and chemotherapeutic option either separately or as a combination treatment [6, 7]. But in recent times, many of the pathways involved in advancing cancer therapies and the approach of the target have been significantly improved, and it has been found that combinatorial approaches which make use of multiple targeted treatment options or conventional chemotherapeutic agents, like taxon and platinum molecules, produce synergistic results [8].

The most popular conventional cancer treatment recommendations involve surgical removal of tumors, and then chemotherapy or radiation treatment using X-rays [9]. Surgery is the treatment option that works best in the early stages of illness development. Healthy cells, organs, and tissues can be harmed by radiation therapy. Almost all chemotherapeutic drugs harm healthy cells, especially those that divide and expand quickly, despite the fact that chemotherapy has decreased morbidity and death [10]. Drug resistance is a problem with chemotherapy that can happen when cancerous cells that were initially repressed by the treatment start to grow back. It is a serious issue because it means the cancer can continue to grow and spread. One of the main contributors to this problem is that the medication is not being well absorbed as it used to be, and the drugs are being released more quickly than usual [11]. Traditional chemotherapeutic methods have many limitations, including difficulty in selecting the right dosage, the non-selectivity nature of these chemotherapeutic agents, fast drug metabolism, and the often undesirable side effects [12]. Conventional chemotherapy lacks specificity, as shown in Fig. 9.1a, why targeted therapy targets more of a specific enzyme, enzyme interaction, protein, or protein interaction, as depicted in Fig. 9.1b. Targeted treatment aims to kill cancer

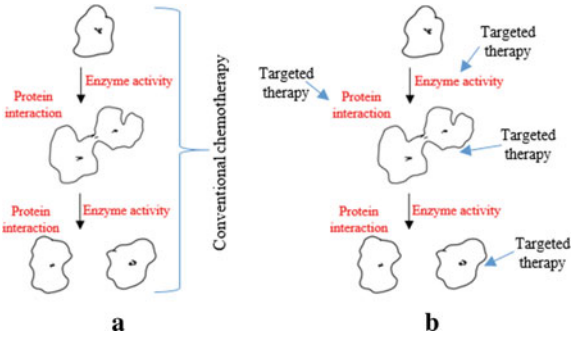

Fig. 9.1 **a** Conventional therapy. **b** Targeted therapy

cells by disrupting the biological processes that give rise to them while causing less harm to healthy cells. As a result, its side effects could be typically more bearable than those of conventional chemotherapy.

9.2 Development of Targeted Cancer Therapy

Targeted cancer therapies are of three main types;

1. Monoclonal antibodies,
2. Immunotoxins, and
3. Small molecule inhibitors.

Behring and Shibasaburo, in their 1890 study of an animal model of diphtheria, defined antibodies as neutralizing substances found in the blood [13]. Heidelberger and Avery recognized antibodies as proteins capable of detecting specific antigens, and Astrid Fagraeus showed in 1947 that plasma B cells from the adaptive immune system form antibodies [14, 15]. The theory of clonal selection that B cell clones carry specific antibodies was proven by Nossal and Lederber [16]. Monoclonal antibodies are therefore antibodies formed by a sole B cell clone. All of them can bind to different regions of the antigen, sometimes called epitopes. Schwaber 1973 discovered techniques for creating human–mouse hybrid cells, and Köhler and Milstein used these techniques to create human-derived hybridomas [17, 18]. Since then, chemotherapeutic monoclonal antibodies (CmAbs) have become the go-to treatment for various human solid and hematological malignancies [19]. Achieving high levels of selective cytotoxicity is difficult because cancer cells and healthy host cells have numerous similarities [19]. Chemotherapeutic monoclonal antibodies were developed with the anticipated benefit of selectivity and function as "targeting missiles" for cancer cells [20]. Chemotherapeutic monoclonal antibodies attach to cell surface antigens linked to cell growth and differentiation to bind to cancer cells [21–24]. Three basic ways are used to destroy cells when chemotherapeutic monoclonal antibodies bind to

the specific target antigen tumor; (a) direct tumor cell death [25–27], (b) immune-mediated tumor cell killing [28–31], and (c) disrupting stromal interactions with cancer cells and vascular ablation [32].

A new class of antibody-binding drugs called immunotoxins is currently being investigated in clinical settings for various cancers. They combine an antibody-dependent targeting domain with a cell-killing bacterial toxin payload. Both dividing and non-dividing cells are damaged by immunotoxins due to their unique method of action, which involves blocking protein synthesis to trigger cell death [33]. Immunotoxins combine some of nature's most poisonous proteins with the focus of antibody treatments to kill cells [33]. The poison is delivered to a cancer cell via the antibody. It then penetrates the cell and destructs it [33]. The earliest immunotoxins were developed in the 1980s, at the time when monoclonal antibodies that interacted with cancerous cells were extensively used. To help in the development of immunotoxins, research was done on the protein toxins produced by diverse bacteria and plants [34]. Immunotoxins consist of bacterial and plant toxins that kill cells by stopping their protein synthesis. Immunotoxins must reach the cytosol inside cells to exert antitumor effects. When an immunotoxin-aiming entity binds to the cancerous cell exterior and produces a lethal effect, the toxin/toxic molecule is taken up into the endocytic compartment [35, 36]. Recent studies of immunotoxins in cancer patients with relapsed and refractory cancers have yielded positive results. Immunotoxins are promising candidates for combination therapy because their mechanism of action differs from conventional chemotherapy and does not share toxicity [34].

The regulatory approval of numerous cutting-edge molecularly targeted cancer therapies during the previous few years has enhanced and elongated the lives of numerous patients. The shift from cytotoxic chemotherapeutics to the development of molecularly aimed anticancer agents has led to a proliferation of effective agents that have positively impacted the lives of many cancer patients. It is well-known that malignancies caused by the corresponding hormones, such as breast and prostate cancers, can be treated with anti-estrogens and anti-androgens, respectively [37]. The majority of acute promyelocytic leukemia patients who had chromosomal anomalies in the RAR (retinoic acid receptor) gene were cured by all-trans retinoic acid, demonstrating the validity of the concept of treating pathogenetic driving anomalies in the clinic with a light chemical [38]. The ABL inhibitor imatinib was discovered and developed and is considered a breakthrough medicine that earnestly recognized the thought of developing minute compound therapies for specific patient populations [39, 40]. Although the inevitable rise of drug resistance remains an issue, the analysis and advancement of targeted new medicines is still very gradual and has an immense rate of failing, especially in end-stage clinical trials [37].

9.3 Computer-Aided Drug Design

Computer-aided drug design (CADD) combines chemical molecular and quantum approaches to identify and create therapeutic chemical agents. Structure–activity connections are the foundation of many CADD strategies (SAR). The primary goals

of CADD are part of a multidisciplinary effort to enhance bioactive chemicals, provide therapeutic alternatives, and comprehend molecular-based biological activities [41]. Computer-aided drug design (CADD) has ratified crucial to numerous initiatives across various contexts and research environments. A significant role has been played by CADD in identifying and optimizing successful compounds that have moved to the end stages of the drug discovery process or the market [41]. CADD encompasses several academic fields, including data mining, molecular modeling, chemoinformatics, and bioinformatics [42]. Bioinformatics, referred to as life science informatics, is a contemporary subfield of biotechnology that provides biologists with an essential tool for more quickly commercializing biotechnology. The field of bioinformatics best illustrates the fusion of biotechnology and information technology. Bioinformatics has long been life science's most crucial technique for mining, analyzing, searching, integrating, and modeling molecular biological data [43]. A relatively recent chemical concept, chemoinformatics, is based on processing information on chemical and molecular structures using computational analysis. These data can be analyzed to examine the connection between molecular activity, chemical characteristics, and structure. It uses an in silico technique, a scientific investigation that is carried out electronically on a computer through software and models. The first step in conventional drug discovery is deciding which disease to target. Next, potential substances and compounds that might be employed to somehow lower the severity of the sickness are sought after. This is done by putting candidates through a number of screening stages, which typically compare how effective they are at thwarting a biological mechanism. Drug design, discovery, and development are one of the main uses of chemoinformatics in research, and it can greatly enhance this process. There are several ways through which this can be accomplished, but it is imperative to use software to compute and display structures [44]. The advancement of theoretical and in silico approaches to model and explore the behavior of molecules, from minute systems of chemicals to enormous bioactive molecules and assembly of materials, is the foundation of the molecular modeling component of CADD. Applications for molecular modeling can be found in computational chemistry, design of drugs, computational biology, and materials science. Also, it is essential to mention that simulation is the fundamental computational method used to carry out molecular modeling. Specific additional processing and software requirements are needed for molecular simulation approaches [45]. Due to CADD's superior selectivity, efficiency, and efficacy, as well as its rapidity, low toxicity, and better ability to match with different pharmacokinetic parameters, the notion of CADD has gained attention for utilization in medicine design, discovery, and advancement process, especially at an early stage [46].

9.4 Computer-Aided Drug Design in Targeted Cancer Therapy

The pharmaceutical industry is grappling with the difficult and urgent problem of how to reduce research expenses while accelerating the discovery of new treatments. Finding new medicines is a challenging, pricey, time-consuming, and expensive endeavor. A new drug typically develops through the regular drug development process in twelve years and for $2.7 billion. The advancement of computer-aided drug discovery (CADD) is a potent and encouraging method for more successful, quicker, and less expensive drug development. We can see that CADD has a lot to contribute to the development of cancer-targeted medicines if we have a better understanding of the background information on targeted therapy and computer-aided drug design. Recent advances in computational drug discovery methods, like anticancer medicines, have shown an enormous and astonishing brunt on anticancer drug design and also have produced informative data about the field of cancer therapy [47].

The rapid increase in computing power, including mass parallelism on graphics processing units and continued advances in tools of artificial intelligence, has recently led to the transformation of basic research into useful utilizations in the medicine exploration field. This has received appreciable consideration for their exceptional work in offering innovative and optimistic suggestions and treatments for life-threatening diseases [48–50].

9.5 Cancer Drug Targets

In discussing targeted therapy development through CADD, one of the essential elements needed is the computational availability of the targets of interest. Approximately, 30,000 genes make up the human genome, and 6000–8000 domains in these genes are considered possible pharmaceutical targets. However, so far, less than 400 protein targets have proven useful in drug development [51, 52]. Currently, there are numerous possible molecular targets for developing therapeutics for cancer, in contrast to many other human diseases [53]. Without taking into account the interactions between medications and proteins, conventional drug discovery generally adheres to the paradigm of "one compound-one receptor-one ailment." However, it has been forgotten that several target proteins are linked to several complex disorders [54–56].

Also, unanticipated drug functions originating from mishits are unintended and uncontrolled activities due to the multi-pharmacological properties of some drugs that can lead to adverse side effects. These are more obvious when taking cancer treatments. Also, there are circumstances when it is useful to target many routes with a particular molecule. As an illustration, sildenafil, which was initially developed as an angina therapeutics, is now utilized for the treatment of erectile dysfunction [57]. A critical step in repositioning and refocusing therapeutics to allow comprehensive

Table 9.1 Cancer target databases

Database	Source
Tumor alterations relevant for genomics-driven therapy (TARGET)	https://software.broadinstitute.org/cancer/cga/target [58]
Therapeutically applicable research to generate effective treatments (TARGET)	https://ocg.cancer.gov/programs/target [59]
Checkpoint therapeutic target database (CKTTD)	http://www.ckttdb.org/ [60]
Non-coding RNAs and drug targets in cancer (NoncoRNA)	https://bio.tools/noncorna [61]
Therapeutic target database (TTD)	http://db.idrblab.net/ttd/ [62]
Protein data bank (PDB)	https://www.rcsb.org/ [63]
The cancer molecular-targeted therapy database (CMTTdb)	https://bio.tools/cmttdb [64]
Cancer drug resistance database (CancerDR)	http://crdd.osdd.net/raghava/cancerdr/ [65]
CanImmunother	http://www.biomedical-web.com/cancerit/ [66]

use of current medicines to treat new disease conditions is the description of all possible new ligand-binding pockets. Accurate prediction of drug targets requires innovative and high-quality bioinformatics target prediction methods [47]. Table 9.1 gives some cancer target databases from which information on protein targets can be obtained for cancer computer-aided drug design research.

9.5.1 Tumor Alterations Relevant for Genomics-Driven Therapy (TARGET)

TARGET is a gene bank containing genes which when biologically modified have direct therapeutic effects in cancer. When predicting drug response or resistance, TARGET genes may be predictive, diagnostic, or a mix of the three. TARGET is a resource for the translational oncology community. It is periodically updated to stay up with new preclinical and clinical discoveries and to retain relevance [58].

9.5.2 Therapeutically Applicable Research to Generate Effective Treatments (TARGET)

This TARGET uses in-depth molecular depiction to uncover the genetic alterations that underlie the development and progression of difficult-to-treat childhood malignancies. TARGET avails its data collection to the research associations to identify

therapeutic targets and prognostic markers in order to design and implement new and more effective therapeutic regimens. There is a need for improved pediatric cancer therapies because.

- Despite rising overall survival, 20% of children with cancer do not respond positively to treatment and eventually die of the disease.
- The statistics of children and teenagers receiving cancer diagnoses are marginally increasing.
- Children who are developing receive extremely harsh treatments today. They frequently have significant short- and long-term adverse effects like infertility, developmental delays, secondary malignancies, and physical and mental health problems.
- Most therapeutic regimens used in current treatment procedures were developed for adult malignancies. Previous genomic research showed that tumors in children could have genetic differences from tumors in adults, emphasizing the need for different therapeutic modalities.

TARGET was developed in collaboration with a sizable, multidisciplinary group of extramural and NCI researchers. TARGET was built around two pilot models examining the genome and transcriptome of substantial-risk subclasses of acute lymphoblastic leukemia and neuroblastoma. The success of two pilot model teams has enabled TARGET to expand its research reach to study more childhood cancers and use higher-resolution genomics techniques. TARGET researchers have molecularly defined subclasses of acute myeloid leukemia, osteosarcoma, some renal malignancies, and several subtypes of acute lymphoblastic leukemia and neuroblastoma. The Children Oncology Group, which is a clinical trials organization focused solely on cancer research in adolescents and youths, is a large part of the TARGET project team.

The accumulated tissue resources and clinical expertise of Children Oncology Group members were made available to the TARGET teams. In order to gather, examine, integrate, and interpret high-quality genomics data, TARGET researchers work together both inside and between groups. By collaborating with the Children Oncology Group in this collaborative scientific environment, we aim to expedite molecular discoveries and translate these discoveries into the clinic more quickly. TARGET data are available to the wider research community for additional research. All patient data provided by means of TARGET will be kept private and confidential. By making the data available to researchers besides the initiative, this data sharing policy increases the chances of developing innovative medicines that benefit children who are suffering from cancer [59].

9.5.3 Checkpoint Therapeutic Target Database (CKTTD)

The CKTTD is a broad database that compiles the checkpoint targets used in cancer immunotherapy and their modulators (small compounds or antibodies) with validated

experimental evidence from the literature. Additionally, a grading system was added to assess the confirmed levels and boost the trust in checkpoint targets for cancer immunotherapy. Due to their importance in tumor-mediated immune evasion, checkpoint targets may represent promising therapeutic targets for cancer immunotherapy. Immune checkpoints are immune system regulators that stop the immune system from attacking cells randomly. Through immunological editing of checkpoint targets or the tumor microenvironment, tumor cells may become immune tolerant [60].

9.5.4 Non-coding RNAs and Drug Targets in Cancer (NoncoRNA)

A database of observationally assisted non-coding RNAs and medicinal cancer targets. NcRNAs, or non-coding RNA molecules, control cellular functions and gene expression in a range of human ailments, like cancer and neurological illnesses. NoncoRNAs, a classically selected library of observationally proven non-coding RNAs (ncRNAs) and drug targets, are a high-quality resource for studying ncRNAs associated with drug susceptibility resistance in a variety of human malignancies. I can provide a data source. NoncoRNA has 8233 entries between 154 drugs and the 5568 ncRNAs found in 134 malignancies. All entries in the NoncoRNA provide comprehensive data on ncRNAs, medications, and malignancies, as well as details on ncRNA expression patterns, experimental detection techniques, treatment outcomes, other targets, and literature references [67].

9.5.5 Therapeutic Target Database (TTD)

TTD is a type of database that provides intelligence on the ailments which are being targeted, also the therapeutic protein, enzymes, and nucleic acid targets that have been identified and analyzed the associated pathways, and the drugs that are utilized to attack each of these targets [68].

9.5.6 Protein Data Bank (PDB)

Protein Data Bank is a foremost openly accessible computerize source in biology for exchanging three-dimensional protein structures. Beginning with seven structures in 1971, Protein Data Bank has quickly grown to 194,011 biological macromolecular structures, enabling breakthroughs in research and teaching [63]. Natural and synthetic macromolecules are entered there. Over 84,000 number of them have microscopic chemical components complexed with them. As a facility for the community

of structural biologists, PDB was initially developed. However, over time, more and more people, including biologists, software designers, computer and other scientists, bioinformatics experts, students, instructors, and the general public have become aware of its importance [69].

9.5.7 The Cancer Molecular-Targeted Therapy Database (CMTTdb)

The Cancer Molecular-Targeted Therapy Database, or CMTTdb, is a comprehensive database that houses pertinent data about molecularly targeted drugs and their combinatorial strategies in clinical trials and studies for cancer treatment. It includes data from open access clinical trials to identify the clinical agents, their related targets, cancer subtypes, biomarkers, clinical characteristics of the patients, and treatment modalities (monotherapy or combinatorial therapies) [64].

9.5.8 Cancer Drug Resistance Database (CancerDR)

The database CancerDR comprises data on about 148 number of anticancer medications and how well they work against almost 1000 cancer cell lines. The bioactivity profiles of these antitumor medications were gathered from Cancer Cell Line Encyclopedia (CCLE) and Catalog of Somatic Mutations in Cancer (COSMIC) databases. CancerDR covers 116 pharmacological targets associated with various anticancer drugs and offers complete details about these targets, including their structure, function, and gene sequences in the associated cancer cell lines. CancerDR allows users to browse medications, medication targets, and cell lines. Finding the drug target mutations that cause drug resistance is one of the database's most important potential uses. This database's multiple sequence alignment features enable users to distinguish between naturally occurring target changes and cancer gene mutations (targets). This information will benefit the creation of biomarkers for many types of malignancies. By classifying cell lines according to their drug sensitivity, users can find those that are resistant to a particular anticancer agent. Users of the clustering facility can also find effective medicines for various cell lines or cancer types. Users can also map or align their sequences with pharmacological target sequences using the mapping or sequence alignment menu. Additionally, it enables users to map or match short reads or contigs (data from next-generation sequencing) on therapeutic targets. As a result, it is feasible to use this database to create personalized medicine, in which an individual's genome or target sequence data can be used to determine a cancer drug's sensitivity or resistance [70].

9.5.9 CanImmunother

CanImmunother is an extensive manual-curated database of resources for immunotherapeutics of cancer. CanImmunother presently offers 3267 analytically verified connections among 484 immunotherapies, 642 biomarkers, 108 targets, and 218 cancer subtypes across 34 body areas through manual curation on 4515 publications. With the help of CanImmunother, physicians and researchers may quickly access and monitor data on cancer immunotherapy associations to find new immunotherapies with more effective cancer-predictive biomarkers. Users can search, explore, analyze, and retrieve association data for precision cancer immunotherapy using CanImmunother's easy-to-use embedded webs. Users can also obtain and provide other associations for inclusion in the future [66].

These databases provide information on protein targets used in computational studies for targeted cancer therapy design and development. Most of these targets are tyrosine kinases that are either receptor tyrosine kinases or non-receptor tyrosine kinases. Currently, targets are being studied using computer-aided drug design for possible inhibition and, when inhibited, alter the cancer pathogenesis mechanism.

9.6 Receptor Tyrosine Kinases (RTKs)

In response to external stimuli, a range of intracellular signaling courses are triggered by the essential cell surface receptors known as RTKs. Many biological functions, including growth, multiplication, translocation, differentiation, and viability, depend on them [71]. RTKs are transmembrane receptors with significant therapeutic value because of the role they play in illness, notably cancer. Numerous routes causing RTK dysregulation have been identified since their discovery, and as a result, numerous cancer types now show an oncogenic addiction to RTKs. Small molecule-based tyrosine kinase inhibitor (TKI) therapies have been made. Over the past 20 years, RTKs have emerged as an important class of targeted therapies due to their clinical approval for a number of cancers. But many of the existing RTK inhibitor therapies eventually cause a quick rise in acquired resistance to a subsequent tumor relapse [72]. Tools and recent technological developments in the computer-aided drug are being utilized to discover and develop new RTK small molecule therapies. With a focus on the receptors and critical cellular components crucial to the RTK signaling cascade, these more recent technologies will be essential for discovering various RTK inhibitors. These computer-aided drug design approaches are vital supplements to conventional biochemical and cell-based analyses, which have been utilized to find the most clinically authorized RTK TKIs.

9.7 Tyrosine Kinases Overexpression in Cancer

A key factor in oncogenesis is the overexpression of RTKs at the proteome level, frequently brought on by localized duplications of genomic areas that include RTKs or polysomy that increases RTK gene copy [72]. RTK clumps or enhanced susceptibility to associated molecules cause constitutive kinase activation with the consequent abnormal signaling when RTK levels are elevated [73]. Epidermal growth factor receptor (EGFR) overexpression is known to cause glioblastoma multiforme (GBM, 57%) [74], breast malignancy (6%) [75], and non-small cell lung cancer (NSCLC) [76]. De novo MET overexpression is implicated in NSCLC (1–5%), gastric malignancies (1–10%), and gliomas (2%) [77]. Fibroblast growth factor receptor 1 (FGFR1) overexpressions are observed in breast malignancy (19% of ER-positive breast malignancy), lung cancer (6%) including 17% of squamous cell carcinoma, prostate cancer (16%), and bladder malignancy (9%) [78]. Breast malignancy (2%) and stomach malignancy (10%) both exhibit elevated FGFR2 [78]. Other instances include human epidermal growth factor receptor 2 (HER2) amplification, which mostly affects breast malignancy (20% of cases), where it is associated with improper diagnosis, growing combativeness, and disease incidence, also stomach (11–16%), pancreatic (2%), bladder (8.6%), and ovarian (7%) malignancies [79]. Table 9.2 gives some other tyrosine kinases implicated in cancers.

Additionally, some NRTKs participate in transmitting signals from external cues and frequently interact with transmembrane receptors. Therefore, they play an essential function in signaling pathways that control essential biological actions like cell differentiation, death, survival, and proliferation. The activity of NRTKs is closely controlled, and carcinogenesis and malignant transformation have been linked to deregulation and/or overexpression of NRTKs. The mechanics of numerous cellular processes, including those implicated in carcinogenesis, have been clarified through

Table 9.2 Some tyrosine kinases overexpressed in cancers

Tyrosine kinases	Cancer types
Hematopoietic cell kinase (HCK)	Leukemia [81], osteosarcoma [82]
Lyn and focal adhesion kinase (FAK)	Prostate [83], endometrial [84], ovarian [85], colorectal [83]
Receptor of activated protein kinase C 1 (RACK1)	Cervical cancer [86]
Lyn and mitogen-activated protein kinase (MAPK)	Melanoma [87]
Pi3k/akt	Oropharyngeal cancer [88]
ABL and Janus kinase and Bruton's tyrosine kinase	Hepatocellular carcinoma [89, 90]
Erbb2	Pancreatic cancer [91]
Cyclin-dependent kinase 12 (CDK12)	Breast cancer [92]

research on NRTKs. It should not be surprising that several tyrosine kinase inhibitors are used to treat a variety of cancers, and more are being researched [80].

9.8 Application of Computer-Aided Drug Design in Targeted Cancer Research

To determine the anticancer targeting effectiveness of phloretin through molecular docking, Arokia et al. used a phytochemical of phloretin and proteins such as the c-Kit receptor protein tyrosine kinase, farnesyl transferase, platelet-derived growth factors (PDGFs), and vascular endothelial growth factor receptor 2 (VEGFR2) to execute induced fit (Schrodinger 2014-2). Numerous poses were created and assessed to understand the docking conformity and general interaction residues between drug molecules and proteins. According to molecular docking studies, phloretin displayed a solid docking interaction model compared to the inherent inhibitor of a known cancer target [93].

The discovery and measurement of pathway crosstalk in different tumor subtypes forms the basis of in silico approaches. To identify drug target pathways in breast malignancy subclasses, Cava et al. improved computer-guided approaches for the discovery of novel pathways for drug targets in cancer subtypes. They used data from The Cancer Genome Atlas to identify many networks of mechanisms for various breast cancer subtypes, which they subsequently verified using data from Gene Expression Omnibus, an independent source of breast cancer information. Scientists then determined using the computer-assisted approach, the outcomes of new or approved medicines on various subclasses of breast cancer by closing respective or merged pathways derived from these types in order to obtain new possible pharmaceuticals or more effective drug combinations. They identified potential pharmacological target pathways for several breast cancer subclasses that drugs could bind to and inhibit to have anticancer effects [94].

To improve our knowledge of convection-enhanced distribution effectiveness and medication delivery, Lambride et al. conducted a computer-assisted inquiry for a brain tumor convection-enhanced delivery therapy simulation. They utilized a brain prototype obtained from clinical imaging information in a three-dimensional definite element conception that predicts drug deposition in convection-enhanced delivery procedures. The approach combines dynamics of biofluid and medicine distribution to the brain parenchyma. Distribution of the drug was looked at under range of pathophysiological circumstances of cancer in terms of the vascular wall pore dimension and hydraulic conductivity of the tumor tissue, and also for medicines of different sizes, from tiny particles to nanoparticles. The effects of the therapeutic agents and the diameters of the arterial wall pores on drug distribution before, during, and after convection-enhanced delivery were documented by a parametric analysis. For the purpose of treating brain cancer, the in silico results provide a useful insight of the typical medicine concentration in the tumor and its spatiotemporal distribution [95].

Imana et al. utilized EGFR protein structure from the Protein Data Bank for research to obtain drug candidates of peptide compounds sourced from PubChem that can play EGFR inhibitory role and have good interactions and therapeutic effects for treating lung cancer using computational approaches. For the computer-based simulation, the preparation of the peptides was achieved using optimization and energy-saving procedures. In determining pharmacophore properties within the EGFR binding site, protein–ligand interaction fingerprint was utilized. The most effective ligands were then put through a computer prediction of their ADME-Tox properties. Rigid and flexible molecular docking was used to simulate screening for both proteins and ligands. According to the protein's binding energy and RMSD value, molecular docking simulation screening revealed that nine substances interacted favorably with the EGFR protein. We discovered the chemicals that interact with the macromolecule via hydrogen bonding [96].

In a study to find potential inhibitors of Lyn tyrosine kinase using computer-aided drug design, Kulavi et al. used AutoDock incorporated in the MGL tools to simulate molecular docking of chosen natural bioactive chemicals against Lyn tyrosine kinase. Following a preliminary screening, substances with higher docking rankings and lower binding free energies than tamoxifen were taken into account for further analysis. The reference medications were some well-known synthetic Lyn tyrosine kinase inhibitors. Based on a pharmacokinetic investigation, toxicity prediction, and in silico Lipinski filter analysis, four chemicals were recommended as potential Lyn tyrosine kinase inhibitors. Additionally, considerable ligand efficiency in energy score was found by molecular modeling investigations employed to evaluate the binding interactions of all suspected Lyn inhibitors. Therefore, the three bioactive suggested compounds may be investigated as possible novel Lyn kinase inhibitors for the treatment of Lyn-associated breast cancer following experimental validation [97].

Utilizing computational approaches like molecular docking, molecular dynamic simulation, and ADMET projection to identify probable phytocompounds that could function as HER2 inhibitors, Lamichhane et al. molecularly docked 1500 selected phytochemicals to the HER2 kinase domain's ATP binding site. Luxenchalcone, rhinacanthin Q, subtrifloralacton D, and 7,7″-dimethyllanaraflavone met Lipinski's rule of five in addition to having significantly higher binding affinities than the standard inhibitor used. The study of the molecular dynamics simulation trajectory revealed that rhinacanthin Q, subtrifloralacton D, and 7,7″-dimethyllanaraflavone formed a reliable and compact complex with little conformational fluctuation. According to an MM/PBSA binding free energy research, HER2 binds strongly to rhinacanthin Q, subtrifloralacton D, and 7,7″-dimethyllanaraflavone. Possible pharmacologically active substances that could inhibit the HER2 protein include rhinacanthin Q, subtrifloralacton D, and 7,7″-dimethyllanaraflavone. To fully evaluate the potential of these phytocompounds, in vitro tests are ultimately necessary. The findings of our study could speed up the development of medications to treat breast cancer that is HER2 positive [98].

Houttuynia cordata Thunb, a traditional medicine herb, is said to contain phytochemicals. The frontrunner phytocompounds from *H. cordata* were found by Das

et al. to have the potential to decrease the overexpression of two kinases, human epidermal growth factor receptor 2 (HER2) in breast cancer and vascular endothelial growth factor receptor 2 (VEGFR2) in stomach cancer. One hundred pharmacologically active phytocompounds from *H. cordata* were examined for their ability to bind to the ligand-binding site of the HER2 and VEGFR2 kinase domains. Using a competitive docking technique, the frontrunner phytocompounds that flawlessly bind to the ATP ligand-binding site were found. Only a few phytocompounds that have binding affinities greater than the ATP ligand found naturally can fit into the ligand-binding site, according to the docking data. Among the frontrunner phytocompounds docked from *H. cordata*, sitosterol and quercetin demonstrated the greatest propensity for interacting with the HER2 and VEGFR2 receptors via hydrophobic and hydrogen associations. This research supported the use of sitosterol and quercetin as possible therapies for breast and stomach cancers [99].

In an effort to reuse benzimidazole scaffolds for HER2-positive breast malignancy therapeutics using computer-aided drug designComputer-Aided Drug Design, mebendazole and albendazole, which belong to the benzimidazole class of medications, were reported to be cytotoxic or have anticancer qualities when taken against certain malignancies. Jubie et al. developed three hydrazone analogs having a benzimidazole motif in their structural framework. They performed both ADMET experiments and in silico binding studies against the HER2 receptor using Accelrys Drug Discovery Studio 4.1. Using the MTT test on HER2 overexpressed MCF-7 cell lines, the in vitro results of the in silico research were verified. One of the substances, 2-[2-(2,4-dinitrophenyl)hydrazinylidene]-2,3-dihydro-1H benzimidazole, showed excellent cytotoxicity effect when compared to a well-known HER2 inhibitor lapatinib [100].

Qin et al. used network pharmacology and thorough bioinformatics methodology to investigate biochanin A in patients suffering from colorectal cancer (CRC) and COVID-19 infection due to the effects of COVID-19 and its vaccine's difficulties in treating people with chronic illnesses, particularly people with cancer. With the aid of network pharmacology method, they revealed two groups of genes implicated in cell proliferation (CCND1, PPARG, and EGFR) and immune reaction (IL1A, IL2, and IL6R) mediated by biochanin A in the CRC/COVID-19 situation. The functional analysis of these two gene assemblage provided additional evidence for the effects of biochanin A on cytokine-cytokine receptor interactions and interleukin-6 production in CRC/COVID-19 illness. Additionally, pathway analysis revealed that biochanin A regulates the PI3K-Akt and JAK-STAT signaling pathways in the treatment of CRC/COVID-19. The results of this research provide patients with CRC with an alternate kind of treatment for combination therapeutic to combat COVID-19 infection [101].

Ibrahim et al. used the QSAR technique to determine new inhibitory effects of derivatives of 8, 9, and 10-disubstituted phenylthio/phenylsulfinyl-9-purines as anti-proliferative drugs. Density Functional Theory (DFT) technique was utilized to enhance the anti-proliferative drugs using the B3LYP/6-31G* level of theory. To create the QSAR prototypes, the Genetic Function Algorithm (GFA) was employed.

The best prototype among those created was chosen and stated due to its statistical fitness using the following evaluation parameters: R2 trng = 0.919035, R2 adj = 0.893733, Q2 cv = 0.866475, R2 test = 0.636217, and LOF = 0.215884. The chosen prototype was additionally evaluated using the VIF, the Y-scrambling test, and the relevant domain, and it was discovered to be statistically relevant. Through the use of molecular docking, the binding form of some selected 2, 9-disubstituted 8-phenylthio/phenylsulfinyl-9H-purine (ligands) in the active site of the EGFR-tyrosine kinase (EGFR-TK) (receptor) was examined. In comparison with the other chosen ligands, molecule 22 was found to have the least binding energy (− 10.4 kcal/mol). This may be due to hydrogen interactions with the amino acid residues MET793 (2.48599, 2.04522) and THR854 (3.76616), as well as hydrophobic/other interactivity with the amino acid residues LEU718, LEU844, MET766, VAL726, ALA743, LYS745, and MET790 (EGFR-TK). The drug-likeness of these preferred anti-proliferative medicines was predicted using the pharmacokinetics profile of the compounds using SWISS ADME. The anti-proliferative medicines were safe to use orally when Lipinski's rule of five was broken just once. This work provided a strategy for developing potent anti-proliferative medications that target their particular enzyme [102].

Sarkar et al. modeled the 3D structure of the vasoactive intestinal polypeptide receptor 1 to uncover double natural compound inhibitors for VPAC1 and EGFR, which are tied to signal transduction pathways related to neuroblastoma, breast, prostate, and lung cancer (VAPC1). To develop acceptable ligand analogs for the concomitant suppression of EGFR and VPAC1, computational homology modeling of VPAC1 and its characterization by molecular interactivity researches has been carried out. Homology modeling was done with the help of the Swiss model, and the projected 3D structure was validated using PROCHECK and RAMPAGE. 92% and 94% of the residues in these two programs' projected structures were in the most favorable region, according to Ramachandran's plot. The ADMET features of compounds selected from the Naturally Occurring Plant-based Anticancerous Compound-Activity-Target (NPACT) database and showing high interactivity with EGFR were additionally examined. Four substances, which include Fisetin, Genistein, Tectorigenin, and Tephrosin, were docked with VPAC1 according to molecular interaction studies, with corresponding spontaneous binding of − 7.1, − 6.98, − 6.9, and − 6.61 kcal/mol. The rotatable bond and reduced molecular weight of fisetin and genistein boosted their drug-likeness more than the other compounds. As a result, concurrent inhibition of VPAC1 and EGFR may also impede the spread of breast cancer. Comparing the data to positive and negative controls further corroborated the conclusions. As a positive control, quercetin had a substantial binding energy with EGFR of − 7.54 kcal/mol, which is coherent with the results of the experiments. When docking with 3-O-cis-p-coumaroyl alphitolic acid was attempted, it was impossible without binding to either EGFR or VIPR1 [103].

MEK1 a kinase that is important for regulating cell proliferation and is a potential target because of its selective extracellular signal-regulated kinase phosphorylation, also features a singular hydrophobic target which has the capacity to store extremely particular allosteric inhibitors. Singh et al. used Poisson–Boltzmann surface area

analysis, durable molecular dynamics (5s), and molecular docking simulation to examine the possibility of quinolines as allosteric inhibitors. For the comparison investigation, four reference MEK1 inhibitors were utilized. The toxicity and drug-likeness of these compounds were investigated depending on their toxicity and ADMET prediction by computer-assisted technology sketch. The results demonstrated that the quinolines (4m, 4o, 4s, and 4n) demonstrated increased stability and spontaneous binding while being harmless in comparison with the standard inhibitors. They inferred that the effectiveness of these quinoline compounds as MEK1 allosteric inhibitors might be supported by experimental study [104].

To create novel, computational designed 9-anilinoacridines (a-z) for their HER2 inhibitory properties. In the Schrodinger suit 2016-2, Kalirajan et al. did docking research on a few medicines against HER2 (PDB id-3PP0). The proposed compounds' molecular docking was simulated using the Schrodinger suit's Glide module. We replicated computational ADMET screening using the QikProp module of the Schrodinger suit. The Glide score was utilized to gauge how well suited the developed compounds were to bind to HER2. The compounds 1a-z, with the exception of 1z, exhibit notable Glide scores in the range of − 4.91 to − 10.59, which contrasts with the typical ethacridine (− 4.23) and tamoxifen (− 3.78). The strongest inhibitor exhibits positive MM-GBSA binding. The findings support the possibility of HER2 inhibitors being 9-aminoacridine compounds with isoxazole substitution. The therapeutic potential of the compounds 1s, x, v, a, j, and r with substantial Glide scores may be revealed by a more in vitro and in vivo study [105].

Kalirajan et al. used the Prime-MM-GBSA module for energy-free binding estimations, the Qikprop module for computational ADMET evaluation, and the Glide module for molecular docking studies to create some novel 9-anilinoacridines substituted with pyrazole moiety (1a-z) and test their ability to inhibit the HER2 enzyme (PDB id-3PP0). Dependent on Glide scoring task, they determined the spontaneous binding of molecules (1a-z) to HER2. In the investigation, the compounds' potent inhibitory activity against HER2 was attributed mostly to the robust hydrophobic and hydrogen bonding interactivity. With Glide scores ranging from − 4.9 to − 9.75, nearly all of the compounds 1a-z showed better spontaneous binding in comparison to the CK0403 (− 4.105) and tamoxifen (− 3.78). The computational ADMET characteristics data made it obvious that the majority of the molecules were in the suggested ranges. The estimates of MM-GBSA binding for the strongest inhibitors were the most promising. They came to the conclusion that the findings provide persuasive evidence that useful molecules in pyrazole substituted 9-anilinoacridines have the ability to behave as HER2 inhibitors and that molecules with large Glide scores may have potent anti-breast cancer effects [106].

Purawarga et al. used a molecular docking simulation to examine the possible use of a few selected phytochemicals in the treatment of breast cancer. They looked at how well they bound to the EGFR, HER2, estrogen, and NF-B receptors. Among the examined phytochemicals, pristimerin, ixocarpalactone A, viscosalactone B, and zhankuic acid A demonstrated higher notable binding affinities toward the desired receptors. The docked complex for pristimerin and the HER2 receptor is stable, according to

molecular dynamics modeling studies. This computer-based work presents a strong basis for advance studies into their anticancer actions [107].

Computer-based research is what makes these and many more studies possible. Before moving on to the phase of drug development known as clinical trials, we first conducted in vitro and then in vivo (in animal) and/or ex vivo tests. However, computer-based research increasingly comes before in vitro and in vivo researches, thanks to the advent of computer-aided drug design and its use in targeted cancer therapeutics, particularly in the creation of tiny molecular inhibitors. Therefore, it is easier to identify a molecule's true intended target and anticipated side effects during the early stages of its conception, design, and development. Within five years of investigation and diagnosis, there were 32.6 million people who have cancer, 8.2 million people lost their lives to the disease, and 14.1 million people developed cancer. More effective therapies are required due to the high mortality rate from cancer. As cancer therapy becomes more and more individualized, cancer is also changing into a group of unusual tumors, with the contrast between them no longer only reliant on histological basis. This phenomenon calls conventional methods to producing oncology medications into question, especially in light of the current economic context, which necessitates careful resource management at all levels of clinical practice, health care, and research and development [108].

9.9 Anticancer Molecule Databases

The applications of computer-aided drug design in medicine design and advancement for different disease conditions, including cancer, have led to the setting up of databases containing drug compounds and compounds yet to be developed as medicine. Some of these databases include both synthetic and natural compounds. These compounds are presented in most of these databases in formats like 2D, 3D, SDF, SMILES, etc., which enables their easy download for computation studies. There are dedicated anticancer molecule databases. Some contain already established anticancer, while others include compounds with potential anticancer activity.

9.9.1 CancerDrugs_DB

The Anticancer Fund's CancerDrugs DB is a carefully managed database of approved cancer medications. The NCI, FDA, EMA, and other data sources provide the source data. The goal is to offer a database of approved medications used to treat cancer that can be easily filtered for researchers, doctors, and regulators. Drugs used for diagnostic purposes are not included, nor are those used to treat cancer symptoms or for other forms of supportive care. Also excluded are investigational medicines and experimental therapies tested in clinical trials [109]. This database also contains the

specific indication and protein target of each drug. The database can be accessed at https://www.anticancerfund.org/en/cancerdrugs-db.

9.9.2 canSAR

canSAR is put in place by the canSAR group. The canSAR team is a chemogenomics and computational biology team in the CRUK Cancer Therapeutics Unit of The Institute of Cancer Research, London, UK, and the Department of Data Science. To produce relevant predictions for drug discovery, canSAR is a comprehensive knowledge base that combines data from various scientific fields, including biology, structural biology, pharmacology, chemistry, cellular networks, and clinical annotations. Making this information available to researchers from multiple fields facilitates translational research and medication discovery. It gives users access to a single knowledge hub that can address complicated, cross-disciplinary queries and support hypothesis formulation. It provides a centralized repository of information to address complicated, multidisciplinary questions like, among many others: Information available about a protein, which tumors express it or have mutations, and what chemical techniques and cell line prototypes may be used to experimentally investigate its activity? What information may be used to explain a drug's unusual bioactivity? What information is available about a medicine's cellular sensitivity profile? To what proteins does the medicine bind? [110] canSAR can be accessed at https://cansarblack.icr.ac.uk/.

9.9.3 CancerPPD

CancerPPD is a one of a kind database that offers comprehensive details on anticancer peptides (ACPs) and proteins that have undergone experimental verification. Data were carefully curated from published articles, patents, and other repositories. Given that structures are crucial to anticancer efficacy, the database includes predicted tertiary structures of anticancer peptides using the cutting-edge PEPstr approach. Secondary structure states are assigned using DSSP. The essential aspect of cancerPPD is that it also details numerous chemical alterations, such as synthetic, D-amino, and modified amino acids like ornithine. To give thorough information about ACPs, the database is cross-linked with numerous other relevant resources [111]. CancerPDD can be accessed at http://crdd.osdd.net/raghava/cancerppd/index.php.

9.9.4 PharmacoDB

PharmacoDB enables researchers to search publicly accessible datasets for cases where a compound or cell line of interest has been profiled. Researchers may also see and contrast the dose–response information for a particular cell line-compound combination from any of the database's studies [112]. PharmacoDB can be accessed at https://pharmacodb.pmgenomics.ca/.

9.9.5 ReDO_DB

A well-maintained list of non-cancer medications with some proof of anticancer efficacy is called ReDO DB. The database's material is comprised of critique articles, medical and therapeutic case records, experimental researches, and clinical trials. All drugs in the database are additionally verified to see if they are off-patent and incorporated on the Essential Medicines List of the World Health Organization [113]. ReDO_DB can be accessed at https://www.anticancerfund.org/en/redo-db.

9.9.6 TIPdb

Taiwan Indigenous Plants Database (TIPdb) is a database of phytocompounds produced from native Taiwanese plants that have anticancer, antiplatelet, and anti-tuberculosis properties. A unique opportunity to develop quantitative models of the relationship between structure and activity for high-throughput analyses of probable anticancer, antiplatelet, and anti-tuberculosis medicines is provided by the database's chemical structures, which are also contained in it [114, 115]. TIPdb can be accessed at http://cwtung.kmu.edu.tw/tipdb.

9.9.7 pdCSM-Cancer

The most thorough platform for predicting anticancer bioactivity to date, pdCSM-cancer, contains expert and confirmed prototypes based on analytical data on the effects of growth inhibition concentration (GI50%) on nine cancer types and 74 different cancer cell lines, including more than 18,000 compounds. Tenfold cross-validation yielded Pearson's correlation coefficients equal to 0.74, while independent, non-redundant blind testing produced identical results of up to 0.67. Using the data from these cell line-specific prototypes, a general predictive prototype was created to uncover chemicals active in about 60 different cell lines. The final prototype performed better than competing approaches in independent non-redundant blind

testing and tenfold cross affirmation, possessing an Area Under the ROC Curve (AUC) of up to 0.94 [116]. pdCSM can be accessed at http://biosig.unimelb.edu.au/pdcsm_cancer.

9.9.8 ACNPD

ACNPD is a web-based tool that enables users to quickly look up the therapeutic mechanisms of all natural anticancer medications. The database comprises information on ten common cancer types, medications, and pharmacological procedures involving the signaling pathways Akt/PI3K, Akt/PI3K/mTOR, Wnt/-catenin, and others [117]. ACNPD can be accessed at http://www.acnpd-fu.com.

9.9.9 NPACT

A well-selected collection of organic anticancer compounds is available through Naturally Occurring Plant-based Anticancerous Compound-Activity-Target (NPACT). It has 1574 phytocompounds with details on each compound's structure, characteristics (topological, elemental, and physical), kind of cancer, cell lines, values of inhibition (ED_{50}, IC_{50}, GI_{50}, EC_{50}), suppliers, molecular targets, and drug-likeness. Only native anticancer molecules derived from plants are the sole focus of NPACT. The biological activities of these natural compounds against several cancer cell lines and also their molecular targets are provided by NPACT, which makes it special [118]. NPACT can be accessed at https://webs.iiitd.edu.in/raghava/npact/index.html.

In addition to these particular databases of anticancer molecules, there are also open databases of approved medications and phytochemicals. The current medication database can be used for in silico drug repurposing investigations in targeted therapeutic research using computer-aided drug creation. On the other hand, new molecular anticancer discovery and development investigations can be aided by phytochemical databases. The databases of current medications include.

9.9.10 DrugCentral

The translational informatics division at the University of New Mexico created and maintains DrugCentral, an online drug information resource, in partnership with the Illuminating the Druggable Genome (IDG) initiative. DrugCentral provides information about pharmaceutical products, active ingredients, chemical entities, medication modes of action, indications, and pharmacologic action [119, 120]. DrugCentral can be accessed at https://drugcentral.org/.

9.9.11 DrugBank Online

DrugBank Online is a vast online resource that is free to access and offers information about drugs and therapeutic targets. It serves as a resource for bioinformatics and cheminformatics by fusing comprehensive drug target data with thorough drug-specific data (chemical, pharmacological, and pharmaceutical data). DrugBank Online is extensively utilized by the pharmaceutical companies, medical chemists, pharmacists, doctors, and members of the general populace. Due to its wide range, thorough reference, and in-depth data descriptions, DrugBank helps to significantly advance the data-driven pharmaceutical sector [121]. DrugBank Online can be accessed at https://go.drugbank.com/.

Some general phytochemical databases that can be utilized for computational target therapy design and development include.

9.9.12 COlleCtion of Open Natural ProdUcTs (COCONUT)

An open-source initiative for Natural Products (NPs) storage, search, and analysis is called COlleCtion of Open Natural ProdUcTs (COCONUT) Online. It is accessible without payment or restrictions and collects information from more than 50 open NP resources. Each item corresponds to a "flat" NP structure and is linked, where accessible, to its many pre-computed molecular attributes, literature, known stereochemical variants, producing organisms, and natural geographic presence. The database contains 407,270 different natural products [122]. COCONUT can be accessed at https://coconut.naturalproducts.net/.

9.9.13 African Natural Products Database (ANPDB)

The African Natural Products Database (ANPDB) is an amalgamation of natural product databases from all over the continent. Currently, ANPDB is composed of the Eastern African Natural Products Database (EANPDB) and the Northern African Natural Products Database (NANPDB) (EANPDB). The NP databases for the remaining African regions are either still being built or have not been integrated into ANPDB yet. The most all-inclusive database of NPs isolated from native African species is called ANPDB. The content currently comprises data sources (covering the years 1962 to 2019) gathered from literature obtained from major natural product journals, M.Sc. and Ph.D. theses available in African university libraries, and chosen searches in regional African publications. The dataset contains 6515 chemicals that were extracted from 1042 source organisms, the bulk of which are plants, as well as microbes, animals (including corals), and marine sources [123, 124]. ANPDB can be accessed at http://african-compounds.org/anpdb/.

9.9.14 Natural Products Atlas

A database of 32,552 naturally occurring microbial products, the Natural Products Atlas, contains references to information on the structure, names of the compounds, source, complete syntheses, and occurrences of compounds reassignment. The database allows searching by physical characteristics, substructures, and structures. The database also provides tools for analyzing the chemical space of natural products as well as dashboards with information on authors and timelines of discoveries. With a centralized network for structure- and property-based searches, these interactive tools offer fresh perspectives on the structural diversity in natural commodities. They also provide a solid information base for finding natural items. It is the first comprehensive, freely available resource of its sort. In order to serve as a central archive for all recognized natural product structures sourced from microorganisms, it was developed as a community-supported resource. The Natural Products Atlas is expected to facilitate the design and development of novel natural product discovery methods and speed up structural characterization for difficult natural product libraries [125, 126]. Natural Products Atlas can be accessed at https://www.npatlas.org/.

9.9.15 Phenol-Explorer

Phenol-Explorer is earliest full-scale database on the presence of polyphenols in food. The database comprises above 35,000 content parameters for 500 distinct polyphenols that can be found in more than 400 foods. These figures were compiled methodically from more than 1300 academic articles, each of which contained more than 60,000 distinct content values. Each of these works was carefully examined before being added to the database. The complete data on food's polyphenol content is available for download [127–129]. Phenol-Explorer can be accessed at http://phenol-explorer.eu/.

Some other databases of importance in computer-aided drug design include ZINC and PubChem, which contain synthetic and natural compounds.

9.9.16 ZINC

ZINC is a free online screening database for substances that are readily available. ZINC offers more than 230 million chemicals in 3D formats that are ready to dock. ZINC offers more than 750 million compounds, making it possible to locate analogs in less than a minute [130]. ZINC can be accessed at https://zinc.docking.org/.

9.9.17 PubChem

The National Institutes of Health maintains **PubChem**, an open chemical database (NIH). The majority of the molecules in PubChem are small and more important compounds like nucleotides, carbohydrates, lipids, peptides, and macromolecules that have undergone chemical modification. It gathers information on a variety of topics on each of the compounds [131]. PubChem site can be accessed at https://pubchem.ncbi.nlm.nih.gov/.

9.9.18 Anticancer Prediction Tool

The continual efforts to design and develop better treatment options for cancers and changes met in the processes have led to the exploration of more possible cancer treatment options. One of such options currently being explored is peptides, referred to as anticancer peptides. Peptides are molecules made up of two or more amino acids, as opposed to proteins, which are long molecules composed of multiple peptide subunits and are also called polypeptides. These small peptides with amino acid sequences known as anticancer peptides are selectively harmful to cancer cells [111]. Because of their high exclusiveness, high bioavailability, and ease of modification, anticancer peptides are a better therapeutic option than antibodies and small compounds [132–134].

The applications of advancements in computer-aided drug design toward the design of targeted cancer therapy have led to the depiction and advancement of computer-based tools that can assist the design of anticancer peptides and also predict the anticancer effect of a peptide. Some of these computational tools include.

9.9.19 AntiCP

AntiCP is a web-based anticancer peptide prediction server. The server relies on support vector machine models. The development of support vector machine models is based on binary profile characteristics and amino acid composition. A total of 225 antimicrobial peptides with anticancer activities make up the positive dataset. Researchers studying anticancer peptides can significantly benefit from this website. Users of this site can create anticancer peptides (ACPs) and their mutants with various physicochemical features. Significant characteristics are peptide design, which enables users to develop every single mutant analog of their peptides and determine whether or not such analogs possess anticancer properties; virtual screening, which allows the user to predict anticancer features in a large number of query peptides; protein scan, which creates all potential overlapping peptides and their single mutant protein analogs that the user has provided. Additionally, it indicates

whether an overlapping peptide or analog is an ACP; motif scan enables users to find anticancer-related patterns in their sequences [135, 136]. AntiCP can be accessed at https://webs.iiitd.edu.in/raghava/anticp/index.html.

9.9.20 Machine Learning-Based Prediction of Cell-Penetrating Peptides (MLACP)

Using four machine learning-based prototypes, a predicting two-layer structure using features derived from the peptide sequence, such as amino acid index, composition-transition-distribution, amino acid composition, dipeptide composition, and physiochemical characteristic. A particular peptide's cell-penetrating peptides or non-cell-penetrating peptides status is predicted in the first layer, and the intake effectiveness of the predicted cell-penetrating peptides is indicated in the second layer. MLCPP is anticipated to be a robust tool for forecasting cell-penetrating peptides and the effectiveness of their adoption, and it may make hypothesis-driven experimental designs easier [137]. MLACP can be accessed at www.thegleelab.org/MLACP.html.

9.9.21 ACPred-FL

Anticancer peptide predictor with Feature Representation Learning (ACPred-FL) is a server for precisely predicting ACPs depending on sequence data [138]. ACPred-FL can be accessed at https://server.wei-group.net/ACPred-FL/.

9.9.22 XDeep-AcPEP

AcPEP is a server for sequence-based machine learning approaches for predicting anticancer peptides (ACP). This site accepts peptide amino acid sequences in FASTA format. It first determines whether the sequences are ACPs before predicting their biological effects on six different cancer types: skin, breast, cervix, colon, lung, and prostate [139]. AcPEP can be accessed at https://app.cbbio.online/acpep/home.

Aside from the above-mentioned anticancer peptide predictors, more others are being developed to improve the existing models.

9.9.23 Chemoinformatics Tools in Targeted Cancer Therapy

Therapeutic chemists and researchers can better grasp the intricate structures of chemical substances using cheminformatics technologies. A newly emerging multidisciplinary subject called cheminformatics focuses mainly on finding new chemical entities, which leads to the creation of novel molecules. Additionally, it is essential for gathering, storing, and evaluating chemical data. The cheminformatics toolkits are computer applications' software development kits for structure–activity relationship investigations, chemical database mining, and virtual screening [140]. In computational drug design, these tools can provide predictive information on the drug-likeness and ADMET of compounds based on their physicochemical parameters.

In screening small molecular compounds for targeted therapy using computer-aided drug design, especially phytocompounds or novel synthetic compounds, there is always a need to speculate the ADMET and drug-likeness of the preferred compounds. This helps to curtail the high incidence of drug failure in the course of clinical trials. These computational-based screenings are made possible due to advancements in computer-aided drug design, which has impelled the design and development of relevant tools for these purposes. Some of these tools include.

9.9.24 DataWarrior

DataWarrior uses chemical structures to predict physicochemical and other attributes, including ADMET directly. By applying a previously published approach to precompiled fragment lists, toxicity concerns are calculated [141]. DataWarrior can be downloaded from https://openmolecules.org/datawarrior/download.html.

9.9.25 SwissADME

SwissADME is an online tool which offers open access to a collection of quick but reliable predictive prototypes for drug-likeness, pharmacokinetics, medicinal chemistry, and physicochemical properties familiarity. These models include internally effective techniques like the iLOGP, BOILED-Egg, and Bioavailability Radar [142]. SwissADME can be accessed at http://www.swissadme.ch/.

9.9.26 pkCSM

A computational server called **pkCSM** utilizes graph-based signatures to create prediction prototypes of key ADMET features for medicine design and advancement.

It is suggested that pkCSM executes equally well or even more than other approaches. It provides a platform that is integrated for swiftly evaluating pharmacokinetic and toxicological characteristics [143]. pkCSM can be accessed at https://biosig.lab.uq.edu.au/pkcsm/prediction.

9.9.27 ADMETlab 2.0

ADMETlab 2.0 is an updated version of the popular ADMETlab that enables the systematic examination of ADMET characteristics, some physicochemical qualities, and medicinal chemistry friendliness. Due to considerable upgrades to functional modules, predictive prototypes, interpretations, and the user interface, ADMETlab 2.0 may now help medicinal chemists in expediting drug research and development [144]. ADMETlab 2.0 can be accessed at https://admetmesh.scbdd.com/.

9.9.28 Molinspiration

Through Molinspiration, a variety of cheminformatics software tools are accessible to facilitate the handling and processing of molecules. The conversion of SMILES and SDfiles, normalization of compounds, tautomer production, molecule division into fragments, estimation of different molecular characteristics for QSAR, molecular modeling and drug design, robust compound illustration, and molecular database tools assisting substructure and similarity investigations is some of the tools mentioned above. Additionally, our technologies offer data visualization, simulated screening using fragments, and bioactivity prediction [145]. Molinspiration has an option for kinase inhibitory prediction, which makes it a good choice for anticancer studies. Molinspiration can be accessed at https://www.molinspiration.com/.

9.9.29 Molecular Docking in Targeted Therapy Studies

Molecular docking is an important approach for understanding how small molecules like ligands and large ones like proteins interact. The molecular forms of behavioral variability for compounds found at a protein's binding site can also be examined via molecular docking, though. Furthermore, molecular docking is a computer-based technique that requires docking programs to complete all of its many responsibilities [146]. These programs include Autodock vina, Flex, Gold, SwissDock, PyRx, etc. Aside from the regular molecular docking and dynamics simulations against tyrosine kinases and peptides against protein anticancer computational studies, there are also cancer stem cell metabolic processes targeted by molecular docking. In this regard, molecular docking is utilized in various signaling and metabolic pathways connected

to cancer stem cells. By shedding light on metabolic pathways, cancer cells can alter their behavior in a way that produces metabolic precursors, allowing them to fulfill their anabolic and energetic needs.

Additionally, the development of tumors and changes to malignant tumors is influenced by various metabolic pathways. Therefore, one of the cancer insignias is metabolic reprogramming [147]. In the conditions of molecular docking applications in metabolic pathways, there are some examples.

In their study to develop novel inhibitors mitochondrial for targeting metabolism of ketone in cancer stem cells by targeting two enzymes: 3-Oxoacid CoA-Transferase 1 (OXCT1) and Acetyl-Coenzyme A acetyltransferase 1 (ACAT1), that offer cancerous cells the capacity to convert ketones into Acetyl-CoA and, thus, to make available more ATP, Ozsvari et al. make use of a computer-based approach to choose a subset of possible promising molecules that dimensionally fit within the binding pocket of these enzymes, depending on their available crystal structures in 3D. These compound libraries were then analyzed based on their observable characteristics for their effect on overall levels of cellular ATP. Metabolic flux analysis confirmed the positive hits. The study revealed that four of these molecules caused inhibition of mitochondrial oxygen uptake effectively. Two of these molecules also caused cancer cells to develop a reactive glycolytic phenotype. They demonstrated, using a mammosphere assay, that these molecules can be utilized to practically terminate cancer stem cell (CSC) activity and advancement. The results of the molecular modeling research demonstrate how these novel molecules are expected to fuse to the OXCT1 and ACAT1 active catalytic sites within their Coenzyme A binding region. They concluded that OXCT1 and ACAT1 represent significant new pharmacological targets for improved medication design and development. They suggested naming this new family of medications "mitoketoscins" to show that they were made to target mitochondrial activity and ketone reutilization [148].

Through computer-assisted molecular docking using survivin, caspase-9, and caspase-3, Wanandi et al. chose andrographolide as the lead compound from the seven examined compounds in their study, which sought to find a natural active molecule with anticancer characteristics that targeted survivin (highly expressed anti-apoptotic protein in breast cancer stem cells [BCSCs]). They found that there exists stronger spontaneous binding between andrographolide and survivin than that between caspase-9 and caspase-3. Andrographolide was applied in vitro for 24 h to human CD24-/CD44 + BCSCs. Human mesenchymal stem cells were used to compare the cytotoxic effects of andrographolide on BCSCs (MSCs). Thr34-phosphorylated survivin and total survivin status were assessed with ELISA and Immunoblotting test, and the production of survivin, caspase-9, and caspase-3 was examined with qRT-PCR. Analyses of the apoptotic activity of andrographolide using flow cytometry and annexin-V/PI were conducted. The findings show that andrographolide had a CC_{50} of 0.32 mM in BCSCs but had no cytotoxic effect on MSCs.

Furthermore, andrographolide inhibited the activation of surviving, while boosting survivin mRNA in BCSCs by decreasing survivin and Thr34-phosphorylated survivin. Andrographolide's apoptotic activity was acknowledged

by an escalation in caspase-3 mRNA and protein and a boost in both the early and late phases of apoptosis. Because of its molecular interactions with survivin, caspase-9, and caspase-3, that cause apoptosis, they concluded that andrographolide could be an anticancer compound that targets BCSCs [149].

In their paper titled "Pharmacological inactivation of Skp2 SCF ubiquitin ligase inhibits cancer stem cell characteristics and cancer progression," Chan et al. found a selective Skp2 inhibitor using accelerated computer-assisted assessment of extensive and disparate chemical libraries. The activity of other SCF complexes is not affected by this Skp2 inhibitor, just the Skp2 E3 ligase. Additionally, it imitates the consequences of genetic Skp2 loss, which includes p53-independent cellular senescence, suppression of survival and Akt-mediated glycolysis, and these effects. They observed that Skp2 plays a crucial part in favorably controlling cancer stem cell populations and their capacity for self-renewal through genetic and pharmaceutical methods. Notably, Skp2 inhibitors work with chemotherapeutic drugs to lower cancer cell survival and exhibit strong anticancer activity in a variety of animal models. Therefore, this discovery offers pharmacological proof that Skp2 is a suitable target for slowing the growth of cancer stem cells [150].

The signaling pathway is the alternative molecular docking-influenced pathway in cancer stem cells. This pathway is helpful for the expansion of targeted CSC therapies [151], embryonic evolution, CSC maintenance, and so on [152]. Some molecular inhibitors of Wnt, Notch, Hh, and other signaling pathways suggest that molecular docking has an essential effect on signaling pathways [153].

It is also important to note that one noteworthy feature of molecular docking performed by natural products is the ability to modulate some target proteins. Natural items can serve as an alternative to pharmaceuticals with several targets. One natural component that has the ability to function as an anticancer lead compound in the process of molecular docking of cancer stem cells is alkaloids [146]. According to in silico experimental data from a study by Mayank and Jaitak titled "Molecular docking study of natural alkaloids as multi-targeted hedgehog pathway inhibitors in cancer stem cell therapy," emetine and cortistatin can inflect the hedgehog (Hh) pathway by binding to sonic hedgehog (Hh), smoothened (Smo), and Gli protein, involved in maintaining cancer stem cells. Solamargine, solasonine, and tylophorine also appeared to be efficient lead compounds for targeting cancer stem cells by changing the hedgehog pathway. In inclusion to solamargine and solasonine, alternative top lead compounds also revealed favorable computer-based ADME profiles. It is possible to correctly modify the anticipated lead compounds to produce a multitargeting cancer stem cell targeted drug and get rid of related resistances [154].

9.10 Conclusion

Computer-aided drug design and its upgrades and advancements have become essential in new medicines discovery, design, and advancement for various diseases. It helps to minimize the cost and time of drug development by ensuring the provision of information by drug candidates like drug-likeness, ADMET, and bioactivity, thereby reducing the chances of drug candidate failure in the course of the clinical trial. The quest to develop better treatment for cancer through target therapies has also inculcated aspects of computer-aided drug design. Even though there are successes already recorded through computational studies in anticancer drug development, like Imatinib, Gefitinib, Erlotinib, Sorafenib, Lapatinib, Abiraterone, and Crizotinib [155], there is also a need to improve on these achievements. As technology keeps improving, researchers are developing more advanced computational means of drug development to enable easier, more precise, faster, and more economical means of developing better-targeted cancer therapies.

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Chapter 10
Leveraging Advancement in Robotics in the Treatment of Cancer

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Contents

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Abstract The limits of lack of specificity, short circulation half-lives as well as limited bioavailability and solubility have necessitated the application of medications in high doses. When drug molecules are administered in large doses, the adverse effects of the drugs are exacerbated. Nanomedicine, or the use of nanotechnology in medicine with clinical applications has made significant strides recently in the detection and treatment of cancer. Artificial intelligence (AI) has a vital role to play in the improvement of nanomedicine and combinatorial nanotherapy to address the issues of patient specificity, timing, and dose dependency of drug administration. AI has developed into a tool that researchers can use to manage complex and huge data, from obtaining complementary results to doing basic statistical analysis. AI improves the accuracy of therapy impact prediction in cancer patients and specifies estimation outcomes. Nanoinformatics is a brand-new area of research made possible by the use of AI in nanotechnology. As a further emerging technology, AI can be combined with nanorobots to create systems for the delivery of specific drugs. AI-based combination therapy can also make it easier to understand cancer patients' diagnosis and treatment, thanks to developments in the field of nanomedicine. In order to give cancer patients a more successful course of treatment, the main goals of this review are to discuss the present trends, opportunities, and future prospects in nanoinformatics.

Abbreviations

ABIS Inc.	American business Intuitive Surgical, Inc.
AESOP	Automatic Endoscopic System for Optimal Positioning
AI	Artificial intelligence
ASD	Atrial septal defects
CAD	Computer-aided design
CAM	Computer-aided manufacturing
CAS	Computer-assisted surgery
CMF	Cytoxan, Methotrexate, and Fluorouracil
CT	Computed tomography
DSA	Digital subtraction angiography
FDA	Food and Drug Administration
IARC	International Agency for Research on Cancer
IMB	Institute of Medical Engineering and Biophysics
IMRT	Intensity-modulated radiation therapy
IORT	Intraoperative radiation therapy
KTP	Potassium titanyl phosphate
LAR	Low anterior resection
LIMA-LAD	Left internal mammary artery and the left anterior descending artery
MAB	Monoclonal Antibodies
MRI	Magnetic resonance imaging
MRIFUS	MR imaging-guided focused ultrasound

NASA	National Aeronautics and Space Administration
NCI	National Cancer Institute
NRC	Nuclear Regulatory Commission
PSMAs	Prostate-specific membrane antigens
RARP	Robotically assisted radical prostatectomy
RFA	Radiofrequency ablation
RST	Radiation surgical techniques
TBS	Thoracoscopy
TECAB	Totally Endoscopic Coronary Artery Bypass Surgery
TURP	Transurethral resection of the prostate

10.1 Introduction

The subject of "what causes cancer" has fascinated humans for centuries. In 1950, the World Health Organization convened an international conference, and the participants were fascinated by the remarkable diversity in cancer kinds observed in various regions of the world [1]. The symposium resulted in the establishment of the IARC, the International Agency for Research on Cancer, was founded in 1965, with the mandate to conduct interdisciplinary research into the etiology of human malignancies [2]. Initially, the IARC's conclusions were based solely on epidemiological evidence; later, the criteria were expanded to incorporate experimental evidence [3, 4].

Cancer is defined as the unrestricted growth and spread of abnormal cells. The spreading of cancer cells is termed as malignancy, in which the cell is characterized by different abnormalities such as cell cycle acceleration, gene alteration, invasive growth, enhanced cell mobility, chemotaxis, and alteration in the cellular structure [5]. The factors which are responsible for triggering cancer, i.e., carcinogens, may be external factors such as tobacco, chemical exposure, radioactivity, and infectious organisms as well as internal factors such as genetic mutations, hormones, and immune conditions. The causes of cancer are diverse and complicated. Many possibilities are yet to be deciphered [6].

With the advancement in technologies and medicines there is a considerable improvement in the field of cancer therapy. Nowadays, chemotherapy, surgery, and radiotherapy are available as the treatment of malignancy [7]. But taken into account of earlier age, it was considered as an incurable disease. The assumption that cancer cannot be treated has remained up to some extent into the twenty-first century. This has contributed to people's dread of the sickness. Even today, some individuals believe that all cancers are incurable and delay visiting a doctor until it is too late for appropriate therapy. Even with the progression in medicine, cancer treatment development was rather slow as the first idea of treatment was surgery. Even in the early days of surgery, complications such as excessive blood loss were common. Until the

early twentieth century, major advances in general surgery and cancer surgery were not made [8].

There were renowned surgeons before anesthesia was discovered. John Hunter, Astley Cooper, and John Warren were lauded for their rapid and accurate surgery. When anesthesia became accessible in 1846, however, the work progressed so swiftly that the subsequent century became known as the "century of the surgeon" [9]. Owned to their valuable endowments to procedure and methods of cancer surgery, three surgeons stand out: Bilroth (Germany), Handley (London), and Halsted in (Baltimore). Their research led to "cancer procedures" meant to remove the whole tumor together with the lymph nodes in the tumor's vicinity [10].

With time, more improvements have been made in cancer surgery, which leads to the term robotic surgery. This phrase refers to the remote manipulation of surgical tools by robot arms and other technologies under the supervision of a surgeon. Robotic lanced systems have been employed in a variety of cancer operations, with radical prostatectomy being one of the most prevalent applications in surgical oncology. Some experts believe that as mechanical and computer technology advances, future systems will be able to remove tumors more thoroughly and with less surgical stress [11].

10.2 Epidemiology of Cancer

The term "Pathology of the Century" is frequently used to describe cancer, giving the impression that it is an epidemic disease that affects people all over the world. According to Roy Porter, it's "the contemporary sickness par excellence" or perhaps "the definitive outcome of modernity" (Siddhartha Mukherjee) [12, 13]. Cancer has overtaken heart disease as the second leading cause of mortality worldwide since the end of the eighteenth century, and these two definitions are generally accepted and supported by this dramatic growth in incidence and mortality [14, 15]. In 2015, 8.7 million people died from cancer, while over seventeen point five million new instances of neoplasia were discovered throughout the world (GBD Mortality Causes of Death Collaborators, 2016). The number of new cancer cases has also grown by around 33% in the decade 2005–2015, despite breakthroughs in the diagnostic, medicinal, and interventional sectors. This is primarily because of the rapid growth in the population and a boost in the average expectancy of life. On the other hand, death rates have remained virtually stable, instead the fact that several countries have seen a decline in cancer mortality despite rising incidence levels [16]. On the other hand, despite improvements in the domains of detection, treatment, and intervention, the number of new cancer cases has risen by nearly thirty-three percent in the decade 2005–2015, mostly because of the rising global population and the corresponding rise in the average lifespan. While a counterpoint, mortality rates have remained virtually stable, even as incidence rates of some types of cancer have increased and death rates have decreased [17]. According to contemporary epidemiological statistics, the National Cancer Institute (NCI) has gathered and analyzed data for the

past 40 years, and the incidence rates of all malignancies have steadily increased throughout that time period. Even though there has been a decline in death rates over the past 20 years, there was a little rise in mortality rates between 1975 and 1995. Because more potent drugs and therapy methods have become available in recent years as a result of advancements in the medical and pharmaceutical fields, the death rate from cancer has decreased [18, 19].

There is a wide variation in incidence and mortality rates for the supposed "major murderers" (i.e., pulmonary cancer; breast cancer; colon; prostate; stomach; liver; cervix uteri; esophagus; bladder; non-Hodgkin lymphoma; pancreatic; melanoma) when epidemiological data are analyzed. Some cancers, such as pulmonary and pancreatic cancer, have remained practically stable in fatality rates since the 1970s. Despite major therapeutic and pharmacological developments in the second postwar era and in the present day, these epidemiological statistics reveal that cancer remains a worldwide health concern and a major challenge in medicine today [20].

10.3 Timeline of Cancer Treatment

In spite of the fact that cancer is viewed as a modern disease, the history of oncology can be traced all the way back to ancient Egyptian and Greek civilizations [21], as shown in Fig. 10.1. Tobacco, soot, and other carcinogenic chemicals were studied for the first time by scientists in the 1700s, which is when modern oncology came into being. On the other hand, the birth of contemporary oncology was made possible by a number of earlier significant discoveries and technologies. As a result of several scientists studying tumors from various angles (anatomical, biological, epidemiological, and therapeutic), a medical, surgical, and interventional revolution occurred between the fifteenth and late nineteenth centuries. From Paracelsus' (1493–1541) discoveries to Percival Pott's (1714–1788) intuitions, this revolution has spanned everything from the invention of the microscope and Rudolf Virchow's (1821–1902) theories of cancer onset, to the first radiation treatment and experimental oncology methods promoted by Marie and Pierre Curie [22].

Solid tumor treatment relied on surgery and radiation until the 1960s. As a result of unchecked micro-metastases, cure rates reached a standstill. Adjuvant chemotherapy following radiation or surgery has been shown to be effective in treating patients with advanced cancer, according to certain recent studies. First, favorable outcomes with adjuvant therapy were achieved in breast cancer; second, this was the first example of multimodality treatment, a treatment technique today used for a wide range of cancers. When adjuvant chemotherapy was first used in the late 1960s, it fundamentally altered the practice of treating cancer locally [23].

In 1978, the combination of cisplatin, bleomycin, and vinblastine resulted in improved cure rates for metastatic germ cancer. Hematologic cancer patients who underwent polychemotherapy learned that various medications have varying effects on tumor cells depending on where in their cell cycle they are [24]. CMF (cytoxan, methotrexate, and fluorouracil) has been a mainstay breast cancer treatment for

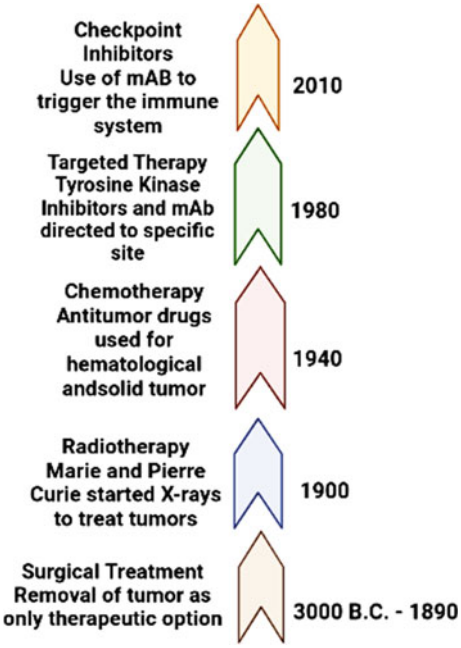

Fig. 10.1 Evolution in the cancer treatment therapy

more than 30 years [25]. After the 1970s, research into the molecular alterations occurring in cancer cells progressed rapidly. Drugs with many different modes of action were launched in the 1980s as a result. Liposomal treatment, which uses liposomes (vesicles composed of lipid bilayers) to encapsulate chemotherapeutic medications, has been developed as a result of these advancements and improvements. One of the earliest stages in nanotechnology-based techniques is liposomal doxorubicin and daunorubicin, which are both examples of liposomal medications. Targeted chemotherapy was first introduced in the 1990s by screening for specific molecular targets [26].

10.3.1 Surgical Treatments

At the turn of the twentieth century, cancer surgery started to develop. Miles did the first abdominoperineal resection in 1908 [27], the first lobectomy in 1912 [28, 29], and Wertheim did the first radical hysterectomy in 1906. All of these surgeries were done based on oncological criteria. Also, the first radical suprapubic prostatectomy was done by Young in 1904. The use of minimally invasive procedures like laparoscopic colectomy (removal of colon cancer) [30], video thoracoscopy (TBS), radiofrequency ablation (RFA), and radiation surgical techniques (RST) like Cyberknife® (radiation ablation) [31] have largely superseded traditional Halstedian

approaches. In order to preserve breast tissue and avoid lymphedema, it has been attempted to use sentinel node obliteration in breast-conserving surgery [32]. For early stage laryngeal carcinoma, another common operation is laryngoscopy laser surgery [33]. When it comes to technology, the Da Vinci® is at the forefront. Cancers of the prostate and kidneys can be removed with robotic surgery [34].

10.3.2 Radiotherapy

Radiation therapy began with the finding of Becquerel, and Rontgen discovered X-rays and radiation in the late nineteenth century. Radiotherapy owes its existence to Marie Curie and her pioneering work in the field of physics. In 1898, the first cancer patient to be successfully treated with radiation was diagnosed [35]. The tomotherapy system was introduced in 2003 as a new variation on the IMRT treatment. As a result of the radiation source circling around the patient, the tumor's morphological limitations may be more easily traced with the beam. Patients with uveal tract melanoma are increasingly being treated with proton or helium ion-based charged particle radiation. Skull base chondroma, chondrosarcoma, and spine tumors can also be treated with this treatment (usually cervical). Fractionated dose administration, technical advancements in X-ray generation and distribution, and improvements in system-based treatment planning are all examples of recent developments in the field of radiation oncology [36]. To combat cancer cells, radiogenic treatment causes the creation of cytotoxic agents. In conjunction with a biological agent, lower radiation doses are employed, and radiation stimulation creates cytotoxic agents. To activate promoters and consequently the production of genes that produce enzymes, this complicated technique was devised. As a result, cancer cells are killed by the activated version of the medicine chosen. Another option is to use radiolabeled molecules, which delivers radioactivity to specific receptor-bearing cells in order to attack cancer. It is possible to target specific groupings of cells with radioactive isotopes that produce Auger electrons, such as iodine-125 or indium-111.

10.3.3 Chemotherapy

Chemotherapy is curative for many advanced malignancies, including acute lymphoblastic and myelogenous leukemia, Hodgkin's and non-lymphoma, Hodgkin's germ cell cancer, small cell lung cancer, ovarian cancer, and choriocarcinoma. Children's malignancies that may be effectively treated include acute leukemia, Burkitt's lymphoma, Wilms' tumor, and embryonal rhabdomyosarcoma. Treatment for these tumors is not usually curative, although overall survival and progression-free survival have improved dramatically. To shrink the size of the main tumor and avoid micro-metastases, neoadjuvant therapy is another option [37]. Compared to more conservative surgical procedures, this sort of therapy is more

effective in preserving organ function. Chemotherapy administered as a neoadjuvant treatment is recommended for patients with some types of cancers of the head and neck as well as those of the anesthetic, breast, bronchial, gastric, rectal, or bladder. Adjuvant chemotherapy had shown a rather beneficial treatment for many different types of cancer; along with new medications and combinations that have become available recently, the likelihood of complete remission is predicted to improve even more. There has been a steady drop in cancer death rates since 1990, despite an increase in the older population in the United States [38].

10.3.4 Targeted Therapy

Selective molecules (e.g., antibodies and their fragments, carbohydrates, peptides, nucleic acids) are used in nanoparticle engineering to accomplish cell targeting and attach to their matching antigen, either an overexpressed receptor or a cell surface carbohydrate in tumor cells. Nanoparticles can also be combined with other biological counterparts, such as folic acid, to take advantage of these cells' fast multiplication. Folate receptors are overexpressed in a wide variety of cancer cell types, including solid and hematological malignancies, which is why these carriers are being combined with folic acid [39].

The bifunctionality of newly synthesized theragnostic nanoparticles has been established. These include perfluorocarbon nanoemulsions, now undergoing clinical studies [40]. Athymic mice bearing human tumors in the head and neck were used to show the targeting abilities of these vectors. It contained single-chain variable fragment antibody directed against the epidermal growth factor receptor and Au nanoparticles, a protein that is often overexpressed in aggressive carcinogenic tumors. Surface-enhanced Raman spectroscopy used the nanoparticles as tags for spectroscopic detection. Tumors have been targeted and visualized using magnetic targeting as a physical technique. The efficiency and discrimination of rat orthotopic 9L-gliosarcoma tumor nanoparticle accumulation were investigated using magnetic resonance imaging (MRI) [41].

Nanocarrier activation by extracellular cues in the tumor environment might be a potential way for accomplishing active targeting. Because of the tumor's acidic pH and out-of-control enzyme synthesis, trigger mechanisms that release just the nanocarriers' delivered payload can be used. These systems are described in detail elsewhere [42]. Another unique technique for minimizing undesired side effects and delivering huge dosages of medications is prodrugs that target tumors and turn on once they get to the cancer cells [43]. For example, development of Pt (IV) encapsulated nanoparticles of PLGA/PEG-functionalized polymers that targeted prostate-specific membrane antigens (PSMAs) in a controlled release manner. The prodrug is converted to cisplatin after decrease in the tumor cells' core [44].

10.3.5 Immune Checkpoint Inhibitors

Recent developments in cancer immunotherapy have been impressive. Monoclonal antibodies targeting immune response-suppressing tumor antigens or T-cell protein receptors have been discovered since 2010. PD-1 and anti-CTLA-4 monoclonal antibodies, which are found on the membranes of T helper cells and cancer cells, respectively, are the two-novel immune checkpoint inhibitors that are being developed. Ipilimumab can be used alone or in conjunction with Nivolumab to treat unresectable or metastatic melanoma [45]. Melanoma patients who had longer PFS and OS in the initial clinical trials had better long-term survival. Other cancers, such renal cell carcinoma, prostate cancer, and non-small cell lung cancer, are also being studied in clinical trials for the therapeutic effectiveness of Ipilimumab, either alone or in combination with Nivolumab [46].

Ipilimumab (Yervoy®) was the first immune checkpoint inhibitor to be authorized by the FDA in 2011. Toll-like receptor 4 (CTLA-4) is a membrane protein expressed on regulatory T cells and is bound by human IgG1 antibody. [The overexpression of CTLA-4 by the tumor microenvironment prevents T-cell surface receptors from activating the immune response against tumor cells by binding to CD80 and CD86 on antigen presentation cells]. Two monoclonal immune checkpoint inhibitor antibodies for the treatment of non-small cell lung cancer, melanoma with metastases, NHL, and urothelial carcinoma have received FDA approval [47]. The two inhibitors that are of concern are pembrolizumab (Keytruda®) and nivolumab (Opdivo®). Both medications are PD-1 antisera that target PD-1 on lymphocytes, specifically human IgG4 antibodies. The down-regulator effect of PD-L1 antigens generated by certain tumors on this receptor renders T cells unable to recognize and kill cancer cells [48, 49]. Patients' PFS and OS may be improved by combining treatments, such as immune checkpoint inhibitors and other chemotherapeutic medicines. Combination treatment with anti-PD-1 and anti-CTLA-4 medicines has been proven to have a more sustained impact than either agent used alone [50].

10.4 Robotic Surgery

An interesting new technique, robotic surgery, is being developed with the advancement of time and technology. That's sweeping the surgical community like a hurricane. Up until this point, everything has gone as planned. However, the market has mostly driven the rush to purchase and utilize this new technology. Moreover, surgical robots have become a prerequisite for admittance into medical facilities, despite the absence of present practical applications aiming to be renowned for expertise in minimally invasive surgery. As a result, robots appear to play a more marketing function than a technical one [51]. During the last few years, robotic surgery has seen significant progress [52, 53]; urologic, gynecologic, thoracic, cardiothoracic, and gastrointestinal procedures are all better served by robotic surgery. Furthermore,

robotic surgery has become normal practice at many big hospices across the United States and around the world [54]. RARP, a robotically assisted radical prostatectomy, has been the gold standard for localized PCA surgery since its inception in the field of robotic surgery. There was an 8.4-fold increase in the utilization of robotic surgery in Michigan hospitals between 2012 and 2018, showing how robotic surgery is becoming more commonplace among conventional surgical procedures [55].

In 2001, the first successful remote robot-assisted surgery was performed, but due to technological constraints, including significant latency, it has not been implemented into clinical practice [56]. Various nations are confronted with the issue of a scarcity of surgeons in rural areas [57, 58]. Patients with cancer who require robot-assisted surgical treatment may face an increased travel burden and treatment delays as a result of robotic surgeons' concentration in densely populated areas. In the 12th place, surgeons in urban settings may be able to do real-time surgeries thanks to telesurgery [59], which makes use of real-time communication and exchange of medical data, including images, audio, and video, through telecommunication networks [60]. Several countries throughout the globe are now in the process of deploying 5G networks. The advent of game-changing technology has allowed doctors to do distant treatments, tele guided procedures, and interactive real-time surgery on cadavers, animals, and people.

10.4.1 History and Background of Robotics' Surgery

Over the past forty years, robotic surgical systems had been under development. The world's first surgical robot, Arthrobot, was designed and utilized in Vancouver in 1983 [61, 62]. In the beginning, this robotic technology was mostly employed in industrial settings. It was in 1985 that Unimation Limited introduced the PUMA 560, an improved and upgraded version of the PUMA 200 [63]. In the past, many image computers used in the biomedical industry have been compatible with this robot's computer, making it programmable. The robotic system was subjected to a variety of tests, including chess and watermelon calibrations. PUMA 560 was used in a neurosurgery operation that required the fixture to be held near the patient's head. Drills and biopsy probes were guided by the fixture, making this the original robot-aided surgical technique for CT guidance do a biopsy of brain tissue. The PUMA 560, though, had a number of shortcomings, including long setup times, inaccurate measurements, and safety concerns [63, 64]. The PUMA 560 was used for the first time in urology in 1988 to do a transurethral resection of the prostate (TURP) [65].

Prostate reconstruction and transurethral prostate excision procedures have been performed with the help of the PROBOT robot, created in England [66]. Integrated Surgical Supplies, Inc.'s ROBODOC became the FDA approved the first surgical robot in 1992. The development of the ROBODOC was primarily focused on hip replacements [67–69].

Computer Motion produced AESOP, the Automatic Endoscopic System for Optimal Positioning, in the late 1980s and early 1990s. AESOP aided professionals

by establishing a stable operating environment free from the danger of an exhausted or inexperienced scope bearer. Initially, a foot pedal was used by AESOP to direct the laparoscope's orientation. Later, voice instructions were used to guide the orientation. In 1994, the FDA authorized AESOP for use in intra-abdominal operations, making it the first robotic device of its kind to get such permission [70]. ZEUS was Computer Motion's second-generation robotic system. It consisted of three robotic arms, a 2D video screen, and instrument fitted with camera control. One of the three arms has a 2D laparoscope attached, while the other two had surgical equipment attached to them.

The surgeon manipulated the instrument arms through a remote console, and the camera could be controlled via vocal commands in the same way as the AESOP could. A computer converted the surgeon's actions into the laparoscopic tools [67]. First transoceanic robot-assisted telesurgery was successfully completed with ZEUS in September 2001. This established remote surgery as a viable option for surgical help in remote places and demonstrated its medicinal potential. On a pig model, a robot-assisted laparoscopic cholecystectomy was performed by transferring signals from the animal model to the physician in New York [70].

This method, created by American business Intuitive Surgical, Inc., was found to be easier to learn and more intuitive in terms of technical motions than ZEUS. FDA authorized the da Vinci robotic system in 2000 for general laparoscopic surgeries [71]. The four-armed surgical robot on the patient trolley is equipped with this system's imaging system as well as the surgeon's master console. It is approved to do many different types of surgical operations [72]. In 2008, four right laparoscopic intercontinental tele surgical nephrectomies in pig models were performed employing the da Vinci robotic technology in the United States. Surgeons in Cincinnati and Denver, both around 1300 miles away from Sunnyvale, performed surgeries on animals in Sunnyvale, California; the animal subjects were located in Sunnyvale. A wired internet connection was used in all four procedures to assess the effectiveness and dependability of remote surgery using the da Vinci surgical equipment. Cincinnati Bell delivered the internet connection, and Polycom and Haivision offered commercial video code/decoding protocols for remote control, imagining, and voice cover. Cincinnati/Sunnyvale and Denver/Sunnyvale have a 3 and 8 Mbps wired connection, respectively. The Denver connection's latency was 450 ms on average and good imagining compared to the Cincinnati connection's 900 ms and poor visibility [72].

The market for robotic-assisted surgical devices is expanding at a rapid rate due to the development and expansion of several different devices, some of which had been used in real-time remote procedures, such as Ion (developed by Intuitive Surgical), NAVIO (created by Smith and Nephew), Monarch (created by Intuitive Surgical), and Mako (created by Stryker).

10.4.2 Current Robotic Surgical Scenario

Robots and robot advancements are being created nowadays. The minimally invasive surgical unit of Eberhard Karls University has created ARTEMIS, a master–slave manipulator system [73]. A template small robo-mechanical device for computer-enhanced colonoscopy has been created by Dario and colleagues at the Scuola Superiore Sant'Anna's MiTech lab in Italy. The surgeon operates the two robotic arms in this system from a control station [74]. The same functions as traditional colonoscopy systems are provided by this device; however, it moves like an inchworm due to the vacuum suction. Since the endoscopist may teleoperate or directly oversee this endoscope, they believe it is feasible and may increase the applications of endoluminal surgery and diagnostics. The authors and other researchers are engaged in to combine visual serving with haptic feedback in surgical robots to improve minimally invasive procedures [75–78]. Prodoc, ROBODOC, and the systems listed below are additional; there are a number of other commercially produced and general surgery robotic equipments that have received FDA approval. AESOP is a voice-activated robotic endoscope created by Computer Motion Inc. of Santa Barbara, California; da Vinci and Zeus are sophisticated master–slave surgical robotic systems made by Intuitive Surgical Inc. of Mountain View, California. There is some overlap between the capabilities of the da Vinci and Zeus systems, but their approaches to robotic surgery are different. Both systems utilize video-assisted visualization and computer-augmented reality to allow for remote control of several arms on a surgical robot. The da Vinci system is an advanced telepresence platform with roots in NASA and the US military [79].

However, there haven't been many papers that have talked about the use of robotic surgery which is used to treat cancers and tumors. To investigate how robotic surgery is used in the treatment of cancer, an extensive study of prior research papers is required.

10.4.2.1 Navigation System

Computer-assisted surgery (CAS), which is based on data obtained from computed tomography (CT), is becoming increasingly significant in many different subspecialties of surgery, including craniomaxillofacial surgery. It is possible to achieve a higher level of precision when defining the resection margins of target lesions by making use of navigation devices, which make it possible to precisely position surgical tools throughout the operation. The ablative operations are also helped tremendously by these approaches. However, more complicated treatments, including rebuilding, continue to be a challenge [80]. A system that makes use of computer-aided design (CAD) and manufacturing (CAM) has been developed as a consequence. With the help of this technology, it is possible to create and use distinctive resection templates that are based on coherent numerical 3D models. For use in neurosurgery, Iseki and his coworkers have developed an overlay 3D image-guided navigation system. This

system has ability to lead neurosurgeons in an appropriate direction while they are performing surgical operations [51, 81–83].

In addition, the utilization of surgical robots in conjunction with triangulation systems that make use of CT [84], MRI [85], and US [86] will enable us to carry out gene therapy procedures that are both more accurate and less invasive (For instance, a local injection).

10.4.2.2 Surgical Robots

Master–Slave Manipulator

A surgeon's desk, a vision cart, and a surgical cart make up most robotic systems. An endoscopic camera is inserted into the patient's body, and the images it captures are shown on a monitor in front of the surgeon. There are master manipulators (also known as "slave manipulators") that may be used by surgeons to command the corresponding surgical or patient-side tools' movements and endoscopic manipulators' movements. It appears as though the surgeon is peering into the operating room and at his own hands as he gazes down at the camera. He grasps the dials with his left and right hands. He then carefully places the tool tips within the patient's body. The manipulators at the patient's side closely adhere to the input motions of the surgeon's console manipulators [87]. As a result, doctors may execute more accurate surgical treatments than previously possible with standard endoscopic techniques. Complex 3D manipulations have already been demonstrated to be possible with remote-access endoscopy and the surgical workspace's sensible arrangement may make such tasks easier to do.

AESOP®

The first robot authorized by the FDA for clinical use in the abdomen was the automated endoscope system for optimum placement (AESOP) (Computer Motion, Goleta, CA). After its first introduction, the AESOP could only be operated remotely via a foot switch or a hands-free controller. The most current iteration AESOP is voice-activated [88].

da Vinci™

Intuitive Surgical, the company behind the da Vinci™ Surgical System, created it (Mountain View, CA). There have been 196 da Vinci systems placed across the world so far. The da Vinci system has been used in general surgery, urology, cardiothoracic surgery, and pediatric surgery. There are three key components to this system: the Surgical Cart, the Vision System, and the Surgeon Console. The Surgeon Console, the Surgical Cart, and the three arms on the Surgical Cart are all directly controlled

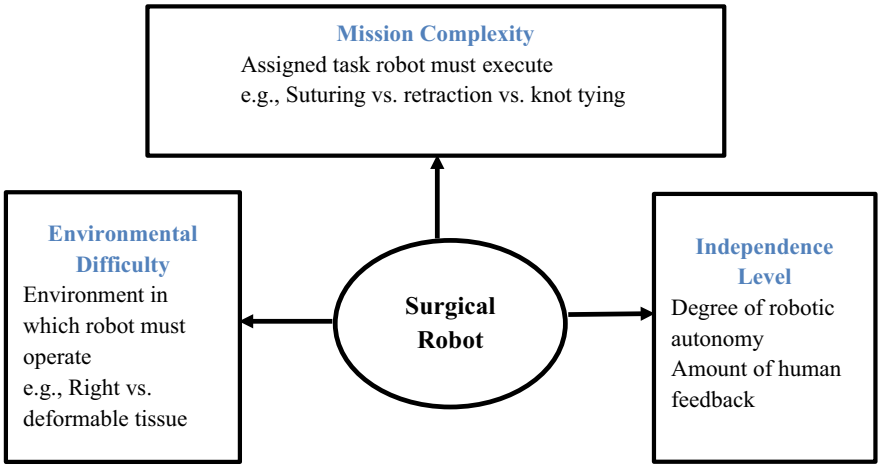

Fig. 10.2 Adapted from the reference model for autonomy levels for unmanned systems, this diagram depicts the operational framework for autonomous surgical equipment

by the Surgeon. The CPU system that controls the entire system is housed in the surgeon console. By using an Endo Wrist™ that mimics human hand movement and a high-quality three-dimensional endoscope, the da Vinci Surgical System is capable of performing complex surgeries with ease.

Scaling and tremor filtering are all included in this system, as is an internal articulated endoscopic wrist that gives the surgeon three more degrees of freedom in addition to an intuitive transition from the movement of the tool handle to the tip. With the da Vinci robot, esophageal tumors, thymomas, retro mediastinal tumors, gastric cancer, and colon cancer were successfully treated [89].

ZEUS®

In addition to creating the AESOP® telerobot, Computer Motion also created the ZEUS® telerobot. Using the AESOP framework, it developed a robot that could do telerobotic surgery. AESOP, the voice-controlled robot in this system, keeps the camera steady. Two more AESOP-like devices had been converted to grip surgical tools. With exception of the internal articulated endoscopic wrist, the ZEUS system performs nearly identically to the da Vinci. With the use of SOCRATESTM (Computer Motion), ZEUS allows surgeons to do remote control surgeries across large distances. Remote surgeons can collaborate with colleagues in the operating theater using SOCRATESTM, a surgical telecollaboration technology. The operating room's core nervous system is HERMES® (Computer Motion). Surgeons and their support personnel can use HERMES® to manage a range of networks, including AESOP®, ZEUS®, and SOCRATESTM [90].

Naviot™

Also recently created in Japan is a brand-new system that goes by the name of the laparoscope manipulator, NaviotTM (Hitachi, Tokyo, Japan). This device is widely acknowledged as the very 1st surgical robot that has ever been created in Japan. This manipulator is built on a five-bar linkage system, and the manipulator's base is equipped with two separate motors. In addition to that, the mechanism that allows the laparoscope to zoom up has been adapted to work with this manipulation system. The angle of movement was approximately 25° in both the horizontal and vertical axes. Using this Naviot, we have successfully completed laparoscopic procedures on a total of one hundred patients as of March of 2004 [91, 92].

10.4.3 Autonomy/AI in Robotic Surgery

ISO 8373:2012 defines autonomy as the capacity to accomplish planned activities without human interference. Autonomy is a scale where human interference is surrendered for full independence. There is a proposed framework for the development of surgical robots. Without the need for human intervention, autonomous surgical robots of the future will be able to "see," "think," and "act" to effectively complete a surgical procedure. An autonomous surgical robot must complete missions that vary greatly in terms of mission complexity, environmental challenges, and human autonomy. As shown in Fig. 10.2, the autonomous robot is equipped with optical and physical sensors to detect its environment, a central processing unit to create outputs, and mechanical actuators to carry out the robot's directives. A clinically viable, flexible autonomous surgical device will require extensive development and integration of control algorithms, robotics, computer vision, and smart sensor technology, as well as lengthy trial periods because soft tissue environments are deformable, hollow organs are present, and tissues are delicate [93].

Understanding robots requires two things: the ability to perform the predetermined the ability to adapt quickly to the constantly shifting circumstances in the operating room as well as the mission of the operation to which it has been assigned. The "surgical skill" of the robot is its capacity to first translate its perception (i.e., sensory inputs) to an estimated environmental state, and then, most effectively, convert that estimate to a future action (i.e., robotic outputs).

The capacity of a computer to learn from past experiences is known as machine learning (ML), a type of artificial intelligence that has been suggested to manage the behavior of autonomous devices. Robots may anticipate an outcome [94, 95] and complete tasks in real-time based on their "experience" when they are given with unique yet comparable data, thanks to appropriately trained algorithms.

Machine learning works best on massive, cumbersome otherwise incomprehensible to humans datasets. The robot's sensors deliver a constant stream of numerical information that will be processed by real-time machine learning algorithms so that

the robot's processors may adjust its behavior in response to changes in its surroundings and if the sensory stream is as accurate as human senses, such analytical algorithms will demonstrate, as shown in Fig. 10.3, to be superior to human perception. Therefore, it is conceivable to anticipate or detect bad events at a level that is beyond human capabilities by utilizing an AI system to recognize "occult" information in sensory input that is ordinarily invisible to person. The success of AI in diagnosing illnesses using one of the first examples of this phenomenon is radiological data. When compared to using AI algorithms to assess a particular type of radiological scan for diagnostic purposes, the challenge of gathering and analyzing multimodal sensory data to replicate a human surgeon's perception in real time is significantly more difficult [93].

Additionally, the robot needs to be taught surgery. The robots might be "taught" by being explicitly programmed (explicit learning), by having it watch a film or a surgeon in real time (implicit learning), or even by training in virtual world. The ability to assess all pertinent sensory inputs, such as the visual and tactile characteristics of the surgical field, and positional information, as well as a database of explicit comprehension on how to proceed safely to achieve the surgical goal, are however also necessary for successfully simulating a human surgeon. Because of this, it is unlikely that either the explicit approach or the implicit method can be employed alone; rather, a combination of the two will be needed, together with continuing reinforcement and correction by subject-matter experts (i.e., human surgeons) [96]. However, with a large enough training database of exercises and lessons, a robot's capacity to learn would only be constrained by continuing reinforcement and correction by subject-matter experts (i.e., human surgeons) [96]. However, given

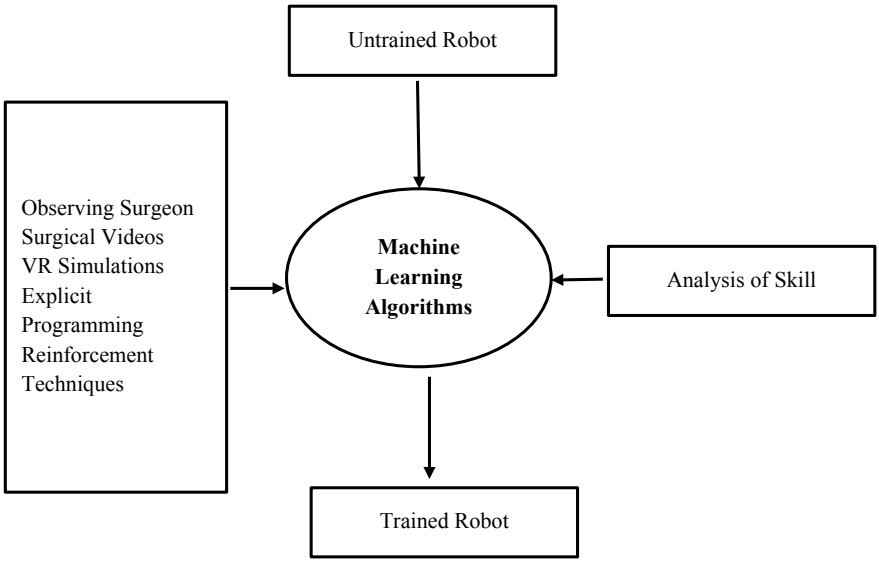

Fig. 10.3 Schematic representation of ML algorithms incorporation into Surgical Robots

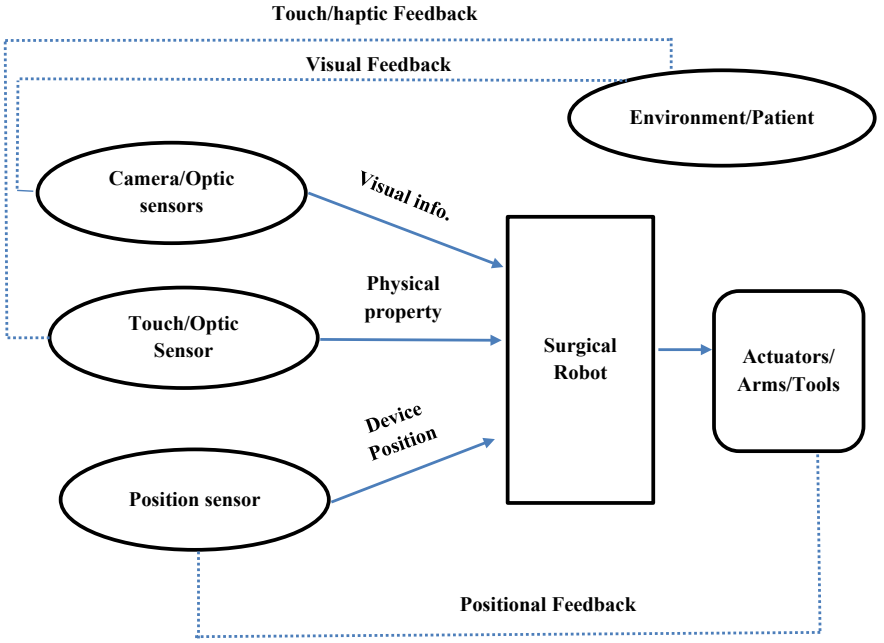

Fig. 10.4 Diagrammatic representation of various sensor incorporated into surgical robot to provide an enhanced work efficiency

enough practice and instruction examples in a training database, a robot's capacity to learn would only be constrained by the capabilities of its hardware and software. However, humans' learning capacities are constrained by physical and mental constraints. Various sensor used in surgical robot is shown in Fig. 10.4.

10.5 Robotics in Surgery of Cancer and Tumors

10.5.1 Neurosurgery

There is a lot of activity in neurosurgery when it comes to robotic surgery. Gamma knife brain surgery without an incision was originally described by Lunsford in his article. It was found that the FDA cleared the gamma knife for sale in 1982 and in 1986, the Nuclear Regulatory Commission (NRC) gave the device its approval. In Pittsburgh, Pennsylvania, gamma knife surgery was first used to treat patients, and it is now possible to have brain surgery without making an incision [97].

Children's thalamic astrocytomas were resected using computer and robot assistance in a study by Drake et al. [94]. Using robot-assisted surgery, the removal of six children with significant benign astrocytomas, ages 2–10, was successful.

Contouring from CT images and digital cerebral angiograms acquired with the BRW stereotactic frame are shown on an interactive, 3D display in this system. Robot PUMA 200 (Westinghouse Electric, Pittsburgh, PA) is used to hold and control the surgical retractor, and a 3D display shows the retractor's location and orientation. This technique makes use of both preoperative planning and simulation. Visually defined margins are used for tumor removal since the brain moves significantly following the removal of the tumor and cerebrospinal fluid [98]. Otolaryngology surgeons can use intraoperative imaging guidance, according to a study by Carney et al. [96]. With a robotic-like joint arm, intraoperative guidance is provided via the ISG viewing wand (ISG Technologies, Mississauga, ON, Canada). Using this technology, surgeons may instantly get 2D or 3D computer-generated reconstructions of CT or MRI scans, allowing them to link anatomical sites in the operating field to their corresponding locations on the reformatted pictures. Resections of the skull, cerebello-pontine angle, and temporal bone have been performed on 14 individuals in this study [99].

Cerebral cavernous malformation resections were performed using interactive image-guided resections by Zamorano et al. [97]. An interactive image-guided excision of cavernous malformations was performed on 15 patients in their study. MRI and digital subtraction angiography were utilized to make the diagnoses (DSA). It was also utilized to verify the position and real-time measurement of the resection's extent with an infrared instrument [100]. Levesque and Parker proved the value of MKM-guided excision for diffuse brainstem tumors. Stereotactic craniotomies were performed on two patients with significant brainstem tumors utilizing an MKM robotic microscope and intraoperative neurophysiological monitoring. Image-guided surgery performed with an MKM microscope enabled the removal of previously believed inoperable large brainstem tumors by injecting surgical outlines into the microscope viewer [101].

NeuRobot is a remotely controlled micromanipulator tool for minimally invasive micro-neurosurgery that was developed at Shinshu University by Hongo et al. [98]. A human cadaveric head was used in surgical simulations using this technique. Micromanipulators (slave manipulators), manipulator-supporting devices, master manipulators, and a three-dimensional display monitor are all components of the system. 3D endoscope and 3D forceps were put in the slave manipulator for remote control with 3 degrees of freedom (rotation and neck swinging) in order to perform complex surgeries. This technique ensured the accuracy of every surgical treatment. It has also been shown that using a micromanipulator in neurosurgery a potassium titanyl phosphate (KTP) laser can be beneficial [102, 103]. Compared to other surgical methods, this system was shown to be capable of cutting, coagulating, and controlling bleeding.

10.5.2 Cardiac Surgery

The da Vinci was designed for a specific kind of coronary artery bypass grafting known as closed-chest CABG [100]. As a result of their practise with the da Vinci

prototype, cardiac surgeons have learned a great lot [101]. The first successful application of the da Vinci system for closed-chest coronary bypass grafting was reported by Carpentier et al. [104] in 1999. Kappert et al. [105] demonstrated that they were able to remove 27 people's left and right internal mammary arteries using the da Vinci surgical system. On 148 patients, Mohr et al. [106] performed coronary artery bypass graft surgery using the da Vinci robotic system. About 15 LIMA blood arteries were harvested using a da Vinci robotic system, and 15 LIMA-to-LAD coronary artery bypass grafts were stitched together using an incision in the middle of the sternum. Twenty-one patients had LIMA-to-LAD bypass grafts following cardiac arrest and chest closure. Recently, utilizing the da Vinci, the LIMA and LAD were anastomosed on a beating heart with the chest closed. The similar procedure was used by Autschbach et al. [107] to operate on 13 patients' mitral valves. At that time, Reichenspurner et al. [108] had successfully used ZEUS on two patients who needed coronary artery bypass surgery [109]. Endoscopic procedures were used to collect LIMA, which was subsequently sutured to the LAD via three thoracic trocars. It was decided to halt the heart's beating with an endovascular cardiopulmonary bypass device. Finally, Boehm employed ZEUS effectively to carry out later that year, three patients underwent closed-chest, off-pump coronary artery bypass surgery (LIMA to LAD) [110]. Patients with beating hearts were successfully bypassed by the same group in 2000 [109]. ZEUS-assisted anastomoses took 14–50 min, and the overall operating duration was 4–8 h (median, 5.5 h) (median, 25). Pericardiectomy and mitral valve surgery are other procedures that make use of ZEUS.

As a result of heart disease's specific features, robotic surgery has not yet been used to treat tumors or cancer. Some of them are mentioned in Table 10.1.

Table 10.1 Surgery with robot assistance in cardiology

Illness	Management
The aortic arch	Reconstruction after resection
The mitral valve is ill	Fixing the mitral valve Changing the mitral valve
Cardiovascular fluid	Coronary window
Cardiomyopathy caused by alcohol	Bi-V pacing epicardial lead placement
Malfunction of the aorta	Dissection of the aorta
Septal defect of the atria (ASD)	Correction of atrial septal defects
CAD	Harvest by IMA The heartbeat TECAB (single vessel) TECAB heart in custody (single and multiple vessel) LIMA-LAD Sternotomy Small thoracotomy bypass with several vessels

Table 10.2 Robotic surgery in pulmonology

Disease	Operation
Medullary thymoma	Thymectomy
Upper limb hyperhidrosis	Sympathectomy
Pulmonary carcinoma	Resection of a wedge Lobectomy

10.5.3 Pulmonary Surgery

For primary pulmonary cancer, one surgeon used AESOP and a mechanism for retracting instruments (UNITRAC; Aesculap, Germany) to accomplish thoracoscopic major resection of the lung by Okada and colleagues [111]. (Table 10.2). An unassisted thoracoscopic lobectomy and dissection of the mediastinal lymph nodes were successfully performed on a 72-year-old woman who had lung cancer. In one incidence of spontaneous pneumothorax, one bulla stitching with fibrin glue was performed. Additionally, five lobectomies, three tumor enucleations, and three excisions were performed utilizing the da Vinci system by Melfi et al. [112]. Some of them are mentioned in Table 10.2.

10.5.4 Mediastinum

Yoshino et al. [113] were able to effectively execute a thoracoscopic thymectomy utilizing da Vinci on a male patient who was 74 years old and had thymoma [99]. A female patient who came with a left paravertebral tumor in the thorax underwent a thoracoscopic resection of a schwannoma using the da Vinci surgical system, according to Ruurda et al. [114]. The patient had previously had a schwannoma removed with thoracoscopic surgery [105].

10.5.5 Mastectomy

In 2000, Kaiser et al. [115] reported that a robotic system may be used for breast lesion biopsy and treatment inside the ROBITOM, a high-field whole-body MRI scanner. The ROBITOM was the name of this device [116]. The ROBITOM robotic system was developed at the Institute of Medical Engineering and Biophysics (IMB) in Karlsruhe, Germany. With this equipment, mammary lesions can be biopsied and treated utilizing interventional methods. A biopsy needle, a laser applicator, a coaxial sleeve, a trocar, and a control and drive unit make up the ROBITOM system. This research used a pig liver cultured in vitro as a model for breast cancer research. There was a total of eight targets used in the in vitro experiments (4 mm in diameter vitamin E capsules), and this robotic biopsy technique successfully hit all eight.

For the process, a 1.5 T whole-body scanner was utilized, and it was carried out immediately in the scanner's isocenter. According to these findings, a robotic system of this kind may make it possible to approach the coordinates of the lesion in the breast while it is being subjected to a strong magnetic field. The researchers Veronesi and colleagues demonstrated the effectiveness of intraoperative radiation therapy (IORT) in patients with early stage breast cancer (103 total) [98].

As a non-invasive treatment for breast cancer, breast-conserving surgery causes the majority of local recurrences, hence MR imaging-guided focused ultrasound US (MRFUS) ablation has recently experienced rapid growth. Gianfelice et al. [117] established the effectiveness of non-invasive MR-FUS ablation in 12 patients with breast cancer [101]. Briefly stated, patients had MR-FUS ablation prior to tumor excision, which involved numerous sonications of the targeted locations while being tracked by MR imaging with temperature sensitivity (Signa™; GE Medical Systems, Milwaukee, WI, USA). Amount of necrotic tissue and remaining tumors was calculated using a histological examination of the resected mass to assess the treatment's efficacy. A MR image analysis, medical exams, and questionnaires were used to measure the procedure's complications. With the exception of two patients who experienced minor skin burns, patients reported no problems with the US ablative (ExAblate™ 2000; In-Sightec-TxSonics, Haifa, Israel). Histological analysis of the excised tumor sections was used to ascertain the extent of necrosis and residual tumor, as well as the visibility of surrounding hemorrhage. In three patients who received treatment with one of the US systems, a mean of 46.7% of the tumor was within the targeted zone, and a mean of 43.3% of the cancer tissue was necrosed. Nine patients received alternative US healthcare, including 95.6% of cancerous growth found inside the intended area of effect, and 88.3% of cancerous tissue had necrosed. Because most of the remaining cancer was located around the tumor's periphery, the treatment zone has to be widened [99]. In addition, the effectiveness of MR-FUS ablation was demonstrated by Huber et al. [118] in a 56-year-old lady with infiltrating ductal carcinoma in the breast [78]. Hynynen et al. [119] also showed the effectiveness of MR-FUS ablation for fibroadenoma [120]. MR-FUS was used to treat eleven fibroadenomas in nine individuals while they were under local anesthetic. Eight out of the 11 lesions treated displayed a full or partial absence of contrast material absorption on post-therapy T1-weighted imaging. Three lesions didn't significantly affect how much of the contrast agent was absorbed. This lack of therapeutic efficacy was most likely caused by a decreased acoustic power and/or patient movement that caused misregistration. With the exception of one instance of brief edema in the pectoralis muscle two days after therapy, there were no side effects reported [115, 120]. Invasive ductal carcinoma, adenocarcinoma, invasive lobular carcinoma, and fibroadenoma are all indications for robotic surgery, according to these articles.

10.5.6 GIT

In March 1997, using telerobotic technology for the first time in a clinical setting, Himpens et al. [121] when they used a da Vinci prototype to conduct a laparoscopic cholecystectomy [122]. In addition, the same group reported using this technique successfully for Nissen fundoplication [122, 123], fallopian tube reanastomosis [124], and telerobotic laparoscopic gastric bypass. Additional research revealed many forms of robotic abdominal surgery [125]. Ballantyne and colleagues used the da Vinci system to conduct a right hemicolectomy for a cecal diverticulum and a sigmoid colectomy for diverticula [126]. The sigmoid colectomy took 340 min to complete, whereas the right hemicolectomy took 228 min. The first two mesh ventral hernia repairs were likewise done by the same team. The first fully intra-abdominal early stomach cancer laparoscopic distal gastrectomy with the da Vinci technology was reported by Hashizume and colleagues. The first stomach splenectomy and devascularization for portal hypertension were also carried out by the same group. According to this paper, telepresence technology makes these operations easier. A robotically assisted Heller myotomy was described by Melvin et al. [127]. The same team also used da Vinci to remove a pancreas.

An intricate cystic growth was discovered in the pancreatic tail of a 46-year-old lady who also complained of back discomfort. The spleen and pancreatic tail were removed together with the lesion using the da Vinci. In a significant clinical experiment with ZEUS, as Marescaux et al. [54] reported, 25 chosen patients received laparoscopic cholecystectomies using ZEUS assistance [111].

Regarding robotic surgery for abdominal cancer, successful procedures include the removal of esophageal tumors, distal gastric resection for stomach cancer, surgical removal of cecum for sigmoid colon cancer, left hemicolectomy for cecal cancer, thymectomy for thymoma, sigmoidoscopy for descending colon cancer, and extraction for retro mediastinal tumor. As a result, robotic surgery may be recommended for practically every form of tumor or malignancy [103]. Some of them are mentioned in Table 10.3.

10.5.7 Urology

Using a da Vinci robot, Abbou et al. [128] described a report on a radical prostate surgery [107]. A T1c tumor was found a 63-year-old male who had one positive sextant biopsy, a Gleason score of 3 + 3, and blood prostate-specific antigen levels of 7 ng/ml before surgery. The da Vinci provided an operational setting that was ergonomic and significantly improved dexterity. The patient spent 4 days in the hospital, and the surgery took 420 min. Three days after the operation, the bladder catheter was withdrawn, and the patient was fully continent one week later. The margins of a pT3a tumor were negative, as discovered by a pathological analysis [110]. Young et al. [129] described a da Vinci surgery adrenalectomy for an adrenal

Table 10.3 Robotic GIT surgery

Disease	Operation
Esophagus	
Gastroesophageal reflux disease	Nissen fundoplication
Achalasia cardia	Heller myotomy
Esophageal cancer	Esophagectomy
Adenocarcinoma	Esophageal mass enucleation
Stomach	
Gastric cancer	Roux-en-Y Gastrectomy Gastric jejunostomy Gastric resection
Colo-rectal	
Colon cancer	Hemicolectomy Colon surgery
Rectal cancer	Low anterior resection (LAR)
Rectal tumor	Rectal tumor ablation

incidentaloma [130]. In this case study, a patient's accidental left adrenal tumor was discovered while being assessed for mediastinal widening. The patient exhibited no signs of an excess of the adrenal glands. The results of preoperative biochemical screening for an active medullary or cortical adrenal tumor were negative.

The da Vinci robotic technology helped to effectively execute a surgical resection. Pathology revealed an uncommon adrenal oncocytoma [131]. Using the da Vinci surgical system, a donor nephrectomy has been carried out during kidney transplantation [130, 131]. Laparoscopic pelvic lymph node dissection with ZEUS assistance in people has been documented by Guillonneau et al. [132]. In 10 patients who had the majority of robotically assisted laparoscopic pelvic lymph node dissection whom had T3 M0 prostate cancer (robotic group). With no unique intraoperative or postoperative issues, all process were carried out in accordance with the specified protocol. There was no conversion necessary, and no technical issues were noted. Renal cancer and prostate cancer are two cancers or tumors for which robotic surgery is indicated. Some of them are mentioned in Table 10.4.

10.5.8 Gynecology

The role of robotic surgery has grown in the field of Gynecology, some of the instances are being mentioned. Mettler et al. [133] evaluated the use of AESOP in 50 patients undergoing routine gynecological endoscopic surgical procedures, and found that it allowed two surgeons to do difficult laparoscopic surgery more quickly than they could have done without the robotic arm [117].

Table 10.4 Robotic assisted urological surgery

Disease	Operation
Kidney	
Kidney ptosis/floating kidney	Nephropexy
Kidney failure	Donor nephrectomy
Kidney cancer	Nephrectomy
Adrenal gland	
Adrenal adenoma	Adrenalectomy
Urinary bladder cancer	Pelvic lymphadenectomy
Ureteral cancer	Ureteroureterostomy
Urinary bladder	
Atrophic bladder, neurogenic bladder	Bladder augmentation
Overactive bladder	Bladder neck suspension
Prostate	
Prostate cancer	Prostatectomy

Eleven patients had robotic hysterectomy and salpingo-oophorectomy, according to Diaz-Arrastia et al. [134]. The 1st clinical surgery of da Vinci robotically assisted endoscopic ovarian transposition technology was described by Molpus et al. [135]. The structural migration of the ovaries from the pelvis to the belly is known as ovarian transposition. Because transposition can enable the keeping of ovary purpose and the retention of aided reproductive ability, it is advantageous for females who are due to receive pelvic radiation. The da Vinci technology can be used to achieve ovarian translocation in certain circumstances [109].

Margossian and colleagues used experimental models to investigate the uses of ZEUS in gynecology in relation to robotic surgery [119, 120]. They showed that four weeks following surgery, all six pigs with uterine horn anastomoses stitched with ZEUS were still alive [139]. In this work, the potential application of robots in microsurgery was emphasized. The same team also employed ZEUS to do five pig hysterectomies [121], with a mean surgical operating duration of 200 min. A robotically assisted laparoscopic ovariectomy for an ovarian serous cyst was carried out using the AESOP system [132]. Ten patients who underwent laparoscopic tubal ligations and had prior tubal reanastomosis were treated with ZEUS by Falcone et al. [136]. All 10 patients underwent the operation satisfactorily; none of them needed conversion to an open surgery. There have been five pregnancies thus far, and a postoperative hysterosalpingogram showed that 17 of the 19 (89%) tubes that were anastomosed had patency [137].

The MR-FUS has also been utilized in gynecology to operate on fibroid tumors and uterine leiomyomas [101, 122]. Tempany et al. [138] state that adult females (over the age of 18), premenopausal status, uterine size of 20 weeks, and absence of a dominant leiomyoma larger than 10 cm in diameter were the recruitment requirements [139]. Nine women with bothersome leiomyomas underwent successful MR-FUS

Table 10.5 Robot-assisted operations in gynecology

Ailment	Surgery
Uterine	Hysterectomy
Endometrial carcinoma	Hysterectomy
Ovary	Hysterectomy
Former tubal ligation patients	Tubal reanastomosis

(age range, 39–51 years; mean, 43.4 years), and the procedure's effectiveness was assessed by having a hysterectomy 3–30 days later. Some of them are mentioned in Table 10.5

10.5.9 Pediatric Surgery

Pediatric surgery now uses robotic surgery frequently as well [123–127]. Some of the cases are being mentioned. Using the da Vinci system, eleven kids, with a mean age of twelve years, underwent bilateral salpingo-oophorectomy for gonad blastoma, a cholecystectomy for cholecystolithiasis, and a Thal and Nissen fundoplication for GERD (limit, seven to sixteen years). A fundoplication took an average of 2.4 h to complete, while cholecystectomy procedures took 2.1 h and salpingo-oophorectomy procedures took 1.5 h, without any incidence of complications [123]. Using a da Vinci robot, Bentas et al. [140] conducted surgery for the removal of adrenal gland for benign adrenal tumors, (adrenalectomy). The same group reported using a da Vinci surgical procedure to clear a blocked ureteropelvic junction (UPJO) [141].

In skilled surgeon hand, a successful alternate treatment for UPJO symptoms is a laparoscopic pyeloplasty. Laparoscopic pyeloplasty with traditional apparatus is complicated, even though patients can benefit from this type of surgery. Eleven pyeloplasty for UPJO were carried out using the da Vinci alone through a laparoscopic transperitoneal route. The average surgery took 197 min (range, 110–310 min). With minimal blood loss and no intraoperative problems, all procedures were finished laparoscopically. At the one-year follow-up, all patients made a quick recovery after surgery and showed outstanding functional outcomes. According to their preliminary findings, robot-assisted Anderson-Hynes pyeloplasty provides a secure and efficient replacement for traditional laparoscopic surgery [142].

Pediatric cardiac disease may be treated with robotic surgery, according to Le Bret et al. [143]. Sixty-five kids with weights ranging from 23 to 57 kg (mean: 12 kg) had their patent ductus arteriosus closed surgically. Where they divided into 2 groups, with twenty-eight patients in group A undergoing visual thoracoscopic procedures and twenty-eight patients in group B undergoing a ZEUS-assisted procedure, the group that received robotic assistance had a much longer operational time.

No thoracotomy was necessary; however, one conversion during video thoracoscopy was essential. Three persisting shunts (one in group A and two in group B) were found during postoperative echocardiography and was addressed by placing a

new clip using video thoracoscopy [144]. Both bleeding and lasting laryngeal nerve damage went unnoticed. In both groups, the average hospital stay was three days.

10.5.10 Dermatology

Robotic and semi-robotic equipments are being used for skin conditions treatment, some of them are mentioned. A robot-assisted port wine stain and other stains can be removed with a scanning laser handpiece, and angiodysplasia conditions were described by Rotteleur et al. in 1988 [145]. A handpiece featuring a scanning feature and a microprocessor-equipped control chamber make up this instrument. The system has its own power meter and is electrically unconnected to the laser. The energy deposit was designed for efficient skin heat dispersion. The robotized handpiece was used to treat 123 patients in total, and no hypertrophic scars were seen. When treating benign cutaneous vascular disorders in children with laser therapy, McDaniel [146] shown that automated robotic laser scanning equipment enables quicker, painless in comparison, and more affordable cure.

A method for computer-supported melanoma identification based on high picture quality of epidermal and dermal profiles was demonstrated by Handels et al. [147, 148]. In a nutshell, profiles are created by using a laser profilometer to sample a 4 mm $\times$ 4 mm area with a high resolution of 125 sample points per mm and a vertical resolution of 0.1 m. Skin tumor treatment is now simpler and more precise thanks to a new image analysis and pattern identification technique [149, 150].

10.6 Pros and Cons of Robotics in Surgery

Due to their ability to circumvent many of laparoscopic surgery's challenges, these devices provide several benefits. They promote vision, hand–eye coordination, and dexterity, as well as posture and ergonomics. Additionally, these devices enable procedures that were previously technically challenging or impractical. In numerous ways, these robotic devices improve dexterity. The capacity of the surgeon to control the tools and subsequently the tissues is substantially improved by instruments with higher degrees of freedom. These systems are made to allow the end-effector motion to be adjusted to account for the surgeons' tremor using the proper hardware and software filters. Additionally, these devices could scale movements, converting massive displacement of the control grips into tiny movements inside the patient.

The return of normal hand–eye coordination and an ergonomic position is another significant benefit. By doing away with the fulcrum effect, these robotic systems improve the intuitiveness of instrument operation. Current systems also eliminate the necessity for the surgeon to twist and spin in uncomfortable positions to maneuver the tools and see the display since they have the surgeon seated at a remote, ergonomically built workstation.

Most people agree that these systems' improved eyesight is astonishing [151]. Comparing the three-dimensional image with depth awareness to the traditional laparoscopic camera views reveals a significant improvement. The surgeon's direct control over a steady visual field with improved magnification and agility is another benefit. All of this results in pictures with higher resolution, which, when paired with the more extent of freedom and improved deftness, significantly improves the surgeon's ability to distinguish between anatomical structures, dissect them, and create micro anastomoses [51].

These systems have a few drawbacks. One among them, robotic-assisted surgery is a recently introduced technology, and its applications and effectiveness are still being studied. Almost little long-term follow-up research has not yet been published; feasibility research has dominated. To enhance the effectiveness and use of robotic arms, other procedures will also need to be modified. However, these drawbacks will probably be eliminated with time. The price of these systems is another drawback. They cost a million dollars, which is almost unaffordable. It is speculative whether the cost of these systems will go down or up. Some people think that the price will decrease as technology advances and as more people get experience with robotic systems. Others think that the cost of these devices will rise because of technological advancements like haptics, faster processors, and more sophisticated software [152]. The topic of system updates is also in question. How much money would hospitals and other healthcare facilities need to spend on improvements, and how frequently. In any event, many people think that these systems need to be widely used across disciplines for the purchase to be justified.

Both surgical equipment's have rather huge physical stand prints and hefty mechanical arms. This is a significant disadvantage in the congested operating rooms of today. Fitting the surgical team and the robo-machine inside the operating area may be challenging. Some contend that reducing the size of the robotic arms and tools will solve the issues raised by their present configuration. Others predict that to meet the additional space needs of robotic surgical systems, bigger operating rooms with several booms and wall mountings would be necessary. These robots are an exceptionally expensive technology because of the expense of building space for them as well as the cost of the robots themselves [153].

Lack of suitable tools and equipment is one of the potential drawbacks mentioned. Lack of some tools makes it more necessary to do some of the surgery with tableside help. This is a temporary setback, though, since new technologies have and will continue to improve upon these flaws. With time and advancements in technology, most of the drawbacks will be eliminated. The effectiveness of these methods will only become apparent over time. It seems improbable that there will be robots in every operating room and that they will be utilized for ordinary operations; if the price needed for these machineries stayed high, it won't be affordable for common people or in general practice.

10.7 Microrobots Approach in Cancer Therapy

Combining the benefits of microrobots with nano size have boosted the potential to a great extent for long- and short-range tumor targeting. It can be achieved by combining the advantages of previous nanomedicines in terms of drug delivery and exhaustion, selectivity, and biocompatibility with active locomotion [154]. Microrobots are defined here as motile microsystems that have been physiologically (genetically), chemically, or physically developed to take use of their actuation for a particular purpose. Additional sensing, tracking, and motion control capabilities might be incorporated into these microrobots. There are primarily three types of microrobots used to combat cancer, and these categories are defined by their construction and means of propulsion. There are three types of microrobots: (1) cellular microrobots (biologically actuated), which are constructed entirely of cellular elements and are designed to express antioncogenic effects; (2) synthetic microrobots (physiochemically actuated), which only contain artificial components, structures, and machineries; and (3) hybrid microrobots, which combine synthetic and biological components (usually biologically actuated). As has been shown for bacterial cells, immuno-components, and semen, and their synthetic analogs in hybrid microrobots [155–157], active motion, which is required in all designed microrobots, is desirable for boosting smooth invasion into the target, and accretion in, tumors/tissues. It is represented in Fig. 10.5. A fascinating research objective for artificial microrobots is enhanced tumor penetration, and encouraging in vivo findings are emerging [158]. Additionally, active movement in entity has the more possibility to improve the loading of delivery vehicles into the cells, as has been shown in regular and malignant-relevant cellular settings using various actuation systems and microrobot sizes [159–161].

It would be essential to tailor microrobot designing to the unique requirements of the specified tumors if attempts to translate robotic technology into the clinic are to be most effectively utilized. The site of the tumor inside the body (which propulsion techniques is more fitted) and the molecular stacking of the tumor (medications which accomplish the highest treatment efficiencies) are important factors to consider. Microrobots must be optimized in prototypical systems that promptly resemble the cancer's in vivo circumstances to be able to answer these concerns. Most of this research has focused on cell-based approaches, including bacterial treatments and cellular immunotherapies. Therefore, it is not surprising that cell-based treatments with precise engineering have made the most headway into the clinic. For instance, the FDA authorized CAR-T cells in the year 2017 as the 1st genetic treatment in the United States, demonstrating the exceptional medical achievement of a particular kind of therapy which is cell based [162]. Additionally, large number of attenuated Salmonella and Clostridia species have been examined before the clinical stage (preclinical) cancer investigations, and several are now being examined in human clinical trials [163]. Future sophisticated hybrid microrobot treatments could be built on these techniques. Model medications rather than genuine chemotherapy have been studied mostly in vitro utilizing two-dimensional or- three-dimensional

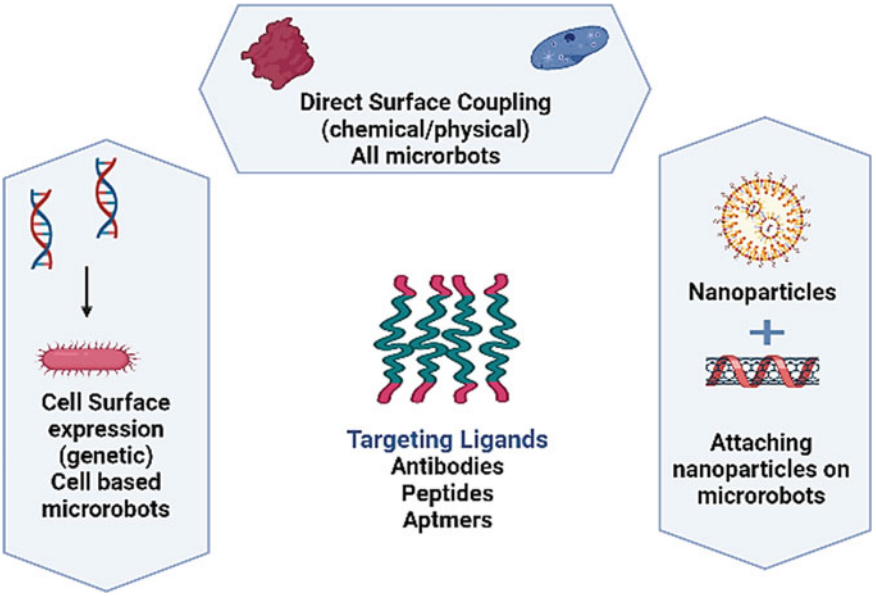

Fig. 10.5 Targeting Tumors using special microrobots

cultures of tumor cell lines, which vary greatly from primary cancer cells, in the more recent disciplines of synthetic and hybrid microrobots. However, research on artificial and hybrid microrobots in live creatures is beginning to emerge more and more. Extending these investigations to different types of microrobots and conducting clinical trials are crucial next steps. To do this, it will be crucial to concentrate research on cancer situations that are typical of patients [164].

10.8 Robotics in Indian Scenario

A new firm that has been operating for a few months in India has installed 9 robotic systems, while an older company in India provides 74 robotic equipment [on personal communication]. The surgeons had to go previously to another nation to receive training in the aforementioned systems. Three training facilities have been built in the last three years where surgeons may get advanced robotic training using either cadaver or live porcine models, which are offered at MSR Medical College in Bangalore and Yenepoya University Mangalore, respectively. The sole constraint of the pig model would be somewhat different anatomy; bleeding would certainly not be present in cadaveric models, and this limitation may be solved in the porcine model. To overcome early lethargy, problems, and unnecessary conversions, more experienced robotic surgeons who serve as proctors hold the surgeons' hands.

The training period is supposed to be shorter since these systems offer intuitive motions. Lower GI surgery, gynecological surgery, and uro-oncological treatments have increased in popularity in India. Additionally, the figures rise yearly. The number of robotic system installations is rather low for a nation with a population of more than 1 billion people due to the financial concerns in the form of greater capital expenditure and operating expenses. Because of the aforementioned factors, the community's reach is rather limited [163, 164].

10.9 Conclusion

Even though it is still in its early stage, robotic surgery has already shown to be extremely beneficial, especially in locations where traditional laparoscopic operations are not feasible. However, whether robotic technologies will take the place of traditional laparoscopic equipment in less technically difficult procedures is still up in the air. Regardless, robotic technology is poised to transform surgical procedures by creating surgical technology, increasing laparoscopic methods, and bringing surgery into the computer age. Furthermore, it may expand surgical therapy possibilities beyond what is now feasible for humans. It will take a lot of studies to figure out whether the benefits of using it will outweigh the costs involved. A greater number of prospective randomized trials assessing effectiveness and safety must be conducted even if the feasibility has essentially been demonstrated. For robotic surgery to take hold, more study is required to assess if it truly outperforms traditional medicine in terms of cost-effectiveness.

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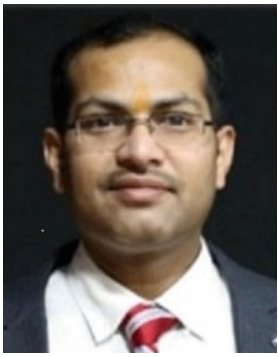

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Chapter 11
Innovative Biomedical Equipment for Diagnosis of Cancer

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Contents

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Abstract Biomedical equipment is crucial for effective disease screening, assessment, therapy, palliation, and recovery. Although cancer fatality may be significantly decreased if instances are recognized and treated early, many avoidable deaths occur due to a lack of appropriate tools to screen, diagnose, and treat illnesses. Several gene mutations have been detected in various stages of cancer, indicating that they are important in cancer pathogenesis. These gene changes are the source of abnormal cell growth. Genetic illnesses caused by hereditary or hereditary causes increase cell proliferation. Significant data has been gathered thanks to molecular and bioinformatics technology breakthroughs, which may be helpful for early diagnosis and treatment. Several biomedical devices, instruments, and methods for cancer diagnostics have been covered in this chapter. The diagnosis of early stage tumours is a potentially valuable method for lowering cancer mortality. X-ray mammography is the most efficient screening method for identifying clinically occult breast cancers. When used in conjunction with commercial microwave equipment, three-dimensional finite-difference time-domain is one of the strategies for diagnosing various malignancies in their early stages. Also mentioned is a breast cancer detection system based on ultrawideband (UWB) radar. For the diagnosis of cancer, non-invasive methods such digital infrared thermal imaging devices are also considered. An equipment integrating Raman, fluorescence, and reflectance spectroscopic modalities have also been described for skin cancer diagnosis. A unique fibre-optic probe has been expressly created to examine cutaneous lesions, and instrument development has focussed on skin cancer applications. Artificial intelligence also plays a crucial role as a cancer diagnostic tool, such as neural networks. Numerous biological applications of THz spectroscopy and imaging are available that help with tissue imaging and macromolecule identification using THz radiation. We have also spoken about a refractive index sensor for early cancer detection based on photonic crystal fibres. It is a unique cancer sensor that uses a dual-core photonic crystal fibre to find cancer cells in the basal, breast, and cervical regions. Using a technique called selective infiltration, the samples are acquired as liquids and injected into the farmed hollow. Colonoscopy plays a significant role in diagnosing and treating lower gastrointestinal lesions. In order to increase colonoscopy quality in terms of both technical and cognitive considerations, several new endoscopic procedures and technologies have been created. In order to identify and treat lung cancer, CADe and CADx systems have become essential study domains in recent years. A sort of computer system known as a CAD system employs a second opinion to assist in the identification and/or diagnosis of disorders. Systems called CADe are created to find lesions in medical photos. Furthermore, CADx systems assess lesions, such as distinguishing between benign and malignant cancers. In this chapter, we have described a variety of modern biomedical equipment, instruments, and approaches for cancer diagnosis. Some of the field's prospects have also been thoroughly discussed.

11.1 Introduction

The "New Medical Equipment and In Vitro Diagnostic Regulations in 2017" were published by the Ministry of Health and Family Welfare of the Indian government. In accordance with these ground-breaking regulations, the ministry's undertaking organization, the CDSCO, published the "Medical Device Rule 2017," which went into effect on 1 January 2018 in India [1]. According to Indian regulation agencies, biomedical equipment must adhere to technology standards set forth by the International Organization for Standardization, the Electro-technical Commission, and the Bureau of Indian Standards (BIS) [1]. Today's medical setting significantly relies on a variety of medical devices for patient diagnosis and care. To protect patients and users from harm, this medical equipment must be in perfect functioning condition. In addition, the hospital needs to execute the appropriate cost controls in the light of the difficult competitive environment and complex healthcare system [2]. A crucial element of contemporary health care is medical equipment. However, the districts' related administration or upkeep is very poor. The deployment rate of medical equipment has greatly outpaced the expansion in management and maintenance capacities. Patient safety, operation performance in cost/efficient analysis, risk assessment, and control are crucial considerations when employing medical equipment in hospitals in addition to standard operational management [3, 4]. A definite biomedical approach is also used for diagnosing specific cancer, such as an adhesion-based approach for detecting oesophageal cancer. This gloomy number is a result of the cancer's aggressiveness as well as the fact that oesophageal cancer generally develops at a late stage [5]. Early identification would probably raise the 5-year survival rate in the case of the latter cause, as it did for more widespread diseases like colon and breast cancer. As a result, an early oesophageal cancer diagnosis is a key diagnostic objective [6–8]. Compared to normal tissue, oesophagus changing tissue undergoes biochemical changes that offer the potential to create tests that can identify oesophageal cancer in situ at an early stage [9, 10]. Additionally, several studies demonstrate that sick tissue may be identified utilizing molecular constructs (also known as ligands) similar to pharmacological targets expressed exclusively in changing oesophageal tissue [11, 12]. Therefore, ligand-target molecule recognition is a viable diagnostic strategy to detect changing tissue inside the oesophagus. The elements governing the selective identification of diseased tissue are undoubtedly one of the most crucial issues to consider whilst building such systems. Therefore, ligand-target molecule recognition is a viable diagnostic strategy to detect changing tissue inside the oesophagus. The elements governing the selective identification of diseased tissue are undoubtedly one of the most crucial issues to consider whilst building such systems. The detected element might be the actual targeted ligand itself or a particle that is conjugated to the ligand. Given that the oesophageal lumen is B2 cm, the ligand-conjugated particle may be a nanoparticle or have a diameter of 10–100 μ [13]. Employing particles, especially those in the micron range, has a number of notable advantages. These upsides include that these particles can frequently be detected by bright field microscopy, that they enable a relatively large volume of imaging material to be

carried per particle, which can enhance identification, that they exhibit little to no non-specific cell uptake, which reduces non-specific signal and off-target distribution, that they are reasonably straightforward to manipulate experimentally, and that they are easy to recognize [14, 15]. One promising technology for diagnosing lung cancer is the electronic nose (e-nose) [16]. The volatile organic compounds (VOCs) produced by cellular metabolism and present in breath can be detected by the e-nose. Volatile organic compounds may be examined to generate a sensory combination akin to a fingerprint by identifying the various chemical components as well as their pattern of expression (breathprint). Either a multimodal platform or the e-nose alone can mimic the multivariate selectivity of the human olfactory system [16]. Contrary to the human nose, which receives gas mixes, the e-nose filters gas combinations before exposing them to many sensors. The signals produced by these sensors are subsequently examined using multivariate analysis and pattern recognition computer programmes [17]. A potential development is the "no touch" early diagnosis of lung cancer made feasible by e-nose technology. The e-nose recognizes "fingerprints," also known as "breathprints," by activating gas sensors when they are exposed to VOCs, which are indications of biological metabolism and are found in both exhaled breath and other organic fluids. Exhaled breath and other organic fluids both include VOCs [18, 19]. Early studies have already demonstrated the effectiveness of the e-nose in identifying an adenocarcinoma histotype, distinguishing between malignant and benign pulmonary nodules, and even determining the status of the EGFR gene [18, 19]. One of the most promising and evolving technologies today is nanooncology for the diagnosis and treatment of cancer. Nanotechnology is used in cancer administration and therapy by a subspecialty of nanomedicine called nanooncology. Thanks to developments in the discipline, nanotechnology now offers a wider range of biological science applications [20]. Nanotechnology makes it simpler to recognize cancer at the molecular level by using small molecules like gold nanoparticles, quantum dots, and other nanomaterials that provide crystal-clear diagnostic indications of cancer. The identification of cancer biomarkers has improved because to nanotechnology-assisted molecular diagnostics; for example, a nano biosensor is incredibly sensitive and can quickly identify multiple protein biomarkers [21]. Nanotechnology-based high temporal and spatial techniques, such as NIR quantum dots, nanoshells, and colloidal gold nanoparticles, can be utilized to precisely track live cells and keep an eye on their dynamic biological processes in tumours. The most important stage in cancer treatment is an early and accurate diagnosis, which CT, MRI, PET, ultrasound, and other imaging tools frequently achieve [22]. It has becoming harder to provide outstanding patient outcomes and therapy as a result of recent investigative and imaging tools' limited ability to provide full clinical information about various types and stages of tumours [23, 24]. Most presently available anticancer drugs do not distinguish between malignant and healthy cells, which causes systemic toxicity and unwanted side effects. Also, a significant issue with cancer is that it doesn't get diagnosed until after it has spread to other body parts. In European males, prostate cancer (PCa) which does not include nonmelanoma skin cancers—has the greatest prevalence of any cancer and ranks third in cancer-related deaths, after lung and colorectal cancer [25]. The incidence of the illness is anticipated to

rise as the population ages, and since 1995, there have been 16% more fatalities from prostate cancer [26, 27]. Prostate cancer differs from many other cancers because it frequently presents as multifocal lesions rather than a single circular mass [28]. Prostate cancer can proceed in various ways, and before it reaches an advanced stage, it frequently goes unnoticed [29]. Digital rectal examination (DRE), which historically served as the primary detection technique, is now regarded as a relatively rudimentary instrument. Because cancer cells have a higher density of cells than healthy cells, prostate tumours are often tougher than healthy tissue [30, 31]. Transrectal ultrasonography (TRUS)-guided systematic biopsy is the gold standard for identifying and diagnosing PCa [32]. A minimum of 10 cores is advised according to European recommendations from 2007. Brightfield microscopy is used to examine the cores, and cancer is found thanks to its unusual morphological characteristics [33]. A systemic biopsy is frequently used as a benchmark; however, because of its limitations, the exact accuracy of the tested procedure cannot be ascertained. A systemic biopsy is not recommended in favour of the histological study of material after radical prostatectomy [34]. Prostate cancer clinical detection rates and diagnostic precision may rise with several MR and ultrasound methods [35]. For a patient to survive, melanoma must be promptly diagnosed and treated whilst it is still in its early stages. Despite intensive study into the many melanoma appearances and morphological traits, the clinical diagnostic efficacy is still not at its highest [36]. The sensitivity for identifying melanoma amongst doctors who specialize in examining pigmented lesions is about 80%, whilst the diagnostic accuracy is about 65% [37, 38]. Therefore, it would seem that roughly 1 in 3 diagnoses for or against melanoma obtained by a straightforward visual examination is inaccurate [39, 40]. Globally, there is a serious public health issue with cancer. In the coming decades, rising global demographics indicate an increase in the prevalence of cancer, with more than 20 million more occurrences yearly anticipated by 2025. GLOBOCAN database estimates 8.2 million cancer-related deaths, and millions of people were infected in 2012 [41]. Cancer is defined by the uncontrollable multiplication of abnormal cells and improper immune system recognition and is known as "a wound which never healed" [41]. Nearly two-thirds of all fatalities globally are caused by high-mortality conditions like cancer and cardiovascular and diabetes illnesses. Early identification and ongoing assessment are essential for improving patient prognostic and minimizing individual burden. There seems to be an immediate requirement for novel diagnostic, biomedical equipment, medical devices, and therapy follow-up techniques that enable earlier pathology diagnosis as well as ongoing physiologic assessment of certain treatments [42]. All stages of cancer treatment involve the utilization of various biomedical diagnostic techniques. Imaging, which can provide information related to morphology, structure, metabolic activity, and function, is a crucial component of oncology diagnostic procedures. Clinical judgement is facilitated by integrating it with another diagnosis technique [43]. The diagnosis ability of TD-EVs could be inferred for earlier and more accurate cancer detection, which was previously unachievable. These exosomes EVs have lately been used to carry biomarkers for diseases such as pancreatic, breast, prostate, ovarian, and lung cancer. Its appearance in human fluids makes diagnosing and tracking a patient's reaction to therapy

simple using non-invasive liquid biopsy techniques [44]. Bubble with ultrasound, owing to its versatility as both a diagnostic device (using sonography) and a therapeutic tool (utilizing high-intensity focussed ultrasound), ultrasound, for instance, is an excellent tool for theranostics [45]. Understanding these nanotechnology-based particle's mode of action and how it interacts with the body's defensive system is crucial for moving from the laboratory bench to the therapeutic side. Quantum dots and carbon nanotubes are highly desired alternatives in investigative nanobiology for cancer cell diagnosis because of their new features. Upcoming therapy plans may be facilitated by Quantum dots and carbon nanotube's capacity to act as multimodal nano-theranostics agents [46]. Applications of multifunction peptide nano assemblies for cancer diagnostics include fluorescence, magnetic imagery, and biosensors for cancerous cell biomarkers. For example, peptide nanoparticles, nanovesicles, and nanospheres are useful in drug load and the release of anticancer medications. In contrast, peptide nanosheets and nanofibers have shown their utilization in photodynamic and photo-thermal tumour treatments [47]. Various biomedical technologies used for cancer diagnosis are shown in Fig. 11.1.

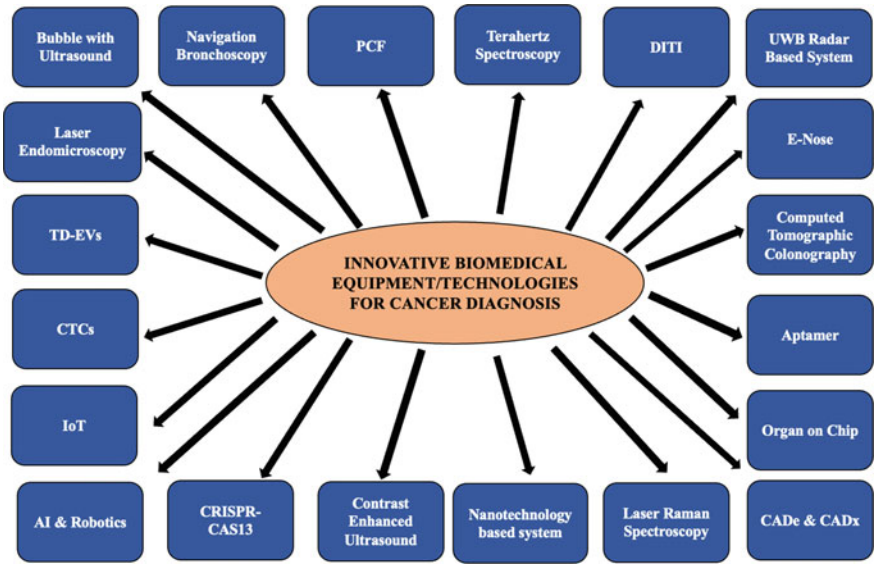

Fig. 11.1 Innovative biomedical equipment/technologies for cancer diagnosis

11.2 Novel Approaches for Cancer Diagnosis

11.2.1 Photonic Crystal Fibre

In photonics sensing applications, photonic crystal fibre (PCF) has proven to have a lot of potentials [41]. Typically made of silica, PCFs have microscopic air holes along the length of each fibre. PCFs offer several advantages over standard fibres, including single continuous mode, big mode area, adjustable dispersion, strong birefringence, tunable enormous optical nonlinearity, and shallow loss, due to their exceptional design flexibility. The analyte components and external variables that govern the sensing property also heavily depend on PCFs [48]. In particular, PCFs have been employed in biological applications for illness diagnosis, treatment, prevention, and detection for improved health. One of the most prevalent illnesses in the world today, cancer is extremely dangerous to human health. It belongs to the class of viruses known as oncoviruses. The impact of cancer existence depends on the virus's size and how it spreads throughout the cell. The next generation of cancer testing technology is urgently needed to detect cancer cells as early as feasible due to the rising cancer rate. It has been shown in a study that the biofluidic detecting technique uses microstructure optical fibre. The suggested approach guarantees improved outcomes with enhanced sensitivity for cancer diagnosis at an early stage. Nanometre-sized cancer samples, such as blood or other fluid from the afflicted region, are treated instead of viruses. We do this by directly analysing the cancer sample samples' refractive indices. As a result, basal, cervical, and breast cancer fluid samples are taken into account and infused into the silica substrate's cancer cell cavity.

11.2.2 Terahertz Spectroscopy and Imaging

THz radiation is the term for the frequency range of the electromagnetic spectrum that is between the microwave and infrared bands and ranges from 0.1 to 10 THz [49]. Modern terahertz TDS, which is used in astronomy, microelectronics, and biomedical science, is a result of the quick development of ultrafast lasers [50]. Macromolecule identification and tissue imaging using THz radiation have advanced significantly. The ability to acquire the spectroscopic characteristics of numerous blood cells in the THz area and the astounding linearity between THz signals and erythrocyte concentrations have demonstrated the technique's promise for determining quantitative cell concentrations. Additionally, the complex dielectric constants of many growing human carcinoma cells may be used to separate them from one another [51]. The THz approach has demonstrated to be more sensitive in detecting minute structural changes in developed cell monolayers when compared to conventional optical phase-contrast imaging and electric cell-substrate impedance sensing [52]. Without the requirement for staining or labelling, THz-ATR measurements may be utilized to do an in situ, non-invasive, real-time examination of the dynamics of

cytoplasm leakage. Human cancer cells' internal water dynamics and the number of hydrating water molecules present may both be assessed via ATR spectroscopy [53, 54]. THz technology allows for the simultaneous extraction of morphological characteristics and inherent properties from amplitude and phase data [49]. Changes in tissue composition and structure are necessary for THz biomedical imaging. These tissues differ from ordinary tissues in terms of their THz absorption. Structural variations: decaying cellular morphologies, changing macromolecules, and altering tissue microenvironments might alter the amount and quality of the picture contrast [55].

11.2.3 Digital Infrared Thermal Imaging

A non-intrusive, non-contact technique for detecting infrared radiation produced by the surface of the body to determine body temperature is called digital infrared thermal imaging (DITI). Although this technology was initially created for the US military's use in night vision, it has a variety of medicinal applications. The reason for its application in cancer is that tumours often have higher blood flow, angiogenesis, and metabolic rate, which leads in bigger temperature gradients compared to nearby normal tissue [56]. Prior imaging methods, however, were unable to distinguish between temperature because of the costly equipment required and the general absence of computer analytical abilities. Since then, infrared thermal imaging technology has substantially evolved thanks to digitalized high-resolution photography and sophisticated artificial intelligence-based neural network image processing. Infrared emission measurement equipment could only distinguish temperature differences of 0.5–1 °C. Some even required patient touch, using an older technology that required a special liquid crystal film to be applied to the patient's breasts in order to measure temperature. Without coming into contact with the patient, current digital infrared thermography cameras can detect temperature changes at 0.08 °C or higher. Currently, DITI has the potential to significantly impact medicine [57]. DITI is non-invasive, painless, does not generate radiation detrimental to patients, poses no patient danger, yields results immediately, and costs not too much money. DITI is significantly less expensive for the patient and the provider than magnetic resonance imaging (MRI), a complementary diagnostic method growing in popularity for breast cancer. DITI can recognize tumours since it is assumed that their biology differs from that of the normal tissue around them. One research discovered a connection between the thermographic hot areas and the microvessel density of breast cancers, offering a mechanistic basis for using DITI in cancer detection. DITI must be conducted in combination with another test, such as mammography or ultrasound, because thermal recordings may only be utilized to evaluate physiologic factors. For example, a breast parenchymal infection or inflammation might affect temperature readings and provide false-positive findings [58].

11.2.4 Ultrawideband (UWB) Radar-Based System

A system for the detection of breast cancer using ultrawideband (UWB) radar is made up of complementary metal–oxide–semiconductor integrated circuits. In terms of waveforms, there are typically two approaches to microwave imaging. One is based on radar and uses an ultrawideband (UWB) transmission. A hemispherical antenna dome was used to mount the antenna array in the multistatic radar-based detection system that Klemm and Craddock et al. Demonstrated [59, 60]. Using a UWB signal-generating circuit, the monocycle Gaussian pulse might be generated and transmitted in this configuration [61, 62]. The signal channel that will in turn excite the UWB antenna array can be chosen by the switching (SW) matrix circuits. By employing comparable high-speed sampling circuits, the incoming signals may be sampled and digitalized. The digitized information is then stored in the computer for later processing to produce the breast image [63, 64]. A tiny UWB antenna array, CMOS UWB signal-generating circuits, switching matrix circuits, and high-speed sampling circuits make up the majority of the system's hardware. The switching matrix generates the GMP signal during the detection and transmits it to a specific antenna. The high-speed sampling circuits capture the reflected signals.

11.2.5 Electronic Nose

In addition to standard types of treatment, the electronic nose is a potential technological advancement that might be utilized to detect lung cancer. The volatile organic compounds (VOCs) produced by cellular metabolism and present in breath can be detected by the e-nose. With the use of analysis, it is possible to generate a sensory combination equivalent to a fingerprint by identifying the precise chemical components of VOCs as well as their pattern of expression. The incredible combinatorial specificity of the human olfactory sense is mimicked by the e-nose. The e-nose filters gas mixtures rather than exposing them to numerous sensors like the human nose does. The signals produced by these sensors are then evaluated automatically utilizing multivariate analysis and pattern recognition algorithms [17]. It can detect "fingerprints" or "breathprints" unique to a given chemical by activating gas sensors when they are exposed to VOCs, which are indicators of biological metabolism and are present in both exhaled breath and other organic fluids. Low-dose computed tomography screening of people with a high risk of getting cancer led to a 20% drop in death particularly attributable to lung cancer, according to studies from the National Lung Screening Trial [65]. Radiation exposure, the likelihood of false-positive findings necessitating unnecessary invasive diagnostic procedures, the alleged over diagnosis of slow-growing cancers, as well as the paradoxical promotion of smoking are all possible arguments against the use of LDCT screening [66]. Monitoring VOCs in exhaled air as a lung cancer screening method has been the subject of several research [67].

11.2.6 Aptamer

Short, single-stranded, non-coding aptamers, also known as "Systematic Evolution of Ligands by Exponential Enrichment," were created in vitro. Because aptamers differ from antibodies in key ways, including high specificity, stability, and non-immunogenicity, they have the potential to be therapeutic agents. They can attach to specific locations with high affinity like antibodies. Aptamers, sometimes known as "chemical antibodies," are also simple and inexpensive to make. Previous years' selections included a number of aptamers that were particularly bound to breast cancer biomarkers and cells. Finding biomarkers is essential for prognosis, early diagnosis, and monitoring the effects of treatment for breast cancer. One of the most important and often used breast cancer-specific biomarkers is HER2, which is used for both molecular classification and clinic-focussed treatment of the disease. A potent technique for identifying breast cancer cells is the use of aptamers, which are produced from both Cell-SELEX and purified target-based SELEX [68, 69]. In a study, MUC1 and HER2 aptamers were used to make dye-functionalized silica nanoparticles with dual aptamer functionalities for the rapid screening of breast tumour cells [70]. This method would be able to capture the target cells by using aptamers that have been modified to attach to magnetic beads and separate them magnetically. A fluorescence signal would be detectable if target cells were collected following magnetic separation and fluorescent stimulation; otherwise, there wouldn't be any fluorescence. Additionally, different aptasensor kinds depending on nanomaterials were investigated for the detection of PSA. Biosensors, which operate primarily on the interaction amongst biological components and nanomaterials processors, identify biological components in most tumours, such as prostate cancer. Because of their rapid speed, outstanding efficiency, and simple processing, aptasensors are suitable for constructing lab-on-chip sensors. Future studies are expected to demonstrate that these nanotechnology-based materials are more effective in aptasensors [71] (Table 11.1).

11.2.7 Computer-Aided Detection (CADe) and Diagnosis (CADx) System

A family of computer systems known as CAD (Computer-Aided Identification and Diagnosis) systems aims to provide a "second opinion" to help in the detection and/or diagnosis of illnesses. Each year, more than 1.59 million people die from lung cancer. To lower this percentage, CAD technologies are being created to aid radiologists in identifying and diagnosing. These technologies must accelerate diagnosis whilst lowering mistake rates and enhancing quantitative evaluation CAD solutions are designed to expedite image interpretation whilst improving radiologists' accuracy. CADe and CADx systems are the two categories into which CAD systems fall. For identifying lesions, medical imaging systems called CADe are used. Furthermore,

Table 11.1 Various aptamers and their targets

Aptamer	Sequence	Target	Cell line	References
MUC1	GCAGTTGATCCTTTGGATACCTGG	Mucin-1	MCF-7 (breast cancer cells)	[72]
H2	GGGCCGTCGAACACGAGCATGGTGCGTGGACCTAGGATGACCTGAGTACTGTCC	HER2	Breast cancer	[73]
AS1411	GGTGGTGGTGGTTGTGGTGGTGGTGG	Nucleolin	HeLa (human cervical cancer cells)	[74]
HB5	AACCGCCCAAATCCCTAAGAGTCTGCACTTGT CATTTTGTATATGTATTTGGTTTTTGGCTCTCAC AGACACACTACACACGCACA	HER2	Breast cancer	[75]
SGC8	ATCTAACTGCTGCGCCGCCGGGAA-AATACTGTACGGTTAGA-(CH2)6-NH2	Protein tyrosine kinase 7 (PTK7) (Cell surface)	CCRF-CEM (T-cell leukaemia cell line)	[76]
TDO5	AACACCGGGAGGA-TAGTTCGGTGGCTGTTCAGGGTCTCCT CCCGGTG-(CH2)6-NH2	IgG receptors (Cell surface)	Ramos cells (B-cell lymphoma cell line)	[77]
GBI-10	GGCTGTTGTGAGCCTCCTCCCA-GAGGGAAGACTTTAGGTTCGGTTC-ACGTCCCGCTTA TTCTTACTCCC	Tenascin-C (Extracellular matrix)	U251 (human glioblastoma cell line)	[78]
A10	GGGAGGACGAUGCGGAUCAGCCA-UGUUUACGUCACUCCUUGUCAAUC-CUCAUCGGC	Prostate-specific membrane antigen (PSMA) (Cell surface)	LNCaP (human prostate cancer cell line)	[79, 80]
A9	GGGAGGACGAUGCGGACCGAAAAA-GACCUGACUUCUAUACUAAGU-CUACGUUCCCAGA	Prostate-specific membrane antigen (PSMA) (Cell surface)	LNCaP (human prostate cancer cell line)	[81]

(continued)

Table 11.1 (continued)

Aptamer	Sequence	Target	Cell line	References
AS1411	TTGGTGGTGGTGGTTGTGGTGGTGGT GG	Nucleolin (Cell surface and nucleolus)	Human colorectal cancer cell	[82]
Xpsm-A9	GGGAGGACGAUGCGGACCGAAAA-AGACCUGACUUCUAUACUAAGUCU-ACGUUCCCAGACGACUCGCCCGA	Prostate-specific membrane antigen (PSMA) (Cell surface)	LNCaP (human prostate cancer cell line)	[83, 84]
CD4 aptamer	AAGUGACGUCCUGAUCGAUUGUG-CAUUCGGUGUGACGAUCU	CD4 (Cell surface)	Jurkat (T-cell leukaemia cell line)	[85, 86]
TTA1	GGAGGACGCUCGCCGUAAUG-GAUGUUUUGCUCCUG	Tenascin-C (Extracellular matrix)	Breast, glioblastoma, lung and colon cancer	[87]
E9P2-2	GGGAGGACGAUGCGGUGCCCACU-AUGCGUGCCGAAAAACAUUUCCCC-CUCUACCCCAGACGACUCGCGCGA	Tenascin-C (Extracellular matrix)	U251 (human glioblastoma cell line)	[88]

CADx systems classify the lesions, for example, identifying benign and malignant tumours [89]. The study found that 98.6% of lung nodules seen over the course of two years of observation have either remained stable or decreased, and just 1.4% of the nodules are cancerous. Systems to improve cancer detection have been created by researchers to provide greater guidance (CADx). Typically, CADx systems use a classifier to determine the malignancy using the image attributes [90]. Significant progress has been made in the investigation of CADe and CADx systems for lung cancer in recent decades. But separate studies are being conducted in these study areas. The fact that CADe systems for lung nodules simply identify them without defining them is one of their main problems. Nodule-finding computer systems are inadequate for clinical usage. Radiologists are in the dark since CADe systems do not yet show the tumour's radiological characteristics. Contrarily, CADx systems do not yet have a high level of automation or the ability to detect tumours [91, 92].

11.2.8 CRISPR-Cas13 System

In recent years, CRISPR's toolkit for modifying the genome and transcriptome has grown significantly. The RNA-targeting CRISPR-Cas13 system is an intriguing tool for cancer research, treatment, and detection due to its distinct biochemical features. Cas13-based diagnostic methods make it possible to detect and track cancer indicators early from liquid biopsy samples without the use of complicated equipment [93]. The current CRISPR-Cas categorization scheme, which is primarily separated into two groups, is based on the design of the effector complexes. In Class 1 CRISPR-Cas systems, effector complexes of various Cas nucleases are organized around a core crRNA skeleton [93]. This idea encompasses exosomes, circulating tumour cells, cell-free tumour DNA (ctDNA), and RNA (ctRNA). Blood, urine, cerebrospinal fluid, and saliva are some of these fluids. Next, the multi-omic analysis is performed [94]. A versatile and effective diagnostic method for identifying cancer early and improving the effectiveness of targeted cancer therapy is liquid biopsy [94]. These expensive approaches need the use of top-notch laboratory equipment, qualified workers, and time-consuming sample preparation procedures. It is impossible to use these technologies outside of centralized facilities due to these limitations. To get over these restrictions and broaden liquid biopsy analysis, rapid, economical diagnostic procedures that are straightforward, sensitive, and specific are needed [95]. By providing rapid, inexpensive, kind, and precise methods without requiring complicated equipment, CRISPR-based techniques increase accessibility for disease detection [96]. Transcriptome engineering techniques and diagnostic tools have both been created using the CRISPR-Cas13 technology. Due to its distinct biochemical properties, Cas13 is a flexible enzyme that may be used in cancer therapy, research, and detection.

11.2.9 Organ-on-a-Chip for Cancer

Cancer and immune-related illnesses are significant drains on our health service, necessitating the immediate demand for efficient, slightly elevated drug development strategies. The cancer microenvironment can be recreated using "cancer-on-a-chip" technologies, which combine cancer organotypic and microfluidic technology. These chips enable a better pre-clinical evaluation of treatment effectiveness by facilitating a more profound knowledge of cancer behaviour in vivo. These models can further explore the relationships of cancer with other organs by connecting various physiologic modules, such as the vasculature [97, 98]. Microfluidic chips provide monitoring of organoid conditions and assist in connecting various modules to represent a variety of organ processes. They are also compatible with online analytic modules. For instance, a "cancer-on-a-chip" system that combined numerous organoid techniques, such as cardiac organoids and liver cancer as well as in order to investigate the fundamental mechanisms causing tumour growth as well as metastasis and test potential anti-metastasis medications, a model simulating bone-specific extravasation of cancerous breast cells was developed. Significant obstacles must be overcome before their widespread usage, such as the accessibility of tissues generated from patients. Not all researchers can easily access microfabrication resources and the associated knowledge [99, 100].

11.2.10 Cancer-on-a-Chip

It recreates the cancer microenvironment by combining cancer organotypic and microfluidic technology. These chips enable a better pre-clinical evaluation of treatment effectiveness by facilitating a more profound knowledge of cancer behaviour in vivo. These models can further explore the relationships of cancer with other organs, such as cardiac organoids and liver cancer, bladder cancer, and breast cancer [101].

11.2.11 Circulating Tumour Cells Technology

It is a part of the liquid biopsy and has enormous potential to revolutionize the current practise of cancer therapy. Numerous studies have shown that individuals with metastatic prostate, lung, colorectal and breast cancer have increased CTC counts indicating a poor prognostic. Technologies for affinity-based CTC enriching either selectively target cancer antigens to collect CTCs (positive enrichment) or selectively target CD45 to reduce hematopoietic cells (i.e., negative enrichment). The only CTC device approved by the FDA as a diagnostic tool for persons with metastasized breast, prostate, and colorectal cancers is the CellSearch® system. Using magnetized beads

coated with antibodies by AdnaTest allows for the enrichment of CTCs in breast cancer and CTC chips for treating metastasis cancers [102, 103].

11.2.12 Tumour-Derived Extracellular Vesicles

The genetic component and certain oncogenic proteins carried by TD-EVs on the membrane surface contribute to the progression of tumours. Also, the ratio of these nucleic acids to the protein differs significantly between EVs generated from malignant and healthy cells. Since TD-EVs are capable of making diagnoses, it is possible to identify cancer earlier and with more accuracy than was previously possible. These exosomes EVs have lately been used to carry biomarkers for diseases such as pancreatic, breast, prostate, ovarian, as well as lung cancer. Its appearance in human fluids makes diagnosing and tracking a patient's reaction to therapy simple using non-invasive liquid biopsy techniques. Furthermore, methods for injecting Genetic material (DNA) into exosomes were being developed in order to target particular cancer cells and induce the appropriate responses in such cells. However, the precise processes of the interaction amongst cancer cells via TD-EVs are still primarily unknown [44].

11.2.13 Bubble with Ultrasound

Owing to its versatility as both a diagnostic device (using sonography) and a therapeutic tool (utilizing high-intensity focussed ultrasound), ultrasound, for instance, is an excellent tool for theranostics. The term "theranostics" refers to integrating therapeutic and diagnostic capabilities in one drug. In addition to being utilized as contrasting image tools, combining bubbles (micro and nano) with ultrasound is also employed to improve drug delivery. Furthermore, as a way of actively destroying tumour cells, high-intensity ultrasound has been utilized as a cancer treatment. However, the bubbles' characteristics are crucial for successful cancer treatment. Using such bubbles for treating cancer is used by utilizing various therapy strategies [45]. Sonodynamic therapy (SDT), an innovative non-invasive therapy approach that combines ultrasound (low-intensity) and sonosensitizers, shows promise for clinical application because of its high capacity to penetrability to cure deeper lesions that are unresponsive to photodynamic therapy (PDT), which has the major drawback of having a shallow depth of tissue permeation and hence play an important role in cancer therapy [104].

11.2.14 Navigation Bronchoscopy

To put it more simply, navigational bronchoscopy is the utilization of technologies to assist the bronchoscopist in precisely navigating the bronchoscope to a location of interest. Despite the fact that ultrathin bronchoscopes are usable, it is very challenging to be specific about a particular location which results in reduced diagnosing yields, the requirement for additional invasive processes, and rising costs. Various navigational bronchoscopy, such as virtual, electromagnetic, and fused fluoroscopy, has been extensively utilized as a diagnostic tool for lung cancer. Presently, computed tomography-guided-guided transthoracic needle biopsy (CT-guided TTNB) is the benchmark for the least invasive peripheral lesions diagnosis (lung cancer). Directly tunnelling, as demonstrated with BTPNA, seems to have the ability to alleviate a few of the challenges with the equipment accuracy manipulation in the extreme lung periphery. Although navigating the lung's irregular branched shape adds additional complexity, robotics' ability to deliver pinpoint accuracy is an incredibly appealing prospective for intervention bronchoscopists [105].

11.2.15 Confocal Laser Endomicroscopy

It is a non-invasive diagnostic tool that can help with real-time, early identification and potentially lessen the need for invasive surgery. For the diagnosis of oral squamous cell carcinoma (OSCC), this Laser Endomicroscopy provides very good specificity and sensitivity. Earlier studies have shown that CLE can accurately and efficiently be utilized as a predictable tool in head and neck cancer with high histopathology assessment repeatability. Recent studies have demonstrated the development of AI algorithms and computational techniques for precisely predicting and diagnosing head and neck cancers.Artificial intelligence technology considerably enhances the opportunities for enhancing OSCC prognosis and screening results [106].

11.2.16 Nanotechnology-Based Biomedical Equipment

Quantum dots and carbon nanotubes are the two nanotechnology-based particles that have attracted the most attention due to their potential use in cancer detection, diagnosis, and treatment. Due to the capacity of quantum dots for penetrating individual cancerous cells, their nanosize, and the fact that they emit light in narrower bands than organic dyes, they can be employed in clinical situations to localize cancerous cells. Carbon nanotubes can help find malignant tumours by affixing antibodies that bind selectively to tumour cells. When subjected to near-infrared light, the cancerous cells can be thermally eliminated using CNTs. Numerous research centres are looking into coupling quantum dots to nanotubes to localize tumour cells in the individual using

QD imaging and then destroy the cells utilizing the release of the drug or treating them thermally. These are highly desired alternatives in investigative nanobiology because of their new features. Understanding these nanotechnology-based particle's mode of action and how it interacts with the body's defensive system is crucial for moving from the laboratory bench to the therapeutic side [46]. One of the fast-growing fields of nanotechnology involves no-wash biosensors. This field has made enormous and substantial development during the last few decades. A variety of potential no-wash biosensors have been found for the in vitro detection of cancer biomarkers, which can include small chemicals, proteins, and even cancer cells. No-wash biosensors' unique physical and chemical characteristics, as well as enhanced optical, electrical, catalytic, and magnetic features, considerably enhance their analytical capabilities, including sensitivity, specificity, and multiplexing capability [107]. The functioning and structure of peptide-based nanomaterials can be primarily modulated through the tailoring design of the sequence of amino acids of the peptide molecules, which has offered significant support for diagnostic and therapeutic malignancies to a large extent. Applications of multifunction peptide nano assemblies for cancer diagnostics include fluorescence, magnetic imagery, and biosensors for cancerous cell biomarkers. For example, peptide nanoparticles, nanovesicles, and nanospheres are helpful in drug load and the release of anticancer medications.

In contrast, peptide nanosheets and nanofibers have been used in photodynamic and photo-thermal tumour treatments [47]. Metallic nanoparticles such as iron oxide nano crystal, which have already been certified for usage in humans as MRI polar compounds, have the unique capacity to function as simultaneously photo-thermal and magnetic agents in colorectal cancer [108]. Both in vivo and in vitro cancer diagnosis uses imaging methods helped by silica-based nanoparticles. The significant uses of silica-based nanoparticles in early cancer imaging are MRI and fluorescence imaging [109].

11.2.17 Computed Tomographic Colonography

The utilization of this colonography for the detection and treatment of colorectal carcinoma, with a focus on monitoring patients following tumour removal procedure and evaluating the colon preoperatively for occlusive cancers. Computed tomographic colonography is the most practicable and technically proficient way to assess the colon close to an occlusive malignancy, which restricts colonoscopic investigation behind the occlusion. This can be done pre or post-metal stent implantation [110].

11.2.18 Laser Raman Spectroscopy

It has been demonstrated that a highly sophisticated biomolecular technique can distinguish between malignant and healthy breast tissue. It is used more frequently in oncogene diagnosis (breast cancer) when paired with other "machine learning" methodologies. Breast tumour diagnosis following surgery utilizing this spectroscopy technique is possible as it is a real-time instrument. Utilizing the biochemical uniqueness of the Raman scattering effect, it is label-free and not destructive. Both kinds of data are automatically provided by artificial neural networks like stochastic backpropagation for each tissue site that laser Raman spectroscopy analyses. It is possible to quickly identify what extra context input is required to enhance networking classifier performance if the network is trained to utilize either humans or unsupervised algorithms [111].

11.2.19 Contrast-Enhanced Ultrasound

By identifying malignant tumours by focusing on a needle biopsy, CEUS enhances the identification of prostate cancer. With this, it may be possible to make a precise diagnosis and administer cutting-edge therapies like focussed therapy. Although thus ultrasound increases the sensitivities of prostate cancer identification, a focussed biopsy solely can result in a greater rate of prostate cancer detection. Furthermore, combining target and systemic biopsies appears to result in a satisfactory rate of cancerous cell detection [112].

11.2.20 Internet of Things

The uses and advantages of IoT technologies, particularly wearable gadgets, in controlling adverse effects, symptoms, life quality, and mortality in patients with cancer ongoing therapy. Mobile health (mHealth) was already emphasized as a crucial aid in managing cancer. Wearable technology makes it possible to collect patient's information in real-time, which helps, for instance, cancer patients manage their side effects whilst seeking medical therapy [113].

11.3 Types of Cancer and Biomedical Equipment Utilized

Biomedical equipment used	Mechanism of action/Technique	Cancer type	References
Tumour-derived extracellular vesicles	The genetic component and certain oncogenic proteins carried by TD-EVs on the membrane surface contribute to the development of tumours. Also, the ratio of these nucleic acids to the protein differs significantly between EVs generated from malignant and healthy cells. Since TD-EVs are capable of making diagnoses, it is possible to identify cancer earlier and with more accuracy than was previously possible. These exosomes EVs have lately been used to carry biomarkers for diseases such as pancreatic, breast, prostate, ovarian, and lung cancer	Pancreatic, breast, prostate, ovarian, and lung cancer	[44]
Terahertz spectroscopy and imaging	THz biomedical imaging depends on variations in water content and alterations in tissue structure. Water content; as demonstrated by MRI, frequency-domain photon migration, and positron emission tomography, malignant or due to increased vascularity or tissue oedema, sick tissues may have more interstitial water. As a result, these tissues exhibit different THz absorption than typical tissues	Skin cancer-Basal cell carcinoma	[55]

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Biomedical equipment used	Mechanism of action/Technique	Cancer type	References
Digital infrared thermal imaging	It monitors infrared radiation generated by the surface of the body to record body temperature as tumours often have increased blood supply, angiogenesis, and metabolic rate, which results in greater temperature gradients relative to surrounding normal tissue	Breast cancer	[56]
Ultrawideband (UWB) radar-based system	The monocycle Gaussian pulse may be created and transmitted using a UWB signal-generating circuit. The signal channel that will each turn excite the UWB antenna array can be chosen by the switching (SW) matrix circuits. The received signals can be sampled and digitized using equivalent high-speed sampling circuits. The computer then stores the digitized data for further processing to create the breast picture	Breast cancer	[61–64]
Electronic nose	The e-nose can assess the volatile organic compounds generated by cellular metabolism and present in breath. Through analysis, it is possible to reconstruct a sensory combination equivalent to a fingerprint by identifying the precise chemical components of VOCs and their pattern of expression. This "fingerprint" can be recognized by an electronic nose	Lung cancer	[17, 65]

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Biomedical equipment used	Mechanism of action/Technique	Cancer type	References
Aptamer	After the target cells had been precipitated by the magnetic beads with modified aptamers on them, the dual aptamer-functionalized dye-doped silica nanoparticles would be introduced as signal generators. If target cells were collected after magnetic separation and fluorescent excitation, a fluorescence signal would be visible; otherwise, there wouldn't be any fluorescence	Breast cancer	[70]
Computer-aided detection (CADe) and diagnosis (CADx) system	Medical imaging lesions can be located using CADe. For example, CADx systems define the lesions by identifying benign and malignant tumours. CADx systems take the picture properties and employ a classifier to calculate the malignancy	Lung cancer	[89, 90]
CRISPR-Cas13 system	Unique biochemical characteristics of the RNA-targeting CRISPR-Cas13 system make it a valuable tool for cancer research, therapy, and detection. Without the need for sophisticated equipment, Cas13-based diagnostic approaches enable the early detection and monitoring of cancer indicators from liquid biopsy samples	Colorectal cancer	[93]

(continued)

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Biomedical equipment used	Mechanism of action/Technique	Cancer type	References
Organ-on-a-Chip	The cancer microenvironment can be recreated using "cancer-on-a-chip" technologies, which combine cancer organotypic and microfluidic technology. These chips enable a better pre-clinical evaluation of treatment effectiveness by facilitating a more profound knowledge of cancer behaviour in vivo. These models can further explore the relationships of cancer with other organs by connecting various physiologic modules, such as the vasculature	Liver, lung, and pancreatic cancer	[97, 98]
Circulating tumour cells technology	Technologies for affinity-based CTC enriching either selectively target cancer antigens to collect CTCs (positive enrichment) or selectively target CD45 to reduce hematopoietic cells (i.e., negative enrichment). The only CTC device approved by the FDA as a diagnostic tool for persons with metastasized breast, prostate, and colorectal cancers is the CellSearch® system	Breast, prostate, and colorectal malignancies	[102, 103]
Tumour-derived extracellular vesicles	The genetic component and certain oncogenic proteins carried by TD-EVs on the membrane surface contribute to the progression of tumours. These exosome EVs have lately been used to carry biomarkers for diseases	Pancreatic, breast, prostate, ovarian, as well as lung cancer	[44]

(continued)

(continued)

Biomedical equipment used	Mechanism of action/Technique	Cancer type	References
Bubble with Ultrasound	High-intensity ultrasound has been utilized as a cancer treatment. The characteristics of the bubbles, however, are crucial for the successful treatment of cancer by actively destroying tumour cells. In addition to being utilized as contrasting image tools, the combination of bubbles (micro and nano) with ultrasound is also employed to improve the delivery of drugs	Cancer	[45]
Navigation bronchoscopy	It involves the utilization of technologies to assist the bronchoscopist in precisely navigating the bronchoscope to a location of interest	Lung cancer	[105]
Confocal laser endomicroscopy	It is a non-invasive diagnostic tool that can help with real-time, early identification and potentially lessen the need for invasive surgery	Head and neck cancers	[106]
Nanotechnology-based biomedical equipment	Due to the capacity of quantum dots for penetrating individual cancerous cells, their nanosize, and the fact that they emit light in narrower bands than organic dyes, they can be employed in clinical situations to localize cancerous cells	Cancer	[46]
Computed tomographic colonography	Computed tomographic colonography is the most practicable and technically proficient way to assess the colon close to an occlusive malignancy, which restricts colonoscopic investigation behind the occlusion	Colorectal carcinoma	[110]

(continued)

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Biomedical equipment used	Mechanism of action/Technique	Cancer type	References
Laser Raman spectroscopy	Utilizing the biochemical uniqueness of the Raman scattering effect, it is label-free and not destructive. Both kinds of data are automatically provided by artificial neural networks like stochastic backpropagation for each tissue site that laser Raman spectroscopy analyses	Breast cancer	[111]
Contrast-enhanced ultrasound	By identifying malignant tumours by focusing on a needle biopsy, CEUS enhances the identification of prostate cancer	Prostate cancer	[112]
Internet of Things	The uses and advantages of IoT technologies, particularly wearable gadgets, in controlling adverse effects, symptoms, life quality, and mortality in patients with cancer ongoing therapy	Real-time cancer management	[113]

11.4 Conclusion and Future Prospects

In recent years, due to their benefits over previous diagnostic instruments, various innovative biomedical devices have been the subject of study in the identification and treatment of breast cancer. These tools make use of several technologies, involving aptamers, e-nose, no-wash biosensors, CMOS integrated circuits, digital infrared thermal imaging, terahertz spectroscopy, photonic crystal fibres, computed tomographic colonography, tumour-derived extracellular vesicles, circulating tumour cells technology, and many others. One of these technologies involves no-wash biosensors. For the purpose of improving clinical early cancer diagnosis, it is crucial to develop a straightforward, practical, quick, and sensitive biosensor platform. No-wash biosensors are a useful and exciting sensing platform for diagnosis when compared to heterogeneous biosensors since these techniques just need integrating the target and recognition probes in solution—not separating or washing them. This considerably

shortens the duration of the test, simplifies the analytical procedures, and minimizes human error in operation. With the help of a variety of professionals, including scientists, engineers, and medical researchers, the development of no-wash biosensors for in vitro cancer diagnostics will therefore make significant advancements in the near future. A number of aptamers have recently demonstrated considerable promise for improving the disease's rates of identification and therapy. Although less stable and repeatable than the older SELEX methods, a number of very successful SELEX procedures have lately been developed. The use of biosensors, which largely rely on the interaction between biological and nanomaterial processors, to locate biological elements in the majority of tumours, including cancers like the prostate. Aptasensors have been discovered to be ideal options for building lab-on-chip sensors due to their quick speed, exceptional efficiency, and simple processing. Future research is anticipated to show that these materials, which are based on nanotechnology, are more efficient in aptasensors. The assumption that tumours have a distinct biology from the nearby normal tissue is what allows DITI to identify tumours. One research discovered a connection between thermographic hot areas and the number of breast cancer microvessels, offering a mechanistic basis for using DITI in cancer screening. Simply put, thermal recordings are a physiological measurement. Hence, due to this limitation, DITI must be performed in conjunction with another test like mammography or ultrasound. For instance, breast parenchyma infection or inflammation might change temperature readings and provide false-positive results. Additionally, those who are morbidly obese and have breasts bigger than DD might not be the greatest candidates for DITI since it is unable to accurately record breast temperature from the inferior side (undersurface). Results from DITI should be compared to those from other imaging modalities because it is not currently indicated or authorized to replace screening mammography. Recent research has shown how AI algorithms are being developed and how computational methods are used to predict and detect head and neck malignancies accurately. Artificial intelligence technology significantly expands the possibilities for better OSCC prediction and screening outcomes. For the purpose of identifying pulmonary nodules in chest CT images, an unique CAD system that incorporates the detection and characterization of nodules into a single system has been proposed. The Watershed methodology was used to segment internal lung structures to distinguish potential nodules from other structures. A Support Vector Machine (SVM) and a rule-based classifier have been employed to reduce false positives further. Photonic crystal fibre has proven to have enormous promise in photonic sensing applications. In particular, PCFs have been employed in biological applications for illness diagnosis, treatment, prevention, and detection for improved health. A three layered dual-core PCF sensor was introduced in order to produce a sensitive PCF-based sensor for cancer cell detection. It has a cavity for cancer cells in the cladding region, which is home to a sample of cancer cells. After the phase matching criterion is met, the optical power is transferred from the cancer cell cavity to the silica core. Nanotechnology and clinical cancer research are linked by a new branch of biology dubbed "cancer nanotechnology". Additionally, it attempts to combine developments in nanoscale device production with cellular and molecular elements related to cancer detection and treatment. It is essential to comprehend these

new technologies to integrate these techniques into therapeutic settings. This cutting-edge method has made it easier to combine nanoscale devices with substances like tumour-specific ligands, antibodies, and imaging probes. Robotics is becoming an emerging concept in cancer treatment by merging them with biomedical equipment, such as in navigating the lung's irregular branched shape adds additional complexity; robotics' ability to deliver pinpoint accuracy is an incredibly appealing prospective for intervention bronchoscopists.

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Chapter 12
Detection of Cancer Biomarker by Advanced Biosensor

Stephen Rathinaraj Benjamin and Eli José Miranda Ribeiro Júnior

Contents

Abstract Early diagnosis and prompt therapy are critical for cancer treatment effectiveness. Advanced biosensors are emerging as inexpensive and portable tools for detecting cancer biomarkers. The technique offers a promising alternative to the time-consuming conventional methods. In order to detect an analyte, a device known as a

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biosensor is used. This sensor takes the biological signal DNA, RNA, or protein into an electrical or digital signal. The introduction of different types of materials into biosensors opens up a great deal of potential as the next-generation sensor. In addition to its great flexibility, it has a wide range of applications. This chapter discusses conventional techniques and advances in developing new-generation biosensors for cancer diagnosis. Additionally, this chapter examines the types of biosensors and the potential use of nanomaterials for cancer detection that can used as reliable diagnostic tools.

Abbreviations

AI	Artificial intelligence
BBB	Blood-brain barrier
COC	Cancer on a Chip
CMOS	Complementary metal–oxide–semiconductor
CNTs	Carbon nanotubes
COC	Cancer on a Chip
CRISPR	Clustered, regularly interspaced short palindromic repeats
crRNA	CRISPR RNA
CTs	Computerised tomography
CV	Cyclic voltammetry
DEP	Dielectrophoresis
DNA	Deoxyribonucleic acid
DPV	Differential pulse voltammetry
ELISA	Enzyme-linked immunosorbent assays
EIS	Electrical impedence spectroscopy
FET	Field-effect transistor
GQDs	Graphene quantum dots
HE4	Human epididymis protein 4
IoT	Internet of Things
LOC	Lab on a chip
LOD	Limit of detection
MRIs	Magnetic Resonance Imaging
OOC	Organ on a Chip
PDMS	Polydimethylsiloxane
PEC	Photoelectrochemical cells
PET	Positron Emission Tomography
PMMA	Poly-methyl methacrylate
PNIPAM	Poly N-isopropylacrylamide
POC	Point-of-care testing
reRNA	Reported RNA
RNA	Ribonucleic acid
SERS	Surface-enhanced Raman scattering

SPR	Surface Plasmon resonance
TAMs	Temperature-actuated Mechano sensors
TFBGs	Tilted fiber Bragg gratings

12.1 Introduction

Disease diagnosis has emerged as a specific challenge in the twenty-first century due to growing public awareness of the importance of this field. People are affected by various diseases, including cancers, HIV, fevers, renal failure, heart problems, brain attacks, and many more. The cancer epidemic is rapidly rising to the top of the list of modern health risks. In 2020, around 19.3 million people were newly diagnosed with cancer globally [1]. Since cancer usually doesn't exhibit symptoms at its earliest stages, it takes widespread population screening to identify localized tumors.

It has become imperative that new materials be developed to detect cancer cells. Cancer research has been greatly aided by genomic technologies such as the detection of biomarkers, biofluids, and nucleic acids. Developing novel tools for direct clinical applications that might benefit medication-targeted therapy in the fight against cancer is possible. It also helps with finding biological markers that may be used for the targeted treatment of cancer. Heart disease, renal disease, multiple sclerosis, and many more disorders may all be diagnosed by detecting certain biomarkers, mostly in blood or other body fluids. Biomarkers are becoming more useful in treatment regimens, and they may also be used to differentiate between genotypic and phenotypic features. These biomarkers' potential to aid in early diagnosis and ultimately save patients' lives are significant. Detecting tumor biomarkers in human bodies, including cancer cells, may benefit from novel materials such as zero-dimensional carbon compounds Graphene quantum dots (GQDs), despite various approaches [2, 3]. Early-stage sensitive diagnostic approaches may identify minute quantities of tumor markers in serum samples, increasing survival rates. It is difficult to detect lumps using physical examination, laboratory assays like enzyme-linked immunosorbent assays (ELISA), endoscopy, imaging techniques like X-rays, MRIs, CTs, ultrasounds, and Positron Emission Tomography (PET), and biopsy because the sensitivity of these techniques is low at early stages, and they are laborious and complex. To address these constraints, researchers are developing biosensors using nanomaterials that are smaller, cheaper, more selective, sensitive, and quicker than current methods. Biosensor arrays able to detect protein expression, and DNA alterations may be built for screening and guided therapy thanks to the many possible biomarkers for cancer detection that have been found due to recent developments in molecular biology.

This review comprehensively composes numerous advanced cancer biomarkers found for cancer diagnoses. Furthermore, the limits and potential of state-of-the-art biosensors for cancer diagnostics were examined, focusing on emerging domains such as the Internet of Things (IoT) and machine learning. According to this report, new cancer monitoring and management developments might one

day lead to "alternative-site" examination, bedside screening, emergency department monitoring, and even home self-testing to decentralize clinical testing.

12.1.1 Biosensors: An Evolution

12.1.1.1 Biosensors: An Essential Concept

Biosensors are analytical instruments that use a biomolecule as the recognition element, with selectivity for a certain analyte and some transduction technology to derive and quantify the interaction between the analyte and the detector molecule. Biosensors may be categorized based on the components or immobilization methods they utilize, such as the transducers and bioactive substances they use (Fig. 12.1). The bio element has high selectivity for the analyte of interest. Optical detection biosensors, thermal detection biosensors, resonant biosensors, ion-sensitive, electrochemical biosensors, and field-effect transistor (FET) biosensors are just a few examples of the numerous varieties of biosensors that may be created based on the transducing mechanism used (Fig. 12.2). It is possible to categorize electrochemical biosensors into conductometric, amperometric, and potentiometric subcategories according to the detectable parameter. Accordingly, biosensors can be grouped into subcategories determined by their components, such as nucleic acid, enzyme, cell, tissue, microbial and immunosensors [4]. Biosensors can be categorized into three types based on the recognition elements: catalytic, metabotropic, and bioaffinity.

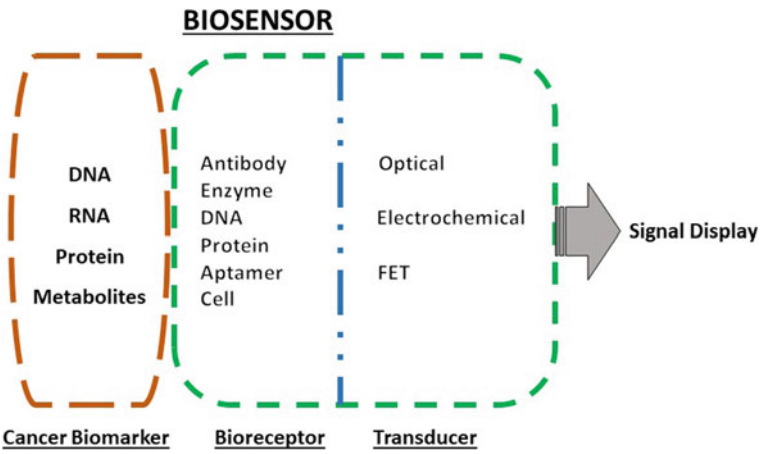

Fig. 12.1 Schematic diagram of cancer biomarker detection by a biosensor

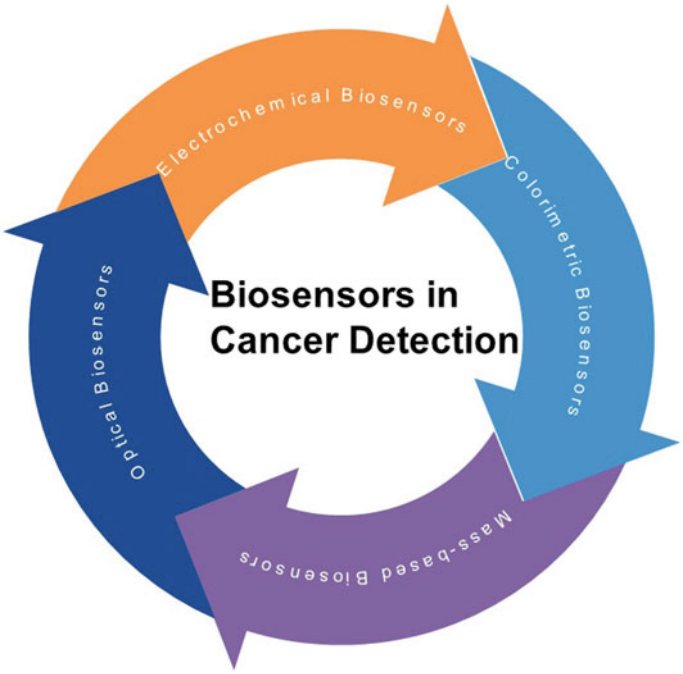

Fig. 12.2 Common biomarkers utilized for cancer detection

12.1.1.2 Electrochemical Immunosensor

Point-of-care testing (POC) is a type of medical testing technology performed aside from a centralized lab, usually at the patient's location. POC testing is described by quantitative or semi-quantitative single measurement systems, a rapid detection time, and analytic instruments that are easy to use. POC testing approaches that use electrochemical sensing have garnered much interest because of their potential for quick analysis, cheap cost, downsizing, and simple control [4]. Electrochemical immunosensors are highly selective for biomarker detection because of the specificity and affinity of antibody-antigen hybridization [5]. However, the labeling required by several of these technologies to provide an analytical signal is a significant time, effort, and money sink. A label-free detection approach does not rely on secondary probes or labels to generate a signal. POC testing with label-free methodology is attractive because it offers simplicity, rapid detection, and reliable results. However, the label-free sensor's limited sensitivity limits its use in clinical settings [6]. Therefore, there is an essential need to design a novel, ultra-sensitive label-free electrochemical immunosensor for use in point-of-care diagnostics.

Label-free immunosensor analysis performance can be enhanced using a substrate with strong conductivity, excellent electrocatalytic signal transduction, and high immobilization of antibodies. Since they meet the criteria mentioned above, recent

developments in nanotechnology have made nanocomposites that imitate enzyme activity (nanozyme) a promising platform for label-free electrochemical immunosensors [7]. Nanozymes made of noble metal alloys can change surface electron states, significantly improving the efficiency of the reaction. Palladium (Pd) and platinum (Pt) alloys have remarkable catalytic activity in various electrochemical processes due to their noble metal composition. Further, by mixing with base metal elements, noble metal alloys may be more active in catalysis, which alters the surface electronic state and interatomic spacing [8]. The demand for biosensing devices that can detect physiological parameters and disease biomarkers, especially cancers with compatible textures and surface characteristics, has been prompted by exploring improved sensing materials, techniques, and device design.

Recently, single-wavelength imaging biosensors have used an innovative data analysis premised on an optimum linear estimation to dynamically recover spectral shift information produced by biomarkers [9]. In breast cancer, exosome-containing extracellular vesicles may be identified in real-time utilizing high-area meta-surface chips arranged using microarrays and paired using microfluidic devices over an optical platform. The optofluidic system's exceptional sensing capability, as measured by its almost 70 1/RIU (figure-of-merit), allows for the average detection of 0.41 nanoparticle/m^2 and the monitoring of the binding of extracellular vesicles to solutions as dilute as 204 femtomolar.

Aside from the chemical biomarker-based cancer diagnosis above, the fact that tumor tissues have distinctively different mechanical characteristics from those of normal tissues may also serve as a foundation for diagnosis [10]. Mok and colleagues [11] created fluorescence-labelled thermo-responsive PNIPAM hydrogel microspheres to evaluate local tissue mechanics at cellular length scales, called microscale temperature-actuated mechano sensors (TAMs). The animals were injected with collagen-functionalized TAMs, distributed inside the tumor that grew following injection of the 4T1 metastatic cancer cell line. The surrounding tumor's remaining elasticity may be mapped by tracking the growth of TAMs. Interestingly, high-rigidity localized regions resulted from cancer cell invasion, opening new possibilities for cancer detection at an earlier stage.

Due to their low number, short duration, and comparable sequences, there is a significant need for an appealing, highly sensitive, label-free technique. In this work, the authors [12] used surface-enhanced Raman (SER) scattering on a substrate of head-flocked gold nanopillars to develop a label-free, ultra-high sensitivity, and selectivity multiplex miRNA assay for the detection of miRNAs linked with cancer. Graphene quantum dots, also known as GQDs, are a potential next-generation carbon material that might be used in various biological contexts, including biosensing with drug administration in the treatment of cancer and other potentially fatal diseases [13]. Due to the identification of the CYFRA 21.1 biomarker in clinical human saliva samples, an innovative portable biosensor for the noninvasive detection of oral cancer was developed on the electrochemical immune platform [14].

Recently, Williams et al. [15] constructed an implanted nanosensor to capture an ovarian cancer biomarker generated locally and transmitted through NIR to an external detector. SWCNTs modulated their inherent NIR emission to detect HE4

(human epididymis protein 4) binding to an immobilized antibody. HE4 was found in the fluids and serum of ovarian cancer patients. After loading nanotube complexes onto a semipermeable membrane, a noninvasive implanted device was developed. In orthotopic mouse ovarian cancer models, sensors evaluated exogenous and endogenous HE4 to distinguish HE4-producing subtypes from biomarker-deficient types.

12.2 Biosensor Sniffs for Cancer, Using Artificial Intelligence

Numerous chemicals in a blood sample are within the detection range of individual nanotube sensors. The system generates an original luminous pattern by integrating the multiple sensor responses. Afterward, the design may be detected by a machine-learning system that has been developed to distinguish between a cancer fingerprint and an average fingerprint [2].

Cancer detection and staging using a chemisensitive nano-based structured film array developed using artificial intelligence [16]. The authors detailed the development of chemical sensor arrays based on nanomaterials that, combined with adaptable machine learning (ML), make for rapid, precise, and user-friendly liquid biopsies of blood samples for the classification and diagnosis of cancer. Nanoarrays with over 84% accuracy, over 81% sensitivity, and over 80% specificity are used in cancer detection methods. In comparison, nanoarrays with over 97% accuracy, 100% sensitivity, and >88% specificity are used to detect metastasis.

Most serum biomarkers lack the sensitivity or specificity necessary for use in cancer screening. Unfortunately, the few available blood biomarkers for ovarian cancer have a high level of specificity but not enough sensitivity to diagnose the disease at an early stage and impact death rates. This research demonstrates that a disease fingerprint derived by carbon nanotubes (CNTs) functionalized with quantum defects emits near-infrared light. Machine learning based on this data may identify high-grade serous ovarian cancer in the blood of symptomatic patients. Current clinical screening tests, including those based on transvaginal ultrasonography and cancer antigen 125, have a sensitivity of 84% and a specificity of 98% [17].

12.2.1 Machine Learning

A biosensor with artificial intelligence (AI) is only valuable if we can make sense of the data it generates, and understanding its meaning is the first step to using it. Learning from data is an important part of AI data processing, and this includes learning how to do accurate analyses, generate valid conclusions, and spot instances of incorrect interpretation. There are two functions of machine learning

that contribute to these objectives. Firstly, using machine learning to reduce the amount of data before wireless transmission, ultra-low-powered AI biosensors may be obtained. The second is concerned with improving data quality by fixing problems like data uniformity, the precision of the monitoring, and the dependability of the data. Machine learning aims to learn from data to identify useful information or modeling techniques for a phenomenon, which encompasses a broad range of approaches, techniques, and assessments of algorithms. There are several variations within the primary concepts of the machine, supervised, and unsupervised learning. Supervised learning involves correlating incoming data with training data that already has labels.

Biomolecular assay development for application in technologies like implantable devices is complicated by the need for conventional molecular recognition components like antibodies. In addition, the production and use of antibodies may be highly time consuming and expensive, particularly for applications requiring multiplexing. Yaari and his colleagues [18] examined a perception-based sensing system for human biofluids. The DNA–SWCNT-based sensing photoluminescent array platform was developed to monitor gynecologic cancer biomarkers such as YKL-40, CA-125, and HE4 in patient fluids and lab samples. According to the classification results from uterine lavage samples, F1-scores for CA-125 and HE4 were 100% and YKL-40 91% for samples collected from cancer patients, respectively.

Oliver et al. [19] provide a case study of integrating cutting-edge live-cell imaging algorithms and AI into COC technology. The utilization of AI, a blood–brain barrier (BBB) within a chip, and 3D-confocal tomography were all used to locate cancer cells that exhibited metastatic brain characteristics. Using their device, the scientists could distinguish between cancer cells in response, intermediate, and a high potential for spreading to the brain. In addition, they could characterize the behaviors of cells taken from cancer patient samples that had established metastatic potential. These findings, combined with AI, allow for the prediction of cancer cell metastatic potential.

12.2.2 CMOS

In biomedical research, CMOS sensors are a crucial equipment component. CMOS sensors are well recognized as a class of electrical devices with applications in image-sensing elements, microprocessors, and storage systems. The CMOS sensors are also suitable for portable clinical diagnostic tools and implantable biomedical systems.

Recent work by Gao and colleagues [20] has resulted in the development a CMOS-compatible SiNW-FET biosensor. This biosensor is capable of real-time, label-free, multiplexed measurement of miRNA and protein, with great selectivity and sensitivity. The SiNW arrays were manufactured with mass reproducibility and cheap cost by integrating them with PDMS chips using an anisotropic wet etching process that included self-stop limiting. With high sensitivity and speed, the biosensor has been shown to detect the lung cancer biomarker miRNA-126 at 0.1 fM and the protein CEA at 1 fg/ml. Using a highly sensitive and reliable CMOS-compatible silicon

nanowire FET sensing device, the cancer biomarker ALCAM may be detected with unprecedented accuracy. The system has a high dynamic detection range (300 fM–30 nM), a quick sensing reaction (30 min), and a LOD (15.5 pg/ml) below important clinical values [21].

Furthermore, Alhoshany et al. [22] described a biosensor-CMOS platform for monitoring biorecognition capacitance. The biosensor can detect and measure a protein present in premalignant tissues. Capacitive sensors have been used for the first time to screen and evaluate spermidine/spermine N1 acetyltransferase-SSAT. However, the baseline capacitance of the biosensor may be lowered by interconnecting a number of series capacitors to maintain a constant analyte concentration. Large sensor areas with low baseline capacitance are used to detect SSAT enzyme sensitivity. Capacitance readings are converted to the digital format using a 0.18 m CMOS 12-bit capacitance-to-digital converter.

SSAT enzyme concentrations are detectable throughout a capacitance range of 16.137 pF with 4.5 fF absolute resolution. As pilot research, the concentrations were chosen, and the capacitive sensor platform showed great sensitivity for SSAT enzymes.

Organ on a chip (OOC) is a novel model system for biomedical study and drug discovery because it can recreate human organs' functional and structural structures in vitro. In oncology research, OOCs are becoming increasingly powerful tools. Cancer on a chip (COC) can reproduce biologically relevant conditions, including dynamic cell–cell, niche factors, cell–matrix interconnections, biochemical gradients, and sophisticated tumor and stromal tissue patterns. Researchers have recently employed osteoblastic cells from mature bone-on-a-chip to investigate bone colonization by metastatic breast cancer cells [23].

Recently, methods depending on clustered, regularly interspaced short palindromic repeats (CRISPR) have become increasingly effective in detecting nucleic acids. Serum samples from patients with brain tumors were analyzed using an electrochemical biosensor and microfluidic chips containing the labelled reported RNA (reRNA), enzyme Cas13a, and target-specific CRISPR RNA (crRNA). The target miRNA in the blood sample triggered "collateral activity" involving the reRNA collateral cleavage and Cas13a/ crRNA complex. Electrochemical biosensors acquired on the shift in reRNA, and the amount of miRNA present in the blood sample correlated negatively with the resulting signal strength. To emphasize, this approach requires less than 0.6 L of sample volume and has a detection time of less than 4 h. Overall, POC testing and individualized treatment benefit from the innovative notion of combining the microfluidic device and the CRISPR technology [24].

Incorporating microfluidics into biosensing allows for enhanced signal-to-noise ratio, multiplex screening, and simultaneous isolation of target biomolecules for identification within the flow, among other advantages. Despite this, antibodies bound to the sensing platform are limited by the shear effects of the microfluidic flow. By employing gold-interdigitated electrodes to detect CA-125 antigens in a biofluid, the work revealed sensitivity variation owing to microfluidic flow [25]. Finally, experimental results showing the capacitance variation during CA-125 antigen–antibody

transduction at various CA-125 antigen concentrations exemplify the employment of gold nanoparticles to increase sensor signals sensitivity.

12.2.3 Lab on a Chip (LOC)

In the recent two decades, LOC systems have received extensive scientific and industrial interest in biomedical applications because of advantages in biological sample processing, high throughput, minimal reagents and samples consumption, rapid analysis time, and multiplexed detection. Particularly, LOC technology has shown potential for increasing the detection of molecular biomarkers by enabling robust and comprehensive readings in a compact device. The application of LOC technology for studying cell-based disease biomarkers and organ-on-chip designs are a cutting-edge field of study. Biomarker testing at the POC may benefit from the recent development of MiSens, a revolutionary integrated and fully autonomous LOC-centered biosensor device. For example, Ulutag et al. [26] performed an electrochemical biosensor integrated into a lab-on-a-chip to quick and sensitive detect the cancer biomarker PSA and obtained a 0.2 ng/mL detection limit. Fang et al. [27] developed microfluidic technology for on-chip immunocapture of exosomes from breast cancer patient cell cultures and human plasma. Immunohistochemical labelling showed that the amounts of HER2 expression in exosomes were equivalent to tumor tissues.

12.2.4 Chip-Based Optical Sensor

This innovative chip-based sensor detects target molecules using light from a microdisc laser embedded in the semiconductor. The laser light changes color (or frequency) as it comes into contact with the biomarker of interest. The researchers included a laser that could perform in liquid environments to analyze urine samples for the presence of toxic substances. Aluminum oxide, doped with ytterbium ions, can be fabricated using lasers emitting in wavelengths outside the range of light absorption in water, which enables precision detection of the biomarker.

Compared with similar sensors, the microdisc laser, instead of ring resonators, allows for unprecedented sensitivity. The sensitivity is due to the narrow lasing linewidth compared to passive ring resonances. Once other interferences, including thermal noise, are addressed, this approach may detect minor frequency changes from biomarkers at low deficient concentrations. Recently, Goede et al. [28] demonstrated biosensing using a microdisc laser incorporated into the system. Due to its low optical losses and emission range of 1020–1050 nm, beyond the range of water absorption. Al_2O_3doped with Yb^{3+} was using a microdisc cavity immersed in water, and a single-mode laser emitting at 1024 nm and 250 kHz was achieved. The ability to detect 300 pM of rhS100A4 in urine illustrates the devices' biosensing capability.

12.2.5 FET

A field-effect transistor (FET)-based sensors have been the focus of most research and development in recent years. These characteristics include low power consumption, accuracy, cheap cost owing to proven chip fabrication technologies, and label-free and simple surface customization. The development of cutting-edge manufacturing techniques has led to bio-FETs, which combine a bio-recognition layer with a transducer [29]. Mandal et al. [30] found that a CD-based microfluidic system can detect PSA levels below 4 ng/mL in serum samples utilizing dielectrophoresis (DEP) and graphene FETs. In order to detect the ovarian cancer antigen (CA125) without using a label, researchers [31] have developed a versatile and ultra-sensitive aptasensor based on MWCNTs/rGO-FET. The sensor is constructed by combining poly-methyl methacrylate (PMMA) as a flexible FET substrate with MWCNTs or aptamers on rGO nanosheets. The proposed aptasensor showed a linear range in human blood samples in the concentration of 1.0 10^9–1.0 U/mL and a LOD of 5.0 $\times$ 10^{10} U/mL.

12.2.6 Optical Fiber Biosensors

Optical fiber biosensors are a fantastic option for quick and affordable diagnostics since they don't need electrical connections and aren't impacted by electric interference like electrochemical biosensors. Optical fiber biosensors have several benefits due to their design, such as being inexpensive to manufacture, tiny in size, resistant to electromagnetic interference, and lightweight. Multiplexed assays employing optical fiber biosensors may detect multiple substances simultaneously. Optical fibers, housed in an appropriately designed medical device, enable in-place, real-time sensing. The optical fiber biosensors' limit of detection (LOD), detection speeds, and high selectivity are also highly appreciated. Surface plasmon resonance (SPR) devices, optical fiber sensors, and interferometers based on gratings are the three types of optical fiber platforms used most often in biosensor applications.

Protein detection can be done with great sensitivity using EIS, DPV, or PEC; however, optical fiber sensors appear to be a more practical platform for use in actual clinical settings. Electrochemical sensors/biosensors may be used for in vivo measurement of electroactive neurochemicals; however, this is often done in neurological fluids/tissues. Electrochemical sensors may be affected by electroactive interference from substances like uric acid, ascorbic acid, and certain drugs in blood samples. On the other hand, optical fiber sensors have great potential since they are both electrically safe and compact. In addition, because of these characteristics, they are suitable for usage in vivo, a setting in which the presence of an electric current poses a danger. Optical fiber as a biosensing platform has several potential advantages, including its cheap cost, chemical and electromagnetic nontoxicity, and the availability of a wide range of surface reconfiguration technologies. Additionally, optical fibers may be shrunk and multiplexed to detect several targets simultaneously.

The CD44 target molecule and two control proteins, such as thrombin and IL-4, were detected using circular optical fiber tips. In varying amounts of CD44 protein, the fully operational sensor records a shift in spectral characteristics the higher concentration of protein results in a greater amplitude. After examining the sensor's spectrum response between 1537 and 1539 nm, it was determined that a feasible LOD of 17 pM. The sensitivity was found to be 1.23 decibels for every tenfold rise in a concentration lower than 0.1 nM [32]. Telecommunications-grade single and multimode optical fibers are used to fabricate Tilted fiber Bragg gratings (TFBGs) for applications like refractometry and biosensing. These fibers are compared and contrasted in terms of their respective performances. Aptamers coupled with TFBGs detect breast cancer indicators like HER2 [33].

12.2.7 Aptasensors

The development of cancer biosensors that use aptamers, synthetic RNA and DNA bio-recognizers with high binding specificity has received a lot of attention. The advantages of aptamers include their relatively low non-specific detecting abilities, high repeatability of manufacture, and versatility of modification (such as by chemical changes or by adding optical tags). Compared to other types of aptasensors, electrochemical aptasensors have several advantages, making them a good choice for POC tests.

Aptasensors, also known as electrochemical biosensors based on aptamers, are an excellent option for use in point-of-care settings. C-MEMS platforms, which stand for carbon microelectromechanical systems, have a low background noise level, a strong capacitance, great stability while exposed to various physical and chemical treatments, biocompatibility, and significant electrical conductivity. In this study, the authors developed label-free platelet-derived growth factor-BB aptasensors by combining bipolar exfoliated (BPE) rGO with 3D C-MEMS microelectrodes. Reduced graphene oxide (rGO) was prepared, reduced, and deposited on 3D C-MEMS microelectrodes using the bipolar electrochemical properties of graphite in deionized water. Electrochemical bipolar exfoliation of rGO removes the disadvantages of the approaches presently employed for the manufacturing and deposition of rGO. These drawbacks include lengthy and costly processes, extensive utilization of toxic chemicals, and challenging deposition methods. Because of the covalent attachment of the amino-tag-ended aptamers to the surfaces of the rGO molecules, the PDGF-BB affinity aptamers were successfully immobilized in the molecules. For turn-off sensing, the areal capacitance was measured using CV plots. A vast linear range (1 pM–10 nM), high sensitivity (3.09 mF cm^{-2} $Logc^{-1}$), and a low detection limit are all features that the aptasensor has (0.75 pM) [34].

A label-free detection technique can be used to measure electrochemical parameters without labels. Eliminating any foreign molecules (such as fluorescent tags, chemiluminescent molecules, or nanoparticles) which are temporally or chemically bonded to a target to perform label-free detection is a technique that may be used to

determine whether or not a target is present or active. Many recent papers have detailed label-free electrochemical aptasensors for sensing PDGF-BB, and their sensitivity has been recognized. In this example, amperometric aptasensors were developed by Jiang et al. [35] utilizing hydroxyapatite (HAP) nanoparticles were modified on a glassy carbon electrode. The amperometric measurement was linear from 0.1 pg mL^{-1} to 10 ng mL^{-1} of PDGF-BB level. Zhang et al. [36] produced a graphene-based sensing probe supplemented with silver nanoclusters. This sensing probe demonstrated a linear response to PDGF-BB across 32.3 fM to 16.61 pM.

Further, PDGF-BB detection was achieved with a label-free C-MEMS aptasensor. The methods used to measure resistance showed the highest sensitivity of 1.65×10^3 Ω $Logc^{-1}$, a detection limit of 1.9 pM, and a linear range of 0.005–50 nM. Both detection methods demonstrated high PDGF-BB selectivity and stability after 10 days, reaching 90.34% [37].

For example, Zhang et al. [38] developed a label-free electrochemical detector that detects CD44 by interacting with ligands and proteins. This sensor was employed in their experiment. The authors detected electrochemical signals of CD44 in cancer cells and human blood samples with a linear behavior from 0.01 to 100 ng/mL and a LOD of 5.94 pg/mL. In addition, the sensor displayed great selectivity, repeatability, and long-term stability for 14 days, with a relative standard deviation of 2.57%. This research would create new kinds of sensors by analyzing the interactions between ligands and proteins, as well as the design of interfaces that would enable efficient detection in a variety of biosystems.

Since its inception, point-of-care technology (POCT) has continuously shown its capacity to reduce medical costs while improving patient care. The lateral flow assay is a typical point-of-care technique that needs to be improved to increase sensitivity, improve cost efficiency, and enhance quantification. Aptamers and nanozymes were recently used for the first time combined to develop an aptamer–nanozyme lateral flow test (ALFA). In this study, a CA125-specific aptamer was used as a capturing reagent, and peroxidase-mimicking gold nanoparticles were used as a label to detect CA125 in blood via competitive ALFA. The ALFA test's precision, recovery, and relationship with CA125 chemiluminescent ELISA were validated and shown to be statistically significant ($P < 0.0001$) with a specificity of 5.21 U/mL [39].

12.3 Protein Biomarkers for Cancer Analysis Using PEC

PEC (photoelectrochemical) bioanalysis represents a unique, dynamically developing method for detecting biomolecules sensitively. The term "protein biomarker" refers to biomolecules that can be used to diagnose certain diseases from their presence or level of expression. The PEC bioanalysis approach combines the advantages of simple, cost-effective apparatus with the ability to separate and detect the signals in different forms. It is an advanced form of electrochemical bioanalysis. Currently, PEC bioanalysis is increasingly applied to cancer and heart disease biomarkers. The

PEC bioanalysis method has recently been increasingly used to detect cancer protein biomarkers in cancer patients.

The vascular endothelial growth factor 165 ($VEGF_{165}$) stimulates the formation of new blood vessels during angiogenesis [40]. Furthermore, numerous cancers, including lymphoma and breast and lung cancer, showed $VEGF_{165}$ overexpression [41]. Accordingly, it is among the most crucial indicators for cancer detection.

The photoactive substance g-C_3N_4 and an aptamer-bridged DNA structure of the network were used to develop a PEC VEGF165 sensor. [42]. The poor PEC effect of g-C_3N_4 and the strong background signals of the sensor hampered the sensor's limit of detection and sensitivity. The single photoactive $AgVO_3$ was used in the PEC aptasensor to produce a near-zero background signal, and exonuclease III was used to amplify the VEGF165 signal for sensitive detection [43]. This sensor is a major improvement over the g-C_3N_4 PEC sensor previously used since its linearity is from 10 fM to 10 nM and LOD of 3 fM. For instance, Zhang et al. [44] constructed a PEC biosensing platform employing multi-signal amplification that was designed to detect VEGF165 ultra sensitively by using tin sulfide-oxygen vacancy Tungsten trioxide nanorods (SnS_2|OV-WO_3 NRs) as the photoactive platform and CdS QDs/TCPP (tetra(4-carboxyphenyl)-porphine) nanocomposites as the sensitizer. The system detected VEGF165 in diluted human serum with a linear response from 0.5 fM to 10 nM and a LOD of 0.34 fM. Consequently, PEC signals were minimized, and the co-sensitization cascade structure was destroyed.

PEC bioanalysis retains excellent sensitivity, which is one of the key reasons for its promise and acceptability, easy equipment, label-free responses, and other benefits of the electrochemical approach [45]. Since many different semiconductor materials may be used as PEC transducing components, it's an easy to design sensor platforms with varying levels of efficiency [46]. The development of a simplified biosensing technology is vital since plasma-free folate binding protein (FBP) is a potentially valuable potential biomarker. Based on these results, an FBP detection for PEC biosensor was proposed, with anti-fouling interface design and specific ligand–protein recognition as key factors. In order to develop the PEC sensing platform, a biomimetic polydopamine (PDA) covering was applied on arrays of TiO_2 nanotubes (NTAs). The macroporous structures resulted in a notable improvement in PEC.

By integrating amino-group-terminated 8-arm poly(ethylene glycol), a remarkable improvement in anti-fouling capability was accomplished. Anti-fouling properties are maintained, and recognition qualities toward FBP are shown, as predicted when folic acid (FA) is included. Good analytical performance may be shown in the built PEC biosensor. There is a possibility that FBP biosensors might be improved by combining ligand proteins recognizing with a PEC anti-fouling interface [47]. The anti-fouling surface for the PEC biosensor was built by the same team of researchers that developed the HA–CD44 interaction to detect soluble CD44 [48]. However, [49] PEC biosensor for CD44 protein detection was developed using the photoactive hybrid composite. The developed PEC biosensor has a detection sensitivity of 1.4×10^2 pg/mL and a working range of 2.2×10^4 ng/mL to 3.2 ng/ mL of CD44.

Recently, Dai et al. [50] developed a potentiometric targeted photoelectrochemical approach as a novel and sensitive sensor for measuring and identifying two

prostate cancer biomarkers. The researchers used a dual disk electrode modified with polyamidoamine dendrimers with cube anatase, titanium dioxide, and mesocrystals to develop a highly sensitive PEC sensor system for human interleukin-6 and PSA. Furthermore, using self-assembled techniques, two light sensitizers were deposited on the surface of the electrode: chitosan (CS) functionalized silver iodide and chitosan-AgI labelled antibodies. The conjunction of g-C_3N_4 at the anode and CS-AgI at the cathode increased the sensor's sensitivity.

12.3.1 A Smartphone-Based Colorimetry Biosensor

Smartphones are a suitable platform for point-of-care diagnostics, notably in contexts where resources are restricted because of their extensive distribution, integrated sensors, and connectivity capabilities. Rapid and accurate disease detection is crucial for treatment planning and lowering disease burden. Colorimetric sensor systems based on smartphones are well suited since they may be used to create medical monitoring and diagnostic systems outside the conventional laboratory [51].

Prostate-specific antigen (PSA) is a commonly utilized marker in detecting prostate cancer. According to Tang et al., photothermal imaging was designed to improve PSA detection accuracy by overcoming background color limitations and autofluorescence. Rolling circle amplification (RCA) was used to amplify the signal to capitalize on the rise in temperature brought on by the CuxS nanocomposites' (CuxS NCs) unique inherent absorption in the near-infrared (NIR) region. This method has two advantages: (a) It gives researchers and patients a wider range of options when diagnosing a disease by allowing them to use less expensive and more versatile testing equipment, and (b) it ensures high sensitivity and reproducibility of the results by using CuxS NCs, which have excellent photothermal conversion efficiency and long-term stability [52]. For instance, Mao et al. [53] presented a quick, rapid, and sensitive colorimetric technique for measuring APN activity, dependent on the ternary structures formed by the host–guest interactions of CB and peptide-functionalized AuNPs (pep-AuNPs). The linear range for APN activity detection is 5–15 ng/mL and a LOD of 0.42 g/mL.

Recently, several developed latent electrochemical interaction probes are capable of very sensitive detection of analytes in turbid solutions, with no interference from additional electroactive biological molecules, and at extremely low serial dilution. The ability of this approach to immediately transform the interaction of analyte probe to electrical signals without the need for any extra device or reduction in sensitivity or selectivity enables it to take full use of its potential for application in small devices, which is one of the many significant advantages of this method in comparison with more conventional analytical methods. In recent years, enormous improvements have been achieved in constructing electrochemical sensors. These sensors have found widespread use in applications related to point-of-care diagnostics.

Aminopeptidase N (APN/CD13) is a candidate biomarker for monitoring cancer recurrence and prognosis after therapy. Earlier this year, Kumaravel and his

colleagues synthesized an electrochemical substrate that targets APN to detect its activity in biosamples in real time. Due to its great sensitivity, the alanine-benzyl alkylated ferrocene carbamate-Ala-AFC probe displayed a detection range of 0.05 to 110 ng/mL and a LOD of 23.18 pg/mL. It was established that APN was the only electrochemical species contributing to the signals and that no other biological species included any electroactive components. In addition, the Ala-AFC probe was used to track and evaluate APN activity in whole blood, tumor cells, and urine in real time [54].

Non-specific adsorption is a critical problem that has to be addressed for electrochemical biosensors that work in highly complicated biological environments. Anti-fouling coatings made of various materials have been included in the sensor surfaces to combat non-specific adsorption. Most biosensors with anti-fouling chemicals and detecting devices interacting at the sensing interfaces reduce either the sensing performance or the anti-fouling capabilities of the biosensor. In addition, Song and colleagues [55], utilized a peptide with anchoring, anti-fouling, and recognition properties, and a simple and dependable anti-fouling biosensor was developed. The proposed peptide features an amine-rich terminal that might bond to a layer of PEDOT-citrate placed on a glass carbon electrode. The other end of the peptide has the possibility of binding to aminopeptidase N (APN) as well as hepatocellular carcinoma cells (HepG2 cells). The anti-fouling properties of the peptide are located in its middle and anchoring regions. This synthetic multifunctional peptide enables the quick and easy construction of highly sensitive and low-fouling biosensing for evaluating HepG2 cells and target APN in a complex biological medium, with LOD of 0.4 ngmL^{-1} and 20 cellsmL^{-1}, respectively (Table 12.1.).

12.3.2 Microfluidic Impedance Biosensors

Since impedance sensors monitor electrical impedance changes, they are sensitive enough to detect even trace amounts of chemicals on working electrodes. This feature has driven exploration into impedance biosensors that can detect data (such as concentrations) from limited sample amounts of targeted biomaterials. These biosensors can detect the information in small amounts of samples. In particular, many studies have looked at the feasibility of merging microfluidic devices with impedance biosensors to reduce the number of samples and the footprint of the sensing system.

Recently, a label-free, microfluidic immunosensor capable of detecting epidermal growth factor receptor 2 (ErbB2) protein at a femtomolar level and with high selectivity has been developed. The sensor was tested using differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) to detect proteins associated with breast cancer ErbB2, an antigen widely expressed in breast cancer cells, can be detected at low levels as 1.0fM and as higher as 0.1 M thanks to the device's GF-nTiO_2 matrix's excellent electrochemical properties [67].

Table 12.1 Analytical characteristics of colorimetric sensors for detection of cancer markers

Type of sensor	Receptors	Marker	Interaction media	Detection limit	References
Immunosensor	Ab/Au-AgNPs	CA 125	3D nitrocellulose membrane	30 U/mL	[56]
ELISA	Ab/ALP/AgNPs	CA 125	Solution	1.75 U/mL	[57]
Aptasensor	Au@DTNB@Ag-cDNA	CA 15-3	Solution	0.1 U/mL	[58]
Aptasensor	Ap/hemin/TMB	CA 15-3	Solution	35 pM	[59]
Immunosensor	Ab/ Cu_2O/Pt NPs/TMB	CEA	Solution	0.026 ng/mL	[60]
ELISA	Ab/HRP/TMB	CEA	Microfluidic Paper	0.015 ng/mL	[61]
ELISA	Ab/ALP/ ZnCP/ $[(Phen)_3Fe]^{2+}$	CEA	Solution	21.1 pg/mL	[62]
Immunosensor	Ab-MoS_2-AuNPs/$NaBH_4$/4-NP	CEA	Solution	0.5 pg/mL	[63]
ELISA	Ab/HRP/TMB	CEA	Distance-based paper	2.0 ng/mL	[64]
Immunosensor	Ab/MGNR/MO	CA 15-3 and CA 19.9	Solution	5.2×10^{-6} U/mL (CA 15–3) 3.5×10^{-5} U/mL (CA 19.9)	[65]
Non-specific optoelectronic tongue	Bi-functionalized AuNPs	CA 125, CA 15-3, CEA, CA 19.9	Origami-based paper	5.3 U/mL (CA 125) 13.4 U/mL (CA 15–3) 4.6 U/mL (CEA) 5.7 U/mL (CA 19.9)	[66]

*ELISA*Enzyme-linked immunosorbent assay, *Ab* Antibody, *Ap* Aptamer, *ALP* Alkaline phosphatase, *AgNP* Silver nanoparticles, *DTNB* 5,5′-dithiobis-(2-nitrobenzoic acid), *TMB* 3, 3′, 5, 5′ tetramethylbenzidine, *HRP* Horseradish peroxidase, *ZnCP* Zinc(II)-based coordination polymer, *4-NP* 4-nitrophenol, *MO* Methyl orange, *MGNR* Magnetic iron oxide coated gold nanorods

12.3.3 Electrochemiluminescence

Electrochemiluminescence, often known as ECL, is a method that generates light through the process of electrolysis. It's a rapid, sensitive, and practical tool for testing nanomaterial surfaces. It is common knowledge that the abundance of surface states on the surfaces of nanomaterials is directly related to certain advantageous properties of nanomaterials. These properties include photocatalytic, electrocatalytic activities, and a peroxidase-like function (many dandling bonds at the surfaces). Therefore, it is crucial to study ECL capabilities for nanomaterials with surface states to screen new nanomaterials for outstanding catalysts and identify novel ECL luminophores for application in chemical sensing.

Most recently, applications of electrochemiluminescence in immunoassays using graphene- and fullerene-like nanostructures of glassy carbon microspheres [68]. In this study, oxidized glassy carbon microspheres were examined for their potential use as a novel ECL material in analytical chemistry by developing an immunosensor for the PSA, which is a model for a cancer biomarker. PSA log concentrations from 5 to 1000 ng/mL are linearly proportional to the strength of the reduced ECL, which can be detected with a LOD of 0.27 ng/mL ($S/N = 3$). Nanomaterial-based immunoassays have been developed; however, traditional methods need a lengthy and complicated nanoparticle manufacturing and conjugation technique, restricting their use. Here, a new immunoassay strategy is proposed that uses biosynthesized nanoparticles with different functions to make it easy and quick to find cancer biomarkers. The biosynthesized quantum dots (BQDs) used in this approach allow for easy antibody attachment, electrode modification, and superior electrochemical and electro-chemiluminescent responses. The BQD-modified electrode's differential pulse voltammetric, (DPV: 1 pg/mL–10 ng/mL; LOD: 0.98 pg/mL),faradaic impedance spectroscopy (EIS: 10 pg/ mL–5 ng/mL; LOD: 3.82 pg/ mL), and electro chemiluminescent (ECL: 10 pg/mL–10 ng/ mL; LOD: 6.86 pg/mL) research shows good specificity and linear detection range for human PSA detection limits are in the picomolar ranges [69]. In 2021, the Zhang group discovered the ECL response of the fabricated biosensor increased proportionally when the concentration of miRNA-21 was increased from 100 aM to 1 nM, demonstrating the LOD value (46 aM) [70]. More recently, Zhang et al. [70] reported ECL biosensor for microRNA-141 detection employing a novel luminescent nanostructured coordination polymer. Furthermore, to investigate the ECL sensor's applicability in cancer cells, Hela and MCF-7 cervical and breast cancer cell extracts were used in an ECL experiment with 10–10^5 cells. The polymer composite developed an ECL biosensor for microRNA-21 detection with longer linear ranges (100 aM–1 nM) and LOD (46 aM).

12.4 Selected Cancer Biomarkers

12.4.1 CD44

The most common malignancies strongly express CD44, an adhesion molecule to the cell matrix, on their cell surfaces. Cancer biomarker CD44 plays a crucial part in cancer invasion and metastasis. As such, CD44 identification and quantification may provide crucial data for the clinical diagnosis of cancer [71]. Label-free tumor cell detection via PEC biosensing interfaces was developed based on ligand–protein receptor interaction. PEC biosensing interfaces have good anti-fouling properties [72]. For the purpose of detecting MDA-MB-231(CD44+), a brand new label-free PEC biosensor mainly based on TiO2 NTs has been developed. This biosensor makes use of the interaction that occurs between the ligand with the protein receptor. The sensor showed a linear range of 0.020–1000 ng/mL and a LOD of 0.015 ng/mL. Table 12.2 indicates the different types of sensors that are described in the recent research.

12.4.2 CA 125

It is commonly accepted that cancer antigen 125 (CA125) is the "gold standard" oncomarker for detecting ovarian cancer. Several months before the onset of symptoms or indications of illness, it is used to track a patient's response to treatment, progression of the disease, and recurrence of ovarian cancer [82]. Recent studies have demonstrated that the electrochemical immunosensor for CA125 oncomarker detection can be developed using gold nanoparticles in chitosan on carbon nanotubes with lactate oxidase as a label to the system. By chronoamperometry (CHA), the newly designed immunosensor exhibited two linear values ranging from 0.01 to 0.5 U/mL and from 0.5 to 100 U/mL, respectively.

In contrast to ELISA, the immunosensor demonstrated outstanding selectivity, repeatability, and stability for detecting CA125 in human serum samples [83].

Recent advances in nanomaterials have improved electrochemical immunosensor sensitivity. These improved nanoscaled materials provide a greater surface for Ab and signal probe functionalization [84]. Additionally, the electrode conductivity was improved, and antibody immobilization was boosted by utilizing polyamidoamine/gold nanoparticles (PAMAM/AuNPs). The electrodes' conductivity and specific surface area were improved; however, incorporating nanotubes reduced graphene oxide on the electrodes. The linear range of the immunosensor is rather wide (0.0005–75 U/mL), and it has a limit of detection at 6 U/mL.

A nanocomposite of P(CTAB-CS)-Ag NPs was mixed on the surface of the modified electrode to increase its effective surface area, facilitating the immobilization of the Horseradish peroxidase-labelled CA 125 antibody. Additionally, CA 125 was quantified by SWV and DPV in the linearity range of 0.001–400 U/mL with a LOD

Table 12.2 Different types of sensors for the detection of CD44 cancer markers

Sr.	Type of sensor, method	Material	Linear	LOD	References
1	Label-free electrochemical, DPV	HA-PDDA-CNTs	0.01–100 ng/mL	5.94 pg/mL	[38]
2	Photoelectrochemical Biosensor	Poly(ethylene glycol)/Hyaluronic Acid (HA)	0.005–500 ng/mL	0.44 pg/mL	[48]
3	EC immunosensor, GCE,DPV and EIS	GO, IL and AuNPS	5.0 fg mL^{-1}–50.0 μg mL^{-1}	2.0 and 1.90 fg mL^{-1}	[73]
4	Photoelectrochemical, ITO electrode, Photocurrent	L-Cysteine modified Ag-$ZnIn_2S_4$ QDs	2×10^2–4.5×10^6 cells/mL	15 cells/mL	[74]
5	Label-free aptamer EIS	Gold –Aptamer	0.1–1000.0 ng/mL	0.087 ng/mL	[75]
6	Electrochemical LSV	Diphenylalanine-AuNPs	0.01 ng/mL–100 ng/mL	2.17 pg/mL	[76]
7	Electrochemical sensor, DPV	Fe_3O_4@SiO_2@HA core–shell particles	1 ng/mL–10 μg/mL	~0.6 ng/mL	[77]
8	Label-free electrochemiluminescence	PEX-14	0.002–250 ng mL^{-1}	0.001 ng mL^{-1}	[78]
9	Label-free electro immunosensor	PdPtCu@BP	100 pg/mL–100 ng/mL	32 pg/mL	[79]
10	Optical biosensor	Silanized ball resonator	4.6 ag/mL–2300 ng/mL	107 ag/mL	[80]
11	Quartz crystal microbalance	HA, polydopamine and polyethyleneimine composite	1×10^3–5.0×10^5 cellsmL^{-1}-M231 5×10^3–4×10^5 cells mL^{-1}-MCF-7	300 cells mL^{-1}-M231 1,000 cells mL^{-1}- MCF-7	[81]

HA-PDDA-CNTs Hyaluronic acid-poly(diallyldimethylammoniumchloride)-carbon nanotubes, *PEG* poly(ethylene glycol), *PDA* polydopamine

of 0.001 U/mL [85]. In addition, the reported gold nanostructure sandwich format immunosensor might be utilized to detect elevated CA125 levels in blood samples, indicative of ovarian cancer [86]. The optimized biosensor displayed a linear response from 20 to 100 U/mL and a low detection limit of 3.4 U/mL.

12.5 Future Directions

Results have demonstrated that these biosensors can detect a wide range of compounds at low concentrations, including biomarkers used in early cancer detection, thanks to the development of many strategies to strengthen their sensitivity. Nanomaterial-based biosensor design strategies have enabled increased signal transduction through a synergistic effect between catalytic properties, conductivity, and biocompatibility, leading to signal amplification over several orders. The importance of these biomarkers for cancer diagnosis cannot be overstated. Concurrently, a method for the simultaneous detection of numerous cancer indicators has been created as a strategy. However, numerous obstacles must be conquered before an accurate cancer diagnosis may be made. First, the described biomarkers have limits for early cancer detection because of the great complexity of cancer cells. Genomic and proteomic profiling and the generation of very sensitive detection technologies like biosensors are still being used to identify new, highly specific biomarkers for diseases like cancer. Cancer biomarkers are present in low concentrations; hence, biosensors with excellent specificity, sensitivity, and stability are needed for early diagnosis. In order to increase the biosensors' sensitivity, multifunctional nanomaterials like zero-dimensional GQDs [3] are used in their construction. Additionally, covalent bonding with biomolecules that identify cancer biomarkers or tumor cells provides sophisticated sensing substrates for POC cancer diagnosis and therapy monitoring.

In addition, multiplexed analytic approaches that simultaneously identify several biomarkers or dual-mode response detection may also considerably improve cancer diagnosis. Final considerations for the clinical use of biosensors should center on their stability in real samples. Reliable detection of cancer biomarkers in whole blood requires increased portability, selectivity, stability, and reduced battery usage. As a result, understanding how to improve the anti-disturbance feature in biosensors is crucial, as it presents a fresh opportunity to create biosensors for reliable clinical cancer detection.

The future of biosensors in cancer diagnosis is to build a device that permits the multi-analysis of established indicators in biological samples. As a result, conventional methods are unlikely to be completely replaced with such devices. Along with the progress made in the design of multiplex biosensors, which are capable of providing a more accurate assessment of cancer biomarkers, there has also been progressing made in the miniaturization of biosensor technology, which makes it simpler to transport the devices. Wearable devices might expand the possibility of testing for analytes near patients. Eventually, wearable gadgets are the present and

future of health monitoring technology, and implantable biosensors are the next logical step. Therefore, coupled advancements in cutting-edge Internet of Things technology of biosensors have the potential to revolutionize cancer care and treatment and lower the worldwide mortality rate.

12.6 Conclusion

This review summarized recent studies that used advanced biosensor devices to identify cancer biomarkers. Despite their sensitivity and precision, traditional procedures are costly, complicated, and time consuming and need sophisticated instruments and skilled personnel. Researchers are studying innovative nanomaterial-based methodologies to address conventional methods' limitations. Scientists are especially interested in biosensors because of their many benefits to the research community, including their high sensitivity, quick operation, low cost, and simple design. This research demonstrates that sophisticated biosensors have the potential to revolutionize cancer biomarker diagnostics and accelerate treatment for patients. In addition, cutting-edge biosensors have the potential to be a trustworthy alternative to traditional approaches in the identification of cancer biomarkers.

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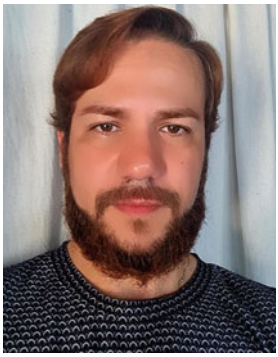

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Chapter 13
Advancement of Nanocarrier-Based Engineering for Specific Drug Delivery for Cancer Therapy

Pankaj Sharma, Vinay Jain, and Mukul Tailang

Contents

Abstract Cancer has progressed to distant organs and is becoming increasingly resistant to chemotherapy; advanced carcinoma is still thought to be an incurable condition. Even though significant therapeutic advances and more efficacious medicines have been made in recent years, long-term incidence rates and unwanted side effects remain the most significant downsides of current clinical procedures. Furthermore, because the majority of chemotherapy medications are hydrophobic,

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they must be diluted in organic solvents that are poisonous, in order to get an appropriate therapeutic dosage. Because of the limits of traditional cancer treatments, nanomedicine, or the medical use of nanotechnology, has been developed to give more safe and effective treatment for cancer. Nanomedicines' ability to overcome resistance, increase solubility, enhance pharmacological character, and lessen chemotherapeutic medication side effects is so highly appreciated. During the last decade, their application in therapeutic settings has risen. At the moment, investigation is being conducted in the domain of innovative nano-pharmaceutical technology, including liposomes, polymer nanoparticles (NPs), lipid vesicles, carbon nanotubes, quantum dots, etc. in order to enhance the effectiveness and longevity of chemotherapy. Until recently, a variety of nanocarriers have been used as treatment solid tumours such as lung, brain, pancreatic, liver, and breast, in a number of trials. Nanocarriers, amongst the several extant nanosystems, have the potential to alter traditional medicine by minimising side effects and allowing for the controlled release of medicinal chemicals. In addition, their compact size makes intracellular absorption easier. Here, we take a deeper look at the therapeutic potential and way of action of nanomedicines in the fight against drug resistance. The importance of cancer cell-specific targeting is also debatable.

Abbreviations

CMC	Critical micelle concentration
CNt	Carbon nanotubes
DDSs	Drug delivery systems
DOX	Doxorubicin
EBC	Enhanced blood clearance,
EMA	European medicines agency
IRP	Improved retention and permeability
LSST	Lower specific stimulation temperature
MWCNt	Multi-walled
NLCs	Nanostructured lipid carriers
NIR	Near-infrared
NPs	Nanoparticles
PDT	Photodynamic treatment
PEG	Polyethylene glycol
PNIPAM	Polymers include poly (N-isopropyl acrylamide)
pHLIP	PH (Low) insertion peptide
PNBs	Plasmonic nanobubbles
QDs	Quantum dots
SERS	Surface enhanced Raman scattering

SiQDs	Silicon quantum dots
SLNs	Solid lipid nanocarriers
SMANCS	Styrene maleic anhydride-neocarzinostatin
SWCNt	Single-walled

13.1 Introduction

In the globe, cancer ranks as the second most prevalent cause of death [1, 2]. The treatment of cancer with precision and free cancerous cells requires chemotherapy [3, 4]. Chemicals are used in chemotherapy to either kill or stop the development of cancer cells [5]. Chemotherapy, which involves using cytotoxic chemicals in their free form to destroy cancerous cells or prevent cancer cell proliferation, is still the go-to medication for solid tumours, especially when it comes to instances of cancer of the breast, hepatic, lung, pancreas, and brain. The reason why antineoplastic medications' therapies are insufficiently successful is because of their non-specific cytotoxicity, and rapid metabolism with poor pharmacokinetics [6–8]. The early diagnosis and treatment of malignancy, meanwhile, continue to be hampered by current technology. Cancer treatment remains far from ideal since it is afflicted by significant shortcomings, despite considerable advancements in traditional treatment choices like chemotherapy and radiation therapy. Current cancer treatments frequently struggle with imprecise systemic distribution of anticancer drugs, insufficient drug quantities accessing the tumour site, unacceptable cytotoxicity, the inability to assess therapeutic outcomes, and the emergence of multi-drug resistance [9–11]. To anticipate effective therapy and treatment outcomes, existing prognostic and diagnostic categories are inadequate [12]. Negative outcomes will keep happening in the type of individual overall survival that are poor and the development of multidrug resistance. Due to such risks, nanocarriers have already been tried as rather promising methods for cancer treatment. Anti-neoplastic may create chemically covalent connections on the exterior of the nanocarrier, become physically deposited there, or be retained inside the nanocarrier [13].

13.2 Cancer Nanotechnology: A New Paradigm in Cancer Treatment

As with any treatment of cancer, the main challenge is to deliver the correct medicinal agent concentration to tumour locations, eliminating malignant cells while causing the least amount of harm to healthy cells. With this perspective, it is essential to develop single drugs that have a great deal of potential to significantly impact

preventing cancer, diagnosis, and therapy. In order to address these issues with traditional chemotherapeutic medications, a number of ligand-targeted drug delivery techniques, radio-immunotherapeutics, such immunotoxins, and pharmaceutical immune conjugates, are being researched. These new tools will be used in the treatment of cancer [14]. Restrictions in their distribution continue to be a significant issue, despite the fact that these concatenated medications have demonstrated effectiveness that is encouraging when compared to traditional chemotherapeutic treatments. Recent developments indicate that nanotechnology is going to have significant influence on illness prevention, diagnosis, and medication. Nanotechnology is the production and manipulation of substances at the nanometer dimensions to produce products that display unique qualities. Cancer nanotechnology is developing as a new area of multidisciplinary study that spans the fields of biology, engineering, chemistry, and medicine. It is anticipated that this research will result in significant advancements in the detection, identification, and cancer therapy [15]. A tempting solution for preferentially eliminating cancer cells while seriously harming healthy cells is the notion of developing more efficient cancer therapies by manipulating them at the nanoscale. One of the most promising areas for cancer treatments is nanotechnology, a multidisciplinary discipline [16]. By creating clever, biocompatible nanomaterials for drug administration, which is the most relevant use of nanomedicine, nanoparticles (the medical use of nanotechnology) has the amazing ability to revolutionize cancer therapies and diagnostics. Nanocarriers have recently been used in an unheard of way, notably in the 10–100 nm size range, as an advent of therapies for the treatment of cancer. The USFDA has authorised the use of liposomes and albumin nanoparticles as two therapeutic nanocarriers in clinical settings. Another instance of an improved retention and permeability (IRP)-based nanovector use for breast cancer treatment is liposomal doxorubicin, albumin-bound paclitaxel (Abraxane1) [17, 18]. These nanosystems have four distinctive qualities that set them against other anticancer therapy: I they can be constructed to carry a significant therapeutic "payload" and also have clinical or diagnostic characteristics taken by individual; (ii) they can be coupled to multivalent targeting moieties to generate higher specificity and affinity for target cells (Fig. 13.1), and (iii) they can facilitate various drug particles to allow combinational chemotherapy. The nanocarriers can concurrently boost against cacerous activity and decrease cytotoxic activity by employing both inactive and active aiming mechanisms to raise intracellular drug levels in cancer cells whilst limiting damage in normal cells [19].

13.3 Nanotechnology Approaches for Cancer Treatment

This chapter describes the various topologies of nanosystems as well as the most current advancements in nanotechnology in the realm of treating solid tumours. By concentrating on this field's efforts, we can create novel therapeutic approaches and enhance the treatment of solid cancers. Nanoparticles, nanocrystals, micelles, nanosphere, carbon nanotubes, niosomes, liposomes, dendrimers, quantum dots,

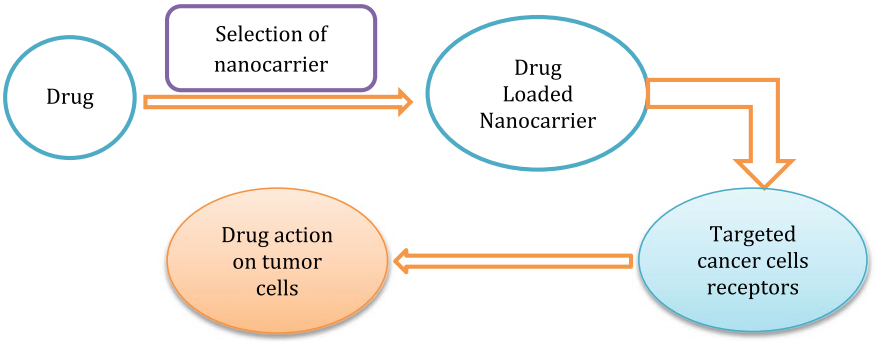

Fig. 13.1 Schematic diagram for representation of affinity for target cells

nanocapsule, etc., are a few instances of the natural and artificial materials who have been employed to create nanocarriers based on nanotechnology [20, 21] (Fig. 13.2). They've shown some really big promise in the therapy of carcinoma by improving drug efficacy and minimising systemic adverse effects.

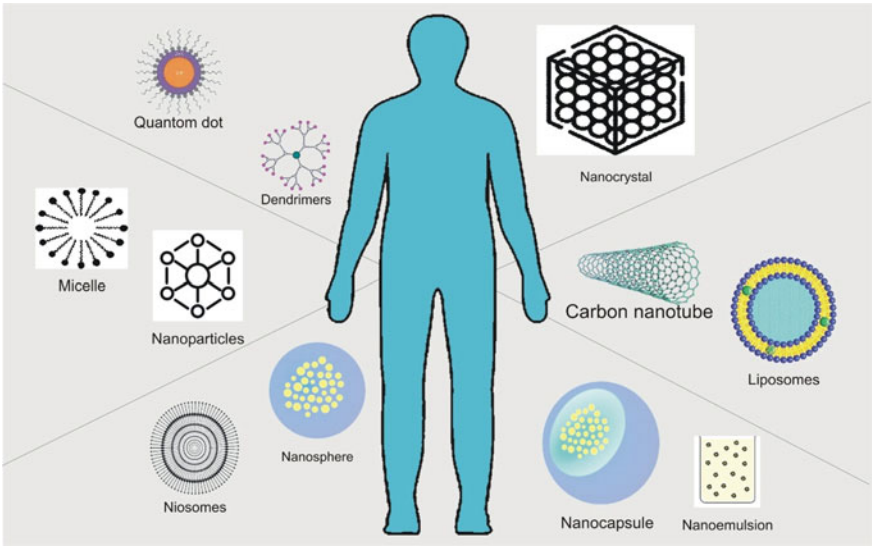

Fig. 13.2 Illustration of the nanocarriers used in intelligent medication delivery systems

13.3.1 Liposomal Nano-Carriers

Amongst the nanocarriers used to treat cancer, lipid-based nanocarriers have made great progress. There are currently different types of lipid-based formulations, such as liposome systems, solid lipid nanocarriers (SLNs), and nanostructured lipid carriers (NLCs) (Fig. 13.2). These lipid-based systems tend to be less toxic than other DDSs, such as polymer NPs, because of their biocompatibility and biodegradability.

A lipid bilayer surrounds a central cavity that may be filled with chemotherapeutic medications for transport to tumour locations to form liposomes, which seem to be spherical drug carrier vehicles created intentionally [22, 23]. As a result of phospholipid's amphiphilic characters as well as the aqueous environment's thermodynamic characteristics, which force the self-assembly into a thermodynamically advantageous orientation with a hydrophobic section contained within the NP core, the formation of liposomes is very simple [24]. Liposomes have by far emerged as the most effective preparation for therapeutic use [25]. Natural phospholipids, cholesterol, and other lipids can form based on the structure of liposomes, which makes them perfect carriers for therapeutic compounds with differing solubilities because hydrophilic substances can be integrated inside the core (for example, Doxil®, entrapped doxorubicin (DOX) table 1) and hydrophobic medicines can be housed inside the lipid bilayer [26]. Lipid vesicles can thereby deliver either aqueous-soluble or poorly soluble medications to a specific place. In addition, they are drug-protective and have minimal immunogenicity and safety. Polyethylene glycol (PEG), a biocompatible and benign polymer, is added to the exterior of liposomes to generate a protective barrier that prolongs circulation by preventing the reticuloendothelial system from being cleared.

There are numerous ways to make liposomes, including solvent injection system [27], reverse phase evaporation, thin layer hydration methodology (also called as the Bangham approach), and detergent filtration [28]. Conventional techniques have several drawbacks. Some unique methods, such as supercritical fluid innovation, supercritical reverse phase evaporation, and the supercritical anti-solvent approach have been developed to overcome those restrictions.

13.3.2 Micelles

Amphiphilic compounds exhibit special self-assembly properties when contacted to a solvent because they include either both hydrophilic and hydrophobic regions. The polar sections of a co-polymer are drawn towards the solvent, whereas the hydrophobic components of the co-polymer orient far from the solvent if indeed the solvent is aqueous loving as well as its quantity surpasses the critical micelle concentration (CMC). Thus, the hydrophilic regions create a corona, and the hydrophobic parts create a centre. Figure 13.2 shows what is known as a straight or normal polymeric micelle [29, 30]. On the contrary side, amphiphilic substances subjected

to a hydrophobic solvent result in the formation of an opposite structure called a reverse micelle. In some kind of reverse micelle, the aqueous loving regions create the core while the aquaphobic sections create the corona. Notable instances of micelles include PG-PCL, PEG-PCL [31], PEEP-PCL [32], and PEG-DSPE [33]. The solubility of the copolymer being utilised determines how micelles are formed. There are two techniques employed for a co-polymer that is largely water soluble: immediate dissolving and film forming. If the co-polymer is not easily soluble in aqua, however, screening or oil in aqua technique is performed [34].

By bridging the CMC, micelles run the risk of premature release of drug. Additionally, contact to blood and unimer uptake to plasma protein can upset the balance here between micelle and blood. A clever micelle is the answer to this dilemma. Micelles are often cross-linked, which is the disulfide-based joining of two polymer chains, to solve the aforementioned issues [35]. The cross-linking systems come in two varieties: central cross-linked polymeric micelles as well as outer cross-linked polymer micelles. Folic acid, sugars, peptides, aptamers, antibodies, and other forms of ligands are employed to adorn the micelle surface so as to aggressively attack cancerous cells. It is feasible to improve the properties the micelle's corona or core in order to release the medication for cancer therapy at the proper quantity. Ultrasound, enzymes, temperature fluctuations, pH gradients, and oxidation are some of the triggers employed in micelle-based delivery system. For the synergistic benefits in the therapy of cancer, the co-delivery method that used a multipurpose micelle is crucial. A heat-sensitive micelle-based co-delivery device was developed by Seo et al. [36] that can transport genes and anti-cancer medications simultaneously. Using an imaging substance, you may adorn the micelle's exterior. Kennedy and colleagues reported on the use of ultrasonography to image tumours while simultaneously administering doxorubicin [37].

13.3.3 Quantum Dots (QDs)

Quantum dots are 2–10 nm in diameter and are inorganic luminous semiconductor nanoparticles made up of 10–50 atoms [21]. Their excellent control over size and shape enables precise manipulation of their emission and absorption characteristics. They are durable for months without deterioration or change and have been extensively explored for optical imaging applications in live systems [21]. For the purpose of marking tumour cells, specific ligands have been added to QDs to enable precise targeting [38]. They are therefore certain to be selected as long-term, highly sensitive, and multi-contrast diagnostic agents used for in vivo detection and diagnosis of cancer [39].

To get over the limitations of cell membranes, several scientists are now concentrating on employing quantum dots as carriers for the transport of genes. Bifunctional silicon quantum dots (SiQDs) were created by Klein and colleagues to be used as a self-tracking delivery method for ABCB1 siRNA [40]. According to Li et al.'s study on the glutathione-mediated liberation of active plasmid DNA with positive

energy CdTe quantum dots, such QDs may be used to selectively and visibly unload cargo in live cells [41]. It has also been looked into if quantum dots may be used to treat cancer via radio- and photo-sensitization processes. Quantum dots, which have electrical levels of energy in the 1–5 eV band, can serve as light sensitizers in photodynamic treatment (PDT), which was recently authorised as a therapeutic option for a particular kind of cancer. According to Juzenas [42], quantum dots have a significant atom and electron abundance that allows them to absorb highly energetic photons and operate as radiosensitizers to harm cancer cells in a limited and targeted manner.

QDs-based tumour detection and therapy may be applied to deeper tissues and provide an optical guidance for organ surgery when combined with near-infrared (NIR) optical imaging technologies. Nevertheless, since they are made up of dangerous heavy metals, QDs' cytotoxicity cannot be disregarded for their uses in vivo. Therefore, in order to assure their safety for future uses to humans, it is vital to rigorously evaluate their toxicity.

13.3.4 Carbon Nanotubes (CNt)

A subclass of carbon allotropes known as fullerene includes carbon nanotubes (CNt), which may take on a variety of shapes, including nanospheres, ellipsoids, tubes, and more. A graphene sheet is termed as a CNt when it is roll down into a flawless circular tube. Both single-walled (SWCNt) and multi-walled (MWCNt) CNt are available [43]. Additionally, nanoparticles between 50 and 100 nm are easily consumed due to the CNt's significant absorption spectra in the near-infrared range. This particle is an ideal match of photothermal ablation. While PEGylated SWCNt can localise in a particular cellular division, MWCNtcan pass over the barriers of several cell organelles. Carbon black and graphite may be heated in a regulated combustion environment to create CNt. The synthetic CNt shape, size, quality, mechanical properties, and purity cannot be controlled by this procedure. Laser ablation, chemical vapour deposition, and electric arc discharges [44] have all been described as solutions to the regulated combustion environment's drawbacks. SWCNTs are more effective than MWCNt in delivering drugs due to their more delineated walls and the comparatively greater number of structural flaws in MWCNt [45].

To make CNt intelligent, they must either be physically or chemically specific job roles [46]. The process of PEGylation is crucial for increasing solubility, avoiding the reticuloendothelial system, and reducing toxicity. Thermal responsive polymers include poly (N-isopropyl acrylamide) (PNIPAM). CNt might be modified for temperature stimulation using PNIPAM because of their lower specific stimulation temperature (LSST). For medication release that is enzyme sensitive, a disulfide bridge centreed on methacrylate cysteine is utilised. The best choice for pH reactive is a polymer (ionizable) with such a pKa value around 3–10. When the pH changes, weak bases, and acids exhibit a shift in the ionisation state [47]. Nanostructures CNt can cross the BBB, according to recent research. CNt has demonstrated

potential for transporting plasmid DNA, antisense oligonucleotides, siRNA, and aptamers. It can be utilised for photothermal therapy of a cancer location in conjunction to gene delivery. As diagnostic instruments for the early identification of cancer, nanostructures CNt can be applied [48–50].

13.3.5 Dendrimers

Dendrimers are synthetic large molecules having tree-like architectures with the elements organised in several limbs and sub-limbs emanate from a core structure [51, 52]. They are made in a series of processes from branching monomer units. As a result, it is feasible to manipulate their molecular characteristics, which rely on the branching monomer units and include size, dimension, shape, and polarity [51]. Due to their void interior holes and exterior moiety, these highly branched structures provide special interfacial and physiological performance benefits. They may thus be changed or coupled with a variety of intriguing molecules and have a huge potential for solubilizing hydrophobic medicines [52]. Dendrimers possess proven considerable possibility in the creation of anticancer medication delivery mechanisms based on their unique characteristics.

The well-defined multivalency of dendrimers is frequently used to covalently attach particular targeting components, such as folic acid, sugar, biotin [52], antibody, and epidermal growth factors, in order to achieve effective medication targeting to tumour tissues. Drugs used for treating illnesses can also be coupled with dendrimers or contained inside them. In order to create grouped molecules for focusing on cancerous cells that exasperate the very dependable folate receptor, Choi and colleagues developed generation 5 polyamidoamine (G5 PAMAM) dendrimers covalently linked to folic acid and fluorescein. These compounds were then connected with complimentary DNA oligonucleotides. The DNA-linked dendrimer complexes may connect to KB cells with specificity, according to in vitro tests, and they might be employed as diagnostic tools for cancer treatment [53].

13.3.6 Niosomes

Niosomes, non-lecithin transporters, resemble liposomes in form but are sturdier. These are nanocarrier systems created by nonionic surfactants, and they offer several benefits including biodegradability, biocompatibility, biodegradability, as well as the capability to encapsulate both lipid-soluble and aqueous-soluble medicines. They were created to get over liposome's restrictions, particularly those brought on by phospholipid oxidation. In addition, the bilayer fluidity and less viscosity of niosomes may be readily controlled, and they have a longer lifespan [54, 55]. Pereira et al. [56] effectively created pH (Low) insertion peptide (pHLIP)-coated niosomes. Niosomes

covered with pHLIP are shorter and much more stable than pHLIP-coated liposomes, with good tumour targeted and pH-dependent cellular uptake. As a result, lipophilic and/or hydrophilic medicines can be successfully delivered to niosomes, which can then enter cells in a pH-dependent way. Niosomes' adaptability improved the oral absorption of drugs. However, because the niosome has a limited capacity for encapsulation, multiple surfactant mixtures are required to encapsulate diverse hydrophobic compounds in its bilayer membrane and preserve the long-term stability of the nanovesicles.

13.3.7 Nanoparticles

The medicinal substance of interest is contained inside the polymer matrices of such submicron-sized colloidal particles, or it could adhere to the surface or be adsorbed. Through surface changes, nanoparticles are directed to certain places where they interact biochemically with specific receptors on target cells [56]. The capacity of nanoparticles to carry medications to the target region while navigating various biological obstacles, including the blood–brain barrier, is another significant function of nanoparticles. After such an intravenous injection, brain scapegoating is made possible by covering the medicine-loaded nanoparticles using polysorbates, which allow them to pass across the blood–brain barrier [57]. Acharya et al. [58] creation of immuno-nanoparticles with increased effectiveness against the MCF-7 breast cancerous cell line included fulfil the required nanoparticles linked to epithelial growth factor antibodies. By precisely directing the medicine to the core of breast tumour cells by combining a core localization motif to the surfaces of the nanoparticles, Misra et al. [56] have increased the medicaments effectiveness of the strong anticancer agent doxorubicin. In order to dissolve curcumin in water phase at clinically meaningful densities, safeguard it against hydrolytic breakdown and then in vivo biotransformation, and produce curcumin in a regulated fashion, Mohanty and Sahoo [19] developed a nanoparticulate delivery mechanism using pluronic F-127 and glycerol monooleate. It is widely acknowledged that the creation of cutting-edge methods for early detection of cancer and efficient treatment would significantly increase survival of patients. In order to fine-tune the emission and absorption characteristics of nanoparticles, novel artificial techniques have been devised [59]. The creation of multifunctional nanoparticles with the capability to deliver drug candidates and image specifically targeted tumours is made feasible by the development of nanoparticles as imaging techniques [60]. Commercially available nanoparticles shown in Table 13.1.

Table 13.1 Typical pharmaceutical products based on nanocarriers available today [65]

Drug	Brand name	Nanocarriers	Indications
Styrene maleic anhydride-neocarzinostatin (SMANCS)	Stimalmer/Zinostatin	Conjugate of protein-polymer	Liver cellular cancer
PEG -L-asparaginase	Oncaspar	Conjugate of protein-polymer	Leukaemia acute lymphoblastic
Anti-CD20 attached to iodine-131	Bexxar	Radio-immunoconjugate	Low-grade, follicular, or transformed non-lymphoma Hodgkin's that is resistant or has relapsed
PEG: granulocyte colony-stimulating factor (G-CSF)	PEG filgrastim/Neulasta	Conjugate of protein-polymer	Preventing neutropenia brought on by chemotherapy
Anti-CD20 attached to indium-111 or yttrium-90	Zevalin	Radio-immunoconjugate	Low-grade, follicular, or transformed non-lymphoma Hodgkin's that is resistant or has relapsed
Daunorubicin	DaunoXome	Liposomes	Kaposi's sarcoma
Vincristine	Onco TCS	Liposomes	Non-lymphoma Hodgkin's with an aggressive relapse
Paclitaxel	Abraxane	Albumin-conjugated paclitaxel nanoparticles	Breast cancer with metastasis
Doxorubicin	Myocet	Liposomes	Combination treatment for Kaposi's sarcoma, ovarian cancer, and recurrent breast cancer

13.3.8 Nanocrystals

Nanocrystals are described as completely solid particles having a crystal-like quality [61]. Nanocrystal formulation has allowed for the recovery of several poorly soluble medications. Because of the fact that nanocrystals are constructed completely of the

drug or payload, they have special properties such as an enhanced ratio of surface to volume, stable rate of dissolution, augmented framework strength, and elevated medicine loading effectiveness. This eliminates the need for a carrier and leads to acceptable therapeutic levels at low doses [62]. Initially, nanocrystals were utilised enhancing oral bioavailability of poorly soluble medicines. Nanocrystal composition has attracted widespread interest for intravenous administration of anticancer medications, even if studies on the medication are still at the preclinical animal stage [61, 63]. Passively administering intravenous nanocrystals delivered to mononuclear phagocytic network rich in cell, tissues such as hepatic, spleen, and lungs due to macrophages' quick digestion [64]. The distribution of nanocrystals in vivo is significantly impacted by the surface, size, and shape functionalization of the particles. Nanocrystal properties such as size, solubility, stabilization, and bioavailability are frequently influenced by the pH of the dispersion media, crystallinity production, and contaminants [62].

13.3.9 Nanoemulsions

When two immiscible liquids are combined, a nanoemulsion is created where one phase is disseminated throughout the other. Typically, they are between 50 and 200 nm in size. Based on their technique of synthesis, nanoemulsions often comprise oil compounds or droplets scattered in an aqueous phase or watery droplets scattered in an oily phase [66]. As a result of their biodegradability, simplicity in manufacture, and regulated drug release, nanoemulsions have received extensive research as drug carriers for lipophilic chemotherapeutics [67, 68]. Additionally, nanoemulsions not only prevent medication deactivation in the gastrointestinal system but significantly improve the solubility of the pharmaceuticals, allowing for better drug absorption and dispersion. Due to the inclusion of additives that are widely regarded as safe, nanoemulsions also have strong biocompatibility. These additives have a high trapping effectiveness for hydrophobic components, physicochemical stability, and increased bioavailability, as well as higher effectiveness and safety.

13.3.10 Nanocapsule

Nanocapsules may be created using two methods: template-based and self-assembly. Because of their hollow spherical shape and capability to self-orientation into the vesicular framework in water solutions, lipid molecules and amphiphilic natured copolymers are excellent antecedents for the creation of stable nanocapsules compositions through a variety of processes. The template technique involves encasing a template component in a polymer shell, which can then be withdrawn to reveal an

unfilled polymeric shell. As an alternative, central particles can be created by traditional emulsion polymerization then removal of the core particles after the introduction of a separate monomer creates a cross-linked shell surrounding the central particle [69].

13.3.11 Nanosphere

In-depth studies have been conducted on nanospheres and nanorods, which are used in a variety of biomedical applications, including administering drugs, photoirradiation-based treatment, bioimaging, diagnostics, and immunotherapy [70]. These entities are optically distinctive in that the nanospheres display a particular plasmon resonance and a high X-ray attenuation coefficient in proportion to their dimensions, while the nanorods show two distinct plasmon absorptions in line with respective aspect ratios [71]. N anospheres, therefore, are interesting materials for bio-imaging and diagnostics, in addition to those coated with molecular structures such as surface-enhanced Raman scattering (SERS) sensors and photothermal compounds. Additionally, nanospheres may be used for photoacoustic treatment due to their optical feature of turning near-infrared light into thermal energy. Nanospheres have a high volume-to-surface ratio and thus are bio-inert, hence numerous experiments have been done to include medicines on their surfaces even though medications cannot be incorporated within inorganic materials. In order to identify and treat diseases in vivo, chemotherapies, genes, even photosensitizer medicines have been mounted on nanospheres [71]. Due to the fact that cancer cells have evolved sophisticated defence system against the body's own immune system, the research of nanocarrier applications in cancer treatment has recently extended to include succeeding immunotherapy. In order to modify the micro-environment and promote tumour growth, cancerous cells convey specific markers and take advantage of a number of immunological procedures, such as controlling the secretions and activities of T and macrophages cells, antigen presentation, and the creation of immunosuppressant mediators [72]. To lessen the potential adverse effects of advertising biodegradable polymeric nanoparticles, vaccines and antibodies have been used as carriers in antigen drug carriers; even so, the possibility of immune rejection of polymeric particulate has been noted, and the method of the immune function is not thoroughly grasped. For instance, despite the fact that polyethylene glycol (PEG) is frequently used during polymeric carriers to prevent protein absorption and reduce non-specific cell adhesion, anti-PEG antibodies have been reported to be brought up on such administering of numerous PEGylated proteins and nanomaterials, that can reduce the treatment effectiveness of the administrated protein [73].

13.4 Cancer Diagnostics and Treatment Using Nanotechnology

Theragnostics (diagnosis and treatment) is an innovative biological approach that combines diagnosis and treatment into one step. The main objective of accurate diagnosis is to specifically site particular (illness) cells or tissues in order to enhance the precision of the diagnosis and treatment. Theragnostic can assist us to combine important phases of a medical procedure, such detection, and treatment, and render a procedure quicker, more protective, and more effective. Nanoparticles have been used as the carriers of therapeutic and diagnostic substances in a number of theragnostic techniques. The creation of biocompatible nanocarrier as cancer diagnostic tools that would allow for unobtrusive detection and accurate cancer treatment is presently underway. These multimodal nanocarrier-mediated approaches show potential for enhancing cure for cancer percentages, decreasing adverse effects, and speeding up the course of treatment. In order to tailor the plasmonic nanobubbles characteristics in a single cell and assess their multifunctionality, Lukianova-Hleb et al. studies of the optical creation and monitoring of plasmonic nanobubbles (PNBs) surrounding gold nanoparticles in single live cells [74] is of particular interest. Magnetic nanoparticles can serve as both drug delivery carriers and detecting imaging techniques tools, according to many recent studies that have covered their engineering drawings, physicochemical characteristics, and therapeutic properties [75]. Shim et al.'s [76] integrated cancer treatment and diagnosis (theragnostics). In their work, the researchers explored the prospect of combining stimuli-responsive multidimensional optical imaging and stimuli-enhanced down regulation by coating tiny polyplexes conjugated to gold nanoparticles through acid-cleavable connections.

13.5 Aspects of Future Scientific Challenges

Every possibility has its share of difficulties. The same is true for nanocarrier. The body's sensitivity to nanocarriers, the system's cost efficiency, the variety and heterogeneity of cancer, as well as the absence of precise regulatory requirements are obstacles to efficient nano-drug delivery systems [77]. Nanocarriers transport and deliver the anticancer medications to specific areas to eliminate the cancerous cell. The outcome of the medicine-carrying nanocarriers is what worries people. Traditional nanocarriers can build up in several types of vital organs like the hepatic, spleen, lungs, kidneys, and cardiac, based on their chemical content, size, specific surface area, shape, surface charge, and existence or lack of a shell around them. Most nanocarriers do not leave the body after use; instead, they build up in the aforementioned essential organs. The toxicity that results from this deposition is a huge obstacle to the development of nanotechnology. There have been a lot of in vitro and in vivo research on toxicities in animal situations, but there has not been much on toxicity in humans. Toxicology research still has a very broad reach

[78]. Cancer is diverse and heterogeneous; hence, the different forms of cancer are yet unknown. Additionally, each person's experience with cancer may be physically different. Consequently, personalising medical care is a significant difficulty as well. Anti-cancer behavioural strategies on DNA/RNA offer a promising future for enhancing pharmaceutical safety and personalization. Consequently, the creation of nanocarriers as carriers of genetic material to eradicate cancerous cells may be a potential study area [79].

Traditional nanomaterials, like the reticuloendothelial system and enhanced blood clearance (EBC), confront several biological obstacles on the path to identifying cancer cells. Traditional nanocarriers are altered utilising a number of techniques, such as PEGylation and ligand patching, to overcome these obstacles. Additionally, the nanocarriers must be designed and synthesized in order to release the medications at specific areas when stimulated. The cost of the finished product increases as a result of these alterations, which result in more production stages. Any product that is released has to have a favourable cost–benefit ratio to be competitive [80].

The biggest obstacle to the commercialization of nanocarriers based dosage form is obtaining regulatory authority clearance. In the review and approval, the European Medicines Agency (EMA) and the FDA play important roles. The amount of FDA-approved nanocarrier-based anti-cancer treatments is quite small, even if there are numerous items in the queue, 23 years since the first nanocarrier-based anti-cancer medication, Doxil, was announced in 1995. Manufacturers must demonstrate the items' short- and long-term security for the human body in order to receive governmental clearance. Consequently, launching an item while doing all the required processes is highly time-consuming and labor-intensive. Sometimes even the clearance process is made more difficult by the lack of explicit rules. To overcome these obstacles, an agreement between scientists, industry, and regulatory agencies is required [81].

13.6 Conclusion

In order to decrease the danger of medication tolerance and specifically target various forms of cancer, the adaptability of NPs allows them to boost pharmacological synergies with better control of medication dispersion in time and space. Several active medicinal compounds have been coupled with a variety of biocompatible nanoscale substance carriers. Nevertheless, the increasing intricacy of the nanoformulation likely results in higher toxicity, more expensive manufacture, and problems in manufacturing procedures. To evaluate the interactions between nanomedicines and biological systems, more sophisticated technologies are required [82]. The primary drawbacks of various NPs distribution channels may be related to the loading percentage and encapsulation effectiveness of effective medicines, the two fundamental metrics utilised to assess nanocarriers. For example, liposomes have a quick release time and a small encapsulation rate. More critically, it can be difficult to rationally combine various medications with diverse mechanisms of action because most

DDS merely combine medications for co-delivery, independent of distributive pharmacokinetics in tumour locations. Additionally, proteins instantly coat the surface of the nanoparticles when they directly interact with live cells in vivo, impacting cellular uptake, inflammation, deposition, and nanoparticles breakdown [83]. The development of several nanocarriers lays the groundwork for its therapeutic use. Although nanocarriers offer many benefits, the FDA has only authorised a small number of them. Innovative nanocarriers' effective transition from pre-clinical to clinical testing requires the precise the identifying of patients appropriate for clinical trial investigation, a complete knowledge of their modes of action, as well as the implementation of effective educational and collaborations between industries at various phases of growth. On an industrial and commercial level, there seem to be a number of evident difficulties, such as repeatability, non-uniform size, uneven framework, sterilisation, and preservation for mass manufacturing [84]. Long-term toxicological hazards arise from the buildup of nanodrugs in undesirable tissues and organs. Consequently, it is important to take into account how NPs are biologically distributed following systemic treatment in preclinical and clinical research.

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Pankaj Sharma have become attracted to Pharmacy as it combines Maths and Science together and shows how these subjects affect lives every day in a positive way. A Pharmacists role is very vital to ensure the right medicines are supplied in an effective way hence it will allow me to work with a range of patients to achieve a lifelong career as a Pharmacist. My pharmacist journey was started just after completing my graduation. My keen interest was explored towards the pharmaceutics after qualifying GPAT and getting scholarship during my M.Pharm.

I have gained valuable knowledge studying Chemistry, Biology and Pharmaceutics which will be beneficial for the Pharmacy course. In Chemistry, I have done a series of experiments which require analytical and evaluative skills such as accurate reading when using burettes. I find the organic Chemistry module rather interesting as I enjoy studying the different reactions of aldehydes and ketones and how these reactions and organic products differ due to the different functional groups present in each compound. Another aspect of chemistry I enjoy is the purification of organic compounds. My Ph.D. guide encouraged me for do something for society so I decided that my Ph.D. research work will be beneficiary for society. With my mentor I worked on "Development of Transdermal patches for malaria treatment" during my Ph.D. This work was also sent for getting patent from SOS, Pharmaceutical Sciences, Jiwaji University, Gwalior M.P., India.

My week of work experience at the local Pharmacy was really inspiring; shadowing the Pharmacist and dealing with the different prescriptions given, was set to be really challenging and showed me how difficult the Pharmacists job can be, including giving advice to the patients and staff management. Other priorities at the Pharmacy included customer service and watching the Pharmacist carefully dispense medicines which I also saw as being valuable. I will be doing more work experience in the October holidays to really get an insight on the Pharmacists role in the Pharmacy.

Dr. Vinay Jain have always been astounded by the medicinal properties that plants possess. To use them in the welfare of mankind has always been there in my contemplation until my graduation (2002), which I completed from SOS, Pharmacy from Jiwaji University, Gwalior. This motivated me to select Pharmacognosy as one of my subjects in Major. I completed M. Pharm (2007) in Pharmacognosy from Barkatullah University Bhopal. Having completed my post-graduation, I started exploring phytochemicals in my professional journey as Assistant Professor in ShriRam College of Pharmacy, Banmore. Concurrently, I got a chance to be the part of training & placement team. This developed my soft skills to a large extent. I successfully defended my thesis entitled "Phytochemical and Pharmacological investigation of *Arisaema leschenaultii*" for Ph.D. I was awarded Ph.D. in Pharmaceutical science and technology from Berhampur University, Odisha. I was Co-convener of Indo-Caribbean conference which was hosted by ShriRam College of Pharmacy in 2018. I was also the organising secretary of national conference on "The Future Prospect in Pharma-Marketing" hosted in association with IPGA. Till date, I have guided more than 20 undergraduate and post graduate students in major projects. My CV today hosts about 30 papers which have been published in distinguished journals/books of international and national repute which are indexed in SCI and SCOPUS. I have presented more than 20 papers in national and international seminars and conferences. I was also privileged to get grants from technical professional bodies like AICTE and MPCST for organising conferences. I am life time member of Indian Society of Pharmacognosy. I always try to keep my staff highly motivated with my open-door policy for grievance redressal and with efficient leadership skills because I believe that only a highly motivated team creates a highly efficient institution.

Dr. Mukul Tailang completed his B.Pharm. (1987), M.Pharm. (1989) and Ph.D. (1997) from Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar (M.P.) a prestigious institute in the profession of Pharmaceutical Sciences.

Prof. Tailang has a vast experience of 33 years of teaching and research in the field of Pharmacy in India and abroad. Specialized in "Pharmacognosy & Phytochemistry" and "Plant Biotechnology", Prof. Tailang has guided 15 Ph.D. students under his supervision and published more than 100 research papers in the Journals of International and National repute and also has three Patents to his credit. Prof. Tailang has also published a book 'Phytochemistry – Theory & Practical' and contributed chapters in 'Text Book of Medicinal Chemistry' and 'Drug Carriers'.

Prof. Tailang has been awarded Prof. M.L. Khorana Award by Indian Pharmaceutical Association in the year 2002 and ILLUSTRIOUS ALUMINUS AWARD—2016 by Dr. H. S. Gour University, Sagar (M.P.) He has been awarded SRF from Council of Scientific & Industrial Research New Delhi in 1993.

He is reviewer of many International and National Journals and is a life member of various societies including Indian Society of Pharmacognosy (ISP), Association of Pharmaceuticals Teachers of India (APTI) etc. He has presented various Research papers in different National & International Conferences and delivered lectures in different Conferences, Faculty Development Programs, National workshops etc.

Presently he is working as Professor & Dean, Faculty of Technology, Jiwaji University, Gwalior (M.P.)

Chapter 14
Nano-Drug Delivery Systems for Tumour-Targeting: Overcoming the Limitations of Chemotherapy

Pooja Mary John, Maria Emmanuel, Jumana Beegum, Franklin John, and Jinu George

Contents

Abstract Nanotechnology has recently gotten a lot of attention because of its ability to accurately identify and manage various malignancies. The track to the commercialization of oncology medications is long and holds significant risk; yet, there is substantial anticipation that nanoparticle knowledge may subsidize the accomplishment of tumour drug development. The step at which pharmaceutical businesses have made partnerships to use patented nanoparticle knowledge has been considerably fast-tracked. It is nowadays familiar that by enhancing the efficiency and permissibility of new drugs, nanotechnology can eloquently contribute to making distinguished products and advance scientific outcomes. Nanocarriers have been inked to avoid the drawbacks of anticancer drug delivery systems, including

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non-specificity, severe intricacies, burst deliverance, and damage to healthy cells. Antitumour medicine's bioavailability and therapeutic efficacy are improved by nanocarriers, which provide favourable accumulation at the target site. Although a variety of nanocarriers have been created, only a few have been clinically authorized for the delivery of anticancer medicines to their designated targets. This chapter mainly includes three parts: the first part introduces diverse nanocarriers and their applications in the delivery of antileukemic drugs; the second section covers typical targeting mechanisms of targeted drug delivery systems, in which both the passive and the active targeting drug delivery systems for tumour therapy; and the third segment describes some selected tumours, such as breast, lungs and pancreatic tumours, as well as implementations of nanocarriers in these tumours. This analysis expands our vision of tumour dealing with the hopeful application of nanotechnology.

14.1 Introduction to Nanocarriers

Tumour is the chief cause of death in the whole world, causing patients physical and mental health suffering [1]. For many years, experts have been particularly interested in the early detection and successful treatment of cancer. Today, one of the areas of science and technology that is the subject of the greatest research is cancer. A body cell is the source of cancer. In order to keep the body's steady-state and homeostasis, cells divide and make new cells to replace the old ones. However, if the genetic code inside the cell is altered, cells can develop abnormally to form tumours. Through lymphatic and blood vascular metastasis, cancer cells spread to other parts of the body and accumulate into the bulk of cells that are tumourigenic. The second utmost common cause of mortality in the whole world is cancer. The best chance for using the proper therapeutic intervention tactics is to diagnose cancer early [2]. The most typical techniques for treating tumours are using therapeutic drugs or radiation, or surgically removing the tumour [3]. Applying therapeutic chemicals is non-invasive and simple compared to other therapies. However, there are still some issues in practice: treatment selectivity falls short of expectations, tumour cells are not eliminated, and side effects are not tolerated. To address these issues, nanomaterials are used to create smart drug delivery systems that allow agents to target tumours more precisely. Tumour tissues differ from typical tissues in terms of evolution, propagation, and metabolism, according to numerous studies. The tumour microenvironment (TME), comprises biochemical variations (such as pH, redox latent, enzyme action, and hypoxia) as well as uncontrolled changes (such as aberrant vascular anatomy and receptor overexpression), which reflects these different properties [4]. These modifications allow drug delivery devices to be more accurately and effectively target tumours. Drug distribution arrangements refer to wisely made-up systems that can single out between normal sites and tumour sites to correctly release the therapeutic agents in tumour sites.

When delivered using traditional drug delivery systems, anticancer chemotherapeutics have several issues, together with meagre specificity, excessive harmfulness, and the creation of drug confrontation. Many anticancer medications lose their therapeutic effectiveness as a result of these roadblocks. By leveraging the differential diagnosis of the lump microenvironment, nanocarrier-based arrangements have enabled the effective transport of anticancer medicines into swellings, dramatically increasing therapeutic outcomes [5].

Nanocarriers are colloidal drug carter systems with constituent part sizes of less than 500 nm. Nanocarriers have been considered intensively in recent times due to their aptitude in the field of medicine transfer. Nanocarriers have the power to change the elementary physical characteristics and bio-activity of medications due to the high surface area to volume ratio. Because of their small size, nanocarriers have unique qualities like quantum effects, a high surface-to-volume ratio, and the capacity to deliver therapeutically effective chemicals to the desired spot [6]. Nanocarriers can offer enhanced pharmacological medicine and circulation, compact toxicities, better quality, solubility and permanency, site-specific transfer of curative remedies, to name a limited benefit. The complete purpose of operating nanocarriers in drug delivery is for treating a sickness effectually by minutest complications. These particles can be categorized as 0-dimensional, 1-dimensional, 2-dimensional, or 3-dimensional nanoparticles based on their overall shape. The surface layer, the shell layer, and the core, which would be essentially the central component of the NP and is typically referred to as the NP directly, make up the basic nature of nanoparticles, which is highly complicated. Deep tissue diffusion of NPs is reported to boost the EPR impact. Additionally, the surface properties have an effect on bio accessibility and half-life by successfully overcoming epithelial opening. By adjusting the particle polymer properties, it is also feasible to maximize the release rate of medications or active moieties. Together, the unique characteristics of NPs control their therapeutic impact in the treatment and prevention of cancer [7]. The transfer of anticancer drugs via a nanoparticle-based channel has many appealing advantages over conventional chemotherapeutics, which would include: (1) enhanced administration of drugs that are not water soluble and distribution of a therapeutic agent into malignant cells at a massive dose; (2) effective security of a drug from extreme conditions before it can approach the targets, resulting to a prolonged plasma half-life of the therapeutic agent in the circulatory system; and (3) improved delivery of a drug from hostile climates before it is able to reach the targets [8]. We can develop innovative approaches for the diagnosis, treatment and prevention of variety of diseases, especially those that are incurable, by understanding the features of nanoparticles and their unique interaction with the microenvironment. According to the findings of numerous cancer scientific studies, nanoparticle-based drug delivery systems with increased bioavailability and fewer side effects exhibit more favourable antitumour effects than free medicines. Different kinds of nanocarriers are used to deliver medications to cancer cells. The majority of nanocarriers are made of inorganic materials, organic materials, or hybrid materials or self-assembled structures designed for their intended function. These include dendrimers, SLNs, liposomes, micelles, virus-like particles,

etc. that enable therapeutic molecules to be encapsulated, chemically coupled, or physiosorbed to overcome solubility problems of drugs [9].

14.1.1 Organic Nanocarriers

14.1.1.1 Solid Lipid Nanoparticles (SLNs)

Solid lipid nanocarriers has showed considerable promise in delivery of drugs, particularly in terms of regulating release of drugs and specifically targeting tissues. SLNs are aqueous colloidal dispersals made of a lipid matrix that is solid at body and room temperatures. While the selection of lipid impacts the properties of medication delivery, surfactants increase their stability. SLNs, a subclass of lipid carriers, are flexible drug delivery systems because they may encapsulate very large volumes of hydrophilic and lipophilic medicines as well as nucleic acids [10]. SLNs are categorized as solid lipid nanoparticles or nanostructured lipid carriers. SLNs is primarily made up of solid at normal temperatures lipids, however SLNs comprises both liquid and solid lipids. One of the most difficult aspects of SLNs creation is ensuring that its carriers can transfer active compounds to the site of absorption and enhance medication absorption. Another problem is integrating functional excipients into the SLNs reliably for target medication delivery [11]. SLNs have several advantages over standard colloidal carriers, including reduced toxicity, wide surface area, delayed drug release, enhanced cellular absorption, and the capacity to boost drug solubility and dissolution rate. It allows for great drug stability, the incorporation both of hydrophilic and also lipophilic medicines, and the improvement of the bioavailability of weakly water-soluble compounds [12]. Schematic representations of solid lipid nanoparticles are shown in Fig. 14.1.

Researchers created SLNs like drug carrier replacement colloid carriers with spherical shapes. The usual dimension of SLNs is between 150 and 300 nm, although depending on the surfactant utilized during synthesis, they can be as large as 1000 nm. The long-term durability, drug loading potential, and drug release characteristics

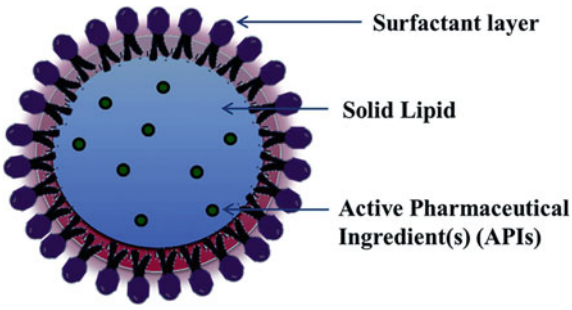

Fig. 14.1 Schematics of solid lipid nanoparticles. Reproduced from [12], Copyright (2020), with the permission of RSC

of SLNs are influenced by their thickness and solid–liquid lipid ratio. As previously stated, SLNs have various advantages, including minimal to no toxicity to living tissue, speed of manufacture in larger units, capability to load simultaneously lipophilic & hydrophilic medicinal agents, and increased drug load-bearing capacity. Oral medicine administration is the most prevalent application for SLNs as nanocarriers. Several medicines, including doxorubicin & idarubicin, camptothecin, and thymopentin have been loaded utilizing SLNs for delivery of drugs [13].

SLN's benefits includes, through the use of biodegradable physiological lipids, it lowers the danger of chronic and acute poisoning and prevents the practice of organic solvents in the creation progression. It increases bioavailability of poorly water-soluble compounds and improves medicine absorption into the skin by using a technique known as site-specific distribution of pharmaceuticals. SLNs regulates both drug targeting and the possibility of drug release. Also, it supports integrated labile chemical manufacturing, trapped bioactive bioavailability, and a strong focus on functional molecule [14]. SLNs have certain disadvantages as well, including deprived drug loading, particularly intended for hydrophilic medicines, a limited solubility in the lipid melt, the possibility of drug outflow, and particle aggregation during polymeric transition throughout packing [15].

14.1.1.2 Liposomes

Liposomes seem to be spherical vesicles composed of one or more phospholipid bilayers containing lipoprotein and organic or inorganic phospholipids. Liposomes are an effective drug delivery mechanism for many therapeutics since they are harmless and biodegradable. By stabilizing substances, removing barriers to cellular and tissue absorption, enhancing drug bioavailability of drugs to target areas in vivo, and lowering systemic toxicity, they have increased the therapeutic potential of drugs [16]. A lipid bilayer encircling a hollow core with a diameter of 50–1000 nm makes up the simplest liposome. Several aqueous layers of multilamellar liposomes or two compartments, such as lipid and aqueous, can each hold a different type of medication. This also enables the sequential release of several drug molecules by separating the layers from the outside layer to the inner core. Liposomes are phospholipid-based nanocarriers that are amphipathic[17]. Typical liposomes have several disadvantages, including instabilities, faster drug dissolution in the plasma, and less circulation time. Liposomes can be made using a variety of practises like hydrating phospholipid sheets, Membrane Extrusion, Solvent Injection Technique, Reversed-phase Condensation Method, Freeze–Thaw Extrusion Process Micro-emulsification surfactant removal method, Column chromatography, and others [18]. A schematic representation of Liposome structure is shown in Fig. 14.2.

Liposomes comprise vesicular nano-systems that are mostly composed of phosphatidylcholine and cholesterol. Because of their low inherent toxicity, little immunogenicity, and biological inertness, liposomes are exceptional in this regard. The very first nanoscale drug, licenced in 1965, was liposomes. "Hydrophobic phospholipid bilayer" and a "hydrophilic core" make up the usual liposome structure. Due to

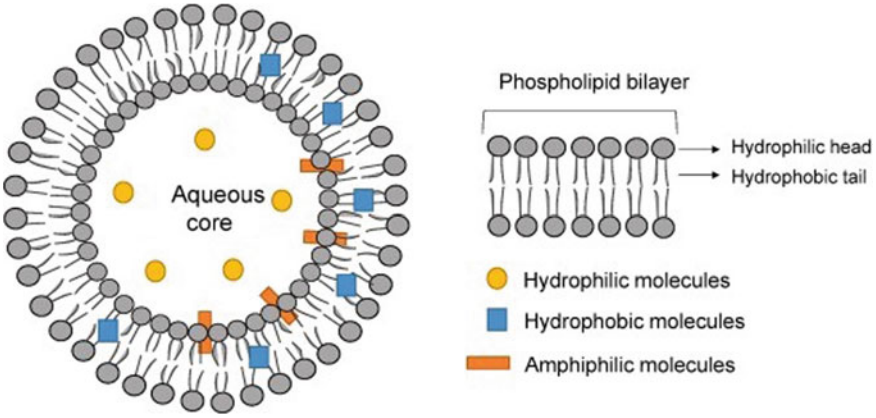

Fig. 14.2 Representation of liposome structure. Reproduced from [16], Copyright (2021), with permission from Elsevier

their distinctive architecture, they can efficiently entrap hydrophilic and hydrophobic medicines to shield the substance that is trapped from circulation-related degradation of the environment. By simply fusing the vesicle well with cellular membranes, liposomes can assist delivery of the drug into the target cell. To increase its stabilization and half-life, several polymers are utilized, such as PEG and poly-lactic-co-glycolic acid (PLGA) [5]. Liposomes used as drug nanocarriers have several advantages, including high entrapment efficiency, nontoxicity, facile surface modification, and also self-assembly production technique. To increase target selectivity, liposomes can be coupled to ligands or antibodies.

14.1.1.3 Dendrimers

Dendrimers are symmetric molecules with a monodispersing structure, which are uniform, nano-sized, & highly branching. Dendrimers have diameters ranging from 2 to 10 nm. Greek words "Dendron" and "meros" are the source of the word dendrimer, which means "tree or branch" and "part", respectively. Poly dendrimers were the very first dendrimers discovered by Buhleier et al. in 1978. Around their central core, dendrimers are made up of a number of branching chains, and their exterior is made up of surface functional groups. Drugs or chemicals can get to the target site through the space between the branching chains within the central core. The outer surface of dendrimers is the major site of contact between the guest molecule and the dendrimer. The kind and quantity of guest molecules which can be integrated into dendrimers are determined mostly by the structure of the dendrimer. By modifying the guest's association with a number of the dendrimer's terminal groups, the loading capability of dendrimers can indeed be significantly increased.

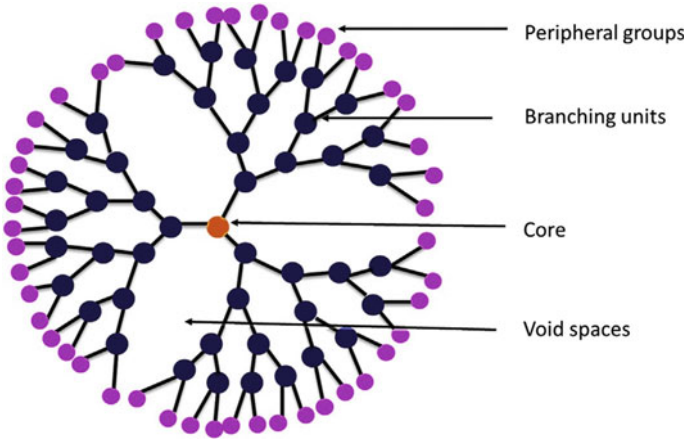

Fig. 14.3 Structure of dendrimers. Reprinted from [21], Copyright (2019) with permission from Elsevier

With each further dendrimer synthesis, more terminal groups are available for interactions with guest molecules [19]. They are the perfect delivery strategy due to their high branching points, 3D glomerular spherical shape, micro/nano-size, lipophilicity, mono-dispersity, and simplicity in penetrating cell walls. They are unique vehicles for the delivery of content material to particular cells due to their small size distributions, availability of numerous functional groups to attach drugs, and sustained control over the liberations of drugs, all of which are implied by their distinct thermophysical properties like solubility, polarity, and net charge. Their number and types of active sites, loading areas, the outside functional groups, and lipophilicity determine how well they attach to the ligands, hormones, antibodies, or liposomes with infused delivery of an active compound, making them a promising unreactive, less toxic, recyclable new therapeutic carrier for medical applications [20]. A simple structure of the dendrimer is shown in Fig. 14.3.

Dendrimers can act by encapsulating pharmaceuticals inside the dendritic structure or by creating electrostatic/covalent interactions between medications and terminal functional groups due to their distinct 3-dimensional structure and numerous surface functional groups. The drug delivery from drug-dendrimer complex is due to either the covalent bonds among both the drug and dendrimer being broken in vivo when the right environment or enzymes are present or a change in the physical parameters' temperature and pH, which are independent of external influences. Dendrimers' physiochemical properties are mostly governed by their generations, end groups, synthesis methods, monomers, and all these factors can be tweaked to give dendrimers specialized properties for various biomedical purposes. Even though there exist disadvantages, they can be controlled by making certain structural changes. Researchers have paid a great deal of attention to the overall advantages offered by dendrimer nano-architectures, not only in terms of drug delivery but also in terms of the identification and treatment of disease. Since early diagnosis increases

the likelihood that an illness can be treated successfully, the practice of dendrimers in disease diagnostics, particularly cancer, is extremely important [21].

14.1.1.4 Polymeric Nanoparticles (PNPs)

Solid colloidal particles known as polymeric nanoparticles (PNPs) can be created as nanospheres or nanocapsules. The drug is largely covered in the cavity of a nanocapsule, which is enclosed by a polymeric membrane. The matrix framework of nanospheres enables homogenous medication distribution. The non-toxic, recyclable, and regulated drug release properties of this polymer open up a plethora of medicinal application possibilities [22]. Natural, semi-synthetic, and synthetic polymers are used to make polymeric NPs. Non-biodegradable polymers such as poly(methyl methacrylate), polyacrylamide, polystyrene, and polyacrylates, were initially employed to create polymeric nanoparticles [23]. In contrast to other nanoparticle DDSs, all natural polymers are fully biocompatible, reversible, and have characterized degradation curves, allowing the drug release procedure of these nanomaterials more customizable. These polymeric NPs can also be directed directly at tumour cells by executing nanoparticle-surface alterations employing various ligands to receptors that are overexpressed on cancerous cells [24].

Polymeric capsules can be created by conjugating targeting ligands, which improves cancer cell selection and intracellular biopharmaceuticals while lowering various side effects and medication toxicity. Monoclonal antibodies or antibody fragments, aptamers, peptides, and other small molecules attached to shell-forming blocks are often used as specific ligands for polymer capsules. The efficiency of polymeric carriers customized with targeting ligands is determined by ligand features such as density and receptor binding affinities, which can improve receptor internalization and drug biodistribution [25]. A schematic representation of a polymeric nanoparticle is shown in Fig. 14.4.

Drug distribution at locations of action can be regulated and targeted by means of polymeric nanoparticles. Additionally, nanoparticle DDSs have outstanding biocompatibility, excellent biodegradability, and minimal toxicity. The high surface-volume ratio of polymeric nanoparticles, which gives them a high capacity for drug-loading and more opportunities to interact with the surrounding tumour environment, is another crucial aspect of these particles. To meet varied therapeutic or diagnostic objectives, nanoparticle characteristics and functionalities can be controlled in a variety of ways. For instance, we can include imaging probes into polymeric nanoparticles to resolve the problem of real-time assessment of biodistribution or clinical efficacy [26]. PNPs have long been employed in the pharmaceutical industry as a delivery system for medicines and active chemicals. Natural or manufactured biodegradable polymers are commonly used. Polymers like Abraxane are utilized in the therapeutic treatment of breast cancer as well as the therapy of numerous other malignancies, including non-small-cell lung cancer [27]. The ability of these polymers to change their physicochemical properties in response to certain changes in the environment enables more precise and programmable medication delivery in the treatment of

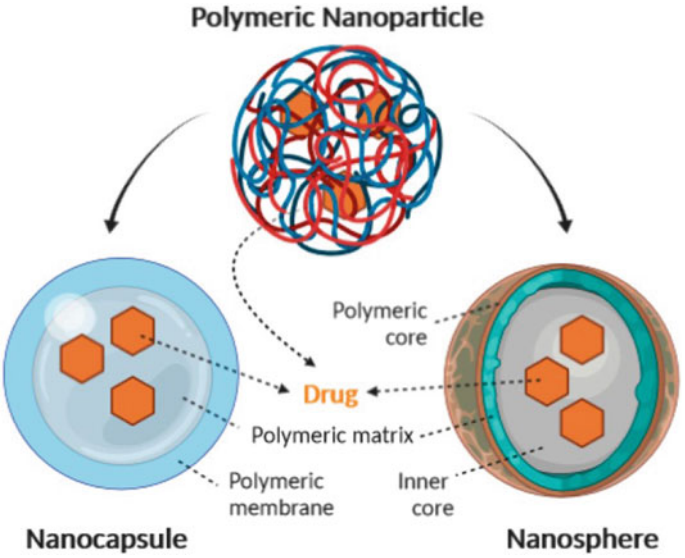

Fig. 14.4 Schematics of polymeric nanoparticles. Reproduced from [25], Copyright (2020) with permission from MDPI

cancer. Several PNPs that have been infused with anticancer medications are currently through various stages of clinical studies. Numerous articles outlining the benefits of PNPs containing anticancer medicines are currently being published [28].

14.1.1.5 Polymeric Micelle (PMs)

Polymeric micelles are nano-sized colloidal particles designed mostly via simple self-assembly of amphiphilic polymers otherwise synthetic amphiphilic di-/tri-block copolymers in an aqueous environment. In the core/shell framework of polymeric micelle, the core is hydrophobic while the shell is hydrophilic. PM's hydrophobic core permits hydrophobic drugs to be trapped and regulates drug release. However, the hydrophilic shell of polymeric micelle assures the solubility of the PMs in aqueous environments and stabilizes the core. The drugs will either be chemically or physically attached to the PMs, entrapping them inside. The micelles extravasate across the damaged endothelial cell junction due to their nano-size and ultimately accumulate in the tumour microenvironment. Polymeric micelles are more effectively absorbed than other nanocarriers such as liposomes and SLNs because extravasation is size-dependent. Furthermore, the vesicles may not be identified by the reticuloendothelial system (RES) or transported to the liver and spleen for clearance due to the hydrophilic PEG coating. Using ligand-linked amphiphilic polymer, micelles' surface could be easily altered, just as other vesicles. Micelles are therefore employed

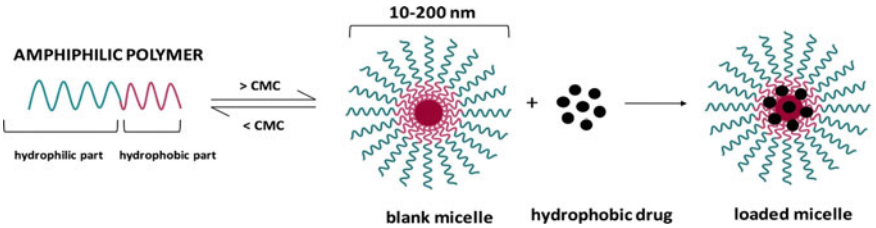

Fig. 14.5 Schematic representation of polymeric micelle. Reproduced from [29], Copyright (2021) with permission from Elsevier

for active targeting [29]. A schematic representation of a polymeric micelle is shown in Fig. 14.5.

In addition to passive targeting, active cancer targeting with PMs is also feasible through the surface modification of PMs with tumour-targeting ligand. Poly-lactide (PLA), poly-caprolactone (PCL), poly-lactide-co-glycolide (PLGA), polyesters, polyaminoacids, and lipids are a few of the often used hydrophobic polymers. Chitosan, dextran, polyethylene glycol, polyoxazolines, and hyaluronic acids are some examples of the hydrophilic polymers utilized to encase the hydrophobic core. Methotrexate, cisplatin, paclitaxel, docetaxel, and doxorubicin are just a few of the cancer-fighting medications that have been successfully incorporated in PMs [30].

14.1.1.6 VNPs/VLPs

VNPs are a new kind of innovative drug carriers that are protein-based nanoparticles and include viruses and protein cages. In contrast to supramolecular structures, which are put together from their protein monomers, protein cages typically self-assemble. VNPs, which are derived from plants and microbes, are non-infectious and non-hazardous to people while also being biocompatible and biodegradable. As a new type of nanocarrier platform, VNPs have a number of appealing qualities, such as biocompatibility, morphological homogeneity, biodegradability, ease of surface functionalization, intrinsic mono-dispersity, and availability in a range of sizes, shapes, and functions [31]. VNPs are able to meeting the demands of drug nanocarriers including biocompatibility, hydrophilicity, and improved drug entrapment expertise and also thanks to the flexibility of chemical and genetic alterations that may be made to their surface. Because they are simple to produce with excellent precision and enable the protein shell to contain medicines in high concentration. Virus-like particles (VLPs), a subset of VNP, can spontaneously assemble into a cage-like structure and are non-infectious proteins because they lack a viral genome. VLPs are useful for delivering targeted drugs, siRNAs, proteins, peptides, and other molecules. Representation of VNPs is shown in Fig. 14.6.

The development of VNP drug delivery systems reduces the harmful effects of medications since the targeted cells may be more effectively reached. As a result, the immune reaction would be less pronounced and a better environment

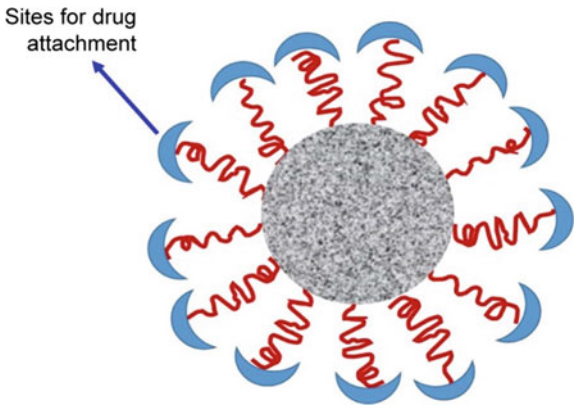

Fig. 14.6 Representation of VNPs. Reproduced from [5], Copyright (2017) with permission from Dove Press

would be created for interacting with human cells. VNP has numerous uses in bio-nanotechnology due to its immunogenicity and versatility. The promise of this nanocarrier system in nanobiotechnology is highlighted by the focus on designing and creating it. These carriers have a generally positive influence on toxicity and unfavourable pharmacological effects. These drug carriers develop interactions inside a particular biological area and emerge from a complicated environment [32].

14.1.2 Inorganic Nanocarriers

14.1.2.1 Carbon Nanotubes (CNTs)

Carbon nanotubes have developed as one of the most sophisticated nano-systems for proficient drug distribution in biomedical sciences and also in bio-nanotechnology due to their interesting physical and chemical properties, with their unique architecture and high loading efficiency, and controlled release rate. Because of their ordered structure, extremely light weight, excellent electrical and high thermal conductivity, and increased upper surface area, carbon nanotubes are regarded as potential drug delivery vehicles. The fact that these delivery systems are not soluble in any particular solvent makes them potentially harmful to human health. However, by making these tubes chemically different and introducing additional functional groups, they become water-soluble and can be employed again for delivery of many different active therapies, including proteins, peptides, nucleic acids, as well as other active molecules. CNTs are made up of one or more graphene sheets wrapped up into a hollow cylinder like single-walled CNT/multi-walled structure based upon the number of graphene layers and belong to the fullerene family. CNTs with magnitudes in the choice from 50 to 100 nm are simple to be swallowed [33]. They can trigger an immunological response since they are carbon-based and interact with any of the immune cells, which will stop the growth of the tumour. To target colon cancer cells, for one, fluorescent

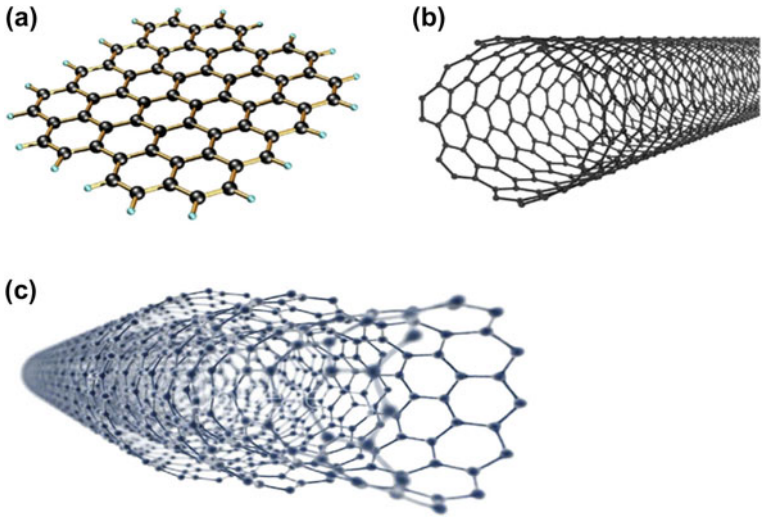

Fig. 14.7 Depiction of **a** Graphite sheet, **b** SWCNTs, and **c** multi-walled CNTs. Reproduced from [34]**,** Copyright (2019), with permission from Elsevier

single-walled CNTs with mAb enclosing doxorubicin are utilized. Doxorubicin is released intracellularly when such CNTs are effectively absorbed by cancer cells, but the CNTs themselves are kept in the cytoplasm. Figure 14.7 Depicts Graphite sheet, SWCNTs and multi-walled CNTs.

For advantages, as we know, carbon nanotubes are strong, very elastic, and highly flexible. They are used as biomedical sensors and also applied in kidney dialysis. Due to its one-dimensional form and extremely large surface area of SWCNTs, drugs delivery can be done efficiently. As for MWCNTs they can be applied in the thermal cancer treatment because after being exposed to near-IR light, these type of CNTs emit a significant amount of vibrational energy that causes localized heating and kills cancer cells [34]. Its preparation can be done by mainly methods like chemical vapour deposition, arc discharge, laser ablation, etc. [35]. According to a study by Yan et al. (2014), carbon nanotubes which were injected into the tissues around a tumour had no adverse side effects on humans, making them a promising nanotechnology for targeted delivery of medications to any tumour tissue. Their characteristics could aid in active targeting in addition to helping them distribute a single or a number of drug units. Prior to recommending carbon nanotubes for normal therapeutic usage, it is crucial to have a thorough grasp of both their pharmaco-toxicological qualities in humans and their numerous appealing features [36]. The characteristics of nanotubes and, subsequently, their use, depend on a number of variables, including size, structure, and form. CNTs can now be used in variety of applications because of their functionalization. One such use that has improved the accuracy of the treatment of cancer and other diseases is drug delivery systems. With

the expansion of research, there are more alternatives for CNTs' usage in medication delivery.

14.1.2.2 Mesoporous Silica Nanoparticles (MSNs)

Because of its cheap synthesis techniques and permeable architecture, silica nanomaterials have seen a surge in medicinal and nanomedicine implications. MSNs allow maximum number of medicines to be loaded, boosting drug accumulation in tumour tissues through passive targeted delivery. MSNs have several appealing characteristics, including strong biocompatibility, a large surface area, and high loading. Although practical transmission of MSNs is still a difficulty, their unique features demonstrate that they are an effective tool for creative biological applications. The problem can be resolved by more effectively treating cancer cells utilizing chemotherapeutics thanks to the special properties of MSNPs, which have a greater surface area enables relatively high loading. Due to its special characteristics, such as the capacity to adjust pore size, high porosity, and morphology, which can significantly impact the mechanism and pattern of drug release, MSNs are the relevant vehicles as drug/gene drug carriers [37]. They are widely utilized in immunotherapy. In a study, camptothecin-loaded MSNs were successfully taken up by colorectal tumour cells. The ability of MSNs to easily surface functionalize for precise and targeted delivery also allows them to improve therapeutic potential and lessen medication toxicity. Large amounts of anticancer drugs can be loaded into MSNs due to their mesoporous structure, which also helps them accumulate in tumour tissues through passive targeting. Additionally, MSNs' suitable surface modification with various site-specific targeting mediators allows them to actively target tumour tissues. The three processes that make up the synthetic pathway for the production of MSNs are: (i) the sol–gel process, that further comprises the hydrolysis & condensation of the silicon alkoxide catalysts under acidic or basic catalysis to produce silica, (ii) the use of surfactants as framework agents to create mesoporous materials and (iii) a modified version of the Stöber method carried out in diluted conditions to produce nanospheres [38]. Figure 14.8 represents mesoporous silica nanoparticles.

The porous nature of MSMs creates voids which can hold and release any wide range of medicinal chemicals and biomolecules. MSNs are increasingly being used as controlled release drug administration nanocarriers due to their adaptability in terms of size, shape, and texture. In this way, MSNs can be created with variable particle dimensions, pore diameters, porosity, incorporated magnetic NPs, or even grown from distinct metallic nanoparticles cores. Through the derivatization of the silanol groups at the interface of the matrix, the composition of the MSNs' surfaces could be easily adjusted. To covalently bind practically any of the functional groups, numerous surface modification techniques have been documented in the literature. Thus, it is possible to customize the host–guest interactions as intended, enabling the fabrication of adaptable nanocarriers. The literature claims that silica-based nanoscale particles are sufficiently biocompatible for use in biological applications. MSNs display clearly defined structures with high targeted surface areas and large pore

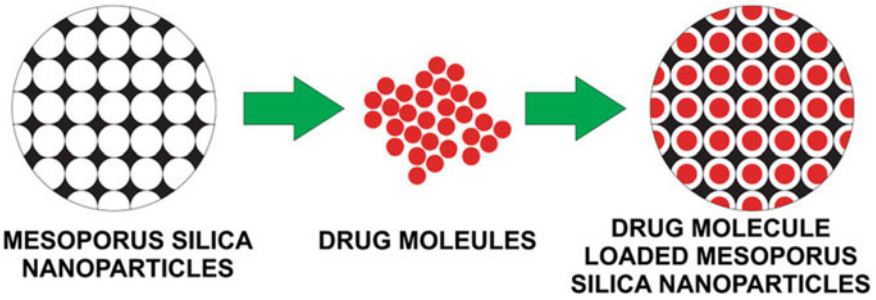

Fig. 14.8 Mesoporous silica nanoparticles. Reproduced from [38], Copyright (2020), with permission from Bentham Open

sizes, which change biological processes as compared to non-porous silica NPs. It's true that these nanocarriers possess many advantages, but they also have some advantages which includes challenge in the preparation of well-ordered, irregular size distribution, development of stable colloidal suspensions [39].

Bulk MSMs' excellent textural qualities and potential for biomedical use were the driving forces behind their development in the nanoscale realm. MSNs were created and are being studied as medication delivery vehicles by numerous researchers across the globe. The principal biomedical applications of MSNs that have been proposed have included therapy of various bone disorders, cancer, and contagious diseases. Since many different pigments and contrast agents can indeed be inserted into MSNs' pores, MSNs may also be utilized as useful image processing agents [40]. Overall, it is clear that the development and creation of MSNs for biomedical field have advanced significantly. However, it is clear that much effort must to be undertaken before clinical adaptation might be accomplished.

14.1.2.3 Quantum Dots

Inorganic nanostructures known as QDs have broad luminous excitation spectra and compact symmetrical excitation spectra that experience significant Stokes shifts. QDs are made of a covering layer to prevent photobleaching and leakage and a metallic core substance that can propagate fluorescence. These carbon QDs are quasi-spherical nanoparticles that can be made using either a top-down or bottom-up approach. The multiple carboxyl components mounted on the surface of CDs are responsible for their extraordinary capacity to either acquire suitable reactive chemical species for complexation or to connect with various inorganic materials, organic materials, polymeric or natural materials for surface passivation. Surface passivation enhances fluorescence while surface modification improves solubility including both aqueous and non-aqueous fluids. These characteristics of CDs put them in a good position to deliver drugs more effectively than before for numerous of applications, among them include electrocatalysis, cell imaging, biosensing, and photosensitizing

treatment. These CDs have been further divided into two categories: man-made CDs and natural CDs (NCDs), which are created using natural ingredients including rice husks, milk, pepper, honey, and curcumin. Due to their wide availability, low cost, safety, and capacity to transform low-value biomass into valuable and beneficial material, NCDs are becoming more and more popular. Natural carbon-based quantum dots are a new type of carbon-based nanomaterials. When related to other common carbon quantum dots, NCDs have received a lot of praise from researchers due to their abundance, eco-friendliness, water solubility, broad functionality, and biocompatibility. Different functional groups, such as thiol, carboxyl, hydroxyl, present on the surface of NCDs offer increased quantum yield and optical properties that support bioimaging, detecting, and drug administration. Figure 14.9 depicts physical state of QD nanoparticle.

Both for management and for cancer diagnosis, QDs offer a lot of potential. Nanocomposites composed of lipids, polymers, or metals, as well as semiconductor QDs, have been created with interesting possibilities in anticancer drugs and early identification. Currently, there aren't much evidence exist on the use of QDs in

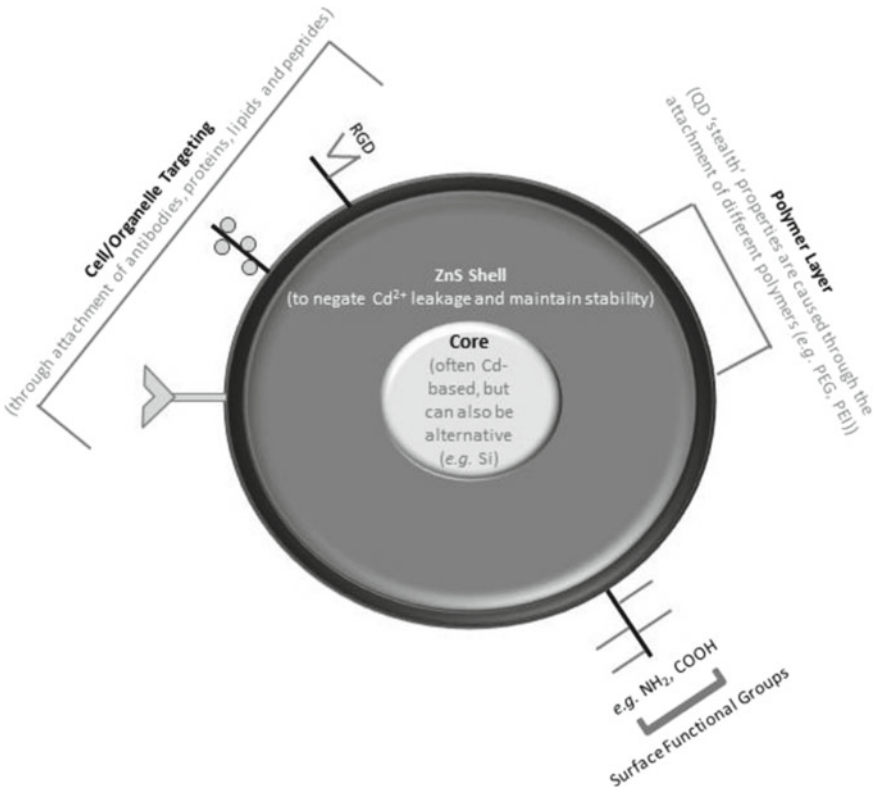

Fig. 14.9 Representation of physical state of QD nanoparticle. Reproduced from [44], Copyright (2012), with permission from PMC

whole-body radiography. Semiconductors containing cadmium as a key component are what give quantum dots their wonderful optical properties. The toxicities of these cadmium-containing quantum dots in normal living cells have not yet been thoroughly investigated, despite the fact that cadmium is likely detrimental. Therefore, it is now of advanced importance to search for non-toxic chemicals with equivalent targeting and optical qualities. For ionizing radiation therapy to effectively treat cancer while minimizing exposure and harm to nearby healthy cells, high precision is necessary. Lately, significant investigation has focussed on creating QDs based on photosensitizers that produce free radicals when bare to visible light waves. Even if visible light is safe, the approach is nevertheless useful to treat only superficial lumps. Their exceptional photophysical qualities and, occasionally, versatile faces are suitable for a variety of biomedical application. With their distinct and steady fluorescence, absorbance and emission spectra, low photobleaching, and steady fluorescence, QDs represent the potential application for medical diagnostics [40]. QDs were employed as detectors for a fair amount of time and have demonstrated their effectiveness not simply in in vitro research but also in both vivo and clinical trials. QDs can be customized with ligands and certain other chemical components on their surface, similar to any other nanoparticle, which will in some manner increase their functionality. The efforts to transition the findings from scientific studies to clinical adaptation have been moving in the correct way, and QDs hold enormous promise for use as therapeutic and diagnostic tools [41].

14.1.2.4 Magnetic Nanoparticles

Magnetic nanoparticles (MNPs) are a novel type of nano-magnetic material, which is not only has distinct implication in physical speculation, but also takes a wide range of implementations in the field of biomedicine. Nanoscale structures of magnetic particles have sizes that range from 1 to 100 nm. MNPs typically have a surface coating next to the active coat and a central magnetic core. MNPs are typically magnetic composite materials made of metals and their oxides, such as iron, nickel, cobalt, and others. Typically, X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy are used to characterize the structural properties of MNPs. The following traits apply to magnetic nanomaterials: The magnetic features of nano-metal particulates make them pretty simple to enrich, separate, transport, and discover navigations. MNPs have such a magnetocaloric action in a high-frequency magnetism, which might subtly destroy tumour cells. They have enormous specific surface areas and can contain a range of tiny chemicals, proteins, RNA, etc. [42]. Due to their distinctive optical, electrical, magnetic, and physicochemical features, magnetic nanoparticles have acknowledged a lot of interest in the past. MNPs are the perfect candidates for surface nanoengineering and the creation of useful nanostructures due to their size. Due to their changes, MNPs can be used as the foundation for a wide range of pharmaceutical and medical applications, including drug delivery and diagnostics, and they hold particular promise for cancer therapy. A number of MNPs are presently undergoing preliminary clinical studies, and several

formulations have already received clinical approval for use in pharmaceutical and therapeutic imaging procedures. In order to address the issue of the body's lack of an effective transport mechanism to carry drugs to the nidus, MNPs as a drug delivery system have attracted a lot of interest [43].

The bio characterization or lethality of MNPs is affected by a number of variables, including the coating, size, and type of the core material. Magnetic nanoparticles made of Ni and Co are harmful because they can be eroded and oxidized by acids. Contrarily, iron oxide nanoparticles are frequently used in biomedical applications due to their strong chemical permanence, mechanical rigidity, and frequency modulated magnetic characteristics. In order to increase the effectiveness of magnetic nanoparticles, a variety of innovative coating and bioactivity procedures are required, either to improve biocompatibility or prevent phagocytosis by RES using surfactants, inorganic compounds (such silica, gold), polymers, and biomaterials [44]. Applications of magnetic nanoparticles (MNPs) for targeted medication administration heavily rely on many factors relating to the size as well as magnetization of the compatible nanoparticles. When combined with an external magnetic field, MNPs are capable of getting around standard DDS problems and transport the API to the desired target location while holding the nanoparticles in place even during drug release. A number of other manufacturers have also entered the market, and magnetic drug delivery is a viable technology for the treatment of cancer. Internal magnets positioned close to the target through minimally invasive surgical procedures will be used to get over the limitations associated with the use of magnetic fields from outside. Future studies have a good chance of realizing the enormous opportunity of MNPs in medical speciality sectors, nevertheless [45]. Superparamagnetic iron oxide NPs linked to LHRH are useful for diagnosing and directing breast cancer. In addition, magnetic hyperthermia uses magnetic NPs to thermally destroy cancer cells. Feridex® and Resovist®, MNPs used to treat colorectal cancer and hepatic metastases, respectively, are two examples of MNPs that are currently available or in clinical trials. The in vivo behaviour of MNPs will then be made clearer as technology advances, while at the exact same time, more complex and useful MNPs can be created. We should point out that even a slight boost in efficiency could spur additional research and method development, which would be beneficial for both the ecology and healthcare [46].

14.1.3 Organic/Inorganic Hybrid Nanocarriers

The advantages of organic and inorganic materials have been combined to create organic/inorganic hybrid nanocarriers. To improve the selectivity and effectiveness of anticancer medicines, certain features of organic components at the exterior of NPs (inorganic ones) were utilized. In a sizable body of literature, the numerous benefits of using hybrid nanoparticles as adaptable nanocarriers for various medications are stressed. By covering the exterior of PLGA nanoparticles with a mucoadhesive polymer called Chitosan, Chakravarthi et al. examined the hybrid chitosan-PLGA

derived particles as a carrier for Paclitaxel with possible applications in the breast cancer treatments. The mucoadhesive characteristics of chitosan were responsible for the 4–10 times upsurge in cellular attachment and a substantial improvement in the cytotoxic effects of hybrid chitosan–PLGA particle loaded with Paclitaxel versus 4T1 cancer cells. The concept of a hybrid formulation through a novel design that combines a vegetable oil with a biodegradable polymer like poly-lactic-co-glycolic acid (PLGA) (NSO) is an example. This study also emphasises how NSO affects the ultimate characteristics of planned systems, including stability, topology, encapsulation/release aspects, and pharmacological activities. Since Izohidrafural (IHF) is a lipophilic stimulant, two properties of NSO were investigated: first, as a key element of the nanocarrier composite, which can develop the ideal micro-environment for incorporating a lipophilic drug; and second, as a potential therapeutic agent, particularly for its antibacterial activity [47].

The most reducing lipid-polymer hybrid nanocarriers (LPNs) for drug administration recently appeared as operational liposomes customized with adaptable polymer with cell-based-biomimetic nanomaterials. Cell-based biomimetic nanoparticles and liposomes modified with polymers are useful for enhancing absorption, controlling release, focussing on and combating multi-drug resistance, and minimizing adverse reactions. Polymer-modified cell-based biomimetic NPs and liposomes are helpful for focussing on and overcoming multi-drug tolerance, minimizing side effects, and improving uptake [48].

A more fascinating development in recent years has been the transition from multi-component, specialized NPs that cross the boundaries of organic chemistry or inorganic chemistry, biomimetic chemistry, and molecular genetics to reasonably simple nanocarriers made from common material categories. The PLGA-lipid hybrid NPs that have been created so far possess a large loading capacity, exceptional biological microenvironment stability, and effective in vivo activities. The hybrid compositions are the most researched carriers due to their versatility as well as ability to be created as co-delivery and otherwise multifunctional systems, creating a brand-new path of "smart instruments" in the creation of innovative, non-invasive arising nanomedicine well with potential to significantly raise patient health outcomes. Another design-related issue that needs to be resolved soon is the difficulty in foreseeing the PLGA-lipid hybrid nanocarriers' usefulness and performances in preclinical and clinical studies. Two major obstacles must be overcome for a hybrid methodology to be successfully designed: first, a thorough analysis of the crucial traits of each component that makes up the hybrid model; and second, a thorough comprehension of how the hybrid nanocarrier interacts with cells to anticipate potential toxicity problems and guarantee its secure clinical usage. As a result, the application of and research into clinical nanocarriers will remain to be an exciting and lucrative area for medical and academic settings in addition to businesses. The continued development of these hybrid formulations, together with the deep expertise of the researchers in the field, will significantly alter and enhance disease treatment and diagnosis and especially their quality of life [49].

14.2 Targeting Mechanisms

The non-specificity, resistance, and systemic toxicity of therapeutic medicines are the biggest challenges in medication delivery. Conventional chemotherapy treatments have harmful and adverse impacts on healthy tissues because of their lack of selectivity, even if they kill tumour cells with such a high level of effectiveness. Nanotechnology, however, offers a new option for tumour focussed therapy [50]. The key properties of a micro-based drug delivery scheme is that medicine can target the cells selectively and specifically. The main goal of using chemotherapeutic medications to target malignant cells is to maximize the lethal effect on cancer cells while minimizing the detrimental consequences to the system. To specifically target malignant cells, researchers are constantly working to modify various medications with the use of nanocarriers. Drug targeting is improved by nanoparticles because they bind or absorb the drug at their surface. Since nanoparticle can be directed in many responsible for transporting at the target region, targeting medications using nanoparticles has huge benefits above other delivery techniques. In contrast to traditional chemotherapy, tumour targeting with nanoscale carriers and other therapeutics has several important advantages, including the potential to be quickly metabolized, to passively collect in tumours, to proactively target cancerous cells, and to traverse biological membranes inside the body. Nanocarriers can be used to target cells in two ways: passive and active targeting which can recognize cancer tissues of complex life forms more precisely and deliver them at harmful and adverse effects on healthy cells are less in malignant tissues [51]. Design of passive tumour targeting and active tumour targeting by nanocarriers is represented in Fig. 14.10.

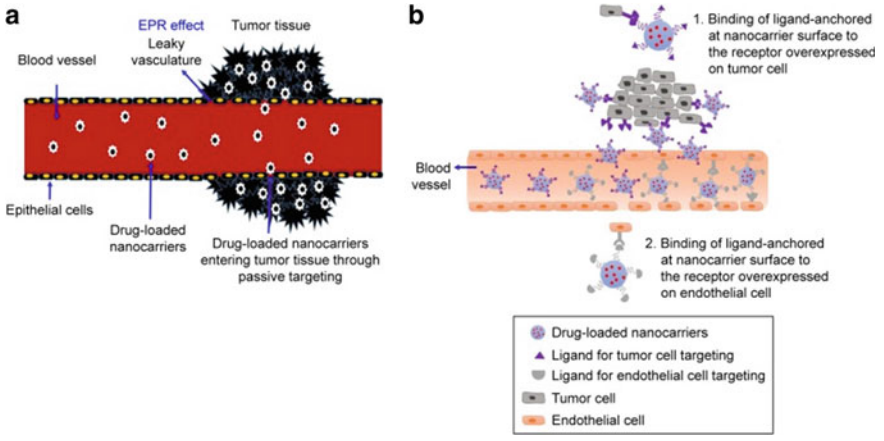

Fig. 14.10 Design of passive tumour targeting (**a**) and active tumour targeting (**b**) by nanocarriers. Reproduced from [5], Copyright (2017), with permission from Dove Press

14.2.1 Passive Mechanism

The most popular preclinical strategy for facilitating the optimal entry of medication into tumours is passive targeting. Drug-carrier complexes or drug delivery routes that can transport the drug straight to tumour cells or matters are often used in passive targeting. The magnitude of nanomedicines and the behaviour of tumour tissue motoneuron play an essential part in passive targeting of cancer nanotherapeutics. Because developing tumour cells have a higher metabolic demand, already existing blood vessels are bare to compression which causes angiogenesis, the formation of new ducts around the tumour. Nanomedicine accumulation in tumour tissues is influenced by interstitial liquefied pressure, which is higher in tumour tissue than in ordinary tissues. Interstitial compression is more in the centre segment and decreases in the periphery, which is in authority for causing drugs to leak out of cells, resulting in drug restructuring in cancer tissues. Nanomedicine build-up in proliferating tissues is inclined by their size, superficial properties, and motion half-life.

Gathering of drug carriers is accomplished by the singularity which is named as the EPR effect. The first phase of EPR effect or enhanced permeability, is distinguished by anomalies in tumour blood vasculature that led to increased vascular permeability. Tumour tissue lacks or has insufficient lymphatic outflow compared to healthy tissues, resulting in inefficient lymphatic drainage from tumour tissue. This situation causes drug delivery schemes to accumulate in the tumour spot, a condition known as increased retention. Drugs released by drug carriers accumulate in tumours, inflammatory regions, and infarcts due to their extravasation through leaky vasculature [52].

The magnitude and form of nanocarriers, besides their charge and surface hydrophobicity, are the major physicochemical factors that will influence the EPR effect. The size of tumour endothelial cell linings varies by tumour type and ranges from 100–700 nm, which is 50–70 times bigger than the regular and distinct endothelium (equal to 10 nm).According to various studies, sub-100 nm sphere-shaped nanoparticles have a higher uptake potency than other forms such as bars, cylinders, and blocks. When the size of nanocarriers was greater than a hundred nanometre, rod like nanoparticles had best acceptance potency among nano-sized particles of other forms. The sub-100 nm spherical nanocarrier were widely explored for nanoscience [53].

The surface charges of nanocarriers can have a major impact on phagocytosis and blood circulation. Negatively nanoparticles have a casual impact on NP blood clearance, whereas positively charged nano-particulates are usually acknowledged as taking a detrimental outcome in vivo stability after plasma revelation. As a consequence of the electrostatic affinity with the anionic cell sheath, positively charged nano-particulates are absorbed by cells more quickly than neutral or negatively charged ones [54]. It was, however, more susceptible to clearance via the reticuloendothelial system (RES), which reduced blood circulation period.

Surface hydrophobicity, in addition to particle dimensions, shape, and charge standing, is a big component in EPR-dependent drug transport. When hydrophobic

nanoparticles join the bloodstream, they are more probable to be coated by specific proteins such immune serum globulin and blood plasma proteins, which are then removed by RES, lowering the competence of drug administration for tumour cure. To fix this issue, nanoparticles were wrapped by means of vastly biocompatible amphiphilic molecules, such as poly ethylene glycol (PEG) and its derivative, which were coupled to the exterior of produced nanocarriers to increase the EPR effect while reducing RES clearance [55].

Polymer-based nanostructures are one type of advanced vesicular nanocarrier that has earned a lot of consideration in recent years because of their exceptional biocompatibility and effortlessness of use; some of them can even breakdown spontaneously in biological environments. As a result, it has been broadly researched as an enhanced drug transport vehicle [56]. For case, poly-lactic glycolic acid (PLGA) was a most implemented biomedical polymer that could not only self-gather into a polymer afterwards being modified with PEG, but self-degrade in vivo. Langer et al. provided a notable example of PLGA-based DDS, wherein they observed that lading the drug into the polymer reduced drug accumulation in the liver also vastly increased blood flow period [57].

The most damaging problems of passive tumour targeting that must be overlooked include exaggeration or misapprehension of the EPR effect, variations amongst the animal prototypical and human affected role, and shortened infiltration of the nanomaterials into the beset tissue and malignancies. In summary, the pairing of the tumour's distinct structure as well as the nanoparticle's design allows anticancer drugs to passively target one specific tumour. Additionally, tumour-associated cells, which function as a reservoir, might cause nanoparticle accumulation. This would guarantee a steady and progressive distribution of the active ingredients, extending the time a tumour is vulnerable to the anticancer medication. Enhancing the carrier's size, accounting for changes in vascular permeability, undertaking a literature review mostly on carrier, and characterizing the histopathological characteristics of each tumour will be necessary for improvements in tumour targeting. This will enable the rational and realistic style of drug delivery mechanisms.

14.2.2 Active Mechanism

One of the main barriers to targeting drug-loaded nanocarriers to tissues other than the spleen and liver is non-specific absorption by RES. The ability of nanocarriers to prevent RES absorption is crucial for achieving the prolonged blood circulation period needed for effective medication delivery. Additionally, passive targeting is primarily useful for effectively localizing nanoscale carriers in the tumour microenvironment. Cancer cells are not able to internalize it very well. As a result, active targeting techniques for nanocarriers are all being investigated [58]. In order to improve the transport of nanoparticle systems to tumour site, active cancer targeting makes use of the attaching targeted moieties. By maintaining their interaction well with targeting ligands, cancer cells' abundantly expressed surface receptors are

utilized through active targeting. The receptors present on tumour cells are easily attached to by the ligands, which can then facilitate the attachment and aggregation of nanoscale particles within the tumour location via receptor-mediated endocytosis, where the drug can subsequently be administered for the healing effect. Targeting precision and delivery efficiency are the two key variables in assessing the effectiveness of active targeting. The structure and chemistry of a nanoscale particle directly affect its capacity for delivery. The conjugation of targeting molecules, such as antibodies, ligands, peptides, polymers, nucleic acids, and others, on the surface of nanocarriers with receptors is an important process. Tumour-targeting compounds on nanocarriers bind to tumour tissues by an endosome-dependent pathway, bypassing the drug efflux pump and resulting in high intracellular absorption. Physical, chemical, and biological factors affect the drug-carrier complex's distribution pattern, permitting it to be acknowledged and accessed only through a certain bio site [59]. Surface amendment of nanocarriers with site-precise directing ligand allows active targeting of specific tumour tissues. The targeting ligands can bind to specific receptors that are vastly articulated selectively by tumour vasculature [60]. Minor molecules, antibodies, antibody fragments, glycoproteins (transferrin), peptides, vitamins, growth aspects, and also nucleic acids are some of the targeting mediators typically utilized to boost the spot specificity of nanocarriers [61]. Nanocarriers with a high surface area to volume ratio can bind several targeting groups, allowing for better targeting of certain malignant cell types. Active tumour targeting is used to lessen chemotherapeutic agent off-target delivery while simultaneously avoiding the limitations of passive tumour targeting and overcoming multiple drug resistance [62]. The intended target receptor obliged to be expressed uniformly in all target cells for active targeting to work, and the specified targeting fraction must only fix to a receptor overexpressed by means of tumour cells. Although there are a number of active targeting tactics being used, they can be effectively grouped into two sub categories. First, there is receptor-mediated endocytosis, in which drug-loaded nanoparticles are interface passivated with unique ligands to determine the specific receptor in the tumour regions for its interaction and consequent cellular absorption. The second method is stimuli-responsive intracellular drug delivery, which depends on a minute alteration in the diseased area's microenvironment before the cargo is released.

In active targeting, the ligand-decorated nanocarrier is directed to either the tumour cells or the tumour microenvironment. Both of these cellular targets have recently received a lot of interest in the arena of active targeting in order to intensify efficacy and diminish the harmful effects of chemotherapeutical drugs. The active targeting of tumour cells employs ligand-receptor interactions to target overexpressed cell surface receptors with ligand-anchored nanocarriers. Following that, receptor-mediated endocytosis (internalization) enhances nanocarrier uptake by cancer cells, resulting in increased intracellular medication concentration with no or minimal tumour cell development [63]. Folate receptor (FR), TfR, epidermal growth factor receptor (EGFR), and cell surface glycoproteins are the most frequently targeted cell superficial receptors expressed on malignant cells in different kinds of cancers. One of the tumour markers may be overexpressed on the surface of a specific

tumour type's cells. Because Folate receptor is found to be highly expressed in breast, lung and colorectal cancerous cells, folate-modified nanocarriers can be used to target these cancer cells. Transferrin can also be used as a ligand for selective administration of anticancer medicines into the brain because TfRs are extensively expressed on the surface of endothelial cells of the blood–brain boundary [64]. In contrast to the previously mentioned technique, actively targeting the tumour vasculature (tumour endothelium) has huge advantages. Targeting the tumour vasculature entails the elimination of tumour vascular endothelial cells, which blocks oxygen and nutrition supply to tumour cells, resulting in cancer cell proliferation. Targeting a variety of molecules on the vascular barrier, such as vascular cell adhesion molecules (VCAMs), $\alpha_v\beta_3$ integrins, matrix metalloproteases and vascular endothelial growth factors (VEGFs) could be used to enable active tumour vascular targeting with ligand-attached nanocarriers [65]. Here are some examples of active tumour targeting by means of diverse nanocarriers that have been surface modified with site-specific ligands. Choi and Park [66] published a work in which they developed docetaxel-loaded nanocrystals with transferrin surface functionalization to give them active tumour-targeting properties. In comparison to docetaxel nanocrystals and pure drug, transferrin-modified docetaxel nanocrystals showed greater intracellular absorption and cytotoxicity in the human lung A549 carcinoma cells. Apatinib-loaded liposomes stayed physically modified with the targeted ligand cyclic arginyl glycyl aspartic acid (cRGD) to precisely recognize the $\alpha_v\beta_3$ integrin [67] in some other study. In comparison to nontargeted liposomes, these cRGD anchored liposomes displayed enhanced cellular absorption and tumour growth suppression in the human gastrointestinal tract HCT116 cancer cell type. There aren't any actively targeted nanoparticles on the market at the moment, but a some are being used in clinical research, including liposome targeted and polymeric nanoparticles. Phase I/II new treatments are confirmed to be ongoing for MBP-426, MCC-465, SGT53, MM-302, BIND-014, CALAA-01, cetuximad-decorated Doxil/Caelyx liposomes, and a retroviral vector. Some of the principal drug applications of these nanoscale particles are HER-2, PSMA, the exterior of gastric cancerous cells, as well as the vascular endothelial growth factor. In numerous investigations, both in vitro and in vivo, active targeting nanostructures of chemotherapeutic medications or conventional/herbal medicines have been shown to increase selectivity of intracellular delivery of pharmaceuticals to cancer cells by receptor-mediated endocytosis and/or cytotoxicity. In comparison with traditional chemotherapeutic medications and nontargeted nanoparticle platforms, they offer a number of benefits, especially in terms of improving treatment efficiency and safety. In cancer therapeutics, active targeting nanoparticles have a number of benefits, including improved drug specificity to cancer cells to prevent adverse effects on normal cells, improved drug storage and anticancer potency in tumour cell, and effective drug distribution control. However, active targeting nanoparticles have some drawbacks, including as their restricted clinical application to malignancies that express particular receptors [68]. The difference between two types of drug targeting mechanisms is shown in Table 14.1.

Table 14.1 Difference between two types of drug targeting mechanisms to tumours

Active targeting	Passive targeting
Active targeting includes alteration or alteration of the drug delivery carriers to revaluates its bio fate	Passive targeting arises because of the body's expected response to the biological properties of the drug carrier system
Quite selective	Least selective
Highly versatile	Limited in use
Folate, transferrin, and galactosamine are examples of targeting ligands that are commonly employed to actively target nanomedicine formulations to tumour cells	Liposomes, antibodies, micelles, nanoparticles and polymers are all examples of nanomedicine applications

14.3 Specific Tumours and Relative Nanocarriers

In all cases, tumours are primarily brought on by mutations or other defects in the tumour suppressor genes, which prevent cell growth and death, or in the proto-oncogenes, which control cellular proliferation. The emergence of malignant cells with their distinct characteristics of unchecked cellular metabolism, inability to stop abnormal cell division, lack of apoptosis, and capacity to penetrate nearby and remote tissues is caused by these altered genes. Radiation, chemicals, physical allergens, inheritance, and viruses are among the risk variables for genetic alterations. Hanahan & Weinberg defined the cancer cells primarily by six distinguishing characteristics known as the hallmarks of cancer. They include maintaining proliferative signal transduction, dodging growth inhibitors, fending off apoptosis, having an infinite capacity for replication, causing angiogenesis, and triggering tumour invasion and metastasis [69]. One of the most lethal illnesses and a major cause of death worldwide is cancer. Yearly, 7.6 million people worldwide pass away from cancer, accounting for 13% of all fatalities. Due to chemotherapeutic medicines' non-selective action on normal cells, the majority of patients receiving conventional chemotherapy experience substantial side effects [70]. Chemotherapy is still chosen as a therapeutic choice among all the conventional ones, although it has a number of drawbacks, including poorer success and poor survival. This is mostly due to the lack of specificity for tumour cells over surrounding cells, which results in insufficient medication concentrations in tumours and cancers that are resistant to treatment. One of the main challenges that researchers and healthcare practitioners have encountered in the sphere of therapy is the absence of a specific therapeutic objective. Targeted cancer therapy has become increasingly popular during the recent decade as a new anticancer treatment. Increased drug accumulation at the tumour location, greater solubility, greater colloidal stability, and improved cellular internalization are just a few of the eye-catching benefits offered by nanocarriers-mediated targeted drug delivery. Due to their nanometre dimensions, vast surface area, and capacity to be functionalized with targeted ligands, medicinal compounds, and passivating agents, nanocarriers are able to achieve these properties. Additionally, nanocarriers

can quickly access various body parts without interfering with their usual operations. Drug particles are often bound, conjugated, or enclosed among nanocarriers and delivered at the target site in response to changes in the surrounding environment, like pH, temperature, light, and ultrasound, among others. As a result, the surface chemistry of nanocarriers is crucial for both the targeting of active drugs and other biomedical applications [58]. Due to their widespread incidence and high death rates, three different types of malignancies—breast, pancreatic, and lung tumours—have been chosen for this analysis, and their uses of respective nanocarriers in these tumours are also covered.

14.3.1 Breast Tumour

Breast Tumour is the most normally detected cancer in women, and it is also the one that causes the most fatalities. It is also termed prostate melanoma which initiates from breast tissue. According to the GLOBOCAN study, there were 1.71 million new breast cancer incidences and deaths around 522,000 globally in 2012. Although the fact that contemporary treatments often provide excellent initial results, 13% of patients experience locoregional relapse within nine years after primary therapy, with 25% patients having distant metastatic pattern at the period of relapse. 60% of individuals with a confined breast lump have a distant, progressive stage malignancy. Standard treatment for these individuals includes neoadjuvant chemotherapy, surgical resection, radiotherapy, and further adjuvant. Breast cancer is a diverse illness with many subtypes depending on HER-2/neu receptor (human epidermal growth factor receptor 2), progesterone receptor and oestrogen receptor expression levels. Breast malignance stem cells show a key function in metastatic breast cancer development and creation [71].

Now, nanotechnology has come up with a promising solution to the breast cancer problem. Many researchers are interested in the mechanisms of action of various nanotechnology-based medication delivery systems. Breast cancer is detected using a variety of nanoparticles, the most popular of which are carbon nanotubes, carbon nanorods, nanowires, and nanobarcodes. Semiconductor quantum dots are a novel nanotechnology innovation; they are nanoscale light-emitting units that outperform fluorescent protein and organic dyes. Semiconductor quantum dots have unique electrical and optical features that make them ideal for biomolecular imaging in cells.

14.3.2 Lung Tumour

With 1.8 M new cases and also 1.5 M deaths worldwide, lung cancer was prophesied to be the most usually identified malignancy and the main cause of death due to cancer among men. It is one of the most deadly malignancies. It has an overall fatality to

frequency ratio of 0.87. The poor prognosis at advanced stages of the disease, as well as metastatic or resistant tumour cell populations, highlights the need for translational transdisciplinary therapies like bio-nanotechnology [72].

The great majority of tumour fatalities are caused by lung malignant cells spreading to secondary place and vice versa. This holds a substantial problem in cancer treatment. One of the biggest issues with current lung cancer treatment is its inefficiency and specificity. To achieve acceptable efficacy and fewer side effects, it is constantly necessary to advance site-specific and targeted medicines. Nanomedicine technology has recently gained a lot of attention as a next-generation anticancer medicine based on integrated imaging & therapeutic responses. Various bio-nanotechnology-derived tools have recently sparked a lot of scientific interest in lung cancer treatment. The most commonly studied bio-nanocarriers for oncologic developments are: macrobiotic nano-systems and bio-nanocarriers, Cell and cell membrane-derived nano-systems, ligand-conjugated nano-systems, and nano-bio-devices.

Sensor-based golden nanoparticles were produced and utilized to identify lung cancer. They were utilized to distinguish lung cancer patients' breath from that of hale and hearty people in a tropical environment. Sensor-based NPs were low-cost, non-invasive lung tumour indicative instruments. Correspondingly, PLGA-based antineoplastic medication-loaded NPs by an typical particle size of two hundred nm were produced, resulting in improved antineoplastic activity in lung tumours. Furthermore, poly (butyl cyanoacrylate) NPs containing doxorubicin were efficient against lung tumours. Liposomes have also been used as nanocarriers for several medications in lung tumour targeting. In vivo and in vitro, the liposomal invention of 9-nitrocamptothecin delivered by atomizers proved particularly efficient against metastatic lung cancers. Patients were given interleukin (IL)-2 liposomes by inhalation, which proved to be quite helpful against pulmonary metastases.

GNPs made of gelatin were also employed to transport hydrophilic & hydrophobic anticancer medicines such as, cisplatin, methotrexate, curcumin, paclitaxel, and resveratrol. GNPs also had higher anticancer activity, longer drug release, and little harmfulness in cells. Doxorubicin-loaded nanoparticles containing gassy molecules as an excipient remained recently produced. These effervescent doxorubicin-containing NPs accumulated deep lung deposits and were largely distributed throughout the lung, with little accumulation in other tissues and methotrexate organs.

14.3.3 Pancreatic Tumour

Pancreatic Tumour is the fourth-largest cause of cancer death in both male and female, with an estimated 53,670 new cases and 43,090 fatalities in the United States in 2017. Pancreatic tumours are immunosuppressive and desmoplastic. Intrinsic physical and metabolic stressors in pancreatic tumours limit drug delivery and distribution, resulting in increased interstitial fluid pressure, vascular compression, and hypoxia. Only 10% of pancreatic tumour patients may be treated with surgical measures if

they were discovered early enough. With a terrible five-year survival rate of 10%, pancreatic ductal adenocarcinoma (PDAC) is one of the most fatal gastrointestinal cancers. Because of the absence of early detection and therapeutic response, pancreatic ductal adenocarcinoma is a very aggressive, fatal cancer. Aggressive metastatic resurgence occurs in some minimally resectable tumours, promoting confrontation to conventional chemotherapy, also radiation medication. The combination of severe fibrosis and immunosuppression poses significant obstacles in the development of pancreatic cancer cure. Nanoparticles have been investigated broadly as delivery platforms and adjuvants for cancer and other illness therapies in this area. Multiple nanocarrier-based formulations have been developed as a result of recent breakthroughs in nanotechnology, which not only includes medication delivery but also boosts immunotherapy-based therapies for pancreatic cancer.

Nanocarriers can improve their dose-dependent efficacy by improving the bioavailability of medicinal or imaging contrast chemicals. Similarly, they are used to selectively target tumour cells to improve picture resolution and reduce chemotherapeutic agent toxicity. These nanocarriers are utilized to treat cancers that have spread throughout the body, such as pancreatic and lung cancers.

A variety of chemotherapeutic drugs were encapsulated, targeted, and delivered using liposomes and also polymeric nanomaterials. Doxil, the first nanoparticle medication permitted by the US Food & Medicine Administration (FDA) was a 100-nm-diameter liposomal formulation encapsulating the anticancer drug named as doxorubicin. The encapsulation of doxorubicin in liposomes significantly altered the drug's pharmacokinetic and pharmacodynamic properties, resulting in increased tumour uptake and improved anticancer efficacy. Doxil is also used to treat a variety of solid tumours as well as platinum-resistant ovarian cancer. Albumin-bound paclitaxel-loaded nanocarriers, also known as nab-paclitaxel, were used to treat pancreatic tumours. Furthermore, location-specific delivery of the NP–siRNA complexes not only suppressed HER-2 expression in pancreatic cancer cells but also increased gemcitabine sensitivity [73], (Table 14.2).

14.4 Conclusion

Traditional therapeutic drugs for tumour treatment have been developed in recent decades, with some of them making significant success. The cure impact, on the other hand, is still insufficient, and the side effects are intolerable. Many new inorganic materials are sensibly selected to construct smart drug transport devices because of the advancement of material science. The carefully constructed drug delivery systems are able to respond to tumoural indicators and deliver medications to targeted areas, resolving the issues that plague traditional tumour treatment. Researchers from all over the world are looking into nanomedicine as a promising strategy for efficient therapies and drug delivery. Nanomedicine has enormous therapeutic potential in the field of cancer research. Nanoparticles' surface, small size, and distinctive shape have been employed as its distinguishing characteristics to be crucial for effective therapy

Table 14.2 Nanocarriers with antitumour drugs for the cure of different cancers [5]

Nanocarriers	Tumours	Benefits
SLNs	Pancreatic, breast and lungs	• A simple double emulsion approach has been successfully used to generate SLN formulations, which provides greater suppleness and reduces process-related strain on the condensed drug. These strategies serve as a starting point for creating SLNs for water-soluble antitumour medicines, such as peptides • Because of the synergetic effect, they have showed cytotoxicity more than the comparable dose of free-drug action
Liposomes	Breast, lungs	• In cancer cells, targeted liposomes have been made known to offer therapeutic advantages over their nontargeting counterparts • Increased drug entrapment results in significant antitumour effectiveness and less cardiotoxicity
Dendrimers	Breast, skin, lungs	• Internalization of drug couples into tumour cells, leading to greater anticancer activity and less toxicity • For anticancer medicines, these conjugates were discovered to have a desirable precise release feature
PNPs	Breast, chronic myeloid leukemia	• The tumour suppression achieved by a sole intravenous shot of doxorubicin conjugated to PLGA NP was equivalent to that achieved by regular injections of free doxorubicin more than twelve days; hence, the NP design was far more effective and longer permanent than free doxorubicin • When compared to the commercial paclitaxel formulation, paclitaxel-loaded PEG-PLGA-based NPs had better in vitro and in vivo cytotoxic effects (Taxol) • When compared to free medication, cisplatin-loaded glycol chitosan NPs showed sustained cisplatin release, enhanced antitumour activity, and lower toxicity

(continued)

Table 14.2 (continued)

Nanocarriers	Tumours	Benefits
PMs	Breast, skin, lungs	• PMs lengthen the time anticancer drugs circulate in the bloodstream • PMs preferentially aggregate in the tumour site due to their reduced size (10 nm–100 nm) and continued circulation durations in vivo, increasing their cytotoxic effect
CNTs	Lungs, breast, skin	• CNTs' needle-like structure permits them to pass through the cell membrane through endocytosis, also known as "needle-like diffusion," and then come in the cell • They have distinct physical and chemical properties, excellent drug frame-up, inherent stability, automatic flexibility, and appropriate surface functionalization
VNPs	Breast, colon, lungs	• VNPs have a number of distinguishing characteristics, including biocompatibility, morphological uniformity, ease of surface functionalization, and a wide range of proportions • The surface of VNPs can be PEGylated to upsurge their circulation time in the mass

and targeting. The use of nanotechnology in therapeutic and diagnostic approaches holds great promise for quick, low-cost cancer diagnosis. Most likely, nanomedicine will be the future of the most effective cancer detection, therapy, and management thanks to the advancements in medical science, immunotherapy, biochemistry, and artificial intelligence. Numerous NP forms, including polymeric, metallic, and hybrid NPs, have demonstrated enhanced drug delivery effectiveness as a result of growing research. The characteristics of the suggested nanoplatforms and the characteristics of therapeutic drugs must be carefully studied by researchers. There are, however, some drawbacks, including the absence of in vitro models that accurately simulate the in vivo stage, carcinogenicity, long-term cytotoxicity, and neurodegeneration. The therapeutic potential of "nano-vaccines" and "artificial APCs," despite they have demonstrated enhanced efficacy in comparison to traditional immunotherapy, is below par. These novel modalities' tolerability and safety must be examined. Anticancer medicines face a difficult and unanswered problem with tumour heterogeneity. Treatment failure is commonly caused by the variation in the quantity and the quality of targets located on the surface of uneven cancer tissue because it prevents ligand-directed targeting techniques from correctly identifying the antigen. Due to the limited effectiveness of anticancer treatments, treating heterogeneous tumour cells just encourages the growth of the most durable and drug-resistant cells. The

one and only way to effectively reduce the variations between tumour cells seems to be to achieve target homogeneity using target amplification methods. Tumour cells are equally accessible and sensitive to anticancer therapies when their homogeneity is restored. It also creates a potent tumour-specific identity that can be used to distinguish between on-target tumour cells and off-target healthy tissue for efficient ligand-directed therapies.

Through this review, we have covered the drug delivery mechanisms, the applications of several nanocarriers in tumour therapy. It is critical for understand tumour pathophysiology and intelligently pick nanocarriers to be able to build better smart medication delivery devices. Various innovative drug delivery systems are currently being investigated, owing to critical medical needs. Combination therapy has been demonstrated to have a greater anti-tumour effect in recent research, and developing multipurpose drug delivery methods has a potential future in tumour therapy. Therefore, it might be inferred from the literature analysis that the nanocarriers created from biomaterials that have been friendly to healthy tissue and coupled with tumour cell indicators are superior to traditional nanocarrier methods for the targeted and enhanced drug delivery at the location of action.

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Chapter 15
Microfluidics and Cancer Treatment: Emerging Concept of Biomedical Engineering

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Contents

Abstract For a long time, cancer research has been at the forefront of scientific and medical study. Cancer is a chronic illness that results from alterations or mutations in genes. In the year 2021, it was estimated that 1.9 million new cancer cases were diagnosed and 608,570 cancer deaths occurred in the United States. Microfluidics offers a lot of promise in cancer diagnosis and therapy, and it's also becoming a preferred way to learn about cancer biology. Microfluidics is the modern science of systems which can manage and modify tiny volumes of fluids in microchannel (from 1 to 1000 um). Due to its low cost, high sensitivity, high resolution, and better control over space and time, microfluidics can be useful in cancer research. Because of the

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microchannel's small size, the fluid flows in a laminar pattern, allowing the concentration of molecules to be adjusted, which is perfect for cancer research. Traditional cancer therapies are intrusive, inflict patient discomfort, and tissue damage, and are typically uniform and invariable, which does not fit all patients. Microfluidics allows us to develop devices that are efficient, effective, quick, and low cost for cancer detection and therapy. In the present chapter, we discuss how microfluidics may be used to design cancer preclinical models, test anticancer medications, and investigate tumor heterogeneity. The functions and current development of the microfluidic chip are highlighted in order to give references for improving cancer detection and treatment.

Abbreviations

ALI	Air-liquid interface
cfDNA	Cell-free DNA
ctDNA	Circulating tumor DNA
circRNA	Circular RNA
CTCs	Circulating tumor cells
EPR	Enhanced permeability and retention
EGFR	Epidermal growth factor receptor
EpCAM	Epithelial cell adhesion molecules
ECM	Extracellular matrix
FRET	Fluorescent resonance energy transfer
GEDI	Geometrically enhanced differential immunocapture
HNCC	Head and neck cancer cells
LDH	Lactate dehydrogenase
lncRNA	Long noncoding RNA
MMPs	Matrix metalloproteinases
mRNA	*Messenger RNA*
miRNA	MicroRNA
MDTs	Micro-dissected tissues
ncRNA	Noncoding RNA
PFF	Pinched flow fraction
PLGA-PEG	Poly (lactic-co-glycolic acid)-polyethylene glycol
PEG	Poly(ethylene glycol)
PDMS	Polydimethylsiloxane
PCR	Polymerase chain reaction
POC	Point-of-care
qRT-PCR	Quantitative Reverse Transcription polymerase chain reaction
RBCL	Red blood cell lysis
SERS	Surface-enhanced Raman spectroscopy
TGF	Transforming growth factor
TME	Tumor microenvironment
VEGF	Vascular endothelial growth factor

15.1 Introduction

Cancer continues to be the primary causes of death worldwide. Estimates indicate that more than 15 million Americans have cancer, and that number is expected to rise and cross the 19 million marks until the year 2024, putting a tremendous financial load of greater than 130 billion dollars annually on the country [1, 2]. According to predictions, there will be 806,590 additional cases of cancer as well as 606,520 tumor-associated deaths in the United States in 2020 [3]. As per American Cancer Society, tumors that develop in the colon, rectum, lung, bladder, breast, and prostate represent the most common kinds of cancer [2]. Uncontrolled cell proliferation from tissue leads to malignant development. Six fundamental characteristics must be maintained for cancer to progress: tolerance to anti-proliferative cues, apoptosis escape, autonomous proliferation, unlimited potential for replication, maintenance of vascularization, tissue invasion, and metastasis [4].

Surgical intervention, radiation, targeted therapy, chemotherapy is all part of the standard cancer treatment protocol. These techniques have strong curative results in the initial time of therapy; however, relapse of cancer frequently happens beyond some treatment time. The one-size-fits-all therapy work distinctly in various people [5]. Each kind of cancer has a different course of therapy [6]. To achieve the correct diagnosis and therapy for each patient, a tool that is effective, quick, and accurate is therefore required. Chemotherapy, surgery, and radiation therapy are some of the common cancer treatments available today. Treatments for cancer frequently cause side effects such as nausea, vascular lesions, atrophy, skin erythema, stomatitis, neurological degeneration, and toxicity to tissues. A typical cancer diagnosis is also strongly linked to collecting cancerous tissues or assessing biomarkers for diagnosis. Most of the these traditional sample techniques are intrusive, which result in tissue damage, restricted access, and intense patient pain [7, 8]. As a result, research is continuously being done to find new ideas for effective methods of cancer detection and treatment [9]. Even though technological advances have made it common to diagnose tumors that are solid, much sophisticated technologies are needed to identify cancers before symptoms appear, monitor the course of the disease, and gauge how well patients are responding to treatment. This will improve prognosis, enhance life quality, lower costs, and increase the likelihood that cancer patients will recover [10].

Microfluidic technology systems are one of the cutting-edge approaches that have been taken into consideration to progress the diagnostic and treatment objective of cancer. This fascinating technology has been evolving swiftly and is recognized for altering reagents inside tiny platforms [11]. At first, the concepts of microfluidics appeared to include the flow of a small number of chemicals through chambers of sub-millimeter dimensions. With the use of microfluidic technology, fluid may be manipulated at the scale of microns between 1 and 1000 m, or roughly the single cell size [12, 13]. These issues are resolved by the microfluidic platform, which has shown to be an effective tool for carrying out difficult, expensive, and time-consuming laboratory procedures on a microchip. Microfluidics technology can carry out complex tasks rapidly and with a small amount of reagent because of its nanoscale

size. Its enormous capacities, time-saving characteristics, and accuracy make it a very effective platform for diagnosis.

Devices of microscale can be designed to address the issue along with ensuring that each cell or molecule is analyzed uniformly, this is crucial when discovering a limited volume of biomolecules and cells. High accuracy level which is not possible with conventional macro-scale processes can be achieved this way. By utilizing scaling laws to produce laminar flow, enhancing forces that are secondary, and defining unique set of geometry to precisely confine, direct, and gather cells and other products, microfluidics may also be utilized to develop wholly novel separation procedures [14, 15]. There has been a lot of interest in microfluidic techniques for cancer detection, like finding cancer cells and its derived compounds. The reagents state that the platform scale causes any fluid passing from a microfluidic chip to experience laminar flow. This flow may be precisely changed and managed to satisfy the individual requirements of the user [16]. Using a microfluidic chip, a technique that can manage factors relevant to cell culture to precisely mimic the microenvironment of cancerous tissue in vivo, is possible. More specifically, micro-fluid control is beneficial to recapitulate fluidic internal environments, the multiplexing microstructures make it simple to carry out high-throughput analysis, the micro structure of the microfluidic chip can sensitively culture cells, and specific properties of material can more accurately mimic the microenvironment. It holds the potential to play a significant supporting role in the development of precision medicine [17], notably in the areas of organoid culture of tumor, screening of drugs for anticancer agents, cancer biomarker identification, and single-cell sequencing.

The manipulation of individual components and complicated interplay in the metastatic milieu are made possible by microfluidic techniques, which have attracted a lot of attention as a powerful tool for therapeutic targeted finding and treatment tracking [18]. The ability to harvest specific tumor cells using a microfluidic device is the most important feature. It also makes transcriptomics, genomics, and metabolomics easier, as well as making it possible to determine the cell clonal backgrounds of specific individuals. The platform may therefore be utilized using a large-scale, unbiased omics approach [19]. Several microfluidic methods have been created to capture uncommon cells, including stem cells, circulating fetal cells and circulating tumor cells (CTCs) [20]. Cancer metastasis, or the spread of cancerous cells, is one of the common and harmful effects of the disease. It is vital to remember that metastasis of tumor accounts for more than 90% of cancer-related fatalities in order to understand the relevance of researching particular mechanisms and possible treatments for metastases [21]. Microfluidics has evolved into a powerful tool for targeted drug development and treatment response assessment due to the ability of adding intricate interactions in the microenvironment of malignant tumors and changing each component [10].

The lab-on-a-chip tool was described in terms of microfluidic technology [22]. In addition to providing novel methods to improve practical cell culture over traditional cell culture techniques, microfluidic systems also tailor appropriate methods to replicate accurately in the extracellular microenvironment for cell-based pharmacological research. With the use of subsequent analysis of these properties, a microfluidic

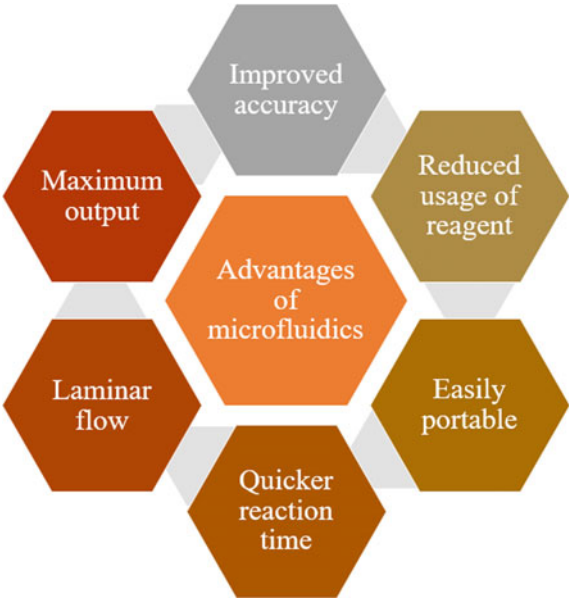

Fig. 15.1 Advantages of microfluidics

chip may detect cellular, molecular, and biophysical indicators of tumor progression [23]. The only known drawback of the method is the absence of an appropriate microfluidic platform that can perform any laboratory experiment on a single chip. Although certain chips can execute several tasks concurrently, like separation as well as identification, no chip at present evaluates and presents data in a way that is easy to comprehend. By eliminating and reducing the restrictions of this technology's detection capabilities, it's possible to detect cancer before it's spread in order to save the life of patient [24]. Advantages of microfluidics are shown in Fig. 15.1.

15.2 Microfluidics

Microfluidic technique enables one to control very small volumes of fluid to flow via tubes that are hundreds of millimeters wide [25–27]. The tiny range of microfluidic devices renders them particularly ideal for applications in biology to research and simulate the tumor microenvironment (TME) since the cell-to-extracellular ratio of fluid volume for tumor and immune cells in the microenvironment of tumor is more than one [28]. Lower Reynolds number which is represented as Re, defined as the proportion of inertial to viscous fluid force, is another characteristic of microfluidics [28]. In microfluidic systems, the flow is laminar if Re is low, the flow of fluid is laminar, which means that transport by diffusion is dominant. This enables the creation of a spatially and temporally variable concentration gradient of soluble components in a microfluidic device.

When optically transparent materials are employed to create perfused hollowed microchannels, which can resemble vasculature, combined with cell biology, and microengineering, these microfluidic devices are known as organ on chip [29, 30]. This organ-on-a-chip typically includes the essential stimuli, a scaffold, and suitable cellular microenvironments for the development of living tissues. Microfluidic technology makes it possible to precisely manage fluids and pressures on a small scale. As a result, it may create three-dimensional scaffolds and regulated microenvironments with reliable bio and physicochemical stimulants when combined with microengineering techniques. These recent methods are currently being used to create physiologically accurate, functional models of organ-on-a-chip, which are essential for biological, pathological, and pharmacological research [31]. According to the data that is now available on cancer research, metastasis invariably plays a part in the mortality of cancer [32]. As a result, certain in vitro models that mimic the natural state are created in order to study metastatic mechanism. Consequently, microfluidics on-chip is a high-performance method employed in cell responses, drug screening, treatments and biomaterials. The creation of a microfluidic system involves a variety of techniques and materials, each with a special scientific use.

Although polydimethylsiloxane (PDMS) is frequently utilized to fabricate microfluidic platforms, a number of polymer for example cyclic olefin copolymer and polycarbonate have now been validated to circumvent some of PDMS' major drawbacks, including the evaporation and adsorption of hydrophobic molecules [33]. Furthermore, along with the advancement of microfluidics as well as the zeal to improve this system to more closely replicate micro-physiological circumstances, several intricate microfluidic designs have now been created for numerous purposes [34, 35]. Materials used for microfabrication is shown in Fig. 15.2.

15.2.1 Microfabrication

Earlier microfluidic chips were created utilizing photolithography with silicon and glass. These operations were costly, labor-intensive, and difficult. The typical materials have drawbacks as well, like being pricy, gas-impermeable, not very physiologically relevant, and opaque [26]. Up until the late 1980s, when the soft lithography technique was created, the disadvantages prevented the advancement and broad use of microfluidics across many sectors. Soft lithography, which is considerably less expensive, made it possible to create three-dimensional microscale objects on a variety of substrates, including polymers, elastomers, and organic as well as inorganic materials. In the 1990s, gas-permeable, transparent, and inexpensive elastomers made of polydimethyl siloxane (PDMS) were applied in conjunction with the new soft lithography microfabrication technique to create microfluidics. As a result, these platforms were available for many fields in science, particularly biology [36]. The fabrication process entails a number of processes, the first and most important of which is building a master mold out of silicon wafers and photoresist using photolithography techniques. PDMS is then poured on the positive mold, degassed, and cured. The

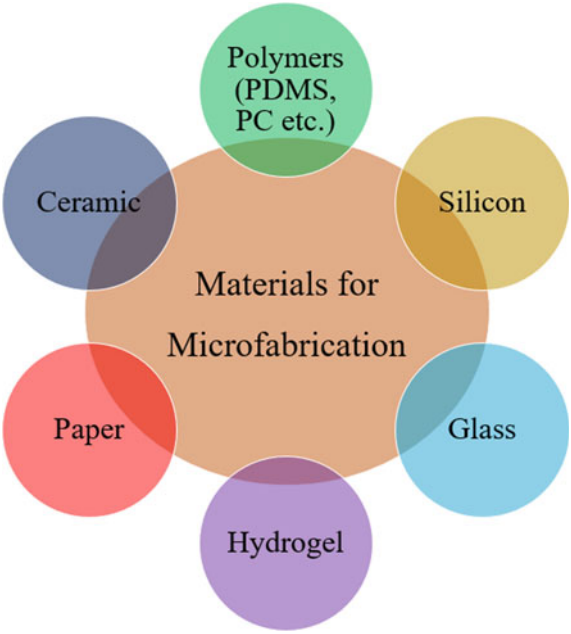

Fig. 15.2 Materials used for fabrication of various microfluidic devices

PDMS cast is then peeled out from the mold to attach with a glass. However, this manufacturing process has significant downsides, including being time-consuming and necessitating the use of a cleanroom [37]. As a result, research into new techniques is ongoing. A different technique is known as hot embossing, which involves positioning a thermoplastic film between two molds, compressing it, and then heating it until it becomes viscous. After doing this, a cast of appropriate mold is created, which can be removed once it's cool. By using such a technique, microfluidic devices are frequently made of the biocompatible and transparent material PMMA. However, hot embossing comes with its own limitations as well, such as the need for expensive and time-consuming mold creation [38]. 3D printing is among the most innovative and promising new alternatives for manufacturing microfluidics. A movable computer-controlled nozzle used in the manufacturing process deposits viscous polymeric layers or the initial polymer solution on top of the prior layer [39]. The simple, quick process is gentle enough to be used with bio-inks like hydrogels. As a result, the use of 3D printing makes it feasible to create organ models for a variety of applications pertaining to biomedicine in the most physiologically appropriate way [40]. However, this technique also has several unresolved drawbacks, including the restricted printable hydrogels and the susceptibility of cells to abrasive operations, which is why the 3D printing manufacturing technique is a topic of an extensively growing number of investigations [41]. Process of microfabrication is given in Fig. 15.3.

In the application of microfluidics, analytics is a crucial topic. The studied molecule might be any of several biomolecules made up of proteins and nucleic acids. To produce exact quantitative results, adequate combination processes, as well

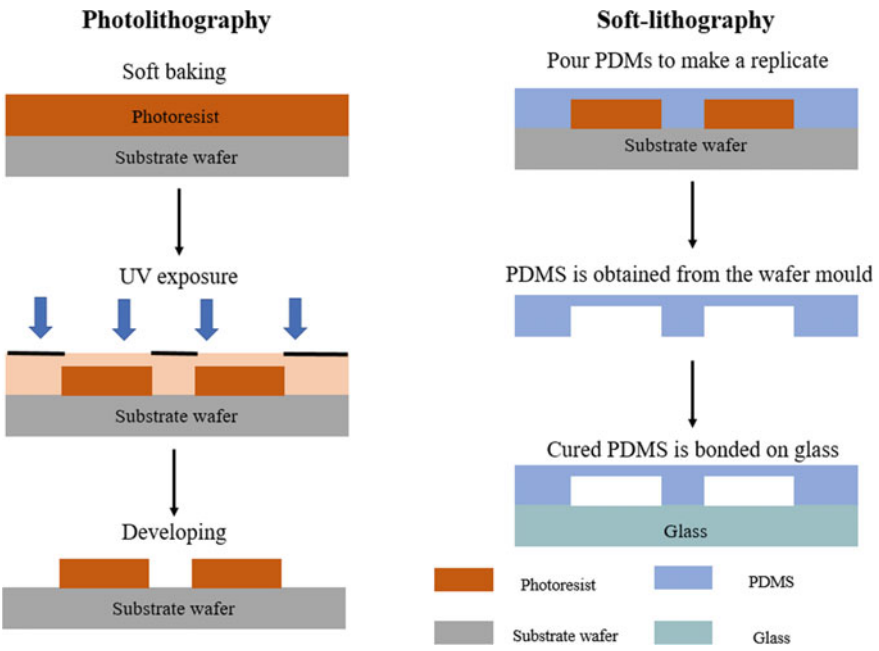

Fig. 15.3 Microfabrication process using photolithography and soft lithography

as highly precise fluid measurements and liquid regulation, are needed. Additionally, mechanization, wearability, mobility, and a wide range of activities are required for the execution of complex analytic procedures. The most challenging tests to design are cellular assays since the cells need to be maintained in a favorable environment to maintain their survival and activity. For assessing the impact of new pharmacological substances on mutagenicity, bioavailability, undesired side effects and toxicity, and at varied dose concentrations, cellular tests are crucial. The most intriguing prospect is to create single-cell analysis assays. Cell-based diagnostics require high throughput systems and little reagent consumption in each test [42].

15.3 Mimicking the Tumor Microenvironment

Cancer is an intricate illness with a diverse character which might cause people with comparable tumors to respond differently to the identical treatment [43]. Consequently, in-depth understanding of tumor biology is essential in the creation of potent cancer treatments. Due to the utilization of incorrect models in preclinical investigations, many cancer therapies experience failures in clinical testing [44]. Traditional preclinical model development has shown to be time-consuming, expensive, and to provide poor predictions of medication responses [45]. Additionally, clinical studies

are frequently carried out on patients who are nearing the end of their illness. As a result, the early stages of cancer, which seem to be crucial for diagnosis and therapy, are not caught. It is essential for an accurate prediction of therapy responses to design in vitro cancer models that can replicate the key features of a tumor in order to handle all of the problems.

The aberrant organ-like formations known as tumors are made up of many types of cells and ECM. It has been shown that the tumor microenvironment is vital for tumor growth, metastasis, and treatment outcomes [46]. Treatment of cancer is greatly hampered by the intricacy of the tumor microenvironment. Numerous distinct types of cells, EMC, blood vessels, and chemical agents make up the tumor microenvironment [47]. The tumor microenvironment is different from the environment of healthy tissues in terms of biophysical as well as biochemical features. Through improper investigation by cancer cells, normal tissue functions such as immunological, stromal cells, vessels can also accelerate the formation of tumors.

A crucial substrate termed the ECM surrounds the tumor cells, other stromal cells along with vasculatures that make up an in vivo TME [48]. The extracellular matrix (ECM) is a substance rich in protein that is released by stromal cells that offers mechanical stability to cells along with several chemokines and growth factors including vascular endothelial growth factor (VEGF) [49]. Consequently, the ECM is essential for several cell functions, particularly during the developmental stages of tumor [50]. Rapidly expanding cancer cells are found far from blood arteries when a tumor develops. Therefore, the cells are forced to go into necrotic phase, which is when cellular death happen, mainly in the center of the tumor, due to lack of adequate nutrition and oxygen [51]. This alleged hypoxic state triggers the production of VEGF and other angiogenesis-inducing proteins. In order to assist the tumor to receive vital nutrients, angiogenesis starts with the budding of new capillaries from the vasculature. After the angiogenesis stage, cancer cells break down the ECM to infiltrate the circulatory system, which leads to metastasis. Once it reaches a secondary place, a secondary tumor starts to develop [52].

Almost all of the body's tissues and organs must be linked to the circulatory system in order to get nutrients and oxygen via means of the blood vessels. Vasculature affiliating with the tumor cells with a high growth rate can promote metastasis and the formation of tumors. Vasculogenesis and angiogenesis are both aspects of vascularization taking place in the tumor's surroundings, which serves as one of the tumor's identifying features. Angiogenesis is the process by which progenitor cells produce new blood vessels [53]. The blood vessel development within the tumor is made possible by the vascularization, which also has a significant effect on the development of tumor cells. In light of this, vascularization might be seen as a desirable target against tumor. However, contemporary studies demonstrate that the use of anti-angiogenesis medications is ineffective because of the use of outdated, ineffective preclinical models.

2D models of tumor, in which tumor cells are grown in monolayers on a plain substance made of plastic, have been the most extensively used traditional models [54]. Both model types, however, have very serious drawbacks. For instance, in

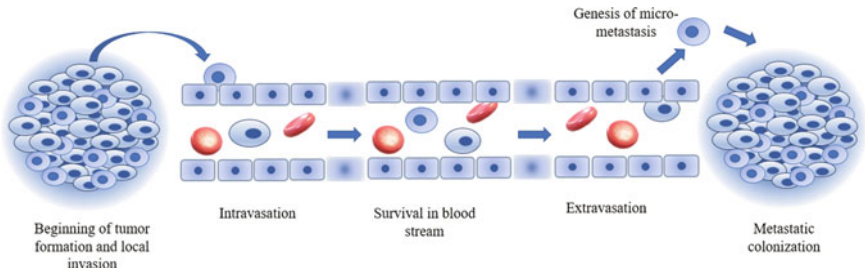

Fig. 15.4 Intravasation, metastasis, and extravasation of tumor cells

terms of elements like cell–cell interactions, cytoskeletal conformations, and cell-ECM interactions, 2D monolayers have little physiological significance [55]. The safety and repeatability of animal models created by injecting human tumor cells into immune-compromised animals are likewise constrained. Hence, there's an increased fascination in the design and development of three-dimensional models, which have the advantages of being simple to manipulate, inexpensive, and less invasive [56]. Intravasation, metastasis, and extravasation of tumor cells are shown in Fig. 15.4.

The key factors—geometry as well as spatial control on the position of various cells, cellular interactions, chemical as well as mechanical characteristics of the ECM—should be taken into account to mimic the complex cancer biology and produce an in vitro model that is physiologically relevant which includes the biophysical and biochemical components. By combining microfluidics with the concepts of tissue engineering, the tumor-on-a-chip has become a potential way to meet these criteria [57]. Compared to traditional animal tumor models and 2D models, tumor-on-a-chip technology offers researchers a number of benefits, including real-time visual analytics, transparency, consideration of cell-ECM and cell–cell complex interplay, governed flow of necessary substances, and accurate manipulation of the geometry of microstructure [58]. Drugs that are in clinical trials fail around 25% of the time because of pharmacological problems such as poor absorption or tumor penetration [59]. Consequently, delivery of drugs to tumor tissue is crucial for antitumor effectiveness, necessitating the immediate need for a microfluidic model that can more accurately mimic the in vivo conditions in the tumor.

The vascularization-on-chips side by side or with top–bottom channels are the most popular and simplest. Endothelial cells adhering to the wall of the scaffold or blood vessels resembling microfluidic channels divided with a membrane are maturely grown to simulate the interface between the extravascular matrix and vasculature [60, 61]. In order to generate shear pressures on endothelial cells that promote the formation of barrier, the monolayer growth of endothelial cells is upside compared to the conventional approach of Transwell. The idea of vasculogenic self-assembly may also be used to create perusable microvascular beds in vitro using microfluidic vasculature. Vasculogenic self-assembly techniques don't need a pre-designed framework to direct endothelial cells toward the formation of the vascular structure. This technique involves seeding endothelial cells onto the apparatus to allow the

3D microvascular network to self-assemble, resulting in a more organic structure. A mixed culture with endothelial cells, spheroids of cancer, and lung fibroblasts, in wells of low adherence can create spheroids that are multicellular which resemble lung tumor thanks to a microscopically patterned hydrogel-based construct housed in elastomer microfluidic devices [62].

Among others, the most likely causes of anti-angiogenic therapeutic failure is tumor heterogeneity. The reactions to anti-angiogenic therapy between tumor-associated blood arteries and normal arteries are structurally and functionally distinct. In order to produce biomimetic blood arteries that are patient-specific from normal endothelial and primary cancer endothelial cells, Jimenez-Torres et al. employed an organotypic microfluidic system in vitro [63]. The biomimetic blood vessels made from normal and patient-derived endothelial cancer cells reproduced characteristics of the tumor and normal vasculature seen in vivo, but the organization of cells was less orderly and there were more openings in the endothelium in the patient-derived cancer-associated vessels of endothelial cell. On the vascular chips, the impact of the anti-angiogenic medicines Sunitinib and Pazopanib were evaluated. The findings showed that tumor-associated vasculature reacted more positively to anti-angiogenic medications than normal cell vessels, and that the responses toward various anti-angiogenic agents varied both within and among various patient-specific models.

In addition to biochemical characteristic changes, the tumor may also experience biophysical alterations due to ECM modifications. The extracellular matrix (ECM) is a complex network of extracellular substances that include polysaccharides, proteins, thrombospondins, and glycoproteins. These substances can aggregate into supramolecular structures including fibrils and sheet-like networks [64]. The ECM exhibits a number of biophysical characteristics in addition to its structural role, including, stiffness, tension, density, and topography [64]. Complicated mechanotransduction cues of cells, such as the mechanical characterization, can also be triggered by interactivity between ECM and cells [65]. The extracellular matrix in the tumor, in contrast to normal tissue, is crucial for invasion and dissemination of cancerous cells as well as drug sensitivity [50, 66].

For instance, aberrant ECM affects cancer growth through influencing cellular change and metastasis. A tumorigenic microenvironment can be created by ECM abnormalities, which can also isolate stromal cells, promote inflammation as well as angiogenesis in the cancer, and also change the stem cell niches to that of the tumor [67]. Collagen was used to replicate the inside body conditions in a three-dimensional microfluidic device in order to understand metastatic mechanisms, like the derangement of the epithelial cellular layer by cancerous cells. It is accomplished by controlling the collective behavior and dynamics of cells using a variety of collagen microchambers. It is pertinent because collagen, which makes up the majority of the ECM, prevents cancer cells from beginning their spread before they enter the blood and lymphatic arteries [68]. When collagen is remodeled by matrix metalloproteinases (MMPs), the ECM's homeostasis becomes unbalanced and the tumor's ECM is extremely dynamic and continually changing [69].

The functions of MMPs in cancer include its participation in the breakdown of the extracellular matrix and their control of non-extracellular matrix components, such as growth factors, adhesion, cytokines, and cytoskeletal proteins that regulate the tumor microenvironment [70]. Sophisticated tumor microenvironment models may be developed to aid in the better understanding of key tumor formation pathways and decrease cancer mortality. The enhanced permeability and retention (EPR) effect, which is characterized by chaotic growth, frequently leads to the development of leaky tumor vasculature and impaired lymphatic outflow in tumor tissues.

A microfluidic-based tumor vasculature-on-a-chip that mimics 3D tumor tissue with rich ECM and leaky tumor vasculature was developed by Wang et al. [71]. The authors were able to use this technology to see the nanoparticles as they moved from extravasation through leaky vasculature to accumulate in cancer tissue. Tumor cells are constantly grouped together to form spheroids, which might lead to an inadequate supply of nutrients to the center region's cells. Recent research has demonstrated that the hypoxic environment conditions affect both the effectiveness of anticancer medications and the motility of cancer cells [72, 73]. In order to imitate the gradient of oxygen tension in the tumor microenvironment, Nam et al. developed a microfluidic device featuring a gradient of oxygen tension [74].

The distribution of anticancer therapeutic agents in tumor tissue is a key factor in determining their therapeutic effectiveness. Molecules must pass the endothelium, the major distribution barrier, in order to reach the tumor tissue. Therapeutic compounds can traverse the endothelium in a variety of methods, including transcellular, transcytosis, and paracellular transport. The capacity of compounds to cross the endothelium has been extensively assessed using animal models and conventional in vitro Transwell models, but their applicability are constrained due to a lack of physiological relevance. In recent years, a number of microfluidic devices have been created to imitate molecules traversing the endothelium [75, 76].

In order to perform physiologically significant measurements of molecule transcellular and paracellular permeability, Offeddu et al. developed microfluidic platform with three-dimensional self-constructed network of microvasculature [75]. The findings showed that the permeabilities to big molecules are multiple folds less compared to those in the Transwell system and equivalent to those seen in vivo. Interstitial flow also plays a significant role in the development and management of tumors in tumor tissue. Increased interstitial fluid pressure inside a tumor has been linked clinically to poor survival [77]. Due to the extracellular matrix's strong resistance, interstitial flow refers to fluid movement within cells that occurs at a significantly slower rate than blood vessel flow [78].

Plasma exiting from blood vessels and draining into the first lymphatics is what frequently causes the interstitial flow to develop. When lymphatics are dysfunctional or obstructed, the cavity circulation can also reassimilate in the segment of microvasculature which reacts most to inflammation [79]. From blood vessels to cells, it may deliver waste and nutrients, which cells can subsequently expel into the blood or lymphatics. Tumors have higher interstitial flow pressure than healthy tissue does [80]. The high permeability tumor arteries and the dysfunctional lymphatics found within tumor tissue are partially to blame for the high interstitial pressure, and the

steep pressure gradient close to the edge is what causes the fluid to penetrate the surrounding tissues with less pressure.

Drug administration into the tumor within is so challenging due to the substantial hurdles that the fluid passing via the tumor interstitium must overcome. The blood-tumor-lymphatics microcirculation mechanism was replicated in a 3D structure by Ozcelikkale et al. to investigate the effects of the anticancer medication doxorubicin [81]. Results obtained with this technology demonstrated that cellular clumps were extremely motile before drug treatment, but that when drug aggregates all around cellular aggregates, cell movements slowed. Ayuso et al. created a microfluidic device that can control parallel as well as interstitial flow [82]. They discovered that a large volume of cultural material displayed an obvious interstitial flow whereas a small volume was perfectly parallel to the core channel. Other crucial elements, like co-culture, pressure, and shear stress are used in microfluidic devices to replicate the tumor microenvironment [83]. Although recreating the TME on a chip is difficult, microfluidics can nonetheless replicate several important tumor traits to simulate tumor development and therapeutic effects (Table 15.1).

15.4 Diagnosis

Cancer-associated biomarkers, such as mRNA expression patterns, free tumor nucleic acids, CTCs, proteins, or associated body fluids are demonstrating bioactivity along with pharmacological responses to therapeutic approaches in patients who have developed the disease. The accurate detection of these biosignatures gives doctors crucial clinical details they need to identify illness states and properly choose a course of treatment. Because of its distinctive qualities in bioanalysis, microfluidics devices have been studied to assess cancer biomarkers. On the contrary, a biochip based on delivery from sample to gene survey was created to concurrently determine several cancer markers of prostate cancer from urine biopsies and serum for the digenetic study of prostate tumor from human body fluids [95]. The biochip device includes various functional components that can perform a variety of tasks, including solid-phase isothermal amplification, electrochemical detection, peroxidase-imitating labeling of nano-enzyme, and electrical lysing targeted cells within a minute to release desired genes. The full detection process may be completed in 30 min using the embedded biologic chip, and it can detect 50 target copies [96].

15.4.1 Microfluidic Circulating Tumor Cells (CTC) Detection

Cancer cells have the ability to disperse from primary as well as metastatic cancer locations and migrate as single or clumped cells through the bloodstream to other locations. The objective of advanced technologies in the CTCs area is to identify

Table 15.1 Microfluidic applications in context of cancer

Application of microfluidics	Interpretation	References
Isolation of CTCs	Separating cancer cells from background blood cells using label-free and label-based techniques 1. Fabricated a densely packed pore array micro sieve silicon lab-on-chip to separate tumor cells from whole blood 2. Cancer cells are separated from white blood cells by using injection molded microfluidic separation device using the pinched flow fraction (PFF) technique 3. Dielectrophoretic lab-on-a-chip device is used for isolating epithelial cancer cells (MCF-7 human breast cells) from colorectal cells	[84–86]
Mimicking tumor microenvironment (TME) on chip	To replicate the environment essential for tumor growth. Like the elements of ECM, biophysical and metabolic variables, etc 1. Fabrication of microfluidic co-culture model to study the cell interaction, and cell migration of stromal cells (HS5) and tumor cells (HuH7) 2. 3D engineered micro tissue models are fabricated on a chip to look into the interactions between healthy cells and tumor cells. The changeover from a healthy to a pathologic status in vitro at the level of the ECM could be seen and quantified in real time, due to the optical convenience of the microdevice and the highly constant features of the 3D tissue model	[87, 88]
Studying metastasis	Creating microfluidic technologies that can replicate the biophysical, biomechanical, and biochemical environment in order to understand the metastatic cascade 1. A microfluidic device with a porous membrane squeezed between two microfluidic channel has been introduced here. This device can be used for studying tumor cell extravasation, adhesion, and trans endothelial migration of metastatic cells 2. Using a 3D tumor, stroma, and a naturally occurring vascular layer structured spatially on a single platform, a novel microfluidic device was fabricated to investigate the impact of intravasation and invasion of tumor-vascular crosstalk	[89, 90]

(continued)

Table 15.1 (continued)

Application of microfluidics	Interpretation	References
Study of angiogenesis and fabrication of tumor on chip with vasculature	For duplicating key elements of microenvironment to transport oxygen and nutrients to cancerous cells 1. To replicate the in vivo TMEs, a tumor spheroid coupled with perfusable vascular network was instigated. Long-term perfusion (>24 h) allows to assess significantly increased tumor cell growth activities, because of the built-in vascular network	[91]
Anticancer drug screening	For enabling controlled drug release, containment, and programmed drug absorption 1. By combining alginate and matrigel hydrogel, they developed a microfluidic droplet device for cell spheroid production that worked satisfactorily	[92]
Organ-on-a-chip	For reproducing an organ's physiological features for replication, the importance of biological, mechanical, and structural elements in comprehending cancer biology and the progress of therapeutic development 1. Created a tumor growth system based on metastasis-on-a-chip that cultivates on a liver ECM, kidney cancer cells in a three-dimensional biomimetic environment to imitate the growth of cancer cells of kidney for monitoring dose response and forecasting treatment effects at various stages of tumor progression 2. Created a microfluidic chip with multi-organ to research the metastasis of lung cancer. This technology allows for the direct, diverse, and quantitative analysis of lung cancer growth, invasion, and metastatic progression	[93, 94]

and isolate clustered or single CTCs from the whole blood of quantities higher than 5 mL and with little stress on cells to reduce their damage [97].

CTCs are tumor cells that solid tumors release inside of the bloodstream. CTCs are strongly linked to cancer metastasis, according to several studies. Because CTCs can reflect the current tumor load and investigate tumor heterogeneity, they are an important cancer biomarker. However, for three reasons, it is very difficult to detect CTCs in blood. First of all, CTCs are extremely uncommon; in the peripheral blood of patients, there may only be single CTC in every 109 blood cells [98]. Second, the size and form of CTCs vary, making it challenging to distinguish them. Third, the identifying process can easily destroy CTCs [99]. According to research on CTCs,

the majority of them show epithelial surface indicators such as epithelial cell adhesion molecules (EpCAM), which blood cells do not. EpCAM antibodies can be used to label magnetic nanoparticles that can be used to extract CTCs using a micromagnetic shift or filtered in a microfluidic chip [100]. Even if other demonstrated surface molecules, such as the human epidermal growth factor receptor 2 (HER2) or the epidermal growth factor receptor (EGFR), can be used to detect CTCs, their expression is incredibly diverse. Due to the heterogeneous expression of tumor associated markers on CTCs, many CTCs that could have been identified using microfluidic technologies and an immunoaffinity method are lost.

CellSearch technology, which relies on affinity of the immune system of cell for cell separation and fluorescence for cell counting of CTCs, is currently the industry standard for CTC separation and counting [101]. Epithelial cell adhesion molecule (EpCAM) is used in this approach as a selection marker for CTCs, and magnetic beads coupled with anti-EpCAM antigen are used to collect and isolate CTCs. The selection of CTCs based on antigen does, however, have significant limitations. First, it has been demonstrated in several investigations that the use of antibodies against EpCAM may change cell metabolism and protein composition [102]. Second, cytotoxic effects may result from antibodies attaching to antigen cell surface [103]. Therefore, selection dependent on physical characteristics of cells, such as their shape, size [104], and electrical impedance [105] has also shown to be successful.

Studies are increasingly combining conventional methods with microfluidic device or image analysis in order to produce more effective CTCs isolation and capture [106]. Because of its multiplexing and simplicity features, microfluidic technology has a lot of potential for use in the capture of CTCs. CTCs are typically isolated and counted using a microfluidic chip. But as more was learned about CTCs, researchers discovered that in addition to counting CTCs to estimate tumor burden, CTCs are crucial for cancer spread and serve as a representation of the heterogeneity of the tumor. Because of this, the downstream study of CTCs after CTC isolation can better give molecular characteristics. For instance, sequencing of single cells can enhance the identification of tumor subpopulations and serve as a valuable resource for individualized treatment. In a different study, nanovectors on a microfluidic device were combined with surface-enhanced Raman spectroscopy (SERS) to recognize the subtypes of CTCs according to the therapeutically suitable surface proteins composites disembodied characteristics [107].

The numerous aptamer-based microfluidic sensors are intended for the detection of CTCs. One class of single-stranded DNA or RNA oligonucleotides known as aptamers can attach to proteins, ions, or tiny molecules with greater specificity and stability than antibodies. They can convert a huge number of analytes into catalytically quantifiable signals at a low cost [108]. The thermo-curable polymer chip used in the high electron mobility gallium nitride transistor-driven microfluidic-based aptasensor has fixed miniaturized sensors that are used to count the number of CTCs present in the solution [109]. These kinds of biosensors don't require further sample processing in order to identify and count collected CTCs at physiological concentrations. Surface-enhanced Raman spectroscopy (SERS) was used by Zhang and colleagues to identify indicators of cell membrane of breast cancer and to quickly

confirm the disease. This was accomplished by first separating CTCs from blood cells based on size differences, and then simultaneously identifying markers on the cancer cells' membrane using several SERS aptamer vectors [108].

Peptide-based sensor technologies have recently advanced quickly and may one day be used in a variety of clinical settings [110]. Due to their distinct optical, biocompatible, structural, optical, and electrical characteristics, screen-printed electrodes and peptides can be used for creating electrochemical biosensors, and nanomaterials can be used for creating microfluidic sensors based on peptides [111]. By utilizing fluorescent resonance transfer (FRET)-based biosensor and a microfluidic peptide-based biosensor, Hassanzadeh-Barforoushi et al. were able to detect from a single cancer cell the protease activity and display it using the signals by fluorescence generated by the breakdown of amino acid chain. Microfluidic biosensors, a cutting-edge technology, may identify CTCs in the bloodstream or in specific cells within tumors [112].

The interplay between antibody and the particular antigen is used in CTCs immunity-based methods. An illustration of a microfluidic device that uses immunoaffinity purification is the CTC-chip. CTC-chips are built upon microposts, which are composed of arrays of flexible silicone vertical cantilever beams that have been functionalized with anti-EpCAM. Unlike other cells, CTCs are caught when the blood passes across the microchannel and they bind to the antibody on the surfaces of the microposts [113]. Microporous nanofibers of varied diameters have been created as a result of the advancement of nanotechnology and development of nanostructures like nanofibers, nanocolumns, and nanotubes, among other structures. Such nanofibers may provide an environment that is conducive to encouraging cell adhesion and development. In order to capture breast cancer cells (MCF7) with high expression of EpCAM, Wu and colleagues created three-dimensional bionic interfaces and electrospun long PLGA nanofibers to cross-link to the surface. This process is known as the development of a biosensor based on cells (cytosensor). MCF cells are then electrochemically detected using EPCAM antibody in a nanochip [114].

15.4.2 Microfluidic Tumor Exosomes Isolation

Cancer cells along with some non-cancerous cells release extracellular vesicles called exosomes. Biomolecules including mRNA, microRNA, lipids, proteins, and metabolites are found in exosomes. At the early stages of cancer, the bloodstream contains exosomes, which are long-lasting and abundant as compared to circulating tumor DNA (ctDNA). Exosomes could be located in most bodily fluids, like saliva, urine, plasma, and amniotic fluid. These characteristics have identified these as potential biomarkers for tumor, and in recent years, these have received great interest [104].

A tumor's state or recent changes in the tumor can be inferred from variations in expression of specific exosome cargoes. The most popular exosome separation technique is ultracentrifugation [115], which requires a lot of time and chemicals. Exosome detection and separation have not changed significantly in recent years,

despite the emergence of numerous innovative techniques [116, 117]. Exosome separation and detection can be successfully completed using a microfluidic chip, and this device's ability to combine these two procedures into one considerably simplifies the process. A microfluidic chip is similar to a framework that researchers can employ as needed for their studies. On chips, exosomes derived from tumor have been detected and separated using a variety of techniques. Immunoaffinity-based separation [118, 119], dielectrophoretic separation [120], nanomembranes filter, separation based on acoustic fluid and lateral displacement are a few of the separation techniques. The detection methods include mass spectrometry, electrochemical detection, and fluorescence detection. In addition, integrated circuits have recently been developed that combine exosome separation and detection. A 2-stage microfluidic device with staggered micropillars of Y shape and an electrode made of Indium Tin Oxide, was created by Xu et al. [121]. By using a novel design of micropillar array to induce anisotropic type of flow and encourage complete adherence of exosomes to the magnetic beads modified by antibodies, this platform was able to separate exosomes. To obtain fast throughput, little damage and high recovery rate [122] for quick detection are the key problems of exosome isolation. Although significant progress has been achieved in exosome recognition on chips in previous few years, there is extensive room for advancement in the methods now in use.

15.4.3 Microfluidic ctDNA Detection

Cancer cells that have undergone apoptosis or tumor exosomes have reached the bloodstream release circulating nucleic acids [123]. Circulating nucleic acid concentrations reflect the presence and progression of tumors [124]. The standard range of cfDNA in the whole blood for healthy people is 1 to 10 ng/mL, however this number can increase in cancer patients. Cell-free DNA, ncRNA, and mRNA are circulating nucleic acids. ctDNA is a sub-group of cfDNA that conveys details about mutations and is frequently discovered in the peripheral blood of patients with cancer. Circular RNA (circRNA), microRNA (miRNA), and long noncoding RNA (lncRNA), and other types of RNA fall under the category of noncoding RNA (ncRNA). Numerous results have demonstrated that ncRNAs are functional regulating molecules engaged in the growth of cancer [125, 126].

The automated platform provided by a microfluidic chip considerably increased the effectiveness of ctDNA extraction. With the goal of high recovery and cheap cost, the cfDNA were isolated from plasma samples of patient using a particular buffer [127]. Additionally, one of the objectives in the creation of the microfluidic chip is point-of-care (POC) cancer detection. Since cfDNA has a relatively short half-life, it is vitally necessary to use quick and automatic methods that separate cfDNA from plasma with little deterioration. A completely automated microfluidic technology was created by Kim and colleagues to quickly isolate cfDNA from the plasma of cancer patients. The time it takes to isolate cfDNA from patient blood has been improved thanks to this technology, which is based on diaphragm valves

that are electromagnetically activated and have integrated functionalities. Numerous research has shown how effectively using the two technologies—microfluidic chips and digital polymerase chain reactions—can be used.

The rationale is that the digital PCR technology on a chip is precise, high output, and time-efficient than the commercial PCR test [128–130]. It is possible to directly examine the relationship between DNA methylation and cancer ncRNA [131] from liquid biopsies using digital PCR onto microfluidic chips. A microfluidic device with multiple qRT-PCR, for instance, was created by Moltzahn et al. to profile the miRNA fingerprint in the blood of prostate cancer patients for prognosis and diagnosis [132]. Wang et al. analyzed miRNAs related to lung cancer using digital PCR [133].

15.4.4 Microfluidic Measurement of Proteins in Cancer

Cancer patients may benefit from the use of biomarkers, such as hormones, growth factors, and chemokines, that are released by cancer cells in unusually high concentrations can be used as diagnostic tools. Therefore, it is essential to create protein panels for detecting small volumes of blood. One of the most sophisticated and ideal solutions in this field that has successfully entered the commercial market is the tracking of biomolecules and proteins in microfluidic systems. Microfluidic devices have made it possible to concurrently test numerous proteins and recover plasma specimens from a drop of whole blood. However, there remain numerous experimental obstacles that microfluidic immunoassay devices must overcome before they can be used in clinical settings to identify cancer biomarkers [134]. It is possible to run into difficulties while measuring proteins using the microfluidic approach, such as instability, aggregation of proteins, and their measurement time. New microfluidic methods, however, can be used to address these difficulties [135] (Table 15.2).

15.5 Treatment

15.5.1 Drug Delivery and Cancer Microfluidics

Drugs can be delivered precisely and locally, in predetermined small doses, using microfluidic tools. These criteria make it easier to administer drugs having a brief half-life or ones with cytotoxic impact when prescribed systemically. Additionally, by designing microneedles or system that is based on injections void of needles, several conventional delivery processes, such as uncomfortable and invasive injections, can profit from these micro technologies [141]. The primary focus of the drug delivery investigation is the operational drug carrier. Such carriers must regulate drug release, modify bioavailability, and reduce side effects. Additionally, they should improve the absorption of unstable and poorly soluble medicines. Oral delivery vehicles must

Table 15.2 List of microfluidic technologies used for diagnosis of cancer

Microfluidic technologies	Properties of chip	Advantages	References
The herringbone chip	The blood cells are adequately mixed by the herringbone grooves in the wall of the chip, boosting the interactivity between uncoated surfaces and CTCs	Increased throughput of volume of blood; excellent purity and effectiveness of capture	[136]
Geometrically enhanced differential immunocapture (GEDI)	The relative obstacle alignment makes use of the shift caused by the collision of cells with barriers to distinguish cells of various dimensions, while the streamline contortion can assist the target CTCs in contacting wall with the immune coating	High cell capture ability; high binding ability and specificity	[137]
NanoVelcro Microfluidic Device (CTC-ichip)	Using determinate lateral displacement to segregate cells with nucleus, aligning cells with inertial focus, and diverting and collecting cells tagged magnetically are some methods for the negative exhaustion of regular blood cells	Automation, high output, and support for single-cell analysis and high quality images	[100]
Spiral chip	Characterized by constant flow in curved channels, this chip produces Dean drag as well as inertial forces needed to separate cells. The physical distinction between CTCs and blood components serves as the foundation for the separation concept	High flow rates, extremely high throughput, stable streamline distribution, simplified helper strategies in clinical trials, and less harm to CTCs	[16]
Straight chip	The straight chip uses flow rate ratio manipulation to segregate CTCs with high purity by utilizing inertial movement of cells in the microchannel	High rates of throughput and recovery, a customizable and predictable cutoff size, and high purity collections	[138]

(continued)

Table 15.2 (continued)

Microfluidic technologies	Properties of chip	Advantages	References
Nanotube-CTC-chip	Red blood cell lysis and preferred adhesion can enhance CTCs separation; CTCs are captured and separated using carbon nanotube surfaces combined with microarray batch production	Great purity and high capture efficiency	[139]
Micro immunoassay chip	Chip with antibody microbeads inside of it, distributed the laminar motion of the liquid sample, which was used to detect cancer antigen CEA and CA15-3 in the breast cancer patients blood samples	Boosted the mass transfer efficiency, dramatically improved antibody binding to the target proteins, magnified the fluorescence signal, and greatly improved detection sensitivity and efficiency	[140]
exoNA-sensing chip	A microfluidic chip (exosomal mRNA sensor) integrated with 3D nanostructured hydrogels to detect exosomal *ERBB2* in the blood	Uses vacuum-driven micropumping method, hence can detect target gene within 2 h. Can detect expression level of reference gene and target gene simultaneously	[84]

also be able to withstand the stomach's acidic environment, be small enough, and coated with the right indicators to pass through the intestinal mucosa and reach the bloodstream. Drug carriers' controllability and adjustability, which are strongly connected with their form, homogeneity, size, and formula, are crucial components of their successful use [142]. For instance, kidneys swiftly filter out nanoparticles smaller than 10 nm, whereas phagocytes, which are immune system cells, can take in large particles of the wrong size [143]. The advancement of drug delivery platforms through the use of microfluidics has produced significantly better drug carriers.

Typically, microfluidic devices may help to handle small numbers of specimens and offer the creation of effective nanoparticles with proportionally sized, shaped, and surface structures to improve the standard of drug delivery and discharge of loaded medication in the course of treatment [144]. Micro-vortices [145], droplet techniques [146], and focusing of flow [147] are frequently utilized techniques for constructing appropriate non-carriers through microfluidic systems. Microfluidics with $Re > 100$ can be used to create nanoparticle for applications in delivery of drugs by controlling micro-vortices. Lipid polymeric nanoparticles were created using micro-vortices at the intersection of the three inlets, which was an adaptation of the 3D micro-vortices model.

By varying the Reynolds numbers, Kim et al. analyzed the nanoparticle diameter and determined that at high Reynolds number the generated particle dimensions are reduced, which resulted in a diameter decrease from 93 to 55 nm with increasing Re numbers [148]. Chaotic advection, which can produce transversal streams that compress and stretch liquid volumes along the microchannel cross-section, is a news method for increasing mixing yield by making use of geometric templates. The herringbone mixer, which uses a configuration of herringbone furrow with multiple channel surface to induce mixing of unstable nature within a constant flow, has already attracted the attention of researchers due to ease in manufacturing and material handling [149]. The reagent is dispersed across the entire cross-section of the microchannel, greatly lowering Taylor dispersion and leading to dispensation of residence time. In a study, mixers of this type were used to create lipid-based nanoparticles, and then the effect of lipid concentrations and the blending procedure was evaluated on nanoparticle sizes. According to the research, a herringbone mixer used to stir low-density lipids could produce lipid particles as small as 30 nm [150]. Herringbone mixers can also be used to create a variety of drug-based nanoparticles, such as siRNA preloaded on lipid nanoparticles and doxorubicin-lipid nanoparticles [151].

Droplet-based microfluidics is the other technique with a maximum throughput for carrier synthesizing and is reliable for producing uniformly sized drug-loaded nanoparticles. This method uses a laminar stream to alter different amounts of liquid in phases that are unmixable. As a result, the ability of microfluidics to create distinct droplets has increased to create particles. A microfluidic device's well-controlled interfaces and flow intensity typically help with this droplet creation. Either PDMS devices or microfluidic capillary tools are used to create tunable monodisperse emulsions [152]. Commonly, the size of the drug carriers has a strong influence on the drug delivery rate; smaller PLGA particles have a higher surface to volume ratio than larger ones, therefore they transport loaded pharmaceuticals more steeply. Hui et al. created a droplet-based process in a microfluidic platform to create Janus nanoparticles in a single step. Two inlets which link to precipitation flow are included in nanoparticle precipitator fluidic devices. They created Janus nanoparticles which are biocompatible that can contain the hydrophobic medication paclitaxel and the hydrophilic doxorubicin on one portion and the other, respectively. In a nutshell, a microfluidic device that uses two side-by-side microchannels that resemble capillaries is permitted to quickly generate and combine two structures with distinct chemical and physical characteristics in Janus nanoparticles for co-drug delivery [153]. Principle of micro droplet synthesis using microfluidics is shown in Fig. 15.5.

Another popular system in microfluidics is hydrodynamic focusing, which is employed when regulated chemical mixing and reaction are inevitable. When two solutions enter the channel side by side and have differing rates of acceleration, the hydrodynamic focusing phenomenon happens. By adopting three channel microfluidics, in which the main stream containing the primary sample is encircled by lateral fluids, in such case hydrodynamic focusing is most frequently administered. Microfluidics' capacity to rapidly mix liquids results in a homogeneous reaction

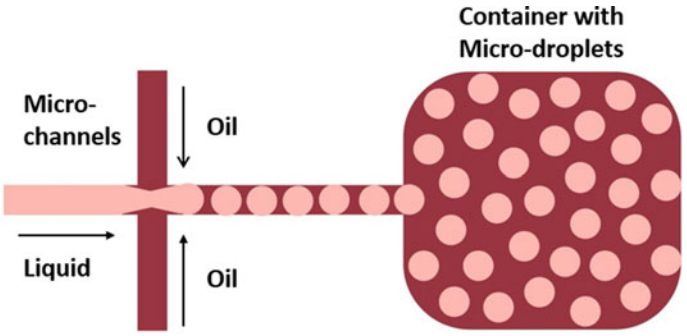

Fig. 15.5 Principle of micro droplet synthesis using microfluidics

and precisely adds reagents to the reaction process. Poly (lactic-co-glycolic acid)-polyethylene glycol (PLGA-PEG) degradable polymeric nanoparticle having specified properties, like optimal size, decreased polydispersity index (PDI), higher drug load rate with slow and controlled release, require quick and customizable microfluidic mixing [154].

Additionally, one layer integrated along with three successive channels for directing vertically accompanied with a typical horizontal directing flow has established a three-dimensional hydrodynamic focusing flow system. It's interesting to note that one issue with 2D flow focusing—the polymer detachment from the walls of microchannel—was solved by the 3D flow focusing pattern. Even when employing high molecular weight PLGA precursors, this type of three-dimensional focusing of flow produced particles over an extended period of time without microchannel clogging, and it allowed those particles to target tumor cells when they were combined with various medications [155]. Micelles of PLGA-PEG which are encapsulated with Docetaxel for lung cancer have been produced using the dialysis process and microfluidics. For the micelles created by the microfluidic approach, a lower size of nanoparticle 72 ± 1 nm having polydispersity index of 0.072 was formed. In contrast, the dialysis approach produced micelles with nanoparticle sizes of the range from 102 to 144 nm with a polydispersity index of 0.390 [156].

Additionally, by adding arginine-glycine-aspartic acid phenylalanine-lysine on micelle surface, created using the microfluidic technique, targeted docetaxel-encapsulated micelles were created. More significantly, the microfluidic approach was associated with greater drug loading efficiency [156]. In order to create a microfluidic platform on silicon for production of Berberine loaded nanoparticles made of chitosan, Farahani et al. used the lithography technique in 2020 for the production of these nanoparticles, they applied a novel ionic-gelation technique in microfluidic devices. It was stated that all created nanoparticles had PDI values below 0.3 and were semi-spherical in shape. Additionally, particle size rose to 200 nm. In addition to increasing the Berberine loading capacity, the microfluidic approach also increased the cytotoxicity and death of Berberine loaded chitosan nanoparticles on

the breast cancer cell lines as opposed to the straightforward mixing ionic gelation route [157].

15.5.2 Screening of Drugs Using Microfluidic Cancer Models

The microfluidic chip can assess the effectiveness, safety, and sensitivity of medicine in addition to offering patients a suitable drug combination administration tailored to their specific needs. These features enable the identification of particular drug classes in advance of the potential formation of drug resistance. Given the small number of prospects that are eventually approved by FDA and the exorbitant expense of drug research, interest in creating three-dimensional models of tumor for better cancer medication development has increased [158, 159]. The volume of reagents needed for microfluidic drug screening may be reduced, and it can be modified for parallelization and future automation.

One method for screening medications and figuring out the best manner to administer them is to set up a microphysiological system. With inhalation therapy, medications are delivered directly to the targeted areas in the body with reduced drug buildup at unintended sites, which is a significant treatment for lung illnesses [160]. For the testing and development of medications for inhalation and intravenous use, a microfluidic platform comprising several organs and a breathable lung chamber has been presented [161]. The liver and tumor compartments were connected to the lung compartment via conduits in this model. It's important to note that scientists altered the common air–liquid interface (ALI) lung model and created a bridge of ALI to simulate breathing of lungs. The device can verify if inhaled therapeutic medicines may be utilized to treat systemic illness through the ALI bridge. Additionally, researchers introduced several cell types and built a 3D structure of the breast cancer tumor using the modified hanging drop technique [161]. Researchers may simply analyze the cytotoxic impact of curcumin given by intravenous injection versus inhalation using this platform. A crucial method for individualized therapy is the use of patient-derived malignant cells for microfluidic-based chemosensitivity assays. Micro-dissected tissues (MDTs), a technique developed by a group, included sectioning patient-derived tumor tissues into submillimeter-sized pieces [162]. Confinement of cells by process of sedimentation can protect MDTs from severe shear stress offering a much secure platform for monitoring and imaging, MDTs were captured by sediment in square-bottom wells. Drug screening on the chip was carried out using a tissue sample from a patient with high-grade serous ovarian cancer. The platform can detect prospective responders since the positive reaction determined by the microfluidic device in vitro was found to be compatible with the patient's clinical response when compared to the clinical follow-up [162]. In fact, during carcinogenesis, the development of dormant microvascular networks always comes first [163]. However, several investigations revealed that when tumor and endothelial cells were seeded simultaneously, excessive tumor development and inadequate vascular expansion took place. Shirure et al. created a microfluidic device

based on organoids derived from patients that can evaluate both anti-angiogenics and chemotherapeutics by varying the seeding sequence of endothelial and tumor cells. Organoids derived from patients were implanted to the area of microvascular networking after 7 days of culture, at which point the microvascular networks were mature and ready to replicate the intravasation of cancer cells [164]. Additionally, drug testing performed on this platform more accurately mimicked the normal distribution of medications to tumors thanks to the microvascular networks.

Hepatic spheroids were used as the material source by Bhise et al. to create liver-on-a-chip utilizing 3D printing technique, which allowed liver tissue to be printed straight into the microfluidic chip [165]. The researchers also demonstrated the viability of using bioprinted hepatic spheroids as a platform for drug toxicity assessment and lengthy culture in devices. Riley et al. described a microfluidic apparatus in different research that can keep slices of thyroid tissue ex vivo for at least 4 days and be utilized to monitor the response of thyroid tissue to radioiodine-sensitive adjuvant therapy [166].

An automated microfluidic system for multimodal and adaptive screening of drugs with tumor organoids of pancreas was described by Schuster et al. [167]. The 200 separate chambers of this microfluidic platform allow for the insertion of thermally sensitive gels and the overlay of a channel layer, allowing the analysis of 20 different fluidic conditions over a period of more than 14 days. A microfluidic system created for drug analysis in a 3D tumor environment was also demonstrated by Pandya et al. [168]. In this concept, devices may be employed for chemotherapeutics testing and efficacy assessments in less than 12 h thanks to the integration of electrical sensing and microfluidics modalities.

15.5.3 Radiation Therapy of Cancer Using Microfluidics

Depending on the kind of radiation used, radiotherapy is one of the essential therapeutic techniques with applications ranging from disease treatment and management to diagnostic imaging [160]. For instance, radioisotopes that emit alpha or beta radiations are used in radiotherapy because the radiation they release is absorbed by tumors. Target locations are frequently impacted by radioisotopes via systemic circulation.

Since only a few organs may receive target-based delivery of radioisotopes, it is helpful to encapsulate or load them onto carriers that are targeting tumor like nanoparticles, micelles of polymer, dendrimers, or liposomes to increase therapeutic efficacy and lessen damage to normal cells [169, 170]. Moreover, the effects of radiation on tissues are extensive and concentrated over several organelles of living tissues and cells, such as nucleus along with essential cellular processes and biochemical pathways. It is necessary to identify platforms that can execute a suitable biological process with outstanding operation and ray investigation in order to explore these effects [171]. Microfluidics can successfully connect the functional strength and the irradiation facility for exploring biological responses since it has the capacity to

maintain biopsies or to mimic the reactions using newly discovered technology called organ-on-chip. An additional area of study for microfluidics in radiobiology examination is offered as biosignature assessment, in addition to the extension of devices for fundamental and biological study. The medical sciences now place a high value on biomolecules and biological elements, which also include radiological examinations. Since they might be utilized to assess radiation sensitivity of tumor cells, gauge the extent to which damage to healthy tissues and other cells will occur, and calculate the optimal radiation dose, traceable biofactors are crucial in the treatment of cancer [172].

Using microfluidic technology, Carr et al. identified radiation-treated apoptotic cells in human head and neck cancer cells (HNCC) by assessing the levels of cytochrome C, lactate dehydrogenase (LDH), and caspase-3-cleaved cytokeratin-18. Before using the microfluidic device for ray study on the HNCC, the system was initially built up using prepared samples of rat liver. Results depicted that the microfluidic device was reliable for preserving liver tissue of rat and HNCC samples without radiotherapy because detectable biomarkers of the apoptosis method, such as LDH and cytochrome C were assessed in the microfluidic platform. However, notable impact was not observed in sensing radiation derived by cytotoxicity in the body tissue. Three days post radiation, in contrast to the untreated tissues, a significant increase in the apoptotic index was seen when caspase-3 cleaved cytokeratin 18 was immunostained. Intriguingly, the study found no discernible difference between the samples which were untreated in standard in vitro cell culture and the untreated sample of tissues with radiation in microfluidic device. The researchers were able to keep the HNCC specimen alive by creating a fictitious three-dimensional in vivo environment in a microfluidic platform. They also used tissue-based variables of apoptosis to present the relationship between the irradiation dosage and radiation-induced cell death [173].

Another research by Patra et al. looked at the effects of on-chip radiation treatment combined with anticancer medication on sarcoma spheroids to assess cell apoptosis. To simultaneously assess the cytotoxicity caused by radiation treatment and chemotherapy, they created a microfluidic apparatus to produce soft tissue sarcoma spheroids. A PDMS-based microfluidic device was built. Then, in order to have more experimental freedom, they created a different apparatus that consisted of five linearly connected chambers, each with cubic trapping for spheroid foundation. Two layers of PDMS were used to fabricate the microfluidic device. Spheroid culture chambers are present in the bottom layer, and a straight channel covers each culture chamber in the top layer. While each chamber may be subjected to a certain radiation dosage, every system holds the potential to be administered by a unique agent. In the devised apparatus, soft STS 117 and STS 93 cell lines were shaped into spheroids of homogeneous size. In addition to chemotherapy, which included doxorubicin at two different concentrations, various radiation dosages were also used. Spheroid culture chambers inside the device were organized in the form of a matrix. Spheroids out of every treatment scenario may be accessed by the gadget independently. Spheroids that had been treated were divided in separate cells and examined using flow cytometry.

Results showed that radiation treatment caused death through various mechanisms whereas doxorubicin-affected STS 93 cell lines perished through apoptosis [174].

15.5.4 Gene Delivery for Cancer Using Microfluidics

Since the discovery of the structure of nucleic acids and the lighting of many illnesses' biochemical pathways, the replacement of defective genes with normal copies has been viewed as a novel treatment strategy known as gene therapy [175]. Expression constructs are used in gene therapy to increase the synthesis of useful proteins within the cells. Additionally, down-regulation of certain genes or faulty genes has demonstrated a significant influence on the treatment of a variety of disorders [176]. Several studies are now looking into gene delivery as a new method of treating cancer. Multiple genomic destructions, such as the inactivation of cancer inhibitors or the regulation of apoptosis-related pathways, as well as P53 mutations, according to some experts, may be a catalyst for the growth of tumors. Therefore, replacing a mutant gene with a healthy one or silencing a dangerous gene may offer a promising prospect for cancer therapy [177]. The following qualities are critical for a successful gene therapy: method for gene expression should be plasmid-based and second, the gene should be able to generate certain therapeutic proteins. Finally, a good delivery method for gene therapy is necessary to protect the plasmid from nuclease activity and to convey it to the site of action [178]. Through a variety of vectors, including viruses and non-viral ones such as cationic polymers and liposomes, the altered gene can enter the cell [179]. The conventional methods for transfecting a gene have some drawbacks, including difficult procedures, poor specificity, poor precision, limited cell survival, and low effectiveness. Microfluidic technology has made remarkable advancements that have opened up new possibilities for gene delivery and treatment. The transmission of genes into cells can be accomplished by electrical transfection. A microfluidic device made for electroporation uses electricity to capture plasmids on the anode and electroporate them into cells to transfer genes. Microfluidic electroporation requires lower voltages for gene transfer than conventional electroporation, which preserves cell integrity. In order to gather cells in one location, electrotransfection capabilities in the microfluidic system contains a layer of metallic electrodes combined with microfluidic device channels. Several chip designs incorporating various materials, microfluidic channels and electrodes have been researched to improve transfection effectiveness. Low direct current voltage boosted efficiency of transmission by almost 60% and life span of a human leukemia cell line by around 85%, according to research by Kim et al. [180].

Hydrodynamic focusing, which has been created before for flow cytometry technique to increase the cell counting accuracy, may also be used for microfluidic approach to enhance the performance of gene delivery. Zhu et al. created a device based on electroporation combining low direct current voltage and hydrodynamic focusing of a microfluidic stream [181]. In the end, optical gene delivery is among the cutting-edge methods that microfluidics may use for gene transfer. In order to

change the amount of energy that light has, it can be simultaneously strengthened and focused to produce lasers. As a result, optical transfection—the delivery of genetic elements into living cells—can be accomplished with less stress on the cells [182]. Different laser sources, including pulsed and continuous wave lasers, have been used for optical transfection. By targeted heating, continuous-wave lasers and nanosecond-pulsed lasers can open a temporary pore in the cell membrane. Additionally, thermoelastic strains and bubbles are produced by these lasers. Without the need for a micropipette to enter the cell, bubble generation can be employed to disrupt the structure of the cellular membrane for further microinjections of biofactors [183]. A microfluidic system was created by Robert et al. employing hydrodynamic focusing to grow single cells with concentrated laser light. With a yield of about 28%, propidium iodide was effectively transported by the microfluidic device into the embryonic human kidney and suggested the potential for continued, high throughput operation [184] (Table 15.3).

15.6 Conclusion

Past few years have seen amazing developments in both microfluidic techniques and tests based on them, which speaks eloquently about the value of microfluidics as a novel approach. Numerous areas of cancer research, including drug screening, diagnosis of cancer, delivery of drugs, and the application of various treatment modalities like radiation therapy, chemotherapy, and gene therapy have made extensive use of microfluidic devices. Microfluidic chips are flexible and customizable, allowing for the development of complex tumor organoids and the cultivation and analysis of patient-derived tumor cells and tissues on a tiny chip. Using microfluidics, it is possible to create drug delivery systems under extremely controlled settings and test the impact of encapsulated medications on tumor. Additionally, microfluidics has the capacity to mimic the characteristics and functions of organs which allows to examine the efficacy and safety of novel treatment and medications before they are used in patients. A successful cancer medicine is thought to cost around $1 billion USD, and 90 percent of drugs that enter the first phase of clinical trials fail to show any therapeutic value and are not subsequently developed. Thus, using 3D culture systems and microfluidic technologies to assess efficacy, ideally using patient-derived cancerous tissues, offers the potential to speed up drug discovery, and lowers cost. Scientists can affordably and quickly diagnose cancer thanks to microfluidic technologies. One of the major advantages of this technology is its high accuracy in evaluating some diagnostic parameters with a lower sample size, making it a formidable challenger to conventional diagnostic testing. Microfluidic chips have rapidly advanced over the last three decades, and numerous advancements in the detection and treatment of tumors have been made. Microfluidic devices should be created for precise point-of-care cancer diagnostics in the emerging age of personalized health care and medicine, offering hope for customized treatment of cancer.

Table 15.3 List of microfluidic technologies used for treatment of cancer

Microfluidic technologies	Properties of chip	Advantages	References
Lung cancer-on-chip	Monitoring the cytotoxicity and estimation of drug compounds in real time	Real-time monitoring showed the comparison of two drugs (doxorubicin and docetaxel) and proved that former caused greater cell death rate after increasing concentration	[185]
Lung cancer metastasis organ-on-chip	Simulating the microenvironment in vivo for spread of lung cancer	The performance of metastasis in the developed platform is validated by the damage in in vivo tests followed by cancer cell metastasis to astrocytes, osteocytes, and hepatocytes	[94]
Lung cancer-on-a-chip	Determining how tumor invasion, metastasis, and medication resistance are affected by the interaction between tumor cells and fibroblasts	A549 cells cultured alongside HFL1 cells demonstrated anticancer treatment resistance, and A549 cells co-cultured with HFL1 and HUVECs demonstrated that the A549 cells might cause endothelial cells to undergo apoptosis, or death	[186]
Liver/Heart cancer-on-a-chip	Duplicating in vitro the negative results of anticancer medications	Assessing the cytotoxicity on both normal cardiomyocytes and cancer cells of an anticancer drug inside the device	[187]
Bone-on-a-chip	Bone metastasis of tumor cells of breast	Observation of previously verified in vivo tests of breast cancer bone metastasis hallmarks	[188]

(continued)

Table 15.3 (continued)

Microfluidic technologies	Properties of chip	Advantages	References
Liver-on-a-chip	Examining the functions of EVs generated from breast cancer in liver metastasis	Breast cancer-derived EVs activated liver sinusoidal endothelial cells, resulting in the breakdown of vascular walls, increased TGF-1 levels, and overexpression of fibronectin, which made it easier for breast cancer cells to adhere to the liver milieu	[189]
Ahepatocellula carcinoma–bone metastasis-on-a-chip	Investigating the anticancer impact on HCC metastasis of thymoquinone (TQ) free and encapsulated form	The extended duration of the inhibitory impact of thymoquinone encapsulated in nanoparticles (anticancer nanoparticles)	[190]
Colorectal cancer-on-a-chip	Studying how the microenvironment affects intravasation	Allows a model system that is applicable to studying early invasive cancer occurrences in humans	[191]
Heart-breast cancer-on-a-chip	Cancer chemotherapy-induced cardiotoxicity: disease modeling and monitoring	The system will enable early identification and forecasting of cardiotoxicity brought on by treatment in specific patients	[192]
Kidney cancer metastasis-on-a-chip	Development of kidney tumor in liver to predict the effectiveness of treatment	Platform showing higher ability to kill Caki-1 cells compared to free anticancer medicine (5-FU) and a linear anticancer relationship between 5-FU concentration and the proportion of Caki-1 cells	[93]

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Chapter 16
Recent Developments in Two-Dimensional (2D) Inorganic Nanomaterials-Based Photothermal Therapy for Cancer Theranostics

Rajkumar Sekar and Shiji Raju

Contents

Abstract In the modern era, two-dimensional (2D) nanostructures have an excessive attraction for numerous scientific utilizations such as biomedical devices, nanobiosensors, nanomedicine, bio-membrane, and energy-storage utensil fabrication on account of superior physicochemical properties, namely extended surface-to-volume ratio, extreme thinness, tuneable surface modification, and quick biomolecules conjugations. In recent years, investigation in the designing and construction of 2D inorganic nanostructures has unwrapped a new golden path for cancer therapy and

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potential selectivity in non-invasive treatments. Including several reported advanced 2D-functional nanomaterials, the nano-graphene (graphene oxide), black phosphorous (BP), MXenes (carbides and nitrides), and transition metal dichalcogenides (TMDCs) have congregated most of the attention in the modern imaging-guided cancer therapy employment because of their readily adjustable physicochemical characteristics. In this chapter, we discuss the current development in the fabrication of 2D inorganic nanosheets, surface modifications, and their potential applications in photothermal therapy (PTT) and combined cancer theranostics.

16.1 Introduction

Prime modern-age issues such as excess consumption of liquor, tobacco, and the explosion of radiation have developed cancer the second-leading deadly disease over cardiac arrest throughout this globe. As stated by the World Health Organization reports, six out of ten death was caused by cancer disease [1]. Many single-modal or combined or synergic modal cancer therapies such as chemo/radiotherapy, immunotherapy, and gene therapy are being extensively applied to specific target cancers of several kinds. Among them, chemo and/or radiotherapy is a traditional therapy with good positive rates because of minimally invasive techniques, specific targeting ability, and quick recovery time compared to other therapies. Chemotherapy is regularly utilized to kill cancer cells by the action of toxic drugs [2]. Though, during the duration of therapy, the development of multi-drug resistance, non-targeting ability, premature release of anticancer drugs, and the lack of diagnosis have led to potential difficulties in chemotherapy [3]. Moreover, heavy doses of toxic drugs affect nearby healthy cells as most of the released drugs initially encounter instantly multiplying cells and lead to cell death [4]. Nanotechnology-based nanomedicine formulations for several cancer cell targeting mechanisms, namely reverse multi-drug resistance and active targeting involving modified surface using targeting agents, have extensively improved the intercellular uptake, which, in turn, potentially enhances the therapeutic efficiency [5, 6]. On the other hand, radiation-based radiotherapy for cancer therapy explores heavy energy waves to kill cancer cells. Earlier reports on radiation therapy, the exhibition of radiation tempts tumor exodus and significantly ablates the tumor also [7]. In some enlarged cell-based organs such as the lung, the tumor is less reactive to radiation and less effective with a maximum cure rate of 25% [8]. Thus, several kinds of tumors remain less sensitive to radiation due to their intrinsic cell resistance and consequently reappear quickly after the therapy because of adequate cell resistance [9]. Additionally, after chemo-radiation therapy, patients suffer potentially with hematological toxicities and mucositis [10, 11]. Regrettably, a continuation of chemo-radiation therapies can lead to a rise in body toxicity, nausea, and vomiting to cancer convalescence. To overcome these restrictions, tumor microenvironment targeting thermal therapy has been developed as an significant method for cancer therapy. Along with cancer-destroying efficiency, thermal therapy can also open a new window for synergetic or combined cancer therapy modalities.

Near-infrared radiation (NIR), radio waves, magnetic fields, ultrasound, etc., have been largely utilized to generate thermal spots and serve the resolution. Compared to other modern therapies, photothermal therapy (PTT) received much attraction for offering outstanding therapeutic efficiency due to its minimal invasiveness, improved therapeutic efficiency, fewer side effects, no need for long-lasting therapy, and quick recovery [12]. A photothermal agent (PTA) can be passed in the tumor microenvironment region and absorbed by the NIR laser lights to obtain kinetic energy that discharges thermal energy on that tumor microenvironment and lead to cell death [13]. Extensive NIR absorption capacity and permission on healthy organs in the range of 650–1350 nm leads to deep interpenetration in the patient's body and develop injury to the tumor [14]. Moreover, this approach rises thermal waves to form trouble in the cell membrane of the adjacent cancer cells [15]. Hyperthermia through absorption of NIR laser limits many issues mainly short inter-penetrating power and strong absorption which leads to backfiring for normal cells developing injury and significantly reducing the therapeutic efficiency. Auspiciously, recent development in nanomaterials has broken such problems. To date, a large number of PTA were designed for phototherapies based on noble metals-mediated nanoparticles (NPs) (generally gold NPs because of the surface plasmon resonance (SPR) [16], carbon family members including graphene [17], carbon dots [18], mesoporous carbon [19], and non-carbon members such as black phosphorous (BP), transition metal dichalcogenides (TMDCs), and polymer-inorganic nanocomposites [20]. Apart from the aforementioned nanomaterials, organic conducting polymers [21, 22] and non-metallic nanomaterials [23] have also been extensively operated as PTA because of the laser absorption characteristics and generation of photothermal conversion efficiency (PCE). In the case of SPR holding NPs, it exhibited that the electrons on the conduction band were localized on the surface of the NPs upon NIR treatments, and subsequently, the localization attaint the target at the resonant frequency to generate SPR, thereafter absorbed NIR transforming into thermal waves [24]. Therefore, it is probable to control NPS via local intravenous injection and consequently, exciting them in the NIR open windows I and II. Additionally, the injected nanomaterials incline to deposits in vital tissues of the spleen, kidney, and liver inducing injury irreversibly [25, 26]. Hence, it is very essential to develop the PTT technique more safely and effectively. Along with most reported spherical and rod-shaped nanomaterials, latest years have shown a potential emerging report on two-dimensional (2D) inorganic nanosheets for a variety of usages in the biomedical, biosensor, environmental remediation, catalysis, and energy-storage devices field [27, 28]. Recently, several reported book chapters and reviews have deeply discussed only anyone type of 2D inorganic nanosheets among several discussed the device fabrication for energy-storage, biosensors to biomedical applications [20, 23, 29, 30]. Moreover, the mechanism behind the formation of thermal energy in parent materials is familiar, while the occurrence of NIR laser reaction with 2D-NSTs and the formation of thermal energy is still unpredictable [31]. Therefore, in this chapter, we focus on the synergetic phototherapies especially PTT-Chemo-PDT with 2D nanosheets, as shown in Fig. 16.1. In the first section, the outline of nanosheets for cancer theranostics, the synthesis, functionalization, and different kinds of 2D-NSTs is introduced briefly. In

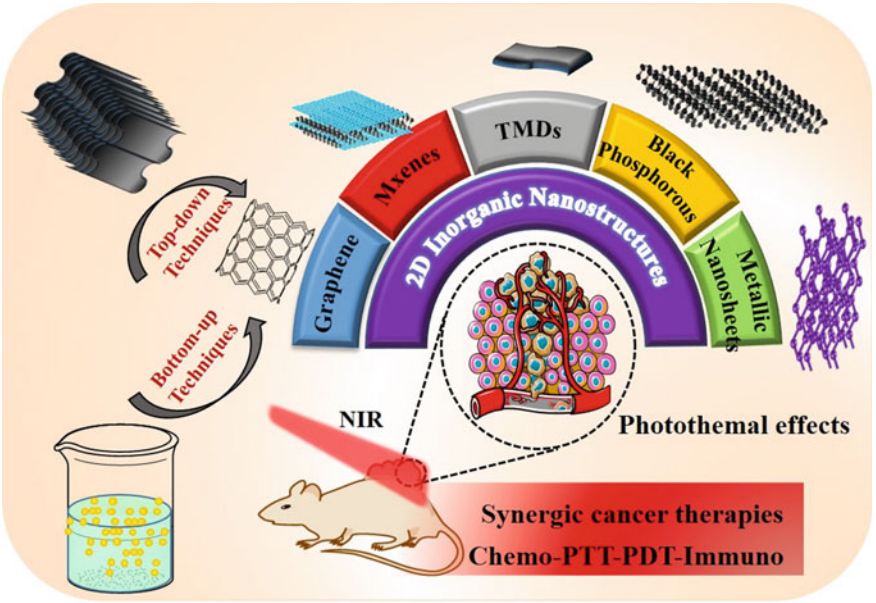

Fig. 16.1 This chapter focus on the synthesis, surface modification, and synergetic phototherapies especially PTT-Chemo-PDT with 2D inorganic nanosheets

the second section, important cases of synergetic phototherapy include PTT-Chemo-PDT with various types of 2D-NSTs. In the second section, properties, mechanism of thermal generation, and working of photothermal effects in 2D-NSTs are explained. In the final section, biosafety perspectives are also discussed. It is expected that this chapter will guide investigations of the fabrication, preparation, and PTT studies of novel 2D-NSTs-based nanomaterials in future.

16.2 2D Inorganic Nanosheets for Cancer Theranostics

2D nanosheets (2D-NSTs) hold merits, namely ultra-thin atomic thickness, high surface area, unique physicochemical properties, controllable size with quick surface functionalization, and high PCE. Remarkably, 2D-NSTs for phototherapies have showed prolonged time blood rotation as well as high accumulation on cancer sites. Surface-modified palladium 2D-NSTs hold a 4.4 nm size range and show renal clearance from the body via urine excretion pathway [32]. The most excellent 2D-NSTs are graphene and its spin-offs for instance nano-graphene oxide (nGO) [14] and reduced graphene oxide (rGO) [33, 34], which show strong NIR laser light absorption and outstanding PCE when compared to the noble metal nanostructures and nanotubes based on carbon derivate. Many investigations on nano-graphene

functionalized with polymers and NPs and therapeutic agents (polyethylene glycol (PEG) [35], palladium NPs [36], gold NPs [37], drug molecules [38], and photosensitizers [39]) for cancer theranostics were stated for delivering of multimodal cancer phototherapies with effects of graphene 2D-NSTs. The unique reduced and oxidized chemical structures of graphene deeply suffer from problems such as cytotoxicity and minimal biodegradation [40, 41]. Spinoffs of graphene are extensively utilized for PTT. For example, graphene, a new 2D of carbon allotrope, showed PCE (upon laser irradiation by 808 nm) of 42% with photoacoustic imaging in vivo mice studies [42]. Similarly, modified graphene hybrid nanocomposites also demonstrated a promising synergistic PTT effect with high therapeutic efficacy [43, 44]. After the graphene family, latest years researchers were widely attracted to the new 2D-NSTs type for PTT the TMDCs, and their nanocomposites showed good performance in biocompatibility and remarkable PCE performances of 62.5%. The TMDCs-based 2D-NSTs are developed by top-down and bottom-up methods; among them, the bottom-up approach is more flexible to control the surface functionalization, aqueous dispersity, regular shape, and size [20]. Even though monoatomic layer 2D-NSTs are very hard through the bottom-up method, which is more desirable for biomedical applications. Emerging next-generation 2D-NSTs of TMDCs have the potential ability to treat cancer through PTT effects due to their good biocompatibility, biodegradability, marvelous PCE performance, less expensive, and extended photo-stability [45]. Commonly, TMDCs consist of a multi-layer of transition metal elements including tungsten, molybdenum, niobium, hafnium, and zirconium, which sandwiched among two outer layers of chalcogen atoms, namely sulfur (S), selenium (Se), and tellurium (Te). Among them, Mo-based molybdenum disulfide (MoS_2) exhibited strong NIR absorption and remarkable biocompatible because Mo exists as the recognizable element for enzymes such as xanthine oxidoreductase, aldehyde oxidase, and mitochondrial amidoxime-reducing and S also present in the body. The MoS_2-based 2D-NSTs were first confirmed in 2013 as a new 2D nanomaterial for PTT effects and exhibited very strong NIR absorbance when compared to carbon group members (graphene and its derivatives) and noble metals group (gold and silver nanomaterials [46]. They have been extensively hybrid with various kinds of organic/inorganic materials to perform as nanocarriers to deliver drug and photosensitizer agents against cancer as well as involve synergetic therapies also [44, 47, 48]. Even though MoS_2 2D-NSTs are limited by design and fabrication difficulties such as long-time consuming processes, using hazardous solvents, and less stability in an aqueous medium. However, it is applied for PTT investigations. The surface of MoS_2 NSTs has been functionalized either by PEGylation or long chain branched polymers to enhance their colloidal stability [49, 50]. Moreover, W-based NSTs have also confirmed their PCE effects in PTT via intravenous injection for combined synergistic cancer theranostics applications [51, 52]. Apart from graphene, one more kind of 2D-NSTs is MXenes with the formulation of Mn + 1Xn ("X" stands for carbon (C) or nitrogen (N) elements and "M" denotes transition metal elements) as an emerging new member in 2D-NSTs series. Ultra-thin MXenes behave like semiconductor metal for the property of band energy, and it denotes that the SPR efficiency is almost close to that of metal/metal oxide NPs [53]. Similar effects are applied to

many 2D-NSTs such as black phosphorous (BP) and metal dichalcogenides. Among them, titanium carbide (TiC) 2D-NSTs are the most extensively investigated materials. An ultra-thin membrane of TiC was synthesized by etching via hyaluronic acid which exposed 100% of PCE with heavy NIR laser absorption than carbon-based nanomaterials [54]. In addition, niobium and tantalum carbide-based 2D-NSTs are also under-investigated in this classification [55, 56]. Many selenides, sulfides, and oxides-based metallic compounds have been studied as PTA for cancer theranostics due to their localized SPR. Moreover, bulk Ti nanosheets prepared by liquid-phase exfoliation (LPE) showed strong NIR absorption at 808 nm laser density and offered a heavy PCE of 73.4% compared to the other 2D-NSTs such as MoS_2 (62.5%) [57], Ti_2C_3 (58.3%) [58], BP (43.6%) [59], and Au nanorods (21%) [60]. In BP, adjusting the band gap of 0.2–3.2 eV, excellent biocompatible nature, biodegradability, and higher PCE made them outstanding PTA agents with nanostructure of 2D-NSTs as well as zero-dimensional nanomaterials. In addition, BP 2D-NSTs are shown high optical properties and large surface area [61]. The BP 2D-NSTs hold photo-stability and high drug encapsulating ability other than the remaining nanosheets [62]. Especially, BP can readily involve synergetic cancer theranostics as it shows strong NIR absorbance. For example, PEGylated-BP 2D-NSTs have efficaciously encapsulated chlorin e6 (Ce6) photosensitizer (PS), then allowed to pass into the cancer region through enhanced permeability and retention (EPR) effect. The prepared BP-PEG-Ce6 nanomaterials irradiated under a NIR laser of 660 nm and consequently showed 43.6% of PCE. In another study, BP 2D-NSTs confirmed their PCE effect in PTT with 39.3% in xenograft tumor mice [63]. Even though the great advancements in the 2D-nanostructures for phototherapies in basic research, still they struggling for big success in clinical practice.

16.3 Synthetic Routes of 2D-NSTs

The designing of well-organized biomaterials aided by 2D-NSTs with an approximate thickness (3 nm) is potentially attracted researchers for several biomedical applications. The 2D thin sheets show higher sizes from 100 nm to 2 μm along some atoms of less than 5 nm of thickness and establish special physiochemical nature, i.e., 2D-NSTs own their monolayers including the poor outward and good inward planes chemistry as atomically arranged linkages. The outstanding physical properties of these nanosheets (ultra-high photo/thermal conversion) make them a hopeful candidate in PTT for cancer theranostics compared to conventional regular nanomaterials. Thus, super advancement of various measurements dependable preparation techniques is available for these nanosheets synthesis. Frequently, the nanosheets were developed by major approaches such as the top-down process depends on the conversion of multiple layers (bulky) sheets to mono layers by exfoliation techniques and the bottom-up process used for developing 2D-monolayers using the corresponding precursor salts as depicted in Fig. 16.2. Though various preparation methodologies affect many chemicals, optical, thermal, mechanical, physical, and

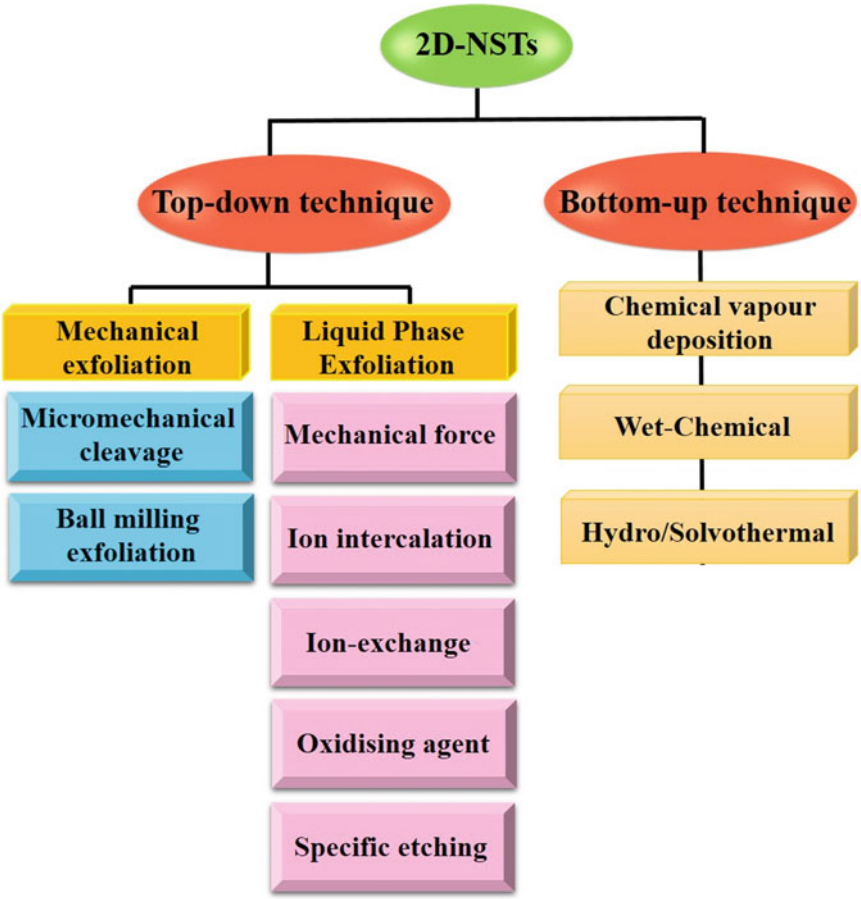

Fig. 16.2 Synthesis tactics of 2D-NSTs based on top-down and bottom-up techniques

electronic properties which can be applied to distinguish the linking between the functional and structural nature of 2D-NSTs. The construction of 2D nanosheets with expected chemical and physical features (ultra-thinness, nano-size, elemental compositions, as well as surface area) is potential factors to comprehend the linkage among structure and functions natures for versatile biomedical applications.

16.3.1 Top-Down Technique for 2D-NSTs

The top-down process is applied for the drive-out controlled synthesis of 2D-monolayer nanosheets from multi-sheets by miniaturizing them while maintaining their originality. Commonly, liquid-phase or mechanical approaches are applied to

achieve 2D-NSTs. The main principle behind the process is to eject poor Van der Waals bonding in the layers of bulky materials. Certain merits of this process are cost-effective and easy product reproducible techniques. So, this approach is a leading favorable one in potential medical science and technology applications. However, the final products appeared with structural defects in the surface, which is the major drawback of this technique.

16.3.1.1 Mechanical Exfoliation (MEX)

In top-down, the mechanical exfoliation method has two major types such as micromechanical cleavage (MMC) and ball milling exfoliation (BMEX). In the MMC process, thin 2D flakes are developed using scotch tape by applying mechanical strength to exfoliate multi-layered bulky materials into a single or combined layer of flakes. The formation of exfoliation by breaking of poor Van der Waals force amid the multi-layers either expansion or decrement of multiple layer bulk sheets and deprived of distress the covalent bonding between individual layers [64]. The merits of this method are crystal quality with a purified surface which is possible, and huge size thickness is a demerit. Conversely, the synthesis of 2D-NSTs by BMEX creates two kinds of force, namely shear and compression, which can separate the bulk multi-layered materials into monolayered 2D-NSTs. The separation is generally generated from the top/bottom surfaces layers as well as corners of the bulk materials and subsequently delivers a clean preparation methodology for surface functionalization due to the great ejection force of BMEX which can support to functionalize exfoliated ultra-thin nanosheets with various advanced-functional molecules that allow bonding reactions without solvents [65, 66]. This is a progressive technique to crack the multi-layers into the monolayers of 2D-NSTs with a size range of <200 nm than other mechanical techniques. Currently, so many reports existing in the literature have exhibited the development of monolayers of inorganic nanosheets by BMEX.

16.3.1.2 Liquid-Phase Exfoliation (LPEX) Process

LPEX process is well-known for the high-scale and effective separation of monolayers from bulk multi-layers and to form ultra-thin monolayer 2D-NSTs utilizing several surfactants and ionic and organic solvents. In this method, the bulk multi-layered sheets are converted into nanoscale individual 2D-NSTs, consequently dispersed the nanosheets in organic/ionic solvents to restrict aggregation. During the exfoliation process, simultaneously, sonication and ball milling (low energy) are applied. Li et al. developed the ultra-thin tantalum carbide (Ta_4C_3) 2D-NSTs using hydrofluoric acid (40%) for engraving, subsequently under probe sonication of parent materials (Ta_4C_3). The mean diameter of nanosheets was 141.8 nm and exhibited an effective PCE of 32.5% [56]. Similarly, [31] fabricated ultra-thin titanium 2D-NSTs with <3 nm thickness and <50 nm size from isopropyl-alcohol dispersion using probe and bath sonication. Various other ultra-thin 2D-NSTs such as BP, TMDCs, MnO_2,

and Pd have also been designed for cancer phototherapy. Further, the LPEX process is subdivided into various types based on supporting processes such as mechanical, ion-intercalation, ion exchange, oxidation, and specific etching. These methods are described briefly in the following section.

(a) Mechanical force supported LPEX: The machine-driven strength in liquid-phase circumstances subsequent in transforming the multi-layered to ultra-thin 2D-NSTs in cost-effective and potential yield by breaking poor Van der Waals interactions. The sonication and shear force methods are applied to achieve the potential single layers. Recently, MoS_2 2D-NSTs were synthesized with 5–15 nm thickness and 40–70 nm size by sonication of bulky micro-materials in a cold condition for 2 h [67]. An ultra-fast shear instrument was utilized for the separation of MoS_2 multi-layer bulk materials through grinding (mechanical), diffusion (sonication), and emulsification, forming a few thin layers of MoS_2 in 50–200 nm size with 65% and 9% of sheets with <4 layers and single layer, respectively [68, 69]. (b) Ion intercalation supported LPE: Addition of cationic ions (K^+, Li^+, Na^+, and Cu^{2+}) with a small radius between the layers of bulk materials to form ion-intercalating molecules and consequently and expand the interlayers spacing and flagging the Van der Waals between multi-layered bulk parent materials [70]. As a result, the multi-layered bulk materials could be exfoliated into two or a few layers 2D-NSTs under minor ultra-sonication in aqueous solvents. In several reports, the addition of Li^+ and Na^+ for thin-layered generation in presence of water can produce hydrogen gas and further prompt quick separation of multi-layers to form the 2D-NSTs [71] (c) Ion-exchange supported LPE: In this process, replacing previously existing small ion by large ion in multi-layered bulk materials through ion-exchange practice. Subsequently, generate expansion in the inter-space of layers of bulk materials and consequently flagging of the Van der Waals interaction. Finally, with the aid of sonication, the ultra-thin layers of 2D-NSTs were achieved. (d) Oxidizing agent supported LPE: In this method, majorly deals with graphite to form graphene nanosheets by utilizing robust oxidizing agents potassium permanganate and concentrated sulfuric acid. The highly rich oxygen functional molecules in the multi-layered graphite result in excellent breaking of the interlayers of graphite through reducing Van der Waals bonding. This prominent approach was used to generate the GO nanosheets in large quantity [72] (e) Specific etching supported LPE: This approach is general way to synthesis MXenes 2D-NSTs. The MXenes exist with metallic coordination amid the interlayers of bulk materials with $M_n + 1AX_{n,}$ i.e., MAX phase, M denotes for transition metal elements as well as others A/X stands for C and N elements. For these nanosheets, octahedral spots of "M" transition elements are closely arranged among carbon or nitrogen, and the layers of "A" are spread like sandwich forms. The M-A bond prefers metallic bonding, while the M-X bond prefers covalent/ionic bonding. For example, Ta_4C_3 2D-NSTs were synthesized by etching from bulk MAX phase materials [73]. The etching method cleared the middle Al layers from Ta_4AlC_3 resulting in monolayer nanosheets. Consequently, aggregation of the nanosheets was discarded using ultra-sonication.

16.3.2 Bottom-Up Approach for 2D-NSTs

Several molecules or atoms depend on processes such as chemical vapor deposition (CVD), hydro/solvothermal preparation, and molecular condensation, which are utilized to generate monolayer 2D-NSTs from atoms by chemical methods. Commonly, this method depends upon the accessibility of selective metal–organic chemicals as precursors for straight preparation of the 2D-NSTs. These approaches are utilized in different kinds of tenders due to their easy reproduction, high-scale production, and cost-effectiveness. Generally, two methods, namely vapor deposition and wet-chemical (hydro/solvothermal) preparation, are routinely applied to achieve 2D-NSTs from the atomic level. These methods are described briefly in the following section.

16.3.2.1 Chemical Vapor Deposition (CVD)

This approach is the prominent path to the synthesis of nano-films and sheets with good efficiency, scale, and quality. Based on this technique, several inorganic thin nano-films on various elements were designed for industrial proposes [74]. Moreover, it aids to customize the 2D-NSTs through adjust the properties such as composition, doping, orientation, phase, size, and morphology [75]. Repeatedly, this method is utilized to develop incessant, pure, large, and uniform thin sheets of nanostructure as essential for wafer-scale materials preparation in practical applications [76]. Recently, indium selenide (InSe)-based uniform thin films were developed with good anti-oxidation properties and more stability through this approach [77]. The vapor deposition process is also applied to the fabrication and preparation of 2D-NSTs with variable thickness, large surface areas, and high quality [76].

16.3.2.2 Wet-Chemical Preparation

The wet-chemical technique is meant for a simple and most potential way to design the TMD-based 2D-NSTs directly from the reaction solution with potential scale-up. This approach can support easy tuning size and physical properties, therefore, developing 2D-NSTs with adjustable morphology with uniform thickness for various fields of applications [78]. This technique also supports designing several monolayer nanostructured 2D-NSTs compared to other techniques. The most general wet-chemical process is hydro/solvothermal preparation. In this method, the growth of 2D-NSTs takes place in an autoclave. Once the boiling point of the solvent was reached, it induces the removal of electrons from precursors to form 2D-NSTs and enhances the crystalline form of the prepared nanosheets. For instance, PVP functionalized MoS_2 2D-NSTs were synthesized via a hydrothermal process involving a chelation reaction to develop the transfer of electron pairs from the carbonyl group of PVP to the empty orbitals of the Mo element [79].

16.4 Surface Modification/Functionalization of 2D-NSTs

Many efficient methods (surface-doping, modifications, and organic molecules loading) have been applied for adjusting band gap energies of nanosheets to generate unique properties. Amid methods, surface-doping is a significant process that plays a potential role in surface modifications of 2D-NSTs by altering the unique characteristics of sub-atomic layers. Commonly, the surface-doping of nanosheets was achieved by several magnetic elements (Nb, Co, and Fe) and nanomaterials ($CoSe_2$, Fe_3O_4, CoS, and CdS) for enhancing their PTT through the synergistic linking method, PCE catalytic activity, and superparamagnetic nature. Meanwhile, physiochemical characters of 2D-NSTs are also significantly adjusted by functionalization through surface decoration through biomolecules, targeting ligands, drugs, and metallic/polymeric nanoparticles to produce the necessary qualities. Surface functionalization of 2D-NSTs by such a process improves the PCE, targeting ability, prolonged blood motion, and intercellular uptake efficiency. Commonly, two approaches developed in the surface functionalization of 2D-NSTs such as doping and surface decoration were briefly discussed in the following section.

16.4.1 Metal Doped 2D-NSTs

The purpose of doping is to bind the metal elements in pure crystalline materials that could alter their physicochemical properties. At the initial stage of the nanosheet preparation, dopants can be introduced as metallic salt precursors in optimized molar ratios. In-plane doping of 2D-NSTs with other metal/elements aids to change the distinct characteristics of the 2D-NSTs such as electron defects, band gap energies, mobility carriers, and conductivity. These doping techniques also enhance the PCE and incorporate supplementary activities to the 2D-NSTs for more accurate cancer phototherapy. For instance, the surface-doped Gd^{3+} acts as a T_1-weighed ligand bonded with WS_2 and $MoSe_2$ nanosheets [80, 81] making them attain to good resolution bio-imaging with improved PCE other than ordinary 2D-NSTs.

16.4.2 Surface-Modified/Decorated 2D-NSTs

The 2D-NSTs are greatly selective for phototherapies because of the extreme PCE at 808 or 1064 nm within NIR windows. Surface decoration/modifications of nanosheets with various moieties such as metal nanoparticles, biopolymers, biomolecules, and radioisotopes help to enhance its biological characteristics for instance low hemocompatibilitv, biocompatibility, drug-loading capability, enhanced tumor-targeting ability, and intercellular uptake. This can be attained through chemical bonding, encapsulation, and weak Van der Waals forces. Table 16.1. The surface

Table 16.1 Surface decoration of 2D-NSTs developed by organic/inorganic molecules

Types of surface decoration	Materials	Physico-chemical changes	Biological functions	References
Polymers	Natural polymers: dextran, cellulose, chitosan	Enhance the biocompatibility	Reduce the attack of macrophages and reticuloendothelial system (RES)	[82–84]
	Synthesis polymers: PEG, PEI, PVA, PGLA			
Nanoparticles	Metals: Au, Pt, Pd Metal oxide: Fe_3O_4, CuO	SPR properties of metal/metal oxides enhances PCE	Attracts more multimodal imaging	[85–87]
Drugs	Anticancer drugs and photosensitizers	-	Synergistic cancer therapies	[88, 89]
Biomolecules	Peptides, antibodies, Enzyme, and DNA	Enhance the stability and specificity	Prompt high binding affinity with cancer cells and receptor mediated endocytosis	[90, 91]
Radioisotopes	Radio isotopic	-	To track the bio-distribution of nanomaterials	[92–94]

PEG Poly(ethylene glycol); *PEI* Poly(ethylenimine); *PVA* Poly(vinyl alcohol); *PGLA* Poly(lactic-co-glycolic acid)

decoration of 2D-NSTs developed by different kind's organic/inorganic molecules has been mentioned.

16.5 2D-NSTs for Synergistic Phototherapies

16.5.1 MXene 2D-NSTs

Pan et al., developed novel 2D $NaErF_4@Ti_3C_2$ nanosheets for NIR-IIb and MR imaging-guided PTT. In this stud, surface modification of Ti_3C_2 2D-NSTs with $NaErF_4$ NPs leads to emission in the NIR-II region (1530 nm) under 808 nm excitation, assisting NIR-II diagnosis and nursing the tumor reduction during hyperthermia. Moreover, the presence of Er^{3+} ions in NPs can also develop potential MR images of the tumor microenvironment. Finally, the designed composites showed a high PCE of 43.62% at 808 nm NIR laser irradiation and express potential tumor ablation with an inhibition ratio of 92.9%. So that 2D $NaErF4@Ti_3C_2$ demonstrated multimodal imaging-guided PTT for cancer [95]. Recently, Wu et al., designed a novel

self-assembled Ti_3C_2 oriented Schottky-junction to control the tumor microenvironment and improve MR imaging-guided chemodynamic/PTT activation through NIR irradiation. The $MnFe_2O_4$ NPs were self-assembled onto 2D Ti_3C_2 nanosheets using chitosan (cross-linker) to form Schottky-junction as TC@Ch-MFO. These nanomaterials can be used to generate O_2 by controlling the catalyzing hydrogen peroxide and diminishing the overexpression of glutathione (GSH) range in the hypoxic tumor microenvironment, which established the CDT via Fenton reaction at 808 nm laser treatments. In addition, Ti_3C_2 nanosheets demonstrated high PCE as PTA and also integrates MR imaging during cancer therapy as shown in Fig. 16.3a. [96]. In other studies, novel Ti_3CN-based 2D-NST was developed for significant photo-hyperthermia in vitro and in vivo [97]. Xu et al., approached iron chelation chemo-photothermal therapy using DOX-linked iron chelator deferasirox (DOXjade) loaded onto 2D Ti_3C_2 nanosheets (Ti_3C_2-PVP@DOXjade). Consequently, the nanomaterials showed PCE of up to 40% and excellent drug-loading efficiency. In this study, an antitumor mechanism was determined by iron depletion-induced TfR protein downregulation. Hence, Ti_3C_2-PVP@DOXjade showed pH-responsive iron chelation-PTT-chemotherapy effect was accomplished both in vitro (HCT116 cells) and in vivo (HCT116 tumor-bearing nude mice) for cancer [98]. Zhang et al., designed a m_eTGCT nanoreactor based on Ti_3C_2 nanosheets with glucose oxidase (GOX), chloroperoxidase (CPO) (chemically conjugated), and tirapazamine (TPZ) (physically loaded) for syngeratic multimodal therapy on cancer. In this study, a brilliant automatic cascaded-enzyme nanoreactor was executed to attain amplified cancer therapy via photo/chemo/enzyme dynamic treatments. In biological (in vitro and in vivo) assessment, due to the biomimetic membrane and CD47 overexpression, m_eTGCT showed potential immune escape and significantly improve specific targeting as well as internalization. After the internalization, GOX and CPO produce HClO to lead the enzyme dynamic therapy (EDT). Moreover, NIR laser treatment could speed up the catalytic reaction to increasing the production of singlet oxygen ($^1O_2$) and hypoxia tumor microenvironments with the reduced oxygen level for promoting EDT [99]. The cobalt nanowires (CoNWs) and DOX incorporated Ti_3C_2 (DOX@Ti_3C_2-CoNWs) nanocarrier heterojunction were recently reported for synergistic chemo-PTT as shown in Fig. 16.3b. In this investigation, the PCE and DOX loading efficiency of nanocarrier were measured to be 34.2% and 225.05%, respectively. The pH-responsive DOX release was explored under pH 7.4, 6.5, and 4.5 in 24 h with 10.24%, 23.59%, and 76.75%, respectively. While under NIR irradiation, the DOX release was 39.3, 40.85, and 89.34% in the above pH range. These results showed that the responsive drug release of DOX was higher than the pH-responsive release from the nanocarrier. The DOX@Ti_3C_2-CoNWs showed magnetic properties due to the presence of Co nanomaterials, and the magnetic saturation (Ms) value of the nanocarrier was 33.026 emu/g. Subsequently, nanocarriers denote magnetic controlled behavior, which aids in directing the nanocarriers toward tumor sites using an external magnetic field. The chemo-phototherapy evaluation of DOX@Ti_3C_2-CoNWs with 50 μg/mL was examined against 4T1 cells with NIR irradiation 808 nm with a power of density of 1.5 W/cm^2. The laser NIR irradiation was applied for 20 min, and after 24 h incubation, <30% 4T1 cancer cells were dead [100]. Zhang

et al., designed 2D Ti_3C_2/graphitic carbon nitride (g-C_3N_4) nanosheets for in situ O_2 generating improved synergistic PDT/PTT. The surface modification of Ti_3C_2/(g-C_3N_4) using triphenylphosphonium bromide (TPP), revealed mitochondria-targeting and enables generate oxygen-independent O_2^- and OH via electron transfer. Consequently, improved PDT is achieved under both normoxic and hypoxic tumor microenvironments. The PCE of Ti_3C_2/g-C_3N_4 nanosheets was measured at 40.8%. [101]. Szuplewska et al., investigated the tumor ablation property of PEG-modified Ti_2C nanosheets with their potential PCE of 87.1% [102]. Synergetic cancer therapy applications of 2D MXenes nanosheets were summarized in Table 16.2.

MRI magnetic resonance imaging; *PA* photoausoctic imaging; *US* ultrasound; *PT* photothermal; *CS* chitosan; *PVP* poly (vinyl propyl); *MSN* mesoporous silica; *RGD* Arginylglycylaspartic acid

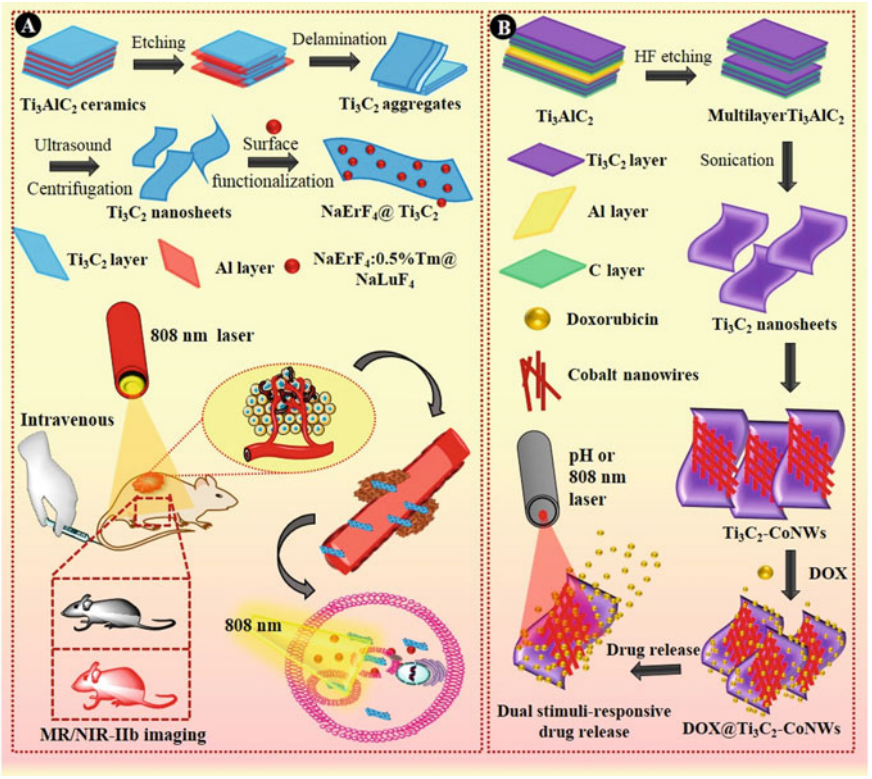

Fig. 16.3 **a** Schematic diagram of the synthetic method of $NaErF_4$@Ti_3C_2 nanocomposites and the dual-modal of MR and NIR-IIb imaging-guided PTT [96]. **b** Preparation process of the Ti_3C_2 nanosheets, intergration of CoNWs and DOX, and their dual stimuli-responsive drug release [100] (Table 16.2)

Table 16.2 Synergetic cancer therapies applications of 2D MXenes nanosheets

Elements	Surface functionalization	Multimodal imaging	PCE (%)	Synergetic therapies	References
Ti_3C_2	$NaErF_4$	MRI/NIR-IIb	92.9	PTT	[95]
Ti_3C	CS-$MnFe_2O_4$	MRI	–	CDT/PTT	[96]
d-Ti_3CN	–	–	36.5	PTT	[97]
Ti_3C_2	Fe^{2+} ions	MRI	26.5	CDT/PTT	[103]
Ti_3C_2	PVP@DOX	–	40	Chemo/PTT	[98]
Ti_3C_2	Triapazamine	–	53.87	Chemo/PTT	[99]
Ti_3C_2	CoNWs/DOX	–	34.42	Chemo/PTT	[100]
Ti_3C_2	PEG/TPP	–	40.8	PDT/PTT	[101]
Ti_3C	PEG	–	37.5	PDT/PTT	[102]
Ti_3C_2	Au@PEG	PA/CT	39.6	Radiotherapy	[104]
Nb_2C	MSN-PEG-RGD	–	28.6	Chemo/PTT	[105]
Ti_3C_2	SP@DOX	PT	–	Chemo/PTT	[106]
Ti_3C_2	MSN-PEG-RGD@DOX	US/PA	–	Chemo/PTT	[107]

16.5.2 Transition Metal Dichalcogenide (TMDC) 2D-NSTs

Existing unique physicochemical characteristics of 2D-NSTs on transition metal dichalcogenide (TMDC) have emerged with a potential scientific notice in materials science applications. Among, molybdenum disulfide (MoS_2) is a typical 2D inorganic nanomaterial. It can capable of cargo a good quantity due to the surface area, robust NIR absorption, and high photothermal conversion efficiency as well is broadly applied in bio-imaging of cancer. The shortage of H_2O_2 and acids in the TME reduces the competence of the Fenton reaction. To overwhelm these problems, phototherapies are applied to strengthen the chemodynamic efficiency as well as enhance combined therapies. Recently, Pidamaimaiti et al., developed MoS_2 nanosheets modified with copper (Cu_2O–MoS_2), which combined improved CDT-PTT for destroying tumors (Fig. 16.4a.). Under NIR laser stimulated Cu_2O–MoS_2 nanosheets performs as strong employ to enhance the synergistic effect on the improved Fenton reaction and PTT activity. In this study, Cu_2O–MoS_2 showed that the PCE was 30.7%, which proved the better photothermal efficiency. Moreover, the thermal produced from Cu_2O–MoS_2 can enhance the performance of the Fenton-like reaction and further catalyze the transformation of endogenous H_2O_2 to free radicals (•OH). In vitro cell studies exhibited that Cu_2O–MoS_2 could significantly generate revered oxygen species in 4T1 cancer cells and promote cell death apoptosis via synergistic with PTT [108]. In another study, as shown in Fig. 16.4b, Wu et al., designed Fe_3O_4 nanozymes functionalized MoO_{3-x} nanoflakes through electrostatic self-assembly and modified with glucose oxidase (GOD) and polyvinylpyrrolidone (PVP) to form MFGP nanomaterials. The MFGP exhibited that PCE was 49.9%, which proved the nanomaterial was the better photothermal agent. The free radicals (•OH) produced by enzymes

stimulating a Fenton-like reaction can destroy tumor cells. GOD devours the glucose of the TME to waste away cancer cells and in situ production of H_2O_2 resulting in required free radicals (•OH) production. Moreover, overexpressed GSH would be significantly consumed through Mo nanoflakes stimulating redox reactions. Notably, due to the strong NIR absorption of Mo, MFGP influenced an outstanding CDT-PTT for cancer [109].

Recently, Cai et al., developed multifunctional MoS_2 nanosheets; initially, Mo-nanosheets were filled with DOX, and then surface-modified with polydopamine (PDA). Further, decorate the surface using thiolated aptamer AS1411 and polyethylene glycol (PEG) to develop DOX@Apt-PEG-PDA-MoS_2 in Fig. 16.5a. In this nanomaterial, aptamer provided the targeting capability to MCF-7 cancer cells. PDA functionalized MoS_2 showed photothermal conversion at 808 nm NIR absorption. Moreover, the acidic nature and NIR stimulate the drug release from the MoS_2 nanosheets. The nanocarrier Apt-PEG-PDA-MoS2 exhibited biocompatibility and synergetic chemo-PTT efficiency [110]. Li et al. reported MoS_2 nanosheets with mitochondria-targeting for improved synergistic CDT-PTT cancer therapy. Initially, MoS_2 nanosheets were functionalized with PDA-Fe^{3+} and further decorated with poly(ethylene glycol) (PEG) and triphenylphosphonium (TPP) to form MoS_2@PDA-Fe@PEG/TPP (MPFPT). Due to the presence of TPP, MPFPT can significantly target mitochondria for cellular uptake. In addition, MPFPT nanosheets showed excellent photothermal efficiency in the NIR-II region and can potentially stimulate the Fenton reaction from hydrogen peroxide to produce more free radicals (•OH) shown in Fig. 16.5b. In vitro and in vivo experimental studies exhibited that MPFPT nanosheets efficiently improve therapeutic effects by PTT-CDT, signifying the ability of the mitochondria-targeting approach [111].

16.5.3 Graphene 2D-NSTs

Graphene family members-based nanostructures are the first reported 2D nanomaterials for the photothermal agent for cancer theranostics because of their outstanding biocompatibility, biodegradability, colloidal stability, and PCE [112–114]. Graphene oxide is the oxidized nature of graphite, comprising honeycomb-like nanostructure enriched in oxygen molecules, which deliver rich reactive sites for the conjugation of biomolecules via covalent, esterification, and epoxide ring opening [115–117]. The high volume-to-surface area ratio including rich oxygenation molecules permits the conjugation of photosensitizers for PDT [118]. Being combined with targeting ligands, anticancer drugs, and photosensitizers, GO is eligible to combine other therapies with PTT with specific targeting for greater therapeutic efficiency [119]. Shi Guo et al. fabricated a multifunctional GO nanosheet for cancer therapy, synergetic photothermal, and photodynamic therapies. Initially, GO was functionalized with folic acid and chlorin e6 (Ce6) to induce tumor-targeting ability and act as photosensitizers for the PDT respectively. Ce6 organic groups can produce ROS under NIR laser light at 660 nm. Polyethylene glycol acts as a linker for grating the folic acid

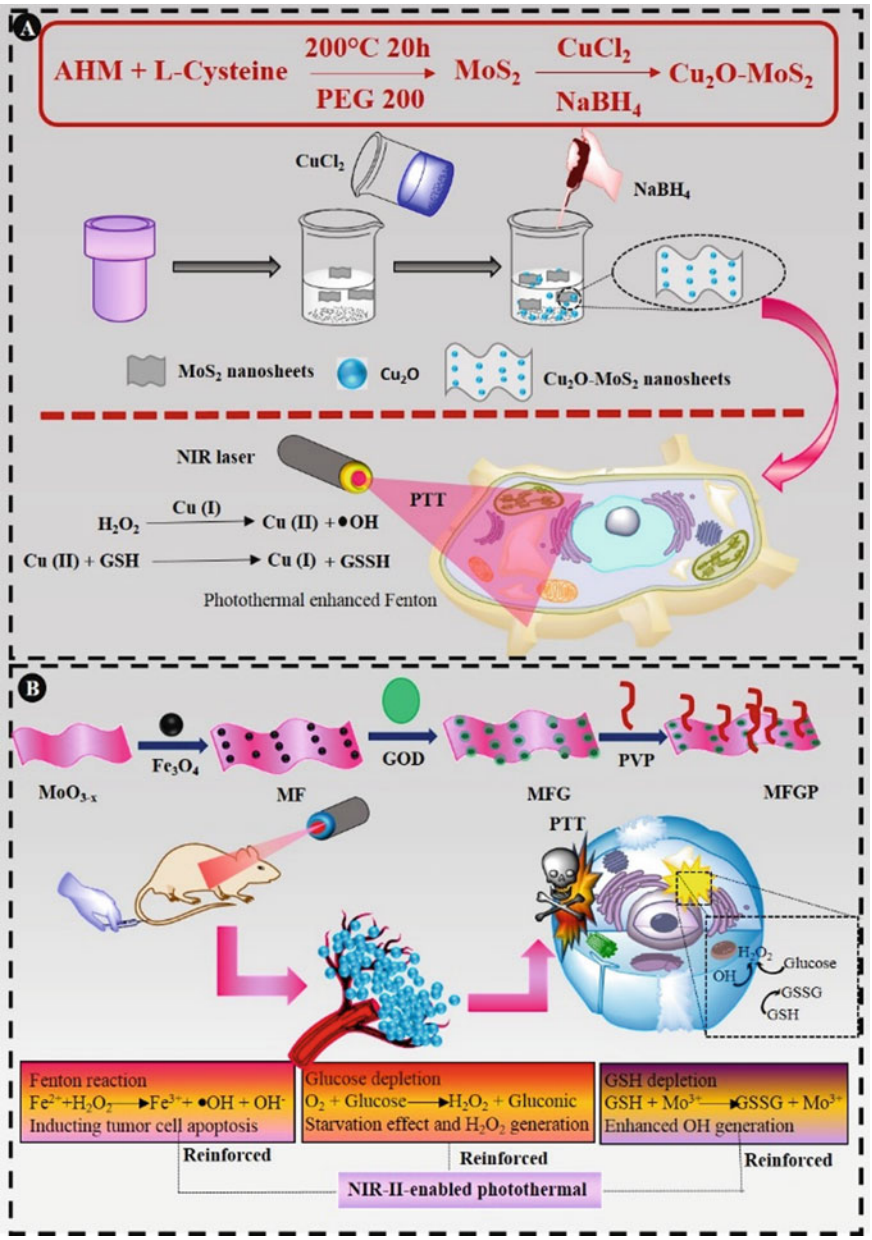

Fig. 16.4 Schematic preparation of **a** MoS_2 nanosheets is modified using Cu_2O for combined improved CDT-PTT to tumor ablation [108]. **b** Fe_3O_4 nanozymes functionalized MoO_{3-X} nanoflakes through electrostatic arrangement and further coated GOD and PVP to form MFGP nanomaterials [109]

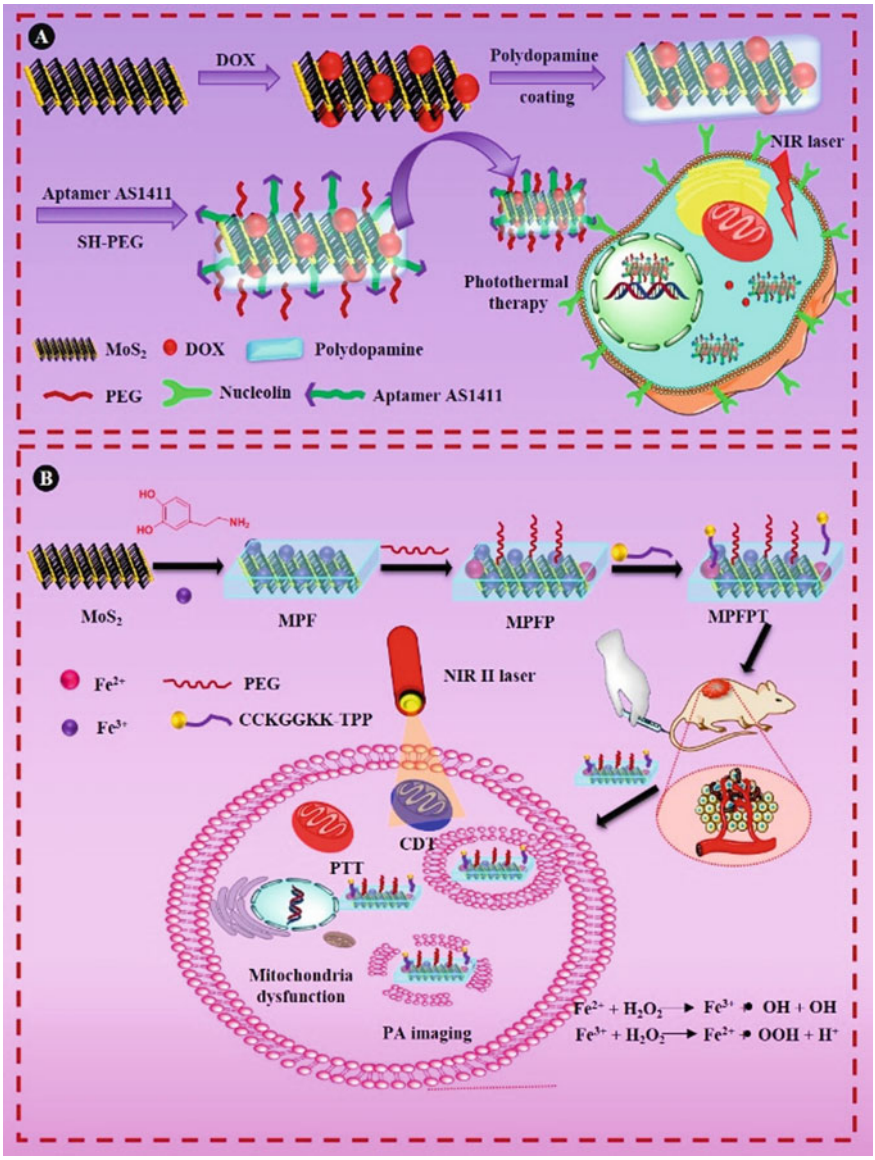

Fig. 16.5 **a** Schematic representation of synthesis of DOX@Apt-PEG-PDA-MoS_2 via Michael addition reaction [110]. **b** Schematic representation of preparation of MoS_2@PDA-Fe@PEG/TPP (MPFPT) and mechanism of generation of free hydroxyl radicals (•OH) from H_2O_2 by Fe^{3+} ions through Fenton reaction [111]

and Ce6 with GO. The prepared GO-FA/Ce6 showed excellent photothermal properties and the capability to produce singlet oxygen. This nanomaterial was competent to target the MCF-7 cancer cells through folate-mediated endocytosis with a better tumor ablation efficiency by phototherapies alone. [120] The real-time usage of reduced graphene oxide (rGO) 2D-NSTs in PTT is still challenging, due to their poor biological dispersion in the tumor microenvironment, irreversible nanomaterials aggregation, and high cytotoxicity. For this reason, Jhang e al. developed rGO 2D-NSTs with a novel reducing and stabilizing agent PCS20k along with PEGylation. 2D-NST. The PCS20k-rGO under 808 nm NIR exhibited PCE ($\eta = 67.7\%$), which is higher than that of GO nanosheets ($\eta = 55.0\%$). As shown in Fig. 16.6a, in vitro studies revealed the PCS20k-rGO ablated MCF-7 cancer cells upon NIR laser light stimulated photo-hyperthermia (over 49 °C) [121].

Recently, Ma et al. developed BSA-functionalized MnO_2 nanoparticles that were conjugated with polyethyleneimine coated reduced graphene nanosheets,

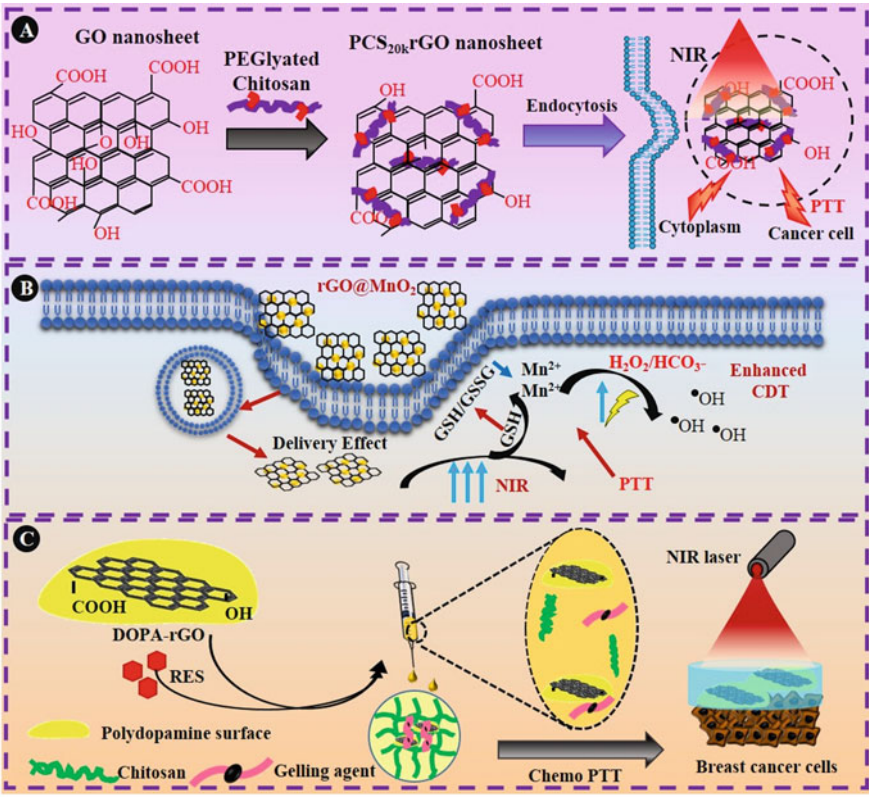

Fig. 16.6 **a** Schematic illustration of synthesis of PCS20k-rGO and way of endocytosis on cells [121]. **b** The mechanism of the synergetic therapies is illustration based on rGO@MnO_2 [122]. **c** Schematic illustration of preparation of the hydrogels DOPA-rGO@Gels and in vitro investagations of the hydrogel under NIR irradation [123]

(rGO@MnO_2) nanocomposites. (Fig. 16.6b). The nanocomposites potentially oxidize cancer cells via GSH and subsequently produce oxidized manganese ions resulting in to transformation of H_2O_2 into free radicals (OH) via the Fenton reaction. Both reduced GSH and free radicals (OH) production rise the singlet oxygen range to increase cancer cells death. Moreover, rGO nanosheets provide PTT under NIR laser treatment, which can improve the killing of cancer cells. In this investigation, rGO@MnO_2 exhibits great therapeutic effects over MnO_2 nanoparticles alone in equivalent concentration, which denoted that successful distribution of MnO_2 inside the cells by graphene nanosheets [122] Approaches to combining nanostructures chemo and photothermal therapy develop a great potential for enhancing cancer nanomedicine. Still, the translation of these strategies has been stuck by the immunogenicity stimulated by certain polymers applied for surface modification of nanomaterials or nanostructures deprived of tumor microenvironment on in vivo injection. To overcome this problem, the design of administrative nanocomposites able to deliver chemotherapy agents and nanostructures into the tumor microenvironment has received great attention. Melo et al., reported a novel dopamine-reduced GO (DOPA-rGO)-photothermal agent and resveratrol (drug)-therapeutic agent loaded on chitosan and cross-linked ionotropically to form injectable in situ hydrogels shown in Fig. 16.6c. This fabricated hydrogels exhibited inject-ability and in situ-gelation as well as better biocompatibility. In vitro, RES-DOPA-rGO@Gel, DOPA-rGO@Gel with laser, and RES-DOPA-rGO@Gel with laser display the MCF-7 cancer cells viability up to 72%, 75%, and 31%, respectively. This result indicates that RES-DOPA-rGO@Gel provides great chemo-PTT efficiency on tumor ablation [123]. Recently, Cui et al., designed and prepared a lipid bilayer functionalized rGO and further modified mesoporous silica through a lipid self-assembly method for significant synergistic chemo-PTT [124]. In another study, Dash et al. synthesized magnetic nanoparticles decorated rGO with surface modification using phospholipid-polyethylene for conjugating gastrin-releasing peptide receptor (GRPR) and anticancer drug doxorubicin (DOX) to form mrGOG/DOX. With potential DOX release in the tumor microenvironment and photothermal production from laser absorption in the NIR range, mrGOG/DOX could be applied for synergetic chemo-photothermal therapy [125].

16.5.4 Black Phosphorus (BP) 2D-NSTs

Black phosphorus (BP), as a new 2D-NSTs, has received material scientist interest due to its excellent optoelectronic properties and extensive applications in various fields. Bulk BP (multi-layers) can readily be exfoliated into nanosheets (single layers) with various thicknesses. Among other 2D-NSTs, BP has a greater surface area to load a high quantity of biomolecules or anticancer drugs, thereby being a significantly good candidate for drug delivery. BP 2D-NSTs have the optoelectronic performance to act as outstanding photothermal agents for PTT due to the high PCE and NIR

extinction coefficient. Thus, the association of biomolecules or drugs with BP 2D-NSTs results in synergetic phototherapy with chemotherapy, immunotherapy, and photodynamic therapy. Hepatocellular carcinoma is heterogeneous cancer that needs compulsory combination therapy, such as chemo-PTT. Recently, cancer immune treatments are promptly emerging and hopeful possibilities to method malignancies. Hence, the association of conventional therapies and immunotherapy in a single stage may enhance the efficiency of hepatocellular carcinoma treatment. In a recent report, Jia et al., developed the gold (Au) this sugar-coated BP nanosheets from BP-Au-thiosugar nanosheets (BATNS) with improved colloidal stability of both BP and Au in various induced physiological conditions in Fig. 16.7a. In this study, BATNS exhibited an extra immune outcome on the hepatocellular carcinoma compared with normal BP nanosheets. The mechanism indicated that the photothermal effect for cancer cell killing is associated with the rise of local NK cell penetration without affecting T cells developed by the heat energy of BATNS of PTT. This work developed a novel approach for stabilizing BPNS and synergetic thermotherapy and immunotherapy for cancer [126]. As shown in Fig. 16.7b, Li et al., developed DOX conjugated poly-l-lysine (PLL) coated BP nanosheets for breast cancer drug delivery systems. The nanocarrier prevents premature drug release due to the presence of disulfide bonds and provides the photothermal effect. In addition, the pharmacodynamic investigations revealed that PLLSS@ DOX-BP is a potential nano-vehicle for synergetic photothermal and chemotherapy [127].

Pan et al., designed and developed ICG-loaded poly(ethylene glycol) functionalized BP nanosheets (ICG@BPNS-PEG). Nanocomposites are prepared to enhance the tumor-targeting ability and real-time fluorescence imaging-guided PTT. In vitro biocompatibility against MCF-7, 4T1 cancer cells and RPE cells exhibited ICG@BPNS-PEG has lesser cytotoxicity and good biocompatibility without laser irradiation (Fig. 16.8a) [128]. Chen et al. developed BP mediated nanocomposites that consist of polypyrrole and Au nanostructure which were coated on BP nanosheets. By gold reduction deposition, the generation of singlet oxygen efficiency was significantly enhanced. In addition, under the external trigger of near-infrared as well as ultrasound, the synthesized nanocomposites exhibited intense PCE and a higher amount of ROS. Moreover, the hopeful synergistic cancer theranostics developed over 82% of cancer cells death (Fig. 16.8b) [129]. The graphene, BP, and TMDs nanosheets based on synergistic PTT were summarized in the Table 16.3.

16.6 Toxicity Performances of 2D-NSTs

The nanomedicine prominence of fabricated functional biomaterials mainly depends on their safety and toxic nature, particularly the in vivo biocompatibility investigations such as hemolysis, histocompatibility, bio-distribution, and urine renal clearances. Consequently, the aforementioned investigations reveal to technology transformation of fabricated biomaterials.

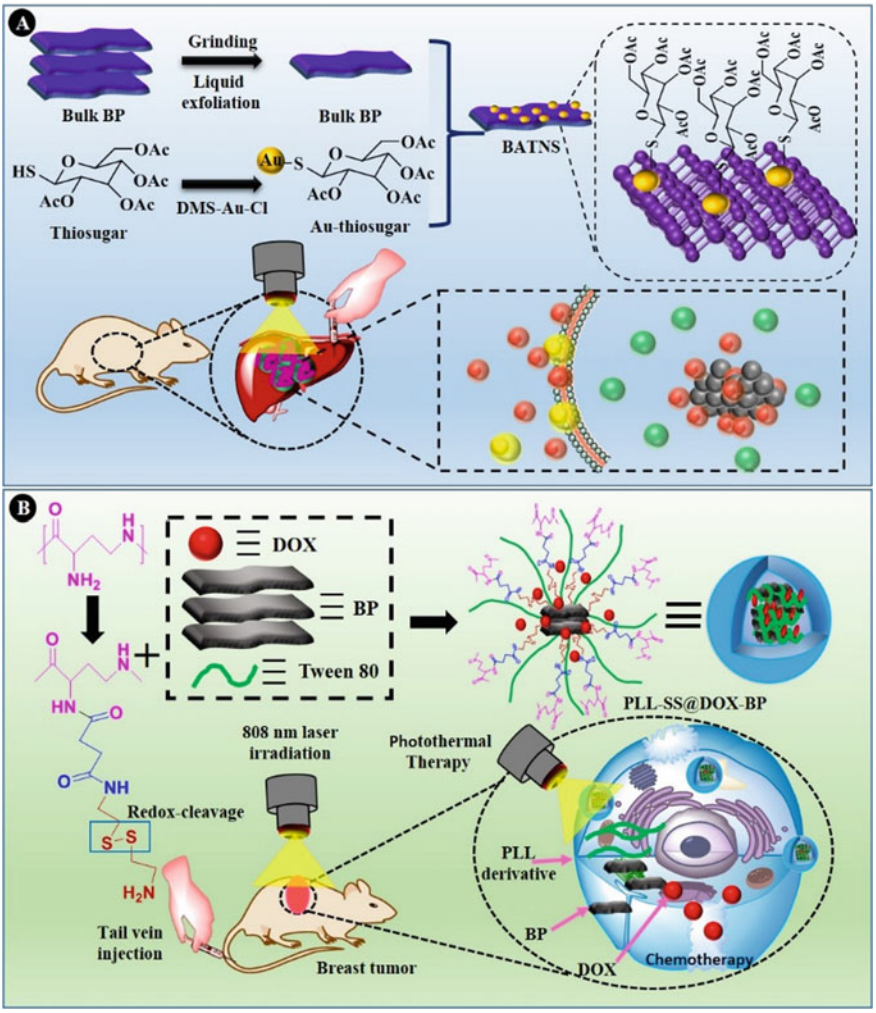

Fig. 16.7 **a** Schematic illustration of synthesis of BATNS as photothermal tempted cancer cell killing agent for hepatocellular carcinoma [126]. **b** The schematic illustration of synthesis of PLLSS@DOX-BP and synergetic photothermal and chemotherapy [127]

The emerging occurrence of 2D-NSTs as a nanocarrier or nanotherapeutic materials in cancer theranostics may begin a deep effect on the body. The 2D-NSTs properties such as nanomaterials shape, colloidal stability, physiological dispersion, and modified surface significantly affect their biocompatibility potential in nanomedicine. The biocompatibility outlook of 2D-NSTs will be vital to receive a comprehensive empathetic of their efficiency, the risk on intake as well as the path of action in the tumor microenvironment. Consequently, it is necessary to do deep research gaps on the suppressed animal exposure determined by 2D-NSTs-mediated

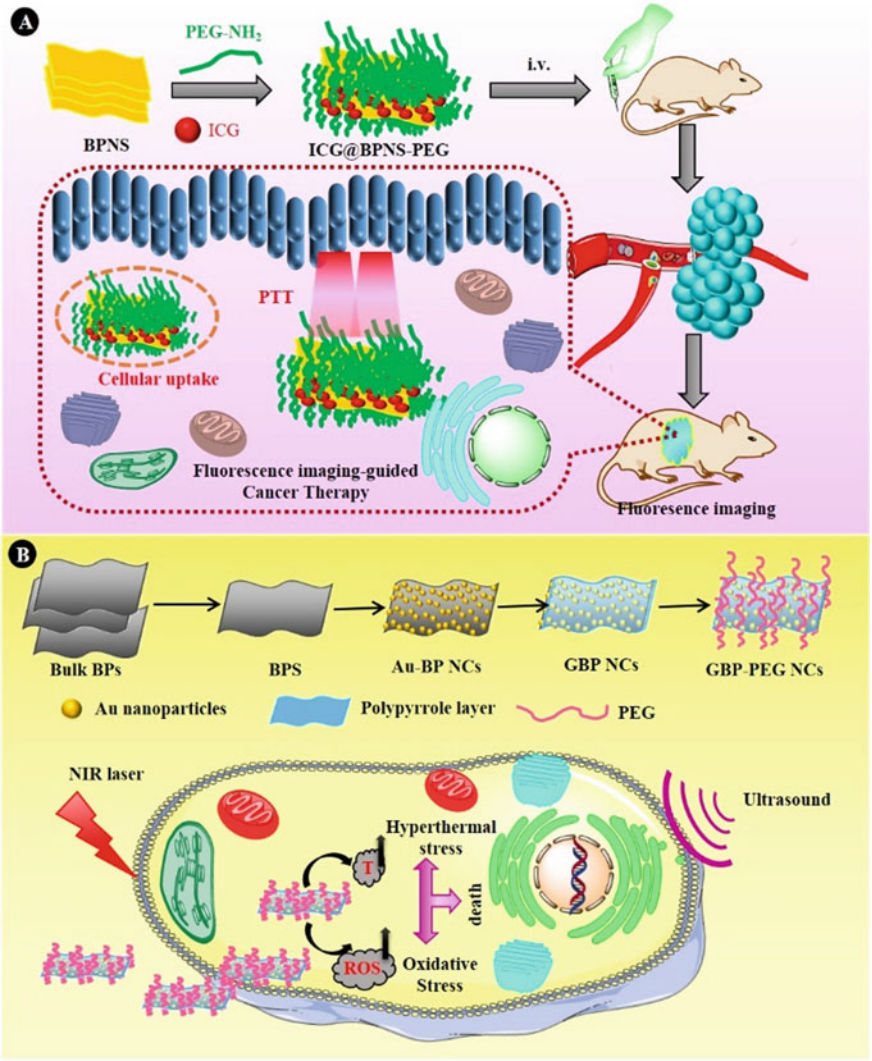

Fig. 16.8 **a** Schematic representation of synthesis and fluorescence imaging of cancer sites of ICG@BPNS-PEG and fluorescence imaging-guided PTT [128]. **b** Schematic representation of the synthesis of GBP-PEG nanocomposites. The synthesized nanomaterials are gathered at the cancer sites by EPR effect. Ultimately, combining sono-dynamic therapy of BP nanosheets and the PTT of polypyrrole for cancer [129]

Table 16.3 Graphene, BP, and TMDs nanosheets-based PTT

2D nanosheets	Surface modifications	PCE (%)	Drugs	Multimodal therapies	Bio-imaging	References
GO	FA/PEG	–	Ce6	PDT/PTT	–	[120]
GO	PEGylation	67.7	–	PTT	–	[121]
rGO	Ga	42.4	–	PTT	PA	[130]
rGO	DOPA	–	RES	Chemo/PTT	–	[123]
rGO	MnO_2	–	–	PTT/CDT	–	[122]
NGO	UCNPs	–	Ce6	PTT/PDT	UCL	[131]
rGO	PLL/PASP	24.3	DOX	Chemo/PTT	–	[132]
GO	PEGylation	–	PplX/DOX	PTT/Chemo/PDT	FL	[133]
GDY	PEG	42	DOX	Chemo/PTT	FL	[134]
MoO_{3-x}	Fe_3O_4-GOD-PVP	49.9	–	PTT	MRI/FL	[109]
Cu_2O-MoS_2	–	30.7	–	PTT/CDT	–	[108]
MoS_2	PDA-Fe@PEG/TPP	34.9	–	PTT/CDT	PA/FL	[111]
MoS_2	Apt-PEG-PPA	–	DOX	Chemo/PTT	–	[110]
BP	Thiosugar/Au	68.3	–	Immune/PTT	–	[126]
BP	PLL	–	DOX	Chemo/PTT	–	[127]
BP	PDA-PEOz	–	BTZ	Chemo/PTT	–	[135]
BP	Au-PEG	42.5	PPy	PTT/SDT	–	[129]
BP	PEG	–	ICG	PTT	FL	[128]
BP	1-PA, RGD	–		PTT	PAI	[136]
BP	PDA-PEG-Apt	–	DOX	Chemo/PTT	IRT	[137]

PCE photothermal conversion efficiency; *PDA* polydopamine; *IRT* infrared thermal imaging; *PAI* photoacoustic imaging; *FL* fluorescence Imaging

biomaterials accurately. Fascinatingly, in vivo safety as well as cytotoxicity investigations on various 2D-NSTs showed lower toxicity toward healthy cells. In addition, the designed nanostructures have further problems for instance non-existence of bioavailability, cytotoxicity, minimum biodegradation, and low colloidal stability subsequently creating anxieties on the in vivo administration. Hence, to overcome such restrictions, the 2D-NST surface is functionalized by various biopolymers, organic layers, and biomolecules. The modifications on the 2D-NSTs surface will alter performances and improve solubility, rise the biosafety of healthy cells, and effects the cell materials interactions.

16.7 Outlooks and Conclusion

Currently, 2D-NSTs play a potential role in biomaterials science because of their excellent properties, including biological compatibility, flexibility, bio-reactivity, surface area, unique planar structure, and effective PCE. The specific PCE characteristics of various 2D-NSTs empowered their extensive applications on cancer

phototherapies. Several top-down and bottom-up strategies have shown the good-scale production of inorganic nanosheets. Intense research on various trustworthy synthetic tactics to convey anticipated physicochemical properties such as surface modifications, number of layers, size, thickness, and composition, and the defect has been crucial things to knowing the correlation between the structure and functions. The most extensively applied 2D-NSTs for PTT are TMDCs as they hold special physico-chemical features and are diagonally equivalent to 2D GO-based nanostructures. Moreover, MXenes have exhibited their significance in PTT by offering 100% percentages of PCE. Black phosphorous is growing 2D-NSTs, and currently, a lot of biomedical applications are derived from BP especially on PTT due to excellent PCE. Recent developments on the aforementioned photothermal agents enhancing the intercellular uptake through intravenous injection with low invasiveness, and the capability to discharge the loaded therapeutic agents on tumor-specific sites using on/off mode NIR irradiation technology creates a novel gateway for cancer theranostics. Therefore, this chapter reports the different 2D-NSTs interactions with the tumor microenvironment and systematic analytical data on their in vitro biocompatibility, the existence of PCE, and biodegradation. Moreover, understanding the basics of nanostructures biological interactions, biosafety, and cytotoxicity is potentially essential for transforming the current advancement of 2D-NSTs into better-improved cancer theranostics in preclinical research. Thus, it is hopeful that these 2D-NSTs will be lightening the attention on biomaterial societies as well as discover the promising path to victory against cancer.

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Chapter 17
Cyclodextrins and Cyclodextrin-Based Nanosponges for Anti-Cancer Drug and Nutraceutical Delivery

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Contents

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Abstract In recent years, cancer has been continuously considered a major problem in society, and unfortunately, cancer cells can increasingly avoid current therapies. Contemporaneously, cyclodextrins (CDs) and cyclodextrin-based nanosponges (CD-based NSs), due to their peculiar features, have acquired great significance in the controlled and/or targeted release delivery systems. CDs and CD-based NSs have been widely considered suitable delivery systems for cancer treatment. CD-based NSs are produced as a result of the chemical cross-linking of CDs. In this chapter, a brief overview of the synthesis, classification, and characterization of CD-based NSs is provided. Further, the potential of the inclusion complexes, formed between CDs and CD-based NSs and anti-cancer drugs or active nutraceuticals, is reviewed. This chapter will construct a theoretical base on existing knowledge for identifying potential gaps in future research in the field.

Graphical Abstract

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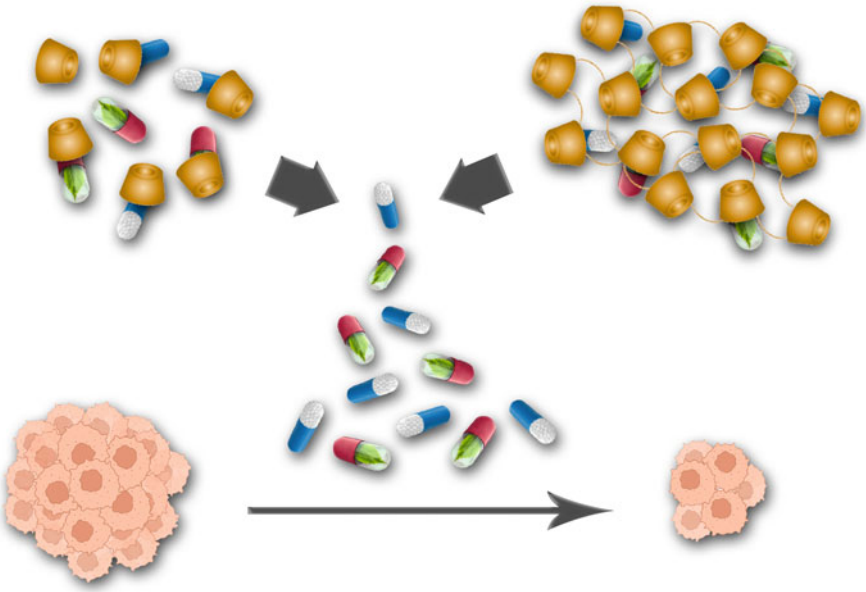

17.1 Introduction

Cancer is a cluster of diseases with similar symptoms such as uncontrolled division and cell migration that are finalized with the death of the patient [1]. There were 10 million cancer deaths and 19.3 million cases in 2020 worldwide as reported by IARC. Although traditional therapies, such as surgery or radiotherapy, are advanced, the use of less invasive approaches opens the gate to novel chemotherapies with the introduction of drugs that can treat cancer cells. In addition, the use of nutraceuticals (the term derives from "nutrition" and "pharmaceutical") in the diet has been proposed as an interesting alternative to prevent and treat cancer [2].

However, the utilization of the aforementioned compounds in cancer therapy is limited due to their physicochemical features such as poor water solubility, oxidation, or poor bioavailability. One of the possible strategies to solve these obstacles is the combination of the drug or nutraceutical with a carrier compound. This carrier can encapsulate the drug or nutraceutical molecule inside it, protecting and improving its different bioactivities [3]. Cyclodextrins (CDs) are acclaimed due to their pharmaceutically relevant physicochemical benefits [4].

CDs are oligosaccharides consisting of six, seven, and eight glucose units known as α, β, and γ-cyclodextrin (CD), respectively, bound by α-1,4 glycosidic linkages. CDs are truncated cone-shaped owing to a hydrophilic exterior and a less hydrophilic interior [4, 5]. The chemical modification of parent CDs generates commercially

available derivatives such as 2-hydroxypropyl-βCD (HP-β-CD) [6], methyl-βCD (M-βCD), and also CD-based polymers. The complex between a drug molecule and CDs is a dynamic equilibrium generated by a non-covalent union, known as the "inclusion complex". The intrinsic characteristics of a great number of drug molecules affect their complexation with CDs. Therefore, researchers developed various materials with improved characteristics, such as insoluble CD-based polymers, known as CD-based nanosponges (CD-based NSs) (Fig. 17.1). They are cutting-edge cross-linked polymers with an amorphous, crystalline structure, and characteristic three-dimensional network. Recent surveys [7–9] indicated their immense potential due to the swelling capacity, low toxicity, etc. [10–12].

CD-based NSs present remarkable benefits in comparison to the corresponding parent CDs. Their particular advantage is the inclusion complexes' formation with non-expected molecules (e.g., macromolecules like proteins). Additionally, CD-based NSs, due to the hindered diffusion, promote slower release kinetics than native CDs [13].

This chapter presents an enlisted of barriers that affect the delivery of anti-cancer drugs or nutraceuticals and the ability of CDs and CD-based NSs to enhance their efficiency by the formation of inclusion complexes. Moreover, the synthesis, classification, and characterization of various CD-based NSs are explained.

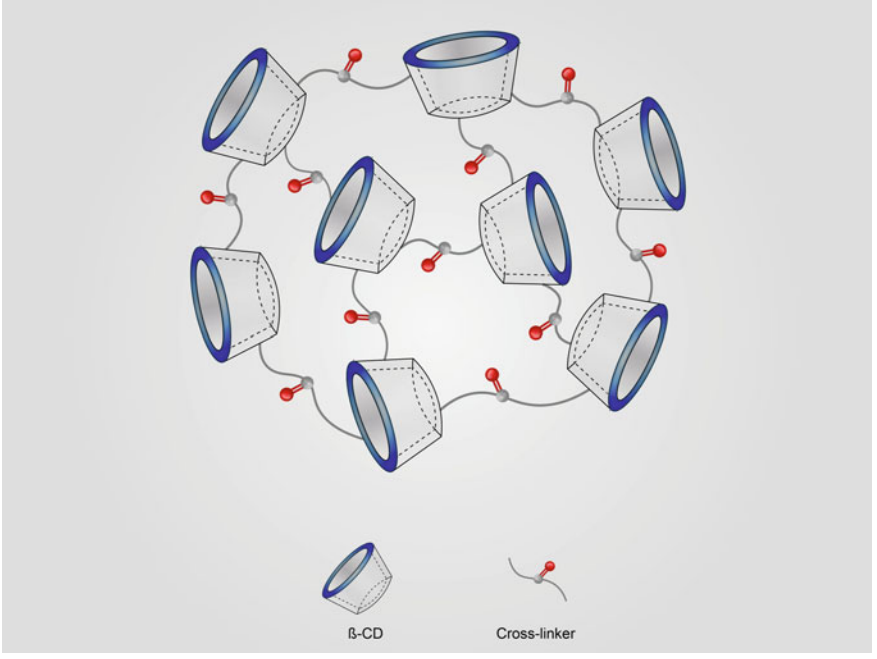

Fig. 17.1 Schematic representation of cyclodextrin-based nanosponges (CD-based NSs)

17.2 Obstacles in Tumor Treatment

17.2.1 Blood

Blood tissue is crucial in the circulation of metabolites in the body, and certainly, the drugs move through the tissue. In blood, diverse phenomena can occur: firstly, as the equilibrium is dynamic, different metabolisms with higher complexation strength can displace drugs outside the CDs [4], thus reducing the efficacy of the treatment. Secondly, as blood contains several proteins, which can generate "opsonization" (an immune process that utilizes opsonins to tag foreign pathogens and eliminate them by phagocytes), PEGylation is suggested [14]. Finally, the size and shape of particles need to be taken into account when the drug formulation is designed to be transported in blood [15].

17.2.2 Tumor Microenvironment

The extracellular matrix (formed by hyaluronic acid, elastin incorporating proteoglycans, and collagen) may decrease the effectiveness of our particle. Depending on the stage of the tumor, the matrix is different. This matrix prevents effective metastasis and is degraded by metastatic cells which release numerous enzymes (metalloproteinases). Therefore, the addition of collagenase or metalloprotease inhibitors to the drug formulations can increase the treatment effectiveness. Moreover, the microenvironment with acidic pH can be used to prepare pH-sensitive materials [16].

17.2.3 Cellular Barriers

The release is accomplished when the particle, considering its parameters such as size, surface charge, or hydrophobicity, interacts with the membrane. A charged particle shows higher interactions with the membrane than a neutral particle that partially prevents entry into the cell. Furthermore, better uptake is observed when the particle size is smaller [17]. To bond targets against specific receptors of cancer cells or to increase the selectivity with cholesterol, the major component of cell membrane in which proportion is increased in cancer cells is considered to be interesting strategies [18].

17.3 Synthesis and Classification of CD-Based NSs

The synthetic conditions of the CD-based polymers and CD-based NSs are tuned by the type of cross-linker, molar ratio CD/cross-linker, and solvent [8]. The final CD-based NSs' structure is strongly linked with the molar ratio CD/cross-linker, the so-called cross-linking density [19].

17.3.1 Synthesis

17.3.1.1 Classical Approach

The classical protocol uses a suitable solvent (organic solvents or water) to dissolve the CD amount. Subsequently, the selected cross-linking agent is added to the solution at room or high temperature under stirring or ultrasonic mixing. Moreover, the addition of a relevant catalyst can increase the rate of the cross-linking reaction [20]. The polymer solution is altered into a gel, and finally, in a solid, monolithic block, that is further purified and ground to obtain a fine white powder [21, 22].

17.3.1.2 Dehydration

The method consists of the use of the Fisher esterification protocol between CDs (hydroxyl groups) and, at least, a desired biacid water-soluble cross-linking agent, adding the catalyst. The solution is heated up and a water molecule is lost as a result of the dehydration. This is also known as a type of condensation process that, under the vacuum, enables polymer formation through the reaction of the functional groups of two molecules with small molecules as by-products [23].

17.3.1.3 Other Strategies

Over the years, diverse possibilities to obtain CD-based NSs are investigated. Among them, the use of interfacial between two immiscible phases, in which the cross-linking reaction occurs in the interphase obtaining a precipitate, is considered [10]. Further, the green alternative approaches to synthesizing CD-based NSs are highly demanded by the scientific community. Mechanochemistry, through the utilization of ball milling and a twin-screw extruder (TSE) to synthesize free solvent CD-based NSs, is acclaimed [24]. Moreover, the petroleum-based solvents are replaced by the so-called natural deep eutectic solvents (NADESs) to synthesize CD-based NSs [25].

17.3.2 Classification

According to CD-based NSs' composition and properties, there are known four generations [8, 13]. The first generation of CD-based NSs is presented by the simple one-step reaction of CDs with various cross-linking agents to synthesize polyurethane, polycarbonate, polyester, or polyether-based NSs. The functionalization of the synthesized CD-based NSs, by adding the desired functionalities, is associated with the second generation. Further, the third generation is represented by stimuli-sensitive CD-based NSs that can adjust their activity according to the environment. The employment of the molecularly imprinted technology to synthesize CD-based NSs, with a high selectivity toward specific guest molecules, is presented by the fourth generation.

17.4 Cyclodextrins and Cyclodextrin-Based Polymers/Anti-Cancer Drugs' Inclusion Complexes, Their Formation, and Characterization

The inclusion complexes between CDs or CD-based NSs and anti-cancer drugs can be obtained by various procedures [13] as it is presented in Fig. 17.2. The classical drug/carrier is mixed in a solvent, commonly in water, for around 24 h to stabilize the complexes [26]. The collected fraction is further purified. The complex can be formed by kneading in a mortar the drug/carrier and an appropriate quantity of solvent. The techniques such as lyophilization, co-evaporation, or spray drying are used to obtain the complex powder.

The characterization of the inclusion complexes is a fundamental step to understanding their physicochemical properties. Therefore, the principles of some characterization techniques of CDs and CD-based NSs and their inclusion complexes with anti-cancer drugs are discussed with a brief introduction to their usage in research.

17.4.1 Microscopic Analysis

Microscopy techniques analyze various features of the sample such as structure and chemical composition, morphology, size and diameter, microstructure and micromechanical properties, interfacial properties, topology [27]. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), and atomic force microscopy (AFM) are techniques exploited to successfully analyze the morphological distinctions between CDs, CD-based NSs, and their inclusion complexes with anti-cancer drugs [28–32].

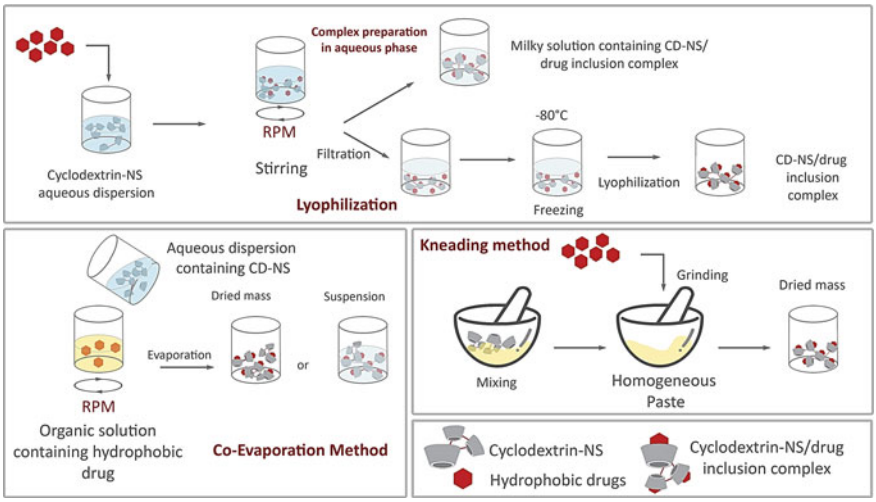

Fig. 17.2 Different methods to prepare inclusion complexes (obtained from [13])

17.4.2 Spectroscopic Analysis

The spectroscopic analysis is useful to identify the interacting species of polymer composite and to better understand the composite properties which make more efficient the processing conditions. Fourier-transform infrared (FTIR) spectroscopy has widely characterized materials through the bands correlated with the functional groups and bond formation in the chemical structure of produced materials. FTIR is molecular spectroscopy that gives information on polymeric systems from their vibrational properties. The spectra are usually obtained, at room temperature, in the 650–4000 cm^{-1} wavenumber range. Nuclear magnetic resonance (NMR) is used to determine the content, purity, and molecular structure of a sample. Therefore, NMR is considered a dominant technique to acquire structural information and investigate the formed inclusion complexes. ^{1}H and ^{13}C NMR spectra are taken to identify the formation of cyclodextrin-based NSs and their inclusion complexes. Ultraviolet–visible (UV–Vis) spectrophotometry is utilized to estimate the drug concentration in a solution that absorbs UV or visible radiations [33–38].

17.4.3 Thermal Analysis

Thermal analysis is also used to confirm the anti-cancer drug complexation with CDs and CD-based NSs. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) are utilized as effective ways to investigate changes in the physicochemical properties of materials [39]. The change of the sample weight as a

function of the temperature, moisture, and residual solvent contents can be studied using TGA. DSC provides quantitative and qualitative information as a function of time and temperature regarding exothermic or endothermic processes or heat capacity changes as thermal changes in materials. Melting point, purity of samples, and glass transition temperature are determined by DSC [38].

17.4.4 Chromatographic Analysis

The chromatographic methods are usually utilized for qualitative and quantitative analyses [38], although sometimes direct UV spectroscopy is enough [26]. The anti-cancer drug content, encapsulated in the inclusion complexes, is determined by a developed HPLC–UV method. The method is usually validated for precision, accuracy, and recovery [40]. Loading capacity and encapsulation efficiency of anti-cancer drugs within the polymer, prone to in vitro and in vivo studies, are determined according to Eqs. 17.1 and 17.2.

The encapsulation efficiency (*EE*) is interpreted as the encapsulated drug mass (m_{en}) over its initial mass (m_{in}) during the loading step, as follows [41, 42]:

$$EE(\%) = \frac{m_{en}}{m_{in}} \times 100 \tag{17.1}$$

Loading capacity (LC) is interpreted as the encapsulated drug mass (m_{en}), divided by the polymer total mass (m_{tot}), as follows:

$$LC(\%) = \frac{m_{en}}{m_{tot}} \times 100 \tag{17.2}$$

17.4.5 X-ray Techniques

The solid structure of plain CDs, CD-based NSs, and their inclusion complexes is characterized by X-ray analysis. Powder X-ray diffraction (PXRD), a non-destructive analytical technique, measures non-crystalline or crystalline materials. When PXRD is not enough sensitive, small-angle X-ray scattering (SAXS) is commonly the ideal option. The SAXS is used to explain the morphology, particle size, and polydispersity index. The material structural changes with temperature variation are studied by SAXS. Further, qualitative and detailed information on chemical elements existing on the material surface is provided by X-ray photon spectroscopy (XPS) [38, 43].

17.4.6 Mechanical Analysis

The mechanical features of the polymer network are investigated by rheology. It analyzes the flow and deformation of the material, by applying a deformation called a strain, and a force called shear stress. In the reaction of the material to the applied force, viscoelastic behavior appeared. The viscoelastic materials' characterization is enabled by the shear modulus, especially, the loss (G") and the storage modulus (G') [19], whereas the solution viscosity can be determined through the measurements carried out in steady flow [44].

17.4.7 Swelling Properties

The kinetics of CD-based NSs, adding them to deionized water at room temperature and monitoring their increase in volume and weight, defines the swelling capacity. The swelling (%S), known also as the water absorption capacity (%WAC), is calculated using Eq. 17.3, as follows:

$$\mathrm{WAC}(\%) = \frac{m_t - m_0}{m_0} \times 100 \tag{17.3}$$

where m_t is the swollen sample weight at a certain time t, and m_o is the dry sample initial weight.

17.4.8 Molecular Modeling Studies

Molecular modeling is used to investigate the possible complexation of anti-cancer drugs with CDs and CD-based NSs. The stoichiometric ratio and orientation of host–drug inclusion complexation can be predicted by the molecular docking approach [45].

17.5 Cyclodextrins and Cyclodextrin-Based Nanosponges as Agents for Anti-Cancer Treatment

17.5.1 Classical Drug Complexes

The encapsulation of anti-cancer drugs by CDs and CD-based NSs has been extremely studied to enhance poor solubility, stability, permeability, and bioavailability, reduce toxicity, and achieve a sustained release and a higher effect of the drug (Fig. 17.3).

The application of Paclitaxel (PTX), one of the most common chemotherapeutic agents, is vastly limited due to its poor water solubility. Therefore, the current formulation of PTX requires the use of other solvents, named Cremophor EL® (polyoxyethylated castor oil). Since these solvents are limited by severe toxicity, the identification of an alternative formulation for PTX is of high significance. In this scenario, nanoparticle albumin-bound PTX (Abraxane®) has been already approved by the Food and Drug Administration (FDA) to be applied to non-small cell lung carcinoma and metastatic breast cancer. The delivery of paclitaxel as a saline suspension of albumin particles has avoided the demand for Cremophor [46].

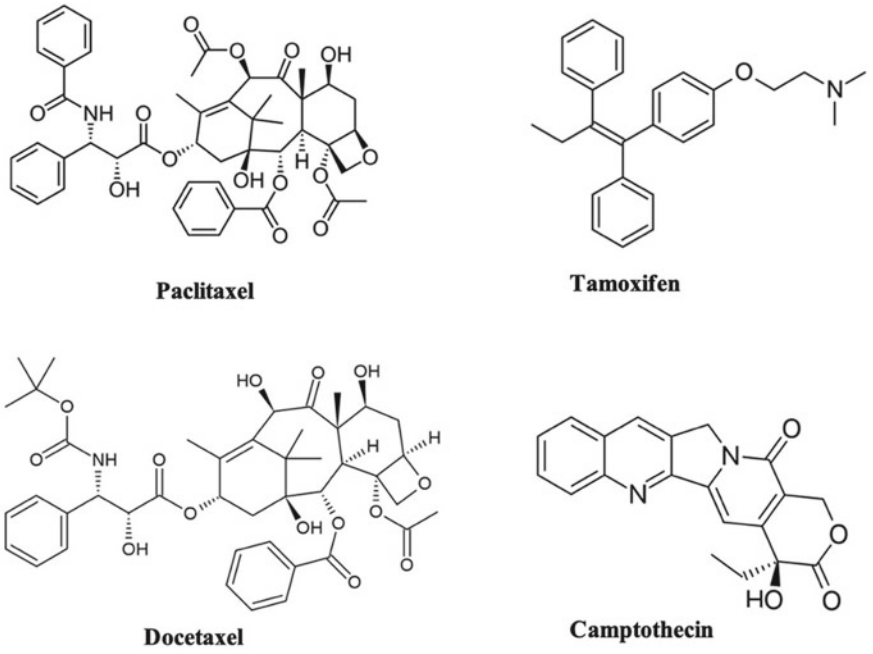

Fig. 17.3 Chemical structure of some anti-cancer drugs that have been complexed with cyclodextrins (CDs) or cyclodextrin-based nanosponges (CD-based NSs)

The development of other Cremophor-free formulations, such as the complexed CDs, is further investigated. Notably, dimethyl-β-cyclodextrin (DM-β-CD) revealed a significant enhancement of the drug solubility [47].

β-CD-based NSs are successfully utilized to produce a Cremophor-free formulation for enhancing the PTX solubility in comparison to native β-CD [48].

Another study demonstrated that novel pyromellitic γ-CD-based NSs loaded with PTX are capable to reduce in vitro and in vivo growth of melanoma cell. Higher efficiency of PTX-loaded NSs, to inhibit the invasiveness of the umbilical vein endothelial cells (HUVECs) of humans, is observed than the free PTX. In addition, tubulogenesis is inhibited at lower concentrations by the NSs in comparison to free PTX [49].

Tamoxifen is a significant anti-cancer drug associated with the class of selective estrogen receptor modulators. Tamoxifen is commercially converted to citrate salt due to its low aqueous solubility. However, the corresponding tamoxifen citrate salt inhibits dissolution rate due to a higher melting point. Hence, to address the aforementioned limitations, tamoxifen-loaded NSs are successfully prepared for oral drug delivery. The solubility of tamoxifen is increased with βCD-based NSs. In vitro cytotoxicity study showed higher toxicity against MCF-7 cells with tamoxifen CD-NSs than with the commercial tamoxifen formulation. Additionally, the plasma concentration of the tamoxifen citrate, at the same dose, is lower than the tamoxifen-loaded NSs' formulation [50].

Docetaxel is another widely used cytotoxic chemotherapeutic agent with poor solubility. To enhance the solubility of this highly hydrophobic drug, the inclusion complexes with β-CD, HP-β-CD, and sulfobutyl ether$_7$-β-cyclodextrin (SBE$_7$-β-CD) are successfully prepared [51].

Additionally, CDs are also known to complex platinum (II) drugs. The low solubility of those drugs compromises therapeutic outcomes and induces side effects. Indeed, oxaliplatin is complexed by α-, β-, and γ-CD, and thus, the anti-tumor activity, against HCT116 human colon carcinoma and MCF-7 human breast cells, is increased [52].

The application of various anti-cancer drugs is moreover limited by their low stability. In this scenario, the encapsulation of the drugs to protect them is revealed.

Several studies presented the improvement of the poor aqueous solubility and low stability of camptothecin (CPT), an inhibitor of DNA topoisomerase-I when encapsulated with particular types of CDs. Indeed, the high chemical instability of the free drug is due to the rapid inactivation by the opening of the lactone ring, essential for the diffusion into cancer cells, and target interaction [53]. βCD-based NSs are demonstrated to protect CPT from degradation, preventing the opening of the lactone ring. Additionally, the poor solubility is improved, and the growth of prostate tumor cells, both in vitro and in vivo, is inhibited by camptothecin βCD-NS (CN-CPT). Moreover, the study extended the effectiveness of CN-CPT, both in vivo and in vitro, on anaplastic carcinoma of the thyroid (ATC), a lethal malignant cancer of humans with median durability of 6 months. The growth, vascularization, and metastatization of orthotopic ATC xenografts in SCID/beige mice are successfully inhibited, with no apparent toxic effects, by CN-CPT [54].

CRLX101, among the CDs with CPT derivatives, reached advanced clinical strategies. CRLX101 is a tumor-targeted system of 30–40 nm that consists of a β-CD-based polymer conjugated to CPT. The conjugation to the CD-based polymer increases the drug's solubility and protects it from degradation. Phase 1/2a clinical trial demonstrated efficacy results, a favorable safety profile, and pharmacokinetics in patients with advanced solid malignancies [55], whereas a currently continuing phase 2 clinical development, over multiple tumor types, is noted. A pilot test is aimed at bimonthly dosing (phase 2 clinical trial recommendation) in patients with progressive chemotherapy-refractory gastroesophageal cancer. However, bimonthly monotherapy demonstrated only minimal activity. The lack of tumoral activity can be due to less drug accumulation in the human tumors than in preclinical models, while the low activity can be explained by the activation of resistance mechanisms. Therefore, frequent dosing and/or combination therapy can be required [56]. Furthermore, the efficacy of CRLX101 combined with enzalutamide is studied in patients with advanced metastatic castration-resistant prostate cancer (mCRPC). The combination of the nanoparticle with the drug is assumed to exceed enzalutamide resistance. A disadvantage of the aforementioned, the combination with CRLX101, is not a tolerable regimen in patients with mCRPC [57].

Doxorubicin (DOX), an anthracycline antibiotic, is a potent chemotherapeutic drug to treat liver and breast cancer. Nonetheless, clinical usage is hampered by serious cardiotoxicity, bone marrow depression, and drug resistance. To overcome these drawbacks, DOX has been studied for a combination with CDs and derivatives. For instance, the anti-cancer efficiency of CD-based NSs combined with DOX is in vitro testing in breast cancer cell lines of mice and humans. The DOX-βCD-based NSs revealed a higher inhibition of cancer cell proliferation than the free DOX. Moreover, the in vivo breast cancer growth in BALB-neuT mice is inhibited by 60% using DOX-βCD-based NSs with a five times dose lower than the therapeutic dose. Successfully, the DOX is highly accumulated in the tumor spot, whereas a low accumulation in the mice hearts is revealed [58]. In addition, DOX-βCD-based NSs are further functionalized with ligands that are bound to specific receptors on the target cells' surface, and thus, the cellular drug uptake is increased. Following this approach, the fluorescent hyper-cross-linked β-CD carbon quantum dot hybrid-based NSs, capable of encapsulating DOX, are synthesized. A pH-responsive controlled release in the microenvironment of the tumor is revealed [59].

Recently, the development of Nisin-Z complexed with CD-based NSs is investigated. The complex can protect the peptide against different agents like the peptidase pepsin. The NSs' complex can increase the intrinsic anti-cancer activity; the breast cancer cells (MCF-7) and colon cancer cells (HT-29) are considered and highly improve the colon cells [60].

Regorafenib (RG) loaded into mannose-modified γ-cyclodextrin (M-γ-CD) is developed to enhance the low solubility, dissolution, and permeability of the drug. Furthermore, M-γ-CD is presented as the RG targeting system. The in vivo study displayed the optimization of the drug pharmacokinetics and biodistribution by M-γ-CD.

In colitis-related cancer and CT26 murine colon carcinoma cell line, the capacity of the inclusion complex to reduce tumor cell proliferation, lesion neovascularization, and remodel tumor microenvironment in a specifically targeted manner is detected [61].

The βCD-based NSs can also protect the encapsulated molecules from enzyme or light-induced degradation. Cavalli et al. incorporated 30% of 5-fluorouracil, a light-sensitive drug, in βCD-based NSs. It is demonstrated that the drug, inside the complex, preserved the cytotoxicity against MCF-7 cells in the in vitro release studies [62].

In recent years, developing siRNA delivery systems is a challenging process associated with limited features. Indeed, naked siRNA is easily degraded by nucleases and the acidic endosome in the cytoplasm. Several CD-based polymers have successfully been studied for entrapping siRNA and protecting it from degradation.

Calando Pharmaceuticals (Pasadena, California, USA) performed the first-in-human phase Ia/Ib clinical trial. A targeted CD-based delivery system, named CALAA-01, is used to administrate the siRNA to patients with solid cancers [63]. CALAA-01 is composed of a βCD-based polymer (CDP), polyethylene glycol (PEG), a steric stabilizing agent, and a transferrin protein (TF) of humans for binding to transferrin receptor (TfR). TfRs are typically upregulated on cancer cells. The CALAA-01 siRNA, within a stabilized nanoparticle, is protected from nuclease degradation. The CALAA-01 delivery system provided the effective targeting of siRNA against ribonucleotide reductase subunit M2, since the amount of nanoparticles, accumulated in the tumor, is related to the dose levels given to the patients [64]. Moreover, its phase Ib clinical trial is stopped in 2013 as a result of the observed dose-limiting toxic events (DLTs). Over phase Ib, a notable increase in acute toxic events and severity, regardless of the general dose reduction, is detected. It is speculated that this can be due to an alteration of the transferrin-targeting agent over a while [65].

The CD-based vectors are well recognized as promising carriers for the delivery of siRNA. The commercialization of CD-based polymer nanoparticles, to be exploited for the treatment of major diseases, has been approved by the US FDA. Therefore, nowadays, the literature concerning the number of surveys about siRNA delivery, through CD-based polymer nanoparticles, is greatly increasing [66].

The above-mentioned investigations are unified in Table 17.1.

17.5.2 Nutraceutical Complexes

Flavonoids, stilbenes, curcuminoids, phenolic acids, coumarins, and essential oils are some examples of families of bioactive compounds that have positive performance in the prevention and treatment of various cancer types (Fig. 17.4). However, similarly to classical drugs, nutraceuticals' hydrophobic nature and low stability can limit their applications. It can be a challenge in the development of suitable formulations to deliver the nutraceutical to the target tissues [67]. This section describes

Table 17.1 Examples of recent studies conducted on cyclodextrins (CDs) and cyclodextrin-based nanosponges (CD-based NSs) for anti-cancer drug delivery

Classic CD monomer	CD-based NSs	Drug	Activity	References
DM-β-CD	–	Paclitaxel	– Improved solubility	[47]
–	βCD-based NSs β-CD-based pyromellitic NSs	Paclitaxel	– Improved solubility – Lower the anti-tumor doses – Increased the effectiveness in inhibiting melanoma cell model	[48, 49]
–	βCD-based NSs	Tamoxifen	– Improved solubility – Increased the effectiveness against MCF-7 cells	[50]
β-, HP-β-, SBE_7-β-CD	–	Docetaxel	– Improved solubility	[51]
α-, β-, or γ-	–	Oxaliplatin	– Improved solubility – Enhanced anti-tumor activity against HCT116 human colon carcinoma and MCF-7 human breast cells	[52]
–	βCD-based NSs	Camptothecin	– Improved solubility and stability – Inhibited the growth of tumor cells both in vitro and in vivo	[54]
β-CD	–	CRLX101 (βCD-based polymer conjugated to camptothecin)	– Enhanced drug solubility and protection from degradation	[55]
–	βCD-based NSs Fluorescent hyper-cross-linked βCD-based carbon quantum dot hybrid NSs	Doxorubicin	– Served as a targeting system for the drug, optimizing pharmacokinetics, and biodistribution – pH-responsive controlled release	[58, 59]

(continued)

Table 17.1 (continued)

Classic CD monomer	CD-based NSs	Drug	Activity	References
–	βCD-based NSs	Nisin-Z	– Improved solubility and stability – Inhibited the growth of tumor cells in vitro	[60]
M-γ-CD	–	Regorafenib	– Enhanced solubility, dissolution, and permeability – Targeting system for the drug, optimizing pharmacokinetics, and biodistribution	[61]
–	βCD-based NSs	5-Fluorouracil	– Protected from light-induced degradation	[62]
β-CD	–	CALAA-01 (βCD-based nanoparticle containing siRNA)	– Protected from nuclease degradation – Delivery system for siRNA	[63–65]

the complexation of CDs and CD-based polymers to ameliorate these problems and enhance the anti-cancer activity of these bioactive compounds in some cases (Table 17.2).

17.5.2.1 Flavonoids

Flavonoids are a large group of polyphenolic compounds that include flavones, isoflavones, flavonols, and other derivatives. They are phytochemicals and share a common structure, consisting of a heterocyclic ring and two benzene rings, which contain embedded oxygen (C6–C3–C6 carbon skeleton). They have antioxidant, anti-inflammatory, anti-cancer, and a large list of more biological activities that make them good candidates for human therapy. Meanwhile, the chemical structure of flavonoids can also result in solubilization problems and limited oral bioavailability.

In this sense, the flavone from honey, propolis, and blue passion flower, chrysin, are loaded in a hyper-branched βCD-based polymer to increase its solubility, photostability, and anti-tumor activity against cervical cancer cells [68]. Moreover, the in vitro bioavailability and chemosensitivity of chrysin, against breast cancer, are improved after its complexation in β-CD by diverse approaches [69].

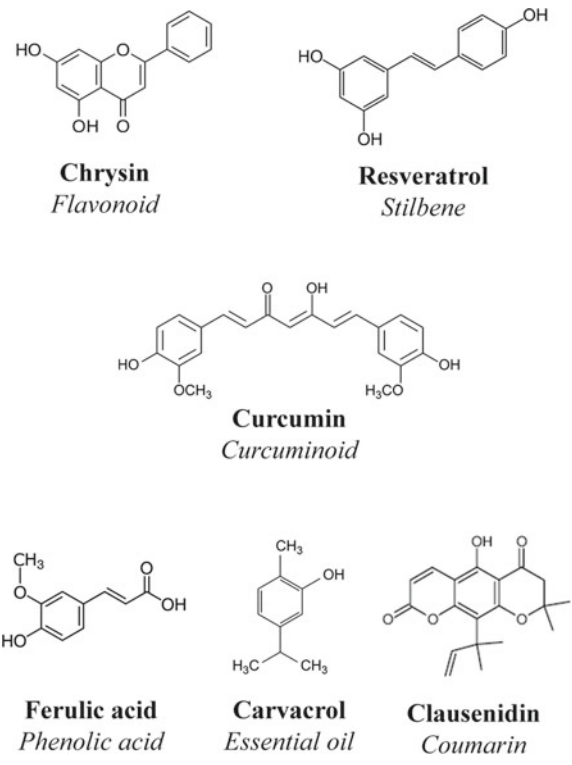

Fig. 17.4 Chemical structure of some bioactive compounds that have been complexed with cyclodextrins (CDs) or cyclodextrin (CD)-based polymers

The isoflavone genistein, which can be isolated from soybeans and other legumes, is encapsulated in a ternary complex of β-CD-D-α-tocopherol polyethylene glycol 1000 succinate to enhance its water solubility, and the final complex can decrease the in vitro viability of breast cancer cells [70]. Besides, the complexation of another isoflavone from soybean, daidzein, with ethylenediamine-modified γ-CD improved its bioavailability and tissue uptake in rats [71]. Finally, in a recent study, a novel derivate of CD-based NSs, using HP-β-CD as a monomer, complexed naringenin to up 8.3 times the antiproliferative effects against MCF-7 cancer cells [72].

In liver cancer, the flavonols fisetin (mainly found in strawberries) and its metabolite geraldol are complexed with β-CD, heptakis-2,6-di-O-methyl-β-CD, HP-β-CD, to overcome solubility problems, and no considerable change in anti-cancer activity, against hepatocellular carcinoma of the liver, is found [73]. Besides, the flavonol quercetin, which is found in citrus fruits, onions, and tea, is delivered to cells on a pH-responsive nanocarrier consisting of citric acid-based α-CD-functionalized Fe_3O_4 nanoparticles. The results revealed that quercetin-loaded nanocarriers have cytotoxicity against cervical cancer cells in vitro, but no inhibition of the normal NIH-3T3 cell growth is observed [74]. In another study, the quercetin complexed with HP-β-CD enhanced its solubility and maintained its bioavailability in a human

Table 17.2 Examples of recent studies conducted on cyclodextrins (CDs) and cyclodextrin-based nanosponges (CD-based NSs) and their inclusion complexes with anti-cancer bioactive compounds

CD monomer	CD polymer or nanoparticles	Bioactive compound	Activity	References
β-CD	–	Chrysin	– Enhanced chemosensitivity through better intracellular uptake and cytotoxicity	[69]
–	β-CD-D-α-tocopherol polyethylene glycol 1000 succinate	Genistein	– Greater antioxidant activity and decrease in MCF-7 viability	[70]
β-CD HP-β-CD	–	Didymin	– Improved water solubility and oral bioavailability – Improved chemosensitivity activity	[77]
–	βCD-based NSs	Ferulic acid	– Increase in solubility and a three-fold decrease in IC50 value of MCF-7 and 4T1 cells	[94]
β-CD γ-CD	–	Thyme extract	– In MCF-7 and MDA-MB-231 cells, the addition of the extract with CDs caused a significant improvement in cytotoxicity, inhibited cell migration, and induced apoptosis, especially using γ-CD in the MDA-MB-231 cell line	[100]
	HP-βCD-based NSs	Naringenin	– The formulation increased 8.3 times the antiproliferative effect against the MCF-7 cell line	[72]
–	Hyper-branched βCD-based polymers	Chrysin	– The complexes exhibited better solubility, photostability, antioxidant, and anti-tumor activity	[68]

(continued)

Table 17.2 (continued)

CD monomer	CD polymer or nanoparticles	Bioactive compound	Activity	References
–	Citric acid -α-CD-functionalized Fe_3O_4 nanoparticles	Quercetin	– Loaded nanoparticles decreased the viability of cancerous HeLa cells with a minimal effect on normal NIH-3T3 cells	[74]
β-CD	–	Carvacrol	– The freeze-drying inclusion complexes gave an increase in cytotoxicity, decrease in proliferation, and inhibition of cell migration in PC-3 cells probably due to the better solubility and bioavailability of the complexes	[98]
–	βCD-based NSs	Resveratrol	– Nanosponges (NSs) improved resveratrol solubility, photostability, antioxidant activity, and cytotoxicity against DU-145 prostate cancer cells	[87]
	GSH-mediated βCD-based NSs	Resveratrol	– Resveratrol-loaded NSs are preferentially uptaken by cancer cells compared to non-tumorigenic cells	[88]
–	βCD-based NSs	Oxyresveratrol	– NSs improved oxyresveratrol solubility, photostability, antioxidant activity, and at the 100 μM cytotoxicity against DU-145 cancer cells	[87]

(continued)

Table 17.2 (continued)

CD monomer	CD polymer or nanoparticles	Bioactive compound	Activity	References
–	βCD-NS	Oxyresveratrol	– CD-based nanosponges showed stronger inhibition of PC-3 cancer cell viability than free oxyresveratrol – CD-based nanosponges showed stronger inhibition of HT-29 and HCT-116 cancer cell viability than free oxyresveratrol	[26]
HP-β-CD	–	Clausenidin	– CDs improved the solubility and cytotoxicity of clausenidin against HT-29 cancer cells, while the viability of normal CCD-18Co cells remained as the control – The complexes can induce apoptosis and cell cycle arrest in the cancer cell line	[97]
β-CD	–	Curcumin	– The complexation of curcumin improved its cytotoxicity against A549 cells, through the induction of cellular apoptosis and G1 phase arrest by the regulation of the MAPK/NF-κB pathway. In particular, these complexes upregulated p53/p21 pathway, downregulated the CyclinE-CDK2 combination, and increased Bax/caspase 3 expressions	[93]

(continued)

Table 17.2 (continued)

CD monomer	CD polymer or nanoparticles	Bioactive compound	Activity	References
β-CD	–	Curcumin	– The administration of curcumin/β-CD complexes in mouse hepatoma H22 tumor led to a reduction of tumor volume, and also, the tumor masses grew much slower (inhibition rate of 34.64%) than the free molecule (inhibition rate of 9.52%)	[93]
β-CD HP-βCD DIMEB	–	Fisetin Geraldol	– The addition of CDs did not modify considerably the effects of these flavonoids in the HepG2 cell line	[73]
HP-β-CD	–	Quercetin	– The complexation increased quercetin solubility while maintaining its cytotoxicity against the T24 cell line	[75]

bladder cancer cell line [75]. In myelogenous leukemia and melanoma cells, the quercetin complexed with HP-β-CD can induce apoptosis in vitro [76].

Didymin, a flavonoid glycoside from citrus fruits that is capable of sensitizing resistant cancer cells against chemotherapeutics, is another poorly water-soluble and bioavailable bioactive compound. Its complexation with β-CD and HP-β-CD is found to overcome both problems, enhance doxorubicin cytotoxicity, and exert chemosensitive activity against breast cancer cell lines [77].

17.5.2.2 Stilbenoids

The secondary metabolites, produced de novo to protect plants from biotic and abiotic stress, are considered stilbenes. They are defined by a basic 1,2-diphenylethylene skeleton with various substituents (C6–C2–C6 carbon skeleton). These compounds can be isolated from different types of plant families, such as *Pinaceae, Poaceae, Vitaceae, Ericaceae, and Fabaceae* [78].

There are many studies, most of them in vitro, that demonstrate the anti-cancer activity of some stilbenes like resveratrol or oxyresveratrol. Nevertheless, their low

aqueous solubility and easy degradation by external factors such as oxygen, light, temperature, or pH indicate some concerns that restrict their potential usage [78]. To solve this, some physicochemical and computational studies, that evaluate the optimal CD cavity to complex resveratrol [79], oxyresveratrol [80, 81], piceatannol [82], gnetol [83], pterostilbene [84], or pinosylvin [85], are performed. However, there can also be considered the interferences of CDs with some methods, like the ORAC method with oxyresveratrol, for measuring antioxidant activity [86].

Besides, Kumar et al. (2019) [87] have successfully integrated resveratrol and oxyresveratrol in βCD-based NSs and tested them in DU-145 prostate cancer cells. The encapsulation in NSs enhanced the aqueous solubility and antioxidant activity of both stilbenes and protected them from UV degradation. In addition, the encapsulation of these stilbenes in NSs resulted in higher toxicity against DU-145 prostate cancer. A selective effect with a focus on resveratrol and GSH-mediated NSs, preferentially uptaken by cancer cells in comparison to non-tumorigenic cells, is noticed [88]. Another study showed that encapsulation with NSs gave a slower and more controlled release of oxyresveratrol. This property increased the capacity of oxyresveratrol loaded in βCD-based NSs to hamper the progress of three various cancer cell lines: PC-3 (prostate), HT-29, and HCT116 (colon) [26].

Piceatannol is also a satisfying anti-cancer nutraceutical. It reduced proliferation or cell survival, enhanced cell cytotoxicity, induced autophagy, and regulated cell cycle proteins [89]. The main problem of this stilbene is its poor distribution, absorption, excretion, and rapid metabolism, thus minimizing its bioavailability. In rats, the intestinal absorption of piceatannol can be increased by using α-CD [90]. Similarly, the piceatannol solubility, in a dose-dependent manner, can also be grown by β-CD [89].

17.5.2.3 Curcuminoids

Curcuminoids are polyphenolic pigments naturally found in turmeric (*Curcumin longa*). Within this group, curcumin is the greatest one for preventing and treating cancer owing to its anti-inflammatory and anti-cancer effects [91]. Its ability to suppress the initiation, progression, and metastasis of several tumors through the regulation of transcription factors, growth factors, or cytokines, among others, is described by some studies [92]. However, this molecule is highly lipophilic, and therefore, it needs a suitable drug formulation to avoid precipitation. The complexation of curcumin in natural CDs (β-CD and γ-CD), CD-based polymers, or CD-based nanoparticles, to target the previously mentioned concern, is considered. Moreover, certain studies observed higher anti-cancer activity after complexation. For example, a reduction of tumor volume and growth rate in mice with hepatoma H22 and an increase in vitro cytotoxicity in lung carcinoma are observed after the curcumin loading in β-CD [93].

17.5.2.4 Phenolic Acids

Hydroxycinnamic acids are considered one of the most abundant types of natural phenolic compounds with great therapeutic potential for human health, and in particular, against cancer. Within this group, ferulic acid, present in several seeds, nuts, and fruits, is trapped in βCD-based NSs to improve its aqueous solubility, in vitro decrease the viability of human and mice breast cancer cells [94]. The complexation of neochlorogenic acid, a less investigated isomer of chlorogenic acid found in green coffee beans, in α-CD and HP-β-CD can raise both aqueous solubility and protection against enzymatic oxidation by polyphenol oxidase [95]. The main component in New Zealand propolis is caffeic acid phenethyl ester. Although it possesses antiproliferative, proapoptotic, and antioxidant activities, it is heat sensitive and easily degradable, thus making necessary its stabilization. Its complexation in γ-CD showed antiproliferative effects in several cancer cell lines such as fibrosarcoma, ovarian carcinoma, cervical carcinoma, lung carcinoma, breast adenocarcinoma, osteosarcoma, melanoma, and neuroblastoma [96].

17.5.2.5 Coumarins

Clausenidin, a pyranocoumarin from *Clausena excavata* with multiple biological activities, is also a poorly bioavailable and aqueous insoluble bioactive compound. Consequently, various researchers encapsulated this molecule in HP-β-CD, to elevate its solubility and cytotoxicity and induct the apoptosis and cell arrest in the M/G_2 phase in a colorectal adenocarcinoma cell line. In addition, fewer side effects, when compared to the viability of normal colon cells, are observed [97].

17.5.2.6 Essential Oils

Essential oil components are a decent example of low-solubility bioactive compounds with limitations in their potential applications. Among them, carvacrol, which is found in oregano and thyme, stands out due to its antibacterial, anti-inflammatory, analgesic, and anti-cancer activities. The complexation of this agent in β-CD, due to the better solubility and bioavailability, reduced the proliferation and cell migration in a prostate cancer cell line [98]. Moreover, in mice with sarcoma 180 in the hind paw, the inclusion complexes can decrease hyperalgesia and nociception (spontaneous and palpation-induced), in comparison to free carvacrol [99]. In breast cancer, the administration of a *Thymus vulgaris* extract (with the main ingredients such as rosmarinic acid, rutin, and kaempferol) in β-CD or γ-CD complexes showed significant cytotoxicity, slight inhibition of cell migration and apoptosis effects, depending on the CD type and cell line [100].

17.6 Conclusions and Future Perspectives

In this chapter, cyclodextrins (CDs), cyclodextrin (CD)-based polymers, particularly cyclodextrin-based nanosponges (CD-based NSs), and their inclusion complexes with anti-cancer drugs and nutraceuticals are discussed. A brief description of the obstacles in drug delivery therapy, to give an open-minded view of the current situation, is provided. The synthesis and classification of CD-based NSs, the formation of inclusion complexes, and their characterization are demonstrated. The way how the inclusion complexes are formed, depending on the nature of the molecule, selection of the best strategy (spray drying, freeze drying, etc.), temperature, pH, and the conditions of the cellular environment, influences the drug or nutraceutical activity. CDs and CD-based NSs increased the drug or nutraceutical solubility, stability, and bioavailability and further improved their delivery and release.

To sum up, the research into novel strategies, to meliorate the medicinal capacity of anti-cancer drugs and nutraceuticals, through their complexations with CDs and CD-based NSs, is fashionable. Be aware of, these complexes can be considered an effective strategy in therapies in the years ahead.

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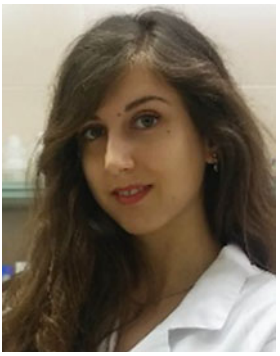

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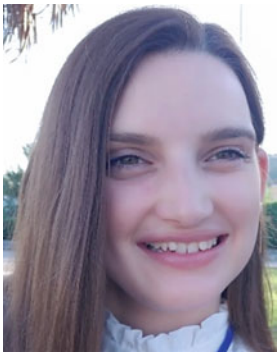

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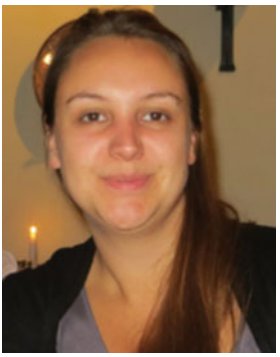

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Chapter 18
Theranostic Approaches for Diagnosis and Treatment of Cancer: An Update

Ruhi Ali, Faraha Ahmed, and Meenakshi Kanwar Chauhan

Contents

Abstract Cancer is one of the most causes of disease-related mortality. One of the major causes of this has been the delay in diagnosis and considerable failure of therapeutic options for cancer. The failure is mainly due to frequent metastasis and a high degree of resistance. The simplest solution to the problem lies in either early diagnosis, efficacious therapy, or continuous monitoring. The identification of sensitive and reliable biomarkers for early screening and therapeutic monitoring has been a thrust area of research. The available alternative, despite the efforts, carries several limitations including high toxicity associated with current therapeutic agents and a

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high degree of genetic variability among cancer types. Thus, there is an unmet need for the development of safer therapeutic alternatives and better diagnostic methods. The field of theranostics has provided an important ray of hope for the advancement of breast cancer therapeutics. Theranostics deal with the targeting receptor for both therapeutic as well as diagnostic purposes. The field of theranostics has been efficiently complemented by the fields of nanomedicine and nuclear medicine. Various aptamers, antibodies, enzymes, and proteins have been conjugated and functionalized to form theranostic nanoparticles and radiopharmaceuticals with the patient-centric approach. The current chapter discusses the concepts and applications of theranostic approaches for the diagnosis and treatment of cancer with a focus on the recent advancement in the field.

18.1 Introduction

Cancer is one of the most common causes of mortality globally [1–4]. It is estimated that approximately 10 million cancer death occurred in 2020 only [5]. Figures also suggest that approximately 50 million population may have been dealing with cancer in the past five years [6]. Some of the common cancers include the brain, colon, pancreas, blood, lungs, nasopharyngeal, breast, and skin. Most of these cancers can be diagnosed by methods like imaging and laboratory testing while early screening is a recommended method.

While radiotherapy, surgery, and chemotherapy are frequently used treatment approaches, the complete cure still remains a challenge due to severe adverse effects, poor prognosis, and high rate of relapse esp. in some of the aggressive cancer types [7].

Chemotherapy alone does not suffice as a treatment and therefore has been combined with either radiotherapy or surgery. Further, chemotherapy has been administered by various methods including the intraarterial route into the cancer blood supply, local injection into the tumor, or the electroporation method [8]. Despite all the approaches, the tissue bioavailability of major anticancer drugs is reported to be low with high peripheral adverse effects. As a consequence of adverse effects, either the chemotherapy cycles are to be followed with longer gaps or the doses are reduced leading to reduced efficacy, the emergence of resistance, or complete therapeutic failure [9]. The undefined biodistribution further complicates the problem and contributes to adverse effects [10–13]. Multidrug resistance to chemotherapy has recently emerged as another important challenge of anticancer therapy. Factors like P-glycoprotein overexpression have contributed significantly to the development of multidrug-resistant cases [4, 14].

Novel drug delivery methods have emerged as a ray of hope for anticancer therapeutics which takes advantage of special features of the tumor microenvironment (TME). The TME is characterized by acidosis due to reduced pH, altered tissue vasculature, the presence of activated immune cells, and enhanced permeability and

retention [15]. These characteristic features of TME can be exploited for cancer cell targeting by utilizing suitable drug carriers [16].

A number of nanomaterials and nanotechnological tools are being exhaustively studied for their role in enhancing the efficacy of existing anticancer agents. These have also been incorporated in the development of newer drug delivery systems in the field of Nanomedicine. Nanomedicine has shown the potential to overcome a number of challenges of the existing chemotherapeutic approaches. The field of nanomedicine utilizes tools to design nanocarriers of varying sizes and employ for tumor cell targeting and consequent improvement of efficacy and safety of existing drugs [17]. The nanomedicines alter the TME in a positive manner. Very recently, it has been argued that these nanomedicines while providing improved efficacy, can also be used to diagnose and monitor the prognosis through what is now known as the theranostic approach [18].

This property of becoming a theranostic has been attributed to the ability of nanomaterials to better coalesce imaging while targeting tumor cells owing to their characteristic surface design, better optical attributes, sizable surface area, and compact size [19].

The present chapter discusses the challenges and approaches in the development of theranostics for cancer therapy.

18.2 Anticancer Drug Delivery: Challenges

18.2.1 Multi Drug Resistance (MDR)

It has been a known fact that some of the blockbuster anticancer agents have become less efficient due to the development of multidrug resistance (MDR). MDR originates as resistance to one drug and later on develops through cross-resistance to drugs of the same or different chemical classes [20]. The major mechanism behind the MDR is the protective mechanism of tumor cells including cellular detoxification, reversal of DNA damage, and protection against apoptosis [21]. The MDR ultimately leads to the failure of therapy and contributed to higher mortality [22].

There are six most commonly identified mechanisms for the development of MDR. These include alteration of drug targets and related pathways, alteration in TME, reduced drug uptake or increased drug efflux, an adaptation of epigenetic changes, and prevention of apoptosis [23–29] (Fig. 18.1).

The ABC family, the transmembrane, energy-dependent proteins which act as transporters, have emerged as one of the most vulnerable targets for the development of MDR [30, 31]. ABC10, ABCG2, and ABCC1 have been identified as the most common contributors to the development of MDR [32]. The simple mechanism includes the pumping of drugs out of cells against the concentration gradient leading to decreased intracellular availability [33]. The ABCC1 has been critical in

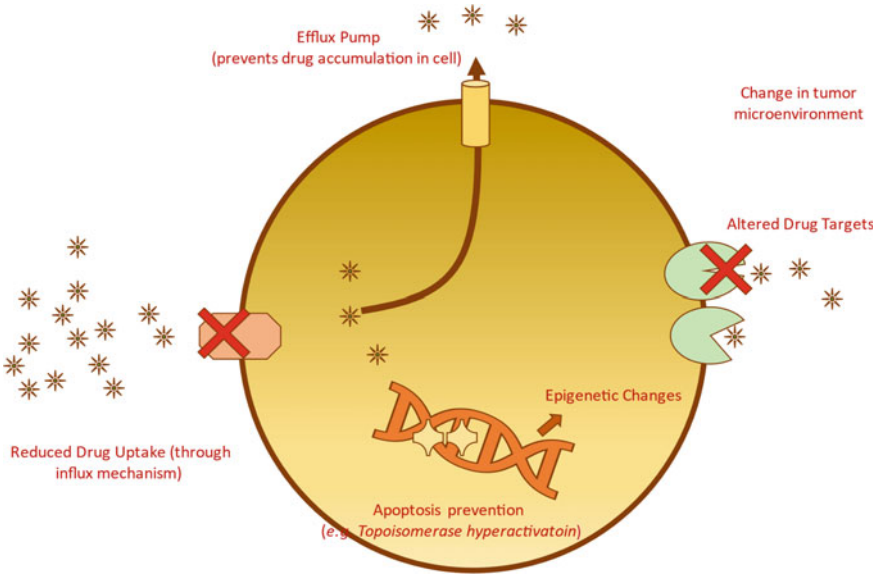

Fig. 18.1 Common mechanisms of drug resistance in cancer (originally created figure, does not require permission)

the development of resistance to anthracyclines and saquinavir [34, 35]. Similarly, vinca alkaloids, taxol, and colchicine are the common substrates for ABCG2 [33].

18.2.2 Biopharmaceutical Properties

Poor aqueous solubility has been the most common challenge with the majority of anticancer drugs. The important reason is that hydrophobicity is an important characteristic of cellular permeability and in turn of anticancer activity. The poor aqueous solubility severely affects both solubility and dissolution of drugs in the GIT post-oral administration and contributed to reduced therapeutic efficacy and causes acute toxicity [36]. The issue is further aggravated by the low therapeutic index of most anticancer drugs which increases the risk of unexpected toxicity or poor efficacy [20]. The low therapeutic index is primarily caused by the low molecular weight of anticancer agents leading to their inefficient retention in systemic circulation and reduced specificity [37, 38].

Nanoparticles can offer the advantage of providing better permeability and retention of anticancer agents thereby enhancing their half-life, improving biodistribution, and providing sustained release in order to manage the concentration within the therapeutic window [39].

18.2.3 Toxicity

Cytotoxicity is the most significant property of a drug to qualify as an efficacious anticancer agent. This property combined with a narrow therapeutic index contributes to the major toxicity of almost all anticancer agents including antimetabolites, microtubule disruptors, alkylating agents, etc. The non-specific nature of cytotoxicity combined with poor biodistribution makes the adverse effects difficult to control [40]. Some of the common toxicities include Myelosuppression leading to reduced immunity and secondary infections. Hepatic and nephrotoxicity, tissue fibrosis specifically in lungs and cardiac tissues, and GI toxicity lead to nausea and vomiting [41–44]. Some of the specific side effects include keratopathy (monoclonal antibodies), onycholysis (paclitaxel), hyperpigmentation (paclitaxel), and development of Mee's [45, 46].

18.3 Scope of Theranostics Nanomedicine in Cancer

The poor hydrophilic character of drugs is a limitation in conventional drug delivery systems. Nano-formulations propose higher dissolution and solubility of drugs by increasing the surface-to-volume ratio. Thus, nanomaterials are of interest to researchers for the development of drug carriers that have higher efficiency, therapeutic, and biological compatibility [47].

The pharmaceutical science that incorporates particles with a size <100 nm (nanometer) is called nanotechnology. This particle range is utilized for designing and formulating drugs as their physical properties like melting point, color, and magnetic characteristics are easily manipulated in comparison to bulk drugs [48]. Nano-formulations have modified pharmacokinetics, drug delivery, and tissue distribution, hence they are delineated to study the enhanced permeability and retention effect (EPR effect) of anticancer drugs [49].

Nano-formulations are fabricated from nanofibers, nanoparticles, nanosensors, nanomachines, and other nano-molecules [50]. Nanoparticles provide a better scope for drug delivery inside the tumor cells and are therefore applied in theranostics applications [51]. Nanomedicines have the capacity to selectively target and accumulate in cancer cells due to the EPR effect [52]. Due to their size, they also serve as potential molecules for the diagnosis and therapy of cancer. For the treatment of cancer, nanotechnology focuses on the selective detection and identification of tumor cells even when hostile cells have small metamorphosis [53].

Broadly two strategies, active and passive targeting are applied for chemotherapy. In active targeting drugs, carriers attach themselves with upregulated biomarkers of cancer cells. Besides, passive targeting involves the EPR effect which is brought about by the accumulation of medicine at the tumor site [47].

The selective treatment of tumor cells minimizes adverse effects and suppresses the harmful effects of chemotherapy. Multiple drug carriers include liposomes [54,

55], micelles [54, 56], natural and synthetic polymer nanoparticles [57, 58], metal nanoparticles, microspheres [59], and direct local delivery using drug-eluting patches and stents [60] exist for site-specific drug delivery. These have been developed to achieve the target concentration of the drug at the specific site [61, 62].

The selection of drug carriers is crucial as it can affect the stability and shelf-life, along with the distribution and bioavailability of the drug. Factors that determine the stability of a carrier are solubility, size, rigidity, charge, and surface phenomenon. The most favored systems in contemporary drug delivery research are Liposome-based and polymer-based nanocarriers as drug delivery systems are the most preferred nanomedicine carriers [63].

Encapsulated nanocarriers and nanocarriers covalently/non-covalently bound to the drug, selectively identify the cancer cells and are hence are used for imaging as well as for treatment of cancer [64]. A single nanoparticle possesses the capacity to incorporate several therapeutic agents. These are called smart drug nanocarriers and can potentially identify the specific biomarkers of tumors and also can target the cancer cells as they can perceive and react to higher pH and temperature at the tumor site [65]. These characteristics of nanoparticles enable enhanced scope of a co-delivery system involving imaging and therapeutic agent together in a formulation for simultaneous diagnostic and curative effects [66] (Fig. 18.2).

Nanoparticles (NP) can be formed from a huge class of materials such as liposomes, polymers, dendrimers, micelles, exosomes, nanostructured lipid carriers, and solid lipid NPs which are organic by nature, and inorganic nanomaterials like carbonaceous NPs, magnetic NPs gold, silver, silica, zirconium oxide NPs, and quantum dots. These organic and inorganic particles can be conveniently orchestrated to formulate a stable and controlled drug delivery to the target molecules at the

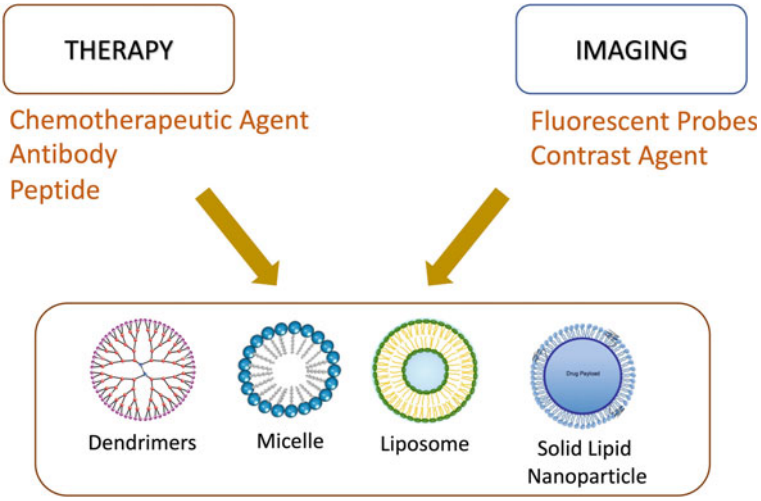

Fig. 18.2 Scope for theranostics development using nanomedicine

effective site. Table 18.1 describes the contemporary research carried out utilizing theranostic nanomedicine in cancer. Additionally, these nano-carriers have enhanced therapeutic effectiveness and depressed adverse effects [67].

18.3.1 Polymeric Nanomedicine

18.3.1.1 Polymeric Nanoparticles

Polymers have been employed for the formulations of nanomedicines as they can overcome the general limitations of existing therapeutic drugs due to their inherent ability. They can decrease the side effects, improve the biological distribution of drugs and attenuate drug resistance. Drug molecules encapsulated in polymers show improved stability and increased plasma half-lives. Further, they also exhibit structured targeting of tumorigenic cells with decreased toxic effects [48].

To achieve enhanced targeting of the tumor cells, therapeutic drugs can either be chemically fused or conjugated with polymer. Furthermore, they can be entrapped in polymeric carriers. Polymeric nanocarriers are of benefit also because they display the retention of drugs passively i.e., even when the target ligand is not present the nanoparticles recruited for a longer duration in the blood circulation and hence accumulate in the tumors passively [87].

Most polymers are biocompatible and biodegradable. Polymers utilized for the formulation of polymeric nanoparticles can be either of natural or synthetic origin. In comparison to the nanocarriers, these exhibit versatile characteristics including the slow and steady release of drugs and stability in the tumor microenvironment. The hydrophilicity and hydrophobicity of the polymer-entrapped drugs can be easily controlled. Often used natural polymers include dextran, albumin, alginate, chitosan, and gelatin. Whereas, glycolic acid, polyglycolic acid, co-polymers of lactic acid, polyethyleneimine, and poly L-lysine are biodegradable polymers to name a few [88].

Polymer NP can be either polymeric micelle or polymeric gene carriers. Polymeric gene carriers are designed to specifically target the malfunctioning genes that are responsible for the development of the pathology of cancer. Polymeric gene carriers target the nucleus and express their therapeusis through transcription and translation [48]. Gene carriers are either viral vectors (retro-viruses and adenoviruses) or non-viral vectors. Viral vectors submit their genetic materials to the host cells and are known to attain greater transfection [89].

18.3.1.2 Polymeric Micelles

Another type of appealing polymeric nanomedicine is a polymer micelle carrier system. After the drug-ligand interaction, micelles are engulfed into the cell through endocytosis. It serves multiple benefits like biocompatibility, efficient targeting,

Table 18.1 Summary of contemporary research carried out utilizing theranostic nanomedicine in cancer

Type of theranostic nanomedicine	Diagnostic agent	Therapeutic Agent	Targeting ligand	In vitro/In vivo model	Outcomes	References
Polymeric nanoparticles	Fluorescence	Doxorubicin	Active targeting	C26 colorectal cancer in BALB/c mice	Increased pharmacokinetics and remarkable anticancer effects	[68]
Polymeric nanoparticles	Boron dipyrromethane	Paclitaxel	Passive targeting through enhanced permeation and retention effect	HeLa cervical cancer cells	pH-depended endocytosis and chemotherapeutic effects	[69]
Polymeric nanoparticles	Boron dipyrromethane	Doxorubicin micelles	Passive targeting through enhanced permeation and retention effect	HeLa cervical cancer cells	pH-dependent chemotherapeutic effect	[70]
Silica (100–200 nm)	Superparamagnetic iron oxide nanocrystals in mesostructured silica	Camptothecin and paclitaxel and	Folic acid through folate receptors	Pancreatic cancer (PANC-1) and human foreskin fibroblasts	Targeted imaging and drug delivery for cancer cells	[71]
Silica (6–7 nm)	Ultrasmall inorganic hybridized nanoparticles	RGDY-PEG-cornel dots	$\alpha_v\beta_3$ integrin	Human melanoma and brain tumors	Precisely targeted drug delivery with low toxicity and considerable pharmacokinetic profile	[72]

(continued)

Table 18.1 (continued)

Type of theranostic nanomedicine	Diagnostic agent	Therapeutic Agent	Targeting ligand	In vitro/In vivo model	Outcomes	References
Silica-gold nanoshell	Nano shell (MR and optical)	Ablation photothermally	Passive targeting through enhanced permeation and retention effect	Head/neck cancer, metastatic lung tumors	Enhanced permeability and retention with greater accumulation at the target site	[73]
Iron oxide (10 nm)	Iron oxide nanoparticles	Anti-EGFRIgG/EGRFvIII	Epidermal growth factor receptor	Human glioblastoma multiforme cells	Specific targeting and improved imaging of infiltrative malignant cells	[74]
Iron oxide nanoparticles	Iron oxide and fluorescein	Azademethylcolchicine	Passive targeting through enhanced permeation and retention effect	MM14-positive MMTV-PyMT breast cancer cells	Necrosis of tumor/antitumor activity while preserving healthy cells	[75]
PEG-Gold nanorod (10 × 40 nm)	Photothermal	Heat	Passive targeting through enhanced permeation and retention effect	Breast cancer in nude mice	Ablation of irradiated tumors	[76]
Quantum dots (30–50 nm)	Quantum dots (Au, graphene, Zn, Cd, Se etc.)	Paclitaxel, doxorubicin, gemcitabine, 5-fluorouracil	CD44, folic acid via receptors	Multiple cancers both in vivo and in vitro	Identify tumorigenic cells and effective for their therapeutics and imaging	[77]

(continued)

Table 18.1 (continued)

Type of theranostic nanomedicine	Diagnostic agent	Therapeutic Agent	Targeting ligand	In vitro/In vivo model	Outcomes	References
Cyclodextrin (70 nm)	Transferrin	RNA interference	Human transferrin receptors (hTfR)	Human solid tumours	Significant gene inhibition	[78]
Gold (27 nm)	Gold nanoparticles	Tumor necrosis factor-alpha	Passive targeting through enhanced permeation and retention effect rhTNF	Human solid tumours	Specific targeting of tumor cells and low toxicity	[79]
Polymeric nanoparticles with iron oxide nanoparticles	Magnetic resonance, homo fluorescence resonance energy transfer	Photodynamic therapy	Passive targeting through enhanced permeation and retention effect	HCT116, CT26, and colorectal cancer in mice	Suppression of drug-resistant cancer cells exclusively	[80]
Inorganic nanoparticles (20–30 nm)	Fluorescence	Calcium phosphate nanocomposite particles	Passive targeting through enhanced permeation and retention effect	MCF-7 breast cancer cells	Enhanced delivery of anticancer drugs to the target site	[81]
Nano graphene sheets functionalized with polymers	Fluorescence	Photothermal therapy	Passive targeting through enhanced permeation and retention effect	4T1 murine breast cancer in BALB/c mice	Significant in vivo photothermal therapy	[82]

(continued)

Table 18.1 (continued)

Type of theranostic nanomedicine	Diagnostic agent	Therapeutic Agent	Targeting ligand	In vitro/In vivo model	Outcomes	References
Magnetic fluorescence nanoparticles	Fluorescence Resonance energy transfer	miR-22 antagomir	AS1141 aptamer	Astrocytoma C6 cells	Improved chemotherapeutic action through selective targeting miRNA	[83]
Hypophilized titanium dioxide nanoparticles	Fluorescence	Sonodynamic therapy	Passive targeting through enhanced permeation and retention effect	HCC7 tumour bearing C3H/HeN mice	15-fold greater suppression of tumour cells	[84]
Glycol chitosan nanoparticles	Fluorescence	Chlorin e6	Passive targeting through enhanced permeation and retention effect	A549 lung adenoma cancer cells	Specific tumor targeting and efficient anticancer effect	[85]
Mesoporous silicon nanospheres	Fluorescence	Doxorubicin	Active targeting through folate	Pancreatic cancer	Higher drug accumulation, lower dose, and enhanced therapeutic effects	[86]

better stability, greater solubility, enhanced drug loading capacity, and extended circulation in the blood in comparison to liposomes. They have a very small size, ranging between 1 and 50 nm and therefore they are a choice of drug delivery for intravenous administration [90].

Structurally, micelles are a cluster of globules composed of amphiphilic lipids. These amphiphilic lipids have a lipid tail, which is impregnated at the core with nanoparticles while the hydrophilic tail encounters the aqueous solvent. Hydrophobic drugs are filled at the core of the micelle while the outer hydrophilic shell, formed with polyethylene glycol enables solubility and stability [48]. Polymeric micelles have several benefits over conventional drug delivery systems. Advantages include (i) administration of hydrophobic drugs due to enhanced aqueous solubility (ii) prolonged half-life of the drug due to preservation from degrading hydrolytic enzymes present in the microenvironment (iii) protection drug from low pH at the tumor site (iv) precise drug targeting (v) decreased toxic effects [91] (vi) greater drug retention time circulation [92]. Polysaccharides, PEG-polyester, PEG-poly (amino acid), and PEG-lipid are generally employed to compose polymeric micelle. Nano-sized polymeric micelles provide the advantage that they can gather considerably at the tumor site and exhibit higher anticancer potential. Polymeric micelles leave healthy and non-tumorigenic cells intact and induce apoptosis exclusively in cancer cells [48].

18.3.1.3 Polymer-Drug Conjugates

Non-viral vector polymers have emerged due to their high safety and capacity to get modified in accordance with the therapeutic requirement [93]. These provide a cheaper and more convenient medium and allow various sizes of genetic material to be transported at ease [94].

The non-viral polymeric vectors are cationic compounds also called polyplexes to interact with DNA through electrostatic forces and yield ionic complexes of nano size. They hold a net positive charge and exhibit enhanced efficacy for transfection. Interaction of these positively charged polyplexes with negatively charged proteoglycans at the cell surface leads to consequent endocytosis. When these polyplexes associate with a negative charge at proteoglycans at the cell surface, subsequently endocytosis takes place [95, 96]. Some examples of non-viral polymeric carriers are synthetic biodegradable polycations, chitosan, cyclodextrins, polyethyleneimine, and poly L-lysine (PLL) [48].

Among various non-viral vectors, polyethyleneimine (PEI) shows the highest transfection efficiency and also displays consistent transfection across multiple cells [48]. Further, it protects the PEI-polyplexes from lysosomal degradation by destabilizing the endosomal membrane [97]. Polyplexes of epidermal growth factor (EGF) and polyethylene glycol (PEG) yield 10–100 times higher greater transfection in most carcinomas [98].

On the contrary, PLL polyplexes show low transfection into the cell. Hence, PLL polyplexes are modified with PEG for better pharmacokinetics and stability [99] and

they showed improved transfection efficiency in human carcinoma cells [100]. Other PLL conjugates for more precise activity include ligands like thioether bond [101], galactosylated PLL [102], histidylated PLL [103], and perplex system-based stearyl PLL [104].

The disadvantage of the cationic polymer is that they do not get metabolized easily in the body and get accumulated in the various cells and tissues. Decreased metabolism and higher distribution in tissues give rise to many side effects. Thus, these cationic polymers are formulated with biodegradable gene carriers such as poly-phosphazene, poly (4-hydroxy-L-proline ester), poly-phosphoester, and poly (a-(4-aminobutyl)-L-glycolic acid). Chitosan is a potent biodegradable non-toxic polysaccharide compound useful for gene delivery [48]. Various chitosan derivates known are galactose [105], low molecular weight chitosan [106], high molecular weight chitosan [107], bile acid complexed chitosan [108], folate [109], quaternized chitosan [110], and chitosan-PEI polyplexes [111]. Cyclodextrin serves as another non-toxic and biocompatible polymer for the delivery of drugs. Cyclodextrin complexes are less cytotoxic, more stable, and have greater transfection efficiency [112].

18.3.1.4 Dendrimers

Dendrimers are polymer compounds that are highly branched, spherical, and symmetrical. Their structure makes them a potential candidate for encapsulation. They are able to deliver chemotherapeutic drugs and hydrophobic compounds [113, 114]. Dendrimers have a number of functional groups, mono-dispersity, good aqueous solubility, and high encapsulation ability [115]. The entrapment of the drug is mediated by the ionic charge at the drug molecule and the opposite charge at the dendrimer. Entrapping of drugs involves physical interactions like hydrogen bonds, electrostatic bonds, and hydrophobic bonds. They can effectively target tumor cells and increase the EPR effect [116]. Applications of dendrimers include magnetic resonance therapy and antisense therapy [117] and provide a drug delivery system with improved chemotherapeutic action [118].

18.3.2 Lipid-Based Nanomedicine

Lipid-based drug delivery systems (LBDDS) can be classified as (i) particulate systems such as solid lipid nanoparticles, solid lipid microparticles, nanostructured lipid carriers, lipospheres, and lipid drug conjugates, (ii) emulsion-based like nanoemulsions, microemulsion, Pickering emulsions, and self-emulsifying emulsions or vesicle based for example liposomes, aquasomes, niosomes, elastic niosomes, aracheosomes, pharmacosomes, phytosomes, colloidosomes, vesosomes, sphingosomes, etc. LBDDS has a number of advantages offers targeted and controlled drug release, a large amount of drug entrapment, dose uniformity, decreased dose,

provides a home for both hydrophilic and lipophilic drugs, it has enhanced stability, lesser side effects biodegradable, biocompatible, economical, allows simple scaling, sterilization and validation and lastly can be given via many routes [88]. Owing to their safety and high biological acceptability, lipid-based nanomedicines owe an advantage over other drug delivery systems. Lipid nanocarriers blended with PEG are very efficient and effective as they form "Stealth" liposomes, which are sterically stabilized. Their mechanism involves enhancing the EPR effect by targeting tumors passively [119].

18.3.2.1 Liposomes

Liposomes are molecules with sizes between 50 and 250 nm with distinct pharmacokinetic characteristics for systemic administration. They can deliver the drug to the tumor through tumor capillaries because of their small size [120]. Liposomes are spheres that have an outer bilayer of phospholipids and an inner aqueous core. They can be categorized as conventional liposomes, pH-sensitive liposomes, long-circulating liposomes, and cationic liposomes. These have higher retention in blood circulation and are distributed by splenic and hepatic systems. Liposomes employ a different mechanism for drug delivery inside the tumor [121]. These are amphiphilic compounds having phosphate groups at the polar ends and the heads of which are oriented toward the solvent. PEG ligation with liposomes imparts steric stability that helps to decrease phagocytic engulfment which increases systemic circulation. Liposomes have several advantages and enhanced solubility of hydrophobic drugs, they can accommodate both lipophilic and hydrophilic molecules together; have low toxicity, and allow surface changes through several chemical groups [65].

18.3.2.2 Nanoemulsion

Nano-sized colloidal dispersions composed of the aqueous phase, oily phase, and surfactant, are termed nanoemulsions. The particle size, physical properties, chemical properties, and stability of the drug are determined by the core of the nanoemulsion [122]. These are highly stable because droplet of nanometer scale is dispersed in another liquid phase [123]. Lipophilic-hydrophilic drug interaction plays an important role in favorable drug encapsulation. Nanoemulsions are generally formulated for drugs having poor aqueous solubility [122]. The encapsulated emulsion also protects the drug from degradation and hence there is an expanded half-life of the drug [124]. Droplets are dispersed with the help of various emulsifying agents which further help to stabilize them. Nanoemulsions of 20–100 nm can easily pass through blood capillaries and are encapsulated with drugs so that they get accumulated in tumor cells. This leads to increased opsonization by the mononuclear phagocytic system and avoids renal clearance [125].

The aim behind the development of nanoemulsions overcome Multi-Drug Resistance (MDR) in cancer. Nanoemulsions have served as effective chemotherapeutic

agents for several cancers like melanoma, prostate cancer, colon cancer, leukemia, breast cancer, ovarian cancer, and lung cancer. They are effortlessly conjugated with antibodies and oligonucleotides that improve chemotherapy. Interaction of nanoemulsions with Bcl-2 inhibitors and P-gp modulators can subdue MDR. An apoptotic molecule, ceramide increases apoptosis of tumor cells and is formulated and administered as a nanoemulsion to overcome MDR. Anticancer class of drugs, taxanes (co-delivery of paclitaxel with baicalein) given as nanoemulsions for therapy of breast cancer and also overcomes MDR. Paclitaxel delivered in vitamin E based delivered in nanoemulsions are efficacious in vitro in paclitaxel-resistant human ovarian carcinoma cell lines [126].

A novel and recent approach that amalgamates nanotechnology for imaging and diagnostic purpose simultaneously are called nanotheranostics. Nanotheranostics aims to formulate nanomedicines having both diagnostic and therapeutic potential. Cancer chemotherapy is the area that takes the most advantage of this technology for prognosis, distinguishing tumors, and formulating specific nanomedicines for discrete cancer treatment [127].

18.3.2.3 Solid Lipid Nanoparticles

As the name suggests, solid lipid nanoparticles (SLNs) are lipids that are solidified at body temperature. The core matrix of solid lipids allows hydrophobic drug encapsulation. Due to its crystalline structure, it has some disadvantages like diminutive drug loading capacity and slow pharmacokinetics [128]. On the other hand, its advantages include that it is cheaper than other lipid carriers, it can be formulated easily, and is biocompatible lipid [129]. Other advantages of SLNs include large-scale production, affinity with both hydrophilic and lipophilic molecules, lower toxicity, and enhanced bioavailability. SLNs can also overcome drug resistance as they can encapsulate numerous classes of drugs, undergo controlled release of the drug, they can disrupt physiological barriers, and can accentuate tumor suppression [130].

SLNs-entrapped drugs have exhibited higher bioavailability and intra-cellular drug delivery thus dose and adverse effects are also decreased. SLNs formulation of several chemotherapeutic drugs of natural and synthetic origin like capecitabine, emodin, docetaxel, and epirubicin have increased bioavailability and intra-tumoral target delivery [131]. SLN of paclitaxel has shown increased drug absorption and decreased breast tumor size in mice [132]. Treatment of mice with methotrexate-loaded SLNs enhanced the life span of animals significantly [133]. Tamoxifen-loaded SLN also has higher cytotoxic potential toward drug-resistant breast cancer cells [134].

18.3.2.4 Nanostructured Lipid Carriers

Nanostructured lipid carriers (NLCs) are composed of several lipid molecules one of which remains liquid at room temperature. This structural modification decreases

the rigidity of the crystalline solid matrix and thus improves the drug-loading ability of the carrier system. These were formulated to curb the limitations of SLNs [128]. Components for the formulation of NLC are solidified, liquified lipids, emulsifying agents, and water. Lipids are the key molecules and NLC is developed with both solid and liquid lipids. The twin matrix comprising both solid lipid matrix offers a better-controlled release of drugs along with high transfection efficiency. It is suitable for gene delivery and with high loading efficiency and lesser cytotoxicity [135].

The advantages of NLC are that it offers expanded drug loading than SLN and thus there are lesser chances of spilling or expulsion of drugs during storage. Further, NLCs are more stable, preserve labile compounds from deterioration and degradation provide controlled drug release, and can be formulated conveniently. NLCs have favorable physiochemical properties; they are compatible with most commonly used emulsifying agents, have the adequacy to incorporate lipophilic compounds, and are also stable pharmaceutical carriers [136].

18.3.3 Inorganic Nanomedicine

Inorganic materials translated to nanomedicines have their unique size and material. The physical and chemical properties of an inorganic compound determine its size which is separate from organic nanoparticles. Inorganic compounds have inert properties, unique magnetic properties, optical behavior, and stability and are easy to formulate. These properties enable these compounds as a potential candidate for diagnosis, imaging, and therapeutic of tumorigenic tissues. Inorganic nanomedicines contain metal oxide having an inorganic core and are coated with an organic shell. This structure is suitable for a bioactive environment and lays out a site for target drug delivery for these molecules [137]. Inorganic formulations have distinct physical and chemical properties, modified surfaces for increased selectivity, a larger surface-to-volume ratio [138], optical properties [3], fluorescence [139], and magnetic properties [140].

18.3.3.1 Gold Nanoparticles

Colloidal gold or gold nanoparticles (AuNPs) are prepared as a suspension of nanoparticles of gold [141]. AuNPs have unique characteristics such as tunability, surface plasmon resonance, and optical activity. The core size of AuNPs can accommodate particle sizes of a broad range varying from 1 to 150 nm. Due to these characteristics, they can encapsulate a variety of genes, drugs, and target ligands and hence can be utilized for multiple applications like molecular recognition, sensing, and imaging [73].

Depending upon AuNP's shape and size, they acquire optical activity after their interaction with light [142]. Additionally, due to porous walls, hollow interiors, and local plasmon surface, the release of drugs can be controlled due to which they are

suitable for therapeutic and diagnostic [104]. AuNPs could suppress malignant cells through their photothermal property and use employed for theranostic applications in photothermal therapy along with optical imaging [143]. These nanomedicines are able to transfer the photosensitizers selectively to the tumor cells. Inside the tumor cells, AuNPs induce apoptosis of tumor cells by enhancing the gene expression of caspase-9 [144]. A novel dual-modal imaging system comprising combined cancer cell membrane-coated upconversion nanoparticles with gold nanoparticles could provide a new dimension of photothermal therapy by invading the immune system and targeting tumors exclusively with high imaging targeting [145].

18.3.3.2 Silver Nanoparticles

In comparison to gold, silver is harder but malleable and ductile. Pure silver is a good conductor of heat and electricity with the least resistance. Silver nanoparticles (AgNP) are nanoparticles of size ranging from 1 to 100 nm. AgNPs also have specific properties because of which they are a choice of nanoparticles to be used for medical purposes like cancer therapy, and molecular diagnostics and are incorporated in medical devices. Like gold, silver also has unique magnetic, electrical, and optical properties that are dependent on its shape and size [146, 147].

AgNPs can help in removing the radiation effect of cancer cells [148] and also exhibit dose-dependent toxicity in numerous cancers [149]. These are also used as radiosensitizers and photosensitizers due to their surface plasmon resonance [150]. PVP-coated AgNPs release silver ions and generate reactive oxygen species, that decrease cell viability and induce apoptosis of tumor cells in acute myeloid leukemia [151]. AgNPs display multiple mechanisms for cellular toxicity. They have the capacity to induce DNA fragmentation, caspase-3 mediated apoptosis, and reactive oxygen species to display toxicity in vitro toward MDA-MB-231 cells [152].

AgNPs can damage the DNA, alter calcium homeostasis, modifies chromosomes for altered genomic stability and induce cytoskeletal stability. All these mechanisms contribute to the anti-neoplastic behavior of AgNPs. Furthermore, they hinder the cell cycle that blocks cell division which adds to their anti-proliferative action toward cancer cells [144].

18.3.3.3 Iron Oxide Nanoparticles

Iron has the inert property of magnetism. So, iron oxide nanoparticles (IONPs) are magnetic compounds that have a magnetic field to exclusively target malignant cells. The magnetic property makes them vulnerable to binding with vector-mobilized biomarkers [153]. Innate magnetism is the prime advantage of IONPs but they also possess other advantages over other NPs due to their innate magnetic property [154]. The magnetic characteristic can be manipulated for high-quality imaging and magnetic targeting [155]. It can also provide confined heat to malignant cells leading to the removal of tumor cells [156]. Additionally, IONPs are biologically

compatible and biologically degradable [157]. The distinct magnetic property of IONPs relaxes the protons in the malignant tissues further awards a clearer image in magnetic resonance imaging (MRI). IONPs are also suited as contrast agents and carriers for drug delivery systems because of their effect on biological tissues both at the cellular and molecular level [158].

When IONPs are combined with the alternating magnetic field, super magnetic iron oxide nanoparticle (SPIONs) is developed. SPIONs possess some advanced characteristics as they can modify cellular metabolism through hyperthermia in a dose-dependent manner modified cellular metabolism by hyperthermic effect dose-dependently. At higher temperatures of >50 °C, they induce cellular necrosis by ablation of cancer cells [159]. These nanoparticles are helpful for translational guidance and are used as theranostics as they provide better imaging for the identification of cancer cells. These have lesser toxicities and higher drug-loading capacity that created better imaging [160].

18.3.3.4 Quantum Dots

Quantum dots (QD) are semiconductor nanocrystals from elements of groups II–VI or III–V. Chemically they are crystalline colloids. Due to their quantum-confined properties, they are semiconductors with photophysical characteristics. Discrete sizes and composition of these elements owe a wide range of QDs can emit different wavelengths in the spectrum of light from visible to infrared. These are widely employed for optical applications due to their greater extinction coefficient and size-dependent luminescence [139].

QDs are 2–100 sized nanoparticles that actively target malignant cells. These are tunable molecules having optical activity. They have 1–5 eV electronic energy due to which semiconductor quantum dots display themselves as organic fluorophores and have photosensitizing capacity. They possess high electron density, they can absorb gamma rays and high X-rays significantly thus behave like radiosensitizers and bringing out the peculiar and targeted deterioration of malignant tumor cells [161]. Certain metal compounds like hafnium, copper selenium, etc., are useful tools as QDs.

Hafnium ($Z = 72$), is an inert transition element that has a high atomic number. It can effectively combine as NPs along with ionizing radiation. Hafnium oxide (HfO_2) is chemically stable, has a large gap in the energy band, has a high melting point, dielectric constant and refractive index, and low toxicity profile [162]. Owing to its radio-enhancing property, it is utilized for delivering the dose of radiation inside the tumor cell. Without spilling, HfO_2 significantly elevates the dose at the target site and doesn't affect healthy cells around the tumor thus offering lesser toxicity [67].

Copper is another element employed as a nanocrystal. Copper in sulfide form acts as a carrier and exhibits photothermal properties and shows synergism in affecting cancer cells through the interaction of chemical and photothermal therapy in vitro [163]. Modified poly acrylic acid-coated $Cu_2(OH)PO_4$ QDs showed an enhanced capacity to absorb infrared radiation. Thus, damaged the cervical cancer cells in

both in vitro and in vivo studies due to their photodynamic and photothermal effects [164].

A much safer and biocompatible alternative for chemotherapeutic drug delivery is Selenium NPs. They lead to apoptosis of the cell cycle by arresting it at the S phase. Through in vitro apoptosis and arrest of the cell cycle, they suppress the malignant cancer cells in human cervical cancer cells [165].

Silicon nanoparticles are also useful as QDs as they are biocompatible, cause lesser side effects, and have high fluorescence characteristics. They can do multimodal drug targeting and imaging, providing an opportunity for efficient and highly therapeutic drug delivery. Matrix of Silica is more efficient and chemically stable and preserves key molecules from enzymatic degradation and photobleaching [166].

18.3.4 Carbon-Based Nanomedicine

Carbon nanomedicines formulated as theranostic agents are believed to be more biologically compatible and safer. Carbon compounds have structural advantages along with unique physical properties including thermal property, optical property, electronic property, and mechanical properties. Carbon nanomedicines are favorable as photosensitizing tools and are used for sensing and imaging malignant cells [167].

18.3.4.1 Carbon Nanotube

Carbon nanotubes (CNTs) are tubular structures that are composed of graphene that is rolled up in the shape to form a cylinder. CNTs are either single-walled structures or multiple-walled structures. Single-walled CNTs have one graphene sheet of diameter between 0.4 and 40 nm. Whereas multiple-walled CNTs contain multiple concentric cylinders of graphene sheets having a diameter between 2 and 100 nm distancing about 0.35 nm from each other in the inner layer. Cylindrical sheets are closed at one end and are arranged in a structural fashion such as nanotubes, zig-zag, chiral, and arm-chair [16, 167]. Nanotubes can be efficiently employed for drugs having elevated systemic toxicity, narrow therapeutic index, poor cellular penetration, and drugs that exhibit resistance. CNTs formulations increase the accumulation of drugs at the target tumor site and decrease its adverse effects [168].

CNTs involve specific cellular targeting with photothermal therapy and damage the cancer-causing cells at a single platform. Single-walled CNTs exhibited an in vivo decrease in malignant cells in mice [169]. Multi-walled CNT has a large diameter and thus can load higher concentrations of the active chemotherapeutic drug. It also has diminutive effect on tumor vasculature by decreasing its density and showing cytotoxicity [170].

Ultra-sensitive doses of radio-isotopes can be precisely delivered for theranostic effects. This is achieved by encapsulation of imaging and therapeutic isotopes within CNTs. This is then followed by activating the surface with a functional group or target

ligand [171]. Neutron-irradiated nanocapsules of ^{153}Sm when given intravenously displayed potency as imaging and therapeutic molecule in vivo for metastasis of lung cancer [172]. CNTs encapsulated ^{153}Sm theranostic compound exhibited remarkable suppression of lung tumors and attenuated toxicities of various tissues such as the spleen and blood [173].

18.3.4.2 Graphene

Graphene is a hybrid allotrope of carbon [19]. Graphene is widely utilized as a nanomaterial for therapeutic purposes. Various nanomaterial forms of graphene include graphene oxide, graphene quantum dots, graphene nanoplatelet, graphene nanoribbons, and reduced graphene oxide. Graphene has a thick carbon sheet that can ensemble a large amount of loaded chemotherapeutic drugs that can efficiently deliver the drugs to the target site [51].

Graphene can also maneuver dendritic cells and macrophages for an improved immune response that further targets to decrease the malignant cancer cells [22]. Graphene oxide exclusively targets tumorigenic cells without damaging normal cells thus they follow the principle of "differentiation." Graphene oxide nanoparticles have various sizes and are capable of selectively inhibiting the growth of cancer stem cells in numerous cancers such as cancer of the brain, breast, prostate, ovaries, pancreas, and lung [174].

Graphene QD loaded with platinum are effective in overcoming hypoxia-induced drug resistance in oral squamous carcinoma cells. This formulation can cause apoptosis and cell cycle arrest at the S phase in both normal and hypoxic cells as it can sufficiently accumulate in tumor cells [175]. Graphene has also the potential to eradicate malignant cancer cells completely due to its photodynamic and photothermal properties [176].

18.4 Clinical Translational Perspectives of Theranostic Nanomedicine

For several decades, engineering and sciences have exhibited attractive integration for the development of novel approaches toward understanding the therapy of cancer and developing novel anticancer drugs [177]. The nanoparticles domain has been well-researched and investigated for the formulation of many medical purposes [16]. Nanomedicines identify the differentiation, pathology, and physiological alterations of multiple diseases for specific and exclusive therapy. Nanomedicines formulated as theranostic agents offer lesser toxicity and targeted drug delivery [39]. However the toxicological and cytotoxic profile of nano-formulations for anticancer needs further

investigation for the development of drugs with enhanced efficacy [22]. Nano preparations require an analytical approach so that they may administer the chemotherapeutic drugs through an advanced drug delivery system and provide novel approaches for better therapeutic [126].

The development of nanomedicines and the expansion of nanocarriers has brought progression in the scientific outlook toward drug delivery for serious life-threatening illnesses. Cancer nano drug delivery system has taken the cancer chemotherapy way forward as a strategy to detect, diagnose, and treat the critical illness. The complexity of various cancers is yet a challenge for complete eradication and hence successful treatment. To deal with such problems, nanomedicines may come out victorious provided that an interdisciplinary approach to cancer chemotherapy is applied with the researchers, medical specialists, physicians, academicians from academia, and the pharmaceutical industry collaborating together [17]. These ligations are necessary because there are many areas yet to be explored to generate more evidence regarding the employment of nanomedicines for the therapy of fatal illnesses like cancer. There is ample scope from the translational perspectives to be investigated. Despite extensive research work done in this domain continues to be an attractive platform for future researchers. Most in vitro and in vivo models have already been explored, thus newer models need to be developed considering the complex nature pathology of diseases. Newer models would also help in understanding the multidrug resistance and effect of permeation and retention in cancer cells. Every type of cancer demands exclusivity for its treatment and therefore also requires a unique diagnosis. But most existing models have differences with human cancer cells. For example, the murine tumor model differs from human cancer cells in several ways including the host's life, metabolic rate, and growth pattern. Therefore, considering the specificity of cancer etiology, the nanomedicines shall be designed [178].

As per FDA, there are no specific guidelines for the production and preparation of nanomaterials. But nanomaterials shall still smartly be designed by precise identification for synthesis and then formulating them further as a carrier. For such implementation, newer insights are required to be worked upon considering nanomaterials [179]. DNA-based newer nanomaterials propose to be a potential candidate for therapeutics and diagnosis of cancer [180]. Succeeding researches require further interrogation of the molecular mechanism associated with the immune system to escalate the application of nanomedicines. Characteristics of nanoparticles required further exploration to identify their limitations, correlation with immune cells, exclusivity of action, target specificity, and toxicological profile. Nanomedicines that have low antigenicity seem to be the future [181].

18.5 Conclusion and Future Directions

Conventional chemotherapeutic drugs possess immense limitations like drug resistance, poor solubility, and bioavailability, lack of efficient and exclusive drug targeting, inadequate distribution of drugs, and a large number of toxic effects.

Nanotechnology is a ray of hope for advancement in chemotherapeutics as they provide scope for diagnosis, therapeutics, and drug delivery of a number of cancers. Nanotechnology provides alternate strategies to surpass unavoidable and critical toxic effects of existing anticancer drugs. Nanomedicines are of better safety and efficacy. A huge class of compounds has been identified as nanomaterials that can serve as substitutes for precise and targeted drug delivery. Molecules which are lipid-based, polymer-based, carbon-based, or based on inorganic material have the potential as candidates for alternate drug carrier systems to surpass the limitations of conventional drug carriers for escalated therapy of life-threatening disease. The smaller size of such compounds (nanoscale range) has different physical properties like absorption, magnetic resonance, fluorescence, light scattering, and Raman Effect than larger particles. Thus, establishing them as exceptional candidates for cancer prognosis, diagnosis, and imaging.

The physiology of tumor cells developing into cancer is intricate to understand for identifying, synthesizing, and thus formulating the nanomedicines for drug delivery. Newer targets can be identified with high reproducibility by understanding the tumor microenvironment. Nanomedicines are well thought out, a well-researched strategy that could be helpful in identifying the loopholes and gaps in cancer chemotherapy. Nanotechnology imparts the development of an incredible drug delivery system that is target-specific, efficacious, and safer.

Translation of nanoparticles for clinical employment demands further clarity and understanding about mechanisms related to safety, and resistance along with research development to prepare an uncomplicated, cost-effective, simple, eco-friendly, and safer methodology for synthesis, formulation, and cognizance for its pharmacokinetics, plasma half-life, and biodistribution. Adequate consideration is desired toward understanding the impact on healthy tissue, tissue damage, immunogenicity, inflammatory outcome, carcinogenic potential, and long-term side effects.

Presently, limited nanomedicines are available for chemotherapy. They also have a higher cost than conventional drugs. Such issues need attention from the regulatory authorities because nanomedicines play a major role in decreasing the morbidity rate in cancer patients by improving their quality of life.

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Chapter 19
MicroRNA Biomarkers for Oral Cancer: A Meta-Analytic Review

Jyotsna Choubey, Olaf Wolkenhauer, and Tanushree Chatterjee

Contents

Abstract MicroRNAs (miRNAs), a small non-coding RNA, are involved in a wide variety of biological processes, and their expression is frequently altered in various cancers including oral squamous cell carcinoma (OSCC). Changes in miRNA expression have been investigated as a potential biomarker in cancer. However, the role of cancer-targeting miRNAs is poorly understood. The use of integrative computational bioinformatics approaches makes it simple to identify miRNAs that may be outliers in cancer. After reviewing the available literature, eligible studies were chosen for meta-analysis. Vote-counting technique was used to examine the data. In the next part, the chapter described computational methods used to determine miRNA–mRNA networks and functional enrichment analysis to identify which pathways were most affected by the altered expression of these miRNAs by obtaining their target genes.

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The strategy proposed in this study combining scientific literature data with innovative bioinformatics methods to determine which microRNAs are associated with a disease and which biomarkers are derived from the targeted genes. By looking at these microRNAs' target genes, the study's clinical value can be inferred. Finally, we suggest future research directions in this field and conclude that miRNAs serve as a promising new biomarker for oral cancer, but more research is needed with larger patient cohorts and standardized guidelines before they can be used clinically.

19.1 Introduction

Oral squamous cell carcinoma (OSCC) is one of the most prevalent cancers that has the vote-counting method highest incidence rate worldwide. The disease is usually identified in its late stages, which contributes to its high death and morbidity rates. At this stage, treatment for OSCC is extremely debilitating. According to most recent GLOBOCON 2020 statistics gathered by the International Agency for Research on Cancer, there will be 19.3 million newly diagnosed cases of cancer across the globe in the year. (18.1 million if only skin cancers other than melanoma are considered.) and cancer-related deaths reached nearly 10.0 million (The number is 9.9 million if only skin cancers other than melanoma are considered.). Globally, oral cancer was diagnosed in approximately 377,713 new patients all over the world, with the highest incidence rates being reported in South-Central Asia and 177,757 cancer deaths occurred in 2020 [1]. OSCC is eighth most frequent cancer globally, which is ranked third in India. It mainly affects middle-aged men. Recent research suggests an increasing trend among younger adults, including females who have never smoked or drunk [2]. In India, OSCC is a significant public health issue caused by tobacco use and affects mainly males (16.2%) rather than females (4.6%), and in the USA, it accounts for 3% with 66,000 cases and 15,000 deaths [3]. OSCC is usually caused by a multistep carcinogenesis process that includes initiation, promotion, and progression. These steps changes cell's metabolome, transcriptome, and proteome and causes alterations in expression of key genes or proteins as well as metabolic and structural pathways to diverge or stop [4]. The fundamental processes that promote OSCC carcinogenesis, on the other hand, are still unknown.

Tobacco (in any form) use is a major contributor to the high rate of oral cancer in low-income countries. Chewing paan, which includes piper betel leaves mixed with areca nut, lime, catechu, and cinnamon, is a major cause of oral cancer, particularly in India's northeastern regions, which have the country's highest cancer incidence rates [5], whereas 74% of OSCC cases in Western countries were found to have a history of tobacco or alcohol use [6]. These risk factors are associated with age, sex, and ethnicity [7] and account for about 90% of the cases. Infection with human papillomavirus (HPV) is one of the reasons that could account for the rising prevalence of this disease in younger people, particularly among young women who do not smoke [8, 9]. In recent years, the incidence HPV-driven oropharyngeal squamous cell carcinoma (OPSCC) has dramatically increased [10]. Plaque build-up and chronic

inflammation in the mouth are also significant risk factors, as are sharp-edged dental fillings that cause permanent damage to the mucosa [11]. Toxic irradiation, malnutrition, and betel nut chewing are all other potential causes of malignant mucosal degeneration [12]. The relative frequency of these risk variables influences the global heterogeneity in OSCC distribution [13].

At early stage of disease, diagnosis is favourable and mortality rate is low hence needs less aggressive treatment. Unnoticed growth and subsequent metastasis are common in oral cancers that originate in hard-to-reach places like the base of the tongue or the oropharynx. Hence, tumour location affects diagnosis and treatment of OSCC [14]. Surgical resection, radiotherapy, and/or chemotherapy are common treatments of oral cancer. Survival rate differs significantly between early- and advanced-stage oral cancer.

Statistics relating to survival after 5 years of OSCC is below 50–60% [15, 16]. Stage I cancers have an 80% survival rate, while stage IV cancers have a 40% survival rate. This difference in survival rate is due to lack of timely diagnosis and high tumour recurrence rates. Only one-third of OSCC patients are diagnosed early [17]. As a result, there is a need for non-invasive, quick, and simple diagnostic methods for oral cancer [18].

Despite tremendous advancements in modern diagnostic technology, full screening of this condition is still lacking. Because the disease's complexity continues to confound modern medicine, more molecular characterization is required [19]. The discovery of novel biomarkers will be crucial in the early diagnosis and prognostication of OSCC.

Biomarkers are biological things expressed in serum or saliva that leads to disease and its progression. They are overexpressed or under expressed cellular components present in body fluid or tumour cells that indicate disease. The primary screening of OSCC can be aided by biomarkers found in saliva, such as circulating tumour DNA, miRNAs, and extracellular vesicles [20]. DNA methylation, histone modifications, and non-coding RNA modifications (miRNAs) have been demonstrated to be significant regulators of oral cancer development and progression [21]. Cellular molecules such as carbohydrates, DNA, mRNAs, proteins, and metabolites serve as biomarkers [22]. The identification of abnormalities that lead to the development of OSCC is made possible by predictive biomarkers, whereas the evaluation of a patient's potential reaction to treatment and their overall prognosis is made possible by prognostic biomarkers [23].

Alteration in cancer-preventing genes that code for protein and/or oncogenes is the primary triggers of tumour development for almost three to four decades [24]. Recent research has led to the identification of thousands of genes, that transcribe non-coding RNAs (including miRNAs), which shows that the cancer biology is far more complicated than was previously believed. In the genesis and maintenance of malignant phenotypes, there are multiple levels of controls at the molecular level (e.g., mRNA, miRNA, and protein) are involved [25]. Since last decade, researchers have focused profoundly on miRNAs in hope that their role in OSCC will enable them to function as potential biomarkers [26].

MiRNAs, also known as microRNAs, are a type of non-coding, single-stranded RNA that typically range in length from 21 to 23 nucleotides. In recent years, it has been suggested that miRNAs could serve as promising biomarkers for the diagnosis and monitoring of cancer [27]. Although transcription occurs in at least 65% of the human genome, only 2% of the human genome encodes proteins. Non-encoded protein transcripts can account for up to half of all mammalian transcripts, emphasizing their significance [28]. Recent research suggests that miRNAs may influence mRNA targets via weaker mechanisms, like binding to non-complementary regions and locations inside the coding sections of transcripts [29]. Gene silencing is induced by binding to the 3'-untranslated region (3'-UTR) of the target mRNA, which then either leads to the degradation of the mRNA or regulates the expression of the target gene at the post-transcriptional level [30]. Since miRNA plays a significant role in a wide range of cellular activities such as proliferation, differentiation, and apoptosis, its abnormal expression has been linked to different types of cancer, including oral cancer [31]. The search for novel, sensitive molecular biomarkers that can be used for diagnostic, predictive, and prognostic purposes, necessitates the identification of aberrantly expressed miRNAs in oral cancer [32]. We concentrated our research on tissue expression profiling since miRNA expression is easier to detect in tissue specimens than in plasma samples or cell lines [33]. Future research to validate candidate miRNAs as biomarkers for OSCC may benefit from our findings. Despite the fact that several researchers have identified miRNAs as potential biomarkers for OSCC detection using expression profiling, the findings of the study are inconsistent due to differences in the way the study was designed, the types of specimens used, the various platforms used for profiling, the amount of miRNA that was available, the methodology, and in some cases the sample number, as well as a scarcity of clinical data. Indeed, various groups may reach different results. Since we wanted to see how accurate miRNAs were in diagnosing OSCC, we conducted a meta-analysis by combining results from multiple studies.

In this study, bioinformatics and integrative meta-analysis were used to identify predictive biomarkers for OSCC. A comprehensive study with a subsequent meta-analysis was carried out with the purpose of determining the prognostic significance of miRNA signatures in OSCC. In the next step, an in silico bioinformatics methodology was applied in order to figure out how miRNA contributes to disease in a mechanistic way. The primary goal of this study to conduct in silico characterization of the miRNA signature and to identify a group of microRNAs and the target genes that are associated with their differential expression. Additional research was conducted to investigate the transcription factors regulated by these microRNAs as well as the target genes. These gene and miRNA signatures have the potential to be used as diagnostic tools for OSCC and lead to the discovery of new biomarkers for OSCC. Also, the results of this research have the potential to be utilized in further investigations into the roles that various miRNAs play in the development of other types of cancer.

19.2 Collection of Supporting Data and Meta-Analysis

In order to investigate the prognostic biomarkers for OSCC, a comprehensive review was carried out, and as a result of this review, various miRNA was discovered to be useful biomarkers. Because microRNA does not contain any coding information, a method was developed to find the genes in oral cancer that are targeted by miRNA. The first part of this investigation consisted of conducting a comprehensive literature search followed by a meta-analysis, and the second part consisted of performing a gene target analysis for using bioinformatics.

19.2.1 Eligibility Criteria for Data Extraction

For this in silico studies, PubMed was used to conduct a comprehensive literature search for studies on microRNA expression. (http://www.pubmed.gov). Published research article was collected using a variety of search terms or any combination of them such as "Expression profiling by array", "Oral cancers", "human oral carcinoma", "miRNA expression profiling of human oral cancers", and so forth. First, the abstract and title of all relevant articles were assessed for their content, then the full text of each study was thoroughly analysed. Original experimental research articles that provide comprehensive comparisons of miRNA expression between oral squamous cell carcinoma and non-cancerous controls in humans were chosen for further investigation. Because of this, we did not include any data on the expression of miRNA taken from publicly available databases such as The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/tcga) and Gene Expression Omnibus (GEO) [34].

Research article that met the following criteria was eligible for inclusion:

First, full text articles available in English; second, miRNA expression profiling studies of oral cancer patients compared to adjacent normal OSCC tissues or healthy control subjects; third, studies reporting lists of differentially expressed miRNAs and cut-off criteria.

Exclusion criteria were set as follows:

(a) article that is unrelated to the OSCC; (b) article that is a duplicate or that has data that is incomplete; (c) if the article were either letters, editorials, commentaries, reviews, or case reports; (d) the research involves animal subjects; (e) studies that did not report any lists of microRNAs that have differential expression, or that only displayed their data on the heat map and did not provide any additional information that could be obtained; and (f) studies conducted on cell lines, and studies that used a variety of patient biological samples (like blood, saliva, or serum) were ruled ineligible for further consideration in this study.

19.2.2 Statistical Analysis of Data

From the studies considered for the meta-analysis, a list of miRNAs with differential expression and the direction of regulation was extracted. The miRbase database was used to map all miRNAs [35]. Studies that consistently report differentially expressed miRNAs have used vote counting methods to analyse the data, which are considered potential biomarkers by the total number of studies reporting these differences in expression [36, 37]. All included studies failed to report fold changes, as a result the process of identifying meta-signature did not take this criterion into account. This study analysed all 18 studies in which miRNAs found in at least three studies were considered differentially expressed. R, a statistical programming language and environment, was used to conduct the analyses.

19.3 Bioinformatics Analysis

To gain insight into the regulatory roles played by commonly deregulated miRNAs meta-signature in OSCC, we applied bioinformatics approach. Target genes and transcription factors that are controlled by miRNAs were studied in greater depth to learn more about their roles.

19.3.1 Transcription Factors, and Target Genes of DE-miRNAs

For the purpose of predicting the upstream transcription factors of screened DE miRNAs, the programme FunRich, which is primarily utilized for the study of gene and protein networks for enrichment of functions and interactions, was applied [38]. The screened DE-miRNAs were used as input in the software to predict top 10 transcription factors for up-regulated and down-regulated miR. Further, the target genes of screened DE-miRNAs were predicted using the user-friendly miRNet database, a repository to find detailed statistical analysis and explanations of how miRNAs work [39].

19.3.2 Functional and Pathway Enrichment Analysis

Web-based programme DIANA-miRPath v3 [40] was utilized to investigate the biological role and establish molecular pathways model of the dysregulated miRNA meta-signature. Functional analysis of commonly dysregulated miRNAs was done for Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways [41] and Gene

Ontology (GO) biological process terms. Unbiased empirical distributions with a microT threshold of 0.7 and false discovery rate correction were utilized for the enrichment analysis approach. When $p < 0.05$, molecular pathways were considered substantially enriched. The DIANA-miRPath v3.0 tool was used to create heat maps of highly enriched routes.

19.4 Outcome of Meta-Analysis and Bioinformatics Analysis

19.4.1 Overview of the Included Studies

After an initial search, 220 articles from OSCC were located; however, after further examination of their titles and abstracts, 202 of those studies were disregarded due to the fact that their content was deemed to be irrelevant. Because these studies empirically investigated the prognostic significance of microRNA expression signatures as an effective biomarker in oral cancer, there were only 80 studies that were deemed to be relevant. During the full text analysis of these 80 relevant studies, 62 of them were subsequently disqualified because they contained insufficient data and were unable to satisfy the necessary criteria. In the end, an aggregate of 18 studies that were pertinent to this meta-analysis was chosen. The flow chart for the process of selecting studies is depicted in Fig 19.1, while the characteristics of the studies that were chosen for this systematic review and meta-analysis can be seen in Table 19.1.

19.4.2 Differentially Expressed MiRNAs

Lists of microRNAs that have different levels of expression were collected from studies that matched the required inclusion criteria. After standardizing the annotations, 18 studies comparing OSCC tissues to normal tissues identified a total of 428 miRNAs that were differentially expressed. 203 miRNAs were up-regulation, 203 miRNAs were down-regulation, and 22 miRNAs showed irregular regulation among the catalogue of identified microRNAs. It was determined that a miRNA was considered to be commonly deregulated if it was found to be dysregulated in at least three different studies. In our study, a total of 55 different miRNAs were found to have been reported in at least three studies. Among the 55 microRNAs that are expressed differently, expression of 44 miRNAs had a consistent direction, of which twenty were found to have an increase in expression (Table 19.2) while twenty-four were found to have a decrease in expression. (Table 19.3). The eleven miRNAs are regulated in a variety of ways. miR-21-5p, miR-31-5p, and miR-155-5p are constantly up-regulated in different studies. miR-21-5p was investigated in eight different studies, while miR-31-5p and miR-155-5p were each investigated in six

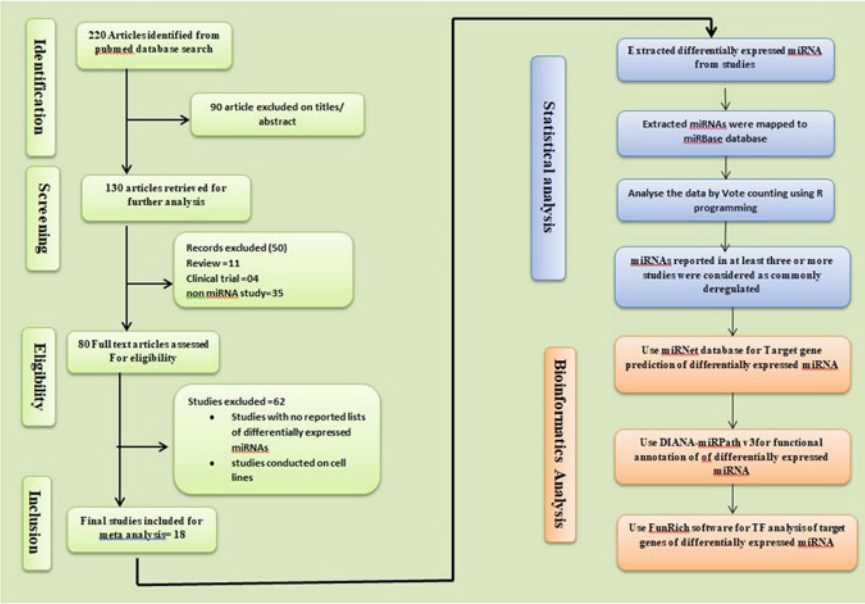

Fig. 19.1 Workflow of study selection process and analysis

different studies. While miR-139-5p, miR-375 miR-99a-5p, miR-486-5p, and miR-30a-3p are constantly down-regulated in different studies. miR-139-5p and miR-375 were found to have their levels reduced in eight different studies, miR-99a-5p, miR-486-5p, and miR-30a-3p were only found to have their levels reduced in five different studies.

19.4.3 Targets Genes and Transcription Factors of DE-miRNAs

It is common knowledge that one miRNA can regulate multiple genes and that multiple miRNAs can exert control over a single gene. These intricate interactions are how miRNAs work together to fine-tune the expression of their targets. To understand the biological system's regulatory interconnection, a network-based visualization techniques can be very helpful. In this study, we used the miRNet database to make predictions about the target mRNAs of candidate DE-miRNAs. According to the findings, a total of 4040 genes were obtained, of which miRNAs studied, 2654 were found to be associated with up-regulation, while 1385 were found to be associated with down-regulation. Then miRNA-target gene network was created which is depicted in Fig. 19.2a, b.

Table 19.1 Characteristics of the studies selected for meta-analysis

S. No.	Study (reference)	Number of tissue samples (cases/control)	Differentially expressed miRNA			miRNA cut-off criteria	Platform	Country
			Total	Up-regulated	Down-regulated			
1	Chang et al. [42]	8(04/04)	09	08	01	FDR = 0	TaqMan rtPCR	Baltimore
2	Latha et al. [43]	05(5/5)	20	16	04	P values < 0.0.05		Houston, Texas
3	Avissa et al. [44]	113(99/14)	12	11	01	Q < 0.001	TaqMan miRNA Assays	Providence, RI
4	Hui Angela et al. [45]	55(51/04)	38	23	15	FDR < 0.3 FC > 2	TaqMan RT-PC R	Toronto, Ontario, Canada
5	Scapoli et al. [46]	15	19	13	06	Delta value 0.78 FDR = 0	qRT-PC R	Italy
6	Nuruls. et al. [47]	12(09/03)	10	07	03	P < 0.05		Taiping, Perak, Malaysia
7	Soga et al. [48]	36(29/7)	23	12	11	p < 0.05	TaqMan low density array (human	Japan, Asia
8	Severino et al. [49]	15(15/15)	62	34	28	p < 0.05	Illumina miRNA arrays version 1.0	Brazil
9	Siow [50]	8(04/04)	19	15	04	FDR < 0.05	Agilent platform Human miRNA microarray	Malaya, Kuala Lumpur, Malaysia;
10	Shiah et al. [51]	80 (40/40	84	33	53	FC > 2 p < 0.05	Human v2 microRNA expression BeadChips (Illumina)	Taiwan, Asia
11	Fukomoto et al. [52]	72 (36/36)	42	NR	42	p < 0.05	TaqMan low density array (Human	Japan, Asia

(continued)

Table 19.1 (continued)

S. No.	Study (reference)	Number of tissue samples (cases/control)	Differentially expressed miRNA			miRNA cut-off criteria	Platform	Country
			Total	Up-regulated	Down-regulated			
12	Ganci et al. [53]	76 (38/38)	78	59	19	FDR < 0.06 p < 0.01 FC > 1	Agilent platform human miRNA microarray	Italy, Europe
13	Shi et al. [54]	04 (2/2)	38	31	7	FC > 2	RNA Seq (Illumina HiSeq 2000)	China, Asia
14	Manikandan et al. [55]	58 (29/29)	39	15	24	SD > 1 FC > 1 p < 0.05	miRCURY LNATM microRNA array (Exiqon)	India, Asia
15	Koshizuka et al. [56]	22 (22/22)	52	NR	52		RNA seq illumina	Chiba, Japan
16	Chen et al. [57]	20 (10/10)	12	7	5	p < 0.05 p < 0.01	TaqMan low density array (TLDA v1.0)	USA
17	Schneider et al. [58]	05 (5/5)	48	23	25	FDR < 0.05	Illumina HiSeq 2500	Poland
18	Cintia Micaela et al. [59]	16	77	35	42	FC ± 2 P values < 0.0.05	Affymetrix miRNA array plate 4.1	Spain

Table 19.2 Consistently reported up-regulated miRNAs ($n = 20$) in profiling studies (oral cancer tissue versus normal)

S. No.	Deregulated miRNAs	Up-regulated: studies (n)	Present in study	Samps(n) a
1	miR-21-5p	8	Latha.2009, Scapoli.2010, Soga.2013, Ganci.2015, Shi.2015, Manikandan.2016, Chen.2017, Schneider.2018	219
2	miR-31-5p	6	Soga.2013, Shiah.2014, Ganci.2015, Manikandan.2016, Chen.2017, Schneider.2018	273
3	miR-155-5p	6	Latha.2009, Ganci.2015, Shi.2015, Siow.2014, Hui Angela. 2010, Chang.2008	223
4	miR-31-3p	5	Soga.2013, Shiah.2014, Ganci.2015, Manikandan.2016, Schneider.2018	255
5	miR-146b-5p	5	Scapoli.2010, Severino.2013, Ganci.2015, Chen.2017, Cintia.2019	142
6	miR-34b-5p	5	Latha.2009, Severino.2013, Shiah.2014, Ganci.2015, Shi.2015	180
7	miR-424-5p	4	Shiah.2014, Ganci.2015, Shi.2015, Schneider.2018	165
8	miR-18a-5p	4	Shiah.2014, Ganci.2015, Shi.2015, Schneider.2018	165
9	miR-21-3p	4	Shiah.2014, Ganci.2015, Shi.2015, Schneider.2018	165
10	miR-455-5p	4	Severino.2013, Shiah.2014, Ganci.2015, Schneider.2018	176
11	miR-142-5p	4	Severino.2013, Shiah.2014, Ganci.2015, Schneider.2018	176
12	miR-20a-5p	3	Ganci.2015, Shi.2015, Schneider.2018	85
13	miR-450a-5p	3	Shiah.2014, Ganci.2015, Shi.2015	160
14	miR-944	3	Shiah.2014, Shi.2015, Schneider.2018	89
15	miR-455-3p	3	Shiah.2014, Ganci.2015, Shi.2015	160
16	miR-7-5p	3	Shiah.2014, Ganci.2015, Schneider.2018	157
17	miR-93-5p	3	Soga.2013, Ganci.2015, Shi.2015	116
18	miR-141-3p	3	Severino.2013, Ganci.2015, Manikandan.2016	149
19	miR-182-5p	3	Severino.2013, Ganci.2015, Shi.2015	95
20	miR-196b-5p	3	Severino.2013, Chen.2017, Schneider.2018	40

FunRich was used to predict transcription factors for candidate differentially expressed miRNAs (DE-miRNAs). Figure 19.3a, b each presents the highest-ranked transcription factors for differentially expressed miRNAs that have experienced an increase in their level of expression. For DE-miRNAs with an up-regulated expression level, the top 10 transcription factors were SPP1, EGR1, POU2F1, SP4, MEF2A,

Table 19.3 Consistently reported down-regulated miRNAs ($n = 24$) in profiling studies (oral cancer tissue versus normal)

S. No.	Deregulated miRNAs	Down-regulated: studies (n)	Present in study	Samples (n) a
1	miR-139-5p	8	Severino.2013, Soga.2013, Shiah.2014, Fukomoto.2015, Chen.2017, Koshizuka.2017, Schneider.2018, Cintia.2019	256
2	miR-375	8	HuiAngela.2010, Shiah.2014, Shi.2015, Fukomoto.2015, Koshizuka.2017, Schneider.2018, Avissa.2015, Siow.2014	316
3	miR-99a-5p	5	Severino.2013, Shiah.2014, Ganci.2015, Koshizuka.2017, Schneider.2018, Cintia.2019	214
4	miR-486-5p	5	Severino.2013, Soga.2013, Shiah.2014, Chen.2017, Koshizuka.2017, Cintia.2019	179
5	miR-30a-3p	5	Severino.2013, Soga.2013, Shiah.2014, Shi.2015, Fukomoto.2015	211
6	miR-204-5p	4	Fukomoto.2015, Chen.2017, Schneider.2018, Cintia.2019	113
7	miR-99a-3p	4	Shi.2015, Koshizuka.2017, Schneider.2018, Cintia.2019	47
8	miR-885-5p	4	Shiah.2014, Shi.2015, Fukomoto.2015, Koshizuka.2017	178
9	miR-376c-3p	4	Soga.2013, Shiah.2014, Ganci.2015, Fukomoto.2015	264
10	miR-30a-5p	4	Severino.2013, Shiah.2014, Ganci.2015, Shi.2015	176
11	miR-125b-5p	4	Latha.2009, HuiAngela.2010, Fukomoto.2015, Schneider.2018	137
12	miR-126-3p	3	Fukomoto.2015, Manikandan.2016, Koshizuka.2017	152
13	miR-29c-5p	3	Shiah.2014, Fukomoto.2015, Koshizuka.2017	174
14	miR-486-3p	3	Shiah.2014, Chen.2017, Cintia.2019	116
15	miR-411-5p	3	Soga.2013, Shiah.2014, Fukomoto.2015	188
16	miR-133a-3p	3	Soga.2013, Shiah.2014, Ganci.2015	116
17	miR-328-3p	3	Severino.2013, Shiah.2014, Chen.2017	115
18	miR-154-5p	3	Severino.2013, Shiah.2014, Koshizuka.2017	117
19	miR-140-5p	3	Severino.2013, Fukomoto.2015, Koshizuka.2017	109
20	miR-376a-3p	3	Severino.2013, Ganci.2015, Fukomoto.2015	163

(continued)

Table 19.3 (continued)

S. No.	Deregulated miRNAs	Down-regulated: studies (*n*)	Present in study	Samples (*n*) a
21	miR-378a-5p	3	Severino.2013, Shiah.2014, Fukomoto.2015	163
22	miR-26a-5p	3	HuiAngela.2010, Fukomoto.2015, Manikandan.2016	115
23	miR-199b-5p	3	HuiAngela.2010, Koshizuka.2017, Cintia.2019	185
24	let-7c	3	HuiAngela.2010, Shiah.2014, Koshizuka.2017	155

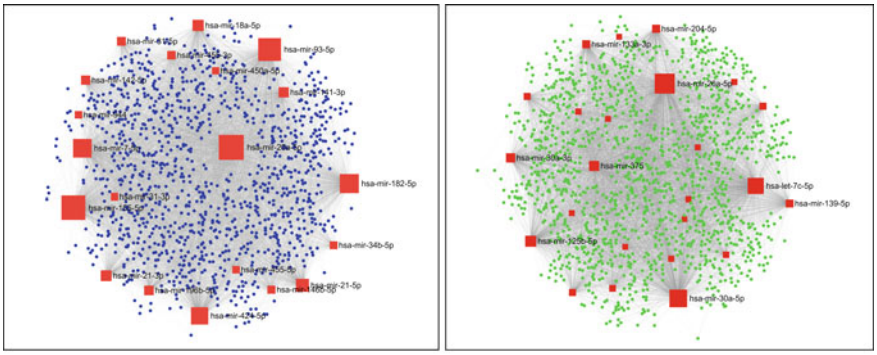

Fig. 19.2 Target genes of miRNAs meta-signature. **a** Down-regulated miRNAs-target genes network; **b** up-regulated miRNAs-target genes network; red square node in both figure is miRNA Target gene are in circular node in green and blue are target gene of down-regulated and up-regulated miRNaA, respectively

FOXA1, NKX6-1, SOX1, ZFP161, and RORA. When looking at DE-miRNAs that were down-regulated, the top ten transcription factors were EGR1, SPP1, SP4, MEF2A, POU2F1, RORA, NFIC, FOXA1, RREB1, and ZFP161.

19.4.4 Functional and Pathway Enrichment Analysis

The ten miRNAs with the greatest fold change in expression were used to perform functional and pathway enrichment analyses, and a P 0.05 cut-off was used. Eleven pathways were significantly enriched in the OSCC miRNA meta-signature, lysine degradation, Proteoglycans in cancer, Hippo signalling pathway,

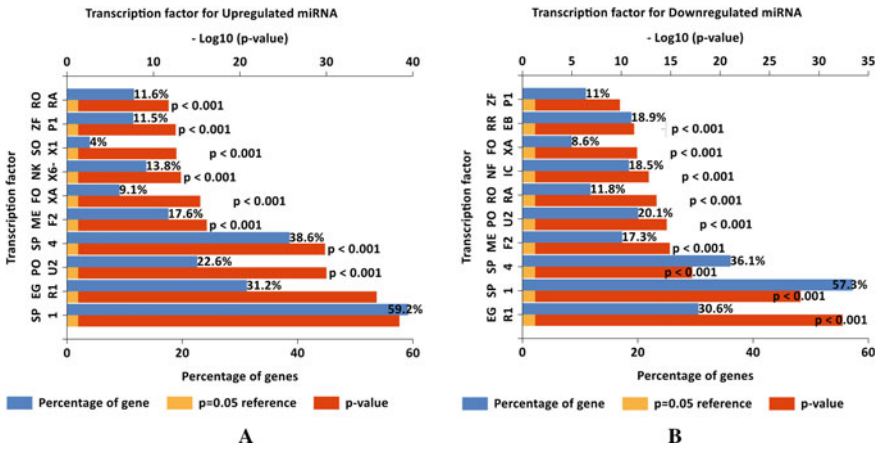

Fig. 19.3 Transcription factors of miRNAs meta-signature. **a** Transcription factors of up-regulated miRNAs; **b** transcription factors of down-regulated miRNAs

FoxO signalling pathway, p53 signalling pathway, Viral carcinogenesis, and Pathways in cancer (Table 19.4). Figure 19.4 depicts the heat maps of the KEGG pathways that are enriched in both the up and down miRNA meta-signature of OSCC. The most enriched GO processes regulated by the deregulated OSCC miRNAs include cell motility, neurotrophin TRK receptor signalling pathway, RNA binding, fibroblast growth factor receptor signalling pathway, enzyme regulator activity, phosphatidylinositol-mediated signalling and intrinsic apoptotic signalling pathway (Table 19.5).

19.5 Discussion

OSCC has a high mortality rate despite being a relatively common cancer. Five-year survival rates for patients with advanced malignancies are below 15%, which is significantly lower than the 90% survival rate for patients with early-stage of OSCC. Despite advancements in surgery and chemo-radiation methods for OSCC, only modest improvements in survival rates have been achieved in recent eras, emphasizing the critical requirement for more efficacious therapeutic options, particularly for recurrent and metastatic cancers. Unfortunately, no reliable method for detecting OSCC in its earliest stages is currently available, which leads to late-stage diagnosis. In this light, microRNAs have been shown to perform an essential regulatory role in the process of carcinogenesis and to have the potential to act as biomarkers for novel treatments of OSCC. As a result, researchers are focusing more on highly reliable biomarkers for the diagnosis of OSCC at an early stage, miRNAs have been found, ushering in a new era in cancer diagnosis. We investigated the present state of knowledge about miRNAs and OSCC in order to devise an approach for

Table 19.4 List of KEGG pathways enriched in oral cancer miRNA meta signature

S. No.	KEGG pathway	p-value	genes	miRNAs	
	Up-regulated miRNA				
1	Lysine degradation	1.90E−14	30	11	hsa-miR-21-5p, hsa-miR-31-5p, hsa-miR-146b-5p, hsa-miR-424-5p, hsa-miR-18a-5p, hsa-miR-21-3p, hsa-miR-20a-5p, hsa-miR-7-5p, hsa-miR-93-5p, hsa-miR-141-3p, hsa-miR-196b-5p
2	Proteoglycans in cancer	<1e−325	116	10	hsa-miR-21-5p, hsa-miR-155-5p, hsa-miR-424-5p, hsa-miR-21-3p, hsa-miR-20a-5p, hsa-miR-7-5p, hsa-miR-93-5p, hsa-miR-141-3p, hsa-miR-182-5p, hsa-miR-196b-5p
3	Hepatitis B	1.55E−15	90	10	hsa-miR-21-5p, hsa-miR-31-5p, hsa-miR-155-5p, hsa-miR-424-5p, hsa-miR-20a-5p, hsa-miR-7-5p, hsa-miR-93-5p, hsa-miR-141-3p, hsa-miR-182-5p, hsa-miR-196b-5p
4	Hippo signalling pathway	8.88E−15	79	10	hsa-miR-21-5p, hsa-miR-424-5p, hsa-miR-18a-5p, hsa-miR-455-5p, hsa-miR-20a-5p, hsa-miR-455-3p, hsa-miR-7-5p, hsa-miR-93-5p, hsa-miR-182-5p, hsa-miR-196b-5p

(continued)

Table 19.4 (continued)

S. No.	KEGG pathway	p-value	genes	miRNAs	
5	FoxO signalling pathway	1.03E−12	86	10	hsa-miR-21-5p, hsa-miR-155-5p, hsa-miR-424-5p, hsa-miR-20a-5p, hsa-miR-944, hsa-miR-7-5p, hsa-miR-93-5p, hsa-miR-141-3p, hsa-miR-182-5p, hsa-miR-196b-5p
6	p53 signalling pathway	1.24E−12	48	10	hsa-miR-21-5p, hsa-miR-424-5p, hsa-miR-18a-5p, hsa-miR-142-5p, hsa-miR-20a-5p, hsa-miR-944 hsa-miR-93-5p, hsa-miR-141-3p, hsa-miR-182-5p, hsa-miR-196b-5p
7	Chronic myeloid leukaemia	2.01E−11	53	9	hsa-miR-155-5p, hsa-miR-424-5p, hsa-miR-455-5p, hsa-miR-20a-5p, hsa-miR-7-5p, hsa-miR-93-5p hsa-miR-141-3p, hsa-miR-182-5p, hsa-miR-196b-5p
8	Glioma	2.46E−11	41	9	hsa-miR-155-5p, hsa-miR-424-5p, hsa-miR-142-5p, hsa-miR-20a-5p, hsa-miR-7-5p, hsa-miR-93-5p, hsa-miR-141-3p, hsa-miR-182-5p, hsa-miR-196b-5p
9	Viral carcinogenesis	7.77E−16	96	8	hsa-miR-18a-5p, hsa-miR-21-3p, hsa-miR-142-5p, hsa-miR-20a-5p, hsa-miR-7-5p, hsa-miR-93-5p hsa-miR-141-3p, hsa-miR-182-5p

(continued)

Table 19.4 (continued)

S. No.	KEGG pathway	p-value	genes	miRNAs	
10	Adherens junction	7.34E−14	48	8	hsa-miR-424-5p, hsa-miR-21-3p, hsa-miR-20a-5p, hsa-miR-455-3p, hsa-miR-7-5p, hsa-miR-141-3p, hsa-mi R-182-5p, hsa-miR-196b-5p
11	Pathways in cancer	2.78E–13	203	8	hsa-miR-21-5p, hsa-miR-424-5p, hsa-miR-18a-5p, hsa-miR-20a-5p, hsa-miR-7-5p, hsa-miR-93-5p hsa-miR-141-3p, hsa-miR-182-5p
12	Cell cycle	7.20E−11	78	8	hsa-miR-21-5p, hsa-miR-424-5p, hsa-miR-18a-5p, hsa-miR-142-5p, hsa-miR-20a-5p, hsa-miR-93-5p, hsa-miR-182-5p, hsa-miR-196b-5p
13	Prostate cancer	4.24E−09	61	8	hsa-miR-155-5p, hsa-miR-424-5p, hsa-miR-142-5p, hsa-miR-20a-5p, hsa-miR-7-5p, hsa-miR-93-5p hsa-miR-141-3p, hsa-miR-182-5p
	Down-regulated miRNA				
14	Proteoglycans in cancer	9.07E−13	106	10	hsa-miR-375, hsa-miR-99a-5p, hsa-miR-30a-3p, hsa-miR-30a-5p, hsa-miR-125b-5p, hsa-miR-126-3p, hsa-miR-411-5p, hsa-miR-140-5p, hsa-miR-26a-5p, has- miR-199b-5p

(continued)

Table 19.4 (continued)

S. No.	KEGG pathway	p-value	genes	miRNAs	
15	Hippo signalling pathway	3.33E−16	73	9	hsa-miR-139-5p, hsa-miR-375, hsa-miR-30a-3p, hsa-miR-99a-3p, hsa-miR-30a-5p, hsa-miR-125b-5p, hsa-miR-140-5p, hsa-miR-26a-5p, hsa-miR-199b-5p
16	Lysine degradation	1.29E−09	23	6	hsa-miR-375, hsa-miR-30a-3p, hsa-miR-30a-5p, hsa-miR-125b-5p, hsa-miR-486-3p, hsa-miR-26a-5p
17	Viral carcinogenesis	8.83E−05	80	6	hsa-miR-375, hsa-miR-99a-5p, hsa-miR-30a-3p, hsa-miR-30a-5p, hsa-miR-126-3p, hsa-miR-26a-5p
18	Pathways in cancer	0.00042	139	6	hsa-miR-99a-5p, hsa-miR-30a-3p, hsa-miR-30a-5p, hsa-miR-126-3p, hsa-miR-411-5p, hsa-miR-26a- 5p
29	p53 signalling pathway	0.007168	37	6	hsa-miR-375, hsa-miR-99a-5p, hsa-miR-30a-3p, hsa-miR-30a-5p, hsa-miR-140-5p, hsa-miR-26a-5p

discovering potential novel biomarkers for prognostic and therapeutic purposes. The inability to examine raw miRNA expression data sets using such a rigorous approach is sometimes hampered by a lack of raw data. Moreover, variations in the number of miRNAs known at different times, as well as the technical platform employed in each investigation, make combining raw data sets more complex. Furthermore, biological results have been inconsistent due to noise in microarray data and a small sample size. As a result, to get over the limitations indicated above, we directly analysed miRNA data sets from published research. As a result, the need for meta-analyses to combine the findings of multiple studies is justified. In this paper, we performed a thorough bioinformatics analysis of eighteen separate profiling experiments to discover cancer-specific miRNAs, followed by vote counting method, method which is a way of merging differentially expressed miRNA lists from several studies. We

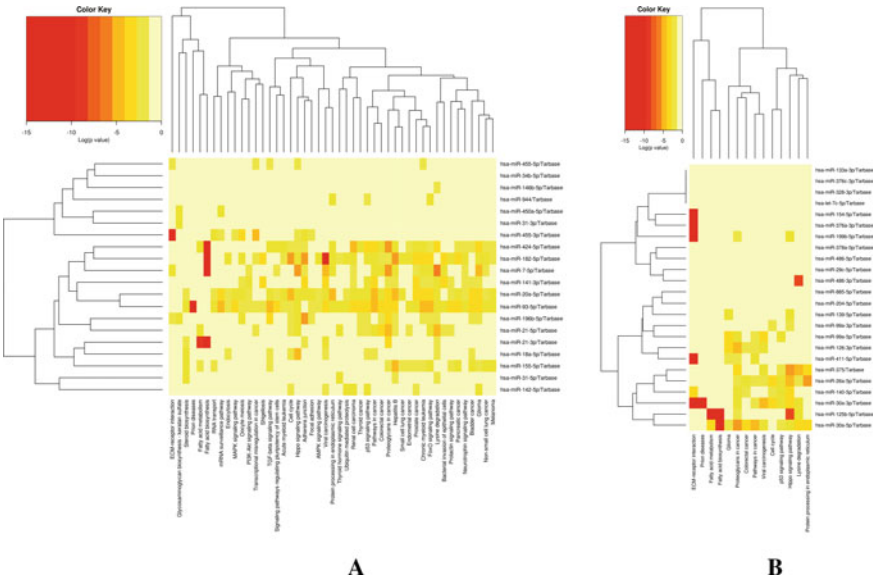

Fig. 19.4 Heat map of KEGG pathways enriched in the dysregulated miRNAs created by DIANA-miRPath v3.0. **a** Up-regulate, **b** down-regulate. The intensity of colour represents the FDR-corrected *p* value. microT-CDS (v5.0) was used for target prediction, *p* value threshold 0.05, microT threshold 0.7. FDR, false discovery rate

identified 44 miRNAs for which the direction of differential regulation was consistent in at least three investigations among the 18 profiling studies we meta-analysed, as well as eleven miRNAs for which the differential regulation was inconsistent. These findings may aid researchers in focusing future research on miRNAs that are more likely to have an effect on the primary stages of disease and/or the development of disease. Different tissue samples, genetic and environmental backgrounds of tissue donors, clinico-pathological characteristics of tissue donors, and expression profiling platforms are all potential causes of inconsistent profile outcomes among research.

The findings of the research on deregulated miRNAs that were included in this study are inconsistent. Low statistical power, difficulties in collecting and storing samples, patient demographics and clinical history, data normalization and analysis, and a lack of consistency in the use of cut-off values for identifying differentially expressed miRNAs are all factors that can contribute to this inconsistency. These observed variations among research could be due to differences in the platforms employed, as well as the types and quantities of probes used. Inconsistency could be caused by molecular alterations associated with carcinogenesis in morphologically normal tissue, including intra-tumour heterogeneity of miRNA expression [60].

Our results revealed that a total of 44 miRNAs, all going in the same direction, have been reported in at least three separate studies, of which 20 miRNAs are up-regulated in OSCC compared with control samples, whereas 24 miRNAs are commonly down-regulated. The microRNAs that we looked at, miR-21-5p showed

Table 19.5 Top ten enriched gene ontology terms identified by functional analysis of the miRNAs in the meta-analysis

GO Category	p-value	genes	miRNAs
Up-regulated miRNA			
Cell motility	0.00018243	87	2
Neurotrophin TRK receptor signalling pathway	5.12E−25	166	15
RNA binding	2.73E−35	998	18
Organelle	6.04E−190	4950	20
Cell death	2.27E−15	452	14
Poly(A) RNA binding	2.39E−18	798	17
Cellular protein modification process	2.35E−57	1216	19
Down-regulated miRNA			
Fibroblast growth factor receptor signalling pathway	4.77E−15	76	9
Enzyme regulator activity	2.77E−13	246	9
Phosphatidylinositol-mediated signalling	3.79E−12	54	9
Intrinsic apoptotic signalling pathway	6.97E−12	37	8
Transcription initiation from RNA polymerase II promoter	1.43E−10	79	6
Immune system process	2.84E−10	382	10
Extracellular matrix organization	3.44E−10	90	6
Platelet activation	2.53E−09	72	8
Extracellular matrix disassembly	2.38E−08	39	7
G2/M transition of mitotic cell cycle	2.51E−08	59	6

constant up-regulation in eight studies. Mir-21 was shown to be up-regulated, which was not surprising, as mir-21 is regarded as an oncomir and hence highly expressed in a variety of cancer types [61]. Mir-21 may serve as a diagnostic and prognostic biomarker for tongue cancer [62]. In recent years, miR-21 has been investigated more frequently as a predictive marker for HNSCC than any other miRNA. In tongue cancer, up-regulation of miR-21 was linked to lymph node metastases, a poorly differentiated tumour, and a late-stage clinical presentation [63]. In SCC-15 cells, miR-21 has been shown to control proliferation by targeting the expression of TNF-α, without having any effect on the cellular apoptosis pathway [64]. miR-155-5p is associated with an effect that is carcinogenic. The gene known as AT-rich interactive domain 2 (ARID2) is a target of miR-155 5p, and research has shown that a lack of ARID2 expression in OSCC patients is significantly correlated with poor survival. ARID2 is a potential biomarker for the identification and management of OSCC with a more targeted approach [65]. miR-31-5p increased the risk of oral squamous cell carcinoma cells while also being significantly up-regulated in oral cancer. Both phosphatase and tensin homolog (PTEN) and p-AKT are target genes of the microRNA miR-31-5p, and the expression of PTEN is increased while p-AKT expression is decreased [66]. OSCC has a significantly increased expression of miR31

in plasma, saliva, and tumour tissue, which contributes to the disease's malignant phenotypes. By binding to its target proteins, miR-31 promotes OSCC by interacting with multiple signalling pathways [67]. Mir-31 up-regulation has been recommended as a biomarker for metastatic oral cancer [68]. miR-146b potentially regulates the spread, movement, and invasion of OSCC cells through binding and down-regulating HMG-Box Transcription Factor 1 (HBP1) expression in OSCC cells [69]. In Tca8113 cells, the microRNA miR-139 can regulate the AKT signalling pathway, which results in the induction of apoptosis. This could result in a more effective method for treating oral cancer [70]. By directly targeting HOXA9, miR-139-5p acts as a suppressor in the carcinogenesis process, inhibiting OSCC cell motility [71, 72].

As a tumour suppressor in most tumour cells, MiR-375 binds to its target genes to control tumour cell proliferation, apoptosis and migration, and invasion. MiR-375 also inhibits tumour cell growth by regulating Epithelial-To-Mesenchymal Transition (EMT)s target genes [73]. In OSCC cells, miR-375 was the most frequently down-regulated miRNA. Down-regulation of miR-375 was linked to a increase in lymph node metastases and decrease OSCC patient survival. Up-regulation of miR-375 in OSCC cells was found to reduce proliferation, cause cell cycle arrest in the G0/G1 phase, promote apoptosis, and increase radio sensitivity, suggesting that miR-375 could be a possible drug target for OSCC patients [74]. When comparing oral cancer cell lines to normal oral keratinocytes, miR-99a another frequently down-regulated miRNAs. By directly interacting with mTOR mRNA, miR-99 has been shown to lower mTOR expression, increase cancer cell proliferation and tumour size [75]. Down-regulated miR-99a was able to inhibit the spreading of oral cancer cells by decreasing the expression of Myotubularin-related protein 3 (MTMR3). It has been suggested that MTMR3 could be used as a therapeutic intervention in the management of oral cancer [76]. Expression of miR-99a-5p in OSCC tissues and cell lines (CAL-27 and TCA-8113) was lower than in controls and correlated with lymph node involvement and clinical staging. OSCC tissues and cell lines exhibited a down-regulation of miR-99a-5p, and overexpression suppressed proliferation, migration, and invasion by targeting isoprenyl cysteine carboxylmethyl transferase (ICMT) [77]. The study also looked at the role of other miRNAs that are part of meta-signature networks. The expression of miR-125b may help to reduce cell proliferation and overcome radio-resistance in OSCC, which could aid in the development of a cure. Furthermore, miR-125b was found to be associated with OSCC survival and radiation response, indicating the possibility that it could be used as a diagnostic marker [78]. PRXL2A protects cells from oxidative stress. PRXL2A is a direct target of miR-125b. Down-regulation of miR-125b protects OSCC tumour cells from oxidative stress [79]. The levels of familial adenomatous polyposis (FAP) were reduced as a result of the down-regulation of the miR-30a-5p gene, which led to a suppression of cell proliferation in OSCC [80]. Apoptosis was induced in TSCCA and SSC-9 cells through the inhibition of BICC1 expression by the microRNA miR-199b-5p [81]. OSCC cell cycle and apoptosis are regulated by miR-376c-3p's suppression of homeobox B7 (HOXB7). SCC-25 cells were also subjected to G1/G0 arrest and apoptosis as a result of its involvement [82]. miR-196b over expressed in OSCC than the adjacent normal tissue samples. According to researchers, epigenetic control of

196b expression plays a critical role in modulating the movement and invasion of cells during OSCC progression [83].

By targeting and reducing the expression of Huntingtin-interacting protein 1(HIP1), miR-204-5p was able to reduce oral cancer cell proliferation, survival, and migration [84]. Researchers discovered that miR-133a-3p controlled COL1A1 expression levels, thereby reducing cell proliferation in a number of different oral cancer cell lines [85]. miR-204-5p enhanced OSCC cell growth and metastasis. miR-204-5p, which was predicted to regulate CXCR4 in OSCC, was found to have an inverse relationship with CXCR4 expression in OSCC [86]. Similar to how down-regulation of miR-376c-3p boosts metastatic spread to lymph nodes in OSCC, it also directly regulates genes for the runt-related transcription factor 2 (RUNX2) [87]. Down-regulated miR486-3p enhance tumour-promoting activity of Discoidin domain receptor-1 (DDR1) tyrosine kinase which is involved in various steps of tumorigenesis in oral cancer [88].

The current investigation identified numerous intriguing miRNAs that have repeatedly been reported deregulated. Their possible targets may shed light on the functions of microRNAs in carcinogenesis and the mechanisms that underpin it. The functional contributions of miRNAs in the development and progression of malignancies have led to the development of cutting-edge diagnostic methods. MiRNAs demonstrated a high level of diagnostic accuracy when comparing patients with healthy humans. Studies on individual miRNA typically use relatively small sample sizes. However, with the help of meta-analysis, we were able to incorporate all the potentially relevant data into a single statistical analysis which is one of the advantages of this study as it increases the sample size, and consequently, the strengths of the study. The combined effect size of all selected miRNA expression and prognosis studies could be applied to future studies. As evidenced by their assessment quality scores, most included studies had acceptable methodological quality. The limitations of the research on miRNA expression profiles are highlighted in our meta-analysis. Firstly, the studies that were included used a range of microarray platforms and validation criteria, implying that more methodological standardization is needed for maximal reliability. Secondly, there was a lot of diversity in the meta-analysis. The following are some possible causes of heterogeneity: The experimental group's age, sex, number of lesions, capsular invasion, lymph node metastases, and stage of papillary thyroid cancer were all different; study design, detection methods, and reagent selection were all inconsistent; and the cut-off values utilized were all different. The significant drawback of this was research that, only a handful of studies were considered in the meta-analysis. The clinical applicability of the analysis is often limited by a small number of studies. A future cohort study should take this into account. Benefits of microRNAs when considering them as diagnostic indicators for OSCC are MiRNAs are easily accessible and stable biomarkers that do not require any invasive procedures to measure their level of expression. In a study researchers found that cancer-causing microRNAs resides in human plasma in a extremely reliable state, and RNase-resistant microRNAs (miRNAs) are produced from tumour-derived microvesicles or exosomes and then used to study a wide range of clinical samples [89]. Researchers has also discovered that miRNAs are abundant in human

serum/plasma/tissue and can be identified using qRT-PCR, making it ideal for use as noninvasive biomarkers in disease surveillance [90].

19.6 Conclusions

In conclusion, our comprehensive analysis of miRNA expression profiling studies identified a set of deregulated microRNAs in OSCC especially miR-21-5p, miR-31-5p, miR-155-5p, miR-31-3p miR-146b-5p were most consistently reported up-regulated miRNAs and miR-139-5p, miR-375, miR-99a-5p, miR-486-5p and miR-30a-3p were most consistently reported down-regulated miRNAs. Some of these microRNAs may serves as potential biomarkers for oral cancer. MicroRNAs have been linked to a variety of OSCC related pathways, and the target genes of these microRNAs form complex networks which highlights their significance in OSCC pathogenesis. When trying to ascertain the practical relevance of these findings and their contribution in the progression of oral cancer, further mechanistic studies as well as external validation studies are required. The findings that microRNAs extracted from different expression profiles have a high diagnostic and therapeutic accuracy for OSCC was a significant. This discovery paved the way for new strategies in cancer research and shed light on previously unknown pathological processes underpinning oncogenic transformation. The approach presented in the chapter has the potential to hasten the identification of novel miRNA signatures for the diagnosis and treatment of cancer, and it should be applicable to the investigation of other diseases as well.

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Chapter 20
Application of Magnetic Nanoparticles in Cancer: Drug Delivery and Therapy

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Abstract Recent years have presented a marked progress in terms of cancer treatment and oncology. In general, nanoparticles have been extensively used in a number of applications, but magnetic behaviors of MNPs make them most promising and contrasting agent to be used as a contrasting agent in magnetic resonance imaging and other hypothermia technologies. Their properties are fully exploited when they are used as an active agent in targeted drug delivery to desired location by applying magnetic field from external side. Earlier diagnosis can be made by magnetic resonance imaging or combination of individual treatment with MRI in order to achieve specific definition and appropriate treatment regimen. The present chapter is focused on the magnetic nanoparticles and their use in targeted drug delivery via active or passive mechanism and, lastly, the therapeutic advantages during treatment. And lastly, the challenges and future prospective in nanotechnology field are also discussed in detail.

20.1 Introduction

Metal-based nanoparticles have proved to be a doorway regarding future of medicine. Although, there exists very little knowledge about the safety and use of these nanoparticles in medicine, but at the same time, these nanoparticles have found their key role in a number of biomedical applications, as for example, in vivo site specific imaging, detection and therapy of cancer, neurodegenerative disorders treatment, human immune deficiency virus or AIDS therapy, and lastly during treatment regimen of various respiratory disorders, and on the basis of their unique properties, these are considered to be active agents as next generation drug carriers [1]. The characteristics such as material, shape, and surface coating attached functional groups determine that whether a nanoparticle is suitable for a specific application or not. Various types of nanoparticles can be utilized in specific manners. However, the diameter and other modifications of magnetic nanoparticles are helpful in determining the effect of manipulating these particles in applications [2]. As for example, size of nanoparticles determines that whether these nanoparticles can pass through the barriers, as for example, blood vessels and membranes, etc. Similarly, the modification of nanoparticle determines biodegradability or biocompatibility along with half-life time of these nanoparticles, thus determining efficiency of drug molecule and family [3]. Targeted approach is either achieved by use of magnetic materials themselves or attaching such molecules or functional groups that are specific to a disease area such as cancer or others. The drug molecules are located on surface of particles encoded with shell and are modified with other molecular arms at same time. The most extensively used magnetic nanoparticles employed in various applications include magnetite and maghemite that have extensive application due to supermagnetic property. During the process of fabrication, one or more forms or iron oxide are formed. So it is very important to control the experimental conditions in order to obtain a single phase of iron oxide [4]. The research on magnetic nanoparticles carrying drugs is very promising candidates. Although the pre-clinical trials are easy to done, but

still, a huge amount of research is needed for observing out the metabolism and toxicity of magnetic nanoparticles for long-term usage. New materials are continuously developing for adding value to this already developed field. These particles are found to have a large number of applications in diagnosis, detection, and treatment of illness such as cancer, cardio vascular, and neurological disorders [5]. Thus, these magnetic nanoparticles are playing their major roles in meeting the needs of tomorrow. The recent trends of using these magnetic nanoparticles in targeted drug delivery and involvement in treatment of cancer have been summarized in this chapter in a better way, thus proving the compatibility of these magnetic nanoparticles with the human system. Tumor imaging technology for cancer has opened the possibility of earlier detection of disease [6]. These modalities include magnetic resonance imaging MRI, computed tomography CT scanning, and near infrared imaging technology. Among all of them, MRI has strongest influence in earlier detection of cancer. Till now, iron oxide nanoparticles have been in used along with MRI; as for example, ferumoxil has the power to increase imaging of bowl disease. Because of smaller size and larger surface area, they are able to reach at site of lesion accurately. Hence, these magnetic nanoparticles are most unimportant candidates, and its applications cannot be ignored as a targeted agent for drug delivery [7]. The property is fully explored when they are employed as drug delivering molecule, thus using external magnetic field enables the localized movement of drugs to a targeted site. They are utilized as drug carriers by binding of antibodies and other chemotherapeutic agents. In the field of cancer therapy, they are used in several ways including chemotherapy, magnetic hyperthermia MHT, photodynamic therapy PDT, and photothermal therapy PTT as given in Fig. 20.1. The current chapter signifies the use of magnetic nanoparticles in medicines such as drug delivery, cancer diagnosis, and therapy. Moreover, nontoxicity has been explored as the most common challenge and further opportunities are predicted [8].

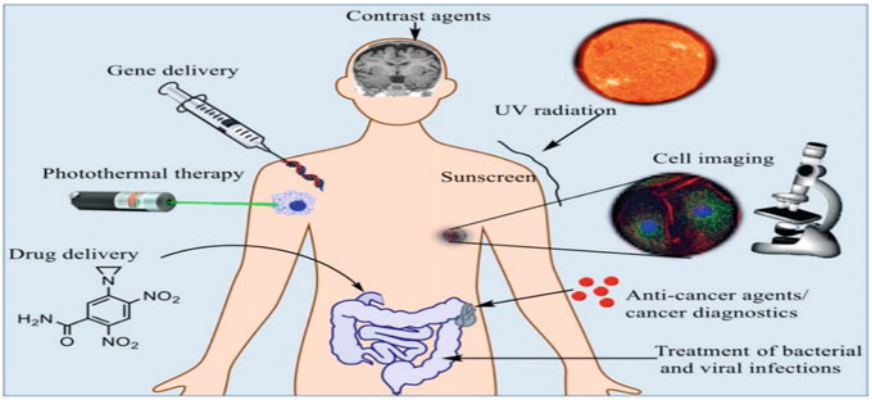

Fig. 20.1 Applications of magnetic nanoparticles in imaging, drug delivery, and cancer therapy

20.2 Overall Synthesis and Characterization of MNPs

Magnetic nanoparticles are usually synthesized from metals such as nickel, cobalt, and gadolinium, but the iron oxide is of special attention in cancer theranostics because of the lesser toxicity and much more contrasting characteristics in imaging techniques as in MRI. These particles are usually synthesized by keeping their shell made of any magnetic material, while their core is coated additionally [9]. The external coating and functionalization increase colloidal properties of nanoparticles, thus causing binding of the therapeutic material easily via covalent bonding providing targeted moieties and playing an important part in tuning the magnetic properties of nanoparticles as in pharma kinetics and pharmacogenomics of a specific agent, cytotoxicity and clearance from system, absorption of a specific protein on surface, and last but not the least release or persistence of drug molecule [10]. Traditionally, magnetic nanoparticles are synthesized by co-precipitation of salt in the presence of any stabilizing agent in addition to hydro or solvo-thermal techniques followed by reverse microemulsion or thermal decomposition [11]. Recently, the trend has been shifted toward the biological synthesis of magnetic nanoparticles or in other words microfluidic preparation. The microfluidic system uses a number of different materials such as glass, silicon, and ceramics in order to form special channels resulting in the development of a proper system for magnetic nanoparticle synthesis. Of all the microfluidic materials that provide certain advantages over all other which include that the process can be monitored easily without any difficulty and it can be made automated eventually [12]. Moreover, reaction time can be increased to a certain time conveniently, and other parameters like temperature and concentration can be controlled according to size, shape, and homogeneity of mixture. As for synthesis of gold and iron oxide nanoparticles without using any surfactants, it has been developed from latex-based microfluidic machine in the absence of organic solvent and other heat treatments. This techniques give the nanoparticles where iron and gold oxide nanoparticles are mixed in such a way that they can give a monodispersed nanoparticles with the core made up of 10 nm iron oxide while they are decorated with 2 nm gold nanoparticles [13]. Additionally for enhancing the doping properties, the zinc metal is doped with iron oxide nanoparticles, thus giving magnetic properties for MRI. The researcher showed a controlled concentration of zinc in iron oxide particles with size smaller than that of 2 nm and enhanced saturated magnetization. Chemical synthesis provides more production, while biogenic synthesis provides the disadvantage of lower yield than normal.

Size, shape, and charge can be tuned for varied applications as in size-dependent hyperthermia treatment. Final charge on nanoparticles aids them in binding to the nucleic acids or protein and to increase time of circulation in blood [14]. Spherical shaped nanoparticles can be produced and have been used mostly in treatment regimen, and further, research is needed for using other shaped nanoparticles in science and technology, i.e., nanorods, etc., in the terms of drug delivery and guided chemotherapy. In the clinical trials, the tumors are evaded by chemotherapy, radiotherapy, and radio dynamical therapy. All of them are helpful in release of

immunostimulatory agents or molecules, thus increasing the immunogenicity of tumors. Immunotherapy can also enhance the strength of immune response [14]. Monotherapies are often involved in removing the tumors completely because of tumor resistance cell population. The long-term use of the chemotherapy agent can further lead to resistance condition. Following radiotherapy in hypoxic tumor tissues, this can cause lesser production of reactive oxygen species which ultimately prevent DNA damage in turn. Thus, the procedure of immunotherapy is effective only in specific patients or a group of patients. Hence in order to bring these therapeutics to tumor area, these nanoparticles have come forward. They act as a transporter molecules or cargo molecules with a number of drugs formulations in combined form to provide combinational and targeted therapy at same time [15].

20.3 Toxicity, Distribution, and Pharmacokinetics of MNPs

These three properties of nanoparticles are of prime importance when considering use of them in human system such as biological distribution, cytotoxicity, and mode of action of drugs. The hydrodynamical size and interaction of nanoparticles with RES are the potent parameters while considering pharmacokinetic and pharmacodynamics inside body of an individual. Size plays an important part because the smaller nanoparticles are easy to pass and exert through renal canal as that of larger ones [16]. The larger ions are taken up by either liver or spleen in order to perform their degradation before their passing to normal excretion route. In the development of the cytotoxicity of these particles, size, surface charge, surface modification, and attached functional groups all contribute to the behavior in vivo. As ion this case, the nanoparticles larger than 200 nm are always captured by spleen and liver while that of smaller than 10 nm are always filtered through renal excretion eventually [17]. Another thing the nanoparticles with no charge or neutral charges have more time of persistence or circulation in system as that of those with either positive or negative charges. Similarly, attachment of functional groups or surface coating also has key role in development of overall behavior of nanoparticle in animals. As for example, polyethylene glycol modified nanoparticles with size of 70 nm have more larger time of circulation than others comprising about 12 h. Apart from these, other factors are also present as their route of synthesis, purity, charge on surface, thus effecting also biodistribution and pharmacokinetics [18]. The toxicity can be exhibited by various mechanisms or phenomenon including Fenton reaction leading to production of reactive oxygen species ROS, direct generation of reactive oxygen species, changes in activities of mitochondria and other organelles by alternation in the cellular signaling. And hence, it is important to determine toxicity of these cells before their use in cancer therapy. As uncoated or dextran coated nanoparticles may cause death of nanoparticles in laboratory conditions that is due to generation of the reactive oxygen species along with SPOINs [19].

20.4 Evolvement of Tumors and Their Distribution

The driving forces in development of carcinogenic are either mutation or clonal selection. The accumulation of mutation in proto-oncogenes and tumor suppressor genes can lead to uncontrollable division of cells or cancerous condition. Some of the mutation associated to it are recognized by the immune system as a foreign agent and are termed as bob self hence are cleared from the body as the result of immunosurveillance [20]. Cells which express only lower amount of antigens are not selected and thus removed. Thus, selective forces are applied only on the tumor cells, thus de-selecting those cells which are less immunogenic. The evasion of the tumors from immune system is usually done by mechanisms as down regulation of MHC1 expression, development of resistance to cytotoxic cells, and lastly the release of immunosuppressive molecules [21].

20.5 Drug Delivery

These nanoparticles are helpful in the improvement of therapeutic properties of a drug and thus reducing side effects caused by conventionally available treatment or drug molecules. These NPs with stabilizing shells have been employed as a contrasting agent in magnetic resonance imaging MRI. On the basis of early diagnosis, the treatment can be started at same time and affectivity can also be improved in similar manner [22]. Hence, they are very essential for drug delivery via bonding of antibodies, chemotherapeutics, or other drugs acting as carrier molecules detection of biomarkers, but at the same time, they can also use to treat cancer because of ability to accumulate to a higher level in cancer cells. As Wang et al. have reported that anti-alpha subunit of adenosine synthase antibody termed as HAI-178 monoclonal antibody has been successfully used for target-based imaging and in vivo imaging of gastric cancer. As in case of human breast cancer, Shanehsazzadeh has shown disappointing results depicting lower accumulation of nanoprobes at targeted site during conjugation of smaller SPOINs to that of C595 monoclonal antibodies. Similarly, a study by Rasaneh and Dadras has also suggested that combination of magnetic nanoparticles with permanent magnet has also resulted in increased efficiency of Herceptin in order to provide phenomenon of enhanced accumulation at cancer target site [23]. For improving phenomenon of therapeutic efficacy, the combination of therapeutic drug molecules and antibodies is greatly increasing attention of scientists. Aries and his colleagues have presented iron oxide nanoparticles being functionalized with CD44 antibody and other gemcitabine derivative, thus providing target-based treatment for efficient treatment of CD44 positive cancer cells. Similarly, Huang et al. have also reported an overlain cell dual targeted therapy having magmatic Fe_3O_4 nanoparticles by using single chain antibody having loaded beta cyclodextrin on it. Thus, these studies provide great examples of combination of antibody with chemotherapeutic agents [24].

20.5.1 Passive Targeting

This type of targeting is very important as it causes the direct detection using magnetic resonance imaging possible. As the fact behind is that the tumors have ability to develop their own vasculature system in order to supply blood to tumor tissues. Angiogenesis can result in the production of abnormal blood vessels which also present, and by using this provided space, the nanoparticles can also accumulate in tumor tissues [25]. The targeting also depends on different physiochemical characteristics of nanoparticles such as size as the particles less than 10 nm can be easily filtered out via kidney while larger ones are catched out by spleen or liver for destruction into smaller ones. Solid tumors are also able to show the enhanced permeability and retention effect termed as EPR effect for nanoparticles of appropriate size and dimension [26]. Thus, consequently, the larger nanoparticles cannot leave and enter tumor cell easily, while at the same time, smaller nanoparticles either enter or leave tumors easily. Hence according to this data, it is suggested that nanoparticles from 1 to 100 nm can easily accumulate in tumor cells as compared to normal tissues. Regarding other properties, positive and hydrophobic nanoparticles have shorter time of circulation than that of negative and hydrophilic ones [27].

20.5.2 Active Targeting

It is advantageous to mention here that active targeting is far more important than passive targeting for targeted drug, gene, and other pharmaceuticals delivery. Thus, it provide advantage of being more potent with limited side effects. The nanosystem involved in active targeting causes more accumulation of drug than that of passive targeting. Moreover after accumulation, the affectivity of drug can be increased by the phenomenon named as active targeting. This is done by the decoration of nanocarriers with the ligands of specific receptors which are later expressed in tumor cells. Among classical examples, transferrin and nicotinic receptors provide the nanoparticle to reach environment of brain tumor [28]. Following this case, the mechanism involved in it is vascular targeting. For active targeting, variety of antibodies and chemotherapeutic agents have been prescribed by researchers. They have been tested both in vivo and in vitro promoting stronger ligand and receptor binding and acting as a potential model for promotion of actively targeting technology. Most of the phenomena involve the tumor cell targeting in general by using certain nanocarriers.

20.6 Chemotherapy Agents

The most common chemotherapy drugs used for purpose of cancer include doxorubicin abbreviated as DOX, paclitaxel, cisplatin, mitomycin C as outlined in Table 20.1. Doxorubicin is the most widely accepted drug employed in targeted drug

Table 20.1 Depicting the names of drugs along with the polymers or functional groups attached and average sizes followed by their use in specific cell lines

Name of drug molecule	Modification of polymer	Overall average size of particles [30, 31]	Use in cancer cell line
Paclitaxel	Chitosan modified	37 nm on average	A5 49
Gemcitabine	Chitosan modified	4 nm overall	MCF-7 SKBR
Mitomycin C	Hydroxy ethyl methacrylate	200 nm approximately	Not known yet
Docetaxel	Cyclodextrin Polymer F127	140 nm	C4-2
Cisplatin	Heparin	46 nm	CP-70
Sorafenib	PEG associated PLGA	206 nm	BEL7402
Bortezomib	Chitosan	7 nm	HeLa cell lines from mouse
Doxorubicin	Polylactic acid PEG Strach g-poly Methyl-metha crylate Chitosan PLGA PEF-PMAM Poly (N isopropyl acrylamide co-IA)	23 nm	HeLA mouse cell line

delivery system. These nanoparticles contain hydrophobic coatings which make them stable in human system. For addressing this issue, a copolymer assembled with SPOINs was developed by scientists in order to deliver drug to target site [28]. The co-polymer made up of polyamidoamine with attached PEG, and dodecyl amine was prepared by Michael addition process. The research has demonstrated that the addition of them to iron oxide magnetic nanoparticles has not only improved cellular penetration in comparison with free DOX and obtained response similar to that of DOX alone. These SPOINs have been investigated thoroughly for their applications for targeted drug delivery system. As for example, mitoxantrone-carrying SPIONs have been utilized in vitro for analyzing different aspects of cellular responses and similarly the persistence or release of drug from nanoparticles [29].

20.6.1 Doxorubicin

The drug having sold under brand name of Ardiamycin is the drug that is especially designed to treat cancer in patients via chemotherapeutic protocols. These cancer types include breast cancer, bladder cancer lymphoma, and acute leukemia. The most common side effects include suppression of bone marrow, vomiting, rash,

and inflammation of mouth. Similarly, serious side effects include certain allergic reactions at site of injection, as for example anaphylaxis, damage to heart, and inflammation at site of injection along with treatment-based leukemia [32]. Discoloration of urine is also seen inpatients with the disease for a very few days. It is actually an antitumor-related family of drugs and usually works by interfering with DNA molecule. It was approved by US drug agency in 1974 included in list of essential medicines and was isolated from bacteria *Streptomyces peucetius.* It is a lipid PEGylated drug which has been specifically used for treatment of breast cancer, ovarian cancer, and AIDS [33]. It can also be used to treat multiple lymphoma or cancer when it is combined with bortezomib [34]. It is the compound which occurs naturally in a large amount as it has been produced from a number of wild strains of *Streptomyces*. But of all, only one naturally occurring specie is involved in producing drug. This drug interacts with DNA by intercalation, thus inhibiting biosynthesis of macromolecule. This in turn also inhibits the action of topoisomerases 2, an enzymes that is involved in the super coiling of DNA during process of transcription [35]. It also stabilized DNA supercoil when it has been broken from a single side, thus preventing double helix structure and stopping overall process of replication meanwhile, thus stopping whole process of replication. It also sometimes results in production of Quinone type radicles causing cytotoxicity to a great extent in biological systems [36]. The aromatic and chromophore portion of molecule intercalates with the double strands of DNA with the six sugars sitting in minor grove of DNA at same time flanking base pairs adjacent to intercalation site as evident from several crystal structures [37] (Fig. 20.2).

In addition, the lipid or liposome PEGylated form of doxorubicin has also been formulated resulting in an appropriate concentration of drug in skin. But at the same time, it also cause serious side effects such as hand and foot disease also termed as PPE [39]. When administered, this drug may leaks from capillaries of hands and soles of feet causing redness, tenderness, and peeling of skin. These side effects can limited use of this drug formulation in comparison with plain doxorubicin in the same treatment regimen. But despite of all these challenges, this encapsulated form is approved for use by Federal drug agency (FDA) [40].

20.6.2 Cisplatin

Cisplatin is a drug molecule that has been used for treatment of various types of cancers, as for example cervical, breast cancer and colon cancer along with lung and brain tumors. Common side effects of this also include suppression of bone marrow, hearing problem, and kidney damage [41]. While serious problems include hearing loss, lack of movement, allergic reactions, and electrolyte disturbance, it is a platinum-based antineoplastic form of medication. It works by binding to DNA and inhibiting its replication. It was discovered in 1845 and was approved for use in 1979 [42]. It is a drug being administered intravenously in normal saline for treating solid and other hematological tumors, thus treating various types of tumors such

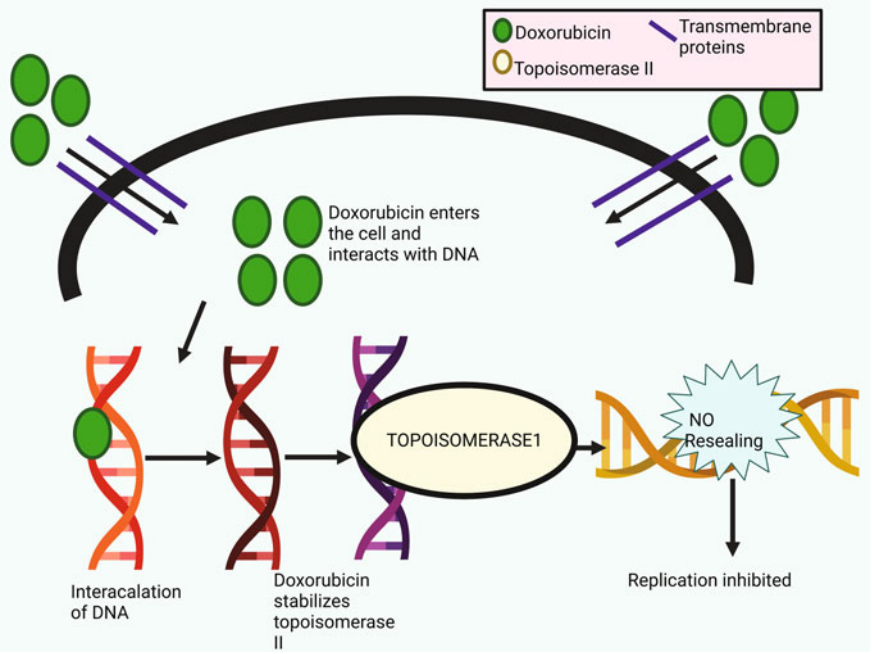

Fig. 20.2 Whole demonstration of molecular avenues occurring during targeted drug therapy in cancer cells [38]

as a sarcomas, lymphomas, and other cervical cancer type [43]. It is particularly important for testicular cancer where it shows its efficiency from 15 to 85% on the basis of its rate of absorption. PRV111 is a nanotechnology-based system being employed for local delivery of chitosan loaded cisplatin particles in human studies, thus playing an important part in treatment of oral cancer in future. Auger therapy has been combined with cisplatin in order for increasing therapeutic efficiencies of cisplatin without causing any toxicity effects [44].

This drug has number of side effects which limit its use in medical science. One of them is nephrotoxicity which is a primary dose limiting condition and can cause kidney damage. It gets accumulated in proximal tubules where it disturbs genetics of mitochondria and endoplasmic reticulum, thus stimulating reactive oxygen specie and other inflammatory cytokines [45]. This can be eliminated by nerve conduction studies before and after applied treatment. The common side effects involve hearing and visual losses that can occur once treatment begins. Triggering apoptosis by intercalating with bases of DNA is the phenomenon used by cisplatin that in turn can trigger other side effects. Similarly, hemolytic anemia can also develop. The pharmacological description includes its interference with replication of DNA, thus killing out fast proliferating cells [46]. Following administration, one chlorine is displaced by water resulting in formation of complex, thus naming process as aquation. Dissociation of chlorine is favored inside of cell in comparison outer surface because the

intercellular surface contains 3–20% of water as compared to extracellular surface. It interacts with DNA in several ways. Primarily through this interaction mitosis is halt and thus in turn apoptosis activation occurs when the repair appears to be impossible task [47]. Most notable changes in DNA include intercalating changes with purine bases inserting intrastrand GpG adducts from at least 90% of all products as these are excised by nucleotide excision repair. Transplatin is another transform of cisplatin, but it does not give the batter form of pharmacological effects as cisplatin [48]. Two mechanisms have been successfully used to exhibit anti-cancer effects of transplatin. At first, the arrangement of chloral agents confers greater chemical reactivity to transplatin, thus causing transplatin to become inactive before it reaches DNA. Secondly, the stereo composition of transplatin is such that it does not cause formation of sufficient CPG adducts in abundant quantity [49].

Cisplatin combination therapy is the central concept of treatment of many cancer forms, but after sometimes, the disease gets replaced with cisplatin resistant disease. For this, a number of mechanisms have already been proposed including cellular uptake, influx system of drug, increase in detoxification of drug, inhibition of DNA damage or apoptosis. Oxalating is another alternative used for the treatment of cancer, and these are the most resistant cancer being used in laboratory. Paclitaxel is used for the cisplatin resistant cancer, but the resistance mechanism is still unknown [50].

20.6.3 Paclitaxel

Paclitaxel abbreviated as PTX is another medicine used in the chemotherapy medication for treatment of number of cancers, as for example, breast or lungs cancer. It has been used as albumin coated formulation and has been administered through intravenous root of administration. The common side effects include loss of hairs, fatigue, diarrhea, and certain allergic reactions [51]. Other side effects are certain heart problems, risk of infection, and inflammation of lungs. Certain concerns occur during pregnancy causing complications. It is taxane form of medicine that also interferes with normal functioning of microbes during process of cell derision. It was first isolated in 1971 and was approved for use in 1991 [52]. It has been prepared from the certain precursors more especially from certain cell cultures. In the terms of mode of action, the drug is the only molecule which targets tubulin [53]. Paclitaxel treated cells have certain problems in mitotic cell division, spindle formation, and segregation of chromosomes. Paclitaxel targets microtubule organizing structures which in turn stabilize microtubule structure and also protect it from disassembly [54]. Chromosomes are thus unable to achieve the shape of metaphase plate. Hence, it blocks the progression of mitosis and also protects the prolonged mitotic phase, thus triggering apoptosis and reversion to G0 phase without any cell division. This property is attributed to suppression of microtubule dimensions [55]. The ability of paclitaxel to inhibit spindle fiber formation is characterized to microtubule dynamics. But at the same time, there are other studies which block the dynamic of microtubule at the concentration much lower than that is needed to block process of mitosis. At the

higher concentration than that of required for therapeutics, it also causes suppression of microtubule detachment from centrosomes. It also binds to beta subunit of microtubules [56]. In the early days, the medicine was roughly produced from the bark of tree Pacific yew that in turn causes death of tree in the processing. In the terms of biosynthesis, it elongates FPP by addition of IPP molecule leading to formation of geranyl diphosphate (GGPP). The whole process is completed in the 19 question which is followed by further several steps. Oxygenation, 2 acetylation, and benzolyation occur at the certain intermediates on certain carbon molecules, i.e., C7, C9, C15, C13, C10, etc.; later on C9, the ketonation occurs at C9 carbon number leading to formation of another intermediate baccatin III [56]. In addition to it, total synthesis is also a process that involves the synthesis from the very starting material or raw materials, and the process was motivated in order to increase chemical understanding than that of the other biological applications of paclitaxel. Other similar compounds include albumin bounded or albumin functionalized with nanoparticles [57]. And the most of the toxicities are associated with solvent ion which these nanoparticles are dissolved in to order to provide the proper drug delivery system. It is approved by FDA for metastasis and non-small lung cancer metastasis and cancer of pancreases [58]. Synthesis approach for paclitaxel has been used for formation of docetaxel, and these have same sort of clinical uses as that of paclitaxel. It can also be used for the production of restenosis that is actually a disease involving narrowing of coronary or peripheral arteries of arteries [59]. The common side effects include loss of appetite, alternation in taste buds, alternation in colors of nails toes, etc.; moreover, other reactions at the site of injection such as redness, pain, or bleeding may also occur. Sometimes, severe reaction such as skin rash and female infertility may also occur by ovarian cancer [60]. Neuropathy may also occur sometimes at some point of life. Severe reactions such as allergic reactions cyclosporine, terpenoids, or the drug containing castor oil may also resulted [61].

20.6.4 Docetaxel

It is the cancer curing drug which has been used to treat a number of types of cancers including head and neck cancers, etc., and has been used for the treatment along with other chemotherapy medications. It is injected as a slow injection in the vein of patient [62]. Data from clinical research has clarified the fact that the dose of DTX has been used for treatment of colorectal, having cytotoxicity against breast cancer, lung, liver or neck cancer, and melanomas. In the terms of refractory hormone cancer, docetaxel improves quality of life and others. Treatment with this drug not only improves survival time in patients with different types of cancers [63]. At the same time, there are some clinical trials that show life survival till three months, while others show more survival times. This shows that the drug slows down progression of metastatic breast cancer, thus leading to disease free survival of a patient [64]. Treatment combination of prednisone with docetaxel showed not only improved rate of survival, but also the quality of life has also been improved in comparison with

treatment with other medications such as mitoxantrone. It also inhibits mitosis by the phosphorylation oncoprotein bcl-2 leading to the apoptosis of breast cancer, thus leading to regression of tumors. Enhanced effects have been observed in mice who were given radiation therapy with docetaxel [65]. The drugs also possess greater uptake of drugs as compared to other having long range of intracellular presence that makes treatment effective with even use of very small dosage. This in turn give very less and fewer side effects than usual in terms of other drugs. Both paclitaxel and docetaxel have comparable efficiency in the therapy for breast cancer [66]. At the same time, docetaxel is prone to drug resistance via different modes of mechanism or actions. The drug is administered with a number of cycles involving one hour infusion and treatment us usually administered in presence of oncologists. The tests which can be performed include complete blood cell count, serum electrolytes, serum creatinine along with fluid retention which are common side effects seen so that the treatment can either modified or terminated accordingly [67]. Pre-treatment using corticosteroids is usually recommended before the administration of dosage in order to reduce side effects such as fluid retention and hyper allergic reactions. Other medications are also provided in order to reduce symptoms and pain of disease. For the breast cancer, it is usually given in combination with cyclophosphamide and doxorubicin [68]. It is also given in combination with capecitabine that is an inhibitor for DNA synthesis. As with all the chemotherapy procedures, the side effects are also very common including hair loss and bone marrow defects. About 90–95% cases have reported neutropenia, anemia, and thrombocytopenia. Pneumotoxicity induced as a result of texane is a rare condition and accounts for 1–5% of all cases [69]. Observations were also made in phase 2 and phase 3. Similarly, a number of side effects have been observed for conjunctive or adjuvant treatment with docetaxel. However, the use of drug is not prescribed for the patients which have neutrophils level 15,000 per ul [70].

The side effects are mostly observed in older aged patients, but at the same time, dosage is not reduced for decreasing side effects. Kidney failure is the most common side effect during docetaxel dosage adjustment [71]. On the basis of research and data available, this drug is prescribed to be safe during second or third trimester of pregnancy. In combination with other drugs, it may cause serious side effects leading to death of embryo as well. Yet, the systemic studies involving human reactions are being lacked in research field. Interaction with other drugs is as a result of the variation in pharmacokinetics and pharmacogenomics [72]. Certain other drugs are administered with docetaxel which are enlisted in Table 20.2. But still, they do not change plasma binding in phase 2 studies. It is mainly metabolized in liver by cytochrome P450 and CYP3A5 of isozyme. In the terms of mechanism of action, the drug binds to microtubules which in turn stabilize them and thus further prevent de-polymerization of calcium ions causing decrease in temperature most probably at positive ends of microtubules [73]. It accumulates at higher level in ovarian carcinoma cells as compared to kidney cells that result in more effective treatment of docetaxel than others. In the terms of cytotoxicity, drug has cytotoxic effects in all types of cancers, and it blocks assembly of interphase microtubules and hence does not prevent entry into mitotic cell cycle by inhibition of spindle assembly. Microtubules formed

Table 20.2 Representing the number of drugs whose interaction with docetaxel gives side effects

Name of drugs	Acute side effects
Cyclosporin	Neuropathy
Doxorubicin	Jaundice
Doxorubicin induced liposomes	Increased exposure
Thalidomide	Thromboembolism
Terfenadine	Diarrhea
Ketoconazole	Fever
Vaccinations for Bacillus	Increased infection

in presence of docetaxel are much larger in size than that of paclitaxel that causes improvement in cytotoxic activity. Its activity is much more in ovarian cancers than that of lungs. As the abundant formation of microtubules caused by docetaxel leading to apoptosis of tumor cells [74].

20.7 Other Drugs for Targeted Delivery

In addition to all these agents, certain Chinese monomers have also been used as a cancer treatment in targeted drug delivery system. A review has demonstrated the use of dendrimers containing magnetic nanoparticles as a drug delivery vector for epigallocatechin gallate. More recent studies have shown that curcumin have been applied in drug delivery system for both breast and ovarian cancer [75]. Mancarella and colleagues have discovered layer-to-layer functionalization of ferrous oxide magnetic nanoparticles by properly coating these particles in dextran and lysine, thus obtaining fully functionalized curcumin for treatment of ovarian cancer. Similarly, ferric oxide coated zirconium phosphate nanoparticles and cyclodextrin nanoparticles are discovered as effective candidate for targeted drug delivery. Similarly in all other cases, oligonucleotides have been applied successfully in drug delivery system [76]. Pourianazar and his colleagues have utilized three layer nanoparticle system where internal core is made up of ferric oxide followed by aminosilane layer and lastly poly-dendrimers that enhanced the overall accumulation of CPG-oligonucleotides in tumor cells in the tumor cells as a novel drug delivery system. Summarizing whole story, these magnetic nanoparticles have provided drug delivery system for achieving direct drug targeting [77].

20.8 Diagnosis of Cancer

The early discovery of cancer at early stage causes improvement of cancer stage actively, and thus, diagnosis proves to be useful. The imaging tumor technology has played its role in diagnosis and choice of clinical treatment options. Moreover, these

magnetic nanoparticles act as a contrasting agents that has been rehearsed and used in cancer imaging [78]. Following imaging, the position of imaging nanoparticles can be determined easily as given below.

20.8.1 Imaging Methods

Of all the imaging techniques, magnetic resonance imaging abbreviated as MRI is considered to be most special and valuable non-invasive type imaging technique because of its higher resolution and capabilities. It has been successfully used as a contrasting agent in magnetic resonance imaging. It is necessary to modify the surface of nanoparticles by induction of magnetic poles and interaction of particles for overcoming of magnetic nanoparticles [79]. As for example, the poly ethylene coated nanoparticles were firstly prepared and then modified with PEG via PEI mediated conjugation chemistry process. The remaining surfaces are modified by acetylation, thus forming functionally stable nanoparticles for magnetic resonance imaging. Magnetic resonance imaging has clearly demonstrated that earlier detection of glioblastoma multiforme in 7 years old boy has been achieved by sensitive imaging of nanoparticles having supermagnetic properties [80]. Other imaging techniques include magneto tomography that utilizes magneto motive force for ion induction of magnetic field or ultrasound in SPOIN labeled tissues, thus providing imaging properties of nanoparticles in vivo within the ultrasound range [81]. Lindemann and colleagues have suggested that dextran coated SPOINs for use in the tumor cell analysis as a tracer materializing MPI. In addition to them, phosphorous imaging has gained attention because of its certain properties and sensitivity in tomography technology. Moreover, photocaustic imaging is also gaining importance because of its increasing applications as an effective imaging technology [82]. A research has developed another technology featuring dextran magneto motion and ultrasound technology, whose imaging production speed is 1000 times greater than that of other photocaustic tracking methods demonstrated previously in research. Similarly, stone and his team also demonstrated a system that can be used for observing nanoparticles in biofilm range using NIR imaging [83]. Scientists also provided breast imaging techniques by combination of NIR with photoacoustic tomography in presence of dye labeled fragments of urokinase plasminogen activator or targeted iron oxide magnetic particles using murine model of mammary cancer. Meanwhile other techniques such as ultra-short echo time UET and MPI also improve detection limit in cancer cells [84].

For the magnetic nanoparticles in addition to MRI, other techniques are also used in combination, and thus, this dual technology improves the era of diagnosis. As for example, dual imaging single photon emission tomography has its applications in breast and pancreatic cancer types. Another study has also demonstrated the synthesis and use of monodispersed iron oxide nanoparticles coated with silica nanoshells providing fluorescence and magnetic imaging of nanoparticles [85].

20.8.2 Imaging Positions

Pancreas cancer is considered to be most dangerous form of cancer because of its late diagnosis and causes increase in rate of morbidity and mortality because of its other risks and late presentation [86]. Hence, its early diagnosis will ultimately lead to increase in cure rate and survival rates of patients. In the case where chitosan coated nanoparticles and surviving antisense oligonucleotides are conjugated to produce Sur –MNPs that in turn leads to targeted localization of nanoparticles ion tumor cells [87]. This can also be used for magnetic resonance imaging of coated nanoparticles at the same time. Considering more sensitive tools for accurate diagnosis of disease for allowing medical imaging of magnetic nanoparticles by use of recombinant human serum albumin leading to development of incorporated iron oxide nanoparticles are also have been developed successfully. Improved targeting and imaging have been demonstrated accurately in mice using SPECT-CT and imaging technology [88]. A study has elucidated technology for designing microacoustic imaging methodology in the case of breast cancer. The results clearly demonstrated that the imaging is very reliable for detection of breast cancer even in presence of lower complexity and if designed according to proper guidelines [89]. Further, liposomal encapsulation also improved accumulation of SPOINs in breast cells of breast tumors providing better options for detecting out breast tumors. The results demonstrated that more cyclic aromatic peptides are present around tumor tissues than that of FMNPs such as arginine, glycine, and aspartic acid [90]. Also it indicated the potential applications of RGD-MNPs as a potential target molecule in MRI and OI of breast cancer. For accurate detection of breast cancer, a compression sensing approach consisting of three dimensional imaging of breast has been suggested. The assay was designed on the basis of basis of maximum accumulation of magnetic nanoparticles in tumor cells or tissues [91]. For prostate cancer, the MRI provides the best resolution for soft tissues and has been used for accurate diagnosis of prostate cancer and for imaging modality for patients with prostate cancer [92]. The examination of lymph nodes in patients with prostate cancer is done by the technique lymphoscintigraphy followed by the injection of tracers that are radio labeled. This method was employed in the first study involving SPOINs in examining prostate cancer using MRI. Another study has also indicated trans rectal injection of SPOINs for magnetic marking found to be a safe procedure and feasibly identifies SLNs or metastasis in lymph node patients. In other cases, diffusional water DW-MRI is predicted to be useful predictor of Gleason score for measurement of prostate cancer aggressiveness. This incorporation monitoring enhances accuracy of pre-clinical trials in transgenic mice of prostate cancer [93, 94].

Similar approaches have been used for increasing the magnetic detection of lungs cancer accurately. In this scenario, the immune SPOINs have been created for detection of metastasis of lung cancer. These SPOINs were coated with a layer of oleic acid and dextran or than conjugated with anti-cluster of monoclonal antibody of mouse [95]. This arrangement proved to be very useful for the accurate detection of mouse tumor diagnosis. In addition to it, the pulmonary inhalation of magneto nanoparticle droplets also facilitates the targeted detection of patients with the lung cancer

Table 20.3 Specific treatment modalities and imaging techniques associated to them

Treatment	Imaging technique	Site of action
Targeted and PDT	MRI	Head and neck
Targeted and PTT, tomography	MRI	Liver, cervical, colon
Photocaustic imaging	Heat ablation	Breast
Fluorescence imaging	Targeted therapy	Cervical, stomach
Chemotherapy	Infrared therapy	Liver
Optical imaging	PDT/MHT	Melanoma
NIR imaging	PDT	Head and neck

along with targeted delivery to site of infection and also presents a variate number of advantages as well [96]. At the same time, there are a number of contrasting agents which are used in pancreas, breast, and prostate cancer in combination with other type of modalities presented in Table 20.3.

20.9 Treatment of Cancer

Acting as a drug carrier, the ultimate goal of nanoparticles is to act as the treatment for cancers.

20.9.1 Magnetic Hyperthermia

The conversion of electromagnetic energy into heat is one of the most important and potential application of nanoparticles that makes them useful for the biological applications such as slower drug release at targeted site, treatment of disease, and controlling functionality of other cell types. But at the same time, there are some drawbacks which limit its applications such as poor conversion properties [97]. But these thermal induction properties can be improved by the use of magnetic nanoparticles by the exchange of properties ion between hard and soft shells of magnet shells, thus maximizing specific loss power which is actually a scale to measure the conversion efficiency [31]. These magnetic nanoparticles have specific conversion values that are of magnitude larger than that of other conventional iron oxide nanoparticles [98]. The trials in the mousse model have suggested that this treatment is superior to that of other treatments being employed as a anti-cancer drug or agent. Thus, the most common way of treating tumor cells to heat them to their threshold till apoptosis. Following this, heating of nanoparticles in presence of magnetic field

depends on several factors, and the optimization of these factors results in improvement of generation of heat even in the presence of lower dosage of nanoparticles. These parameters include size, concentration, viscosity, frequency, and field strength of nanoparticles [99]. An increase in viscosity leads to lower rates of heating in nanoparticle solution which provides evidence for the involvement of hypothermia in drug delivery and treatment at same time,. it presents an active area for development of magnetic nanoparticles in cancer treatment using MHT procedure, but at the same time, in vivo tumor treatment in the presence of low frequency magnetic field has not been demonstrated yet [100].

Magnetic nanoparticles under precise controlled magnetic field are able to transduce energy showing their inherent ability in therapy of diseases such as cancers. Till now, the induced death of nanoparticles by applying low-frequency magnetic field by oscillation of magnetic nanoparticles has only performed and observed under laboratory conditions [101]. Following this, the nanomagnets align themselves in the plane of magnetic field creating a mechanical force that in turn disrupt surface of cancer cell surface leading to programmed cell death or apoptosis [102]. This treatment has specifically reduced brain tumor of transgenic mice having brain disease glioma xenografts in the absence of any side effects. Similarly, loss of hysteresis is also observe that increase heating efficiency. On a general note, these all MHT procedures enhance the heating efficacy of the chemotherapeutic agent following induction in human system [103]. They also disclosed that hypothermia efficacy can be improved by the separation using magnetic field. On the other hand, combination of SPOINs impregnated with multivalent pseudo peptide (N6L) along with doxorubicin has resulted in enhancement of MHT treatment in cancer patients. In addition, the research has also found that MHT has enhanced radiation therapy in marine models of human prostate cancer cells. Genetic engineering or gene delivery also plays its significant role in MHT procedure [30].

20.9.2 Photodynamic Therapy (PDT)

This is an externally activated system that is non-invasive type treatment acting as a therapy for cancer. The mechanism of PDT involves the number of drugs, named as photosensitizers, that involve PSs in the tissues via use of light of specific wavelength and sensitivity. Hence, a multifunctional magnetic nanoparticle approach with magnetic resonance imaging and photodynamic therapy is proved to be an effective allowing targeted delivery of drug along with the monitoring of specific responses, thus leading to diagnosis and treatment of cancer lastly [104]. A hybrid functionalized nanoparticles whose core is made up of ferric oxide and shell is made of photosensitizer heparin surface found to be a multifunctional carrier in PDT. A conjugate of heparin composed of heparin–pheophorbide with an attached thiol is proved to be a macromolecular photosensitizer and thus introduced into nanoparticle surface via gold–thiol interaction. The results indicated that photoactive activity is suppressed by presence of iron or gold nanoparticles. But at same, their productivity

can be restored in glutathione rich intracellular environment thus give rise to GSH-mediated hybrid activity in hybrid photosystem [105]. The animal models proved it to be an photodynamic treatment for treating cancer along with longer persistence and much higher therapeutic efficiency in comparison with other free phAs. In addition, in vivo studies have also elucidated, and these particles can prove to be an effective contrasting agents in magnetic resonance imaging and also the guiding star for dosage to a patient at a specific time period [106]. The PS moves from ground to excited state by absorbing the light of specific wavelength and power in aerobic condition, and electrons move top nearby oxygen atom resulting in production of oxygen free radicle and excited singlet oxygen molecules in turn. Hence, the substances or atoms thus produced are named as reactive oxygen species abbreviated as ROS which are reactive or dangerous and can lead to damaging of cancer cells. for enhancing effects of photosynthesizes the targeted drug delivery has become a point of interest in terms of cancer [107]. A study has suggested that cobalt coated ferric oxide nanoparticles $CoFe_2O_4$ termed as cobalt ferrite that are in turn functionalized by coating them further with HP in order to introduce $CoFe_2O_4$-hematoporphyrins (HPs) and thus has been targeted to cancerous cells. Other studies have also demonstrated efficacy in showing photodynamic activities and anti-cancer activities on cancerous cells most specially in prostate cancer and some other cell lines such as MDA-MB-231 in meanwhile. A novel chlorin photosensitive system pyropheophorbide was prepared for the PDT during cancer therapy. The experiment revealed the fact that prepared magnetic nanoparticles showed stronger photodynamical activity in the presence of lower toxicity. Additionally, it was observed that viability or spread of Hela cervical cancer cells was reduced to 17% following treatment episode with PDT [108].

20.9.3 Photothermal Therapy PTT

Photothermal therapy is based on the heat generation and killing of cancer cells using infrared light. For this strategy, NIR absorbents are found to work for better heat production during treatment. By use of DSS type near infrared radiations during PTT, the usual procedure is followed by enhancement of tumor with administration of absorbent. However, other parameters such as rate of fluency and time of radiation are usually determined before application of NIR radiations [109]. But using this procedure, it is impossible to get accurate information such as tumor size, difference in tumor tissues, and lastly distribution of NIR radiation in tumor cells [110]. The procedure uses photothermal transduction agents that can convert the light energy into heat that usually leads to increment of temperature of tumor area and ultimately abortion of tumor cells. These agents must have some specific characteristics such as efficiency in terms of photothermal phenomenon, biodegradability, and much stronger absorption of light in wavelength ranging from 615 to 1300 nm which is referred as near infrared region. They are in use because of much deeper penetration into tissues and also lesser absorption in the biological tissues. They can be inorganic or organic in nature. In organic, PTAs include carbon nanotubes, certain 2D materials,

and longer time for photostability. Smaller dye molecules, as for example, cyanine and porphyrin, are limited in use because of their deeper absorption of NIR rays [111]. However, other conjugated or complex polymers are discovered to be biodegradable but they can be degraded by photobleaching. They can be easily combined with other magnetic imaging techniques such as MIR for diagnosis effects, thus providing the combined but selective therapy of certain tumor cells also facilitating targeted drug delivery. The co-polymers used in PTT include polyanaline, polydopamine, polythiophane, etc. [112]. The prominent example of this technique using magnetic nanoparticles is that these MNPs have proved to be best photothermal agents for tumor cells because of heat bearing properties. First of all in the history, the cluster of magnetic nanoparticles was used in photothermal mechanism in both in vivo and in vitro trials. In comparison with ferric oxide nanoparticles alone, the clusters of magnetic nanoparticles were more effective in absorption of NIR [106, 113]. It was found that at wavelength of 808 nm, the NIR was more toxic against the cancer cell line A549 at higher temperature [114]. Most of times, MIR and PTT procedures are done in combination simultaneously. However, the smaller clusters of ferric oxide nanoparticles have more international properties than that of larger ones, thus giving out greater sensitivity and better results in in vitro trials. The diameter of the nanoparticles for optimum activity was 120 nm for both PAT and MRI. Hence, size is considered to be an important factor regarding treatment of cancer [115, 116].

20.10 Conclusion

From all above discussion, it is cleared that magnetic nanoparticles have been used as a potent agent in adjuvant therapy. But due to toxicity in animal and human systems, their use has been limited or restricted. Surface coatings in this scenario using functional groups have increased the utility of these nanoparticles clusters. The larger the size of nanoparticles, more will be accumulation in the in vivo environment. Hence by controlling physiochemical properties, it is possible to improve the magnetic behavior of nanoparticles while reducing cytotoxicity. Inspite of many successful attempts to use nanoparticles for cancer targeted drug delivery, there are certain challenges which need to be addressed. Thus by improving drug loading capacity and increasing interaction between particles and tumors, these become suitable for using in cancer in near future.

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Chapter 21
Vehicles for Delivery of Therapeutic Agent for Cancer Therapy

Ramakant Joshi, Rajendra Chauhan, Wasim Akram, Pawan Kushwah, Hemant Mourya, and Navneet Garud

Contents

Abstract Cancer tends to have a high fatality rate over the world. Cancer is defined by anomalies in cell cycle regulatory processes, which result in the survival and

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growth of malignant cancer cells. Chemotherapy, radiation, and surgery are being used to treat cancer. Chemotherapy now confronts concerns such as cytotoxicity, the occurrence of multi-drug resistance, short half-life, poor solubility, stem-like cell growth, and lack of specificity, with treatment regimes focusing on chemotherapy, which has convinced drawbacks such as negatively harming healthy tissues. Some of the drawbacks of standard cancer therapy administration, such as low oral bioavailability, chemoresistance, reduced drug solubility, restricted therapeutic indices, and systemic toxicity, have been solved by nanomaterial-based drug delivery methods. Various advances in nanomaterials useful in cancer therapy such as nanoparticles, liposomes, solid lipid nanoparticles (SLN), dendrimers, carbon nanotubes, quantum dots, micelles, hydrogel, silica nanoparticles, magnetic nanoparticles, and gold nanoparticles are vehicles for specific drug delivery. To produce highly efficient drug delivery vehicles with enhanced delivery, bioavailability, and safety profiles, the concept of integrating smart drug delivery with extended-release has recently been developed. Herein chapter, we will describe the various kinds of constituents used in employing chemotherapeutic agent delivery vehicles, as well as their organizationalfeatures that expand the therapeutic efficiency of their drugs. We will also discuss current scientific breakthroughs in the area of smart extended-release delivery systems for better patient compliance and treatment options in cancer patients.

21.1 Introduction

In general, gene mutations are thought to be the cause of cancer. 9.6 million people died from cancer in 2018, while an estimated 18.1 million additional cases occurred of the disease. By 2030, the Global Cancer Observatory (GCO) predicts that 30 million people worldwide will succumb to cancer every year [1]. Cancer has a high death rate, but it also imposes a heavy financial cost on the general public and cancer sufferers' families. As a result, efforts to prevent, diagnose, and cure cancer are crucial. Although medical science and technology have made considerable strides, there are still few effective cancer treatments. Cancer metastasis and recurrence have a significant role in disability and death, although the precise processes are still poorly understood [2]. Radiation therapy, chemotherapy, and surgery are currently available cancer treatment methods, as well as combinations of these. Conventional chemotherapy largely kills rapidly expanding and dividing cancer cells by impairing DNA synthesis and mitosis. The agents can cause significant unwanted side effects, for example, nausea and appetite loss, because they areunrestrictive and hazardous to healthy normal tissues as well. In reality, one of the primary factors in the high death rate among cancer patients is the significant deleterious effects that chemotherapy medications have on healthy tissues and organs. Additionally, larger dosages are needed since these medications' bioavailability to tumor tissues is generally limited. This results in increased toxicity in normal cells and a rise in the occurrence of multi-drug resistance [3].

The shortcomings of traditional chemotherapy, for instance, its limited bioavailability, deprived therapeutic index, non-specific targeting, and need for high drug doses, have been successfully solved by the improved drug delivery vehicles [4]. Through the development of nanotechnology, nanomedicines used in cancer treatment may lessen the negative effects of chemotherapy, and substantial research has been done in this area [1]. Some of the most well-liked kinds of nanocarriers for the administration of chemotherapeutics include liposomes, polymeric nanoparticles, dendrimers, nano-shells, inorganic, nucleic acid-based, and magnetic nanoparticles. In addition to increasing drug efficacy by maintaining steady-state therapeutic levels of drugs for a longer period, controlled drug release from nanoparticles also reduces drug toxicity and enhances the pharmacokinetics of drugs by increasing drug concentrations. This is because nanoparticles have a higher therapeutic index of loaded chemotherapeutic agents than drugs delivered via conventional dosage forms [5]. Herein chapter, we will describe the various kinds of constituents used through chemotherapeutic agent delivery vehicles, as well as their organizational features that expand the therapeutic efficiency of their drugs. We will also discuss current scientific breakthroughs in the area of smart extended-release delivery systems for better patient compliance and treatment options in cancer patients.

21.2 Limitations in Cancer Therapy

Clinical trials are used to assist the USFDA in approving anticancer medications according to their strengths and weaknesses. Uncertainty on whether these drugs are better to the current standard of care, whether they get better survival or quality of life, or all three limits the scope of nonrandomized clinical trials that demonstrate tumor shrinking in reaction to novel therapy [6]. Limitations of interest in randomized clinical trials (RCTs) can relate to trial design (e.g., using a control arm that is deemed suboptimal or using crossover inappropriately) or result (e.g., failure to demonstrate an overall survival (OS) benefit when a surrogate endpoint improvement is achieved) [7]. The use of surrogate endpoints and the frequency of single-arm studies that result in medication approval have been documented in earlier studies. We recently evaluated the prevalence of subpar control arms. These studies, however, did not assess crossover mistakes or the overall % of these constraints coexisting in the same survey. For instance, what proportions of FDA approvals are based on better survival in a trial with no restrictions? When a patient randomized to the control arm receives the investigational therapy after the disease progresses or has harmful symptoms, this is known as a crossover in cancer RCTs (unidirectional crossover). There are two crossover errors in trials. The first occurs when the investigational agent is used instead of the control arm without the investigational agent's efficacy being recognized. Crossover from the control arm to the investigational treatment in these circumstances might skew interpretations of end goals, including OS, and may even result in phony survival gains [8]. For instance, crossover led to fewer patients receiving docetaxel and only at a delayed time point in a study of a novel,

untested cancer therapeutic vaccination for prostate cancer, which may have affected the control arm [9]. Any changes in survival in this situation may result from either a successful therapy or injury to the control arm.

The second mistake is eliminating crossover when a trial aims to advance the agent to the frontline setting. The medicine has demonstrated efficacy in a succeeding line of therapy. For instance, omitting crossover led to fewer patients receiving pembrolizumab, an immune checkpoint inhibitor, which had been accepted for use in the second-line setting, after progression on the control arm in a study evaluating the drug for previously untreated metastatic non-small cell lung cancer expressing planned cell death ligand 1 (PD-L1) molecules [10]. Crossover is necessary for this situation, and its absence could provide the mistaken impression that early administration is better than the current standard of care (i.e., sequential treatment). Using a comprehensive database, we set out to do a single study that looked at all four of the constraints above in a contemporary cohort of cancer medication approvals and predicted the likelihood that these flaws would frequently occur in the same trials.

Essential cancer therapies include chemotherapy, radiation, or combinations of the two. These treatments are frequently accompanied by various side effects, ranging from discomfort to the growth of additional tumors and severe damage to numerous systems, including the immune system. Growing research suggests that precisely adjusting nutrition may selectively make cancer cells susceptible to traditional cancer therapies while shielding normal cells from their harmful effects. Dietary nutrient modification enhances cancer immune surveillance such that severe immune suppression can be avoided and that patient microbiota remodeling can boost the immune response or immunological-based cancer therapy [11]. This discussion of recent advancements in cancer therapy is focused on the metabolic pathways within cancer cells and the tumor environment (such as the microbiome, immune system, and tumor microenvironment) that are significant in cancer progression and resistance, as well as cancer cell death. Finally, using information from the entire body of literature, we created a plant-based moderate ketogenic diet as a dietary intervention that might be used in future preclinical studies on cancer therapy.

21.2.1 Cytotoxicity

Cytotoxic medications can remove tumors, improve the results of radiotherapy or surgery, lessen metastases, and treat cancer symptoms. Cytostatic can kill tiny tumors that have evaded detection in tests and work outside the significant tumor. All dividing cells, including those in healthy tissue, are affected by cytotoxic medicines. However, cancer cells are particularly susceptible to cytostatic because they frequently divide noticeably more quickly than normal cells. Normal cells are less severely affected, and healthy cells also recover more quickly. With the advancement of pharmacological therapy, the importance of cytotoxic medications in cancer treatment has marginally decreased. They are still commonly used, nevertheless.

In cancer treatment, a variety of cytotoxic medication classes that collectively have various effects are used. The most frequent approach is to provide a cocktail of multiple cytotoxic medications. The type of tumor, its makeup, rate of growth, and the fraction of cells in the dispersion stage all affect how effective chemotherapy is [12]. Cytostatics are occasionally given as high-dose chemotherapy. Leukemia, certain lymphomas, and pediatric brain tumors are all treated with this. In addition, since high-dose chemotherapy can destroy bone marrow, stem cell transplants are necessary. Stem cell transplants can be used after chemotherapy to restore bone marrow function. The patient's stem cells or those from a donor can be used [13].

21.2.2 Harmful Effects of Cancer Therapy

The adverse effects and long-term sequelae of anticancer chemotherapy continue to be a substantial cause of concern for both patients and professionals despite the enhanced efficacy and extended survival durations offered by current treatments. The majority of current treatments for side effects associated with chemotherapy are ineffective, habitually disregard potential long-term implications, or even bring on new side effects that only make patients feel worse. The present research area concentrates on this topic and identifies various areas of advancement. New methods to increase tolerance and decrease the side effects of cancer chemotherapy are urgently required.

Among cancer chemotherapy patients, the most dreaded side effects are nausea and vomiting. Although most patients respond well to therapies for chemotherapy-induced nausea and vomiting (CINV), delayed CINV is extra challenging to treat. It emphasizes that even when acute CINV is properly managed, this symptom is usually underestimated and ineffectively controlled. The synthesis of substance P and its effect on neurokinin-1 (NK-1) receptors is one of the critical phases in the formation of delayed CINV. Describe preclinical studies using ferrets and house musk shrews to demonstrate the effectiveness of one NK-1 antagonist as an antiemetic (i.e., not only for CINV). In reality, the introduction of this specific medicine to the clinic was made possible by these investigations. Rodents lack the emetic response, which is one of the problems with using them as test subjects for the creation of antiemetic medications. But indirect indicators could be utilized [14]. Describe a novel indirect marker of nausea-like behavior based on observation of the rat's facial expression. These authors demonstrated that the eye-opening index (or ratio between longitudinal and axial eye dimensions) reduced after cisplatin treatment and that conventional antiemetics prevented this effect.

There are numerous additional gastrointestinal side effects of cancer chemotherapy that can be harmful to patients and upsetting. In the review, the pathobiology and treatment of the mucosal injury caused by cancer treatment are covered [15]. Localized ulceration and pain may be brought on by oral and gastrointestinal mucositis, which can also raise the risk of sepsis and induce anorexia, malabsorption, weight loss, anemia, and fatigue. It is crucial to remember that, despite extensive

earlier studies on oral mucositis, there is still a lack of safe and efficient therapies and preventive measures. This underlines the fact that mucosal injury most likely contributes to other gastrointestinal problems brought on by chemotherapy and probably represents the complexity of the pathobiology of gastrointestinal mucositis [16]. Explain the pathogenesis, present, and future therapies for chemotherapy-induced diarrhea (CID) and constipation (CIC), which are both prevalent and may necessitate lowering doses, delaying, or even stopping treatment. Rehydration, loperamide, and octreotide are currently available treatments for CID, which are potentially fatal due to dehydration and an electrolyte imbalance. But it also mentions preclinical and clinical studies on potential CID treatments. These include calcium-activated chloride channel blockers, glucuronidase inhibitors, antibiotics, and probiotics. Cannabinoid agonists are also included. One common cause of CIC is the overuse of anti-diarrheal medications for CID, but the exact mechanisms by which it occurs are unknown. Laxatives and certain prokinetic medications are frequently used as current therapy. However, agonists that target intestinal guanylate cyclase C or chloride channels provide hope as prospective targets for CIC research in future.

The profile of toxicities linked to well-established drugs, like platinum-based chemotherapies, is constantly expanding due to the increased usage of anticancer treatments in various patient populations. As an illustration, it consider the prevalence of carboplatin hypersensitivity reactions in kids receiving treatment for solid tumors like low-grade gliomas [17]. According to studies, this drug can cause hypersensitivity reactions in up to 47% of youngsters. The prevalence rises with more infusions rather than just the quantity of the medication, and those at higher risk include younger children, girls, and people with additional allergies. Cisplatin, another platinum-based chemotherapy, may make cancer survivors more susceptible to cardiovascular disease [18]. Used five weekly intraperitoneal doses of cisplatin in male Wistar rats to investigate potential explanations underlying this. At lesser amounts, their model showed signs of vascular endothelial alterations, while at the maximum dose, there were effects on cardiac function. Contrary to cardiovascular damage, nephrotoxicity brought on by cisplatin is widely known. Reveal positive findings for a plant called *Emblica Officinalis* and possible preventive effects (Indian gooseberry). Pretreatment with *E. officinalis* reduced the inflammation and oxidative damage brought on by a single intraperitoneal dose of cisplatin, protecting male Wistar rats from nephrotoxicity. A growing body of research indicates that chemotherapy-induced chronic subclinical skeletal muscle damage significantly affects the long-term health of a substantial number of cancer survivors. Unfortunately, thorough mechanistic studies examining the possible influence of anticancer drugs on skeletal muscle are currently lacking. It is the first to talk about how lean muscle responds to repeated oxaliplatin doses, including aspects of mitochondrial activity. Additionally, they show that the small molecule BGP-15 protects against oxaliplatin-induced muscle atrophy, muscle collagen deposition, and changes in muscle mitochondrial function in their model, which uses male BALB/c mice given six intraperitoneal doses spaced out over 12 days [19].

Anticancer medication-induced central and peripheral neurotoxicity can significantly lower cancer survivors' functional capacity and quality of life years after

their treatment. A review of clinical trials on biological indicators linked to cognitive dysfunctions during and after chemotherapy in cancer patients. This research topic includes. The authors cited research demonstrating alterations in several circulating variables and components of the cerebrospinal fluid connected to chemotherapy-induced persistent cognitive dysfunctions. These variables and genetic polymorphisms may be utilized as predictive indicators to find individuals who are more likely to experience mental problems due to chemotherapy [20].

Numerous anticancer medications, such as platinum-based medicines, vinca alkaloids, taxanes, proteasome, and angiogenesis inhibitors, produce chemotherapy-induced peripheral neuropathy (CIPN). Long-term CIPN is linked to high morbidity, including sleeplessness, ataxia, and depression [21]. Particular chemotherapeutic agents bring on the symptoms, pathophysiological mechanisms, and risk factors of long-term CIPN. However, there is an urgent need for well-developed long-term CIPN preventive and treatment techniques. Thus, the inclusion of two novel research on this subject in this research topic is pleasing. Limb hypothermia to avoid CIPN brought on by paclitaxel in breast cancer patients is being evaluated in a preliminary clinical trial. The activity of many sensory and motor nerves was monitored using nerve conduction tracing before, during, and after treatment. According to the study's findings, some patients undergoing paclitaxel treatment may benefit significantly from continuous-flow limb hypothermia because it can retain specific nerve conduction characteristics. Another pilot study that shows limb hypothermia may be able to help breast cancer patients with the symptoms of peripheral neuropathy brought on by the drug paclitaxel supports these findings [22]. Tempol, a reactive oxygen species scavenger that was previously proven helpful in a rat model of cancer-induced bone pain, was given systemically. This treatment also reduced and stopped the neuropathic pain that paclitaxelinduced in the rats. There is proof that chemotherapy-induced enteric neuropathy may be a factor in cancer survivors developing chronic gastrointestinal dysfunction. Therefore, additional research on neuroprotective drugs to prevent this kind of neurotoxicity may also be necessary.

Finally, the effectiveness of chemotherapy against cancer can be increased while minimizing its adverse effects by combining natural bioactive chemicals with conventional chemotherapeutic medications. In some circumstances, the addition of bioactive substances may be able to break through the cancer cells' chemo or radioresistance. The synergistic effects of nutraceutical compounds such as flavonoids, stilbenes, terpenes, and curcumin are discussed in this research subject. The research on colorectal cancer cells, animal models, and clinical trials examined by the authors provides the most up-to-date information on the mechanisms of action of these drugs. However, using unapproved drug combinations and untested treatments may result in serious side effects and sometimes fatal toxicities. Give a case study on the deadly toxicity of the variety of dichloroacetate and the artemisinin derivative artesunate. Both medications have anticancer activity in vitro and in vivo, and they have been tested on a small number of cancer patients. However, taking both medicines together caused the patient to experience severe liver and bone marrow damage. The writers go over the research on these medications' adverse effects [23].

21.2.3 Polypharmacology

Cancer still poses a serious threat to world health despite advances in basic, translational, and clinical research. People in affluent nations today have a near 50% lifetime risk of having cancer. During the next 20 years, the number of cancer deaths globally is expected to increase to 13 million annually [24]. These startling statistics show how vital it is to hasten the development of new cancer treatments [25]. Our understanding of oncogenesis and cancer development has greatly expanded in recent years [26]. Allowing for the gradual replacement of the universally effective cytotoxic chemotherapy medications with more individualized, secure, and targeted cancer therapies that take advantage of addiction-related oncogene and no oncogene weaknesses [27]. However, despite notable increases in survival rates for some cancer types, many single-agent-focused treatment responses are only momentary. Molecular research and deep sequencing increasingly reveal incredible genetic complexity, which helps to explain why an overly straightforward and single-targeted medicine strategy for treating cancer had very little effectiveness in terms of extending survival. There is an urgent need for solutions to the chemotherapeutic and molecularly targeted medication resistance that is impeding disease control and treatment. Cancer is becoming recognized as a complex and adaptive system from an evolutionary standpoint [24].

The problem of cancer drug resistance has been addressed using many different tactics. First, since just 5% of the more than 500 cancer-causing proteins discovered to date are currently targeted by treatments, one may more fully utilize the druggable cancer genome [28]. Even though this is significant, the average cancer tumor contains two to eight harmful mutations [25]. Therefore, focusing on a particular mutant protein may not be the best course of action, especially if the target is limited to sub-clonal branches of the evolutionary tree of cancer. For this reason, any increase in cancer proteins that have been medicated needs to be accompanied by more intelligent applications of the medications in question. A different and widely acknowledged treatment for polygenic cancer drug resistance is rational combinatorial targeted therapy, which has already produced numerous authorized pharmaceutical combos. Unfortunately, testing every drug combination is impractical due to the exponential growth in the number of possible combinations; hence, sophisticated techniques for ranking and evaluating combinations are urgently needed [29]. Additionally, new data indicates that a significant percentage of cancer-driver genes are altered at very low frequencies [30]. To optimize the potential benefits of combinatorial therapy, it may not be cost-effective to produce a specialized medicine for each one, and it may be difficult to drug the entire cancer genome [31]. The creation of network medications, which can block several cancer-related cellular signaling pathways, is a third suggested remedy for overcoming or preventing resistance. Overall, a combination of these tactics, together with promising novel medications like immunotherapies, will help improve cancer treatment because drug resistance is the most significant single factor impeding progress [32] and is likely needed to achieve long-term survival and

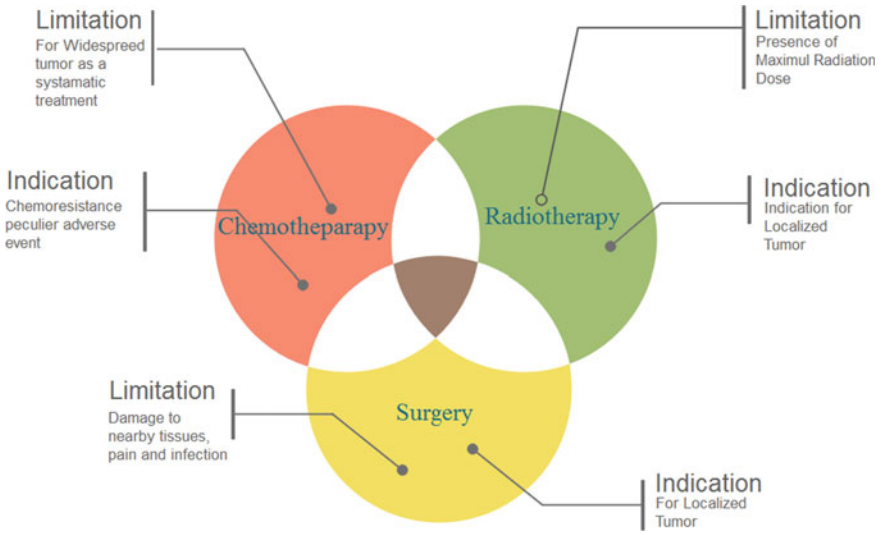

Fig. 21.1 Limitations and indication of the three major cancer treatment modalities

cure. Limitations and indication of the three major cancer treatment modalities are represented in Fig. 21.1.

21.3 Advancement of Nanomaterials-Based Formulations in Cancer Therapy

There are several major categories of advanced nanomaterials being used in cancer therapy. These nanomaterials, which target cancer cells, and the tumor microenvironment, have been customized for a variety of cancer therapies to reduce adverse effects and lack of selectivity, as well as to improve bioavailability and drug loading capability. Despite an increase in the number of studies, very few nanocarriers have been approved in recent years. More research on the targeted delivery of drugs through nanocarriers to improve permeability, retention effects and decrease systemic toxicity, is required to enhance clinical translation. One main advantage of chemotherapy based on nanomaterial is targeted delivery when compared with free drugs. Recent advancements in nanomaterial-based targeted delivery have been made. The concept of targeted delivery of drugs aims for specific targeting of specialized cancerous cells, which can be accomplished through active or passive targeting. Passive targeting employs the enhanced permeability and retention effect (EPR), whereas active targeting employs conjugation with aptamer, antibodies, small molecules, and peptides. Targeted drug delivery reduces systemic adverse effects in healthy cells, prevents the degradation of drug, solubility, and drug loading capacity, and increases half-life.

21.3.1 Nanoparticles

Nanoparticles are small particles with nanoscale dimensions. Polymeric nanoparticles (PNPs), extracellular vesicles, monoclonal antibody-conjugated (mAb) nanoparticles, and metallic nanoparticles are among the nanoparticles (NPs) that have gained a lot of attention. Polymeric nanoparticles are colloidal large molecules with submicron sizes ranging from 10 to 1000 nm. PNPs, as drug carriers, transport drugs and acquire sustained and controlled cancerous sites [33], and a nanocapsule or a nanosphere is formed when drugs are surrounded or linked to the outer layer of the nanoparticles. The components of nanoparticles have evolved. At first, nanoparticles were created using non-biodegradable polymers including polyacrylates, polystyrene, polyacrylamide, and polymethyl methacrylate (PMMA) [34]. PNPs developed by these materials must be excreted from the body promptly to prevent toxicity and chronic inflammation. Difficulty in degradation and slow rate of excretion of these nanoparticles made from polymers lead to the deposition in the tissue to the toxic level. Biodegradable polymers have been developed to strengthen drug release kinetically, raise biocompatibility, and decrease toxicity. Polylactic acid (PLA), poly (amino acid), poly lactic-coglycolic acid (PLGA), and polycaprolactone (PCL) are examples of these polymers [35], and natural polymers include gelatin, albumin alginate, and chitosan. Because of their characteristics and structure, these polymeric nanoparticles have distinct advantages. PNPs aid in the stabilization of volatile pharmaceutical agents. PNPs offer an additional route of administration for chemical drugs which includes oral and intravenous administration, as well as greater loading capability to protect drugs from degradation, which aids in the reduction of undesirable toxicity to healthy cells; for example, PNPs containing cisplatin, such as α-tocopheryl succinate or dexamethasone, have been used in the chemotherapy to prevent cisplatin associated ototoxicity.

Monoclonal Antibody-conjugated (mAb) Nanoparticles

Current advancements in mAb nanoparticles have been made. Monoclonal antibodies (mAbs) are extensively employed in targeted therapies due to their precise targeting capabilities and anticancer activity. Furthermore, in recent times, mAbs have been used in the development of novel anticancer nanoplatforms and have been at the top of the list of the research area. To improve the therapeutic effectiveness of chemotherapeutic agents, mAbs are conjugated with cytotoxic agents, which are known as antibody–drug conjugates (ADCs). By guiding the drug complex with specific antigens differentially expressed in cancerous and healthy cells, greater precision and low toxicity can be obtained. Trastuzumab is a monoclonal antibody which is used in the treatment of breast cancer with positive expression of human epidermal growth factor receptor 2 (HER2). Trastuzumab has been studied in the antibody–drug conjugates system, and the results indicate enhanced treatment effectiveness over the conventional Trastuzumab alone. An antibody–drug nanoparticle has been developed with a core containing paclitaxel and an outer layer containing trastuzumab. Two HER2-positive cell lines and one HER2-negative cell line have

been treated individually with this newly developed NP, paclitaxel, and trastuzumab, and the results were encouraging: The NP complex demonstrated better antitumor activity than paclitaxel or trastuzumab alone, as well as lesser toxicity in epithelial cell of the human breast. Trastuzumab nanoparticles based on the ADC system are emerging as promising nanoplatforms in cancer therapy, and extensive research is currently underway [36, 37].

21.3.2 Liposomes

Liposomes as a nanocarrier system for the delivery of drugs offer stability to the formulation and enhanced pharmacokinetics, as well as "passive" or "physiological" targeting of malignant cells. Liposomes are categorized into two types based on these characteristics: unilamellar vesicles and multilamellar vesicles (MLV). Small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV) are two types of unilamellar vesicles (LUV). Multilamellar liposomes have an onion-like structure, whereas many unilamellar vesicles can form inside other vesicles to form multilamellar concentric phospholipidspheres separated by water molecules [38]. According to exhaustive investigation on nanocarriers, the latest liposomes have a wide range of distinctive features and characteristics; consequently, novel applications depending on liposome materials have emerged. Primarily, three main problems were identified and addressed during the advancement of liposomes. Researchers have been struggling to overcome biological barriers and avoid fast clearance. As previously stated, biological barriers have been serious technological hurdles for nanocarriers to resolve. Liposomes are phagocytized by the mononuclear phagocyte system, which is primarily found in the spleen and hepatic cells. One of the most successful strategies for extending liposome half-life is membrane modification. Covering the membrane with polymers, peptides, proteins, or other molecules improves the capability to avoid the mononuclear phagocyte system and thus aids in the achievement of prolonging the circulation time of liposomes. Such kinds of liposomes are generally referred to as stealth liposomes. Later, it was discovered that polyethyleneglycol conjugated liposomes have a prolonged half-life than the other modified liposomes. Based on these findings, doxorubicin-loaded PEG-liposomes were used for the treatment of Kaposi's sarcoma in a patient with HIV. Loading of drugs and controlled release of liposomes are also critical issues that must be addressed while designing liposomenanocarrier. Drug effectiveness in cancer chemotherapy is influenced by bioavailability. Since the bioavailability of doxorubicin-liposomes is less than that of free doxorubicin, increasing the bioavailability should be taken into account while developing liposomes. Controlled release and co-delivery are two main uses of liposomes [39, 40].

21.3.3 Solid Lipid Nanoparticles (SLN)

Solid lipid nanoparticles (SLNs) are made from lipids that are solid at both room and body temperature. They have emerged as an effective, less toxic, and multifunctional system for the delivery of anticancer agents. Liposomes, SLNs, and nanostructured lipid carriers (NLCs) are three major categories of nanomaterials that are receiving a lot of interest in the latest studies and clinical trials. Liposomes were accepted in 1965 as the first enclosed microscopic phospholipid bilayer nanosystem. Liposomes are spherical particles composed primarily of unilamellar and multilamellar phospholipids, with sizes of range from 20 nm to 1 um. Liposomes are made from a hydrophobic phospholipid bilayer and a hydrophilic core. This structure allows the encapsulation of both hydrophobic and hydrophilic therapeutic agents. Liposome prevents the degradation of the drug embedded in the core cavity from environmental factors while circulating in the bloodstream of the human body. SLNs are colloidal nanomaterials having a size of 1–100 nm, and there are two most crucial factors (size and number of bilayers) that can influence the amount of loading drug and systemic half-lives of the drug. Due to the strict size limitations, SLNs are known as zero-dimensional nanomaterials, as they are at least one dimension smaller than other larger nanomaterials. Unlike liposomes, solid lipid nanoparticles contain solid materials like solid lipids, water, and emulsifier. Lipids used in SLNs include triglycerides, PEGylated lipids, partial glycerides, waxes, steroids, and fatty acids. There are distinctions and similarities between both SLNs and traditional liposomes in aspects of function and structure. The outer layer of lipid and chemical drug delivery function in both have lot of similarities. Apart from traditional liposomes, which are made up of lipid bilayers which encircle by an aqueous pouch, some SLNs lack a contiguous bilayer and just form a micelle-like arrangement and drugs entrapped in a non-aqueous center. The lipid material of SLNs at body temperature is solid and has greater stability and longer release than the liposome. However, due to their crystalline structure, SLNs have restrictions such as unexpected gelation and inherent low incorporation rates [41, 42]. Over the last two decades, NLC carriers have been developed as a better generation of both liposomes and SLNs. NLCs are developed as a system that consistsof a core matrix containing both liquid and solid lipids to increase stability and drug loading capabilities while sustaining protection function, non-immunogenicity, and biocompatibility. NLCs can be administered orally, parenterally, inhalational, and intravenously. NLCs have received a lot of interest in recent decades since so many drugs used for the management of cancer are lipophilic.

21.3.4 Dendrimers

Unique macromolecules known as dendrimers have a hyperbranched, well-defined architecture. Dendrimers' highly branched and adaptable surfaces are their most noticeable feature. These dendrimer polymers typically have sizes between 2 and

15 nm; however, some particularly large dendrimers can have diameters between 14 and 20 nm. The dendrimer molecules consist of three main structural parts: an exterior surface conjugated with substances useful in the treatment of cancer, a central core that encapsulates therapeutics through noncovalent loading, and branches that make up the interior dendritic system, such as 5-ALA (5-aminolevulinicacid), polypropylenimine (PPI), poly(ethylene glycol), bis-MPA (2,2-bis(hydroxymethyl) propionic acid), polyamidoamine (PAMAM). Dendrimers have several benefits over other nanomaterials, including a specified molecular mass, flexible, customizable branch, a low polydispersity index (PDI), and remarkable hydrophobic drug solubility and bioavailability. Because of their capacity to bind to nucleic acids and form complexes due to their cationic nature and positively charged surfaces, dendrimers are useful nucleic acid nanocarriers. Two extensively investigated dendrimers with diverse application methods are PAMAM and PPI. Using fluorescence imaging, a PAMAM dendrimernanohybrid was created to manage MDRs and monitor cancerous cells at the same time. Separate complexes were made for each. Blue-emitting carbon dots(CDs), an antineoplastic drug, and noncovalent connections between them made up the first component, a CDs/DOX complex. The other component is G5-RGDTPGS, which targets the cyclic arginine-glycine-aspartic (RGD) peptide by combining the therapeutic efflux inhibitor d-tocopheryl polyethylene glycol(TPGS) 1000 succinate with generation 5 (G5) PAMAM dendrimers. An electrostatic connection between two components created a dual therapeutic-loaded nano-hybrid framework. The luminescence of CDs enabled in vitro fluorescence, and the existence of RGD ligands that target v3 integrin receptors overexpressed in tumor cells enabled targeting selectivity [43, 44]. The outcomes demonstrated that TPGS significantly inhibited cancer cells.

Additionally, completely different materials can be delivered using the dendrimer's co-delivery capability. To treat colon cancer, people frequently use doxorubicin (DOX). The death receptors DR4 and DR5 are highly expressed in numerous types of cancers and can bind to the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which is an essential part of the apoptotic pathway. The Pishavar group combined the TRAIL and DOX plasmids into a dendrimernanocarrier that displayed bigger doxorubicin and a more powerful antitumor effect than reconfigured carriers containing only the TRAIL or DOX plasmid. A PAMAN nanocarrier based on dendrimer was developed to treat liver cancer cells using chemotherapy and photothermal therapy. Although unmodified PAMAN dendrimers have drawbacks like poor cellular internalization, low transfection efficiency, and unstable encapsulation, the nanomaterial exhibits strong potential in combination therapy because of its competitive contrast properties [45].

21.3.5 Carbon Nanotubes (CNTs)

Carbon nanotubes (CNTs) are well-ordered, hollow, and have a variety of characteristics. Some of these include a large surface area, a high aspect ratio, and an amazingly low weight. CNTs are usually categorized as either single-walled (SWCNT) or multi-walled (MWCNT). Based on the temperature at which they were created, a single carbon layer with a diameter of between 0.4 and 2 nm makes up SWCNTs. A larger diameter has been seen to result from a higher growth temperature. In contrast, MWCNTs generally consist of multiple cylindrical carbon layers with inner tubes that are 1–3 nm in diameter and outer tubes that are 2–100 nm in diameter. According to some research, the basic carbon configuration of SWCNTs and MWCNTs has different structural relationships. SWCNTs have structures organized in helical, chiral, armchair, or zigzag patterns. On the other hand, the arrangement of the graphite sheets allows for the division of the structure of MWCNTs into two groups. The first is a "Russian doll"-like structure in which the graphite sheets are loaded one on top of the other, and the second is a parchment-like model in which a single graphite sheet is wound around itself. Carbon black and graphite can be heated in a controlled flame environment to make CNTs. This process results in irregularities in the size, shape, quality, purity, and mechanical strength of the CNTs. These problems have been addressed by methods like laser ablation, electric arc discharge, and catalytic decomposition of hydrocarbons. Different types of CNT with distinct features could be created based on the method of synthesis. The best fabrication method can be used depending on how the CNTs will be used. For instance, SWCNTs should be utilized instead of MWCNTs when CNTs are needed for electric transportation. Because MWCNTs are metallic and SWCNTs can only be semiconducting, this is the case. SWCNTs are known to be more effective than MWCNTs for the delivery of drugs. This results from the one-dimensional structure of the SWCNT and its efficient capacity for drug loading as a result of its extraordinarily large surface area. It has been demonstrated that an SWCNT-anticancer drug complex has a considerably longer blood circulation half-life than the anticancer drug alone, resulting in a more extended and sustained accumulation of the drug by cancerous cells. This is because of the enhanced retention and permeability effect. Numerous reports claim that after the medication is released into a specific location by the functionalized SWCNT, it slowly leaves the body through the biliary pathway before passing through the feces. As a result, it can be concluded that SWCNTs are promising nanoplatforms for potential future cancer therapeutics and suitable drug delivery candidates. SWCNTs may be employed for scanning as well. The fluorescence and structural characteristics of SWCNTs can be examined using Raman spectroscopy and single-molecule fluorescence spectroscopy methods.

Contrary to most single molecules or semiconductor nanoparticles, the results show that SCWNTs do not experience spectral or intensity fluctuations. Even identically constructed individual nanotubes exhibit various emission energies and line widths in their fluorescence spectra, and these differences are most likely brought on

by inadequacies in the immediate environment. MWCNTs are found to be more effective for the thermal treatment of tumors as compared to SWCNTs. This is because after being exposed to near-infrared light, the MWCNTs release a large amount of vibrational energy. This energy can be used to kill cancer cells when it is released inside a tissue, where it causes localized heating [46–48].

21.3.6 Quantum Dots (QDs)

Quantum dots are being extensively studied as biomedical imaging probes because of their unique electronic and optical properties. Quantum dots are semiconductor crystals with a typical nanometer scale and are frequently utilized to boost the efficiency of fluorescent markers in bioimaging. Quantum dots (QDs) differ from organic fluorophores in that they exhibit distinct electronic and optical characteristics, including size and composition of QDs that allow for tunable fluorescence emission from visible to infrared wavelengths, as well as high luminance and photostability. Three most widely used QDs made of carbon are nanodiamonds, carbon diamonds, and carbon quantum dots (GQDs). Carbon QDs are most frequently used in bioimaging, which can be employed for carcinoma detection and imaging. Because of their excellent biocompatibility, quick excretion, and inherent grand surface that are appropriate for molecular conjugation, GQDs are recognized as novel nanomaterials in chemotherapy and biosensing. For biocompatibility and pH sensitivity, a photoluminescent glycodendrimer with terminal cyclodextrin molecules system was developed. The surface on which PAMAM could grow was produced by GQDs. The emission spectra from GQDs-PAMAM-CD and GQDs were recorded after 365 nm UV light excitation. The result showed significantly greater effectiveness in destroying cancerous cells than DOX alone had because it contained GQDs. It could then be used as a photoluminescent imaging agent. GQDs' fluorescent properties were also utilized in the development of a novel carrier system for the delivery of drug at the target site.

To create folic acid-doped graphene quantum dots (FA-SGQDs), pyrolyzing citric acid (CA), folic acid (FA), and 3-mercaptopropionic acid were all that was required (MPA).The complex fluoresced blue and generated an emission band at 455 nm when excited at a wavelength of 370 nm. This demonstrated that TA-SGQDs entered FR-positive tumor cells through a non-immunogenic FR-mediated endocytosis process. Applications for GQDs including PTT and PDT were being researched in addition to biosensing and bioimaging. A precisely modified GQD with strong (1070 nm) NIR-II region absorption was created. The so-called 9 T-GQDs showed the potential of GQDs in PTT because of their tunable fluorescence, high photothermal conversion efficacy (33.45%), and uniform size distribution, which made them efficient for removing tumor cells and reducing tumor progression when exposed to NIR-II radiation. A combined photodynamic-chemotherapy DDS focused on carbon quantum dots. A mono-(5-BOC-protected-glutamine-6-deoxy)-cyclodextrin (CQD-glu-CD) moiety was added to 5-aminolevulinic acid (5-ALA), and so these compounds were

coupled with DOX-loaded CQDs. High cytotoxic effect and morphological changes were present in MCF-7 cancer cells; in addition, 15 min of 635 nm (25 mW cm^2) radiation induced ROS, which had greater therapeutic effects. With regard to targeted therapy, PDT, cancer imaging, and the induction of antitumor immunity, CDs and nanodiamonds were also investigated in the management of cancer. Research on carbon QDs has advanced more recently as compared to other carbon materials. Major obstacles to the clinical application of QDs include a lack of standardized protocols for high-quality QD fabrication and their concise reaction pathway and formation process [49–51].

21.3.7 Micelles

Micelles have been shown great efficiency as nanocarriers to deliver drugs owing to their advantageous properties such as small particle sizes ranging from 5 to 100 nm, prolonged circulation time, excellent biocompatibility, and enhanced permeability and retention effect. They are spherical or globular colloidal nanoscale structures created by self-stabilization of amphiphilic block co-polymers directly solved in an aqueous medium at a specific temperature and concentration known as critical micelle concentration (C.M.C) [52]. The hydrophilic shell keeps the hydrophobic core stable and makes both polymer and hydrophobic pharmaceuticals water soluble. The hydrophobic core functioned as a reservoir for the hydrophobic agent. Hydrophobic drugs with poor water solubility have been linked to therapeutic issues including reduced absorption, low bioavailability, and complications associated with drug aggregation like Embolism. On the other hand, many medications, particularly those used to treat cancer, are linked to poor water solubility. Polymeric micelles have shown the potential to enhance the solubility of the drug in water by 10 to 5000 times and have been extensively investigated as nanocarriers via injectable route for those anticancer drugs that have poor water solubility [53]. Drugs can be incorporated into the micelle through chemical, physical, or electrostatic interactions. Paclitaxel-loaded pH-sensitive and fluorescent micelles are stable and have the potency to release the drug in a controlled manner with a favorable toxicity profile. The anticancer ability of micelles was found to be enhanced as compared to the paclitaxel alone [54]. Polymeric micelles loaded with two hydrophobic drugs, curcumin, and 5-fluorouracil (5-FU) formed by using pH-responsive poly (2-vinyl pyridine)-b-poly (ethylene oxide) block co-polymer can prevent these drugs from the photo-chemical degradation and permit UV sterilization of the micelle system loaded with the drug without reducing drug anticancer efficacy of drugs [55]. Poly (lactide-co-glycolide acid) (PLGA) and polylactic acid (PLA) both are biocompatible and biodegradable polymeric materials and have received FDA approval. It has been shown that PLGA-PEG micelles exhibit a higher therapeutic efficacy than a free drug by delivering all-trans retinoic acid (ATRA) and programmed death ligand 1 (PD-L1) for the management of oral dysplasia and oral squamous cell carcinoma

[56]. The co-delivery approach is crucial for the synergistic benefits in the treatment of cancer when using a multifunctional micelle. The co-delivery system based on the temperature-responsiveness nature of micelle has developed with the capability to transport anticancer drugs and genes simultaneously [57]. To prevent the premature release of the drug and enhance its stability, a multipurpose star-shaped micelle was developed. These micelles are formed from the four-arm poly (ethylene glycol)-poly(ε-caprolactone) (PEG-PCL) co-polymer linked to folate ligands, which display good stability and prolonged release of drug from micelle while allowing the immediate release of drug in acidic environments [58].

21.3.8 Hydrogel

Hydrogel has been largely investigated for the development of a drug carrier for the controlled release of drugs at the tumor site. A hydrogel also referred to as aquagel is a thermodynamically compatible three-dimensional (3D) framework of hydrophilic polymers that contains a large number of aqueous phases that cannot solubilize the network due to the existence of interconnections known as crosslinks [59]. Drug delivery systems through hydrogel can be used in various types of formulation, including oral, rectal, ocular, epidermal, and subcutaneous administration. Hydrogels are classified into three types based on their size: nanogels (200 nm), microgels (0.5–10 m), and macroscopic gels. The various functions of the hydrogels and the route of delivery by which they are administered for the treatment of tumor are determined by their different sizes and structures [60]. Hydrogels have become more popular among the other vehicle for cancer therapy due to their four main characteristics such as biodegradability, awesome biocompatibility, eminent drug-encapsulating abilities, and controlled release of the drug. Therefore, these features have been extensively used to develop novel drug delivery systems for cancer therapy. Various types of synthetic and natural polymers, for example, chitosan, polyesters, hyaluronic acid, and polyphosphazene are employed to manufacture hydrogels for cancer therapies that are biodegradable. Because hydrogels can be loaded with a wide range of molecules from micro to macromolecules such as proteins and DNA, as well as hydrophilic and hydrophobic drugs, they are promising candidates for the release of drugs at the required site controllably. Hydrogels are considered to be intelligent or smart hydrogels due to their ability to produce beneficial effects against various intrinsic or extrinsic stimuli such as pH, temperature, electric field, magnetic field, and light. Upon receiving the stimulus hydrogels alter their physical and chemical nature for the smart delivery of entrapped drugs in a controlled manner [61]. Drug-hydrogel systems are susceptible to the temperature and acidic pH of microenvironments of the cancer cell, allowing the separation of polymer networks and swelling or breakdown, resulting in targeted drug release [62]. Hybrid microgels were prepared that release multiple drugs when activated by the unique conditions of tumor cell microenvironments (low pH, high temperature, and raised glutathione levels), which could be an effective approach for the treatment of cancer

by more than one drug [60]. Self-assembled hyaluronic acid nanogels were designed to deliver doxorubicin and cisplatin simultaneously for the management of osteosarcoma. Cisplatin served as a crosslinker as well as an anti-carcinogen. As a result, doxorubicin was not released prematurely, and a synergistic anticancer activity was obtained [63]. A further study, methotrexate, was combined with chondroitin sulfate to develop a self-assembled nanogel for the delivery of the antitumor drug. These nanogels have been shown to enhance drug solubility, increasing anticancer activity while decreasing methotrexate side effects [64].

21.3.9 Silica Nanoparticle

Mesoporous silica nanoparticles (MSNs) have evolved as an impressive nanomaterial for anticancer drug therapy. According to the IUPAC, mesoporous materials have pores with diameters ranging from 2 to 50 nm. Mesoporous silica nanoparticles have a honeycomb-like porous silica configuration. They are the perfect platforms for designing multifunctional nanosystems because of their advantageous properties, such as large surface area, tunable size (50–200 nm) and shape, high pore volume, robustness, and ease of surface modification and biocompatibility. Tunable particle size is a necessary criterion for being a smart carrier of nanomaterial, and tunable pore size facilitates the adhesion of drugs with a wide range of molecular shapes on MSNs [65]. MSNs have earned a lot of attention owing to their special characteristics, such as porosity, dual or multiple drug loading capabilities, biodegradability, biocompatibility, and controlled release of drugs. But besides such distinguishing characteristics, early release of drug and hemolysis of RBCs, are major drawbacks of mesoporous silica nanoparticles that restrict their successful applications and necessitate additional appropriate measures. To overcome these problems, a lipid coating was applied to the MSNPs which gives a gatekeeping effect to sustain the therapeutic agent within pores and prevent early release, as well as increased the carrier's cellular uptake [66]. Mesoporous silica nanoparticles are reasonably stable, allowing for a wide range of chemical modifications. Mesoporous silica nanoparticles are classified into three types: ordered MSNs, rattle or hollow MSNs, and shell or core MSNs. Ordered MSNs have homogeneous pore sizes with a well-organized pore structure. Due to excessive drug loading and ease of surface functionalization, hollow-type MSNs are preferred over ordered MSNs. The functionalization of mesoporous silica nanoparticles can be enhanced by attaching them to solid cores such as platinum and gold. However, gold is primarily used to functionalize the outer layer of the mesoporous silica nanoparticles for numerous applications [67]. Numerous studies show that when MSNs are used as acarrier for the delivery of antitumor agents like drugs and siRNA, tumor growth is significantly reduced [68]. Because of hemolysis of RBCs, non-specific binding to human serum albumin (HSA), and phagocytosis, traditional MSNs have limited systemic half-life; however, the circulatory half-life of mesoporous silica nanoparticlescan be enhanced by PEGylation [69]. The opening of pores of smart mesoporous silica nanoparticlescan be governed by implanting co-polymers onto

their surfaces. The co-polymers grafted on the surface of the MSNs function as a gatekeeper which inhibit the premature release of drugs from the nanoparticles. Folate, transferrin, and peptides can be used to modify the surface of MSNs for active targeting off to the target cell. PEGylation can be employed to produce stealth behavior. Drug can be unloaded from the mesoporous silica nanoparticlesin response to a variety of stimuli such as a change in pH, enzymatic modification, alteration in temperature, redox reaction, light, and magnetic field. Enhanced transfection efficiency delivery of genes can be achieved by employing positively charged MSNs. Hsiao et al. prepared an MSNs-based theranostic drug delivery system that could be utilized for tumor imaging as well as delivery of drugs to the targeted site. The epithelial cell adhesion molecule (EpCAM) is a cell surface receptor that is ubiquitously expressed in colorectal tumor tissues in contrast to neighboring healthy tissues [70], making it an excellent colorectal biomarker in active targeting. Therefore, the therapeutic efficiency of doxorubicin was enhanced against SW620 colorectal tumor cells by modifying MSNs with a DNA-EpCAM aptamer and demonstrated substantially increased doxorubicin toxicity as compared with the non-targeted MSNs [71].

21.3.10 Magnetic Nanoparticles

Magnetic nanoparticles (MNPs) are nanomaterials made up of material obtained from natural or synthetic sources that has magnetic properties and sizes ranging from 5 to 100 nm [72]. MNPs typically have a magnetic core in the center and a surface coating adjacent to the functional coat. Iron, nickel, manganese, cobalt, and other pure metals are used to create magnetic nanoparticles. Iron oxide nanoparticles are mostly employed in biomedical applications. Nanoparticles are categorized as paramagnetic, ferromagnetic, anti-ferromagnetic, diamagnetic, and super-paramagnetic materials based on their magnetic properties. Diamagnetic materials show zero magnetic moments when there is no external magnetic field applied whereas, in the presence of an externally applied magnetic field, diamagnetic materials are repelled. However, without a magnetic field, paramagnetic materials lost their magnetic characteristics and create a weak magnetic moment. Moreover, despite there being no externally applied magnetic field, ferromagnetic materials retain their magnetized property which is attributed to unpaired electrons. A significant magnetic moment is produced when their domains align with the magnetic field direction. Due to the equal magnitude and opposite moments of magnetic moments in anti-ferromagnetic materials, there is no magnetization. Because ferri-magnetic materials are made of two distinct ions with unequal opposing moments, they exhibit a net spontaneous magnetic moment. In the presence of magnetic fields, super-paramagnetism functions as a paramagnet. The biocompatibility or toxicity of magnetic nanoparticles is affected by several variables, including the coating, size, and kind of core material. Numerous innovative coating and functionalization techniques are required to increase the effectiveness of magnetic nanoparticles. These techniques can either improve biocompatibility or prevent phagocytosis by the reticuloendothelial system

(RES). Magnetic nanoparticles have been created and are now being used for the delivery of antitumor drugs at the targeted site. A powerful permanent magnet is used to develop a gradient magnetic field to hold the drug-coated particles at the desired location after they have been injected into the systemic circulation of the patient. Additionally, magnetic nanoparticles coated with a therapeutic anticancer drug might be administered intravenously, moved to and kept at specific places, making them a suitable candidates for drug delivery. Targeted imaging and simultaneous therapy with antibody-bound fluorescent magnetic nanoparticles were used for gastric cancer, and doxorubicin delivery with self-assembled reducible polyamidoamine-magnetic iron oxide nanoparticles was used for cancer therapy [73]. Microbubbles and nano-liposomes coated with magnetic nanoparticles can improve the effectiveness of the nano-microcarriers' drug delivery to the plaque [74].

21.3.11 Gold Nanoparticles

The present era of gold nanoparticle (AuNPs) synthesis started about 150 years ago with the work of Michael Faraday, who was perhaps the first to notice colloidal gold solutions possessing properties different from solid gold. Gold nanoparticles have emerged as an excellent candidates as vehicles to deliver cancer therapeutics at the target site because of their distinct chemical and physical characteristics. Among metallic nanoparticles, AuNPs are the safest and least toxic agents and have unique characteristics such as optical, electrical, and magnetic properties with a large surface area. Gold nanoparticles are made up of a gold core surrounded by an organic ligand outer layer. However, while developing an efficient drug delivery system, various factors must be taken into the consideration including gold nanoparticle size, surface chemistry, and charge, which have been proven to influence their absorption and subsequent intracellular fate [75]. Gold nanoparticles have been utilized to deliver a wide range of payloads from small molecular drugs to big biomolecules, e.g., proteins, nucleic acids (RNA or DNA) to the target cell [76]. External stimulation (X-ray, light, and laser) or even internal stimulation (redox condition, pH, and matrix metalloproteinase) either could be used to release the drug from AuNP. GNPs have generally synthesized by three methods including chemical, physical, and biological methods. Drugs and other molecules can be linked to nanoparticles either actively or passively for the targeted drug delivery. Easy accumulation of nanoparticles in the tumor is achieved in passive targeting through the enhanced permeation and retention (EPR) effect which makes the tumor's vasculature usually leaky. Whereas in the case of active targeting ligands like monoclonal antibodies or peptides are attached to the nanoparticles. These ligands bind to their respective receptors present on the malignant cells, enabling endocytosis and drug delivery [77]. Smart nanocarriers should have the capability to be chemically stable in biological fluid. Gold nanoparticlesthat have not been modified are unstable in blood and are more likely to be taken up by the reticuloendothelial system (RES). PEGylation of gold nanocarriers is required to overcome these limitations. These PEGylated gold nanoparticlesexhibit improved

both solubility and stability under physiological conditions. AuNPs have greater penetration capability than as compared with conventional drugs and exhibit fewer risks when used in both diagnosis and treatment. AuNPs as a vehicle for drug delivery can improve pharmacokinetics and reduce systemic adverse effects of the drug as well as deliver a high concentration of the drug the at target site. The safety, biodistribution, and pharmacokinetics profile of dextran-coated gold nanoparticles were found to be enhanced in a mouse model, and also it has been demonstrated that the AuNPs primarily accumulate in the liver and spleen but do not exhibit hepatic or renal toxicity [78]. A chemical synthesis approach was used to manufacture hesperidin-loaded gold nanoparticles (Hsp-AuNPs). These nanoparticles displayed high cytotoxicity and apoptosis increase in human breast cancer cell lines [79]. Vehicles for specific drug delivery in cancer therapy are shown in Fig. 21.2.

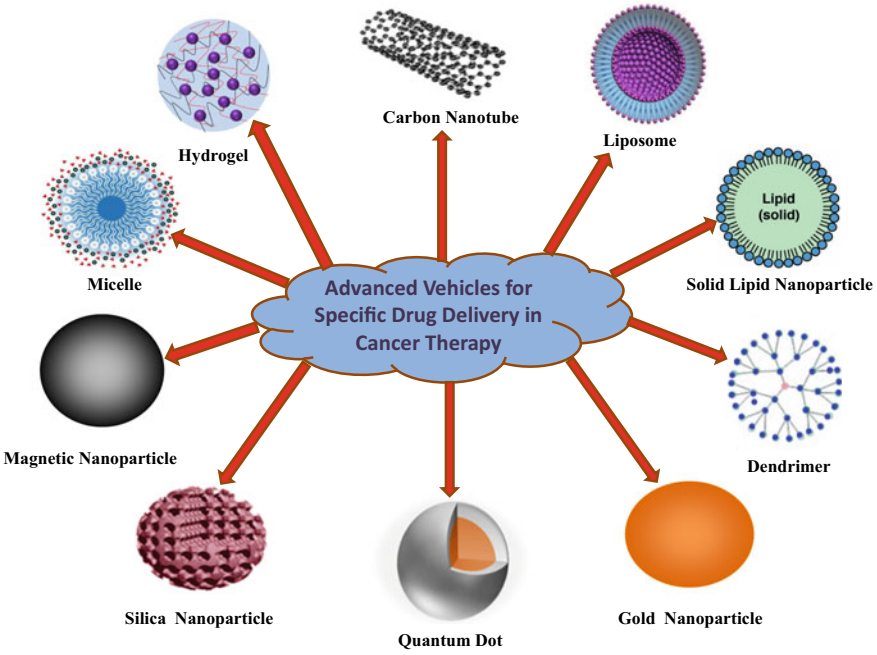

Fig. 21.2 Advanced vehicles for specific drug delivery in cancer therapy

21.4 Strategies Involved in Nanomaterials Targeting Cancer Therapy

21.4.1 Nanomaterials Involved in Cancer Cells Targeting

By focusing on cancerous cells, you can treat cancer naturally. Improved retention, permeability, and active targeting enable the delivery of chemical medicines or biomaterials to cancer cells using modified nanocarriers such as nanoparticles and dendrimers [80, 81]. These platforms frequently employ antibodies that target particular antigens that cancer cells overexpress on their surface. According just on encapsulated cargo, chemical medications that are endocytosed by cancer cells either demonstrate cytotoxicity or result in cell death. Advances have remained achieved now the supply of nucleic acids, and much research is being done on nano-drug delivery systems based on exosomes [82, 83], polymeric nanoparticles, liposomes [84], and dendrimers [85] to treat cancer.

A. **Passive Targeting**

In the late 1980s, it was noted that a small number of macromolecules accumulated preferentially in cancer cells. The first macromolecule identified as accumulating in the tumor was Poly (Styrene-co-Maleic Acid)-NeoCarzinoStatin (SMANCS), according to Matsuura and Maeda [86]. According to additional research, fenestrations in the weakened tumor blood vessels and insufficient lymph drainage, these, when combined form the "enhanced effect on retention and penetration," are the causes of this preferential distribution. The endothelial layer of blood arteries develops extra permeable in specific circumstances like hypoxia or inflammation [87]. In hypoxic conditions, rapidly expanding tumor cells often engulf or activate more blood vessels to keep up with their demands. Neovascularization is the term for this action. The wide holes in these new blood vessels cause them to be less permselective of tumor blood vessels than normal blood vessels, making them leaky. Its tumor microenvironment varies according to the type of cancer and the location of the malignancy [88, 89]. These enormous pores often referred to as fenestrations, range in size from 200 to 2000 nm [90]. This blood vessel's fast and faulty angiogenesis offers very little protection against extravasation, allowing nanoparticles to diffuse from it and eventually gather inside cancer cells. Extracellular fluid (ECF) draining into lymphatic arteries occurs frequently in healthy tissues with a normal flow rate of 0.1–2 m/s, maintaining continual drainage and renewal [91]. The lymphatic system is disrupted when a tumor develops, resulting in little interstitial fluid intake. Because they are not removed and accumulate in the tumorinterstitium, this property helps to keep nanoparticles, in the body longer [92]. This method indicates the increased permeation and persistence effect's better retention component. This unique property does not apply to chemicals that circulate quickly and are immediately flushed out of cancer cells [93]. To just provide tumor choice, enhance pharmacokinetics, and lessen adverse effects, it is, therefore, usual practice to encapsulate these minuscule

molecules in nanoscopic drug carriers to address these situations. Tumor microenvironmentis essential to passive targeting in addition to the increased permeation and retention effect. This increases the acidity of the surroundings and provides the main energy source for cell multiplication [94]. The pH-sensitive nanoparticles, which release medications at low pH, could take advantage of the tumor microenvironment's lower pH [95]. Passive tumor targeting is the name given to this method. Passive targeting mostly relies on the carrier's properties (size and circulation time) and the various tumor biology (vascularity, leakiness). There is no ligand specific to a particular subset of tumor cells in this type of tumor targeting. Fundamental aspects of tumor biology have a significant impact on the enhanced permeability and retention effect, including:

- Lymphangiogenesis and angiogenesis intensity or extent
- How far a perivascular tumor has invaded or spread
- Intratumor pressure.

The effectiveness of the nanoparticle medication delivery system is determined by these elements working along with the physicochemical properties of nanoparticles.

B. **Active Targeting**

The use of certain ligands or compounds such as folate and transferrin that bind to particles or receptors that stay selectively communicated or overexpressed on the target cells is necessary for active targeting. Ligand-mediated targeting is the name given to this method of targeting [96]. To increase affinity, the target must be adjacent to the nanoparticles that have ligands with particular actions, like holding and uptake [97]. This tactic increases the likelihood that nanoparticles will bind to cancer cells, which in turn increases drug penetration. By grafting antibodies onto the surface of liposomes, the first proof of this was found in 1980 [93]. Peptides and aptamers are then followed by many different types of ligands. To boost crosstalk without altering the overall biodistribution, the main technique attempts to increase nanoparticle interaction with the target [98]. The target substrate receptors' identification of ligands is a key component of active targeting; for example, ligands might be any of a wide proteins among a multitude of other compounds, peptides, antibodies, nucleic acids, carbohydrates, and tiny compounds like vitamins [99]. The transferrin receptor, folate receptor, glycoproteins, and epidermal growth factor receptor (EGFR) are the receptors that are most frequently researched. Through receptor-mediated endocytosis, ligand-target contact causes the membrane to infold and nanoparticles to become internalized. There are several processes via which active targeting occurs. Nanoparticles that target tumor cells, in general, carry out most tumor targeting. Their cell penetration is improved by this technique. One of the extensively investigated receptors is transferrin as was already mentioned. A specific subset of serum glycoproteins aids in the uptake of iron by cells. That receptor is known to be highly expressed in the majority of tumor cells, especially solid tumors, while they are expressed at a lesser level in healthy cells [100].

As a result, we can alter the nanoparticles with linked ligands that aim solely at transferrin. Targeting cancer-associated cells, such as angiogenic endothelial cells, is another possible strategy. Such cells are also close to the blood arteries within the tumor. By decreasing the blood supply to the cancer cells, this method enables the production of hypoxia and necrosis. Tumor tissues have been discovered to be more acidic than healthy tissues. Warburg effect has been used extensively to explain this [101]. This is why the metabolism of cancer cells switches to glycolysis, which produces lactic acid. The cell perishes as lactic acid builds up in it. As a response, the cells begin to overexpress proton pumps, which can increase the amount of lactic acid released into the extracellular space raising its acidity. Hence, a pH-sensitive medication delivery method based on liposomes has been researched. Target cancer cells are more likely to interact with ligand-coated nanoparticles because of the multivalent nature of the nanoparticles. Nanoparticles require multipart design because the chemistry of the ligand-target and the nanoparticle architecture affect the overall method's effectiveness. The effectiveness of the system is also influenced by other elements like the administration route, the size of the nanoparticles [102], and physicochemical characteristics like ligand density [103].

21.4.2 Nanomaterials Targeting the Tumor Microenvironment

Another approach is focused here on tumor microenvironment which contains tumor cells. As was already established, almost all cancers have active angiogenesis as a result of unchecked cell multiplication, which requires a lot of energy. Findings from research on this particular trait were encouraging. Sengupta created a nanoparticle system that uses combretastatin to specifically target aberrant tumor angiogenesis. Additionally, doxorubicin was co-encapsulated within the PLGA core [104]. The doxorubicin was able to be effectively absorbed more by tumor as a result of the combretastatin-induced quick shutdown of the malignant arteries, which improved both the total treatment efficacy and toxicity. Extracellular matrix (ECM), in addition to aberrant vasculature, has been studied in the treatment of cancer. Cancer proliferation, migration, invasion, and angiogenesis are guided by the extracellular matrix [105]. Collagen, hyaluronic acid, and different enzymes are a few of the key substances causing these malignant characteristics. The extracellular matrix's main structural protein is collagen. Helps tumor cells create migration pathways, whereas hyaluronic acid raises interstitial fluid pressure (IFP), which inhibits medication diffusion and penetration [106, 107]. Matrix metalloproteinases (MMPs), a type of enzyme, can control tumor microenvironment by modifying the function of Non-extracellular matrix substances like cytokines, receptors, and growth regulators [108]. One factor to consider while designing nanocarriers is the extracellular

matrix. Recombinant human hyaluronidase a PEGylated version that aims for extracellular matrix hyaluronic acid has therapeutic efficacy when combined with traditional chemical medicines, notably in those who express hyaluronidase at high levels [109]. The capacity of chemical therapies placed on nanocarriers to penetrate solid tumors has been improved by coating the carriers with hyaluronidase (HAase).This straightforward method exhibits improved antitumor activity [110].

21.4.3 Nanomaterial's Targeting for Immunotherapy

The growth and spread of cancer cells depend on the immune system. The advent of immunotherapy has revolutionized cancer care. It has been discovered that nanoparticles can be employed in conjunction with immunotherapy in addition to aiding in the targeted administration of chemotherapy. Immune checkpoint blockade therapy, cancer vaccine therapy, chimeric antigen receptor (CAR)-T cell therapy, and immune system modulator therapy are a few immunotherapy techniques used to activate the immune system against cancer cells [111]. "Nano-vaccines," "aAPCs (artificial antigen-presenting cells)," and "immunosuppressed tumor microenvironment targeting" are examples of nanoparticle-based immunotherapy. Tumor-associated antigens and "adjuvants" are delivered by nano vaccines to antigen-presenting cells like dendritic cells (DCs) [112]. Additionally, these can be used as adjuvants to improve "APC antigen presentation" and encourage DC maturation, which stimulates cytotoxic T cells with antitumor activity [113, 114]. It was discovered that liposomes, poly (lactic-co-glycolic acid) nanoparticles, and gold nanoparticles could deliver tumor-associated antigens into the cytoplasm of dendritic cells (DCs) [115].

The most popular inorganic nanoparticle, mesoporous silica, has demonstrated an adjuvant function that stimulates immunological response [116]. Artificial antigen-presenting cells directly engage T cell binding major histocompatibility complex (MHC)-antigen complexes. They also interact with co-stimulatory molecules, which activate T cells by binding to co-stimulatory receptors [117]. Another way to use nanoparticles in immunotherapies is to target the immunosuppressed tumor microenvironment. This is accomplished by focusing on crucial tumor microenvironment cell types like "myeloid-derived suppressor cells (MDSCs)," "tumor-associated macrophages (TAMs)," and "regulatory T cells." Chemoimmunotherapy has also demonstrated efficacy in the treatment of cancer. Consider a study that revealed that co-loading the chemotherapeutic Nutlin-3a with the cytokine GM-CSF in "spermine-modified acetylated dextran (AcDEX) nanoparticles" enhanced the proliferation of cytotoxic $CD8^{(+)}$ T cells and triggered an immunological response [118]. Some of the crucial immunological checkpoints include "programmed cell death protein 1 (PD-1)" and "programmed cell death ligand 1 (PD-L1)." Therefore, these are targeted by utilizing nanoparticles and immune checkpoint inhibitors. A study found that PD-L1/PD-1 conventional immune checkpoint drugs produced erratic results. Immunological checkpoint inhibitors and immune checkpoints are more likely to combine when poly (amidoamine) dendrimers with several charges are employed. These

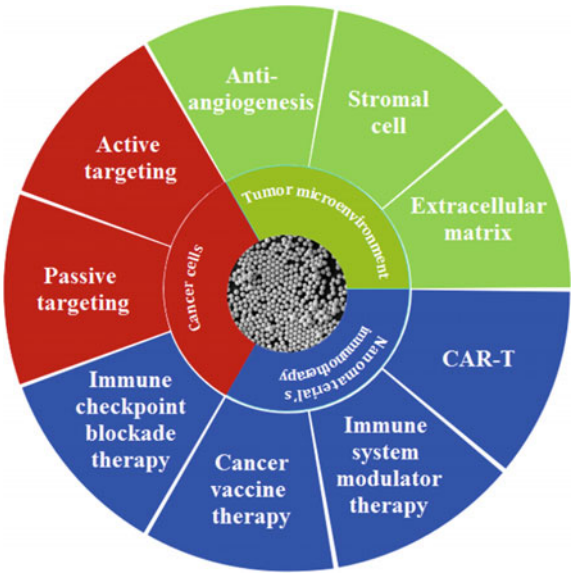

Fig. 21.3 Nanomaterial-based methods for cancer treatment

dendrimers showed enhanced PD-L1 (programmed cell death ligand 1) suppression as well as higher drug accumulation at the tumor site [119]. Cancer treatment based on nanomaterials is shown in Fig. 21.3.

21.5 Conclusion

Without a doubt, one of the main causes of death is cancer worldwide, but because of significant developments in nanomaterials used in cancer therapy, its long-lasting consequences are being lessened. A new field of study in biomedical engineering called nano-biotechnology offers the possibility for the targeted treatment of cancer employing a variability of nanoparticles with effective pharmacological and pharmacokinetic profiles. Researchers have looked at a variety of nanocarriers, and by doing so, they have been able to avoid some of the drawbacks of conservative chemotherapy by making the drug more soluble in free form and reducing its toxicity to healthy tissues. Drugs packaged in nanocarriers may now be actively and passively targeted to the illness site due to advancements in the creation of multifunctional nanocarriers. When nanocarriers are properly built, they can avoid renal clearance and circulate in the body for anextended time. The introduction of nanocarriers has enhanced the anticancer medication dosage restriction; however, new difficulties

have emerged. Nanoparticle-drug loading competence, the durability of nanoparticles through attached ligands, ideal receptor-ligand connections, and the length of time the targeted receptor are expressed are hurdles in nanocarrier design.

This chapter has provided a summary of a range of materials that are either now being employed as drug delivery systems for cancer therapy are currently being developed. Due to their special qualities, physicians may now give them as fresh treatments (monotherapy) or as supplements to current therapies (collective treatment) to boost therapeutic efficacy. Several novels and promising materials that are now being developed show significant potential, giving courage intended for innovative therapeutic choices in the nearby forthcoming, even though some of these constituents have not been effective in their clinical transformation.

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Chapter 22
Photothermal Therapy for Cancer Treatment

Sumit Sharma, Sonali Batra, Meenakshi Kanwar Chauhan, and Vikas Kumar

Contents

Abstract Photothermal therapy is a technique which utilizes light-absorbing materials and converts light energy into heat energy to damage tumor cells. In particular, near-infrared region starts from 800 to 2500 nm is used to induce cell death through photothermal effect. To achieve efficient heat production at tumor site this therapy is facilitated by near-infrared absorbents such as gold, copper sulfide, copper

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selenide, carbon nanotubes, nanographenes, and dyes like indocyanine green. Generally, fluence rate and irradiation time are mainly two key parameters that influence the anti-tumor activity through photothermal effect. But sometimes it is impossible to determine the true information about the tumor size and the effective distribution of near-infrared absorbents as a consequence therapeutic effect of photothermal therapy remains noneffective. Another important consideration in photothermal therapy is heating tumor tissue at optimized temperature and minimizing the collateral damage to normal tissues that surrounds the tumor tissue. Commonly tumor tissue is heated to a temperature of 50 °C or above to induce inflammation, necrosis, and ultimately cause thermal damage. Moreover, biocompatibility with biological tissue and biopersistence of near-infrared absorbents are also important to treat cancer. Most of the inorganic materials used as photothermal agents remain in the body for a longer period of time therefore eventually organic conductive materials like porphysome, polypyrrole, etc., have recently triggered extensive attention because of their biodegradable nature. However, considering the wavelength of laser light for exciting photothermal absorbers also contributes as a promising candidate to ensure deep tissue penetration of heat. Amalgamating with nano drug delivery technology these organic materials cause better penetration into the tissues and tumor-homing ability which further ensures accurate laser exposure and superior tumor ablation. Despite of aforementioned challenges and important critical considerations with photothermal therapy it has emerged as an attractive non-invasive technique for tumor ablation.

22.1 Introduction

Cancer metastasis is one of the serious challenges in the society and contributes a lot to mortality. Cancer metastasis is a multistep complex process that leads to wide diversity in clinical characteristics. These varied characteristics lead to spreading of cancer cells from primacy tumor tissue to surrounding non-cancerous cells and/or to systemic circulation. Once these cells entered into circulation the metastasis occurs at different secondary sites or organs and becomes complicated to cure and often turns out to be fatal. Currently, the available treatment includes surgery, radiotherapy, and chemotherapy for curing and preventing tumor cell metastasis. However, all these treatments are associated with undesired limitations that sometimes limit the use of these techniques, for instance, surgery is a choice of treatment in case of removal of macroscopic tumor tissue but it is subject to uncertainty because if cancer cells entered into the circulation or permeated into non-cancerous tissues it becomes difficult to cure and turn out to be fatal. Moreover, during removal of macroscopic tumor tissue, some of the small lesions may go unidentified and may not be removed thus, leaving behind a strong probability of relapse. The challenge with radiotherapy is low selectivity and limited curative effects. On the other hand, chemotherapy is an invasive technique and is associated with systemic side effects. Therefore, the society needs rapid, sensitive, and effective reliable cancer therapy

with less risk-to-benefit ratio that will help to increase the survival rate every year worldwide [1, 2]. Photothermal therapy is a light-mediated method which has been emerged as an attractive non-invasive technique for tumor ablation. Photothermal therapy is a technique which utilizes light-absorbing materials and converts light energy into heat energy to damage tumor cells. In particular, near-infrared region starts from 800 to 2500 nm is used to induce cell death through photothermal effect. To achieve efficient heat production at tumor site this therapy is facilitated by near-infrared absorbents such as gold, copper sulfide, copper selenide, carbon nanotubes, nanographenes, and dyes like indocyanine green, etc. Generally, fluence rate and irradiation time are mainly two key parameters that influence the anti-tumor activity through photothermal effect. But sometimes it is impossible to determine the true information about the tumor size and the effective distribution of near-infrared absorbents as a consequence therapeutic effect of photothermal therapy remains noneffective [3, 4]. Another important consideration in photothermal therapy is heating tumor tissue at optimized temperature and minimizing the collateral damage to normal tissues that surrounds the tumor tissue. Commonly tumor tissue is heated to a temperature of 50 °C or above to induce inflammation, necrosis, and ultimately cause thermal damage [5]. Moreover, biocompatibility with biological tissue and biopersistence of near-infrared absorbents are also important to treat cancer. Most of the inorganic materials used as photothermal agents remain in the body for a longer period of time therefore eventually organic conductive materials like porphysome, polypyrrole, etc., have recently triggered extensive attention because of their biodegradable nature. However, considering the wavelength of laser light for exciting photothermal absorbers also contributes as a promising candidate to ensure deep tissue penetration of heat. Amalgamating with nano drug delivery technology these organic materials cause better penetration into the tissues and tumor-homing ability which further ensures accurate laser exposure and superior tumor ablation. Despite of aforementioned challenges and important critical considerations with photothermal therapy it has emerged as an attractive non-invasive technique for tumor ablation. This chapter provides an in-depth analysis of scientific field to elucidate molecular mechanisms of photothermal therapy, state-of-the-art bioengineering design for developing targeted therapy, challenges or critical attributes associated with PTT, and the recent advancement to tackle the challenges for the better modality against cancer metastasis.

22.2 Molecular Mechanisms of Photothermal Therapy

Photothermal therapy employs high-energy irradiation to induce photothermal effect that leads to cellular death by inducing artificial elevation of intracellular temperature as shown in Fig. 22.1. This therapy takes an advantage of sensitivity of cells against heat and further promotes killing through apoptosis or necrosis. In order to produce photothermal heat in tumor tissues this technique utilizes photothermal agents which absorb light energy at specific wavelength on irradiation. As a consequence, the

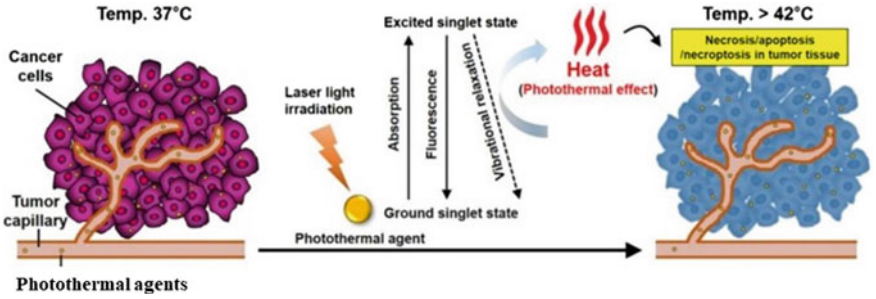

Fig. 22.1 General mechanism of photothermal therapy inducing cancer cell killing. Reused from Han, H.S.; Choi, K.Y. Advances in Nanomaterial-MediatedPhotothermal Cancer Therapies: Toward Clinical Applications. Biomedicines 2021, 9, 305. https://doi.org/10.3390/biomedicines9030305 [6]

agents get excited from ground state to excited state and then return to ground state resulting in libration of heat to neighboring tissues. The cause for generation of heat energy when excited state undergoes vibrational relaxation is the increase in kinetic energy in the photothermal agents. Earlier it was understood that photothermal therapy has higher risk-to-benefit ratio as there is a significant damage to normal tissues also along with tumor tissues due to nonspecific localized heat generation. With the advancement in technology now photothermal agents can be used to target specifically tumor tissues as well as enhance the heating effect at the local region. Photothermal therapy also influences the tumor microenvironment, for instance, the vasculature and permeability of tumor tissues get increased than usual due to heating effect. This effect results in deeper absorption of photothermal agents and causes targeted and effective cellular necrosis. Also, it opens the gateway for combinational therapy along with photothermal therapy like chemotherapy, radiotherapy, photodynamic therapy, immunotherapy, and surgery [6].

It is also important to know about the exact mechanism of cell death that will be followed after the PTT. Because immediate release of pro-inflammatory or growth factors during necrotic pathway of cell death promotes metastatic ability of remaining cancer cells post-PTT that renders the therapeutic efficacy of PTT ineffective [7]. Therefore, it can be said that type of cell death mechanism has a profound effect over the fate of remaining cells at the tumor site. Studies have explained that temperature has a significant role in defining whether the tissue undergoes necrosis or apoptosis as discussed in previous section. However, apoptosis is widely accepted pathway for cell damage through photothermal therapy because it is immunologically silent and anti-inflammatory. But apoptosis has a drawback i.e. a complete cancer cell death cannot be achieved because cells often circumvent the cell death mechanism and probable cause may be a poor immunogenic response of anti-tumor treatment [8]. On the other hand, necrosis is immunogenic but this pathway of cell death is uncontrolled which often leads to pro-tumorigenic inflammation that subsequently causes cancer by inhibiting anti-tumor immunity [9]. In general, a threshold temperature range for tissue damage is 42–47 °C [10]. It is reported that above 45 °C the

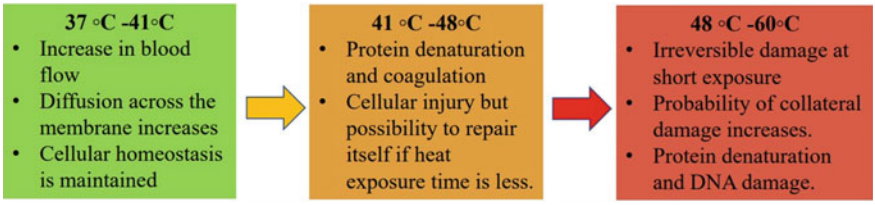

Fig. 22.2 Illustration summarizes the biological effects in tumor influenced by temperature adapted from Jaque et al. [13]

molecular events of inflammation initiate and resulting in cell death by necrosis. And with optimized photothermal conditions it is possible to control unnecessary heating of neighboring normal cells and prevent necrosis. Figure 22.2 depicts the various biological impacts on tumor tissue influenced by range of temperature during photothermal therapy. Apart from apoptosis and necrosis, another terminology has emerged as necroptosis which comprises a programmed cell death with necrosis-like morphological changes initiated by receptor-interacting protein kinase-1 and -3. In necroptosis when tumor cells undergo cell death they release damage-associated molecular patterns which subsequently stimulate production of several immunostimulatory cytokines [9, 11]. Necroptosis also includes leakage of cellular contents due to increased permeability of plasma membrane which is mediated by mixed kinase domain-like protein channels. All these reasons make necroptosis as highly immunogenic and pro-inflammatory. Morphologically, necroptosis is distinguished by organelle swelling and increased permeability of plasma membrane [12].

22.3 Factors Influencing Anti-Tumor Activity of Photothermal Therapy

22.3.1 Temperature

Photothermal killing shows different patterns of tumor cell killing mechanism including DNA damage and protein denaturation depending upon varying temperature. In literature, it has been reported that tumor cells when heated up to 42 °C the undergo apoptosis and necrosis. At 42 °C cells undergo irreversible tissue damage through thermal effect and heating up to 46 °C initiates apoptosis and necroptosis. However, higher temperature i.e. more than 49 °C promotes necrosis [6]. Cancerous cells are more thermosensitive than normal cells and are more susceptible to cell death because of irregular and poor blood flow by virtue of which there is a slow dissipation of heat. However, the major concern of utilizing hyperthermia effect for the ablation of tumor cell is nonuniform distribution of heat within the tissue. This drawback can be overcome with PTT where the photothermal molecules are used to localize the

hyperthermia effect within the tissue. Thermotolerance is another problem associated with PTT. It is a physiological homeostatic phenomenon which induces heat shock response through up-regulation of heat shock proteins using heat shock transcription factor 1. Thermo-tolerance is developed by cells under mild thermal stress where mainly cancer cells have high heat shock response due to elevated levels of heat shock proteins and proteasome activity that results in increased thermal tolerance as compared to normal cells. Further, these heat shock proteins are anti-apoptotic proteins and ultimately enhance the survival of cells under heat stress mainly induced by heating at low-temperature range (~39 °C - ~ 41 °C) or short time exposure at high-temperature range [14, 15] (Table 22.1).

22.3.2 Photothermal Agents

It is well understood that nonuniform distribution of heat into the tumor tissues has more risk due to the significant thermal damage to neighboring normal cells. Therefore, photothermal agents are used in PTT which gets heat up under the exposure of specific wavelength of laser radiation particularly in near infra-red region. Hence, under the influence of light, these photothermal agents get heat up and dissipate the heat energy to the close proximity which renders little thermal damage to normal tissues. An appropriate molecular design of photothermal agent is important for imparting ideal characteristics to photothermal agent while employ for PTT. Ideal characteristics of photothermal agents should be good sensitivity to near infra-red irradiation, good amount of absorbance ability, effective efficiency of photothermal conversion, biodegradable, and excellent photon stability [21].

Photothermal agents used in PTT are generally classified as organic and inorganic materials. Both types of materials have their advantages and limitations but clinically organic molecules are preferred. This is due to the ease of constructing safer in terms of biodegradability and less cytotoxic material as compared to inorganic materials. Moreover, organic materials provide enormous susceptibility to impart and tune certain characteristics considering formulations aspects that permits targeting to cancer cells such as active targeting, passive targeting, and stimuli-responsive targeting. On the other hand, most of the inorganic materials are non-biodegradable, difficult to structural modification, and induce cytotoxicity which sometimes provides barrier to effective treatment by PTT. Although inorganic materials can be coated with certain polymers that contribute to targeting but still lack of biodegradability remains the serious issue as it enhances the cytotoxicity and affects the normal tissues also [22].

Photothermal agents may also have poor ability to aggregate in tumor tissues and due to favoring high irradiation density it may cause severe thermal damage to normal tissues and provide barrier to several translational efforts clinically. Also, the deep penetration of these agents is also important in order to prevent the relapse of cancer. This can be achieved through functionalization of materials that aid in specific targeting and deep penetration. Table 22.2 summarizes the materials exploited for

Table 22.1 Summarizes various studies demonstrating the effect of temperature-dependent tumor reduction in various cell lines

S. No.	Cell lines	Temperature	Morphological changes	References
1	B16-F10murine melanoma cells	45 °C for 30 min	• Cell lines showed low tolerance to hyperthermia at 45 °C and significant decrease in cell adherence was observed in initial 24 h • The cells in S phase decreased significantly immediately after the shock treatment as confirmed by flow cytometry • Alterations in F-actin cytoskeleton, condensation of nuclear material and shrinkage of cytoplasm were observed after 24 h of the treatment	[16]
2	B16F1 murine melanoma cells	43 °C	• Detachment and alteration in cell shape, i.e., from slender shape to rounding up was observed • Cells regained their normal morphology after 24 h of incubation in fresh culture medium after the hyperthermia treatment • After repeated cycle of hyperthermia treatment cell viability was not able to reduce further from ~ 15%	[17]
		45 °C	• Cells were not able to regain their normal morphology and irreversible structural alterations were observed • Repeated cycle of hyperthermia treatment showed almost 100% decrease in cell viability	
		47 °C		

(continued)

Table 22.1 (continued)

S. No.	Cell lines	Temperature	Morphological changes	References
3	B16-BL6murine melanoma cells	43 °C	• No effective cell killing was observed • Cells undergo approximately 10% apoptosis, 18% necroptosis, and 17% necrosis	[2]
		46 °C	• Necroptosis pathway predominates significantly and increased to approximately 46%	
		49 °C	• Necrosis mechanism predominates to approximately 50% and showed significant cell death • The percentage of apoptosis and necrosis for cell killing dropped significantly	
4	• B16 melanoma cells • A375 and MDA-MB-435S human melanoma cells	42–45 °C	Thermosensitive HSPB1 protein silencing combined with hyperthermia treatment increased the sensitivity of cytotoxicity against cells treated with hyperthermia only	[18]

(continued)

PTT as photothermal agents. Another important consideration for photothermal agents particularly the nano size materials is their ability to avoid leakiness from tumor vasculature. The size reduction usually in nano range is preferred for targeting tumor tissues but very small nano size materials have tendency to permeate deeper into dense tumor tissues and sometimes may rapidly cleared out. Hence, retention is a serious challenge while developing a nano size material and it is important to optimize a suitable nano range which can overcome the penetration into tumor tissues and simultaneously address retention limitation by avoiding clearance from tumor extracellular matrix and lymphatic vessels of tumor tissue [23].

Table 22.1 (continued)

S. No.	Cell lines	Temperature	Morphological changes	References
5	SH-SY5Yhuman neuroblastoma cells	43 °C and 47 °C	• Autophagy found to be dependent on intracellular temperature • Importantly, autophagy contributes to tumor suppression but abnormal autophagy may initiate tumorigenesis • Mild hyperthermia at low temperatures has protective effect over cancer cells through autophagic response whereas mild hyperthermia at higher temperature has enhanced cytotoxic effect • Moreover, importantly using autophagy inhibitors can be more beneficial at low temperature as compared to high temperature as despite of the high temperature with autophagy inhibitor cells were able to form new colonies	[19, 20]

22.3.3 Wavelength of Laser Light

To understand this, consider a light-absorbing molecule inside the tissue, and laser light with a specific energy is incident over the tissue surface with specific wavelength. Some of the light is reflected back, whereas some amount of light penetrates the tissue. Further, some part of the penetrated light is scattered within the tissue (intercellular and intracellular areas) and some part is absorbed by the molecule. Hence, considering the situation one important factor needs to be understood here is that the total energy given through laser light does not reach to the light-absorbing molecules and molecules are not able to convert complete incident light energy into thermal energy. Therefore, the actual light energy absorbed by the molecule depends upon two coefficients, i.e., absorption and scattering coefficient. To simplify this, consider an equation: $Q = \mu I S$; which represents the heat generated inside the tissue is equivalent to the product of attenuation coefficient, light intensity also represented as fluence and tissue thickness. The attenuation coefficient here is a sum of absorption coefficient and scattering coefficient. Both of these coefficients represent the energy

Table 22.2 Summarizes various photothermal agents explored for PTT

S. No.	Photothermal agents	Characteristics or considerations	Remarks	References
1	Gold nanomaterials	• Efficient photothermal conversion ability • Size influences maximum absorption spectrum • Biocompatible	• Due to nano size gold nanoparticles have enhanced penetration and retention effect • Long-term residence in the tissue could be an important concern on safety prospects • Plasmon effect varies depending upon the size and shape of gold particles which means scattering and absorption of light by gold materials are influenced by physical dimensions. For instance, 22 nm colloidal gold showed maximum absorption spectrum at 517 nm whereas 99 nm colloidal gold showed red shift and maximum absorption spectrum was around 550 nm	[24, 25]
2	Carbon nanomaterials (Graphene oxides, carbon naotubes, nanohorns)	• Excellent photothermal ability • Hydrophobic and poor solubility in water • May form agglomerates in aqueous suspension • Non biodegradable	• As a consequence, it becomes critical when water is essential to be used as dispersion medium. But functionalization with oxides, folic acid, and hyaluronic acid may improve their solubility and biocompatibility • May produce reactive oxygen species which can cause inflammation, lipid peroxidation, reduce mitochondrial membrane potential, and may cause genotoxicity by reacting with DNA	[26]

(continued)

Table 22.2 (continued)

S. No.	Photothermal agents	Characteristics or considerations	Remarks	References
3	Indocyanine green functionalized with cellulose nanocrystals	• Indocyanine green has efficient photothermal activity but poor water solubility, rapid photodegradation, and rapid blood clearance • Rod shaped organic nanomaterials	• May flocculates in aqueous suspension but can be prevented through suitable structural modifications • Functionalized indocyanine green with cellulose nanocrystals showed improved thermal stability and non toxic nanomaterial • Compared to spherical-shaped nanomaterials rod-shaped nano materials have enhanced penetration ability into tumor tissues • Inherent hydroxyl groups on cellulose nanocrystals allow variable functionalization for targeting or variable biological functions	[27]
4	Prussian blue nanoparticles	Biologically safe with efficient photothermal effect and good photothermal stability	16 ppm concentration of prussian blue nanoparticles has shown < 10% cell viability of HeLa cells	[28]
5	Porphyrins conjugated with phospholipids	• Biodegradable and biocompatible • Free porphyrins have longer life of flourescence	Excited state lifetime significantly reduced	[22]

loss per unit length of the tissue penetrated by the light [29]. However, the specific laser wavelength in near-infrared region is chosen to avoid scattering of light as much as possible and maximum absorption into the tissues to ensure deep penetration. As the tumor tissue is deeply seated so the effective cell death demands deep penetration of light. Hence, to achieve maximum penetration deep into the tissue depends upon the wavelength of incident laser light. As we know wavelength is inversely proportional to energy and as discussed earlier generally near infrared region is utilized for PTT, therefore laser light with short wavelength in near-infrared region is exploited

for PTT. Shorter wavelength provides high-energy light which further able to penetrate deeply into the tissues. But this does not hold completely true when it comes to the practical implications of PTT as discussed later in this section. Moreover, from the above equation, it is also clear that heat generation also depends upon the thickness of the tissue which is also variable at different sites and influences the light penetration. Hence, determining the appropriate wavelength before applying PTT is important to ensure the effective light penetration into the deep tissues.

In general, for cancer treatment, PTT uses 700–2000 nm wavelength range of near-infrared optical radiation [30]. This is because majority of the biological components like hemoglobin, melanin, proteins, and water have minimal absorption. Hence, ensures maximum light reaches to photothermal agents for plasmon effect or conversion to heat [31]. In a study, the effect of different near-infrared windows was explored to determine the superiority of wavelength among the near-infrared range in terms of better penetration into the tissues and cell ablation. Two biological windows are identified in near-infrared region, i.e., NIR-I (700–900 nm) and NIR-II (1000–1700 nm). The NIR is preferred over visible light range due to less scattering and maximum absorption into the tissues. It has been observed that NIR-II is found to be superior for better tumor cell ablation as compared to NIR-I because ability to penetrate deeper into the biological tissues. Furthermore, the NIR-II is split into two sub-windows, i.e., NIR-IIa (1300–1400 nm) and NIR-IIb (1500–1700 nm). Among these two sub-windows NIR-IIa spectra range is preferred as it avoids the absorption of light by water molecules in the tissues. Moreover, it is reported in literature, compared to 808 nm, 1275 nm has less absorption by melanin, hemoglobin, and other human tissues [32, 33]. Xunzhi Wu et al. found the superiority of NIR-IIa wavelength in terms of deep penetration while studying the effect of laser illumination by comparing transmittance of different wavelengths (808 nm and 1275 nm) and temperature distribution over varying thickness of porcine muscles. The conclusion was based on less scattering of light in tissues and more focused diffusion of temperature in the exposed tissue region. They observed that tissue exposed at 808 nm had more diffuse temperature distribution, whereas 1275 nm showed more focused temperature [33].

22.3.4 Fluence Rate

Fluence rate is one of the parameters that have to be determined before the initiation of photothermal therapy. Since it is difficult to dig out precise tumor-related information such as heterogeneity, size of tumor, and distribution of photothermal agents inside the tumor tissue, therefore, it is impossible to ensure maximum therapeutic effect of PTT with predetermined irradiation conditions. Hence, it is always suggested the irradiation conditions which provide the maximum fluence rate is considered to be optimum for the effective PTT. However, often a higher fluence rate has been observed to cause collateral damage to surrounding normal tissues also due to excessive rise in temperature (>60 °C) which further promotes necrosis pathway of cell death.

As discussed earlier, the necrotic pathway has certain drawbacks like unregulated inflammation that may cause tumor metastasis.

In a study using ICG lactosome as NIR absorbent in PTT, researchers suggested that fluence rate is not reliable determinant for effective PTT in tumor size reduction specifically if the surface temperature exceeds 43 °C. However, in such condition, thermal dosimetry is more reliable for predicting effective PTT using NIR absorbents [34].

22.3.5 Irradiation Time

Irradiation time used in the photothermal therapy depends on many factors which need to be critically analyzed and optimized. Frequency of the laser beam irradiated, extinction co-efficiency of the photothermal agents, efficiency of the matrix used along with the temperature involved is certain factors which need to be strategically defined so as to strike the maximum result in the defined irradiation time [35]. It has been generally observed that regardless of the factors mentioned, the irradiation time of the laser beam cannot be more than 10 min [36]. Increase in irradiation time indirectly exposes the cancer cells to long-temperature exposures, which in turn induces the hyperthermia of the cancer cells and development of thermo-resistance. The sequence of non-reactive cancerous cells might not stop here, but also generate heat shock proteins, consequently, leading to insufficient apoptosis, and re-occurrence of tumor [64]. Hence to maximize the effect of photothermal agents, within the small irradiation time period, different approaches can be practiced. For instance, 17-AAG–C12 elastin-like polypeptide–gold nanorod matrices were prepared by Huang et al., in which the heat shock protein inhibitor was used along with, had significant effect (>90%) on the death of cancer cells, in the irradiation time of 8 min [37].

22.4 State-Of-The-Art Targeted Photothermal Cancer Therapy

22.4.1 Nanomaterial-Mediated Photothermal Cancer Therapies

A successful PTT not only kill tumor cells but also prevent normal cells from thermal damage and ensures 100% tumor cell death leaving behind no residue of metastaic cells. Generally, PTT therapies mainly involve the use of photosensitizers, irradiation time, and near infra-red radiation but these are not sufficient for complete tumor cell ablation. This is because tumor is associated with heterogeneity which leads to inappropriate distribution of photosensitizers and hence thermal effect. As discussed earlier, low temperature may result in thermal homeostais by releasing

heat-protecting proteins which further cause tumor cell survival even providing PTT for long period of time at high fluence rate or temperature. This happens due to inappropriate distribution of heat to the neighboring cells particularly residing in depth. As tumor cells are deeply seated and photosensitizers are not able to penetrate deep into the tissues leaving behind metastatic cells. Moreover, some of the photosensitizers also cause inflammation due to their on cytotoxicity and rapidly cleared by immune response. All these issues can be resolved satisfactorily by combining PTT with nanotechnology. Nanotechnology provides advantages like (1) Ability to absorb deeply into the dense extracellular matrix of tumor. (2) Ability to modulate and optimize size and shape which has significant effect in absorption and clearance from microvasculature. (3) Nanomaterials along with better permeability also act as verstaile carrier to photosensitizers which further may impart photostability and increase their shelf life. (4) Nanomaterials are also studied to enhance the photothermal effciency of photosensitizers. (5) Ability to target tumor tissues by conjugating with overexpressed receptors in tumor cells like folic acid and biotin etc. Considering all advantages of nanotechnology in PTT some important considerations need to be addressed while exploiting nanotechnology. For example, reduced retention into tumor tissues of nanomaterials is one of the major hurdles with nanotechnology as very small-size nanomaterials are susceptible to cleared out rapidly through blood vessels or leaky vasculature of tumor tissue and further may easily eliminated by macrophages in blood. On the other hand, large size materials may penetrate up to subcutaneous level and not able to permeate further leading to ineffective PTT. Hence, it is very important to optimize the size and shape of nanomaterials simultaneously considering the heterogeneity of tumor. Moreover, apart from nano size range, multifunctional nano carriers are desirable for targeting but the synthesis of such multifunctional nanocarriers and ligand attachment is very difficult due to unavailability of binding sites on the carrier. Dendrimers are one of the nano carriers with highly branched 3-D architectural design polymeric nanomaterials and allows multiple functionalities on the surface. Also due to high-density internal cavity and variable multifunctional surface allows heavy payload which can support higher loading of photothermal agents and simultaneously facilitate targeting by binding with various ligands [38–40].

22.4.2 Conjugation for Targeting and Deep Penetration into Tumor Tissue

Mostly photosensitizers used in PTT are hydrophobic and have poor aqueous solubility by virtue of which these agents may form agglomerate in aqueous medium. Therefore, this usually restricts the permeability of photosensitizers up to the subcutaneous level. Also, non-selective accumulation of photosensitizers in the tissue may result in collateral damages to normal cells. Another limitation is less retention time of photosensitizers in tumor tissue which further cause rapid clearance and does not

allow the PTT to be performed for extended period of time. Interestingly, conjugation with suitable ligands or loading of photosensitizers in suitable functionalized carrier systems may improve aqueous solubility and allows deep penetration with targeting of tumor cells. Targeting of photosensitizers either active or passive can improve the therapeutic efficacy of PTT and reduce collateral damage during PTT (Table 22.3).

Table 22.3 Various conjugations explored for targeting photothermal agents in PTT

S. No.	Conjugation	Remarks	References
1	Chitosan conjugated with Pluronic F68	Gold nanorods and chlorin e6 as photosensitizers were loaded in thermosensitive Pluronic-based nanogel conjugated with chitosan and has shown improved tumor targeting and circulation time	[41]
2	Thiol-modified AS1411 aptamers conjugated gold nanostars	• Enhanced targeting of gold nanostars (photosensitizers) due to affinity of AS1411 aptamer conjugated with gold nanostarsfor overexpressed nucleolin in HeLa cells • Efficient photothermal conversion with good photostability and less cytotoxicity	[42]
3	IR1048 conjugated with mannose-modified zwitterionic polyester-based nanoparticles	• Prolong circulation time due to polyester • Approximate 44% photothermal conversion efficiency • Reduced specificity of near-infrared II small molecule was enhanced by mannose as it binds to overexpressed mannose receptors • Zwitter ionic co-polyester provides the pH responsive property thereby promotes targeting of cytotoxic IR1048 dye at tumor microenvironment with low pH	[43]
4	Hyaluronic acid conjugated on carbon nanomaterial	• Hyaluronic acid has the affinity to bind with overexpressed CD44 + receptors in tumor • The hydrophobic characteristic of hyaluronic acid promotes deep absorption into thick tumors and transdermal delivery in skin melanoma	[44, 45]

22.5 Combination of PTT with Other Anti-Cancer Therapies

Due to shortcomings of monotherapy in complete tumor ablation, it is usually recommended to practice combination therapies. Despite of certain advantages like non-invasive, patient compliant, and easily available PTT alone has some shortfalls such as not able to completely eradicate the tumor and prevent metastasis ultimately leave the probability of relapse. Despite of enormous clinical advantages, these shortcomings of PTT which become the main obstacles for its clinical application can be resolved by synergizing the therapeutic efficacy of PTT with combination therapy. Therefore, realizing the urgent demand for a robust anti-tumor treatment, researchers have proposed several possible combinational therapies with PTT and also proved significantly effective in tumor ablation and overall improved efficacy as compare to PTT alone.

22.5.1 Combining with Chemotherapy

As discussed, a monotherapy with PTT cannot eliminate tumor completely because of residual tumor mass even after the PTT. Also, we cannot modulate the treatment to aggressive conditions such as excessive irradiation time, very high temperature and fluence rate. Aggressive photothermal treatment has low level of patient tolerance and high risk-to-benefit ratio. Therefore, in order to develop a single robust anti-tumor treatment one promising strategy is to commingle chemotherapy with PTT. It is reported that chemotherapy induces anti-tumor immunity and in combination with PTT is able to upregulate immune responses. For instance, Nam et al. has demonstrated experimentally the upregulation of MHC-II and CD40 in the lymph nodes of tumor, expression of CD8 + T-cells, and production of NK cells at both primary and distal tumor site in mice bearing CT26 colon carcinoma when treated with doxorubicin along with PTT. Also, increased levels of IgG (which specifically binds to CT26 cells) during the treatment was observed. Furthermore, the team has also reported that this treatment is not only eradicating the tumor during treatment but also build long-term immunity to prevent relapse. The conclusion was based on when the group of animals was rechallenged by CT26 tumor cells after being recovered by the combined treatment of doxorubicin and PTT, the animals rejected the tumor cells and tumor had failed to develop. On the contrary, the group of animals which were previously not exposed to CT26 tumor cells and any treatment had developed tumor within 30 days after being challenged bt CT26 tumor cells. Thereby, concluding that the chemotherapy through doxorubicin along with PTT induces innate as well as adaptive immunity in primary and distal tumor hence increasing the therapeutic efficacy of PTT [46].

Chemotherapy alone is widely used non-specifically to treat cancer irrespective of the heterogeneity in cancer. However, the therapy is associated with various side

effects due to non-specific distribution of cytotoxic agents in normal and cancer tissues. Moreover, inability to use high doses of cytotoxic agents to kill cancer cells is another challenge with chemotherapy. Researchers have suggested two possible ways to overcome the aforesaid concerns with chemotherapy i.e. (1) Targeted delivery of chemotherapeutic agents to cancer cells. (2) Combination therapy. Combination therapy with PTT allows the use of cytotoxic agents at low doses with enhanced therapeutic efficacy. Also, targeted delivery of cytotoxic agents with photothermal agents at tumor site prevents non-specific distribution of cytotoxic agents as well as heat in local region [47]. Interestingly, it was observed experimentally that increase in temperature has an influence on increasing anti-cancer activity of various cytotoxic agents and thus providing synergistic effect. Mild hyperthermia during PTT in the range of 40–45 °C increases the sensitivity of tumor against cytotoxic agents [48]. This can be attributed to increase in the vascular permeability of undeveloped blood vessels of tumor which permits the increased accumulation of cytotoxic agents at tumor site. Additionally, this influence allows the use of cytotoxic agents at low doses with enhanced therapeutic efficacy. This minimizes the non-specific distribution of cytotoxic agents and reduces the major side effects associated with such agents with normal cells [49]. Simultaneously, chemotherapy also minimizes the adverse effects of PTT such as improved therapeutic efficacy prevents the use of high radiation time and exposure to very high temperatures thus increasing the patient compliance to anti-cancer treatment.

22.5.2 Combining with Radiotherapy

The success of radiotherapy depends upon the presence of oxygen in the tumor area. It becomes challenging in case of hard tumor due to reduced oxygen supply resulting in hypoxia and limited blood vessels. As discussed earlier the hyperthermia induced by PTT increase the blood flow at tumor site and increasing the permeability in blood vessels thus resulting in increase in tissue oxygenation. As a consequence, potentiate the effect of radiotherapy by modulating the hypoxia condition at tumor site.

Apart from this, there are certain sensitizers such as gold, super paramagnetic iron oxides, bismuth, and platinum that respond to X-rays and NIR as well. It was observed that these metals produce reactive oxygen species in the presence of X-ray irradiation in human colon carcinoma cells which further results in cancer cell cycle arrest, senescence, and apoptosis [50]. On the other hand, these agents also act as efficient photosensitizers with high photothermal activity through surface plasmon resonance process. This implies that these agents can be used as a single sensitizing agent for both the therapies together.

The important consideration in combination therapy of PTT with radiotherapy is the order of treatment. The PTT is preferred initially and radiotherapy is applied after the PTT. This can be explained as X-rays ionizes water molecules and produce reactive oxygen species which leads to cell death through apoptosis or necrosis but due to limited tissue oxygenation and blood supply in hard tumors, radicals are unable

to cause effective cell damage and renders the treatment ineffective. PTT reduces the obstacles for radiotherapy like hypoxia condition, reduced blood flow, and deep penetration particularly in solid tumors as discussed earlier. Thereby, potentiating the therapeutic efficacy of radiotherapy and in combination provide synergistic effect [51].

A study concluded experimentally the enhanced anti-tumor efficacy by combining two different mechanisms of killing cancer cells, i.e., damaging DNA by radiotherapy and inhibiting further damaged DNA repair by mild hyperthermia using gold nanoparticles as dual sensitizer to radiotherapy and PTT. Study has revealed that after damaging DNA through radiotherapy, cancer cells have the tendency to repair itself by the expression of Rad 51 proteins. Rad 51 proteins play important function in DNA repair through exchanging homologous strands. Therefore, after radiotherapy, a mild hyperthermal treatment by PTT downregulates the expression of Rad 51 proteins which prevent the radiation-induced damaged DNA repair and improves the outcomes of the treatment [52, 53].

22.5.3 Combining with Surgery

Surgery is always been a preferred treatment for removal of tumor. However, complete removal of cancer cells even through surgery is not possible always particularly in case of aggressive tumors and the multiple solid cancer. Also, a precise surgical removal of cancerous tissue is also very difficult that may be due to tumor at multiple sites or at deep and critical locations ultimately leading to relapse a common concern in surgery also. PTT is known to cause irreversible damage to cancer cells but due to bypassing the exposure of cancer cells to PTT because of certain reasons as discussed earlier, PTT also demands promising adjuvant therapy with different mechanism to kill cancer cells. Considering the issues with surgery despite of its incomparable advantages of successful removal of cancer tissue, PTT as adjuvant treatment to surgery is suggested as one of the promising combination therapies in literature. The major challenge associated with surgery is insufficient elimination of cancer cells which is particularly a major concern in infiltrative tumors or malignant tumors. PTT has the potential to remove the residuals of cancer cells remained after the surgery because of ability to penetrate deep into tissues and specific cell binding affinity with cancer cells as discussed in Sect. 22.4. On the other hand, PTT has a challenge of inability to completely damage large tumor tissues or sometimes requires large exposure time, temperature, and power density of irradiation which may not be patient compliant. Therefore, in such cases surgery supports to remove the cancerous mass and further PTT can be applied to remove the residuals of cancer.

The order of the treatment is also important in this type of combination therapy. As discussed in previous subsection radiotherapy is preferred to be performed after the PTT but unlike in this case, PTT is preferred after the surgery for better outcomes. Marina Simon et al. evaluated the combination therapy using PTT with surgery to treat fibrosarcoma. Fibrosarcoma was selected to evaluate the potential of combination

therapy due to the characteristics like (1) malignant type (2) highly invasive soft tissue sarcoma (3) reduced sensitivity to chemotherapy and radiotherapy (4) ability to grow in deep tissues and relapse is a common issue. All these factors are actual challenges if PTT and surgery are performed as standalone to eliminate tumor completely and prevent recurrence. Researchers, injected HT1080 human fibrosarcoma cells in mice and developed orthotopic tumors. The mice were incised and approximately 75% of tumor mass was removed through surgery and after tumor removal the tumor site was exposed to PTT. The gold nanoshells were used as photothermal agents which were injected a day before to surgery and five minutes exposure of near-infrared irradiation was applied. Interestingly, a group exposed to both treatments has shown higher surface temperature (~50 °C) as compared to the groups not exposed to gold nanoshells. This is attributed to the conversion of light energy to heat energy through gold nanoshells. Moreover, higher temperature was observed even in small tumor remnants and this was attributed to ability of nanoshells to accumulate in deeply seated infiltrative tumor growth through passive targeting and enhanced permeability and retention effect which is commonly observed in malignant type fibrosarcoma [54].

22.5.4 Inhibiting Heat Emergency Proteins

One of the biggest advantages of using PTT in cancer therapy is the low-temperature conditions which can be used to target and position the tumor. Hyperthermia generated by PTT for the ablation of cancer cells, utilizes near-infrared light along with photothermal agents has been the potential curative measure for cancer [55]. Due to its high specificity and less collateral damage to the normal cells, this technique has been most widely accepted [35]. But, thermal damage produced by laser irradiation, can cause expression of specific proteins which are termed as heat shock proteins leading to thermos tolerance of cancer cells. The function of heat shock proteins is to restore the thermal damage caused by PTT and hence cancer cells develop tolerance to thermal damage and thus there are high chances of recurrence of cancer in such cases [56]. Different of cancer cells release different types of heat shock proteins. Some are similar, and some of them are specific to the type of cancer developed. HSP 90 or HSP 70 are few heat shock proteins which are generated commonly. The aim to increase the efficiency of PTT is to deliver the heat shock protein inhibitor consequently with near infrared photosensitizers. Studies have shown that some, siRNAs have been used to inhibit the synthesis of these proteins [57]. Also, 17-AAG (17-allyl amino-17-demethoxy geldanamycin) has been a typical inhibitor for the heat shock proteins. Various strategies have been developed to deliver 17-AAG, like the use of nanoparticles, nanorods and others along with photothermal agents so as to enhance the efficiency of PTT. In one of the study, Zhang et al.; utilizes gambogic acid; which has dual role, i.e., it is a natural anticancer drug and secondly, heat shock protein inhibitor [58]. Taking advantage of dual properties of gambogic acid, the only aim was to deliver, this poorly water-soluble drug with an effective carrier. The gambogic

acid along with near infrared sensitizers (IR780) was thus encapsulated in albumin nanoparticles with MnO_2 deposition on the surface. The purpose of using albumin in this was the hydrophobic and hydrophilic domain which 3D structure of albumin carries, thus making it a naturally safe carrier [59]. The firm nanoparticles surfaced with MnO_2 effectively catalyze the overexpression of hydrogen peroxide which is produced in the microenvironment of tissue. Both in-vitro and in-vivo have shown to decrease the production of hydrogen peroxide, thus decreasing the hypoxia.

22.5.5 Combining with Immunotherapy

It has been observed that PTT induces immunogenic response by triggering tumor-related antigens which further has anti-cancer effect and prevents metastasis to some extent. When tumor cells are killed, the remains of cancer cells and released cytokines are phagocytosed by immune cells and then presented by antigen-presenting cells. This further leads to an activation of cytotoxic T-lymphocytes with tumor-specific receptors. The produced cytotoxic T-lymphocytes are transported to tumor sites where they recognize the binding sites on tumor cells and kill them. But this induced immune system is not very aggressive and usually in large tumor tissues and malignant cells can easily evade the aforesaid immunogenic cell killing mechanism. The other possible reasons for this evasion are (1) loss of antigenicity of tumor cells. (2) Tumor microenvironment prevents the infiltration of cytotoxic T-lymphocytes and antigen-presenting cells into the tumor which ultimately prevents immune response. Therefore, tumor cells with high immunogenicity are easily eliminated by immunity whereas cells with low immunogenicity and advanced tumor will survive. This is because the tumor cells may express PD-L1 (immunosuppressive molecule usually express on T-lymphocytes or release immunosuppressant cytokines such as interleukn-10 and TGF-β. The survived tumor cells with low immunogenicity may infiltrate in blood vessels and become malignant. This leads to a particular interest among the researchers that if this specific immunogenic cell death can be influenced additionally along with PTT then it may enhance the therapeutic efficacy of PTT. The examples of such immunotherapies are anti-tumor monoclonal antibodies, anti-cancer vaccines and immuno-modulators, etc. [60–62].

For instance, Zhang Da et al. artificially designed natural killer cells which have the ability to specifically bind with hepatocellular carcinoma. The treatment was given with PTT to eliminate any residual of cancer cells after the PTT and avoid the relapse. A 2-dimensional manganese-based coordinated nanosheets was developed which has excellent light to heat conversion efficiency. Further, TLS11a aptamer was designed where natural killer cells were adsorbed over the surface that could specifically actively target the hepatocellular carcinoma and eliminate any residual left during the PTT. The active targeting of artificially designed natural killer cells was examined in mice after 24 h intravenous injection of labeled artificially designed natural killer cells through confocal laser scanning microscope. The results have shown excellent active targeting in excised tumor. Additionally, DNAzyme was attached over the nanosheet

which has utilized Mn^{2+} as co-factor and prevent the action of HSP through HSP70 gene silencing. The inhibtion of heat shock proteins has shown significant anti heat survival ability and also the group treated with artificially designed natural killer cells after PTT has shown better results in HepG2 tumor size reduction. However, the best results were observed in combination therapy of three times intravenous injection of artificially engineered natural killer cells on TLS11a aptamer after PTT with DNAzyme attached over 2-dimensional manganese-based coordinated nanosheets [63].

Considering all clinical potential of combining immunotherapy with PTT, the immunotherapy is also associated with certain drawbacks that indeed need to be addressed. For example, (1) There is no universal immunotherapy which can be given to every cancer patient because of differences in the immune response among individuals, (2) Heterogeneity of tumor, (3) Detailed understanding of developed tumor that will recommend the use of suitable immunotherapy or helps in designing specific monoclonal antibodies. (4) Some patients may also suffer from serious side effects caused by immunotherapy particularly in HIV patients and in long-course treatment.

22.6 Summary and Conclusion

PTT has been observed to be a promising therapy clinically as it has not only shown positive outcomes if applied standalone but also has the possibilities as multi modal therapies. Combining with other therapies like chemotherapy and radiotherapy where dose adjustment of toxic drug molecules and sensitizers is an important concern, PTT has shown its dominancy of being used as adjuvant therapy. The primary reason is PTT allows the use of these toxic agents which may cause serious collateral damage at low levels leading to higher therapeutic index. Moreover, PTT allows various variable factors to modulate the treatment according to the need of the patient such as irradiation time, fluence rate, irradiation power density, and wavelength. PTT also allows opportunities for targeting cancer cells both by active and passive pathways through ligand binding, enhanced permeability, and retention of photothermal agents in cancer cells and nanotechnology. Despite of various advantages, PTT has some drawbacks also such as not able to eliminate cancer completely in some cases and lead to recurrence. Moreover, cytotoxicity of some photothermal agents and their inability to biodegrade after the treatment leads to collateral damages. However, in literature sufficient studies have suggested promising solutions to these issues and lead pathways for further advancements in PTT.

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Chapter 23
Tool and Techniques on Computer-Aided Drug Design for Targeted Cancer Therapy

V. G. Niveditha, V. Sindhu, Moni Philip Jacob Kizhakedathil, I. Shanmuga Sundari, and Malathi Balasubramaniyan

Contents

Abstract Cancer is a highly debilitating disease affecting millions around the world. The current drugs marketed are incapable of eradicating the same. Hence, there is a dire need to find a new drug with extraordinary therapeutic properties. Though there are lots of new advanced drugs currently in research and development, the majority of them fail to resurface in clinical trials due to ethical and financial constraints. Hence, to minimize these difficulties, computer-aided drug design (CADD) can be used. CADD is an in silico technique used in drug modeling through simulations. It involves bioinformatic tools like software and various databases to model a molecule (ligand) that has therapeutic activity. The in silico method of drug discovery is advantageous over in vitro and in vivo methods as it prevents the use of animal/human models and controlled environments, thereby reducing the complexity, time, and expenses involved. In addition, it is done at a level of biological networks allowing personalized

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treatments. This chapter gives an overview of CADD basics with an emphasis on fine-tuning cancer drugs, pharmacokinetics, pharmacodynamics, and drug repurposing.

Abbreviations

ABCB1	The ATP cassette transporter G2
ACE2	Angiotensin Converting Enzyme-2
ACFIS	Auto Core Fragment in silico Screening
ADAM10	A Disintegrin and metalloproteinase domain- containing Protein 10
ADME	Absorption, Distribution, Metabolism, and Excretion
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
AI	Artificial intelligence
AKT1	Serine/Theonine kinase 1
ATC	Artemis Comparison Tool
ANN	Artificial Neural Network
APC	Adenomatous polyposis coli
APLP2	Amyloid Precursor-Like Protein 2
ALK	Anaplastic Lymphoma Kinase
AR2	Androgen Receptor 2
ARH1	ADP-ribosyl arginine hydrolase 1
ARI 1	Acute Respiratory infection
ATP	Adenosine Triphosphate
BACE-I	Beta Site Amyloid Cleaving Enzyme
BC	Breast Cancer
BCL2	B-cell Lymphoma 2
Bcl-xl	B-Cell Lymphoma-Extra Large
BCR-Abl	Breakpoint Cluster Region protein- Nonreceptor tyrosine kinase (d)
BCRP	Breast cancer Resistant protein
BMA	Biomedical Mutation Analysis
Cag A	Cytoxin Associated Gene A
CDK	Cyclin Dependent Kinases
CHARMM	Chemistry at HARvard Molecular Mechanics
CLK1	CDC Like Kinase 1
CK1	Casein Kinase 1
Co A	Coenzyme A
CT	Computed Tomography Scan
CTD	The Comparative Toxicogenomics Database
CNS	Central Nervous System
COX1	Cyclooxygenase
CPDB	The Carcinogenic Potency Database
CRC	Colorectal cancer

CRISPR-Cas9	Clustered Regularly Interspaced Short Palindromic Repeats
CXCL1	Chemokine (C-X-C motif) Ligand 12
CycD	CyclinD
DBD	DNA Binding Dependent
DGID	The Drug Gene Interaction Database
DNMT	DNA methyltransferases
DSEA	Drug-set enrichment analysis
DSSTox	Distributed structure-searchable Toxicity
DYRK1B	Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1B
EBS	Epidermolysis bullosa simplex
EGCG	Epigallocatechin gallate
EGFR	Epidermal Growth Factor Receptor
EGF	Epidermal Growth Factor
EMT	Epithelial-mesenchymal Transition
ESFT	Ewing's Sarcoma family tumor
FDA	Food and Drug Administration
FSCN1	Fasin actin -bundling protein 1
GEN UI	Generator User Interface
GO	Gene Ontology
GNM modelling	Gaussian Network Model
GROMACS	GROningen Machine for Chemical Simulation
GROMOS	GROningen MOlecular Simulation
GSK3	Glycogen-Synthase Kinase 3
HAT	Histone acetyltransferases
HDAC6	Histone Deacetylase 6
hERG	Human ether-a-go-go-related gene
HMGCR	3-Hydroxy-3-methylglutaryl-CoA reductase
HMMR	Hyaluronan Mediated Motility Receptor
HER2	Human Epidermal Growth Factor Receptor 2
HDAC	Histone deacetylases
HTS	High-Throughput Screening
HIF-1 α	Hypoxia-inducible factor 1-alpha
HPIP	Hematopoietic PBX-interacting protein
HPV16	Human Papillomavirus 16
IDH1	Isocitrate dehydrogenase 1 gene
IEDB	Immune Epitope Database
JHDM2	JmjC domain-containing histone demethylase 2 family
KEGG	Kyoto Encyclopedia Of Genes and Genomes
KO	Knockout gene
KNN	K nearest neighbour
KRAS	Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
LAMPS	Large-scale Atomic/Molecular Massively Parallel Simulator
LINCS	The Library of Integrated Network-Based Cellular Signatures
LSD1	Lysine-specific demethylase
MBDP	Methyl-binding domain protein

MCODE	Molecular complex Detection
Mfold	Multiple folds
MET	Mesenchymal-Epithelial Transition Factor
MD simulations	Molecular Dynamics simulations
MDM2	Mouse double minute 2 homolog
MGMT	O-6-Methylguanine-DNA Methyltransferase
MHC	Major histocompatibility complex
MLL1	Mixed Lineage Leukemia 1
MOE	Molecular Operating Environment
MIF	Macrophage migration inhibitory factor
MM	Molecular mechanics
MMP2	Matrix Metalloproteinase-2MMP9 - Matrix Metalloproteinase-9
mRNA	Messenger RNA
MRI	Magnetic Resonance Imaging
MVD	Mevalonate Diphosphate Decarboxylase
MVK	Mevalonate Kinase
MYC	Myelocytomatosis(d)
NAMD	Nanoscale Molecular Dynamics
NCI	National Cancer Institute
NOX	Nicotinamide Adenine Dinucleotide Phosphate Oxidase
NSAID	Nonsteroid Anti-inflammatory Drug
NSCLC	Non-Small Cell Lung Cancer
PARP	Poly-Adp Ribose Polymerase
PBPK	Physiological-based pharmacokinetic modeling and simulation
PDAC	Pancreatic ductal adenocarcinoma
PDGFR	Platelet derived growth factor receptor alpha.
PET	Positron Emission Tomography
PFRED	PFizer RNAi Enumeration and Design tool
PGK1	Phosphoglycerate kinase 1
pH	Potential of hydrogen
PHD fingers	Plant Homeodomain
PHPS1	Phenylhydrazono pyrazolone sulfonate 1
PK tutor	Pharmacokinetics and metabolism software
POLE2	DNA polymerase epsilon 2
PPI	Protein–Protein Interaction
pROTAC	Proteolysis targeting chimeric technology
PTEN	Phosphatase and TENsin homolog deleted on chromosome 10
QSAR	Quantitative Structure–activity Relationship
RAR-β2	Retinoic acid receptor beta
RBM10	RNA binding motif protein
ROCK	Rho- Associated Protein Kinase
ROS1	C-ros oncogene 1
RNAi	RNA interference
SAM	S-adenosyl-L-methionine
SDTNBI	Substructure-Drug-Target Network-based inference

SHAFT	SHApe-Fea Ture Similarity
Shp2	Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2
SiRNA	Small Interfering RNA
SIDER	Side Effect Resource
SILCS	The Site Identification by Ligand Competitive Saturation
SLC	Solute-carrier gene
SOM	Self Organizing Map
STAT 3	Signal transducer and activator of transcription 3
STITCH	Search Tool for Interactions of Chemical
STRING	Search Tool for the Retrieval of Interacting Genes/ Proteins
SVM	Support vector machines
TCF	T cell factor
TCGA	The Cancer Genome Atlas
TET	Ten-eleven translocation 1
TG2	Tissue Transglutaminase
TLR4	Toll-like Receptor 4
TNBC	Triple Negative Breast Cancer
TOP2A	Topoisomerase II Alpha
TP53	Tumor Protein 53
VEGFA	Vascular Endothelial Growth Factor A
VEGFR	Vascular Endothelial Growth Factor and its receptor
WHO	World Health Organization
XIAP	X-linked inhibitor of apoptosis protein
5fmc	Formylcytosine

23.1 Introduction

Cancer is a group of diseases that develops when cells start to divide uncontrollably, bypassing senescence, and apoptosis. The WHO states that it is the second highest cause of death (the leading death is due to ischemic heart disease) which leads to 1.6 million deaths worldwide. Based on pathology, tumors are classified into two types: benign and malignant tumors. A benign tumor is a localized group of cells that are dividing endlessly. They will not spread or invade other tissues. A malignant tumor is a group of cells that divide abnormally and spread to neighboring tissues, causing them to divide uncontrollably. They can also reach distant tissues by using the circulatory and lymphatic systems. This condition is called metastasis. Cancers are divided into four main groups: sarcoma (affecting connective tissues like muscle, joints, etc.), carcinoma (affecting epithelial tissues), lymphoma (affecting immune cells), and leukemia (affecting blood-forming cells). Further down the line, they are classified based on the origin and type of cells involved like adenocarcinoma, colorectal lymphoma, etc. [1]. Stomach, prostate, liver, colorectal, and lung cancers

Fig. 23.1 Process of cancer development

are prevalent among men. Lung, thyroid, breast, colorectal, and cervical cancers are predominant among women. The single cell does not prerequisitely possess the properties of tumor cells but rather tumor development is a multi-step process. This process is shown in Fig. 23.1.

A normal cell becomes a cancer cell to avoid the stress of senescence and apoptosis. A cancer cell can not only escape senescence but can also develop from one using the pro-inflammatory and pro-proliferative factors secreted by the senescent cells. To evade apoptosis, the cancer cells impair death receptor signaling, reduce caspase function, and disrupt the balance of pro-apoptotic and anti-apoptotic proteins. In addition, cells reach immortality by inhibiting telomerase enzyme and PARP [2]. One of the reasons that explain why cancer is dangerous is that within the same tumor group of cells, we can find genetic diversity. This leads to multidrug resistance. The second reason is that cancer cells hide from the innate immunity responsible for evading them. The exact mechanism is unknown. Besides, targeting cancer cells is difficult. Further, there is little development in epigenetic profiling of cancer [3, 6]. Owing to these reasons and more, the development of cancer treatment should be a progressive one with the ability for enhancements within a short period. The existing treatments are quite capable of tackling the robust nature of cancer. But, they all possess some disadvantages. The details are mentioned in Table 23.1.

Having established that the above-mentioned treatment strategies have their limitations (particularly side effects), it is essential to bring forward a relatively new treatment strategy, computer-aided drug design (CADD). This method aims at simplifying the complex process of drug discovery. Although it has to be mentioned that the drugs are not developed in silico, the applications of CADD are in constructing disease models, identifying the drug targets, screening for a viable chemical molecule as a therapeutic intervention, and deciding the experimental program. The construction of disease models makes it possible to develop a drug that has minimum possible side effects. Computational methods also make it reliable to identify the effects of mutation on receptors and drug resistance, moving researchers a step forward in designing drugs. The implementation of CADD keeping β-amyloid protein, BACE-I, ROCK, NOX, and HDAC6 enzymes as therapeutic targets is beneficial in treating Alzheimer's and various other neurogenerative disorders [9]. Also, CADD is applied in COVID-19 therapy keeping the spike S protein and the ACE-2 receptor of the host as the target [8]. Further, various therapeutic marine molecules like Meridianin-A targeting CLK1 (P49759), GSK3_(P49841), DYRK1B (Q9Y463),

Table 23.1 Treatments available for cancer

S. No.	Existing treatments	Mechanism of action	Challenges	References
1	Surgery	An invasive/non-invasive method that removes localized mass of tumor	Effective only in localized mass of cells. Cannot cure metastasis	[4, 5, 146–157]
2	Chemotherapy	It uses one or more drugs in combination for treating cancer. Since drugs can travel through bold, and they can treat metastasis	• Drug resistance • Harmful side effects • Low specificity • Rapid drug metabolism.	
3	Radiotherapy	Radiation uses particles or waves targeted at tumor mass to induce damage to the DNA of cancerous cells	Damage to healthy cells, organs, and tissues	
4	Stem cell therapy	This therapy focuses on restoring the blood-forming cells when they have been destroyed by chemotherapy/radiotherapy	• Therapeutic dose control • Low cell targeting • Retention in tumor sites • Not durable	
5	Immunotherapy	This therapy promotes the formation of immune cells enabling them to fight cancer	Potential side effects like swelling, weight gain, diarrhea, fever, chills, fatigue, etc.	
6	Anti-angiogenic therapy	It prevents the flow of blood to the cancer cells	• Long-term therapy leads to tumor hypoxia • Genotype alterations • Affects the cells of the microenvironment • Tumor blood vessels normalization	
7	Hormone therapy	It involves synthetic hormones to prevent the flow of natural hormones to the cancerous tissues	• Weight gain • Hot flashes • Fatigue • Bone loss • Memory problem	
8	Gene therapy	It replaces the defective gene with a normal gene that does not lead to cancerous division	• Selection of the best delivery mechanism • Integration into genome • Limited efficiency in some • Neutralized by the immune system	

(continued)

Table 23.1 (continued)

S. No.	Existing treatments	Mechanism of action	Challenges	References
9	siRNA-based approach	siRNA prevents the tumor from growing via RNAi to decrease the expression of the M2 subunit of R2 (ribonucleotide reductase)	• Dosage correction • Variabilities between individuals	
10	Natural drugs	With a wide chemical diversity, they serve as therapeutics for the treatment of cancer.	• Limited bioavailability • Toxicity.	
11	Targeted therapy	It targets the proteins that control cell division, senescence, and apoptosis	Long-term side effects	
12	Ablation therapy	It removes tumor mass by vaporizing them. It is of three types: microwave ablation, cryoablation, and irreversible electroporation	• Acts only on localized tissues • Low penetration power	
13	Nanomedicine	It modulates the distribution and release of drugs administered. It involves biocompatible nanoparticles, liposomes, etc., as carriers for the release of drugs	• Low biocompatibility • Possible toxicity • Environmental disposal problem	
14	Extracellular vesicles	Extracellular vesicles (EVs) are a group of secretory vesicles with cell-derived membrane and contents. They are used in drug delivery as they are rejected by the immune system	Lack of pre-clinical procedures for isolation, quantification, storing, and drug loading	
15	Radiomics/pathomics	It collects data from images obtained from various scans like CT, PET, MRI, etc., and the collected data are utilized by clinicians to know about the state of the tumor, its location, and other relative information. It is used in personalized treatment	• Description of parameters and computational/statistical methods to set robust protocols for the generation of models for therapy • Definition of univocal data acquisition guidelines • Standardization of procedures to facilitate clinical translation	

and CK1_(P48730), Hodgsonal, Dendrinolide, Rossinone-A, Apliacyanin-A, and Polyrhaphin-A have been identified using CADD in treating various neurodegenerative like Parkinson's, Alzheimer's, and cardiovascular diseases [10]. Most importantly, this method is also accounted as fast and reproducible. It aims in reducing the time, resources, and expenses involved in designing a therapeutic molecule. With these strengths, it can be extrapolated that CADD has tremendous application in the treatment of cancer.

23.2 CADD Methods

Computational methods make the earlier stages of the complex drug discovery process less time-consuming and inexpensive. Typically, CADD is involved in the stages as listed in Fig. 23.2.

Virtual screening is done using known compounds as a foundation and then identifying new compounds similar to the existing compounds. This step leads to the identification of potential hits of therapeutic value. After this, the specificity of target–ligand interactions is known using the molecular docking technique. Ligands specific to the receptor are further filtered by checking their potency, ADME, and toxicity properties using QSAR methods. These phases optimize the molecules that have therapeutic value for drug design [11]. Important properties to consider include solubility, efficacy, safety, bioaccumulation, CNS bioavailability, permeability, metabolic stability, hydrophobicity, potency, and selectivity [12]. The process of drug design using CADD can be classified into two types depending on whether or not the 3D structure of the target is available. If the structure of the target protein or the analogue is obtained, then we can find ligands that spatially fit the target using functional groups. This is called structure-based drug design (SBDD). If the structure of the analogue is known, then we can model the target protein with more than 35% similarity. Two strategies can be employed for SBDD—de novo design of ligands, where small pieces of molecules are connected to form a model, and molecular database

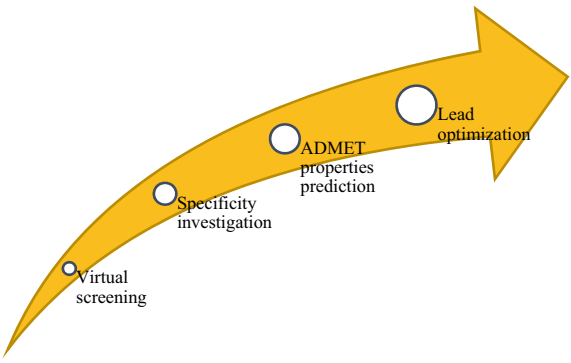

Fig. 23.2 Phases of CADD

mining, where the analogues of previously active ligands to the known receptor are identified. Docking is the preferential method of database mining as it gets high rates of results. The drawback of docking is that it cannot identify molecules of high activity. The second one is molecular dynamics simulation. This method involves analyzing the physical movement of atoms and molecules. It allows the atoms and molecules to interact for a definite period and then numerically predicts the dynamic evolution. The identified ligands can be converted to molecules of biological activity (hits). If the structure is not known, then we can use a known active ligand as a base and find similar compounds using virtual screening. This strategy is called ligand-based drug design (LBDD). Three methods can be employed in LBDD—building pharmacophore models, QSAR analysis, and 3D-QSAR analysis. A pharmacophore can be positively or negatively charged atoms, donors, or acceptors or cyclic groups. They have a particular property, but they are separate pieces. Pharmacophore modeling involves building them together to get the desired ligand of known specificity. QSAR determines the activity of these pharmacophores by determining the correlation between the activity and the structure of its homologous analogues. 3D-QSAR goes a step further and can determine the activity of compounds of another chemical class. It can also show the regions of high and low binding [13]. The analogues identified can be optimized to leads based on the ADMET properties to become drug candidates. The old and new techniques and software involved in CADD are listed in Table 23.2.

Computer-aided drug designing process is enhanced by augmenting with the technologies in the silicon world like machine learning (ML), big data, and artificial intelligence (AI). CADD involves the use of multiple databases to get information from different perspectives. These databases generate huge amounts of data. Hence, the big data concept comes into the picture. This also faces challenges from the multiple Vs like Volume (data scale), Velocity (data growth), Variety (data sources), Veracity (data quality), Validity (data authenticity), Vocabulary (data terminologies), Venue (different data platforms), Visualization (data patterns), Volatility (duration of usefulness), and Value (data value). Machine learning is a part of artificial intelligence that deals with remembering information from past data. It helps overcome the value challenge of big data by reducing drug attrition. It also has huge applications in QSAR, de novo drug designing, and artificial neural networks. New modeling algorithms, better web portal designs, database curation, experimental protocols, quality control, and transparent data reporting are the keys to eliminating the challenges faced by big data [14]. Despite these technological advancements and tremendous applications in drug design, CADD faces some drawbacks like low efficiency of virtual screening, an under-developed field of chemogenomics, less number and quality of computational resources, weak design of drugs for multiple molecular targets, diminished prediction of toxicity and side effects, weak augmentation with other technology that enables optimized screening of biomolecules [15]. In addition, CADD can be further developed by understanding the modeling errors and uncertainties. This can lead to new algorithms and statistical testing grounds [16].

Table 23.2 CADD methods and the free and commercial software available

S. No.	Method	Software available	References
1	Molecular dynamics simulation	• CHARMM, GROMACS, AMBER, LAMMPS, GROMOS, CP2K, OpenMM-PLUMED, NWchem, NAMD, Ascalaph Designer, CPMD, Abalone, Desmond	[158–181]
2	Homology modeling	• MODELLER • SWISS-MODEL • RaptorX • GalaxyWEB	
3	Molecular docking	• AutoDock • AutoDock Vina • CDOCKER, Discovery Studio LibDock, DOCK 6, rDock, ZDock, Schrödinger Docking Tutorial, SnugDock, FlexAID, Glide, LeDock, ClusPro, FireDock, ClusPro, PatchDock, HawkDock	
4	Database screening	• Program pharmer	
5	SBDD and LBDD methods	• Discovery studio • Schrodinger • Open eye • MOE	
6	De novo design	• Gen UI • SYNOPSYS	
7	Pharmacophore modeling	• Pharmer • DrugOn	
8	QSAR	• QSAR-Co • Coral	
9	3D-QSAR	• Cloud 3D-QSAR • AutoGPA	
10	In silico ADMET prediction	• Swiss-ADME • ADMET-SAR	
11	Molecular similarity	• eSHAFTS • GraphSimTK	

23.3 Targeted Cancer Therapy

Targeted therapy is a type of cancer treatment, where small molecules developed as drugs are used to target disease-related genes or proteins. It is more effective than chemotherapy as it reduces side effects and is more specific. Various genes, mRNA, and proteins are set as a target. Kinase enzyme class has established to be one of the most targeted receptors of all time. Kinase receptors such as receptor tyrosine kinase, non-receptor tyrosine kinase, serine–threonine kinase are the most sought after. In addition, epigenetic targets, BCL-2 targets, hedgehog pathway targets, PARP, and

proteasome targets are also used in developing small molecule inhibitors as drugs [17].

23.3.1 Targeting Oncogenes

See Table 23.3.

23.3.2 Targeting Cancer-Related mRNA

A new compound 3-(4-amino-1H-benzo[d]imidazole-2-carboxamido)-4-oxo-3,4-dihydroimidazo [5,1-d] [1–3, 5] tetrazine-8-carboxamide has been designed by computer-aided drug design and tested its efficacy against breast cancer and glioma cells. This has also been proved that it impacts various coding and non-coding RNAs using in vitro experiments [26]. Emerging technologies in gene therapies include antisense drug design which uses artificial antisense oligonucleotides to target mRNA and control gene expression. Prediction of antisense oligonucleotides (AOs) against target mRNAs includes two methods. The first is by constructing a neural network by setting a wide range of input parameters. A database of 490 AO is collected using sequence information from the literature. This was used to construct the neural network. This model can predict AOs with a success rate of 92%. In addition, it predicts 12 AOs per 1000 AOs. The second method uses thermodynamic and structural motifs. This work constructed artificial neural networks based on 11 parameters to predict oligo as opposed to non-oligo nucleotides. This model showed an accuracy of 92.48% with 91.7% sensitivity and 92.09% specificity [27, 28]. For designing AO, a software called PFRED is used. It designs, visualizes, and analyzes siRNAs and AOs. It is an open-source code with an intuitive user interface [29]. To reduce the uncertainty in AO selection against multiple mRNA targets, several parameters have been taken into consideration, and the targets are integrated. By computational methods, AO prediction then takes place against the integrated target. The efficacy depends on the structure properties of the target. The 5' and the 3' end of the target are also important in determining efficacy [30]. Another way of AO selection is based on computational secondary structure analysis. This is done using the program Mfold, S-fold, and OligoWalk. This program utilizes the free energy data of base pairs' formation and structural features of helices and loops to determine the structure [31, 37, 145]. Various databases are also developed for the antisense oligonucleotides (ODNs). Until 2005, 700 ODNs against 26 target mRNAs are documented in this AOBase. This can be accessed using TargetSearch and AO Search web interfaces. Another database called therapeutic target database (TTD) has information of 649 antisense drugs with structure and sequence [35, 36]. Several works have contributed to the antisense drug design. Compound like Genasense, GTI 2040, is to be used against hematological malignancies and solid tumors, AP1261, and several S-ODN

Table 23.3 CADD methods in targeting druggable gene receptors for various forms of cancer

S. No.	Cancer form	Gene targets	CADD method	Description	References
1	Breast cancer	31 genes including AKT1, EGFR, MYC, and VEGFA	• Virtual screening • Target prediction • Pathway enrichment analysis • Molecular docking	The therapeutic potential of *Prunella vulgaris* is explored through a network pharmacology-based approach. Computer-aided drug design helped the study identify the potential druggable gene targets for breast cancer therapy	[18]
2	Bladder cancer	CXCL12, FGF2, and FSCN1	• Text mining using GenCLiP tool • Pathway analysis using GO and KEGG • PPI network construction using STRING • Modular analysis using MCODE	Genes shared by bladder cancer and Crohn's disease were to be identified by the CADD methods mentioned. A total of 296 genes were identified by text mining tool and were filtered down further. Finally, three druggable gene targets were identified. In addition, 26 drugs are found to be treatable for bladder cancer	[19]
3	Colon cancer	20 genes including HMGCR, MVK, MVD	• Network analysis • Fuzzy optimization network	This work identifies anti-cancer agents using fuzzy optimization network. Using this method, druggable gene targets, metabolite, and reaction centric targets are identified	[20]

(continued)

Table 23.3 (continued)

S. No.	Cancer form	Gene targets	CADD method	Description	References
4	Colorectal cancer	KRAS, APC, SMAD4, TP53, and others	• Virtual screening • Molecular docking • Homology modeling	This review compiles the driver genes essential for colorectal cancer, focusing mainly on the receptor–ligand interactions	[21]
5	Leukemia	Bcr-Abl, KO, Bcl-xl	• Qualitative network modeling using BMA software	This study using the BMA tests all possible combinations of drug targets with gene network that regulates the disease to develop drugs	[22]
6	Gastric cancer	435 genes are involved	• Deep belief network–graph transformer network	A disease similarity network and a gene interaction network was built using the graph transformer network, and the deep belief network is used to reduce the dimension of features	[23]
8	Pancreatic cancer	ADAM10, APLP2, EGF, and others	• Virtual screening • PPI network analysis • Modeling • Molecular docking • GNM modeling	Cancer genes and anti-cancer druggable targets are identified by combining three different datasets obtained from PPI network and microarray	[24]
9	Pancreatic ductal adenocarcinoma	PGK1, HMMR, and POLE2,	• 'Spectral Clustering for network-based target ranking' (SCNrank)	A new method designed SCNrank that combines three types of data: expression profiles from tumor tissue, normal tissue, and cell-line PDAC; protein–protein interaction network (PPI); and CRISPR-Cas9 data to prioritize potential drug targets for PDAC	[25]

candidates are involved in antisense drug design [32–34]. Another emerging strategy of controlling gene expression is post-transcriptional gene silencing (PTGS). This strategy includes degrading of mRNA whose gene expression is to be reduced. This process involved computational approaches in many of the steps like RNA secondary prediction (MFold tool), molecular graphics (ViewerPro), site-specific mutagenesis, etc. [37].

23.3.3 Targeting Oncoproteins

Targeting enzymes that play a crucial part in tumorigenesis is one of the strategies. Cyclooxygenase-based enzymes form two types: COX-1 and COX-2. COX-2 targeting is done by NSAIDs with gastric side effects. In this study, in silico approaches including virtual screening, molecular docking, homology modeling, and 3D-QSAR lead to the identification of new inhibitors of COX-2 [54]. Human DNA ligase is the enzyme that is important in DNA repair mechanisms. In silico approaches like virtual screening, molecular docking, and MD simulations identified 192 compounds that can bind to the target. Adjoining in vitro assays narrowed down the inhibitors list to five [55]. Farnesyl transferase adds a farnesyl group to the -SH group of cysteine residue at the terminal of the target to form a thioether linkage. Computational methods including virtual screening, molecular docking, ADMET profiling, density functional theory, molecular interaction field studies were done to identify small molecule inhibitors against this enzyme. The inhibitors SCH226734, BMS-214662, L-778123, and U49 have better effects than the drugs tipifarnib and lonafarnib [56]. The deregulation of kinases poses a threat by inducing various stages of cancer. Different types of kinases are involved in cancer like transcription SE targeting therapies including CDKs 7, 8, 9, 12, and 13; receptor tyrosine kinase signaling EGFR, KIT, PDGFR, MET, and HER2; and some protooncogenes. Compounds like SY-1365, CCT251545, BCD-115, NVP-2, BAY1251152 are found to inhibit the transcription-related kinases important in cancer [57]. Topoisomerase II is an important enzyme in DNA topology. Inhibiting this enzyme leads to cell apoptosis. Targeting topoisomerases using CADD has been done by both SBDD and LBDD. Variety of compounds with inhibitory effects have been identified so far [58]. Protein tyrosine phosphatases, targeting Shp2 was effective against the cancerous effect. Methods were applied, and several small molecule inhibitors like isatin, PHPS1, PHPS4, etc., were screened and designed [59]. Targeting cell cycle proteins, essentially enzymes, are another strategy for cancer therapy. MDM2 is a protein that is important in regulation of the expression of the p53 protein. If this protein is inhibited, p53 is not regulated, and the cell undergoes apoptosis. Structure-based drug design approaches were undertaken to identify small molecules that inhibit MDM2 protein. This includes pyrazolidinedione sulfonamide, tryptophan

derivatives, norbornane derivatives, *cis*-imidazoline derivatives, 1,4-benzodiazepine-2,5-diones, and synthetic chalcones [62]. Myc is one of the proteins that regulates cell growth if over expressed leads to cancerous outcome and drug resistance. Targeting Myc involves identifying small molecule inhibitors of two types: (i) targeting the dimerization of Myc with Max and ii) targeting Myc–Max interaction with DNA. Structure-based drug design approaches were utilized in identifying the said inhibitors [43]. Cyclin-dependent kinases (CDKs) are essential in regulating cell cycle. Computational methods used to screen molecules that are capable of inhibiting the CDKs reveal a benzodiazepine derivative NSC 664704. It inhibits CDK2 [63]. Structure-based drug design measures were undertaken in discovering small molecule inhibitors of CycD–CDK2 interaction. This approach leads to the discovery of a new and potent molecule O^6-cyclohexylmethyl-2-(4'-sulfamoylanilino)purine [64]. There is another class of proteins that are closely linked to diseases like cancer. They are called intrinsically disordered proteins (IDPs). These proteins have trouble folding under human physiological conditions. CADD approaches such as HTS, Vs, and reverse docking have been employed in determining the ligands that have inhibitory effect on this target [82]. In addition to the bioinformatic approaches, interdisciplinary methods like AI, big data, and machine learning have also been at play in identifying therapeutic molecules targeting oncoproteins. AI-assisted virtual screening, AI-assisted reverse docking, 3D therapeutic alignment structural target prediction, and deep learning models help discover therapeutic molecules against specific targets, repurpose drugs for other diseases, identify the drug binding pocket in a receptor in human and animal models, and predict ADMET profiling, respectively. Big data approaches are utilized in huge databases like PubChem, Protein Data Bank, and DrugBank. Computational approaches like these help researchers combine the databases and strategies to discover or design a new therapeutic molecule. Machine learning methods improve the screening and designing processes in drug design. It also helps scientists in integrating various bits of information to design a drug de novo [65, 66]. Furthermore, other oncoproteins (including a variety of cell structural proteins) like canonical Wnt-b-catenin, tubulin, cyclophilin A, fibroblast growth factors, HSP90, KRASG12D, membrane proteins and soluble proteins, MIF, SLC, TLR4, and other transcription factors are also targeted using CADD (mostly SBDD) strategies to inhibit them in some way or other to prevent their downstream effects on other proteins leading to metastasis [71–81]. Various cancer forms' targets are addressed using computational means by identifying therapeutic small molecule inhibitors as listed in Table 23.4.

23.3.4 Targeting Epigenetic Receptors

While genetic changes affect the transcription and translation of genes, epigenetic changes affect the expression of genes. Epigenetic changes like DNA methylation, DNA hydroxymethylation, and histone modification are covalent and can affect DNA or histone residues turning a normal cell into a cancerous one. Protein families that

Table 23.4 CADD methods targeting druggable oncoproteins for various forms of cancer

S. No.	Form of cancer	Target protein	CADD methods	Description	References
1	Lung cancer, anaphylactic large-cell lymphoma, neuroblastoma	Anaphylactic lymphoma kinase	Structure-based drug design	Small molecule inhibitors of the insulin receptor–tyrosine kinase were identified based on SBDD and were approved by FDA. They are crizotinib, ceritinib, alectinib, brigatinib, and lorlatinib. In addition, pROTAC method was found to be useful in developing ALK degraders	[38]
2	Lung cancer	• KRAS • RBM10 • VEGFR • BRAF • EGFR	Structure-based drug design	Curcumin, resveratrol, sulforaphane, epigallocatechin, fistein, and vemurafenib were identified to have inhibitory effects on various lung cancer receptors	[39]
3	Lung cancer	ROR1	• Homology modeling • Virtual screening • Docking	ROR1 is a receptor tyrosine kinase highly expressed in NSCLC. It also confers drug resistance to the tumors. Combining in silico and in vitro methods, it is found that ARI1 inhibits ROR1	[40]
4	Lung cancer	ROS1	• Molecular docking • Virtual screening • Pharmacophore studies • ADMET • Boiled-egg plot	DB-125503 was found to inhibit the ROS1, a receptor tyrosine kinase inhibitor via computer-aided drug designing	[41]

(continued)

Table 23.4 (continued)

S. No.	Form of cancer	Target protein	CADD methods	Description	References
5	Breast cancer	Estrogen receptor-α	• SBDD • LBDD	By targeting EBS, AR2, EBS-DBD, and other portions of the receptors using computational method, the authors have been able to identify potential drugs and other ligands that can inhibit the cancerous effect	[42]
6	Breast cancer	Myc–Max dimer	• HTS	Myc is one of the un-druggable targets in breast cancer. It was found that on targeting the Myc–Max dimer, compounds 10058-F4, 10074-A4, and 10075-G5 are able to inhibit them	[43]
7	Colorectal cancer	p53	• Molecular docking • Virtual screening • QSAR modeling of target	p53 is one of the most expressed proteins in colorectal cancer tissues. Virtual screening and molecular docking led to the identification of dihydroergocristine, a small molecule inhibitor against p53. This has been further developed and tested using in vitro assays	[44]
8	Colorectal cancer	• Cyclin D1 • PTGS2	Ontology combined with network assisted gene ranking	This method predicts new drug targets in addition to the FDA approved ones	[45]
9	Colorectal cancer	TCF-driven EMT	Structure-based drug design	TCF-driven EMT enhances drug resistance and metastasis. Computational methods reveal the ATP competitive inhibitors of TOP2A. Unlike the other TOP2A inhibitors, it does not damage DNA and is not cytotoxic	[46]

(continued)

Table 23.4 (continued)

S. No.	Form of cancer	Target protein	CADD methods	Description	References
10	Melanoma	V600E BRAF protein kinase	• QSAR modeling • Docking • Virtual screening • ADMET profiling	Compounds 39, N1, N2, N3, N4 are able to inhibit the V600E BRAF protein kinase that is expressed in melanoma tumor	[47]
11	Melanoma	S100B-p53 association	• HTS • SILCS	Compounds SC0067, SC0332, SC0844, SBi132, SBi1, SBi279, SBi523, SBi4211 identified through computational methods are able to inhibit the association between S100B-p53 and initiate cell death	[48]
12	Pancreatic cancer	• TG2 • MMP2 • MMP9	• Virtual screening • Homology modeling • Molecular docking	Phytochemicals—Bacoside A3 and myricetin have been docked against and known to inhibit TG2. Nitric oxide donors—S-nitroso-N-acetylpenicillamine and nitroglycerin have been docked against and known to inhibit MMP2 and MMP9	[49]
13	Acute myeloid leukemia	STAT3	• Network pharmacology • Molecular dynamics simulation • AI algorithm models • 3D-QSAR	ZINC20816625 has the ability to inhibit STAT 3 protein and the ability to be developed into the drug	[50]
14	Gastric cancer	Cag A	• Virtual screening • Molecular docking • Molecular dynamics simulation	Cytotoxin-dependent antigen A was expressed in gastric cancer tissues. Through in silico approaches, it was found that ZINC153731, ZINC69482055, and ZINC164387 efficiently bind to and inhibits the Cag A protein	[51]

(continued)

Table 23.4 (continued)

S. No.	Form of cancer	Target protein	CADD methods	Description	References
15	Cutaneous T cell lymphomas	Tox protein	• Virtual screening • Docking	Thymocyte selection-associated high mobility group (HMG) box protein (TOX) protein was found to be associated with cutaneous T cell lymphomas. To find the small molecule inhibitors against the TOX protein, in silico approaches were utilized. At the end of the experiment, 18 compounds including 190,444, 190,414, etc., from the ZINC database were found to inhibit the TOX protein	[52]
16	Ewing's sarcoma	EWS-FLI1 fusion protein	Structure-based drug design	Ewing's sarcoma family tumor (ESFT) cells contain the EWS-FLI1 fusion protein as a cancer biomark were identified.	[53]
17	Bladder cancer	H-Ras p^{21}	• Virtual screening • Molecular docking	In silico methods were applied in identifying small molecule inhibitors against H-Ras p^{21}. This protein is present in about 30% of tumors. Compounds like 3-aminopropanesulfonic acid and hydroxyurea showed better binding affinity than its native ligand Raf	[60]

(continued)

Table 23.4 (continued)

S. No.	Form of cancer	Target protein	CADD methods	Description	References
18	Pancreatic cancer	Lipoxygenase (LOX)	• Molecular docking • Molecular dynamics simulation • Homology modeling • Structure-based lead optimization • QSAR • Pharmacophore modeling • Scaffold hopping • Pseudo receptors	Lipoxygenases' overexpression is identified in pancreatic cancer cells. To inhibit this enzyme, CADD approaches were undertaken to develop small molecule inhibitors against LOX	[61]
19	Pancreatic cancer	HIF-1α	• Virtual screening • Molecular docking	Raf kinase inhibitory protein (RKIP) has metastasis suppressing properties that is used to inhibit this receptor. Computational approaches have been used to evaluate the protein–protein interaction	[67]
20	Breast cancer	HPIP	• Virtual screening	TXX1-10 has anti-cancer activity through inhibiting this target and reducing metastasis	[68]
21	Cervical cancer	HPV 16	• Virtual screening • Molecular docking • MSA and phylogenetic analysis	The target has been associated with 99.7% of human cervical squamous cell carcinoma. Natural inhibitors like EGCG, curcumin, theaflavin, and other molecules are identified using virtual methods that hold promising value	[69]
22	Lung cancer	Rab39a	• Virtual screening • Homology modeling • Docking • ADME profiling	Computational methods were involved in identifying hetero molecules with amine and amide groups with therapeutic potential against this target	[70]

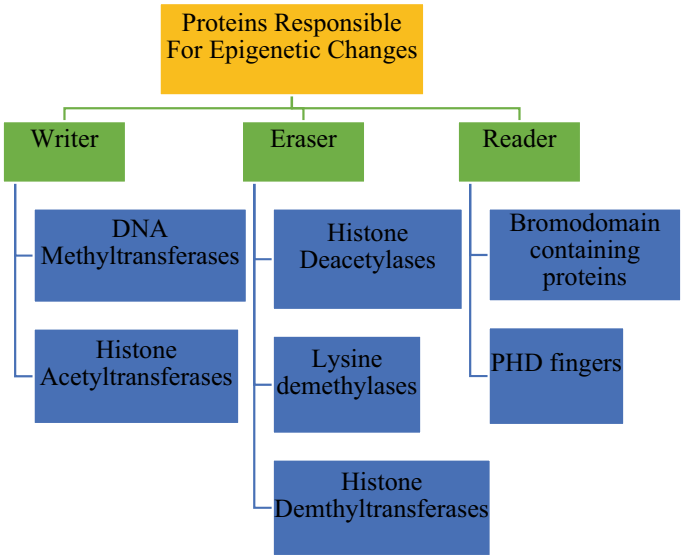

Fig. 23.3 Protein families causing epigenetic changes

cause these epigenetic changes are broadly classified into three types depending on their relationship with the change: writers, erasers, and readers. Writers are the group that causes the covalent modification in DNA/histone proteins. Erasers are the group that removes the covalent modification. Readers are the group that identifies the covalent modifications and involve essential molecular chaperones to rectify the issue. The different types of protein families along with the CADD methods employed to assess them are furnished in Fig. 23.3.

Methylation, brought about by DNA methyltransferases and the association between MBDP and HDAC interfere with the transcription of genes and silence certain cell cycle regulators (p16, p21, p27, p53), DNA repair protein (MGMT) pro-apoptotic gene (ARH1), and differentiators (RAR β2). Drugs that demethylate the tumor suppressor genes are effective against this change. Hydroxymethylation of cytosine residues by the TET family proteins serves as an epigenetic change. It affects the mutations in IDH1 and 2 and leads to various malignancies. The product 5-hydroxy methyl cytosine (5hmC) can be further oxidized to 5-fmC and 5cmC by TET proteins. They add to further methylation. This is a stable modification of DNA. 5hmC is found across various cell cycles and has various promotors. This adds to distinctive downstream effects [83]. Lysine residues and arginine residues of histone are prone to mono-, di-, and tri-methylation. This is brought about by SAM-dependent methyltransferases, 2-oxoglutarate-dependent methylases, and lysine-specific histone demethylase 1. It does not affect the structure of chromatin but affects its binding sites where other proteins bind to hold a nucleosome together. Histone acetylation is a change where an acetyl group from acetyl Co-A is transferred to the ε-amino group of lysine residue of histone, especially 16th lysine residue

of 4th histone protein (H4K16). This is brought about by histone acetylases protein family. The histone deacetylation done by histone deacetylases protein family is the reverse of histone acetylation. It removes the acetyl group from histone protein [88]. Histone demethylation is removal of methyl group from lysine or arginine residue in the histone protein. It is done by histone demethylases protein family subdivided into LSD and JHDM. Mutation in the expression of this protein family leads to various forms of cancer, particularly leukemia [85]. Examining and analyzing them using wet lab techniques are a hard and expensive process. The in silico approaches that analyze the protein families are mentioned in Table 23.5.

Drugs like azacitidine, decitabine, guadecitabine, hydralazine target DNA methyltransferases to treat various cancers. Drugs like pracinostat, belinostat, entinostat, vorinostat, panobinostat, romidepsin target histone deacetylases to treat various cancers. Drugs like tazemetostat, CPI-1205, MAK683, Molibresib target bromodomain extraterrestrial to treat various forms of cancer [87]. There are challenges in epigenetic treatments. Docking fails to identify important parameters in a molecule like solubility, etc. It has low accuracy. Further research has to be developed based on targeting new targets like HAT and PPI. Reproducibility of results in different laboratories is difficult, and overinterpretation seems to be problem [84].

23.4 Tackling Multidrug Resistance in Cancer Using CADD

Multidrug resistance (MDR) is a phenomenon where tumor cells are unresponsive to different types of therapies. It is acquired by the tumor cells because of a complex number of reasons. First, the expression of p-glycoprotein and other transporters induces the efflux of drugs outside the tumor cells. Second, selective multiplication of MDR-attained cells is also observed. Third, tumor cells are now immune to drug-induced apoptosis. Fourth, the microenvironment of tumor cells provides them with an MDR phenotype [111]. There have been lots of approaches proposed in handling MDR. CADD is among them. Different types of targets and corresponding ligands for MDR therapy are discovered. ABCG2 is an ABC transporter involved in breast cancer MDR. Using CADD methods, phenyltetrazole derivatives are identified as inhibitor and the specific functional groups responsible for MDR therapy are also discovered. Several machine learning methods are also involved in identifying inhibitors for the BCRP/ABCG2 protein responsible for breast cancer [112, 113]. Tyrosine receptor kinases have also been identified as potential target for MDR therapy. In this regard, CAD methods have been utilized, and N-substituted isatins' class of compounds has been identified as inhibitors to this receptor [114]. QSAR has been used to identify MDR reversal agents. The structure–activity relationships between 609 compounds have been identified in this study [115]. SBDD methods have been utilized in determining target mutation-induced MDR. Challenges in MDR have been overcome by this method [116]. An anti-cancer drug cisplatin has been used for tackling MDR. It is a platinum complex which induces apoptosis in tumor-induced cells. Cisplatin

Table 23.5 CADD strategies, methods, and tools available for targeting druggable epigenetic receptors

S. No.	Strategy	Method	Function	Tools available	Target	References
1	Structure-based drug design	Homology modeling	It predicts structures of protein based on its sequence	• MODELLER • SWISS-MODEL	DNA methyltransferases	[84–86]
2	Structure-based drug design	Docking	It determines the binding energy values of receptor-ligand complex	• AutoDock • AutoDock Vina	DNA methyltransferases	
3	Structure-based drug design	Molecular dynamics simulation	It predicts the movement of biomolecules in an interaction	• AMBER MD • GROMACS	DNA methyltransferases and histone deacetylases	
4	Structure-based drug design	Fragment-based drug design	It develops therapeutic molecules from small fragments of low molecular weight and low affinity to receptors	• ACFIS	DNA methyltransferases	
5	Ligand-based drug design	Chemoinformatics	This branch deals with handling chemical information and solves chemical problems	• DNMT focused libraries • NCI diversity set	DNA methyltransferases	
6	Ligand-based drug design	Structure similarity search	It identifies molecules with similar structures to perform the same function	• ChemMine • MadFast	DNA methyltransferases	
7	Ligand-based drug design	Pharmacophore modeling	It gets information from the interaction of 3D structures of receptor and ligand	• Pharmer • PharmMapper	Histone deacetylases	

(continued)

Table 23.5 (continued)

S. No.	Strategy	Method	Function	Tools available	Target	References
8	Ligand-based drug design	QSAR	It finds correlation between the structure of a biomolecule and its activity through a mathematical equation	• QSAR-Co • QSAR Toolbox	Histone deacetylases	
9	Ligand-based drug design	Combining virtual screening and similarity searching	It combines both the mechanisms of virtual screening and similarity searching	Tools available for individual functions	DNA methyltransferases	
10	Ligand-based drug design	Virtual screening based on pharmacophore	This screens for compound based on the pharmacophore	Tools available for individual functions	DNA methyltransferases	
11	Ligand-based drug design	Virtual screening based on pharmacophore and docking	This screens for compounds with pharmacophore have a good binding score with the target	Tools available for individual functions	DNA methyltransferases	
12	Ligand-based drug design	Druggability prediction	It classifies known gene families with small molecules that have previously been targeted with drugs	• SiteMap • XGB_DrugPred	Bromodomain and BET	
13	Ligand-based drug design	Scaffold hopping	It creates therapeutic biomolecules similar to known molecules based on its bioisosteric replacement of core motif within molecules	• SHOP • Click Scaffold Hop	Menin-MLL1 interaction	

(continued)

Table 23.5 (continued)

S. No.	Strategy	Method	Function	Tools available	Target	References
14	Ligand-based drug design	Quantum chemistry calculation	It is used to understand non-bonding interactions	• GAMESS • ChemShell	Bromodomain	

has also faced resistance by tumor cells. Combining different therapies along with using cisplatin seems to treat MDR [117].

23.5 Pharmacokinetics of Small Molecule Inhibitors

Drug discovery is a complex process starting from identifying therapeutic molecules to administration. There are a lot of methods both in vitro and in silico to identify potential hits that can be used as treatment. In addition to the therapeutic properties, a drug must also contain certain properties that will make it convenient for the body to metabolize it without any concerns. Those properties are called the ADMET properties—absorption, distribution, metabolism, excretion, and toxicity. ADMET profiling will help reduce the side effects during clinical development. In accordance with the 'fail early fail cheap' saying, in silico approaches of ADMET profiling of a molecule will help identify the associated risks immediately, without much time, and reduced complexity. Hence, the computational methods of ADMET prediction is becoming popular these days. The various tools available for in silico ADMET prediction are furnished in Fig. 23.4.

The physiochemical (PC) properties of a compound include molecular weight, ionization constant, topology, solubility, and lipophilicity. There is a strong connection between ADMET properties of a drug and its PC properties. For solubility, Klopman solubility model [138] is used. It removes charged atoms from the molecule to estimate the parameter. This limits its application. Lipophilicity is a property that determines the compound's permeability across membrane, solubility in lipids, potency, and selectivity. For its prediction, ab initio models, sub-structure-based models, and property-based models are used. It is estimated by the term log $P_{O/W}$. $P_{O/W}$ is the ratio of the compound in water and octanol phases at equilibrium. The log P term [140] is also used to determine the hydrophobicity parameter. The ionization constant affects drug diffusion and distribution in blood. Since transport in blood depends on the physiological pH and the charge of the drug, ionization constant plays a mighty role. There are a lot of mathematical models used in in silico *methods* that is used to estimate the PC properties. Although they are difficult to model using conventional QSAR approaches due to chemical diversity of data, less quality, and difficulty in reproducing data, in silico approaches have taken over. Pattern recognition methods are a statistical method used to recognize patterns in any given data. In QSAR, the set of descriptors indicating property X (activity) is empirically related to descriptors indicating property Y (structure). Statistical regression methods provide a linear relationship between two properties X and Y. This is used for ADME properties like lipophilicity and solubility. When one ADMET property has a lot of nonlinearly related descriptors contributing, instead of assigning a numerical value, the classification models assign it to two or more classes. Methods that do this model include: kNN methods [135], decision tree [134], and LDA method [133]. Artificial neural networks [136, 139] get an output Y when X is given as an input. It is inspired from the human central nervous system. It is used to predict models for molecules with X input and

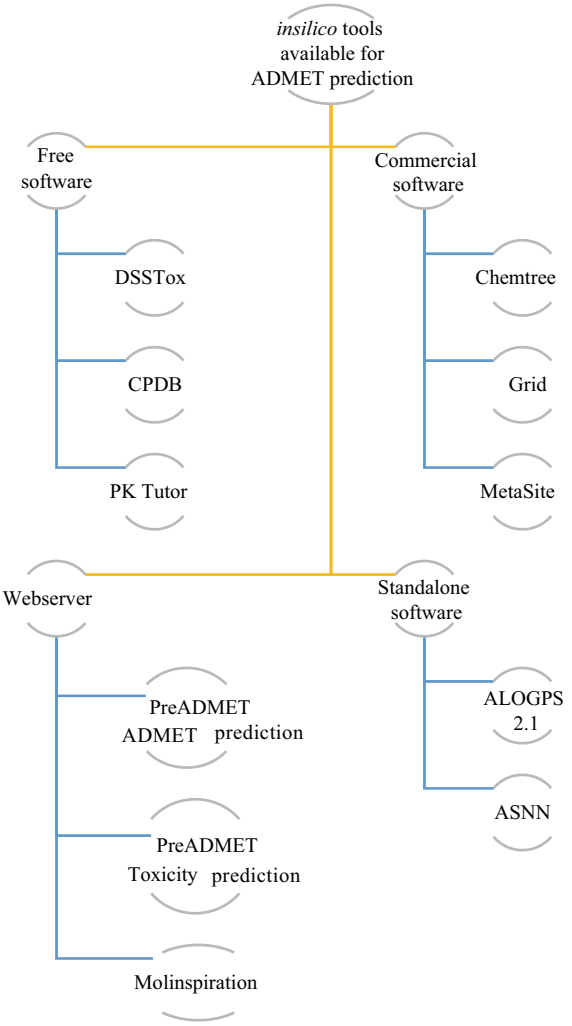

Fig. 23.4 Classification of computational tools available for ADMET profiling

Y is unknown. The factors linking X and Y represent the DNA. This method is used to train artificial neural networks (ANNs). It is also good at finding different solutions to the same problem, but quality varies depending on the parameters. Several ADME-based models have also been developed. The human intestinal absorption model is based on determining drug bioavailability orally. Models were built that use human intestinal absorption data [141, 142] and genetic algorithm [137]-based ANN function, and a classification model was created based on polar surface area. It is important to identify binding capacities of blood–brain barrier candidates. Models have been reported based on 45 data points, experimentally determined descriptors, and 3D descriptors. An important factor of drug is bioavailability. Models have been

proposed based on fuzzy adaptive least squares method and square regression analysis, and a nonlinear-based model has also been reported. Error rates of these models are high. Metabolism is essential as the body needs to process the drugs efficiently. Models that predict the metabolism rates have been mentioned in literature based on semi-empirical quantum mathematics [143], dynamics MM, and simulations based on homology modeling [144].

Prediction models, metabolite prediction models, membrane transporters-based models, and PBPK models are involved in metabolism-related prediction. Several toxicity prediction models like acute toxicity, genotoxicity, hERG toxicity, and systems toxicology are also utilized [89, 98]. Various anti-cancer studies have been made that have included in silico ADMET prediction using the above-mentioned parameters and models. The details have been mentioned in Table 23.6.

Despite the merits, there are challenges and limitations in in silico ADMET prediction: (i) the difference between global and local models of prediction, (ii) application domain of models, and (iii) model validation techniques [99].

23.6 Drug Repositioning

Drug repositioning is the process where existing drugs used for the treatment of a certain disease are analyzed and checked whether the same drug can be used for treating other diseases in addition. The main purpose of this method is to reduce the time and expenses involved in the drug design protocol as opposed to de novo drug design. In the former, we can skip the identifying hits to optimize lead steps and go straight to the clinical trials. Further, the drugs obtained by repositioning tend to be safer than the new drugs developed at the same time. Thus, it reduces drug attrition during clinical trials. The adverse effects of the drug reduce when the severity of the new disease is less than the original disease. It is becoming a growing trend in recent years, particularly for the drug design of cancer, as new types of treatment are required to tackle the genetic alteration leading to multidrug resistance and the fast metastasis that over paces the treatment in a short period. Drug repositioning is limited to the drugs already on the market and the bioactive molecules that have therapeutic value but have been removed from the clinical phase owing to safety concerns. Changes in the formulation, dosage, composition, route of administration, etc., will be seeking re-examination of the drug profile [100]. Since drug repositioning depends mainly on the data of the drugs and the disease it cures, computational approaches make this process much simpler. This process is classified into two strategies: (i) if the data of the drugs are known and (ii) if the data of the disease are known (Fig. 23.5).

Drug-based strategies are based on the phenomenon that when two drugs have the same chemical properties, mechanisms of action, and ADMET properties, then the two drugs are considered strong candidates for curing the same disease. Two main types are involved: (i) genome-based strategy and (ii) chemical structure and molecule information strategy. The genome is the total set of genes in a given organism. The large amount of data obtained from a genome is used in this strategy.

Table 23.6 Anti-cancer studies that screen molecules based on ADMET prediction

S. No.	Cancer form	Study	ADMET prediction	References
1	Breast cancer	Activity prediction, SBDD, molecular docking, and ADMET properties' prediction of quinazoline derivatives were done in silico against MDA-MB231 cell line	The derivatives were bioactive, compatible, and followed Lipinski's rule of five. Swiss-ADME and pkCSM online servers were used	[90]
2	Breast cancer	ADMET prediction of anilinopyrimidine derivative compounds	The compounds passed the pharmacokinetic parameters and Lipinski's rule of five	[91]
3	Breast cancer	QSAR, LBDD, and pharmacokinetic analysis of parviflorons	The compounds passed the pharmacokinetic parameters and Lipinski's rule of five and are prepared for pre-clinical trials	[92]
4	XIAP-related cancer	Structure-based pharmacophore modeling, Vs, docking, and ADMET prediction of molecules against XIAP protein as a target	ZINC77257307, ZINC247950187, and ZINC107434573 were found to be active, biocompatible, and less toxic	[93]
5	Targeting microenvironment of various cancers	Comparing new therapeutic molecules and drugs in silico and performing ADMET prediction	Qikprop tool was used for ADMET prediction, and it was revealed that AGSPBM131, AGSPBM130, and AGSPBM134 was safe, which passes Lipinski's rule of five and bioactive	[94]
6	Targeting microenvironment of various cancers	Vs, docking, ADMET prediction of Garcinia caged xanthone derivatives	1G molecule was less toxic, bioactive and passed Lipinski's rule of five	[95]
7	Lung and breast cancer	Vs, docking, ADMET prediction of 3,5-dimethyl-1,3,4-hexanetriol, and dodecanoic acid derivative	Compounds 10 and 12 were selected as best hits with less toxicity, active, and biocompatible	[96]
8	Breast cancer	Docking, drug likeliness, and ADMET prediction of chromen-2-ones analogues	Compounds 6, 8, 11, and 12 are safe, bioactive, and biocompatible	[97]

The latter strategy offers data about the profile of drugs and similarities between two different drugs. Chemical structure similarity is analyzed using two ways: 2D topological fingerprints and 3D conformational fingerprints. Chemical structures can also be used to identify the targets of drugs and model disease-related phenotypes. Further, molecule information like bioactivity and drug-disease relationships can be obtained from chemical structures as they influence the properties. The second

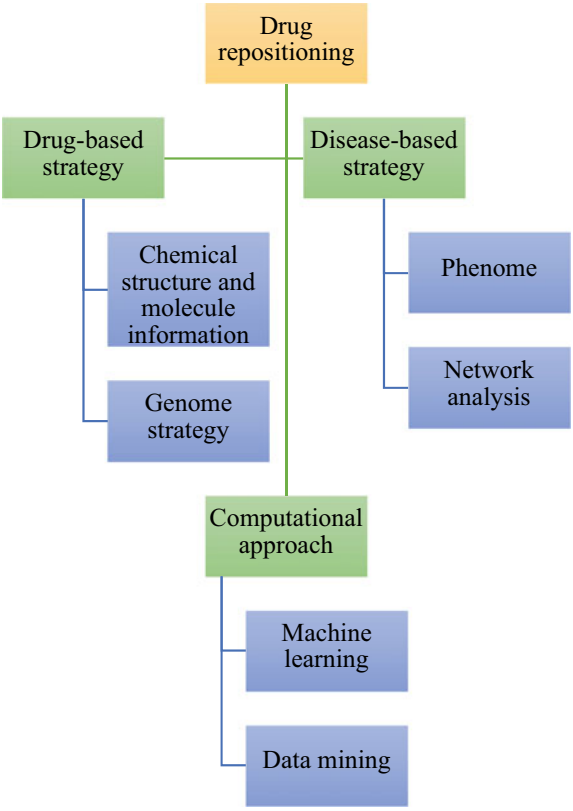

Fig. 23.5 Classification of strategies in drug repositioning

strategy is based on the data on diseases. It involves two strategies: (i) phenome-based and (ii) network analysis. Phenome is the term used to define all the phenotypic traits of an organism. It is important in drug repositioning because it tells us information about the physiological effects of the drug and the unknown side effects. Modeling a disease/drug network and analyzing it give us new and previously missed therapeutic values of a bioactive molecule that can be used in repositioning. Network analysis is possible by constructing bipartite graphs, clustering, and utilizing network centrality measures [101]. The networks usually constructed for drug repositioning studies are drug–target networks, PPI networks, chemical reaction networks, disease–drug networks, drug–drug interaction networks, protein structure networks, chemical compound networks [109]. Additional to the bioinformatic techniques, multi-disciplinary computational methods can also be used to strengthen drug repositioning. The technologies involved include (i) data mining and (ii) machine learning. Data mining is the technique where researchers get a piece of information from a hidden voluptuous system of information. Obtaining information involves two sub-types of techniques. The first one is text mining, where we can check the co-occurrence of two different drugs and arrive at a conclusion. For

example, if drug A follows network A and this network is responsible for disease A, then drug A can be a candidate for disease A. The second one is semantic technology where a combination of information from various data sources is used to identify new therapeutic values for existing drugs. The use of machine learning for building associations between different drugs and diseases has been a growing trend. This technology has been found superior to conventional statistical measures. Methods of machine learning include KNN, random forest, SVM, and deep neural networks for value prediction, binary classification, and multi-class classification [110]. The tools involved in computational drug repositioning and their mechanism are mentioned in Table 23.7.

There has been a new development in the study of cancer hallmarks serving as molecular targets. This data have been provided by TCGA. The Cancer Genomic Atlas has extensive data of over 33 cancer forms and 11,000 tumors. Its goal is to identify the cancer alterations by combing the genomic, epigenomic, and transcriptomic data from various forms of cancer. This public project makes the comparison of different cancer types and concludes similar molecular pathways among them possible. The omics studies that presented the target-related data through TCGA can also provide disease-related data by studying mutations in the oncogenes and inhibition of tumor suppressor genes. Oncogenes like KRAS, c-MYC, and the β-Catenin are studied for this purpose. Tumor suppressor genes like p53 tumor suppressor and PTEN tumor suppressor genes are also studied to provide disease-related data in drug repositioning [101]. The method of drug repurposing for various forms of cancers is furnished in Table 23.8.

After the identification of repurposing drugs, validation is carried out to check the efficacy. Such models are classified into seven types: in vivo model, in vitro model, electronic health records model, leave one out and cross-validation model, benchmarking previous models, case studies, literature cross-referencing models, and domain experts cross-referencing models. The validation model may not be accurate as the computational model. Hence, choosing the right validation model is essential for success in this research like K-fold cross-referencing model which is used for machine learning-based studies [109]. Despite reducing the time, cost, and the complexity of drug design, computational drug repositioning has its own disadvantage like the poor intellectual property protection and patenting problems associated. This discourages the research on drug repositioning [100]. In addition, the dosage required to administer for the candidates of repurposed disease is not clearly defined.

23.7 Immunoinformatics

Cancer immunotherapy is gaining impetus as a potential therapy for the cure and prevention of cancer. Unlike traditional treatment procedures like chemotherapy and radiotherapy, this method makes use of the body's natural defense mechanism to fight cancer. It can operate the body's defense system to identify and wipe out lethal

Table 23.7 Tools for computational drug repurposing

S. No.	Tools	Strategy	Mechanism	References
1	PREDICT	Drug properties	Identifies new drug–disease relationships by combining drug's properties, side effects, and the disease–disease similarity examined through HPO	[101, 118–132]
2	SDTNBI	Drug properties	Identifies new disease targets by obtaining chemical structures of the drug from ChemBL database and drug targets from CDK fingerprint database	
3	ChemMapper	Drug properties	By combining pharmacophore and 3D structure similarities, it predicts the polypharmacology and mode of action of small molecules	
4	SIDER	Drug properties	It is a database for drug repurposing that includes 1430 drug structures, 5880 drug adverse reactions (ADRs), and 140,064 drug–ADR pairs	
5	STITCH	Drug properties	It predicts the interactions between chemical–chemical and chemical–protein. The structures are obtained from PubChem database, and the interactions are predicted by assessing molecular pathways and other experimental databases including protein sequencing and high throughput assays	
6	ATC	Drug properties	It categorizes known drugs based on their medical applications. It is used by the WHO	
7	Pharo	Drug properties	It is a web interface that explores Target Central Resource Database	
8	DrugBank	Drug properties	It is a collection of chemical, pharmacological, and pharmaceutical data for 13,363 drugs	

(continued)

Table 23.7 (continued)

S. No.	Tools	Strategy	Mechanism	References
9	CMap	Drug treatment	It is a project where a collection of genome expression profiles induced by drug effects is collected	
10	LINCS	Drug treatment	It is a library of integrated network-based cellular signature	
11	MANTRA	Drug treatment	It is a network-based analytical tool which finds the similarities between transcriptional responses and thereby identifies drug neighborhoods	
12	DSEA	Drug treatment	It uses gene expression data for identifying common pathways of various drugs	
13	Gene2drug	Drug treatment	It identifies drugs that target a common set of pathways	
14	PharmGKB	Drug treatment	It identifies the genetic variations that occur as a response to a drug effect	
15	DrugMatrix	Drug treatment	It provides toxicogenomic profiles of > 600 cells of various tissues	
16	TG-GATE	Drug treatment	It provides toxicogenomic data derived from in vivo and in vitro exposure to 170 compounds at multiple dosages and time points, together with gene expression profiles	
17	NetPredATC	Disease properties	It is a tool that is used for classification modeling tasks	

cells leaving the normal cells unharmed [182]. Immunotherapy involves the use of peptide vaccines consisting of B and T cell epitopes, DNA vaccines, viral vectors, dendritic cells, whole tumor cells, and antibodies, which enable the body's immune system to fight against the cancer cells. Immunoinformatics is an in silico technique that is employed in designing promising cancer therapeutic agents [188].

In general, immune system is complex and diverse involving thousands of molecules and the related networks and pathways. Currently, in immunoinformatics, many computational tools, databases, and software are available which can store, process, and analyze the clinical, functional, and epidemiological data. Immunoinformatic involves the study and design of algorithms for mapping potential B cell and

Table 23.8 Methods of drug repurposing for various forms of cancer

S. No.	Cancer	Method of drug repurposing	Mechanism of action	References
1	Adeno cortical carcinoma	Heter LP	It identifies new drug–target, drug–disease and disease–target relationships and discovers new drugs and targets for ACC	[102]
2	Breast cancer	GraphRepur	GraphRepur integrated two major classes of computational methods, drug network-based, and drug signature-based. The differentially expressed genes of disease, drug-exposure gene expression data, and the drug–drug links information were collected	[103]
3	Breast cancer	Network-based integration approach	It identifies single or pair of drugs that treats all the sub-types of BC including the sub-type TNBC by combining the properties and relationships between drug, genes, and diseases	[104]
4	Colorectal cancer	Specific studies like CORECT, CFE, MECC, GECCO, and other general cancer-related databases and projects	It integrates data of over 50,000 CRC patients	[105]
5	Chordoma	ksRepo	It compares the Chordoma tissue samples' data with the pharmacogenomic interactions data available in the CTD	[106]
6	Gastric cancer	Computational reversal of gene expression approach	This method is based on the effects of gene expression signatures. Data required are downloaded from various resources, and candidates are determined using reversal gene expression scores	[107]

(continued)

Table 23.8 (continued)

S. No.	Cancer	Method of drug repurposing	Mechanism of action	References
7	Soft tissue sarcoma	Method using survival associated gene signatures	STS survival associated genes were generated using univariate cox regression analysis and applied to three databases—L1000FD, CMap, and DGID to identify candidates for drug treatment	[108]

T cell epitopes, to understand pathogenesis of infectious diseases, diagnosis, immune response, and computational vaccinology. Bioinformatics methods are employed to study and assign functions to uncharacterized genes, which could serve as a potential vaccine target. Immunoinformatics is advantageous as it reduces the cost and time required to analyze the potential vaccine candidates. Selection of antigen in vaccine production for cancer appears to be censorious as it has to be designed in the way to target malignant cells and not the normal healthy cells epitopes leaving space for less errors. This method enables the scientists to design new vaccines using reverse vaccinology techniques based on the potential binding sites [189]. Literature suggests that the multi-epitope vaccines designed using immune informatics methods exhibit high specificity, safety, stability, storage, and ease of production [190].

23.8 Conclusion

In addition to in vitro and in vivo methods, in silico methods have been becoming popular in drug design as it is simple, inexpensive, and less labor consuming. Among a wide range of diseases, drug design for cancer forms is tricky as it must deal with metastasis and MDR. Computational approaches, namely CADD, is very suitable in the early stages of drug development where therapeutic small molecule inhibitors are identified from a wide range of sources. Structure-based and ligand-based drug design approaches are available. Targeted drug discovery is when the approaches target a mutant molecule and try to downregulate its effects. This review focused on oncogene, mutant mRNA, oncoproteins, and various epigenetic targets that cause cancer. The drug likeliness properties (ADMET) of the discovered compounds are also evaluated computationally. Furthermore, strategies that allow repositioning of various commercially available drugs are also discussed.

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Chapter 24
Importance of Gut Microbiome-Based Therapeutics in Cancer Treatment

Mohd Rabi Bazaz, Ziaur Rahman, Insha Qadir, Tulasi Pasam, and Manoj P. Dandekar

Contents

Abstract Increasing cases of cancers have become worrisome as it is globally considered the second prime origin of death. While surgical procedures,

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chemotherapy, and targeted drugs are available for cancer treatment, most of the patients suffer with drug resistance, disease recurrence, and unwanted side effects due to these interventions. Thus, use of biopharmaceuticals which are more selective and less toxic has gained more attention. The host's gut microbiome reported to play an important role in the efficacy of anticancer drugs and development of resistance to chemotherapy. The tumor-operator mutations are also synchronized by the microbiome present in the gut indirectly through manipulation of immune system. In this chapter, we focus on how changes in the gut microbiome signature contributes to tumorigenesis and the treatment of cancers. We also describe the gut microbiome-based mechanisms underscore inflammation and cancer treatment. However, additional preclinical or early clinical studies with prebiotics and probiotics may contribute awareness toward the importance of microbiome-deployed therapeutics in the cancer treatment. Furthermore, depletion of bacterial metabolites as a consequence of cancer development acts as a contributing factor for the aggravating the tumor growth. Thus, identification of bacterial metabolite-based interventions may be useful in the cancer prevention.

Abbreviations

CC	Colorectal carcinoma
CTX	Cyclophosphamide
CRP	C-reactive protein
DMH	1, 2-Dimethylhydrazine
DNA	Deoxyribonuclic acid
FMT	Fecal microbiota transplantation
GC	Gastric carcinoma
GSH	Glutathione
GST	Glutathione S-transferase
HC	Hepatocellular cancer
HDACi	Histone deacetylase inhibitor
IL-6	Interleukin-6
LAB	Lactic acid bacteria
NAFLD	Nonalcoholic fatty liver disease
NGS	Next-Generation Sequencing
PD-L1	Anti-programmed death ligand 1
TNF	Tumour necrosis factor
TNF-α	Tumor necrosis factor-α
TLRs	Toll-like receptors
SA	Sodium azide
SCFA	Short chain fatty acid
WHO	World Health Organization

24.1 Introduction

The World Health Organization (WHO) had announced cancer as a primary cause of death globally, which is a physiological state of an abnormal cell division. Several studies unveiled the importance of gut microbiota in different pathological conditions, including cancer [1]. For instance, due to the dense population of gut microflora in the gastrointestinal tract, its role in the regulation of metabolic, inflammatory, and immune responses may not be precluded. The gut microbiota, particularly fungi in gut and their receptor recollection, C-type lectin receptors provide conceptual basis for controlling cancer and understanding carcinogenic potential of fungi [2]. The gut microbiota constitutes a new era of comprehensive cancer treatment. Gut microbiota therapy not only acts as supportive therapy to relieve symptoms of cancer treatment but also influences the progression of cancer [3]. Microflora of intestines has a prospective responsibility in the expansion of cancer in humans [4]. Whereas in the past, biomarkers expecting therapy reaction have been tumor-centric and involved characterizing tumor mutational load, metabolism, and sensitivity to immune effectors; the intestine microbiota is new, non-tumor-centric vicinity with healing potential. It is important to comprehend how the intestine microbiome may affect tumor development [5].

The intestine microbiota consists of approximately one hundred trillion microbes that interact with immune system of host. An enormous impact of gut microflora has been documented in human well-being and disease [6]. The comprehensive knowledge of gut microflora interlinked with axis of immune system can be traversed to expropriate cancer development as well as identify innovative treatments. The cancer growth and development is influenced by carcinogenic effects of microorganisms and their products. The levels of circulatory metabolites modulated the immune system and inflammatory mediators that are involved in tumor growth [7]. Most of the studies support that the microflora of gut plays vital role in the treatment of cancer [8]. The localized alterations of microbiota have been shown to improve the progression of cancers concerning organs that harbor commensal organisms (neck, cervical, head, and lung). Gut microbiota plays salient role in various neoplasms of the gastrointestinal tract. In relation to this, the mechanistic connection of the gut microflora has been documented in the cancer of colon and rectum [9]. More than 450,000 individuals have been affected with esophageal disease and gastric cancer, and it is 6th most elevated death pace of all tumors [10]. Gut fungi are associated with gastrointestinal carcinoma as they reside in gastrointestinal tract, wherein they play a salient role in the innate immune response [11]. Mucosal microbiota adheres itself to the polysaccharide matrices of intestinal epithelium that makes it more stable than its luminal counterpart [12].

Current studies have revealed that gut microbiota manages mediators of physiological homeostasis, including gut barrier function, immune system, and pathways of disease susceptibility [13]. The dysregulated dating among microorganisms and their metabolites with the intestinal mucosa can negatively changes the mucosal permeability that allows entry of carcinogenic compounds [14]. The entangled relationship

between microorganisms and their metabolites with the gut mucosa lead the way to changes in permeability of mucosa that increases confined exposure to a broad assemblage of prospective carcinogenic compounds and hence leading to a state of chronic inflammation [5]. The gastrointestinal lot fills in as a permanent place to stay for intestinal microbiota, and hence, connection between fungi of intestine and lump in gastrointestinal tract has been essentially considered, explicitly in colorectal carcinoma. Gut microbiota helps to prevent these cancers by generating metabolites and byproducts. It also reduces chances of cancer development and progression [10].

Gut microbiome signatures have been associated with projection and development of progressive melanoma and thus contemplated a biomarker of therapy for immune entrapment. Countless gut inhabiting bacteria, known as probiotics, are being recognized as safeguard in case of the commencement of malignancy. Given their potentiality of preserving homeostasis of gut, probiotics have been presently tested to aid in fighting dysbiosis in carcinoma patients subjected to radiotherapy and chemotherapy [15]. Therefore, preservation or reimposition of microflora and composition of metabolites is considered to be a therapeutic or precautionary target in case of censorious illness. Fecal microflora transport as well as augmentation of probiotics are microflora-established treatment techniques that are somewhat finite in terms of confirmation-based effectiveness [16]. Various studies focus on the significance of exchange between sepsis and the gastrointestinal ecosystem to culminate peculiar microflora-selected therapies to improve the effect of sepsis therapy [17]. The development of colorectal cancer is linked with gut microflora has been investigated by various studies. The imbalance of gut microbiota upgrades the progression of cancer in colon and rectum, via numerous mechanisms including invigoration of carcinogens, inflammation, and damaging DNA of host. Activity and composition of gut microbiota is being altered by various therapeutic methods, including symbiotics, prebiotics, and probiotics [18]. These kinds of therapeutics provide benefits for colorectal cancer patients. The modern way of regulating the microbiota of gut includes fecal microbiota transplantation [19].

The conventional microbiome therapies have prompted superior malignant growth therapies now and again; in any case, issues, for example, guarantee injury to the cooperative microbiome, and dependability of these therapy techniques has prompted new mechanical improvements planned explicitly for disease microbiota crossing point [20]. Thus, prosperousness of nanomaterials in disease counteraction has prompted the possibility that nanomaterials can adjust the malignant growth causing microbiota and their metabolites as well as modify the malignant growth microenvironment. Accordingly, nanomaterials can be utilized as original methodologies to treat malignant growth [21, 22].

24.2 Why to Target Gut Microbiota in Cancer Treatment?

In recent past, accountability of gut microflora in cancer progression had been largely reported [23, 24]. Gut dysbiosis indicated the unfavorable alterations of the microbial phenotypes comprising gut microbiome, metabolites, and gut-related physiological conditions. Gut impairment and specific microorganisms in the gut can perpetuate cancer or advance malignant growth pathway by enacting cancerous mechanism, prompting inflammatory cascade, and degrading host DNA [25, 26]. Numerous bacterial proteins encourage the dissociation of β-catenin from E-cadherin and activate the β-catenin signal pathway, which is important in the development of cancer. Short-chain fatty acids derived from bacteria are produced less frequently as a result of intestinal dysbiosis. Through raising the cell production of proinflammatory molecules and consequently promoting carcinogenesis, intestinal dysbiosis causes inflammation. This is accomplished by microorganism-associated molecular patterns by Toll-like receptors (TLRs). Many bacteria have the capacity to damage DNA through the release of certain metabolites in addition to causing inflammation, which aids in the development of cancer. Unexpectedly, certain microbiome species modify the effectiveness of cancer treatment [27], significantly affecting the clinical result of cancer patients. Therefore, greater apprehension of the link between internal bacteria and cancer can lead to the development of effective treatment and diagnostic approaches.

A range of cancers such as gastric carcinoma (GC), colorectal carcinoma (CC), hepatocellular cancer (HC), pancreatic carcinoma, breast carcinoma, and melanocarcinoma have been linked to the altered gut microflora configuration. In contrast to healthy persons, patients with tumors (GC or CC) showed specific abnormalities in the gut bacterial flora [28, 29]. When conventional or germ-free mice were fed a carcinogen, the fecal microbiota from CC patients stimulated carcinogenesis, demonstrating the carcinogenic characteristics of the CC microbiome. According to growing epidemiological research, chronic antibiotic administration alters the configuration and reduces the heterogeneity of the gut microflora, augment the risk of CC, as well as pancreatic, breast, gastric, lung, and prostate cancers [30]. In line with these findings, chronic antibiotic usage was significantly associated with accelerated colorectal tumor progression in the ApcMin/ + mouse, a genetic model of human adenomatous polyposis [31]. There are conflicting results for escalating use of antibiotics and chance of developing cancer. In mice model of colon cancer xenograft, oral metronidazole treatment lowered the load of *Fusobacterium* and colorectal tumor size [32]. Additionally, use of antibiotics might dissolve biofilms and get rid of microbial sulfide, safeguarding the colon mucous barrier, and stopping epithelium hyperproliferation [33, 34]. Additionally, a range of studies demonstrated that intestinal tumorigenesis may be prevented by loss of gut microbiota using mixture of antimicrobial agents [35–37]. To find the underlying molecular mechanism of cancer patient prognosis after antibiotic exposure, more research is necessary.

24.3 Bacteria that Improve Anticancer Drug Efficacy

Numerous investigations have documented the role of commensal gut microflora in cancer treatment, as efficiency was negatively affected in gut microbiota disrupted conditions. Cyclophosphamide (CTX) has been shown to change the intestinal microbiota of mice and encourage the translocation of particular gram-positive bacteria into secondary lymphoid organs, promoting the generation of "pathogenic" Th17 cells that exhibit characteristics of both T helper 1 (Th1) cells and Th17 cells [38, 39]. On the other hand, antibiotic-treated or germ-free animals displayed therapeutic resistance to CTX [38]. Contrarily, the intricate interactions between the host, bacteria, and fluoropyrimidines, antimetabolite medications frequently used to treat cancer, were clarified using genetic models made up of *Escherichia coli* and *Caenorhabditis elegans* [40]. Through bacterial vitamin B6, B9, and ribonucleotide metabolism influence the effectiveness of fluoropyrimidines in *C. elegans.* Both immunotherapy and chemotherapy effectiveness are affected by commensal microbiota. An early investigation in mice given an antibiotic cocktail supports the idea that CpG-oligonucleotide immunotherapy and platinum chemotherapy are compromised by changed microbiota. In the presence of a healthy microbiome, tumor development is inhibited by immunotherapy through CD8 T cell response and myeloid cell production of tumor necrosis factor (TNF). In contrast, administration of antibiotics inhibits the ability of immune cells such macrophages, monocytes, and dendritic cells to produce TNF and cytokines, which in turn slows tumor regression in mice receiving immunotherapy. These findings suggest that commensal bacteria trigger the production of inflammatory cytokines by tumor-associated myeloid cells in response to immunotherapy. This is accomplished via activating the toll-like receptor 4 (TLR4) receptor [41]. Designing microbiological consortia that will increase the effectiveness of cancer therapy is encouraged by more mechanistic insights into the roles played by the interactions between commensal bacteria and the host.

24.4 Pathogenic Microbes Contributing in Cancer Development

A range of harmful bacteria have been identified that contribute in the emergence and progression of cancer. Several microorganisms serve as etiological factors in roughly 20% of all malignancies worldwide, including the human *Helicobacter pylori (H. pylori)*, papillomavirus, and the hepatitis B virus [42]. The majority of research on various bacterial species, synthesis of toxic metabolites, and modification of the intestinal milieu suggested tumor-promoting abilities. For instance, *H. pylori* is known to cause chronic gastritis and gastric carcinogenesis, which by secreting virulence proteins and activating a number of signaling pathways can promote tumor growth [43–45]. The enterotoxigenic strain of *Bacteroides fragilis*, which produces the toxin *Bacteroides fragilis*, can cause deoxyribonucleic acid (DNA) damage

and intestinal inflammation, conducive for the development of CC [46]. Compared to normal tissues, the majority of the colon tumor tissue in CC patients contain *Streptococcus gallolyticus subsp. gallolyticus (Sgg)*, an opportunistic, gram-positive pathogen [47]. The *Sgg* has also been demonstrated to encourage the growth of colon tumors in animals fed a carcinogen by activating the β-catenin signaling pathway [48]. A number of toxins, such as cyclomodulin, play a role in tumor formation, can be produced by pathogenic *Escherichia coli* (E. coli) [49]. Recently, it has been discovered that the *Fusobacterium nucleatum,* an oral gram-negative commensal, is more prevalent in colon tumor tissues and facilitates progression and cancer metastasis [50–52]. *Fusobacterium nucleatum* also increases tumor recurrence rate and causes autophagy in cancer cells, which promotes resistance to chemotherapeutic agents [53]. The considerable difference in the diversity of gut microbiota among cancer patients and healthy people shows the potential for both predictive and diagnostic use of certain pathogenic microbes in cancer. For instance, increasing abundance of *Fusobacterium nucleatum* abundance was noted in colorectal adenoma and CC patients, indicate its potential use in the early identification of CC [54]. Moreover, inclusion of fecal immunochemical test with *Fusobacterium nucleatum* abundance might increase the sensitivity and precision of diagnosing progressive adenoma and CC [54, 55]. The presence of *Fusobacterium nucleatum* in CC tissue not only aids in diagnosis but also predicts patient survival. Furthermore, targeting mechanisms pertaining to specific infectious microbes causing cancer may lead to the advent of biomarkers for cancer diagnosis and prognosis.

24.5 Prebiotics and Probiotics in the Management of Cancer

The word "probiotics," which was first used in 1965 by Lilley and Stillwell, is Greek in origin and means "for Life." Probiotics were initially described by Nobel laureate Elie Metchnikoff, who postulated that altering the gut flora by host-friendly bacteria could impact positive well-being effects. Probiotics have drawn interest because of their capacity to influence cellular and immunological responses as well as cancer signaling by modifying the malignancies via (a) activation of apoptosis pathway, (b) decrease in genotoxic activity, (c) expression of oncogenes is suppressed, (d) triggering autophagy, (e) suppression of kinases, (f) tumor suppressors being activated again, and (g) avoidance of metastasis. They may be utilized to ensure as adjuvant treatment for tumor patients supplemented with chemotherapeutic agents, according to a growing body of research [56]. The World Health Organization and the Food and Agriculture Organization describe probiotics as potent bacterial strains when ingested in sufficient amount influence positive impact on the host health. In addition to remodeling of gut microbiome, probiotics, prebiotics, and synbiotics may also operate as antimutagens by modifying the host metabolism. It has been anticipated

that these beneficial effects of probiotics mediate via restoration gut microbiota and regulating the gut ecosystem.

Recently, several clinical investigations have been run with the purpose of examining the gut microflora's therapeutic potential in cancer patient [57]. Many in vitro studies also demonstrated the success of probiotics in controlling the growth and death of cancerous cells [58]. *Lactobacillus*, *Bifidobacterium*, *lactococcus*, *Streptococcus,* and *Enterococcus* are most usually seen in human nutrition [59]. The most studied probiotic in oncology is *Lactobacillus rhamnosus* GG (LGG). Several studies have suggested that LGG is a viable lead to be described as a potential supplement in combined antitumor therapy. Goldin and Gorbach [60] observed that diet supplemented with *Lactobacillus* declined an occurrence of colon cancer (0% versus 77% in controls). Since 1907, probiotics were available for human consumption, among them *Bifidobacterium (longum, breve, adolescentis, animalis, and adolescentis*) and *Lactobacillus (casei, gasseri, plantarum, rhamnosus, fermentum, johnsonii,* and *salivarius*) are mostly used species. Many other species are known for their role in patient well-being such as *Akkermansia*, *Faecalibacterium,* and *Roseburia* along with other potent probiotic strains such as *Bacillus, Enterococci, Clostridiales, Candida, and Weissella* as antitumor agents. Thus, gut microbiota-based therapeutics like probiotics may prevent or halt tumor growth [61]. While probiotics are generally considered to be safe, its prescription to immunocompromised cancer patients may raise issues about the transmission of resistance toward antibiotics as well as threat of opportunistic infections.

Prebiotics consumed in the form of fibers or indigestible constituents positively impact the host's health. The known prebiotics are inulin, wheat and barley unrefined, breast milk, and cumin. The habitual prebiotics constitute lactulose, inulin, fructooligosaccharide, and starch which may halt the metastasis progressive environment by pH changes in colon, regulating xenobiotic metabolizing enzymes, differential gene expression in the feces and caecum. It has been demonstrated that dietary fiber possesses prebiotic effect, suggesting its association in declining the incidence of colorectal cancer. The ingestion of asparagus, artichoke, onion, and garlic was reported to raise the levels of short-chain fatty acids (SCFAs) and *bifidobacteria* in the colon and improve the colorectal cancer. Inulin and oligofructose consumption also lessened the severity of 1,2-dimethylhydrazine (DMH)-developed tumor in colonic region of mice. The prebiotics consumption reported to improve immunological function, which has an antitumorigenic impact. The anticancer effects of prebiotics may be ascribed to the increase levels of butyrate. Prebiotics found to be more effective when concurrently given with probiotics as a synbiotics [62].

While nondigestible fermented oligosaccharides that induce modifications in the intestinal microflora constitution and/or activity confer health benefits, synbiotics frequently produce synergism that encourages cell growth to inhibit tumorigenesis [63]. According to Rowland [64], combination of inulin and *Bifidobacterium longum* reduced azoxymethane-induced aberrant crypt foci more effectively. Regulation of gut environment was stated by Roller and colleagues showed the synbiotic recipe

of *Bifidobacterium lactis*, oligofructose-enriched inulin, and *Lactobacillus rhamnosus*, possess impact against cancer impact. Synbiotic treatment also offers prevention against azoxymethane-induced decrease of action of natural killer (NK) cells in the region of payer's patches; this was not seen with the specific probiotic and prebiotic therapies. Outcomes recommend that synbiotic therapy will be beneficial for CC therapy [65]. Overall, research using a variety of animal and in vitro models shows that synbiotics, prebiotics, and probiotics have antitumor activity; their intake helps to treat cancer that has already developed as well as prevent its onset. However, there is still little proof based on human investigations [66]. The synbiotic mixture of this compounds facilitates the apoptotic cascade activation to DNA damage by colon cancer and further investigation to prove for its potential benefits to prevent colorectal cancer. Combinations of prebiotics and probiotics may be active toward CC [67]. Due to higher rise in antioxidants linked with increased grade of weakening of DMH-induced carcinogenesis, the utility of synbiotics is an improved preventative approach than utilizing the probiotic and prebiotic alone [68]. Inhibiting cell development and promoting apoptosis and also promoting cell differentiation were the markers for cancer prevention that were regulated by the fermentation of wheat aleurone [69]. NK-cell activation was increased in aged C3H and C57BL/6 mice using a rice bran-derived arabinoxylan. Aged mouse NK activity is increased by MGN-3, which may be helpful for improving NK function in elderly people [70].

24.5.1 Prebiotics and Probiotics Treatment in Colon Cancer

Malignancies of the digestive system account for 25% of all cancers globally, and account for 9% of all gastrointestinal (GI) carcinoma. Study has established that prebiotics and lactic acid bacteria (LAB) can inactivate genotoxic carcinogens. LAB has the potential to act as chemoprotective agents from a molecular perspective [71]. LAB strains may be appropriate substitute for designing therapeutic hit for colon cancer; its probiotic function was developed by isolating the infant feces for alternate inclusion in the management of tumor in colonic regions [72]. Due to its immunomodulatory, anti-inflammatory, and anti-carcinogenic capabilities, LAB exerts a variety of strain-specific health-promoting effects. *Lactobacillus casei* ATCC 393 inhibits the evolution of colon tumor in experimental model. Thus, probiotic LAB strain has positive tumor-inhibitory, antiproliferative, and pro-apoptotic properties [73]. The antimutagenic effects of *Lactobacillus casei* BL23 following oral ingestion are good for reduction of inflammation and cytotoxic elements [74]. *Lactobacillus casei* BL23 also showed antitumor effects in cancer mice model and TH17 cells [75]. Studies have proven that *Lactobacillus casei* ATCC 393 is useful, for its growth-inhibitory activities against experimental-induced colon cancer [73]. *L. acidophilus* and *L. casei* strains also displayed enhance potency of 5-flouroruracil to induce apoptosis [76]. Clinical study with *L. casei* that habitual use of LAB lowers the incidence of bladder cancer and relapse of superficial bladder cancer [77]. According to the findings, significant potential of *L. casei* BL23 to cure colorectal cancer has opened up new

research avenues for the investigation of the immunomodulatory properties of probiotics**.** *L. fermentum RM28* and *E. faecium RM11* probiotics restricted colon cancer cell multiplication rates 21–29% and 22–29%, respectively. According to results, combined bacterial flora might be exploited as prospective probiotics in useful foods or biological products for acting against tumor of colon [78].

Lactobacillus plantarum (AS1) regulated the growth of DMH-induced rat colon carcinogenesis [79]. In addition, *L. plantarum* delayed the development of tumor mass and improved the resistant [80]. Administration of *Lactobacillus rhamnosus GG* and *Lactobacillus plantarum A7* produced anticancer effects by heat-killed cells and supernatants free from cells [81]. *Lactobacillus rhamnosus* strain found valuable dietary entity, as it has protective effect against colorectal carcinogenesis by inducing apoptosis and decreasing inflammation [82]. During experimentally induced tumorigenesis of colon, *Lactobacillus rhamnosus* GG (LGG) and *Lactobacillus plantarum* (AdF10) probiotics offered defense against reactive oxygen species and apoptosis-related protein dysregulation [83]. *Lactobacillus rhamnosus* in normal diets was utilized to inhibit the methylnitronitrosoguanidine-induced colon tumor by dramatically increasing glutathione (GSH) and lowering glutathione S-transferase (GST) gene activity [84]. Consumption of 6 probiotic microorganisms of *Lactobacillus* and *Bifidobacteria* strains for 4 weeks by patients with colorectal tumor showed the declined levels of pro-inflammatory cytokines in the colonic environment [85].

High intake of diet linked with yogurt was strongly associated to downgrade the risk of colorectal carcinoma [86]. Kimchi, a dish produced in Korea from fermented vegetables, yielded *Bacillus polyfermenticus* KU3, showed reduced levels of proinflammatory cytokines and nitric oxide indicating effect against inflammation [87]. Administration of *B. polyfermenticus* halted the growth of colon cancer and preneoplastic lesions by an antioxidant mechanism in male F344 rats [88]. The anticancer properties of *Bacillus polyfermenticus* are mediated by inhibition of ErbB2 and Erb3 [89]. The nutritional probiotic for *Lactobacillus acidophilus* KFRI342 was examined in traditional Korean dish kimchi. *L. acidophilus* showed potential probiotic action as a symptom-inhibiting agent for DMH. *L. acidophilus* obtained from kimchi has been used as a probiotic for human use [90]. Probiotic formulation (containing *Lactobacillus acidophilus* and *plantarum*, *Saccharomyces boulardii,* and *Bifidobacterium lactis*) effectively reduced the issues of postoperative risk in patients undergoing colorectal surgery [91]. *B. subtilis* and *C. butyricum* prevented proliferation of colorectal cells induced by DMH which halted the cell cycle and provoked programmed cell death by reducing inflammation and enhancing the immunological homeostasis [92]. A study conducted on immune system response of rats has shown that *Bifidobacterium lactis Bb12* and *L. rhamnosus GG* as well combination of both resulted in a decrease in colon tumors. By modifying the gut-associated lymphoid tissue, combination therapy might help to decline the progression of colon cancer [93].

Lactobacillus acidophilus utilized to produce exopolysaccharides antitumorigenic against HT-29 cells through stimulation of autophagy process [94]. *Atopobium minutum*, a significant novel gut bacterium, was compared to *Bifidobacterium lactis, Lactobacillus rhamnosus*, *Escherichia coli K-12 strains,* and *E. coli* gut flora

for causing apoptosis in Caco-2 cells [95]. *Lactobacillus acidophilus, Bifidobacterium bifidum*, and *Bifidobacterium infantum*, (LBB), have shown the modification of microflora by improving the probity of the gut barrier by suppressing the inflammation and apoptosis, thereby inhibiting the progression of colon cancer by declining tumor incidence, multiplicity/count, and volume [96]. After being injected with 1,2-dimethylhydrazine, *L. salivarius Ren* was used to regulate the structure of the colonic microflora thereby reducing the incidence of tumorigenesis [97]. Resistant starch type-3 (RS3), Novelose 330, enhanced the proportion of goblet cell population in the distal part of colon that primarily contained acidic mucin while having no impact on the overall no. of goblet cells. These findings suggest that RS3 inhibited colon mutagenesis by increased death of injured cells as well as modifications to the colonic mucosa's dedifferentiation characteristics [98]. Intake of *Bifidobacterium longum* was linked to possibly advantageous alterations in physiology of caecum and metabolic activity of bacterium with reference to risk of tumor and the occurrence of preneoplastic lesions in the colon. These outcomes suggest that combination of probiotics was successful in reducing colonic lesions [64].

24.5.2 Prebiotics and Probiotics Treatment in Breast Cancer

Combination of *Lactobacillus rhamnosus LC705* and *Propionibacterium freudenreichii* offered the therapeutic benefits in treating breast cancer [99]. Study with *in-vivo* model of breast cancer was investigated to determine the *Lactobacillus acidophilus*' immunomodulatory effects suggesting its role in regulating the incidence of tumor [100]. Treatment with *L. lactis NK34* inhibited the cancer growth and also inflammatory cascade [101]. Two fast-acidifying *S. thermophilus* strains also displayed an anticancer and probiotic effects in vitro [102]. In Japan, regular consumption of *Lactobacillus casei Shirota (BLS)* drinks containing isoflavones showed an inverse correlation with the occurrence of breast cancer in women [103]. Oral administration of *Lactobacillus acidophilus* in 0.5 mL solution was started two weeks prior to the implantation of a malignant tumor and went for 30 days after with 3-day intervals. This treatment can enhance immune responses by promoting the induction of proinflammatory cytokines like interferons and interleukin-10. Thus, a prominent spike in the survival tendency among the *L. acidophilus* group collated to controls [104]. Animal studies have demonstrated that administration of *L. acidophilus* by an route showed anticancer in breast cancer model [105]. In ainvestigation, were mice orally recived 0.5 mL of slurry, containing *Lactobacillus casei*, influenced the NK-cell cytotoxicity by activation of Th1 production in mouse spleen cell culture [106]. In animal carrying breast cancer, oral intake of milk containing fermented *Lactobacillus helveticus R389* produced an immunoregulatory response, suggesting its potential utility as an immunological adjuvant therapy to prevent cancer [107]. Interferons and IL-2 levels were observed higher in mice given selenium nanoparticles loaded with *Lactobacillus plantarum* and also displayed increased NK-cell

activity [108]. Selenium nanoparticles enriched with *Lactobacillus brevis* produced the antitumor effects in mouse model [109].

24.5.3 Prebiotics and Probiotics Treatment in Hepatic Cancer

Primary malignancy in population with cirrhosis and other chronic liver conditions is hepatocellular carcinoma (HC). Specific probiotic mixture reported to increase the levels of anti-inflammatory mediators such as IL-10, IL-13, and IL-27, and down-regulated the angiogenic factors and receptors [110]. Human and animal studies highlighted role of probiotics in the reversal gut dysbiosis [111, 112]. Treatment with *Lactobacillus plantarum*, a strain obtained from traditional Chinese fermented foods, increased the excretion of aflatoxin in feces and managed the antioxidant insufficiency of the defense system in a mouse model [112]. As aflatoxin exposure remains a health problem in developing countries, probiotic yoghurt consumption constituting *Wiessella cibaria, Lactobacillus rhamnosus, Streptococcus thermophiles* may reduce aflatoxin poisoning in Kenyan children [113]. In a rat model, chlorophyllin and probiotic fermented milk decreased the levels of cmyc, Bcl-2, rasp-21, and cyclin D1 and subsequently tumor growth by 40% [114]. Patients with nonalcoholic fatty liver disease (NAFLD) benefited with supplements containing *L. acidophilus* and *Bifidobacterium lactis* probiotics [115]. In a study population with obesity and NAFLD, probiotic treatment has shown decline in body weight and fat content. Probiotics also lowered liver tenderness in patients having obesity by inhibiting the proinflammatory mediator tumor necrosis factor [116]. The hepatoprotective and anti-inflammatory action of probiotic bacteria also altered the gut physiology. Probiotics restored the depletion of gut microbiota diversification, intestinal tight junction function, and resistance toward colonization in rats which were fed with high-fatty diet and rich in sucrose diet as well. Lowering blood lipopolysaccharides (LPS) levels and decreasing TLR4 regulated hepato-inflammation slowed the progression of NAFLD in response to the re-establishment of microbiota and gut barrier function [113]. Numerous clinical studies have examined the usage of probiotics as a cutting-edge and successful strategy to cure or avert HC and chronic liver disease. Probiotic VSL#3, a blend of *lactobacilli*, *Bifidobacteria*, and *Streptococcus thermophilus*, may shorten the length of hospital stays for patients with hepatic encephalopathy and liver cirrhosis. The patients suffering from liver cirrhosis and hepatic encephalopathy, probiotic VSL#3, a blend of *lactobacilli*, *Bifidobacteria*, and *Streptococcus thermophiles* may reduce the length of their hospital stay [117]. According to a randomized controlled multicenter trial including 117 patients with alcoholic hepatitis, those who got probiotic therapy using *Streptococcus faecium and Lactobacillus subtilis* had reduced blood levels of lipopolysaccharide than those who received a placebo [118].

24.5.4 Role of Bacterial Strains in Several Cancer Models

Enterococcus faecalis CECT7121 probiotic enables the evolution of new leads to beneficial approaches against oncology therapies [114]. Kefir, a grain product made with novel probiotics fermentation technology (PFT), is a natural blend predominantly made up of *Lactobacillus*, a particular strain of *L. kefiri* with distinct development features. PFT's apoptosis-inducing impact in human multidrug-resistant (MDR) myeloid leukemia suggests that it may be a possible therapeutic for MDR leukemia [115]. Using *P. freudenreichii*-fermented milk, researchers were able to increase the cytotoxicity of the stomach cancer medication camptothecin [116]. Additionally, *L. paracasei IMPC2.1* cells were shown to be a valuable element of a functional diet strategy for slowing the progression of cancer [119]. *L. reuteri*-regulated effects on apoptotic cascade may also assist in the evolution of regimens including probiotics for inhibition of tumor and irritable bowel syndrome (IBS) [120]. Probiotic VSL#3 exhibited improve alkaline sphingomyelinase, vitamin D receptor (VDR) expression, and antiangiogenic factor angiostatin, and showing that initial treatment with probiotic can decrease numerous autoimmune parameters, thus providing its vital therapeutic utility in patients suffering from chronic colitis [121]. Studies have proven the significance of probiotic-curd for its antitumor properties [122]. Chicory's containing fructans are reported to have antitumor effects in preclinical model [123].

24.5.5 Probiotics as Antimutagens

Ingestion of *Lactobacillus alimentarius*, *Lactobacillus reuteri*, *Bifidobacterium bifidum,* and *Enterococcus faecium* reduced intestinal cancer indicators and fecal mutagens when administered orally to goats. These probiotics halted the mutagenesis caused by sodium azide and benzopyrene [124]. Few strains of the genera *Lactobacillus* and *Bifidobacterium* were examined for their antimutagenic properties against benzopyrene sodium azide (SA) which displayed either no impact or low antimutagenic activity against benzpyrene. Complex antimutagenic properties of *lactobacilli* supernatants were seen against SA [125]. Heat-killed *Lactobacillus* strains produced an antimutagenic effects against acridine orange [126]. Antimutagenic properties of viable and non-viable cells from nine strains of *bifidobacteria* and six strains of *Lactobacillus acidophilus* were assessed using eight strong promutagens and mutagens. A mutant of *Salmonella typhimurium* was used to determine the Ames TA100 assay's results for the mutagenic activity of these carcinogens and the antimutagenic activity of bacterial flora against the mutagens. The antimutagenic activity of probiotic bacteria's containing live cells was stronger, and they were more active in preventing the mutagens than dead bacterial cells [127]. Another study has proven that combination of *Bifidobacterium longum PS*, *Bifidobacterium longum PS+*, and *Bifidobacterium longum* improved antitumor activity against the potent mutagens [128]. To investigate the antimutagenic effects

of milk, cultured in lactic acid bacteria containing 71 strains from the genera *Lactococcus*, *Lactobacillus*, *Bifidobacterium,* and *Streptococcus* on the mutagenicity of N-methyl-N'-nitro-N-nitrosoguanidine has proven its antimutagenic effects [129]. The Ames test was used to determine the antimutagenicity property of *Lactobacillus acidophilus L. helveticus, Streptococcus thermophilus, Streptococcus salivarius, Lactobacillus delbrueckii* and *L. bulgaricus.* All these have demonstrated considerable dose-dependent activity against both mutagens [130]. The ability of *Lactobacillus gasseri, Streptococcus thermophiles, Lactobacillus confuses* to possess antimutagenic activity has been assessed [131]. Antimutagenic ability of the Korean *Lactobacillus plantarum KLAB21* strain against 4-nitroquinoline-1-oxide (NQO), N-methyl-N-nitro-N-nitrosoguanidine (MNNG), 4-nitro-O-phenylenediamine (NPD) and aflatoxin B1 was tested using the strains TA100 and TA98 of *Salmonella typhimurium.* Its antimutagenic potency was verified using a *Bacillus subtilis* spore-rec test [132]. Studies have shown that the 76 lactic acid bacteria strains in the cultured milk sample proven their different antimutagenic effect against Trp-mutagenicity of Trp-P2 [133]. Another study examined the antimutagenic properties of milk cultured with *Streptococcus thermophiles* and *Lactobacillus bulgaricus* using an in vitro test method utilizing strains of *Salmonella* [134]. To ascertain their proteolytic activity in yoghurt, studies were conducted on *Lactobacillus* species of *casei*, *acidophilus,* and *paracasei* [135]. The short-term bacterial assay chromo test was used to investigate the antigenotoxic activity of probiotic bacteria against furazolidone using *Escherichia coli* as the test organism. The best genotoxicity inhibition was demonstrated by *Lactobacillus acidophilus* (81.9%) and *Bifidobacterium lactis* (92.0%) [136] whereas probiotic *L. rhamnosus* also provided in vivo defense against colon damage brought on by N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) by converting MNNG into less toxic metabolites [137]. Numerous mutagens are produced during the cooking process of meat, primarily in the form of heterocyclic aromatic amines (HCA). It has been suggested that LAB works through direct binding mechanisms to defend laboratory rodents and people against the carcinogenic effects of HCA [138]. The mutagenic and antimutagenic activity of *Lactobacillus plantarum*, an isolated lactic acid bacteria (LAB), was evaluated using the Ames test. *L. plantarum* would therefore be a good candidate for the development of functional foods as a supplement to delay the incidence of colon cancer [139]. Therefore, for optimum health, species-specific probiotics must be developed to select isolates in order to build a species-specific probiotic [140].

24.6 Role of Short-Chain Fatty Acids (SCFAs) in Cancer Treatment

Numerous SCFAs are produced by the gut microbiota from indigestible and fermentable carbohydrates, including dietary fiber [141]. Propionate, acetate, and butyrate are the principal SCFAs whose total intestine concentration exceeds 100 mM

[142]. Recent evidence suggests that SCFAs may have an impact on the progression of a number of illnesses, including inflammatory bowel disease (IBD), diabetes, atherosclerosis, and CC [143–145]. The impact of SCFAs on CC has received most of the attention from researchers [60, 146]. Studies have revealed the reduced number of bacteria producing SCFA and fecal SCFA levels in CC patients [147, 148]. Some bacteria have been shown to produce metabolites like secondary bile acids, favoring tumor growth in contrast to other bacteria that produce tumor-impeding metabolites like SCFAs [149, 150]. Considering an important role SCFAs, an extensive epidemiological research revealed that patients with diets fewer in SCFAs or fewer fecal SCFA levels had an increased risk of inflammatory diseases and cancer, especially breast and stomach malignancies [151]. By preventing cell growth and migration, decreasing histone deacetylases (HDAC), and onset of apoptosis, SCFAs in the inhabitant gut and other areas might effectively ameliorate carcinogenesis and help prevent and treat gastrointestinal and lung cancers [152]. In addition, epidemiological revelations indicate that high-fiber diets are related to lower incidence of cancer than diets heavy in red meat, which have been shown to increase the risk of cancer [153–155]. It has also been shown that increased synthesis of SCFAs such as butyrate through the action of the microbiota is the key for high-fiber diets to exert anticancer impacts [150]. Here, we reviewed the most recent research findings that examined the correlation between SCFAs produced by the gut microbial community and certain malignancies.

24.6.1 SCFAs and Intestinal Cancer

The research reveals that SCFAs help to keep the intestinal barrier intact and balance of the microbiota, as well as to prevent cancer and inflammation. According to Hu et al. [156], gastric cancer has depleted pathways that contribute to SCFA formation, which suggests that the disease is more inflammatory and has dysbiotic bacterial groups. In distinction to these findings, 16S rRNA analysis in two studies [29, 157] confirmed the involvement of gastric mucosa-related pathways in the production of SCFAs in patients suffering with gastric cancer.

Sodium acetate, a SCFA, is frequently employed in the food industry to regulate pH [158, 159]. Numerous studies have also examined the cytotoxicity of sodium acetate [158]. According to reports, sodium acetate promotes the apoptosis and differentiation while inhibiting the growth of CC cell lines [158]. Acetate's ability to cause apoptosis or necrosis in CC cells by mitochondria is where its anticancer properties come from. Additionally, earlier studies have shown that sodium acetate, at a concentration of 12.5 mM, stimulates the growth and viability of gastric adenocarcinoma cells in a concentration-dependent manner. However, sodium acetate inhibits cell proliferation in a concentration-dependent manner at concentrations > 12.5 mM [158]. Additionally, cells exposed to sodium acetate for 24 h showed increased expression of TNF-, IL-1b, and IL-8, the effects of which have been demonstrated in mice [158]. Numerous studies have been done on the cytotoxic effects of sodium

acetate in colonic epithelial cells. The biological toxicity of sodium acetate, however, cannot be restricted to the colon and can reach out to other parts of the body once it is initiated through oral route in the human body, it reaches organs like the stomach. Reference [158] pointed out the cytotoxicity of food containing high doses of sodium acetate in this regard.

Preservation of the cellular colony, elimination of cancerous cells, and the regulation of apoptosis primarily require the Fas receptor (FasR)/Fas ligand (FasL) complex [158]. This complex is present in most human cancer cells. On the surface of tumor cells, the interaction of FasR and FasL triggers apoptotic signaling, stimulating caspases, which then interacts with cytoplasmic signaling proteins to activate apoptosis [160]. After the addition of 12.5–50 mM sodium acetate, [158] Xia et al. showed that FasL and FasR's mRNA expression increased in a concentration-dependent manner. Following Fas-initiated apoptosis, subsets of caspases are activated. The Fas-induced cascade of apoptosis is initiated by caspase-8 [161].

24.6.2 SCFAs and Hepatic Cancer

Hepatic carcinoma (HC) is becoming more common, and both genetic and environmental factors have been proposed as potential causes [162]. In contrast to preneoplastic injuries associated with hepatitis C virus infection, certain hepatotoxins, like organochlorine compounds, aflatoxin, and organochlorine compounds, are significantly involved in the progression of HC [163–165]. Additionally, recent research has shown that *Helicobacter hepaticus* can cause HC in mice that is promoted by aflatoxin, which necessitate the production of pro-proliferative and proinflammatory cytokines [166]. These investigations support the findings of human studies indicating *Helicobacter spp.* could be isolated from the liver and intestine [167, 168]. As a consequence, liver cancer initiation and progression are influenced by microbial metabolism of ingested food and byproducts as well as immune responses to the bacteria.

The main risk factors for the progression of HC are thought to be obesity and a high-fat diet, but the precise molecular mechanisms are still unclear [169]. This pathogenetic pathway may be affected by changes in the intestinal microbiota [169]. Hepato-carcinogenesis in obese mice was studied by Yoshimoto et al. [170]. They showed that antibacterial drugs could control dysbiosis and the subsequent release of pro-carcinogenic and proinflammatory factors, thereby lowering the risk of HC development in mice under treatment. Notably, fermentable and nondigestible carbohydrates may help to regulate the tumor growth outside the digestive system [171]. In animal models of breast or liver cancer, it has been claimed that oral administration of inulin-type fructans (ITF), as nondigestible carbohydrates, decreases the tumor size [172, 173]. Inulin-type fructans can be fermented by saccharolytic bacteria, which boosts the production of SCFA in the murine caecum [174]. ITF, a prebiotic nutrient, modifies the gut microbiome's activity and composition and regulates the host's immunity and metabolism [175–177].

24.6.3 SCFAs and Colorectal Cancer

The CC comes under top three malignancy that results in death worldwide [178, 179]. Diet is one of the important factors which influences the development of CC [180, 181]. Butyrate is a major metabolite of SCFAs that controls dietary fiber-mediated CC reduction [180]. Butyrate, in contrast to other SCFAs, is metabolized primarily by colonocyte cells and causes epigenetic changes, where it increases the rate of histone acetylation by inhibiting histone deacetylases [159, 180]. Butyrate enhances differentiation, decreases proliferation, and triggers programmed cell death in CC cells [180, 182]. It is noteworthy that butyrate is metabolized in colonocyte and is thought to be responsible for its impact on cell apoptosis and proliferation [183, 184]. The beta-oxidation of butyrate, that is influenced by exogenous agents, is thought to be carried out by colonocytes, according to numerous studies [185, 186]. Additionally, butyrate exposure causes noncancerous colonocytes to proliferate more and cancerous colonocytes to proliferate less [180]. Because cancerous and noncancerous colonocytes have different metabolic characteristics, butyrate has a diverse metabolic effects on both of them.

Interestingly, a strong correlation has been noted between an increased CC risk and the altered gut microbiota, decreased SCFAs, and elevated inflammation [187]. In addition, McLoughlin et al. [188] studied the impact of SCFAs, prebiotics, and synbiotics on systemic inflammation in healthy persons, overweight, diabetes, kidney disease, obesity, cancer, bowel, and liver diseases. Meta-analyses revealed that supplementation of prebiotics and synbiotics diminishes systemic inflammation by depleting C-reactive protein (CRP), IL-6, and TNF-α even though the association was stronger with precise supplement types (particularly oligosaccharides) [188]. Furthermore, Nomura et al. [189] assessed both plasma and the fecal SCFAs in patients with solid cancers receiving programmed cell death-1 inhibitors (PD-1i). They discovered that PD-1i efficacy can be correlated with fecal SCFA levels, indicating that SCFAs can be linked with PD-1 efficacy. Fecal examinations can be used for routine patient monitoring because they are non-invasive; as a result, they should have a promising future in the treatment of cancer by enhancing SCFA formation through modulating gut microbiota. Additionally, dietary fiber, whole grains, and risk of colorectal cancer were examined by Aune et al. [190] in a systematic review and dose–response meta-analysis of prospective studies. Numerous epidemiological studies have also shown that dietary fiber is effective in preventing colorectal cancer. In this regard, several mechanisms for fiber's anticancer effects have been identified such as a shorter transit time of feces in intestine reduces the exposure of mucosa to luminal carcinogens, absorption of bacterial toxins, biogenic amines and bile acids, and formation of SCFAs like butyrate [191–203].

Different foods leave remnant in the intestine after being absorbed and digested [204]. Both intestinal epithelial cells and gut microflora are impacted as food travels through the colon because the gut microbiota helps in fermentation of dietary fiber leftovers and produces the SCFAs [203, 205]. The idea of symbiosis between intestinal epithelial cells and gut microbiota is supported by research showing

butyrate as the primary energy source for intestinal epithelial cells [206]. Multiple mechanisms suggest that butyrate may slow the development of CC and enhance intestinal health [204].

Intestinal epithelial cells can use butyrate as an energy source and produces anti-tumor and anti-inflammatory effects [204]. The decreased levels of butyrate transporter protein in the cancerous tissue are the proposed mechanism for the effect of butyrate molecule on the development of CC [207]. Numerous gastrointestinal disorders have been linked to butyrate metabolism dysfunction [208]. It is well reported that the impairment of intestinal epithelial barrier stimulates the tumor-related macrophages, production of inflammatory cytokines, and tumor development is linked to the pathogenesis of CC [209]. Butyrate is reported to improve the tight junctions by stimulation of cAMP-activated protein kinase (AMPK) [209]. Moreover, it can preserve the functionality of the intestinal epithelial barrier by inducing MUC2 expression in the human colon cancer cells LS174T [210–212]. Additionally, it can reduce the intestinal movement, which will impact the incidence of CC and delays the intestinal transport. Various studies have suggested that butyrate can have an impact on the internal nervous system and can control excitable neurons, which reinforces the action of extracorporeal contraction [213, 214].

In this manner, butyrate plays a pivotal role in preserving the homeostasis of the intestinal microbiota and colon health. Additionally, several studies have shown that butyrate may prevent development and progression of CC through a variety of mechanisms [204]. Through β-oxidation, butyrate is transformed into acetyl-CoA and then oxidized in the Krebs cycle [204]. However, butyrate is typically not metabolized in cancer cells because of the Warburg's effect. However, it may build up in the nucleus as an HDAC inhibitor (HDACi) and then affect the expression of downstream target genes [204]. In tumor cells, HDAC enzyme expression varies depending on the type of tumor, and these enzymes are essential for controlling gene expression [183]. For example, HDAC1 is mainly present in prostate, gastric, lung, esophageal, and breast cancers whereas HDAC2 is mainly present in gastric, cervical, and colorectal cancers [204].

Additionally, HDAC3 and HDAC6 are primarily found in breast and colorectal carcinoma, whereas HDAC3 and HDAC6 are primarily found in neuroblastomas [215, 216]. The Wnt and extracellular-signal-regulated kinase (ERK) genes, as well as the protease system and the activities of several enzymes like protein kinase C, are affected by acetylation in the majority of tumors, which results in altered expression of these genes [217]. HDACi can cause cell death via a number of mechanisms, including changes in histone modifications, gene expression, and epigenetic changes [218]. Chromatin lysis happens after histone acetylation is blocked, which improves DNA exposure [204]. The slowing down of tumor cell growth has been associated with decreased HDAC activity [219]. Butyrate has variety of roles in histone acetylation, and eventually triggering the cell cycle arrest and programmed cell death.

24.6.4 SCFAs and Breast Cancer

Around 2.4 million people are affected by breast cancer and cause > 500,000 fatalities worldwide in female populations [220]. In the United States, 3.4 million breast cancer cases survived in 2015, and this number is again growing [221–223]. About 90% of breast cancer patients survive at least five years after diagnosis [221], and the disease is currently regarded as a chronic, allowing survivors to live longer [221]. However, consumption of high-fat diet, less fiber food, and lack of physical exercise raises the risk of breast cancer. Diet makes a notable impact on how the gut microbiome and estrogen metabolism interact, which in turn affects breast cancer metastasis and relapse. The growth of harmful bacteria with high levels of glucuronidase is accelerated by the typical American diet. The glucuronidase enzyme catalyzes estrogen and elevates its systemic levels, which increases the cancer prevalence that responds to estrogen. The production of SCFAs such as propionate, butyrate, and acetate, which can prevent "leaky gut syndrome," is decreased by this diet. This syndrome accelerates the progression and recurrence of breast cancer by increasing the flow of harmful inflammatory mediators into the bloodstream [221]. Leptin and insulin resistance are factors in the modulation of carcinogenesis and are increased by inflammatory proteins [224]. The interaction of insulin molecules with steroid hormone-binding globulin (SHBG) leads to increased estrogen accessibility and concentrations [225], which aids in the development of breast cancer [225, 226]. Insulin resistance is exacerbated by low levels of adiponectin and raised levels of the cell-proliferating hormone insulin-like growth factor 1 (IGF-1).

A high-fiber diet, on the other hand, promotes "healthy" microbiota [221]. Along with the fecal excretion of estrogen, decreased glucuronidase activity raises SGBH and lowers estrogen levels [221]. The mucosal layer of the colon is protected from leaky gut syndrome, inflammation, and the emergence of cancer after an increase in SCFAs [225, 227, 228]. After being conjugated in the liver and secreted into the digestive tract, estrogen molecules are then broken down into free estrogen by microbial glucuronidase and reabsorbed into the bloodstream [221]. This process is aided by a variety of bacterial species, but it is still debatable which ones are best able to produce large amounts of glucuronidase [221]. The major bacterial players involved in the metabolism of fiber and polyphenols belong to the *Firmicutes* and *Bacteroidetes* phyla [221]. Regarding these phyla's impact on obesity, a notable risk factor for breast cancer—researchers have reported a variety of findings [229, 230]. A high-fiber diet may reduce the inflammatory symptoms of leaky gut syndrome by promoting the production of intestinal alkaline phosphatase and SCFAs [231]. For the intestinal endothelia to remain intact, intestinal alkaline phosphatase is necessary [232–234]. Along with SCFAs, gut alkaline phosphatase strengthens the tight junction of the colonic mucosa to stop the leakage of pathogenic bacteria and their cancer-causing effects [232–234]. Through its effects on self-replication and programmed cell death, the gut microbiota may exacerbate prolonged inflammation [235, 236].

The estrobolome, an assembly of enteric microbial gene products, regulates how estrogen metabolism is genetically predisposed. These bacteria primarily obtain their energy from fiber. When eating a diet rich in fiber, the estrobolome stimulates the metabolism of estrogen and subsequently its excretion from the body. A fiber-rich diet eliminates estrogen, reducing the availability of breast cancer cells, as estrogen is thought to be the primary cause of about 70% of breast cancer cases. Consuming dietary fiber, as "common sense" advises, reduces inflammation in breast cancer patients [221]. Long-term consumption of fiber and polyphenols increases the likelihood of surviving breast cancer [237, 238].

Deregulation of epigenetic pathways, like post-translational histone modifications, has been linked to women's breast cancer progression, as a result of the silencing of essential genes involved in tumor suppression [239]. Epigenetic changes, in contrast to genetic changes, are reversible and have been investigated for their role in cancer treatment [240]. DNA methylation and histone acetylation can be modified by bioactive dietary components with anticancer effects [241, 242]. Food ingredients may be used to reactivate epigenetically silenced genes, which could be used to combat cancer [243]. Butyrate is an important dietary HDACi because it has an antineoplastic effect and has been used in phase I clinical trials to treat cancer [244, 245]. The fermentation of dietary fiber by the gut microbiota results in the formation of this SFCA [246]. Butyrate is considered to be the promising SCFA for breast cancer treatment [247–249]. Butyrate appears to act as an HDACi and promote the formation of the cyclin-specific kinase inhibitor p21 in order to restrict the growth of breast cancer cell lines [248, 250, 251].

Few studies have examined the efficacy of the retinoid and HDACi combination in the breast cancer treatment [252]. Prior findings revealed that trichostatin (a synthetic HDACi) has enhanced restrained effects on breast cancer cells of human origin [253]. More significantly, by reactivating the RA receptor beta (RAR-β), the synthetic HDACi made breast cancer cells more susceptible to the inhibitory effects of RA [253]. Few studies have examined the effectiveness of similar type of treatment, especially for preventing breast carcinoma, despite the antineoplastic effect of HDACi in combination with retinoid [252]. It is interesting to note that while vitamin A does not prevent MCF-7 cells from proliferating, it does potentiate the growth inhibition caused by butyrate [253, 254]. The cell cycle arrest in G2/M phase of MCF-7 cells may be responsible for this combinatorial effect [253, 254]. This improves the effectiveness of dietary HDACi butyrate and retinoid in the prevention of breast cancer [255]. However, the interaction between butyrate and vitamin A has no additive effect on preventing the growth of MDA-MB-231 cells that lack the estrogen receptor [255]. This suggests that the type of breast cancer should be considered for the co-administration of butyrate and retinoids for cancer treatment.

24.7 Fecal Microbiota Transplantation (FMT) in Cancer

Modification of gut microflora is anticipated to be a unique approach to treat diseases related to intestinal dysbiosis. The gut microbial communities can be modified using diet, prebiotics, probiotics, FMT, and antibiotics. FMT is the process of transferring the gut microflora from lively donors to unwell patients in order to reimpose the heterogeneity of the internal microbial population [256, 257]. Since this intervention reverses the "pathobiome" phenotype of gut microbiota, FMT is acknowledged as the most inventive and pragmatic approach. About 1,700 years ago, a renowned physician Ge Hong first noted the use of feces for treating food poisoning or severe diarrhea [258]. In 1958, Eiseman made the first use of FMT to treat severe pseudomembranous enterocolitis [259]. However, this method was not widely employed until 1983, when Schwan reported the first known case of *Clostridium difficile* infection (CDI) treated with FMT [260]. According to 2013 guidelines [261], FMT has been approved as a therapeutic treatment for treating recurrent CDI, and its clinical efficacy has reached about 90%. Additionally, growing evidence suggests that FMT is effective in treating inflammatory bowel disorders, unremitting functional constipation, and other conditions [262, 263]. Additionally, the reported intestinal dysbiosis in cancer raises awareness of the possible benefits of FMT in the treatment of the disease.

While fecal donors could be relatives, family members, or people with no relation to one another, it is preferable to obtain fecal sample from a centrally controlled stool bank when available from a healthy unrelated individual [264]. Donors in advance of FMT should be screened in accordance with a specified methodology to reduce the possibility of unintentionally transferring an infection [265]. Frozen fecal material offers the benefit of easier maintenance as compared to other techniques of preserving feces [266]. The diversity of microorganisms in frozen material appears to be less than in fresh feces [267]. Frozen fecal material performed less well than fresh material in a recent double-blind investigation of CDI patients [268]. The donor microbiota may be challenging to keep despite retention enema's low cost and safety [269]. The best method of administration is still being researched. In contrast to Terveer et al., [24] who came up with an idea of centralizing stool bank that could provide the storage and safety of fecal samples and allow the remainder of the FMT protocols to be performed in hospitals. The European conference on FMT published in gut strongly advocated the creation of FMT centers [270, 271]. FMT can be delivered via different routes and formulations such as capsule, colonoscopy, nasogastric tube, nasoduodenal tube, or enema [272]. Administration through the oral route is more readily accepted by patients due to increased satisfaction, even if administration through endoscopic means permits direct examination of the intestinal mucosa [273]. The intervention of FMT in cancer management has been demonstrated in several preclinical and clinical studies.

24.7.1 FMT and Gastrointestinal Cancer

Changes in microbial diversity and abundance in gastric cancer patients also revealed a dysbiotic gut [274]. H. pylori and certain oral bacteria, notably *Fusobacterium nucleatum*, *Parvimonas micra*, and *Peptostreptococcus stomatis*, are linked to the progression of stomach tumors [275]. Gastric cancer is associated with a prominent enrichment of *Parvimonas micra, Peptostreptococcus stomatis*, *Dialister pneumosintes, Slackia exigua, Streptococcus anginosus* [29], *Clostridium colicanis*, and *Fusobacterium nucleatum* [276] and a significant reduction of *Helicobacterium* [277]. The risk of stomach cancer reported to decrease by *H. pylori* eradication therapy [278, 279]. These findings suggest that the gastric microbiota is implicated in the progression of gastric cancer. Moreover, gut microflora has a role in colorectal carcinogenesis as there are several microorganisms in close proximity to the colonic epithelial cells. In fact, several bacterial species can cause CC by bacterial translocation, exposure to harmful substances, persistent inflammation, and damage to the mucosal barrier. By generating toxic compounds, pathogenic bacterial species like *enterotoxigenic Bacteroides fragilis* can impart pro-tumorigenic characteristics [280–282]. A CC-specific bacterial signature was also observed in clinical trials, which showed substantial alterations in the composition gut microbiota between healthy people and those with CC [28, 283]. The CC patients display reduced abundance of *Lactobacillus* and *Bifidobacterium* and increase in *Staphylococcaceae*, *Fusobacteria*, and *Peptostreptococcus anaerobius* in stool samples compared to healthy individuals. And fecal microbiota analysis could be adopted as a non-invasive method to increase the accuracy of early CC identification [53]. Some probiotics also showed preventative effects against CC. Both *Clostridium butyricum* and *Bacillus subtilis* are renowned butyrate-producing bacteria, which prevented colon cancer in rats [284]. Administration of *Lactobacillus caseiBL23* also prevented the CC in mice by remodeling the gut dysbiosis [285]. Furthermore, *Bifidobacterium* tri-multiple viable oral probiotics were found to be effective in treating small intestine bacterial overgrowth and reducing gut dysbiosis in CC patients [286, 287]. The development of CC was influenced by deoxycholic acid-induced intestinal dysbiosis, a secondarily carcinogenic bile acid. In this connection, the fecal transplantation from deoxycholic acid-injected mice reported to show an increase in intestinal tumor development [37]. The conclusion was intriguingly confirmed in patients in an investigation and the fecal microbiota from CC patients encouraged intestinal tumor growth and decreased microbial plethora in germ-free and conventional mice administered a carcinogen.39 Additionally, Rosshart et al. reported that FMT from wild mice donors demonstrated an improved resistance to CC and reduction of inflammation [288]. Taken together, FMT may prove to be the potential therapeutic intervention for CC.

24.7.2 FMT and Hepatic Cancer

The liver makes communication with gut bacteria through portal circulation that carries toxic deoxycholic acid and lipopolysaccharide produced by bacteria in the gut [289, 290]. Although an altered intestinal microbiota contributes to liver diseases, the underlying mechanism is yet undisclosed. The gut-liver axis describes the intimate association of anatomy and function between the gut and liver. Bacterial metabolites may facilitate the onset of chronic liver dysfunction and hepatic cancer via dysregulating the gut-liver axis, which are frequently linked to intestinal dysbiosis [291, 292]. Transplanting the microbiota of chronic liver diseased mice resulted into a greater liver damages in the recipient animals [293]. Moreover, mice became more susceptible to chronic alcoholic liver disease after receiving FMT from individuals with severe alcoholic cancer hepatitis [294]. Injection of penicillin or dextran sulfate sodium in mice showed severe hepatotoxicity due to microbial dysbiosis [295]. The colonization of *Clostridium* species, which affect bile acid metabolism, accelerated the liver tumor formation in mice lacking gram-positive bacteria [291]. These findings offer concrete evidence that microbial dysbiosis may be a key factor in the liver illness and cancer.

Recent studies have shown that FMT has potential for managing the liver diseases. In mice, FMT reduced lipid metabolism and liver damage inflicted by a high-fat diet and enhanced the richness of gut flora. FMT improved the survival and resolved ascite in a pilot trial of individuals with severe alcoholic hepatitis [296]. A case of severe alcohol-related hepatitis in a young male patient with corticosteroid-nonresponsiveness was described by Philips et al. in 2017 [297]. FMT quickly reduced hyperbilirubinemia and improved appetite. Notably, patients with persistently positive HBeAg underwent FMT [298] showed clearance of HBeAg, indicating that controlling the gut microbiota may be helpful for the treatment of chronic hepatitis B. In patients with advanced liver cirrhosis, FMT reversed antibiotic-induced microbial dysbiosis [299]. Furthermore, animal and clinical studies proved an impact of FMT on hepatic encephalopathy. In animal studies, FMT improved hepatic encephalopathy by reducing cognitive dysfunction and preventing hepatic necrosis [300]. In a patient with hepatic encephalopathy, FMT significantly improved the patient's life quality and serum ammonia [301]. FMT also helped hepatic encephalopathy patients with their cognition and hospitalizations [302]. Given the efficacy in treating chronic liver disease, FMT's advantages for patients with hepatic cancer should be studied.

24.7.3 FMT and Pancreatic Cancer

Latest research reveals the impact of microbiome on the progression and management of pancreatic cancer [303]. Lipopolysaccharide-induced (produced by several gram-negative bacteria) inflammation, in mouse model, has been shown to accelerate the proliferation of pancreatic cancer by increasing TLR4 levels in immune cells [304]. In one of the published study, 113 people with pancreatic ductal adenocarcinoma (PDAC) showed intratumor bacterial positivity in 76% of individuals [305]. While the antibiotic ciprofloxacin was able to overcome the resistance, some of the bacteria that were found, such as *Gammaproteobacteria*, may increase resistance to gemcitabine, a chemotherapy medication frequently used to treat PDAC.

Previous research has demonstrated the difference in oral microbial makeup between individuals with pancreatic cancer and those who are healthy. *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* showed a significant increase among pancreatic cancer groups, and decreases phylum *Fusobacteria* and genus *Leptotrichia.* These findings suggest that oral microbiota may be used as a non-invasive and specific clinical diagnostic marker for pancreatic cancer [306]. Furthermore, a worse prognosis was shown to be independently correlated with a high abundance of *Fusobacterium* species in pancreatic cancer tissue [307], suggesting that *Fusobacterium* species may prove to be a useful indicator of pancreatic cancer prognosis. The development of tumors in germ-free mice was sped up by the transfer of the microbiota from PDAC-affected animals but not from healthy mice [308]. Together, these investigations suggest that pancreatic cancer management with microbiota-based therapy may be beneficial.

24.7.4 FMT and Breast Carcinoma

Hill et al. In 1971, proposed a theory on the link between gut microflora and genesis of breast cancer, taking into account the epidemiological similarities between colon and breast cancer [309]. The research on the possible link between gut microbiota and breast carcinoma is still in its infancy. As in one of the study, comparison of the 48 pretreated postmenopausal breast cancer patients and 48 healthy controls showed significantly less alpha homogeneity and changes in the configuration of the fecal microflora using Goedert et al. analysis [310]. Studies have been done on potential processes, including the metabolism of estrogen, immunological control, obesity, and so on [311]. Animal studies have shown evidence that probiotics can prevent breast cancer by regulating the gut flora. *Lactobacillus acidophilus* as an oral supplementation was found beneficial.

24.7.5 FMT and Melanoma

Gut microbiota also plays an essential role in the progression and management of melanoma. The rate of melanoma and how it responded to anti-programmed death ligand 1 (PD-L1) immunotherapy were different between JAX and TAC mice with varied gut microbial assemblages [312]. 16S ribosomal RNA sequencing revealed that *Bifidobacterium* supports the effects of PD-L1 therapy [312]. The efficacy of immunotherapy has recently been linked to the presence of microbes, as per the study on 39 patients with metastatic melanoma receiving immune checkpoint therapy [313]. The bacteria *Faecalibacterium prausnitzii*, *Bacteroides thetaiotaomicron*, and *Holdemania filiformis* were prevalent in the gut of cancer patients receiving immunotherapy [313]. FMT also reported to improve the efficacy of immunotherapy in mice that were given responsive melanoma patient feces [314]. FMT from PD-1 responders into the digestive tracts of melanoma nonresponders is being tested in a clinical research [315]. FMT appears potential for boosting antitumor immunity in melanoma patients by transplanting a favorable gut microflora.

24.8 Conclusion

With the emergence of next-generation sequencing (NGS) technology, role of gastrointestinal microbiome has evolved from being a mere commensal to the therapeutics approach. Furthermore, computational technologies augmented the therapeutic utility of the gut microbiome. The accountability of gut microflora has been demonstrated in several cancer studies. Due to a close association of gut dysbiosis and cancer incidence, gut microbiome-based therapeutic is gaining a tremendous attention. In this regard, several studies documented the anticancer activity of gut microbiome and use of certain bacterial strains as an adjuvant therapy for the management of cancer. However, sufficient clinical information is unavailable to dictate the ability of gut microflora as an established form of intervention. Therefore, additional studies are warranted to reveal the anticancer action of certain bacterial species and finding their underlying mechanism. Additionally, arbitrary, double-blind, placebo-controlled clinical trial needs to be done for confirming the efficacy of FMT, probiotics, prebiotics, and synbiotics as a substitute or adjuvant to cancer treatment (Tables 24.1, 24.2, 24.3, 24.4, 24.5, 24.6).

Table 24.1 Showing the list of genotoxic and antigenotoxic probiotics along with their source of origin

Antigenotoxic /Microorganism	Origin	Genotoxic	References
Lactobacillus alimentarius and reuteri Bifidobacterium bifidum Enterococcus faecium	Goat milk	Benzopyrene and sodium azide	[124]
Lactobacillus and Bifidobacterium	ATCC	Benzo[a]pyrene and sodium azide	[125]
Lactobacillus rhamnosus	Infant feces	Acridine orange	[126]
Lactobacillus acidophilus and Bifidobacteria	ATCC	4-nitro-O phenylenediamine	[127]
Bifidobacterium lactis and longum	Milk	Benzopyrene	[128]
Streptococcus, Lactococcus, Lactobacillus and Bifidobacterium		MNNG	[129]
Lactobacillus helveticus, delbrueckii, bulgaricus and acidophilus, Streptococcus salivarius	Fermented milk	MNNG and 3,2'-dimethyl-4-amino-biphenyl	[130]
Bifidobacterium breve and Bifidobacterium longum	Human infant stool	N-methyl-N'-nitro-N-nitrosoguanidine	[131]
Lactobacillus plantarum KLAB21	Kimchi (Korean Fermented vegetables)	4-nitro-O-phenylenediamine	[132]
Lactobacillus acidophilus and lactis	Milk	3-amino-1-methyl-5H pyrido [4,3-b]indole (Trp-P2)	[133]
Lactobacillus bulgaricus and Streptococcus thermophilus	Milk	2-(2-furyI)-3-(5-nitro-2-furyl) acrylamide and 4-nitroquinoline-N-oxide	[134]

(continued)

Table 24.1 (continued)

Antigenotoxic /Microorganism	Origin	Genotoxic	References
Lactobacillus acidophilus, casei, and Paracasei	Yogurt	1,1-diphenyl-2 picrylhydrazyl and 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)	[135]
Bifidobacterium lactis Bb12 and Lactobacillus acidophilus		Furazolidone	[136]
Lactobacillus rhamnosus	Vaginal mucosa	N-methyl-N0-nitro-N-nitrosoguanidine	[126]
Lactobacillus helveticus	Milk	Heterocyclic aromatic amines	[138]
Lactobacillus plantarum	Fermented durian	Sodium azide (NaN3) and 2-nitrofluorene (2-NF)	[139]
Lactobacillus plantarum CM4	Thai fermented food products	Heterocyclic amine binding and N nitrosamine	[316]
Lactobacillus paracasei subsp. tolerans	Pepper leaves Jangajji	2-nitroflourene and nitroquinoline-1 oxide	[108]

Table 24.2 List of probiotics eliciting their anticancer activity in various established models

Probiotic	Animal	Activity	References
Lactobacillus casei	Mice	Anti-inflammatory and apoptotic activity in cancer cells via JNK pathway	[57]
Lactobacillus rhamnosus GG (LGG)	In vitro tumor models colorectal APC/min mouse cancer model dimethylhydrazine-induced colon tumor model in rats	Colorectal, ovary, breast, cervical, hepatic tumor models decreases colitis-linked cancer in mice reduction of tumor mass by downregulation of proinflammatory molecules (anti-inflammatory, anti-metastatic, and antiproliferative effects)	
Lactobacillus johnsonii and *Enterococcus hirae*	Cyclophosphamide, coupled with oral bacterial administration Combined activity of lactobacilli with Cisplatin	Improving cyclophosphamide efficacy in tumor-bearing mice Mice have shown targeted response to therapy	
Alistipes shahii	Tumor mice treated with antibiotic	Significant improvement is observed enhancing the TNF synthesis	
BifidobacteriumLongum (BB536) and *Lactobacillus johnsonii (La1)*	Double-blind study involving patients as subjects	Bacterial strains were able to attach to colonic mucosa, which decreases the mass of gut flora	
Lactobacillus acidophilus LAC361 and *Bifidobacterium longum BB536*	Randomized double-blind controlled trial	Significantly decreasing moderate and severe treatment-induced diarrhea during pelvic radiation	
Colon Dophilus (mixture of 10 different probiotic strains)	Clinical trial evaluation irinotecan-based chemotherapy	Diarrhea is prevented in population who were with metastatic CRC Decline in the occurrence and intensity of diarrhea	
Combination of prebiotics and probiotics	Double-blind, randomized trial	Population with CRC resection may relive irritable bowel syndrome	

(continued)

Table 24.2 (continued)

Probiotic	Animal	Activity	References
Symbiotic (probiotic and prebiotics)	Rats	DMH-induced cancer treatment by using synbiotic therapy has 100% survival, whereas rats supplemented with only carcinogen had 70% survival	[61]
Enterococcus faecium RM11 and *Lactobacillus fermentum RM28*	Tumor of colon	In vitro study has proven the antiproliferation activity in colonic tumor	
Live *Lactobacillus casei (L. casei)* ATCC393	Mice	Antiproliferative, inhibition of tumor growth, and pro-apoptotic effects have been proved in this study	
Dairy strain of probiotic live *L. casei* BL23	Female mice of 6–8 weeks age	Potent anti-inflammatory and antitumor effects upon oral administration	
Probiotic L. plantarum AS1	1,2-dimethylhydrazine-associated tumor of colon in male albino Wistar rats	Alleviation of rat tumorigenesis through antioxidant dependent pathways	
Oral administration of probiotic Lactobacillus acidophilus	Breast cancer model in mice of 8–10-week-old BalB/c female	Decline in the tumor mass via immune response modulation and spike in the proliferation of lymphocytes, guarding the TH cells and triggering the antitumor cells	
(L. lactis) NK34 with a dose 106 CFU	Human lung carcinoma cell line, human colon adenocarcinoma cell line, human stomach adenocarcinoma cell line, and human breast adenocarcinoma cell line, adenocarcinoma cell line	Vital antitumor and anti-inflammatory actions by forbidding the multiplication of cancer cells	

Table 24.3 Significance of live bacterial strains in preclinical and clinical experimental setup

Live bacterial strain acting as probiotic	Cell line adopted	References
E. faecium	Caco-2 cells	[72]
L. casei	In vivo	[285]
L. cocktail	HT-29	[285]
S. thermophilus *Recombinant L. lactis IL-17A*	TH17 in vivo Animal	[75]
E. faecium *L. casei*	CT26, HT-29	[73]
L. plantarum AS1	In vivo	[79]
Lactobacillus and Bifidobacteria strains	Human	[85]
Yogurt probiotics	Human	[86]
L. acidophilus	In vivo animal	[100]
L. lactis NK34	HT-29	[101]
S. thermophilus	HT-29	[102]
L. casei Shirota	Human	[103]
E. faecium	In vivo animal	[105]

Table 24.4 Mechanism of probiotic strains on tumor cell lines

Probiotics	Cell Line	Effect	References
Lactobacillus rhamnosus GG Bifidobacterium lactis Bb12	HT-29 induction	Activation of apoptosis	[69]
Pediococcus pentosaceus FP3 Lactobacillus salivarius FP25/FP35 Enterococcus faecium FP51	Caco-2	Inhibition of cell spread and activation of apoptosis	[72]
Lactobacillus casei ATCC 393	HT-29 and CT26	Activation of apoptosis	[73]
Lactobacillus acidophilus CL1285 Lactobacillus casei LBC80R (in the presence of 5-FU)	LS513	Apoptotic cascade increment by 40%	[76]
Lactobacillus rhamnosus GG	HGC-27	Inhibition of tumor mass and activation of apoptotic cascade	[77]
Enterococcus faecium RM11 Lactobacillus fermentum RM28	Caco-2	Cell division inhibited	[78]
Lactobacillus plantarum A7 Lactobacillus rhamnosus GG	HCT116, SW1116, Caco-2 Caco-2, HT-29	Decline in the intensity of cell growth	[81]
Bacillus polyfermenticus KU3	LoVo, HT-29, AGS	More than 90% decline in cell build up	[87]
Bacillus polyfermenticus	NMC460	Decrease in the colony mass	[89]
Lactobacillus acidophilus SNUL Lactobacillus casei YIT9029 Bifidobacterium longum HY8001	SNUC2A, SNU1, NIH/3T3 and Jurkat cell	Decline in the cell growth	[91]
Clostridium butyricum ATCC Bacillus subtilis ATCC 9398	HCT116, SW1116, Caco-2 Caco-2, HT-29	Decrease in the intensity of cell proliferation	[92]
Lactobacillus acidophilus 606	HT-29	Control of cell growth	[94]

(continued)

Table 24.4 (continued)

Probiotics	Cell Line	Effect	References
Lactobacillus rhamnosus GG Bifidobacterium lactis Bb12	Caco-2	Triggering of apoptosis	[95]
Bifidobacterium adolescentis SPM0212	Caco-2, HT-29, SW480	Inhibition of tumor mass	[99]
Enterococcus faecalis ↑CECT7121	LBC	Inhibition of cell spread and activation of apoptosis	[114]
Lactobacillus kefiri P-IF	MDR	Activation of apoptosis	[115]
Propionibacterium freudenreichii ITG P9	HGT-1	activation of apoptosis	[116]
Lactobacillus paracasei IMPC2.1 Lactobacillus rhamnosus GG	DLD-1, HGC-27	Inhibition of cell spread and activation of apoptosis	[119]
Lactobacillus reuteri ATCC PTA 6475	KBM-5	Apoptotic pathway activation	[120]
Lactobacillus pentosus B281 Lactobacillus plantarum B282	Caco-2 and HT-29	Decline in cell proliferation and arrest of cell cycle at G1 phase	[317]
Lactococcus lactis NK34	HT-29, LoVo, AGS	More than 80% decline in cell multiplication	[101]
Lactobacillus rhamnosus GG	Caco-2	Decrease in the level of IL-8	[318]
Propionibacterium acidipropionici CNRZ80	HT-29	Inhibition of tumor mass and activation of apoptotic cascade	[319]

Table 24.5 Action of bacterial strain on cancer-induced animal models

Probiotic Strain	Model	Effect	References
Lactobacillus rhamnosus GG Lactobacillus acidophilus	SD rats	Decline in cell growth and rate of proliferation	[68]
Lactobacillus plantarum	BALB/c mice	Necrotic cascade activation and decrease in tumor volume	[80]
VSL#3 (Probiotics mixture)	C57BL/6 mice	Decline in the rate of oncogenesis and decrease in the growth of abnormal cells	[81]
Lactobacillus rhamnosus	SD rats	Decline in tumor incidence, tumor volume and multiplicity of cells and increase in apoptotic mechanism	[82]
Lactobacillus plantarum and Lactobacillus rhamnosus GG	SD rats	Decline in cell proliferation	[83]
Bacillus polyfermenticus	CD-1 mice	Decrease in tumor growth	[89]
Lactobacillus rhamnosus	Rats	Decline in GST and rise in GSH levels	[92]
Lactobacillus acidophilus	F344 rats	Decrease in the aberrant crypt foci (ACF) formation	[90]
Lactobacillus plantarum	BALB/c mice	Decrease in rate of tumorigenesis and stimulation of apoptotic pathway	[87]
Bifidobacterium lactis	SPF C57BL rat	Decline in cell multiplicity	[94]
Lactobacillus acidophilus Bifidobacterium bifidum Bifidobacterium infantum	SD rats	Decrease in the tumor generation incidence	[96]
Lactobacillus salivarius Ren	F344 rats	Decline in tumor growth formation	[97]
VSL#3 (Probiotics mixture)	SD rats	No development of CRC in animals	[121]

(continued)

Table 24.5 (continued)

Probiotic Strain	Model	Effect	References
Lactobacillus acidophilus, Lactobacillus casei Lactobacillus lactis biovar diacetylactis DRC-1	Rat	Decrease in proliferation of cells	[122]
Bacillus polyfermenticus	F344 rats	Antioxidant mechanisms Decrease in the ACF formation	[132]
Pediococcus pentosaceus	Swiss albino mice	Decline in tumor progression and activation of apoptosis	[320]
Lactobacillus plantarum	Wistar albino rats	Decline in the action of bacterial enzymes	[188]

Table 24.6 Prebiotics in the management of cancer

Prebiotic	Study type/Animal model	Effect	References
Consumption of *B. lactis* and resistant starch	Azoxymethane induced in rats	Increase in apoptotic activity	[67]
Synbiotic treatment	Azoxymethane-associated suppression of NK-cell activity in Peyer's patches	Synergistic effect	[93]
Starch type-3 Novelose 330	Induced apoptosis in damaged cells of rats	Decline in the rate of carcinogenesis	[98]
Inulin and *B. longum*	Azoxymethane-induced aberrant crypt foci (ACF)	Significant reduction in ACF formation	[64]
Consumption of modified Arabinoxylan rice bran	NK cells	Activation of NK-cell activity	[115]
Inulin and oligofructose	DMH-induced colon cancer in rats	Oncogenesis rate is reduced	[123]
Inulin-type fructans, present in foods such as garlic, onion, artichoke and asparagus	Colorectal cancer	Increase in the level of SCFA levels, antitumor action is associated with increase in the concentration of butyrate	[123]
Consumption of fiber	Meta-analysis	Consumption of 27 g of fiber per day has shown 50% decrease in CRC compared to consumption of 11 g of fiber	[321]

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Chapter 25
Computational Tools for Drug Discovery of Anticancer Therapy

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Abstract The use of computers in cancer diagnosis is increasing continually powered by new modalities of imaging and advanced image processing methods. The use of bioinformatics, chemoinformatics, mathematical models and artificial intelligence is some of the critical fields but not limited to employing computers for drug design. Medical imagining remains to be notable factor in the management of cancer in patient which is currently at the rise with the clinicians due to an increase in cancer incidences. This increase availability of data with the medical practitioners has the potential for using computers or algorithms for cancer diagnosis for elevation of patient outcomes, reduction in toxicity and lowering the clinical burdens. Cancer data used for diagnosis and treatment will always be on the rise due to the advanced treatment options such as adaptive radiotherapy (RT) planning and image mining of quantitative nature. Machine learning particularly is used to build statistical models based on the past data sets collected having the self-learning knowledge from collected data sets for making future predictions. It has been shown that a trained deep neural network is able to detect malignant cancer lesions form skin lesions photographs giving a rivalry to well-trained dermatologists. Mammographic lesions can also be detected by trained deep neural networks producing rivals to certified radiologist which shows the immense accuracy and profitable use of computer-aided methods in cancer diagnosis. Designing new anti-cancer therapeutic or helping in the process of developing such therapies to decrease the failure rate and approval time is the most exciting potential of CAD in cancer such as autoencoders. Precise clinical and preclinical, which lead increased likelihood for clinical success, may be achieved by using computational methods to predict correct mechanism of action. Effective combinations of drugs, which remain to be a combinatorial, can also be solved using AI-based methods as the number of anti-cancer drugs is increasing day by day. An increase in the growth of complex experimental data sets has lead researchers today to welcome sophisticated algorithmic and computational tools to analyse the results and study basic biological questions including oncology. Recently, some of the prominent applications of CAD in cancer diagnosis include tumour detection through medical image analysis, histopathological characterization and quantifications, clinical diagnosis through computers, selection of treatments, planning treatment and using multimodal clinical data, anti-cancer drug development and surveillance of cancer in populations. These steps collectively help to achieve precision oncology which may increase the efficiency of early screening and improve treatment levels. In this chapter, computer methods employed in most of the critical fields of oncology such as radiation oncology, diagnosis and detection of cancer and optimization of cancer treatment are discussed.

25.1 Introduction

The conventional use of empirical techniques used in drug designing lately and high throughput screening (HTS) has played immensely in the identification of potential hits. Computer-aided drug designing (CAD) has further enhanced the efforts currently made in the early drug discovery process fuelled by conscientious docking and simulations of free energy perturbations [1]. Structure-based drug design (SBDD) evolved to be more practical and reliable with the ever-increasing NMR, X-ray and Cyro-EM structures, and intuitive visualization by CAD provides a complete picture of the complex interactions between ligand and binding site residues. Various machine learning techniques, molecular graphics, scoring functions and docking have improved the CAD which have accelerated the drug designing procedure in different stages [2, 3].

CAD is considered as an advancement to HTS as it involves minimal prior knowledge for compound designing to yield potential hit compounds from which promising candidates can be selected for further analysis. Screening large compound libraries into smaller clusters to predict active compounds are the first role for CAD which later may be carried for lead optimization through improving the biological properties such as ADMET and affinity, thereby building chemotypes starting from a nucleating site by addition of fragments with optimized function. When compared to traditional HTS, CAD is more directed towards the generation of "hits" with efficient management of time and cost when applied in various stages of drug designing such as identification of target, validation, designing molecules and drug target identification [4].

Structure-based CAD is more suitable for soluble proteins which can be crystallized, while ligand-based CAD is for compounds exhibiting high binding affinity towards the target with no off-target effects and can be designed with good drug metabolism, minimal free energy and ADMET/pharmacokinetics [4]. Occasions where little information on the structure is available specially in the case of membrane proteins, CAD remains to be the best option. Major contributions have been made by CAD for the molecules in drug development which are in clinical use or clinical trials. In this chapter, we describe the various methods applied in CAD procedure and the challenges which are associated with traditional and novel CAD, and its impact on the cancer diagnosis field is shown in Fig. 25.1.

25.2 Binding Site Prediction for the Targets

Identification and understanding of binding site are highly essential which are available from X-ray crystallized structures of protein and ligand for SBDD. Binding site characteristics and the interaction of protein–ligand can be observed using user-friendly softwares such as PASS and QSite Finder with tools such as MOE SiteFinder [2, 5, 6]. Drug discovery is also enhanced by building binding pocket based on known

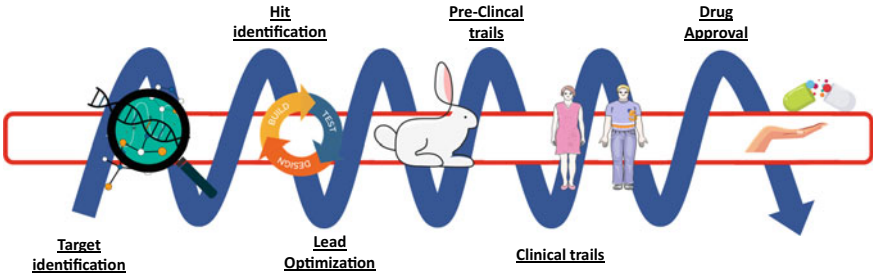

Fig. 25.1 Computer-aided systems for speeding up anticancer drug discovery

compounds by considering their ligands, solvation energies and binding orientations. These types of binding site have the potential to build up the consistency in binding poses with the help of softwares such as Quasar [7] and SKELGEN [8]. Improved hits can be suggested by SAR and pharmacophore-based models via lead optimization based on the previously known hits with FEP + and ADMET properties [1, 8–10].

25.3 Keystones for CAD Diagnosis

In order to perform CAD and to pick the appropriate method, medical image processing requires preliminary information on the content and the nature of image. A high level of efficiency and accuracy can be achieved for automated diagnosis, and it is important to adopt methodical image processing approaches within the critical steps of CAD. The following steps are important for CAD diagnosis [11].

25.3.1 Pre-processing of Image

This step is essential for procedures such as ultrasound for enhancing the image and to reduce the noise with little distortion in image distortion in some CAD system is devoid of this step. Image pre-processing plays a critical role in obtaining the desired outcome for the other stages of CAD. Removal of noise, defects during image capture, image resizing and increasing image intensity is performed using in this pre-processing step [12].

25.3.2 Segmentation of Image

Efficient development of CAD systems requires to segment the image, and the main reason behind this is the separation of region of interest (ROI) with the essential

properties [13]. This image segmentation is the important component for pattern recognition and computer vision, and the success of computer procedures depends highly on accurate image segmentation. Selection of segmentation method depends on specific modality of application as increase in resolution and dimension of images makes it difficult for manual examination with a huge amount of image information. Specific tumour regions and other organs can be highlighted using segmentation methods for further medical examination. Computed tomography (CT), 3D ultrasound and magnetic resonance imaging (MRI) produce 3D images as these images are more favourable for segmenting volumetric imagery.

Fully automatic and semi-automatic are the two type of methods which are the segmentation methods. It is favourable to have an automatic algorithm in medicine to detect abnormalities and low rate of false positive and false negative is important. Hence, for practical aspect of CAD, evaluation methods in segmentation algorithms are an important dimension. Based on the properties of image, segmentation approaches are of two types: similarity-based approach and discontinuity-based approach, wherein the former divides the image according to some predefined criteria, while the later divides the image based on intensity [14]. Similarity-based methods are again divided into cluster-based, region-based and threshold-based [15], and discontinuity-based approach is again narrowed down to edged-based method. A comparison of the different segmentation techniques is given in Table 25.1.

Table 25.1 Comparison of different segmentation methods

Methods	Specifications	Merit	Demerits
Cluster-based methods	Objects are clustered based on similarity	Easy implementation	Difficult to demarcate clusters
Region-based methods	Pixels are grouped based on seed points into homogenous regions	Simple, good at handling noise and multiple options can be chosen at one point	Dependent on seed points, expensive computational cost
Threshold-based methods	Peaks and valleys of the histogram give the threshold	Can accommodate low computation complexity and simple threshold calculation	Close spectrum images do not work
Edge-based methods	Sharp discontinuity in the image defines the method	Easy human readable forms, good with high contrast image	Low contrast and smooth change in images do not works and noise sensitive

25.3.3 Similarity-Based Approach

Cluster-based methods

These are popular segmentation techniques used for medical image segmentation and are generally divided into partitional and hierarchal clustering where the former are iterative procedures further classified into fuzzy and hard clustering, while the later are recursive in nature, capable of finding nested clusters both in bottom-up and top-down method [16]. A set of members are allotted in different levels for each element and each element can belong to more than one cluster in fuzzy clustering, while in hard clustering, each object occurs only once in a cluster. Fuzzy C-means and K-means are the two main well-known procedures for both fuzzy and hard clustering beneficial for segmentation of clinical images as it was applied for MRI breast scans [17, 18]. Reconstruction algorithm development has been current interest due to reconstruction of 3D images from its parent image which has its wide application in anatomy, diagnosis and surgery [19]. Segmentation and reconstruction of 3D images from MRI scans and mammogram have proved themselves to be the best fit for detection of breast cancer [20, 21].

Region-based methods

It is a method in which the image is divided into homogenous regions with connected pixels depending on the previously set texture, intensity or colour and is divided into growing region and split and merging regions, where selection of seed is made in the growing region [22]. The initialization of seed point is important and effective on the result of segmentation as split and merging regions are a top-down approach. Initial step for this starts with a whole image and then is partitioned to arrive for more homogenous regions and other regions with similar criteria are then merged into one region. Region-based segmentation algorithms are becoming popular for breast cancer detection CAD systems as evident from this study where region of interest (ROIs) were extracted using region-based segmentation [23]. Another study showed that a region-based segmentation scheme was made using the combination of maximum likelihood analysis, hybrid assessment analysis and maximum gradient analysis [24], and another study used seed selection with the help of an adaptive region procedure using a split and merge algorithm [25].

Threshold-based methods

It is a segmentation method effective in distinguishing the foreground image from the background image by selecting appropriate brink based on image properties and then assigning pixel images to specific areas. The background information on the intensity, object sizes and object types present in an image is required for automatic threshold value selection [26]. Wide use of this method is found in the development of CAD systems for extracting important areas for detailed analysis. Al-Bayati and El-Zarrat [27] showed the combination of different thresholding comparing them

to segmented mammogram images, and Saha et al. [28] achieved a 97% accuracy in nucleus segmentation by developing the method based on images of breast histopathology employing histogram-based threshold [28].

25.3.4 Discontinuity-Based Approach

Edge-based method

These structural techniques are used to detect pixels or edges among different regions of images which have sudden change in intensity and also work efficiently with non-noise and high contrast images [29]. Laplacian from Gaussian, Canny, Prewitt, and Sobel are some of the edged-based methods [30]. The prime application of this method is used for recognition of organs, CT scan of lungs containing salt-and-peeper noise [31].

25.3.5 Extraction and Selection of Features

Based on the characteristic, lesions obtained from images features are extracted from the image, and these features are generally large and selecting the best feature is highly critical for the next step. Feature extraction marks the computation of feature from image to minimize the data volume, and features represent the area of interest or the whole image. Factors such as robustness, memory size, classification accuracy and computational cost are influenced by the selected features and this selection also depends on the application. Descriptors are divided into three dimensions using three axes, namely density, spectra and pattern and shape [32]. Three categories exist in image descriptors, namely textural, shape-based and colour-based descriptors, where the shape descriptor category is critical for human interpretation of colours and texture and also to discriminate objects. Quantifying image properties such as regularity, smoothness and coarseness are the texture features used to analyse and interpret images taking into consideration the differences in intensity [33]. Exact method of textured feature extraction is as follows: grey-level co-occurrence matrix, grey-level run-length, texture based on fractal dimension and windowed Fourier filters which are widely used in medical image analysis [34]. Critical visual clue is provided by colour-based descriptors for object recognition and image retrieval [35]. Dermoscopy, cervicography, gastrointestinal endoscopy and fundus photography are the new release in medical imaging involving colour descriptors and have the potential to be explored for cancer diagnosis [36].

Shape-based features

This feature extraction method is used for applications like shape recognition, retrieval, alignment, classification and registration and is considered as the most

important for processing of medical imaging particularly breast cancer detection. The roughness of contours is measured in breast cancer mammograms to classify them as benign or malignant, and the ROI area, moments, compactness and Fourier descriptors are extracted for accurate results [37]. A challenging task faced by physician is to distinguish the differences in the shape of benign lump from malignant within a mass of mammographic images [38]. Three new shape features circularity range ratio, irregulatory ratio and convexity ratio are also used to classify mammograms with 87.5%, 94.5% and 88%, respectively [39].

Textural descriptors

Many areas of computer vision and pattern recognition have convenient acceptance for texture features which is an important clue for processing of medical images and are widely used in the CAD for designing extractors. The recent work shows the use of texture-based descriptors in breast cancer diagnosis using fuzzy support vector machine for classifying mass using ultrasound images [40]. Employing grey-level co-occurrence matrix (GLCM) and grey-level run-length matrix (GLRLM), an automatic breast cancer diagnosis method on cytological images obtained from fine needle material biopsy which resulted in an 90% accuracy in detecting malignancy [41]. A set of texture features such as energy, entropy, GLCM features, inertia, Horlick's correlation, moment difference, cluster prominence and shade are used to carry out analysis of 3D morphology for benign and malignant tumours from breast MRI scans [42]. Another method is the particle swarm optimized wavelet neural network (PSOWNN) developed based on texture features extracted from mammogram images [43]. Differential Evolution Optimized Wavelet Neural Network (DEOWNN) is another CAD for automatic detection of breast cancer from mammograms with an accuracy of 92.4% [44].

25.3.6 Classification

The abnormalities in images are differentiated and labelled through classification which is the last stage of CAD and is important for medical imaging. It is divided into two groups: supervised and unsupervised where supervised classification goes through a large number of unknown data and assigns them onto classes based on their characteristic, while unsupervised classification does not require previously determined class. However, supervised classification techniques are used for clinical image processing. Multilayer Perceptron Neural Networks (MLPNNs), Support Vector Machines (SVMs), Decision Trees (DTs), Linear Discriminant Analysis (LDA) are some of the classification methods used in supervised classification.

Several approaches are presented at the functional and data level to handle imbalanced data sets [45] such as kernel transformation techniques and biased penalties are used for SVMs [46]. The selection of the best-fit classifier is important for distinguishing benign from malignant tumours, and a comparison study showed that SVM shows as compared to other NN and k-mean clustering [47]. Another comparison

study for the classification rate of k-NN, SVM and CART (classification and regression tree) showed CART and SVM scored higher rate than k-NN for distinguishing normal and abnormal lesions, and further, a computer-aided diagnosis method was developed [48].

25.3.7 Evaluation of Performance

Performance evaluation of the classifier is the last stage of CAD based by producing a 2×2 confusion matrix (Normal/Abnormal) for the binary outcome of the classifier. Various metrics such as accuracy, specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) are used to measure the performance. However, main performance evaluations are best estimated by metrics such as area under receiver operating characteristics (AU-ROC), sensitivity, specificity, receiver operating characteristic, accuracy, true positive fraction and false positive fraction [49–51]. The visualization of a classifier performance in all possible threshold value is carried out by the most advantageous ROC curve which has two interpretation type: numerical and graphical but the most popular being AU-ROC [52, 53].

25.3.8 Rule of ABCD

This rule states for A (asymmetry), B (border), C (colour) and D (diameter) for the lesion image. To calculate the A, input image is segmented to a perpendicular axis so that the lowest asymmetry score possible is given such as 2 for asymmetry with both the axes, 1 if asymmetric with one axis and 0 if no asymmetry is found at all. B is calculated by dividing the image into eight and it is checked for abrupt and sharp changes after which it is scored, where 1 represents sharp cut or otherwise 0. Black and brown colours are used for cancer detection but red, white or pink are also used and then the colours are counted. D is the diameter of the lesion which is carefully checked, and if the diameter is larger than 6mm, it is classified as melanoma [54].

25.4 Recent and Indicative Studies in Cancer Diagnosis

During the last five years, around 192 studies were done using machine learning (ML) technique-based pipelines made on conventional methods for performing diagnosis for cancer types such as lung, breast, colon and pancreatic cancer. Most studies used data from imaging such as CT, X-ray, MRI and positron emission tomography (PET) to develop automatic diagnosis architecture. Convolutional neural network (CNN) models were used to detect early breast cancer through the analysis of histopathological images which is more accurate as compared to other ML methods

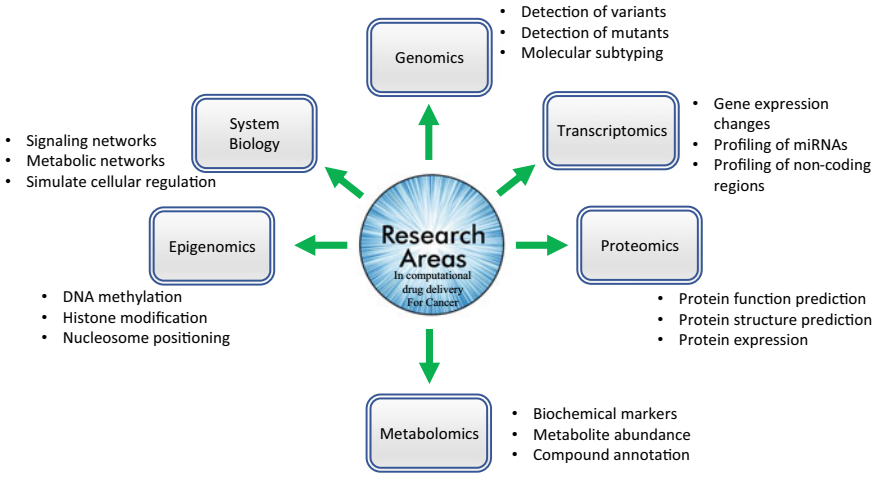

Fig. 25.2 Research areas in the field of CAD

[55, 56]. Similar procedures were also proposed by many studies for using deep CNN in diagnosis by going through imagining slides [57] as shown in Fig. 25.2. These studies showed an average accuracy of 90% showing the author's positive contribution towards the clinicians in their diagnosis procedure. Based on CNN architecture, deep learning (DL) framework was developed for analysis of CT and dermoscopy images of liver and skin cancer [58]. Gaussian mixture model (GMM) algorithm was used to separate the lesions, and deep neural networks were then employed for automatic diagnosis work [59]. The most important factors for skin cancer are identified using optimization and feature selection, and deep CNN was used for detection of melanoma with 90% classification accuracy [59].

ML algorithms of conventional type such as Random Forest (RFs), Decision Trees (DT), k-Nearest Neighbour (k-NN), Artificial Neural Networks (ANNs), Support Vector Machines (SVMs) and Gradient Boosting Machines (GBMs) were used in medical oncology for automatic diagnosis of cancer [60]. Potential cancer biomarker was identified for the onset of pancreatic cancer using supervised and unsupervised methods to transcriptomic data [61]. The recent studies on artificial intelligence (AI)-based cancer diagnosis for cancer types such as brain, breast, lung and skin exhibit fast and great accuracy as compared to human specialist. 27.5 million new cases could be diagnosed globally per year by 2040 as estimated by Cancer Research, UK, which means that radiological data will grow at an exponential rate as compared to the number of radiologists. This has increased the work load of radiologist, and a study shows that at an average, one radiologist analyses one image in 3–4 minutes in a workday of 8-hour in order to meet the workload which makes human error inevitable [62, 63].

Breast cancer screening programmes exist worldwide, but the interpretation of mammograms is influenced by high rates of false positives and false negatives. A

novel breakthrough AI algorithm was recently reported capable of outperforming radiologist for diagnosis of breast cancer from huge number of mammograms data set from the UK and USA. This study had many shortcomings which are common to many computers-assisted diagnoses [64].

25.5 Future Advances and Challenges

Among all the architectures developed, CNN performance remains to be the best as shown by winning the ImageNet Large-Scale Visual Recognition Competition (ILSVRC) in 1998 which was LeNet with a seven-level architecture. AlexNet in 2012 won which is also a successful version of CNN. This architecture is the winner from 2012 to 2015 competition with ResNet, AlexNet, GoogleNet/ Inception V1, ZFNet and VGGNet displaying the success of this architecture. The difference being in this architecture with only the difference in its performance evaluation and the best performance being shown by ResNet by reducing the errors to 3.7% beating the human-level performance.

One of the major challenges for implementing CAD is the lack of available data sets but efforts are being made by picture archiving and communication society (PACS) to make archives of medical images maintaining the confidential images of patients. Cancer image data can be obtained from hospitals and cancer research organization for using these data for algorithm execution [65]. Another way of eliminating this lack is the use of data augmentation that includes techniques such as cropping, rotation and filtering in order to increase the existing data in number. The problem of overfitting can also be surpassed by using transfer learning; signal-to-noise ratio (SNR) and low contrast images are responsible for lowering the performance of a model, and decreased performance is also observed when a model is trained on multi-institutional data. Unequal distribution of training set data is another challenge as when positive data sets are more as compared to negative, the system automatically tends to be biased, thereby giving only positive results. This equality aspect is very important for training data sets which was previously ignored by researchers. The size of the target object, in an image is another problem in CAD as with the size of the object training the model to learn about the size change become difficult but methods such as standard pooling operation aided with multi-crop pooling [66].

25.6 Conclusions

This chapter explains the critical aspects of CAD in cancer and the different methods employed for cancer diagnosis. In addition to the standard CAD regarding to the trusting, the process is the implementation of Findable, Accessible, Interoperable and Reusable (FAIR) principles. The complex nature of cancer and its multi-step of cancer progression makes data collection difficult from a single data centre. Also,

the combination of omics and CAD for precision oncology will have the potential for promoting global analytical method. The recent years have shown that CAD systems are able to automatically detect breast cancer and lesion classification as benign or malignant in methods such as ultrasounds, MRI and mammography. CAD has helped to increase the radiologist's performance in discriminating normal and abnormal tissues and have a lot of future potential for cancer diagnosis.

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Surovi Saikia is seeking International experience and enhancement of my personal goals of achieving excellence in the field of Bioinformatics and Artificial Intelligence. She is working as a postdoctoral fellow in your university will allow me to learn recent most techniques in my area of research and explore the applications in the field of artificial intelligence and bioinformatics with special focus on healthcare and other applications. They are currently engaged as a Dr. D. S. Kothari Postdoctoral Fellow at the Department of Biotechnology, Bharathiar University, Coimbatore, India. They have completed my Ph.D. (Bioinformatics) from Dibrugarh University, Assam in 2020 and UG and PG from Tinsukia College and Dibrugarh University. They have 8 years of research experience with 16 publications in peer reviewed national and international journals. Currently, I am working on algorithmic based computational work.

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Chapter 26
Stem Cell Therapy in Cancer

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Contents

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Abstract Metastatic cancer cells cannot be removed from body using traditional methods such as surgery, radiotherapy and chemotherapy because the reoccurrence of disease is the common thing following treatment. But the therapies involving use of stem cells are proving to be a promising approach for treatment of cancer. They are the novel because they can provide homing to as well as targeting tumorous foci. Stem cells as a platform to deliver drugs to targets not only decrease volume of tumor but also enhance rate of survival in pre-clinical trials. They can also be employed as viral and other carriers in order to enhance the efficacy of drugs and providing enhanced permeability and retention after effects the same time with the least side effects. These stem cells can be applied in regenerative medicine, cancer cell therapy and immune cell-based therapy, and lastly for screening of anti-cancer drugs. Technically, treatment of cancer using stem cells is proved to be feasible but still there are certain challenges which need to be addressed such as durability of treatment and tumorigenesis. The recent chapter is focused on the stem cell review including its types, sources, applications, challenges and affectivity in terms of clinical trials which also aids in refining future.

26.1 Introduction

Cancer is the major cause of increased rate of mortality and morbidity in developing as well as under developing countries which in turn puts pressure on the medical fields due to increment in population and aging in a geometric manner. Usually, cancer is treated by conventionally available methods such as radiotherapy, chemotherapy and surgical operation [1]. The type of treatment employed relies on the specific class of cancer, its progression, pathogenicity and the objective of treatment. As for example, surgery involves direct removal of tumor tissues from any part of body specifically. Radiotherapy involves the damaging of DNA of cancer cell thus being involved in tumor cells removal. On the other hand, chemotherapy involves use of toxic chemical substances that results in slowing down profession of cancer based on their mode of action [2]. Similarly, immunotherapy has become an important

point of discussion in research and investigation because they employ monoclonal or polyclonal antibodies, certain inhibitors and adoptive cellular transfer that has provided improved clinical outcomes in patients. However, these treatments have more side effects as compared to that of advantages which include off targeting drug delivery, resistance to a particular drug and other therapeutic side effects [3]. These disadvantages cannot be eliminated using already available therapies but because the chances of reoccurrence of cancer are far more than that of other diseases discovered so far. Hence, the researchers have shifted their attentions to the treatment regimen which is less toxic and compatible with biological environment [4].

Regarding this, stem cell transplant is one of the strategies that involves restoring of stem cells in patient body whose cells have been destroyed by radio or chemotherapy being employed for certain types of cancer. Blood cells are of prime importance in this regard as they have the ability to differentiate into various other cell types eventually [5]. This stem cell therapy has provided a hope to fight against battle of cancer. And also, it can reduce off target effects of other therapies by providing targeting drug delivery events. There has been a number of stem cell-based strategies which provide promising as well as challenging situation in pre-clinical trials [6]. Hence, further investigation is required for their effective use in clinical trials. As these stem cells have unique characteristics such as they have the ability to regenerate and differentiate into any other cell type effectively. Also they are effective in the migration toward the desired area having tumor cell accumulation thus reaching there and secretion of certain chemical bioactive compounds and resulting in immunosuppression of that targeted cancer tissue [7]. Thus, the aim of this chapter is to provide an overview of stem cells, their sources, properties, classification as well as the available treatment opportunities regarding stem cells and certain challenges associated to their use in clinical systems.

26.2 Sources of Stem Cells

The stem cells have ability of self-renewal to an indefinite level, can be derived from single cell and differentiated into multiple cell types. This self-renewal property has aided in regeneration of stem cells and maintenance of homeostasis [8]. Certain cells are sources of stem cells which include.

26.2.1 Embryonic Stem Cells and Induced Pluripotent Stem Cells

Embryonic stem cells are pluripotent in nature and can be divided into any cell type except those present in placenta and hence can be used as a gold standard for the development and evolution of pluripotent stem cells in the laboratory conditions

[9]. But here are certain ethical considerations which are present for development, production as well as use of these stem cells in human clinical trials because of which their employment as therapeutics has been restricted. Hence, for this purpose, the embryonic stem cells have been replaced by use of induced pluripotent stem cells that can be reprogrammed according to will into any type of cell body such as that of skin fibrosis on the basis of certain pluripotency factors; thus, these are the cells which do not require destruction by embryonic cells [10]. iPSC are the different from embryonic stem cells in the generation of immunogenic environment and other ethical limitations; hence, their use is more applicable than that of other cell types.

26.2.2 Mesenchymal Stem Cells

These are multiple stem cells and can be divided especially into skeletal tissues as for example cartilage and bone which is actually a layer of fat present around bone. With the passage of age, these cells are usually converted into the fat accumulating tissues only. Other types of tissues it can be differentiate into are connective tissues such as tendons, muscles and stroma. These cells not only can be easily isolated but also can be used in the variety of treatment regimen [11].

26.2.3 Hematopoietic Stem Cells

These are multipotent stem cells which are majorly divided into either lymphoid lineage or myeloid progenitors and occur to be present in a number of organs such as peripheral and umbilical cord blood and lymphatic linage [12]. These are basically most primitive cells found in bone marrow and result in production of mature blood cell types when they proliferate. For the last four decades, the transplant procedure using these cells has been performed effectively adding value to this type of treatment [13].

26.2.4 Neural Stem Cells

These are the cells which have potential to renew and further differentiate into different progenitor cells that is in generation of neuron cells such as glia and astrocytes, oligodendrimers, etc. that have been employed in treatment of brain, breast and prostate cancers. They are classified according to presence of certain markers such as nestin and Sox2 in the presence of rich culture media containing growth factors such as epidermal growth factors [14].

26.2.5 Endothelial Progenitor Stem Cells

These are humanized cells which can be cultured and contain surface markers or receptors which have close similarity to vascular endothelial cells. They are prime derivers of vascular regeneration and have been proved to be used in cancer therapy by a number of previous researches and scientists [15]. This is followed by attachment or coupling to anti-tumor or angiogenesis drug molecules or inhibitors. Thus, their role has been shifted to EPC providing benefits pathogenesis and intervention of disease. Reports on the use of EPC cells in the clinical trials are still rare because of limited research [16].

26.2.6 Cancer Stem Cells

These are the limited type of cells being derived from the solid or liquid tumor and have contributed to expansion, distribution and metastasis after therapy. On the basis of surface markers, these are stem-like cancer cells being derived from tissues or cells of cancer patients effectively [17]. These cells not only have appropriate genes but also can be differentiate into other non-stem cell and can be renew at same time. These are highly resistant to conventional treatments available for cancer. They can also initiate other types of cancers. The traditional treatments can only kill non-stem cells but cannot kill cancer cells. Hence, the tumors can reoccur or relapse upon the passing of certain time period. So these cells are mainly important for solving problems related to resistance and reoccurring of cancer [18]. Different types of stem cell are shown in Fig. 26.1.

26.3 Properties of Stem Cells

In addition to property of regeneration, these stem cells possess certain other properties such as they can act as an immunosuppressive agent and also anti-tumor for a number of tumorous cells [19]. These stem cells have growth factors and other cytokines required for the development and maintenance of innate and acquired immunity of host; hence, they can be utilized as the targeted gene delivery agent as well as initiating specific immune response. These stem cells are also helpful in secretion of other factors such as CCL2/MCP-1 which can not only interact with tumor cells directly but also act as a mean to provide functions of anti-tumor agent [20]. In addition to it, variety of human cells have tumor tropic characters that have been originated from interaction of chemokine and tumor cells. The very first report where the stem cells have been used as a xenograft in humans was performed on mouse that have abilities to home tumor tissues. For this purpose, stem cell differentiation mechanisms have been studied in detail [21]. Hypoxia is the phenomenon that

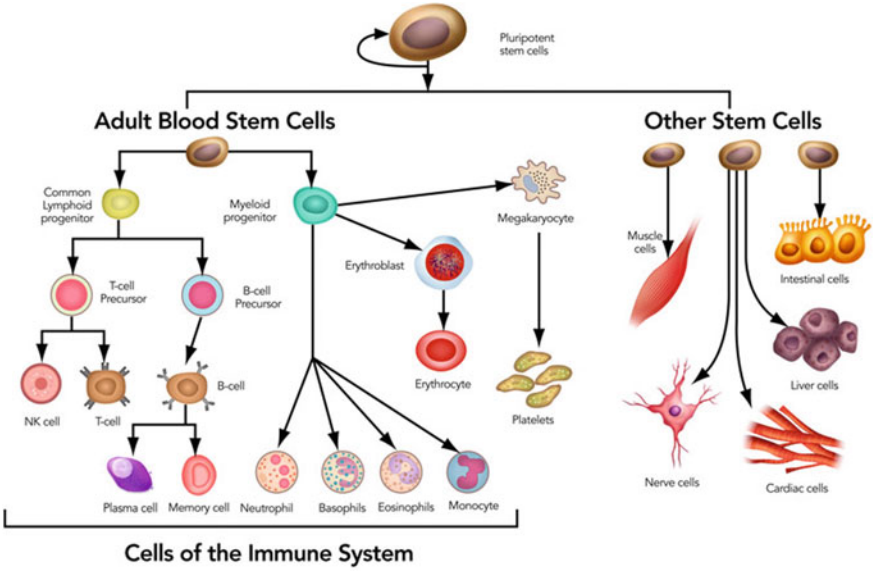

Fig. 26.1 Different types of stem cells which can either be adult stem cells and others classes as represented

is involved in activation of chemoattractant which in turn causes movement or transport of neural tumor stem cells to tumor nucleus. On the other hand, the movement of hematopoietic stem cells, i.e., directional movement includes stronger interaction between the receptors and CXCL12 [22]. There have been discovered a number of tumor-based receptors that involve use of multiple stem cells further causing homing of tumor cells. Stromal derived cell factor plays a key role in almost all types of the stem cells toward targeted tissue which in this case is cancer or tumor cells. In order to improve efficacy, sensitivity and homing of cells these cells are further engineered with certain chemokine molecules [23]. Or in other case, the target tissues are activated to release chemokine on attachment. As the research demonstrated by PARK and his colleagues has demonstrated fact that the multipotent cells which over express CXCR4 have shown better rate of migration than that of other cells considered as control. Moreover, release off chemokine in a controlled manner from the bioactive compounds also raises chances of recruitment toward stem cells. Hence, these all techniques not only improved the homing of stem cells but also increased the efficiency of treatment [24].

26.4 Types of Stem Cells Involved in Treatment of Cancer

On the properties such as migration, pattern of proliferation and differentiation, the cells used in cancer therapy are classified as.

26.4.1 Adult Stem Cells

Adult stem cells have ability to differentiate or divide into other cell types, tissues and organs. And of all them, hematopoietic stem cells, mesenchymal stem cells and neural stem cells form majority of them being especially employed in cancer treatment as discussed above. They usually resides in bone marrow and can be regenerate into any other blood cell type [25]. The blood cells obtained from cord blood are the only source of blood cells discovered so far that is also approved by Federal drug agency FDA for treating out leukemia, myeloma and other types of blood disorders. These multipotent stem cells are located in different organs and cell types thus playing their function in regeneration and repair [26]. They also can replicate and regenerate different tissues in laboratory environment such as osteocytes, adipocytes and chondrocytes. These cells have very unique and specific biological characteristics which proves them an appropriate agent for use in therapies used in cancer subtypes. Neural stem cells which are present the central nervous system can be self-renewable and can be generate into glia and other neuron cells. They are helpful in treatment of primary metastasis, breast, lungs and brain cancer classes [27].

26.4.2 Pluripotent Stem Cells

These include embryonic stem cells being derived from inner mass of undifferentiated embryo and can give rise to almost any type of cell except that of cells presents in placenta. But the challenge here is that their use in clinical settings has been prohibited because of presence of certain challenges [28]. 2006 was an important year in evaluation of biology when induced pluripotent stem cells were invented based on the Yamanaka factors. These induced pluripotent stem cells were having same specialties than that of embryonic cells but it only removes barrier of ethical considerations. Hence, these both cells are involved in the induction process of effector cells and also in development of vaccines against battle of cancer worldwide [29].

26.5 Mechanism of Action of Stem Cells in Cancer Therapy

Followings are the phenomenon or mechanisms in the targeting of tumor containing stem cells by use of stem cells:

26.5.1 Signaling Process in Cancer Stem Cells

It has been clearly mentioned in the literature that molecular pathways such as Hedgehog, PI3K/PTEN, JAK/STAT and NOTCH are primarily performing their functioning in proliferation of stem cells. Any mutation or persistent type variation in signaling pathways can lead to development of subsequent development of cancer stem cells and other types [30]. They contain a huge capacity of regeneration and development into other cells based on type of tumor and level of tumor growth, reoccurrence and lastly metastasis. Moreover, they have also widened the options for treatment of tumors in specific cells and prediction of effective therapeutic capacity [31]. They are found to exist in almost all of tumor containing cells as for example that of brain, lungs, prostate and gastro-intestinal being identified using certain techniques and imaging procedures as that of presence of surface biomarkers and other functionalities [32]. These cells are usually originated from totally normal cells as described for all other cells. Surface biomarkers as CD13, CD44 and Ep CAM are specifically utilized with the objective to characterize these cancer cells from that of other cells of different types because they have ability to be expressed efficiently in normal tissues as well [33].

As previous reports have depicted that CD133 has been isolated well from brain, lungs, colorectal and gastric cancer and they exhibited the power of self-renewal and also the starting of tumor formation in body of organism [33]. The properties, which are identical to former in this scenario, include their evolution from normal cells along with the further biological characters. Other tumor markers such as CD44, CD133 and CD24 have been studied well for overall determination of cancer cell population of all tumors. In NOD or SCID, mouse model of breast cancer lowers amount of CD12, and CD44 is present in very small amount but it still has the properties such as tumor growth, development, differentiation and spread. Similarly, these tumor cells are further identified by their functional and metabolic activities [34]. For this purpose, aldehyde dehydrogenases that trigger the oxidation of aldehyde molecules in various substrates and toxins, the value of which is often measured and taken as record. The flow cytometry assay has shown higher level of this ALDH enzyme in consistent with the cells having higher metabolic activities at the same time. These positive cells are found in high tumor environment of breast cancer in comparison with lower population of these markers [34].

Addition to all of these cancerous stem cells are also identified by presence of higher glycolic activity, lower division or growth rate and enhanced therapeutic or immune resistance at same time. Other receptor acting as antigens such as cancer/testis antigens is also expressed in germ cells or embryonic cells serving as a hotspot for the for serving as a targeted molecule in immunotherapy [35].

26.5.2 Secretion of Paracrine Factors Leading to Differentiation

The pieces of evidences collected from a huge collection of research have clearly demonstrated that the stem cells perform their function by release of paracrine factors, i.e., extracellular materials and soluble materials thus effecting differentiation and proliferation of tumor cells in various organs. Among all of them, nanosoized cells exhibit regulators for developing cell to cell interactions [35]. They are mainly produced by the exocytosis process of intracellular multivesicular body that in turn contains a number of biological molecules such as DNA, RNA and proteins [36]. On the release from MSCS, they can target any of cancer cell niche upon internalization by cancer cells by endocytosis or receptor ligand interaction releasing whole cargo in the receiver cells. Moreover, stem cells can also respond to stimuli produced by a huge collection of receptor cells and soluble factors, as they have capability to release immunosuppressive, anti-angiogenesis, anti-inflammatory and anti-apoptotic factors [37].

Similarly, the differentiation capacity of all stem cells plays an important role in management of tumors. As transplanted cells can give rise to any type of blood cells, so the clinical outcomes will be comprised of the quality of blood cells and types such as HSCs. Similarly, the neural stem cells play their function by replacing neural and glia cells in brain tissues. And induced pluripotent stem cells serve as a way for induction of effector tumor cells in the terms of specifically targeting a tumor tissue [38] (Fig. 26.2).

26.5.3 Bone Marrow Homing Mechanism

When chemotherapy is performed, it results in the damaging of the potent tissues such as blood cells and leucocytes in order to remove all sort of cancer cells. Following this condition, a patient is usually given the intravenous injection of autogenously HSCs [39]. This will ultimately lead to homing process leading to differentiation of stem cells in bone marrow. After entering into bone marrow, the transplanted HSCs undergo engulfment process that can lead to specialized types of blood cells. The bimolecular mechanism behind the homing of HSC involves a direct interaction between receptors and gradients released from bone marrow cells niche [40]. On the other hand, the remaining molecular signaling processes involve ceramide phosphate, sphingosine phosphate, extracellular ATP or UTP, calcium and hydrogen ions. Transmigration through blood vessels require direct interaction with endothelial cells and also secretion of matrix degradable enzymes at same time [41].

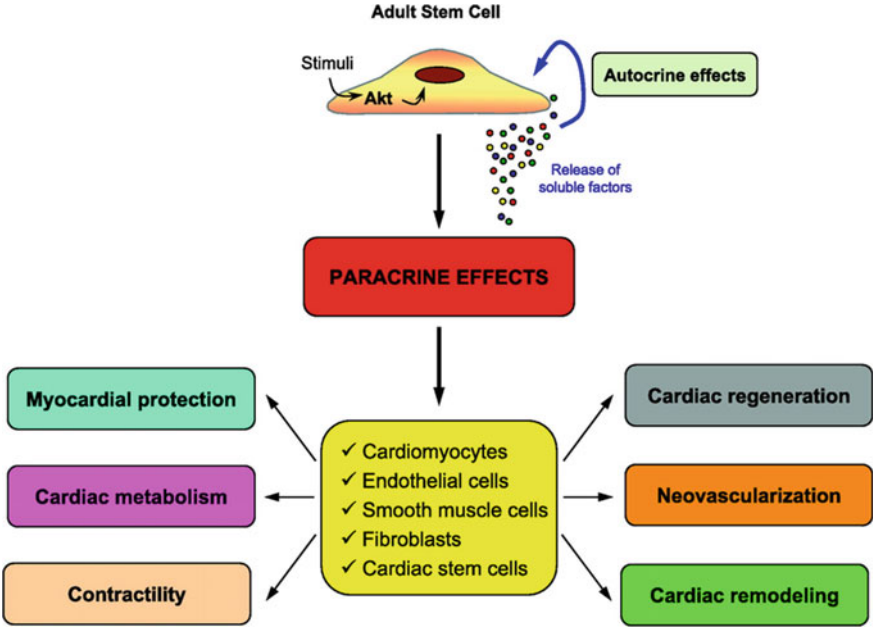

Fig. 26.2 Elucidation of the mechanism where secretion of paracrine factors leads to phenomenon of differentiation

26.5.4 Tropic Effects Induced by Tumor Cells

Microenvironment of tumor cells consisting of extracellular proteins and paracrine factors are present are all involved in growth and invasion via directional migration of cells as endothelial cells, MSCs and immune cells [42]. Tumor cells are considered as chronic wounded tissues further stained by hypoxia, immune response and other such events but tend to never heal at any point. Hence, the movement of thee MSCs to tumor site is very similar to that of movement toward wound site as described in detail [43]. Actually, both tumor cells and tumor associated pathways involve the process of secretion of chemoattractant XCL16, SDF-1, CCL-25 and IL-6 being secreted from prostate, liver, lungs and intestinal cancer types. The pro-inflammatory secretions from tumor-induced immune cells play their role as well in migration of MSCs toward or within tumors. In the tumor cellsm it further differentiates into myofibroblast contributing to tumor development [44].

26.6 Choice of Stem Cells Bone Marrow/Peripheral

The sources of stem cells can either be peripheral blood or be bone marrow. However, the protocol for aspiration of stem cells is exposed to be very complicated and can

cause certain complications, i.e., fracture, wounds and infections. At the same time, the procedure for isolation of pluripotent stem cells is much less complicated, and chances of morbidity are also less [45]. Hence, the pluripotent stem cells are considered as a proper source of stem cells but this is under investigation is under controversy for reaching a proper conclusion. As the chances of graft versus host reactions are also different in both BM and pluripotent stem cells, studies show a summary of the stem cell transplantation performed time now providing a brief comparison and guide for use of either BM or PSCs thus providing different outcomes or results [46]. Some of the results suggest the idea that pluripotent stem cells will generate more counts for lymphocytes but at the same time, another group of scientists observed no difference in the number of cells at all but instead he explained the presence of cytotoxic substances [47]. Similarly, the studies suggested different sort of controversial results on use of either of these types of stem cells and resulted allergic reactions such as graft versus host reaction in research and study [48]. Double stem cell transplantation showed better and improved results as compared top single stem cell transfer. Granulocyte-colony stimulating factor [49] aids in the differentiation and proliferation of hematopoietic stem cells progenitors. There are other factors that can also enhance metabolic activity of stem cells given as docetaxel, and recombinant methanol human stem cell factor [50].

26.7 Role of Purging in Isolation of Stem Cells

The most preferred method involves the acquiring of stem cells from allogeneic donors. But still, only 30% of the donors are eligible for this process because of the age restriction problems. These stem cells are easy to approach but they may cause the problem of co-existing of hematopoietic progenitor with their malignant counterparts that lead to phenomenon of the relapsing of cancer later in life [51]. In the patients of breast cancer, the pluripotent stem cells are given to patients which result in engraftment and proved to be less contaminated than that of the bone marrow stem cells. No betterment in the survival rate has been observed regarding this or related studies [52]. The retrieving problems of stem cells with that of attached stem cells are also a major problem as reported by the previous studies but their effect on the clinical outcomes is caused to be less problematic in nature. During isolation of stem cells, purging technology is used for their removal or separation of stem cells from that of related contaminants [53]. A number of the techniques have been employed involving use of monoclonal antibodies, fractionation, pulsed electric field, immunoadsorption technology and lastly hyperthermia. The chemical or drug amifostine has been used to protect normal progenitors from damage by the use of alkylating agent during purging of stem cells [54]. Double procedure that may use positive value for CD34 and negative value for CD19 reported to be better than that of single procedure for purging purposes during diagnosis of life-threatening disorders. At the same time, it also increases the risks of acquiring other hospital-induced nosocomial infections [55].

26.8 Lifespan of Adult Stem Cells

The most limiting thing in the use of the stem cells is their half-life or the lifespan. In this trend, embryonic stem cells are considered to be best because they can show division or differentiation to an infinite level being attributed to the expression of telomerase. The practical use of embryonic stem cells at clinical is still restricted [56]. The adult stem cells usually do not possess a huge amount of telomerase activity and hence they cannot bear the loss of telomeres; with the each cell division, the telomerase activity begins to slow down and it tends to cease at the critical phase with aging. Thus, the generation of enough stem cells for performance in clinical system is a critical problem [57]. One of the best strategies is to use the genetic manipulation that in turn causes the increment of replication span of stem cells by the control of genes involved in process of replication. In humans, this is performed by taking control over the process of senescence which is the mechanism of division where cell ceases to divide [58]. This can be done by controlling gene telomerase hTERT gene. In these studies where the control is done, the cells continue to divide for a longer period of time and hence maintain their ability to proliferate in a finite manner. The hematopoietic mesenchymal stem cells being immortalized with HPV16 E6/E7 in laboratory conditions have been performed without the presence of any neoplastic changes. This in turn paves the ways for the successful application of these cells in clinical fields where the quantitative amount of stem cells is not a prognostic factor acting as a future strategy [59].

26.9 Applications of Stem Cell Therapy in Relation to Cancer

A number of strategies have been proposed for using stem cells in cancer therapy which include hematopoietic stem cell transplant, MSC infusion for post cancer treatment, stem cells as carrier of drugs, generation of immune effectors and vaccine at last [60]. These cells have shown more level of T and CD4, CD8 cells in addition to neutral killer cells. Hence, stem cells from peripheral tissues are considered as safe, however, still multiple complications are resulted from various other studies following a detailed comparison of peripheral and bone marrow tissue types [61].

26.9.1 Hematopoietic Stem Cell Transplantation

For multiple myeloma, leukemia and lymphoma, the transplantation procedure is used mainly after the exposure to radiotherapy or chemotherapy. Moreover, the treatment has been defined as the major breakthrough following combination with either radiotherapy or chemotherapy at same time for treatment of cancer types such as

that of brain cancer, neuroblastoma, sarcoma and breast cancer [62]. But at the same time, there are glances of development of graft versus host infection which remains a point of concern when allogeneic source of disease remains a challenge which can be further treated by use of immunosuppressive drugs that are preferred to use because of side effects and less effectiveness [63].

26.9.2 Stem Cells as a Therapeutic Carrier

The rationale behind using stem cells as therapeutic carrier is to prevent degradation of the bioactive compound present in drugs, reduction of side effects related to system and lastly increase in overall level of therapeutics for enhancing our targeted effects of stem cells. The anti-tumor effect depends on the number of stem cells being localized into tumor microenvironment [64].

26.9.2.1 Nanocariers for Oncolytic Viruses

Oncolytic viruses tend to show selective mode of replication in cancerous cells but at the same time, it has been postulated that these viruses not only induce tumor cell lysis but also cause the activation of immune system for killing out the remaining tumor tissue present in close proximity [65]. For this purpose, naked oncolytic viruses are more easily recognized by immune system and are removed. Here, stem cells play their role and they act as a carrier of oncolytic cells for protecting and delivering OVs to tumor tissues. For example, human neural stem cells being transfected with CRAd-Survivin-pk7 OV in combination with the chemotherapeutic agents and ionizing radiations have shown possibility of enhancing toxicity and also increased the chances of mice in vivo bearing GBM [66].

Similarly, the MSCs are found to load attenuated measles virus or oncolytic HSV that in turn not only reduced the pathogenicity of hepatocellular carcinoma and GBM in mice but also increased chances of survival. Subsequently, stem cells acquired from ovarian cancer patients have shown comparable potential of showing greater proliferation as a carrier obtained from healthy donors. The whole procedure was performed after thawing process of freeze cells which show their potential application in the cancer treatment [67].

26.9.2.2 Genetically Modified Stem Cells

The approach is followed by the involvement of MSCs and NSCs aimed to enhance the overall secretion of soluble factors which are either the enzymes having capacity to convert the substances into soluble factors. They are known for its name and function as suicidal genes or gene drug enzyme producing therapy [68]. The enzymes released from the viable cells have ability to convert these prodrugs into more active

form which are more toxic to cancerous cells. As for example, 5-fluorocytosin is converted into 5-fluorouracil that is a toxic to tumor cells which are expressed by either MSCs or NSCs. Similarly, another compound irinotecan can be metabolized into less toxic compounds in the presence of carboxyl esterase enzyme. In the mouse model of neuroblastoma, grafts having co-administration of irinotecan and NSCs were found to be more effective as compared to the single drug or drug alone [69]. The single trials involving GDEPT have been tested. The patients having high-grade glioma are injected with neural stem cells which express CD receptors and 5-FC was injected in oral form. Additionally, leucovorin was also used for enhancement of the tumor killing effects of 5-FU. Stem cells expressing Herpes simplex thymidine kinase was injected in the patients with gastrointestinal cancer. Certain substances such as HSV-TK can convert ganciclovir monophosphate into much toxic form, i.e., triphosphate having more cytotoxic activity than before. The combination of prodrugs and multiple stem cells was explained to be more potent enabling the stabilization of cancer status in the cells [70].

Moreover, these stem cells which are genetically modified can also show chemokine or cytokines as a receptor system. Following this, tumor-toxic TNF-α-related apoptosis-inducing ligand (TRAIL) exhibits more sensitivity to tumor cells than that of other drugs at the same time for enhancing the chances of survival of the mice with brain tumor treatment [71]. This has been used to treat lung adenocarcinoma following clinical trials of gene in mouse model. Co-expression of the suicidal genes or cytotoxic genes prevents tumor growth also promotes apoptotic effect. Thus, stem cells show enhanced activity for increasing efficacy of already available treatments [72]. Application of nanoparticles in stem cell therapy is shown in Fig. 26.3.

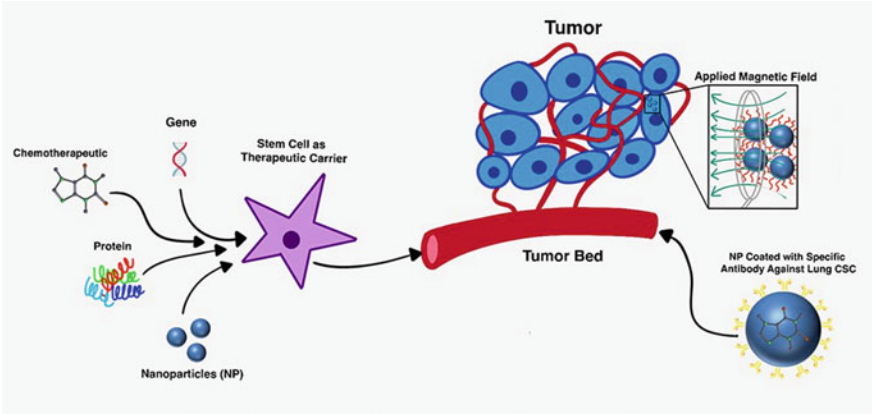

Fig. 26.3 Use of nanoparticles in stem cell therapy providing targeted action on tumor tissues [73]

26.9.2.3 Nanoparticles Carrying Stem Cells

Nanoparticles have been employed for carrying out drug molecule for a longer period of time and are preferred because of enhanced permeability and retention effects [74]. But at the same time, there are certain challenges which include leakage from body, less targeting effect and uptake by non-cancerous or normal cells reduce their utility. Stem cells however can serve as an effective transporter for carrying drug molecule to site of cancer [75]. For this purpose, the cells are either loaded or conjugated to cell surface. Intake of nanoparticles can either be performed by active or passive endocytosis depending on the size, surface charge and lastly incubation time; the major point of concerns is either drug loading capacity or potential to cellular control. Moreover, uncontrollable exocytosis of nanoparticles accidently can lead to release or reaching of nanoparticles to infect normal cells [76]. Roger and his colleagues employed a statistical method where PLA or lipid nanoparticles are loaded onto MSCs without having any sound effect on cellular viability or functionality [77].

In the terms of brain cancers, these MSCs serve as a pathway for transferring nanoparticles containing drugs to site of injection. Another study has also exhibited the more localization of nanoparticles containing paclitaxel-loaded NPs on the multiple stem cells providing more advantages than nanoparticles alone in mice with lungs tumor [78]. Firstly, the nanoparticles reach parenchyma of lungs and then move to site of tumor infection. The approach that increased uptake of nanoparticles not only enhanced targeted drug delivery but also improved treatment affectivity. Nanoparticles are directed toward cancer tissues by anchoring involving interaction with amine or thiol groups. Recently, a study has explained the use of nanoparticles conjugated with cyclooctyne on MSCs forming triazole at room temperature [79]. Using this method, the content of trizol was increased to about 98 pg per cell which is far more than that of other techniques, i.e., 1–20 pg per cell while maintaining phenotype or physical appearance of cell at same time. These platforms have also increased retention and enhanced effect at tumor site. In spite of rapid increase in the rapid cell engineering, no clinical trials are present which use nanoparticles on carriers [80].

26.9.2.4 Stem Cells-Based Exosomes as a Therapeutic Agent

Exosomes can also intake or load therapeutic agents such as anti-cancer agents as for example micro-RNA, proteins or proteins. These natural encapsulating agents have provided more benefits than that of synthetic ones that is it can be more biocompatible, can load more drug molecules and sustainability with more retention into tumor cells at same time [81]. They can easily functionalized with any molecule such as protein for enhancing targeting in tumor microenvironment. Using this technology, miRNA and other anti-tumor gents can be loaded onto exosomes. Similarly, they can be attached to specific ligands molecules such as protein for increasing out drug loading capacity [82]. The techniques for the packing of genetic material in exosomes are followed to be traditional one. As for example, Katakowski and colleagues collected

exosomes released from 156mi RNA expressing stromal stem cells. In the brain of the mice grafted with brain tumors when these stem cells were injected, they exhibited a rise in glioma cell production [28]. Another study involving hepatocellular carcinoma model, the injection of exosomes from 122miRNA having MSCs raised anti-tumor effects of sorafenib. Similarly, MSCs-based exosomes were able to deliver siRNA to bladder cancer cells thus silencing out polo-like kinase 1 gene [83].

Similarly, small drug molecules can also be loaded into exosomes using appropriate mechanisms as firstly priming with exogenous materials, stem cells can easily uptake those packaged materials and later or can release to external environment by exocytosis. Those exosomes found to suppress the leukemia and myeloma cell lines in different patients [84]. Likewise, other drug agents used as anti-cancer doxorubicin, gemcitabine and cisplatin can be paired up with exosomes for delivering out desired outcomes [85]. Therapeutic drug can also be loaded using post loading methods. When they are released from culture medium, these exosomes are usually forced to release out drugs by mechanisms as dialysis, extrusion and electroporation. This aids in loading of both hydrophilic and hydrophobic drugs providing control over drug loading more efficiently and lastly increasing out encapsulation efficacy [86].

26.9.2.5 Stem Cells for Production of Immune Cells

For cancer immunotherapy, neutral killer (NK) cells and chimeric antigen receptors are used successfully. These cells are extracted from the patient's own body, inserted into construct or vectors, expanded and then later or reintroduced to body of patient. But at the same time, quality and quantity of the immune cells remains a huge challenge in the patients with higher ages and who have undergone immunotherapy recently [87]. Anti-tumor activity of these tumor cells remains a point of concern because they can rapidly differentiate into the other cells. So there is utmost need to get these CAR cells from other sources which show expansion of immune-based therapy in larger number of patients [88]. For this purpose, the selected cells can either be induced pluripotent stem cells or be embryonic stem cells which can be derived from unlimited sources [89]. The process of differentiation involves growing of stem cells in a medium containing neutral killer cells or cytokines factors which can be IL-3, IL-17 or IL-15. The type of medium may also vary as in the case of hematopoietic embryonic stem cells, and bone marrow cells are cultured in a medium containing IL-7, SCF or FLT3L. Surprisingly, induction of HAR on hematopoietic stem cells increases their efficiency in treating out cancer cells. Transplanted cells will lie in bone marrow and will continue to generate immune cells having CAR receptors. Thus, the whole combination provides a stronger key to kill cancer cells [90].

26.9.2.6 Vaccines Based on Stem Cells

The role of cancer stem cells in the progression and spread of cancer is undeniable so the therapies which target these cells will be hallmark in providing effective treatment for cancer. Among the various treatments, anti-cancer vaccines hold prime importance because of their higher immunogenic effects [91]. These vaccines can be raised from oncofetal peptides of CSC, ESC or iPSCs. The production process involves loading of antigens on to the cells which generate primary T cell responses in vivo and engineered T cell therapy in vitro following adoptive therapy [92]. But the point of concern is that the only use of peptide vaccine does not provide desired immune response because of heterogeneity of tumors and escape mechanisms. So the anti-tumor vaccine consisting of whole cell lysate is more advantageous than that of others. Despite the rapid progress and identification of cancerous stem cells, their isolation from body still remains a challenge which hinders their use as vaccine source further [93].

Like all others, vaccines produced by embryonic or induced pluripotent stem cells are more useful and in larger amount than others [94]. The risk for this treatment can either be autoimmunity where the own cells of body become responsive to the injected cells or teratoma formation. In the mouse model, the autologous and allogeneic cells showed better response. But still at this point, these vaccines can be used as prophylactic treatment rather than therapeutic one. For enhancing level of immunity induced by this method, it is necessary to combine them with other treatments such as surgery, chemotherapy, adjuvants and immune inhibitors [95].

26.9.3 Mesenchymal Stem Cells After Treatment

Usually, treatment of cancer involves invasive removal of tumor followed by higher dose therapy that also causes damage to normal cells. These are clear evidences which show infusion of MSCs and proliferation of HSCs thus enhancing an overall level and quality of treatment [96]. Additionally, the MSCs with immunomodulatory effects can reduce strong immune responses in patients having GVHD. Co-transplantation of HSCs and MSCs has shown more promising outcomes with no or very little side effects. The process also known to facilitate the recovery of injured organs and also enhance tumor killing effect by enhancing tolerance to high-dose chemotherapy [97].

26.9.4 Secreted Agents

Stem cells can functionalize as the internal factories for release of anti-tumor agents for an extended period of time that in turn can overcome the other barriers such as higher systemic cytotoxicity and shorter drug half-life. Of them, tumor necrosis factor alpha can induce tumor cell apoptosis and is one of the most powerful agents

used in therapeutics. But its short half-life in animals can reduce the chances of being employed as an effective agent. This can be done by combining of trial stem cells with synthetic extracellular matrix which in turn that is introduced into glioblastoma cavity after surgical debunking phenomenon [98]. The treated cells can release these agents at resection margins thus avoiding growth of malignant or invasive brain tumors and increases rate of survival in mouse model. These stem cells are also regulated to release inhibitory compounds IFN-β that render tumor growth [99]. Ling and his colleagues studied infiltration of mesenchymal stem cells which express IFN-β at the higher level in comparison with primary breast tumor cells. Interferon beta was secreted at higher level in the tumor environment at higher level as that of normal circulation of blood. This in turn suggests the expression of IFN-β in abrogated cancer cells via inactivation of transducer activator transcription factor 3 [100].

26.10 Side Effects/Potential Risks of Stem Cell Therapy

26.10.1 Adverse Effects as a Result of Allogeneic Transplant of HSCs

Hematological and lymphoid cancers can be treated very easily upon the use of hematopoietic stem cells transplant. But it can induce long-term side effects in a large number of patients including that of GVHD, tissue-related infections, recurrence and secondary cancers, and lastly quality of life in patient. Because only extension in survival is not the objective of treatment but providing health recovery and social relationship improvement is also included in it. For improving outcomes, studies must be concern for the source of HSC transplantation. It is conceived that use of coed or umbilical blood can reduce the incidence and severity of disease. More interestingly, transplantation of MSCs showed improvement in GVHD and other side effects related to transplantation [101].

26.10.2 Toxicity and Resistance of Drug

Use of stem cells in drug and gene delivery is wholly dependent upon the number of cells being localized in tumor cells. But from investigation, it is clear that only 2 to 5% of stem cells can reach the tumor tissues after initial injection which later becomes stable over time of observation [102]. The intravenously injected cells are firstly entrapped in parenchyma cells and then move to lymph nodes, liver and spleen with every passing period. This can raise issues that include non-targeted therapy can induce toxicity to the normal cells and organs. Moreover, presence of drugs in the tumor sites not only increases chances of drug resistance but also reduces sensitivity. Finding out the method that can provide the targeted action of stem cells to tumors without effecting surrounding cells can improve efficacy [103].

26.10.3 Sudden Immune Response and Autoimmunity

The application of allogeneic stem cells obtained from donors can result in abrupt immune responses. Because the research has proved that the introduction of host antigens can result in the production of B cell and T cell humanized antibodies. Although it is safe to perform initial transplant to have idea but later on already available memory responses will degrade the transplanted cells and cause graft rejections or related infections. Induced pluripotent stem cell-based vaccines can increase risk of autoimmunity. It contains both normal and cancerous stem cells associated antigens. Hence, it is possible to induce immune response against normal tissues. Nigel and his research group has investigated autoimmunity generated as a result of stem cell transplant and hence further investigation needs to be carried out for providing safety approaches during treatment [104].

26.10.4 Tumorigenesis

Normal and cancerous stem cells possess their key biological role in the number of signaling pathways. The variation or mutation in the microenvironment of normal stem cells can lead to alternation or conversion of normal cells into tumor cells. In case of humans, endogenous stem cells are monitored on the basis of their surrounding stem cells so that they can work in right way as that of normal cells. However, the transplanted cells are exposed firstly to external environment of culture before their transplant and it can induce change in genotype and phenotype respectively [105]. After one month placing in the culture, they gets converted into tumorous or malignant cells. This procedure is a main point of controversy for scientists but the culture conditions must be controlled enough so that it does not produce any negative side effects in the stem cells. Pluripotent stem cells are more tumorigenic in nature than that of adult stem cells. Additionally, stem cells can also promote and differentiation of existing tumors [104].

26.10.5 Viral Based Infections

Viral infection is most general and used method for using and modifying stem cells so that they can act as a carrier of gene or drug subsequently. But at the same time, it also introduces chances of infections to carriers or recipients. The problems associated to it include the strong immunogenicity of induced viral particle which can induce terrible immune responses, production of toxins and even death in most of cases. Hence, viral vectors must be carefully chosen and modified in order to delete specific sequencing causing toxicity [106]. Challenges to use of stem cells with advantages are shown in Fig. 26.4.

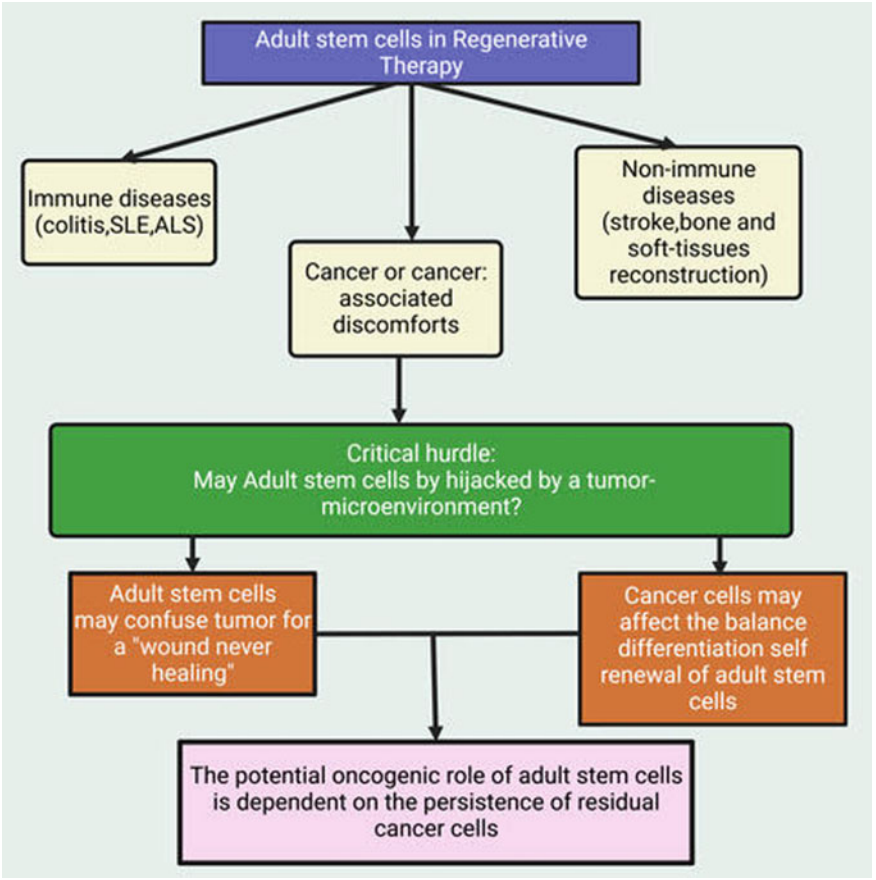

Fig. 26.4 Challenges to use of stem cells especially adult stem cells in cancer therapy along with benefits

26.11 Other Applications of Stem Cells in Cancer Therapy

26.11.1 Anticancer Drug Screening

In addition to all cancer cells, induced pluripotent stem cells can be used for screening of anti-cancerous drug molecules. These induced pluripotent stem cells are more useful and viable and related to humans as compared to currently available screening methods for traditional cancer cell lines, xenograft of mouse and other tumors. In addition to it, hepatotoxicity also becomes a barrier for application of drugs from being clinical application and has also been used for originating hepatocytes from induced stem cells derived of various sources having a proper genetic background

Table 26.1 Various adult stem cells in different tissues along with their stability

Adult stem cells	Tissues/cells	Extent of stability
Induced pluripotent stem cells	Reprogrammed cells of body	Maximum stability
Embryonic stem cells	Obtained from blastocyst of embryo	Maximum
Multipotent adult progenitor stem cells	Either brain or bone marrow	Better
Mesenchymal stem cells	Gathered from bone marrow	Good
Neural stem cell	Major source is brain	Limited
Umbilical stem cells	Umbilical cord of baby	Excellent
Cardiac muscle stem cells	Heart tissues	Limited
Liver muscle stem cell	Hepatic tissues	Limited
Adipose tissue stem cells	Bone/cartilage	Fair
Epithelial stem cells	Intestine	Better
Skin stem cells	Hair follicles	Good
Amnion stem cells	From the amnion membrane of embryo	Good
Hematopoietic stem cells	Umbilical cord blood or BM cells	Very good

[107]. The extent of stability of various adult stem cells in different tissues along with their stability is elaborated in Table 26.1.

26.11.2 Development of Regenerative Medicine

Based on their differentiation and self-renewal capacities, stem cells are employed for use as a regenerative medicine after undergoing chemotherapy. After the treatment using high-dosage radiotherapy or chemotherapy, transplantation of hematopoietic stem cells result in the hematological recovery [108]. The treatment results in the development of bone marrow and other marrow failure conditions in order to treat blood disorders and also work by the supply of stem cells which differentiate into

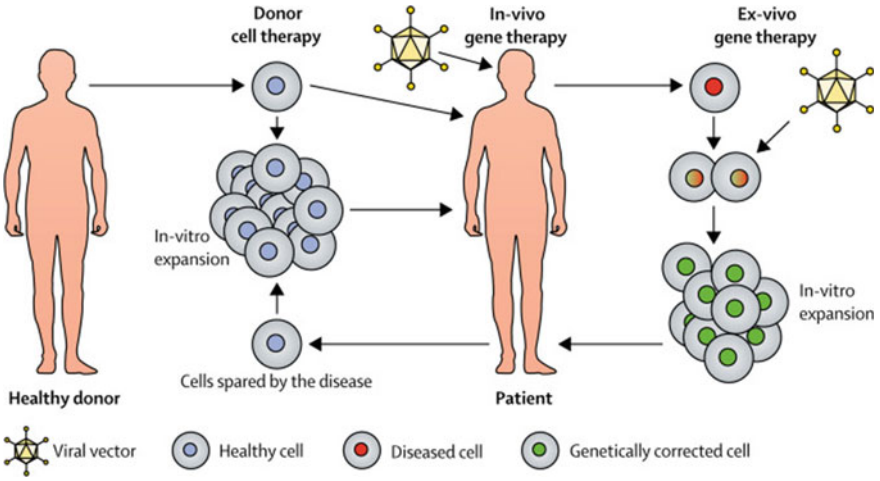

Fig. 26.5 Stem cell therapy and regenerative medicine in treatment of cancer and its subtypes

any specific cell type in short. Additionally, transplantation and grafting have been successfully potential to reconstitute stem cells in the patients [109]. Healthy induced pluripotent stem cells can be theoretically employed during regeneration of tumor or treatment based injured tissues. A number of other tissues can be reproduced through regenerative, medicine technology by use of induced pluripotent stem cells. They can also be used for repairing of cancer patients for repairmen of tissues damaged by other traditional treatments such as chemotherapy, radiotherapy and surgery. However, it requires engagement of induced pluripotent derived tissues in the system of animals, i.e., in vivo environment. Only a few types of induced pluripotent stem cells such as hepatocytes have been successfully used in animal models [110]. Stem cell therapy and regenerative medicine in treatment of cancer and its subtypes are shown in Fig. 26.5.

26.12 Factors Effecting Stem Cell Therapy

26.12.1 Type of Stem Cell

All of the stem cells possess similar properties but their effects may also differ in different types of tissues. The first comparison was made between neural stem cells and mesenchymal stem cells in the terms of adenoma virus in glioma cells. Both of the cells not only supported the replication of adenoviruses but also more ratio of cells was released from neural stem cells as compared to that of mesenchymal stem cells. The intracranial administration of virus loaded NSCs prolonged the time of survival in animal models of orthotropic glioma mouse model. Similarly, NSCs showed more

therapeutic effects in comparison with that of MSCs instead of similar migrate properties. In terms of cancer, treatment depends on the sort of stem cell being employed. Autologous hematopoietic stem cells are routinely developed for hematological and non-hematological malignancies in order to prevent hematopoiesis after treatment of chemotherapy. These stem cells are better than stem cells for assessment of toxicity induced by drugs [111].

26.12.2 Route of Transplantation of Stem Cells

The route of treatment also plays a major role in anti-tumor therapy. The appropriate method used involves accessing of target, benefits and risk to patients and objectives of therapy. Contralateral injection into tumor site can be achieved in murine model of GBM. Intracranial injections are an invasive method and cannot be used for repeated applications. Hence, they are delivered intranasal for providing efficient migration to tumor tissues. Hence, it can reduce the risk caused by intravascular delivery system such as hurdle caused by the blood brain barrier, embolism and infractions. Semisolid injection of substrate improves affectivity by providing an opportunistic support and reduction of metabolic stresses. Poor rates of survival of NSCs can be optimized by use of certain biocompatible devices [112]. Following this, Hansen et al. proposed three-dimensional substrate being purified from skin cell culture via a novel administration of grafted cells thus giving an opportunity regarding expression of NSCs thus retaining their uncommitted level of differentiation [113].

26.12.3 Number of Cells and Timing of Transplantation

Outcomes of treatments are affected by the conditions of transplantation, i.e., number of transplanted cells or the timing of transplantation. Insufficient transplant of the stem cells in patients with hematological disease can lead to replacement of hematopoietic stem cells and more relapsing of disease. However, more large number of transplantations in a given time causes formation of teratoma formation and more ectopic engraftment. Heave the number of transplanted cells must be optimized for proper functioning of transplanted cells. It also depends on the timing of administration as well. As it is suitable to give this medicine before radiations and chemotherapy. When the drug loaded, NSCs were given to patients with GBM prior to any other treatment has increased days of survival in patients to about 9 days in patients receiving dosing. In the terms of oncoviral therapy, there is an appropriate need to lead these cells into tumor beds. The viral particles are released at that spot thus allowing the functioning of those viral particles at that spot. Research has cleared that the maximum viral progeny has been released from NSCs after their loading in in-vitro conditions. After implantation, it takes about 24–48 h to reach tumor site. Hence, these cycles must be appropriate for homing of tumor abilities.

26.13 Clinical Uses of Stem Cells in Treatment of Cancer

The stem cells have been improvised in the treatment of breast cancer using high-dosage therapy and stem cell support. This has been done in the presence of the high-dosage chemotherapy which itself is a main problem causing a huge range of toxicity in the human biological system. However, the stem cell transplantation in the presence of certain conditions can cause cytoreduction and reduce chances of graft versus host allergies. The presence of cancer stem cells is like a miracle in patients with pre-existing prostate cancer having pre-resistant ADT-resistant cells that later give rose to CPRC as they show both the properties of self-renewing and the tumor propagating capacities as they lack receptor-based androgen expression. Moreover, the samples obtained from chemotherapy show persistence of cancer stem cells thus showing their critical part in chemoresistance [114].

In the ovarian cancer, the use of HSCT in combination with chemotherapy is proved to be very useful. When the infusion of stem cells increases GVY effect, the progression of stem cells is likely to propagate meanwhile. Allographic injection of the HSCs with the three factors has shown to enhance GVT response thus decreasing metastasis and duration of survival in patients for the renal cancer. Similarly, autologous chemotherapy has been combined with the chemicals of chemotherapy for giving relief from lungs cancer where the enhancement of survival rates allogeneic implantation of RIST has been employed in tee patients with leukemia [115].

26.14 Conclusion

Despite the utmost success in clinical and pre-clinical trials, many of the challenges related to stem cells need to be overcome by providing specific solution to them. The further research needs to be done in the signaling of stem cells and metastasis in stem cell growth thus leading to choosing a specific strategy for engineering of stem cells. These technologies may pave the ways for the cancer therapy. Stem cells move toward the specific tumor site and thus deliver specified anti-tumor agent at that site overcoming the short lifespans and other challenges of other traditional treatments. But this requires further research in this field for maintain a normal relationship between cancer cells and normal cells of body. So, a better understanding of underlying stem cell mechanism can help in their improvement of stem cell-based regenerative medicine or anti-cancer drug therapy for wide range of clinical use.

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