Targeting Cancer Stem Cells by Nanoenabled Drug Delivery

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Abstract

Resistance to chemotherapy and radiotherapy is commonly seen in cancer cells due to various reasons like mutation in drug target or their overexpression, drug inactivation, or drug removal from the cell, thereby rendering a problem in cancer management. The cancer stem cells (CSCs), which are responsible for cancer metastasis, are far reached from conventional therapies as these approaches are unable to eradicate the drug-resistant CSCs, and a novel approach for targeting these CSCs is warranted. Nanotechnology has occupied a huge space in drug delivery due to their unique photophysical properties and large surface area to volume ratio compared to their bulk counterparts. Targeted drug delivery can be achieved using nanoenabled drug delivery as the different nanostructures can be functionalized to tag different molecules which can identify specifically the CSCs. Moreover these nanostructures can also be used as cargo for carrying the chemotherapeutic drugs and delivering them to the target site. This chapter discusses the different types of nanocarriers used for targeted drug delivery as well as the progress in research for targeting the CSCs and destroying them.

Keywords

Cancer stem cells · Nanoenabled drug delivery · Bionanotechnology · Nanotheranostics

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17.1 Introduction

New therapeutic and diagnostic strategies for treatment of cancer have made enough progress in its preclinical and clinical research on cancer [1], but the metastasis in cancer is life-threatening as it spreads the cancer cells to other tissues from the origin [2]. The tissues found in cancer consist of heterogeneous cells with different states of differentiation and contain "tumor-initiating" cells formed by normal stem cell mutations [3, 4]. These "tumor-initiating" cells were termed as "cancer stem cells" (CSCs) which exhibit similar properties like other stem cells, self-renewal, can differentiate into any cell, and can proliferate to enhance malignant cells [4]. The strategy for cancer therapy includes the balance between self-renewal and differentiation of these CSCs to prevent formation of cancer.

In recent times, many new drugs are being invented with outstanding pharmacokinetic and therapeutic properties, but delivering those new drugs to target effectively becomes a challenge. Once targeted to the specific molecules, it can show its potential activity. Many nanotechnology-based drug delivery systems have been introduced and successfully commercialized like oncology drugs based on solid nanoparticles, liposomal formulation, conjugates of proteins and polymers, or drug-polymer conjugated nanoenabled drug delivery systems. However, the bioavailability of these drugs is dependent on several factors like size of the drug, dosages, difference in solubility of water-soluble and fat-soluble drugs, and their clearance from the blood stream. The drug designing also involves the target cells because in case of cancer it is desirable that the drug should affect the malignant cells only, not the benign ones, thereby warranting certain drug carriers which can encapsulate the drug and release them in only tumor microenvironment. Modern medical bionanotechnology has enabled us to design such nanocarriers which can target cancer cells. Targeting CSCs is much more relevant in cancer research because there are many drawbacks associated with conventional treatments using radiation and chemotherapy. But in cancer some CSCs can escape this treatment and migrate into new place through metastasis and start developing fresh tumors, relapsing the disease [5–7]. The different types of nanocarriers and their role in targeting CSCs will be discussed in this chapter.

17.2 The Different Types of Nanocarriers

The nanocarriers are helpful for the solubilization of lipophilic drugs, give protection to drugs which are fragile from degradation by enzymes or pH, and can target the drugs to be released at specific sites [8]. The different types of nanocarriers are discussed below.

17.2.1 Nanobots

Nanobots are nanorobots or nanomotors which are self-driven with submicron dimension, biodegradable nanodevices composed of bionano materials that can transport the cargo to deliver them in target sites. Zinc-based nanobots, named as PEDOT/Zn micromotor, were used to deliver payload in the stomach of a mouse model, which gradually dissolves the nanobot in the stomach acid and delivers the payload [9]. Single-molecule-based submersible nanomachines in solutions were activated using UV light, and single-molecule fluorescence correlation spectroscopy (FCS) was monitored. Designing such nanobots, which were non-unidirectional rotating motor, provided 10% enhanced diffusion, and we could monitor the behavior of these motorized molecules in solution [10]. DNA origami-based nanobots were designed to deliver payloads by designing outer functionalization using a DNA aptamer which can bind nucleolin, an endothelial cell tumor protein, and in the inner cavity had thrombin, the blood coagulation protease. They demonstrated that when these nanobots were injected intravenously, they delivered thrombin to the blood vessels associated with tumor and could induce thrombosis intravascularly. This resulted in necrosis of tumor and inhibited tumor growth [11]. Nanoactuators have also been designed which get activated using light by binding temperature-responsive polymers over gold (Au) nanoparticles which are charged. This stores the elastic energy which can be released rapidly under light for repeated isotropic nanoactuation. When the nanoactuator was heated above critical temperature ($T_c = 32\ ^\circ C$) using light from incident laser, the coating expels water and gets collapsed into nanoscale within a microsecond which is million times fast compared to the base polymer. This phenomenon triggers a small number of nanoparticles to get tightly packed into clusters. When the nanomachine is cooled below T_c, the strong van der Waals force between the cluster particles is surmounted as the expansion of polymer takes place giving rise to nanoscale forces of several nN. The intensity of the large force is dependent on van der Waals attractions between the Au cores existing very large in collapsed polymer state which sets a tightly compressed spring of polymer that can be triggered further into inflated state [12]. Nanoswimmers were designed which can be applied to swim in bloodstream to deliver the drugs. Multilink nanowire-based chains of diameter 200 nm were used to make a composite which exhibited planar undulations induced, using a planar-oscillating magnetic field. The chains were constructed by an elastic polypyrrole tail like eukaryotes and rigid nickel links which were magnetic in nature connected by hinges made up of flexible polymer bilayer. This multilink design showed high swimming efficacy and thereby could be used as a vehicle for drug delivery in body fluids [13]. These nanotechnological developments can enable nanobots useful for drug delivery.

17.2.2 Nanoneedles, Nanoclusters, and Nanobubbles

To facilitate the entry of drugs into the cell cytoplasm directly, nanoneedles are used because the biological membranes do not facilitate the drug entry into the cells. Nanoneedles are mainly used in atomic force microscopy but are applied for drug delivery to cells where they make small temporary perforation in the biological membrane and deliver the drug without perturbing the biological functions [14].

Metal nanoclusters are usually of the size of 10 nm prepared by self-assembly of polymeric or small organic molecule-based nanoparticles, cross-linked together with plasmonic metals like gold and silver or magnetic nanoparticles. These nanoclusters exhibit molecule-like properties and fluorescence; they are used for tracking the drug carried using these clusters to the target site and imaging. Peptide-protected gold nanoclusters (Pep-AuNCs) were used for self-regulated loading and release of drug vancomycin (van). The antimicrobial activity of van loaded in Pep-AuNCs was comparable to van alone, and the van released by Pep-AuNCs was proportional to the number of bacteria present [15].

Nanobubbles, on the other hand, are nano-sized spherical structures filled with gas which are usually stabilized using polymeric/lipid shells. The nanobubbles are used in combination with ultrasound, thermal, or magnetic sensitivities for efficient application in drug delivery and imaging, because of their higher stability and long time of residence in systemic circulation. For the purpose of diagnosis and therapy done together, theranostics has come into field, and researchers have developed plasmonic nanobubbles (PNBs) for tunable theranostic applications. The PNBs were designed by gold nanoparticle exposed to laser after delivering it intracellularly which generated transient photothermal vapor nanobubbles. The action of PNBs was tuned inside the individual cells from noninvasive, at lower laser fluence, to cell membrane disruption at higher fluence. The imaging was also captured with 50-fold amplification of optical scattering amplitude, and PNBs were established to support diagnosis, therapy, and image guidance at the cellular level in a single process [16].

17.2.3 Nanoghosts, Nanoclews, Injectable Nanoparticle Generators (iNG), and Nano-Terminators

Nanoghosts are based on a technology to form nanovesicles isolated from natural functionalized membranes of mammalian cell surface of complete biological cells like mesenchymal stem cells (MSCs) which do not contain cytoplasm or any organelles. These are smart delivery vehicles for drug or gene delivery. As they are derived from natural source, they do not pose difficulties related to drug loading, adverse immune response related to evading tumor etc. Moreover, it provides improved nanoparticle stability and gives a superior drug release profile. Nanoghosts were successfully isolated from cell membranes of MSCs (MSC-NGs), and in vitro and in vivo tumor targeting properties were also retained and were cleared from blood-filtering organs. These MSC-NGs were biocompatible, and drug-loaded MSC-NGs showed 80% inhibition of prostate cancer cells after systemic administration [17]. Negatively charged plasmid cDNA (pDNA) was loaded on a nanoghost

derived from MSCs which retained their unique surface-associated tumor-targeting properties. These engineered nanoghosts which were loaded with gene that is toxic to cancer cells could inhibit the growth of orthotopic lung cancer which has metastasized. These nanoghosts also proved to be effective in subcutaneous prostate cancer models and were shown to improve the survival of animals [18]. Nanoghosts derived from monocyte cell membrane were used along with doxorubicin-loaded PLGA core to make core-shell nanoghosts. The size of the nanoghosts was nearly 200 nm and was stable in serum for 120 h. These core-shell nanoghosts showed higher cellular uptake and cytotoxicity in MCF-7 cell lines compared to non-coated nanoparticles [19].

A nanoclew or nanococoon is made up of a single-stranded DNA which self-assembles to form a cocoon or yarn or a clew-like structure. The DNA amplification takes place by rolling circle model, and these nanococoons are highly biocompatible nanodrug delivery system. Sun et al. [20] first described a cocoon-like DNA-based nanocomposite as a drug delivery carrier which was associated with "caged worm" of deoxyribonuclease (DNase) that can undergo self-degradation thereby releasing the drug inside the cells. The DNA structure was a nanoclew made by weaving of DNA amplified by using rolling-circle model, and the self-assembly was facilitated by incorporating a palindromic sequence. The loaded drug was doxorubicin (DOX), and the targeted tumor delivery was achieved by folic acid (FA) conjugation with a nanoclew complementary DNA which gets hybridized to the DNA nanoclew. For self-degradation after reaching the tumor site with acidic environment, an encapsulated DNase I in single-protein-based nanocapsule (NCa) having a thin positively charged polymeric layer shell made up of cross-linkers which were acid degradable was used. This NCa which was positively charged was embedded into the nanoclew through electrostatic interactions forming a DOX-loaded DNA scaffold which was self-degradable. Under physiological pH, the DNase I was not released by the cage, but as soon as the nanoclew entered the cancer cell, the acidic microenvironment degraded the nanoclew as the pH-sensitive polymer releases the DNase I, thereby releasing the encapsulated drug DOX exhibiting higher anticancer efficacy [20].

Injectable nanoparticle generator (iNG) was first described by Xu et al. [21] and was made up of a polymer loaded with DOX which had multiple strands enwrapped over a nanoporous silicon material which is biodegradable. When these drug-loaded nanocarriers were intravenously injected, they got accumulated in tumor cells due to natural tropism. Then the silicon material slowly degraded and released the drug polymeric strands. Spontaneously, these strands formed nanoparticles which were taken up by the cancer cells, and the acidic microenvironment inside the cancer cells made the polymeric stands to trigger drug release. The iNG-based drug delivery system could cross the multiple biological barriers, and the dimensions and geometry of the silicon core could be tuned for targeting precise anatomical locations like the lung and liver. Moreover instead of DOX any other anticancer drug could also be loaded in the engineered iNG [21].

Nano-terminators were developed by Lu et al. [22] that were nanodroplets made from liquid metal loaded with drug which were absorbed by the tumor cells when

injected. In acidic tumor environment, it released the drug because the nanodroplet made from liquid phase eutectic gallium-indium core and a thiolated polymeric shell equipped with hyaluronic acid got dissolved. This nanodroplet was a core-shell nanosphere loaded with DOX and the hyaluronic acid acted as a tumor-targeting ligand. This nanoformulation when used in chemotherapy was shown to inhibit tumor in xenograft tumor-bearing mice in a much superior way than conventional chemotherapy [22].

17.2.4 Exosomes, Liposomes, and Niosomes

Exosomes, typically of the size from 30 to 120 nm, are used for transferring information from one cell to another and can be used as a natural vehicle for targeted drug delivery. The mode of transport using exosomes is depicted in Fig. 17.1.

The exosomes can be isolated from the patient's own cells which are healthy, as they can interact with its own cellular membranes when used for drug delivery without any hindrance. The exosomes have a unique property called "cell-specific tropism" which means that they can target specific cells by expressing specific receptors on the membrane, toward the cells from which they are isolated. This property can be utilized to convey drugs, microRNAs, or proteins loaded in these exosomes. Since the origin of these exosomes is biological which contains natural lipid bilayers, the immunogenicity and issues regarding clearance of drug can be reduced. Moreover, these exosomes can also cross the blood-brain barrier overcoming the challenging situation for drug delivery in the brain and for designing personalized medicine. Encapsulation of natural products and RNA has been accomplished in exosomes for the treatment of many solid tumor cancers like pancreatic,

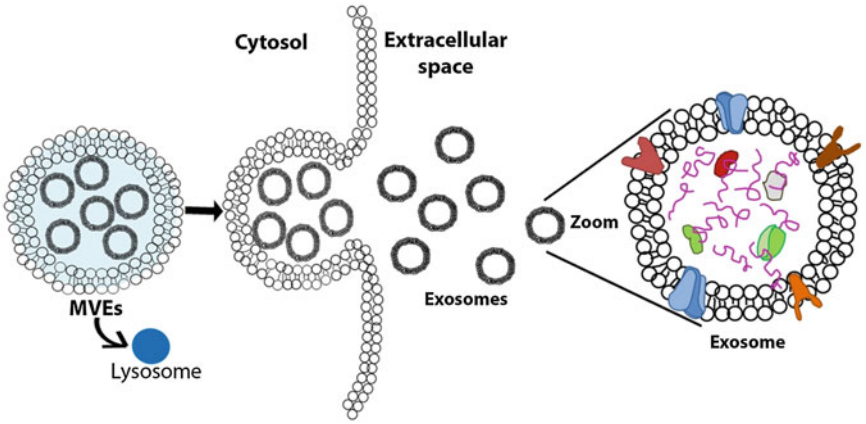

Fig. 17.1 The formation of exosomes. The multivesicular endosomes (MVEs) encompass the exosomes and these MVEs can fuse with plasma membrane to release the exosomes to the intercellular space or fuse with lysosome for their degradation. Once the exosomes are released, they can be isolated and used as a vehicle to carry DNA, RNA, protein, drugs, etc.

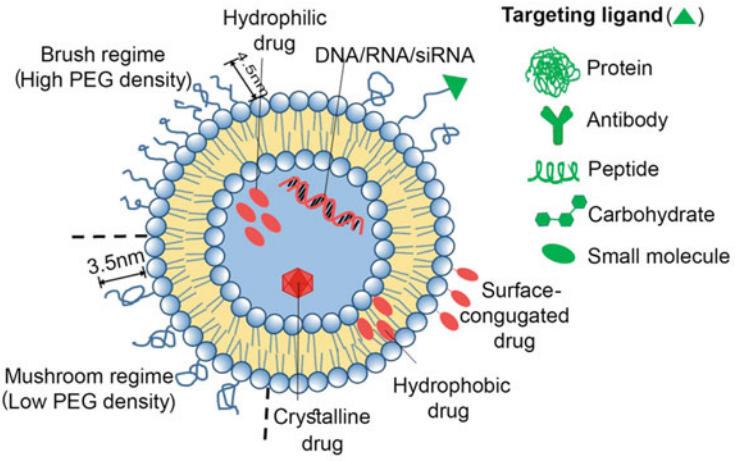

Fig. 17.2 The schematic diagram showing a liposome and the possible drug loading capacity of different types of drugs. The different surface functionalization is also illustrated for targeting the different types of cells

breast, prostrate, lung, and glioblastoma [23]. Three means in the exosomal targeted therapy can be achieved: (1) by targeting the peptides to the exosomal surface, (2) by encapsulating specific genes within the exosomes and transferring them to tumors, and (3) by targeting the exosomes that contain tumor-associated antigen. These are elaborately discussed in review by Wang et al. [24].

Liposomes are formulated spherical vesicles comprising of an aqueous core and surrounded by a lipid bilayer, used for improving the bioavailability, drug absorption, and reducing toxicity. The unique feature of liposomes is their ability to compartmentalize as well as solubilize both hydrophobic and hydrophilic materials, thereby opening a vast encapsulation capability. The different possibilities of using liposomes as drug carrier are given in Fig. 17.2.

A co-delivery system was developed based on fusogenic liposome that encapsulated chemotherapeutic agents with ATP-responsive elements and a liposome that contains ATP. When these two liposomes fuse together, there is triggering of ATP-mediated drug release. The design of the fusogenic liposome is a protein-DNA complex core consisting of an ATP-responsive DNA scaffold with DOX which could release DOX by a change in conformation of aptamer/ATP duplex in the presence of ATP. To achieve cancer cell targeted delivery, the fusogenic liposomal membrane was coated with a peptide which can open when acid-triggered to fuse the two liposomes under cancer acidic microenvironment. Thus, a pH-sensitive anticancer drug delivery system was achieved [25].

Niosomes are nonionic surfactant vesicles available in different sizes that range from 20 to 50 μm. They can be constructed by self-assembly of monomers of hydrated nonionic surfactant and are capable of encapsulating a variety of drugs [26], and their typical structure is shown in Fig. 17.3.

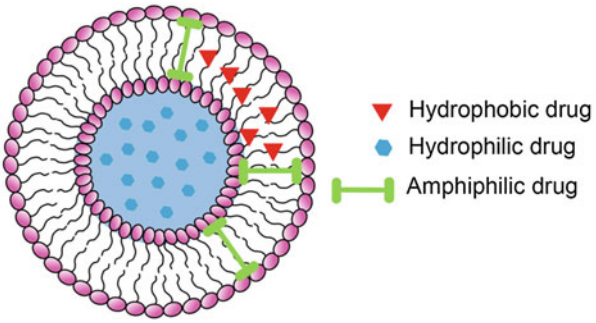

Fig. 17.3 The structure of noisome made from nonionic surfactant and cholesterol used for drug delivery. All the types of drug, hydrophobic, hydrophilic, and amphiphilic, can be loaded in the niosomes

Due to the stability problems found in liposomes, niosomes are introduced as an alternative drug delivering vehicle. Niosomes also possess the capacity to encapsulate both hydrophilic and lipophilic drug substances. The efficiency of entrapment increases with the increase in lipophilicity and concentration of the surfactant which is used to make them. Compared to liposomes, niosomes have different chemical compositions of its bilayer making them more advantageous. The components of liposomes are based on phospholipids, whereas surfactants are used to make niosomes which have improved chemical, physical, and biological stability. Moreover, by modulating the noisome bilayer composition, enhanced drug entrapment can be achieved. The industrial manufacturing of niosomes is also less expensive as they do not need special handling methods as well as storage conditions due to their high stability. The mostly used nonionic surfactants for niosome preparation used for drug delivery are alkyl ethers, sorbitan fatty acid esters, alkyl glyceryl ethers, and polyoxyethylene fatty acid esters. The correct selection of the surfactant plays an important role in designing nonionic vesicular systems. The stability, size, pharmacokinetics, entrapment efficacy, pharmacodynamics, and targeting properties of the vesicular systems are affected by the molecular structure of the surfactant used [27]. The different types of niosomes used for cancer drug delivery are well discussed in the review by Bondar et al. [28].

17.2.5 Dendrimers

Dendrimers are polymers having a well-defined structure with a core at its center made up of an atom or molecule. Branches emerge from its core comprising of repeated units of the constituent polymer with the branch junctions, known as generations [29]. There can be first-generation, second-generation, third-generation, or fourth-generation dendrimer emerging from a single core. The branching makes multiple functionalization and many molecules can be attached thereby on a single core. Dendrimer framework can be controlled and can be utilized as a good drug carrier, and their functionalizations are used for conjugation with drugs or DNA/RNA. Dendrimers can enhance the solubility and bioavailability of the drugs that are hydrophobic. The entrapment of drugs can happen in the intramolecular

cavity of dendrimers or can be conjugated to the functional groups attached at their surface [30].

17.2.6 Graphene and Carbon Nanotubes

Graphene is a two-dimensional nanostructure of carbon with one-atom thickness made from densely packed sp2-hybridized carbon atom network arranged in a hexagonal crystal lattice structure exhibiting unique nanoscopic properties [31–35]. It has profound usage in materials science as well as biomedical science [32, 35]. The graphene nanoparticles can exhibit various structural features, biological responses, and physicochemical properties based on their manufacturing methods [36]. The different types of graphene nanoparticles are graphene nanoribbons (stacks of ribbon-shaped graphene synthesized by the unzipping of the multiwalled carbon nanotubes), graphene nano-onions (spherical shaped layers of graphene which are concentric having both sp2 and sp3 hybridizations), and graphene nanoplatelets (irregular or disc-shaped multiple layered graphene nanoparticles which are synthesized from graphite, also named as graphene oxide (GO)) [36]. The promising applications of graphene in imaging, therapeutics, and drug delivery are attributed to their unique physical and chemical properties [35, 37–39], and so it is considered as a multifunctional nanoparticle. The surface of graphene nanoparticles can be functionalized covalently or noncovalently with anticancer drugs as well as functional groups that can target the cancer cells or tissues for improving the treatment efficacy. The physicochemical properties of graphene nanoparticles can be utilized to assist stimulus-responsive therapy as well as drug delivery. Scientists have targeted CSCs using graphene nanoparticles without causing any harm to normal cells [40].

Carbon nanotubes (CNTs) are made from carbon graphite nanomaterials arranged in an ordered array and hollow structure. CNTs have high surface area, ultralight weight, high aspect ratio, and high tensile strength with tube diameter ranging from 1 to 100 nm. The end of the tubes is usually capped with half-fullerene molecules on both ends and exists as one or several coaxial layers of graphite having diameters in nanometer range. Every carbon atom in CNT is joined to their three neighbors with sp2 hybridization just like graphite that gives the molecules huge strength. CNTs are classified into two types depending on their structure: single-walled carbon nanotubes (SWNT) and multiwalled carbon nanotubes (MWNT). In the field of drug delivery, CNTs have a number of advantages to deliver the drugs at specific locations in our body suggesting that CNTs may overcome the difficulties of nanoparticles. Since the CNTs have a huge inner volume, it allows more drug molecules which can be encapsulated. Moreover, these volumes are easily accessible because the fullerene caps at the ends can be removed easily and they can have different functionalizations for inner and outer surfaces [41]. CNTs can be chemically modified to attach a variety of molecules on its surface such as proteins, DNA, drugs, peptides, ligands for targeting cells, etc. which enable them to be appropriate candidate for targeted drug delivery. Although one of the drawbacks of CNTs is that

they are evidenced to show oxidative stress both in vitro and in vivo causing inflammation and damage to cells in the liver and lungs [42]. To overcome these, nitrogen can be doped in CNTs in the form of various functionalities like pyrrolic nitrogen, pyridinic nitrogen, oxidized nitrogen, and graphitic nitrogen. Further alterations of these functionalities by means of chemical reactions can be done to get desired nitrogen species [43]. When the nitrogen atoms are incorporated into the graphitic lattice of the CNTs, an additional strain to the structure of CNT results in forming "stacked cups" [44]. These stacked cups are held together with weak van der Waals forces, and when these weak interactions are disrupted, individual or short-stacked nanocups are obtained. These short-stacked nanocups are corked with gold nanoparticles, thereby yielding sealed nanocontainers for cargo delivery [45]. In this way a much biocompatible, sealed drug delivery system can be obtained and can be used to deliver drugs at targeted cells. In cancer immunotherapy also CNTs are used as an artificial substrate. Expansion of T cells isolated from mice was done using CNT-polymer nanocomposite, as an artificial antigen-presenting cell. The antigens were attached onto bundled CNTs and complexed with polymer nanoparticles which contained magnetite and interleukin-2 (IL-2), a T-cell growth factor. The results obtained were very promising, and the T cells obtained could delay tumor growth observed in murine melanoma model. Thus, CNT-polymer platform could generate a huge number of cytotoxic T cells which can be used for cancer immunotherapy [46].

17.2.7 Nanodiamonds

Nanodiamonds are carbon-based nanoparticles with 2–8 nm diameter having truncated octahedral structure which gives them multiple facets. These nanodiamonds are not recognized and carried out by the transport proteins which usually pump the drugs outside the cells, and thus the drugs attached to these nanodiamonds remain inside the cells. The synthesis of nanodiamonds can be done using chemical vapor deposition (CVD), detonation, or high-temperature-high-pressure process [47]. Nanodiamonds have good chemical stability, structural rigidity, octahedral symmetry, large surface area, and low production costs [48, 49]. The two types of nanodiamonds used in medical applications are detonation nanodiamonds (DNDs) and fluorescent nanodiamonds (FNDs). In cancer chemotherapy, nanodiamonds are coupled with chemotherapeutic drugs that enable sustained release of the loaded drug for a period of 1 month. Epirubicin, a chemotherapeutic drug, was attached to nanodiamonds of nearly 5 nm diameter to make a nanodiamond-epirubicin drug delivery complex (EPND) which could specifically kill the CSCs apart from killing normal cancer cells [50]. The other applications of nanodiamonds in cancer therapy are discussed by Gupta et al. [51] and Ho et al. [52].

17.2.8 Whole Cells

Drug delivery mediated by whole cells involves specific cells as vehicles for the drugs to deliver them into targeted sites. Therapeutic drugs or imaging molecules are loaded inside these cells and further released into the diseased sites. The cells usually used for cell-based therapy include leukocytes, red blood cells, stem cells, etc. and these cells act like a Trojan horse. The payload is carried inside the cells and gets transferred to the diseased tissue from the circulating blood. During this process these cells retain their original properties because of which they mimic the migration behavior of certain cells for carrying the drug to targeted site when administered in vivo [53]. Mesenchymal stem cells (MSCs) are recently being used as drug carriers as reviewed by Cheng et al. [54] apart from the use of genetically modified MSCs for the delivery of different pro-apoptotic, antiangiogenic, as well as therapeutic proteins to various types of tumors. Jiang et al. [55] have induced overexpression of CXCR4 in human adipose-derived stem cells and used these cells as a potential vehicle for targeting hypoxia in tumors. Paclitaxel (PTX), a potent chemotherapeutic drug, was successfully delivered using nano-engineered MSCs which acted as a tumor-specific drug delivery vehicle with improved anticancer efficacy compared to conventional chemotherapeutic drugs [56]. A hybrid spheroid/nanomedicine system was constructed from MSC spheroid entrapping a drug-loaded nanocomposite. The spheroid formulation increased the tumor tropism of MSCs and allowed the loading of various types of drugs. The system altogether acted as drug delivery platform tested in glioblastoma model integrating the properties of cell- and nanoparticle-mediated drug delivery along with tumor-homing features of MSCs, resulting in advanced combinational therapy for cancer [57].

17.2.9 Photodynamic Therapy

Photodynamic therapy (PDT) is a mechanism of killing cells using a photosensitizer, oxygen, and appropriate wavelength of light. The photosensitizer is specifically delivered into the cancer cells using a vehicle. The photosensitizer when activated moves to their excited state and generates reactive oxygen species (ROS) by two different ways. In primary photochemical reaction (type I), the electrons are transferred to oxygen or other molecule which forms a radical. This radical further reacts with molecular oxygen forming superoxide anion. In secondary photochemical reaction (type II), the main pathway involves energy transfer to molecular oxygen which further forms the ROS. Both type I and type II mechanisms can take place simultaneously, and the proportion of the two reactions is dependent on the photosensitizer type used, the substrate concentration, and the amount of oxygen present. If the accumulation of the photosensitizer can be made selective at the target site and there is delivery of focused light, it can reduce the damage to the normal cells and can eventually enhance PDT efficacy [58]. The destruction of cells by the reactions of PDT is mediated by either necrosis or apoptosis [59]. The commonly used

photosensitizers which can be administered intravenously for PDT are very rapidly cleared from our circulation, thereby rendering them safe for usage. However, the hydrophobic nature of these photosensitizers renders them to aggregate in aqueous solution and thereby reduces the efficacy of PDT. This drawback of the photosensitizer hinders their delivery inside our body and also causes a decrease in singlet oxygen formation due to self-quenching at the excited state [60]. Thus, to overcome this difficulty, to maintain the photosensitizer in their monomeric state, to give them protection from aqueous environment, and to increase the safety as well as efficacy of PDT treatment, different pharmaceutical carriers and drug delivery systems have emerged. These delivery systems for photosensitizer include liposomes, micelles, oil-based emulsions, polymeric nanoparticles, etc. [58].

For the eradication of cancer, PDT is used in combination with conventional therapy to yield superior outcomes, and nanoenabled therapy for cancer gives higher specificity for cancer cells, lowers side effects, and destructs the cancer cells with high efficiency both in vivo and in vitro. PDT is suitable for treating the types of cancers which cannot be cured by surgery, and moreover the nanoenabled drug delivery system can reach the CSC niche, thereby killing cancer cells and destroying the CSCs which are drug resistant. These nanomediated therapies give 100-fold high therapeutic efficiency compared to free drugs against the drug-resistant cancer cells. The different types of photosensitizers used in PDT are hematoporphyrin, photodithazine, methylene blue, curcumin, chlorins, hypericin, and phthalocyanines [61–63]. To enhance the therapeutic efficiency of the photosensitizers, improvements are being made through conjugation with other molecules. Previous applications of PDT were restricted to surface applications only because of the inapproachability of light in the deeper areas. It has been shown that PDT can also be used in deep-seated cancers including brain tumors and liver cancers using a wireless device capable of activating the photosensitizer inside the tumor [64]. Bakalova et al. have shown that the photosensitizer trifluoperazine when loaded in anti-CD90 antibody conjugated with water-soluble CdSe core-shell nanocrystals was delivered directly to CD90 + leukemia CSCs and could kill the CSCs when exposed to UV light via apoptosis [65]. The different aspects of PDT in targeted cancer therapy have been discussed by Crous et al. [66], but only future perspective of targeting CSCs has been discussed. Till now, not many potential PDTs have been developed to target CSCs.

The different treatment strategies involving nanoenabled drug delivery were discussed so far. Regarding the treatment of cancer, research is still ongoing concerning the target cells, vehicles for drug delivery, and their outcomes in combating the disease. Targeting CSCs becomes the most effective way of controlling the tumor outbreak and metastasis. The different targets that can be utilized for CSC destruction are given in Fig. 17.4.

The CSCs actually act as seed for the initiation of tumor, transition from epithelial to mesenchymal cells, and resistance to chemotherapy thereby resulting in metastasis [67]. A combination drug therapy can help in improving the clinical outcomes, by combination of inhibitors of CSC with conventional cytotoxic agents which can kill both CSC and bulk tumor cells simultaneously [68]. The combination therapy not

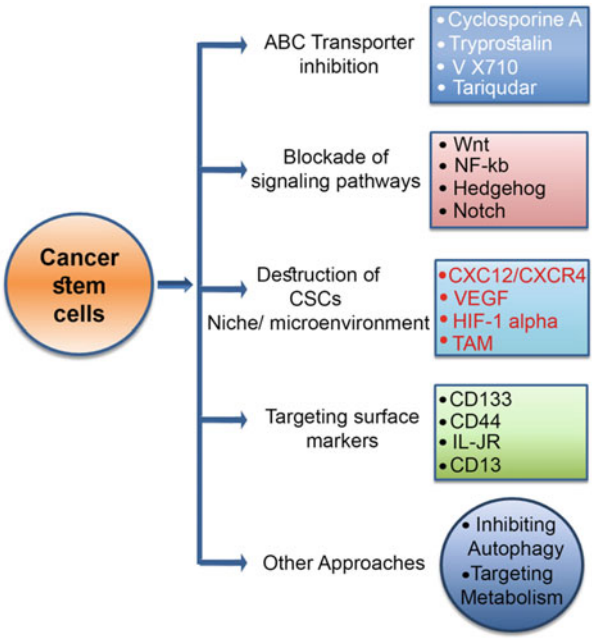

Fig. 17.4 The destruction of CSCs can be achieved by interrupting various pathways as depicted in this figure like inhibition of ATP binding cassette (ABC) transporter, blocking different signaling pathways, destruction of CSC niche, targeting the CSC markers and autophagy inhibition or targeting metabolism

only delays or suppresses the adaptation of cancer, its mutation, and progression, but it eventually decreases the individual dose and hence the side effects [69–71]. The targeting of cancer cells or CSCs can take place in two different ways:

1. Passive delivery systems: This system is based on the enhanced permeability and retention effect (EPR) in case of solid tumors. In this phenomenon, due to the increased permeability of the vasculature around the tumor tissue, low molecular weight molecules (up to 40 kDa) can enter into the tumor space, and the suppressed lymphatic filtration also allows these molecules to accumulate [72–75].
2. Active targeting system: Active targeting systems can be achieved by associating the cancer cell-specific affinity ligands to the nanostructure-based drug delivery systems [76, 77].

17.3 Nanoenabled Treatment of Cancer Stem Cells

Chemoresistance is the major cause of failure in treatment of cancer and also a common trait in the tumor-initiating CSCs. CSCs escape the chemotherapy and have enhanced tumor initiation capacity. Targeting cancer stem cells for effective therapy of cancer is being studied in the last few decades [78–80]. Targeting the CSCs using different cell markers gives a strategy for the targeted drug delivery. For example, there are several markers for ovarian cancer stem cells like epithelial cell adhesion

molecule (EpCAM), CD117 (c-kit), CD44, CD133, and aldehyde dehydrogenase isoform 1 (ALDH1) [81]. The most common cell markers of CSCs are CD44 and CD133 which are generally used for targeting the CSCs using nanovehicles. The progress in research using nanocarriers for targeting CSCs is discussed below.

17.3.1 Nanodiamonds as Drug Carriers

A nanodrug delivery platform based on nanodiamonds, to deliver epirubicin, a chemotherapeutic drug, has been used to impair the growth of tumor that is developed from chemoresistant CSCs. The nanodiamonds were attached reversibly to epirubicin through physical adsorption (nanodiamond/epirubicin ratio = 5:1) to make epirubicin drug complex (EPND). The drug complex, EPND, was characterized (size and surface charge) and found to be capable of passive targeting with enhanced permeability and retention property. The cellular uptake and cell killing capacity of EPND were monitored showing higher chemotherapeutic killing in both CSCs and normal cancer cells [50]. Previous studies have suggested that there can be covalent and noncovalent methods for functionalization of the nanodiamonds which make them more biocompatible and superior than the other carbon-based nanomaterials like SWNT, MWNT, and carbon blacks [82]. Several applications of nanodiamonds in drug delivery system for cancer have been reviewed by previous researchers [83], but very few reports exist on targeting the CSCs.

17.3.2 Polymeric Nanoparticles

Polymeric nanoparticles have also been applied for the drug delivery [84] for targeting cancer stem cells. Yang and his team [85] prepared functional micelles that were self-assembled from the mixture of polyethylene glycol (PEG) and acid-functionalized polycarbonate to make a diblock copolymer (PEG-b-PAC) and a PEG and urea-functionalized polycarbonate copolymer diblock (PEG-b-PUC) through hydrogen bonding. These synthesized micelles had high stability because of the hydrogen bond presence (urea-urea and urea-acid) and had the ability to accumulate preferably in the tumor tissues due to EPR effect [86]. They also exhibited high loading capacity for the chemotherapeutic drugs like DOX [85–87]. Phenformin is another chemotherapeutic drug, with two guanidine groups that can form hydrogen bond with urea group and can have ionic interaction with the acid group present in the micellar core. A self-assembly of PEG-b-PUC and PEG-b-PAC mixture was made and loaded with phenformin, and the drug-loaded micelles were characterized for its size, stability in serum containing solution, and drug release properties in vitro. Lung cancer cell line H460 was analyzed for its cytotoxicity using only phenformin and micelle-loaded with phenmorphin which showed promising results for the micellar form. Further the CSC population in the tumor tissue after treatment with only phenformin and micelle-loaded with phenmorphin was monitored. The

tumors posttreatment with only phenformin and micelle-loaded with phenmorphin were excised and dissociated to make a single-cell suspension and were analyzed for CD133-positive cells using flow cytometry. The results showed that free phenformin did not reduce the CSC subpopulation, whereas, the phenformin-loaded micelles could significantly reduce the CSC's population in the tumor cells compared to the control. The reason behind this may be due to the EPR effect of drug-loaded micelles in the leaky tumor tissues that led to preferential accumulation of these micelles in the tumor tissues [88]. In another study, salinomycin-loaded poly(lactic-co-glycolic acid)-polyethylene glycol nanoparticles were used for conjugation of CD133 antibodies (CD133-SAL-NP) for the purpose of elimination of CD133+ ovarian CSCs. The size of the polymer-loaded drug-antibody conjugate was 149 nm and had the property of sustained drug release with high efficient binding capacity to CD133 + ovarian cancer cells. An increased cytotoxicity was observed in CD133+ ovarian cancer cells compared to nontargeted SAL-NPs and only salinomycin. There was a reduction in the CD133+ ovarian CSCs in the ovarian cells compared to only salinomycin and SAL-NP treatment showing that the polymer-loaded drug-antibody conjugate was effective in targeting the CSCs. The nude mice were taken which bore ovarian cancer xenografts and were treated with CD133-SAL-NPs, showing enhanced therapeutic effects demonstrating that CD133-SAL-NP can be a promising target for killing ovarian CSCs [89]. Actively targeting CSCs was achieved using a multilayered core-shell polymeric nanoparticle using hyaluronic acid (HA) in place of PVA as a drug loading vehicle [90]. HA can specifically bind to CD44 antigen which is commonly overexpressed at the surface of several types of CSCs [91, 92], and the HA-decorated nanoparticles can co-deliver many drugs specifically into the CSCs. The four drugs used for co-delivery were doxorubicin hydrochloride (DOX, hydrophilic), curcumin (CUR, hydrophobic), indocyanine green (ICG, hydrophilic), and irinotecan or camptothecin (CPT, hydrophobic), using nanoparticles prepared from four polymers: pluronic F127 (PF127 with and without chitosan modification), poly(D,L-lactide-co-glycolide) (PLGA), HA, and chitosan. These polymers are approved by the US Food and Drug Administration (FDA). The combination of PLGA and PF127 yielded more uniform size and high stable nanoparticles compared to the one obtained using PF127 or PLGA alone. Chitosan was also found to bind specifically to CD44 which was overexpressed in the CSCs [93]. Drug repositioning is another strategy used for targeting the CSCs because it helps to overcome some limitations of conventional drug therapies like poor drug solubility, toxicity at off-targets, etc. A transcription factor, named STAT-3, can regulate the genes which are involved in the renewal of stem cells and has become a novel target for cancer therapy. Breast cancer stem cells' (BSCs) studies were highly correlated with STATs [94], and STAT-3 has been documented for its role in invasion, survival, and promotion of cell proliferation in tumors, immunosuppression, angiogenesis, obesity, inflammation, as well as premetastatic niche formation [95]. Moreover, STAT-3 also plays an important role as potent immune checkpoint responsible for immune response for multiple tumors that are present in tumor microenvironment for promoting tumor progression [96–99]. Thus, any therapeutic approach that can block STAT-3 can be effective in the treatment of cancer. Drug repurposing strategy was

done for delivering suitable STAT-3 inhibitor, niclosamide, incorporated in a polymeric nanoparticle (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)) and conjugated with CD44-targeting peptide yielding CD44-tagged niclosamide-loaded nanovehicles (CD44-NIC-Veh). This drug-loaded nanoparticle was used for efficient targeting of BSCs and was found to be promising in breast cancer stem cell killing capacity by altering the gene expression and protein translation. There was downregulation of CSC marker genes like MYC, BCL2, IL10, MCL1, IL11, MMP9, MUC1, EGFR, COX2, IFNG, and VEGF in the mouse xenograft tumors that were treated with the CD44-NIC-Veh in comparison to the nano-Veh-treated controls. The researchers have also found that the CSC populations were significantly decreased, as evidenced by the reduction in CD44+/CD24$^-$ expressing cell population. This showed that the "stemness" characteristics were reduced in the CSCs and the CD44-NIC-Veh could deactivate STAT-3 [100]. HA-functionalized ethylenediamine conjugated bovine serum albumin (eBSA) encapsulating all-trans retinoic acid (ATRA) (HA-eNPs) was used as a drug delivery vehicle for the targeted drug delivery to CD44-enriched B16F10 cells. In vivo imaging experiments showed that HA-eNPs could accumulate in the lungs of the tumor-bearing mouse. The ATRA-laden HA-eNPs could exert better killing ability to B16F10 cells as seen from the cytotoxicity assay compared to free drug or normal nanoparticles exposed at the same dose. Moreover, the tumor growth was inhibited significantly by HA-eNPs/ATRA as seen in the lung metastasis tumor mice. Thus, HA-eNP-loaded ATRA can be a superior drug for controlling the CSCs [101]. Active targeting of breast and colon CSCs was achieved by targeting the stem cell surface marker CD44. PLGA-co-PEG loaded with PTX micelles was used for targeted drug delivery to BCSCs and colon cancer cells showing promising results [102]. N-Isopropylacrylamide, vinylpyrrolidone, and acrylic acid polymer mixture was used in the molar ratio of 60:20:20 to encapsulate curcumin and was found to reduce the brain tumor size and also reduced the number of CD133+ stemlike cells [103, 104]. The Hedgehog (Hh) signaling pathway was interrupted by delivering GLI inhibitor through PLGA-PEG nanoparticles and showed inhibition in metastasis in hepatocellular carcinoma models [105, 106]. Anthothecol encapsulated in PLGA could alter the fate of pancreatic CSCs by inhibiting CSC proliferation and inducing apoptosis [107]. Polyethyleneimine/polyethylene glycol conjugated with mesoporous silica nanoparticles was used to load the TGF-β inhibitor, LY364947, for inhibiting the TGF-β signaling pathway of BCSCs and also to deliver siRNA to the CSCs. These nanopolymer-based delivery of siRNA caused the accumulation of the siRNA in the tumor and reduced the CSCs [108, 109]. Targeting the different CSC killing pathways using nanoenabled drug delivery is discussed in detail by previous researchers [7, 110–113].

17.3.3 Liposomal Nanocarriers

Liver cancer stem cells (LSCs) are responsible for the initiation of liver cancer, invasion, recurrence, metastasis, and further chemoresistance. Like other cancers,

targeting LCSCs using nanoenabled drug delivery can show some insight in liver cancer treatment and prevent their recurrence [114]. Nanoliposomes were prepared using a lipid mixture of hydrogenated soybean phospholipids (HSPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(PEG)-2000] (PEG-DSPE) in the ratio of 85:10:5. The nanoliposomes were used to make salinomycin-loaded nanoliposomes (SLN), doxorubicin-loaded nanoliposomes (DLN), as well as a combination of salinomycin and doxorubicin (SDLN) for targeting both normal liver cancer cells and LSCs. The mole ratio of DOX/salinomycin sodium at 1:1 had the optimum synergistic combination index value, and the same ratio was taken in SDLN. The percentage of LSCs in vivo was significantly decreased after treatment with SDLM and SLN + DLN post 12 h treatment [115]. Liposomal nanoformulations for targeting the prostate cancer cells and prostate CSCs have been engineered using cabazitaxel (CBX)- and silibinin (SBL)-loaded liposomes made from cationic phospholipid *N*-[1-(2,3-dioleoyloxy) propyl]-*N,N,N*-trimethylammonium chloride (DOTAP) and cholesterol. For specifically targeting prostate CSCs, HA was coated atop the cationic liposome that had affinity for the CD44 cell surface receptors overexpressed in CSCs. The in vitro results showed that these surface-functionalized liposome-encapsulated drugs could exert specific cytotoxicity against CD44+ cells. Thus, the results showed the potential of CBX-SIL co-loaded liposomes for eradicating prostate cancer stem cells [116].

17.3.4 Exosomes as Drug Cargo

Exosomes are natural nanovehicles derived from cells and are widely distributed in body fluids for the cell-cell communication. They are involved in multiple diseases, including cancer, and they contain receptors above their lipid bilayer membrane. They carry lipids, proteins, miRNAs, mRNAs, and small DNA fragments within them to protect the degradation of these molecules [117–120]. The exosomes have specific surface markers like TSG101, Alix, Flotillin-1, CD63, and CD9 and are present in different cell culture-conditioned media as well as body fluids like saliva, synovial fluid, urine, semen, blood, and breast milk [121–124]. Different treatment strategies have been proposed for controlling the proliferation and differentiation of CSCs. Since the cell surface marker, CD44, is highly expressed in hepatic CSCs, it has been targeted for liposomal drug delivery to control hepatic CSCs [125], and exosomal delivery of anti-CD44 antibody can be a future aspect for targeting the CSCs. Similarly other CSC markers like CD24, CD133, epithelial cell adhesion molecule (EpCAM), and CD200 can also be attached to the surface of the exosomes for targeting the CSCs. Multiple clinical trials are going on for targeting the cancer stem cells using exosomes as nanocarrier [126].

17.3.5 Nanoporous Materials

Mesoporous silica is the most commonly used nanoporous materials and is synthesized using tetraethyl orthosilicate which reacts with micellar rod templates. The porous form of silica thus synthesized is a collection of rods or spheres of nano-size filled with numerous pores arranged regularly. They are usually of two types, MCM-41 and SBA-15. Mesoporous silica based-nanoparticles, encapsulating γ-secretase inhibitors (GSIs), were used to control the notch signaling driven stem cells and enhance the tumor reduction in medulloblastoma [127]. Notch signaling inhibitors were loaded in mesoporous silica to deliver the drug in BCSCs which are susceptible to more glucose consumption. The results showed reduction in CSC population as well as size of the tumor both in vivo and in vitro [128]. The typical porous nature of mesoporous silica is shown in Fig. 17.5.

Thus, the different nanoenabled drug delivery systems used so far for targeting the CSCs have been discussed, and the outcome of such treatment strategies was also reviewed in the above sections.

17.4 Conclusion

The major problem in addressing the chemoresistance and multidrug resistance in cancer therapy is the inability to combat the CSCs through any drug. These CSCs migrate to a different site and initiate different tumors, thereby spreading the cancer. This warrants the need of targeting specifically the CSCs and delivers the chemotherapeutic drug to these populations and killing them. The CSCs have been known to exhibit different cell surface biomarkers as well as pathways of internal signaling which are involved in their self-renewal and the drug resistance. CD44 and CD133 are commonly identified in many cancer types. If these biomarkers can be targeted using some novel drug delivery system, it can improve the CSC killing thereby improving the drug resistance, eradication of CSCs, and possible cure for cancer.

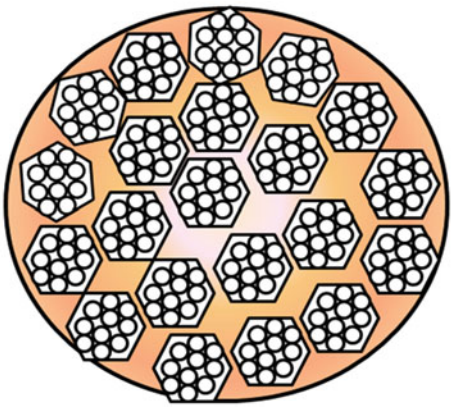

Fig. 17.5 The porous structure of mesoporous silica which enables the drug to be loaded in the small pores to reach the target site

Nanostructure-based therapeutic strategy has been recently evolved for effective cancer treatment due to their specific properties like (1) sustained drug release, (2) designing and development of personalized medicine, and (3) improved bioavailability of drugs and use in multifunctional therapy. In this chapter we have discussed about the different nanoenabled drug delivery systems and their possible therapeutic research done so far to target the CSCs. The nanodrug delivery systems could deliver a single or multidrug to the CSCs with particular biomarker targeting molecules attached to their surface for the CSC targeting. The targeting of CSCs was done using small chemical ligands, peptides, lipids, polysaccharides, and surface markers which have selective affinity for the CSCs and attaching them with the nanocarrier along with the chemotherapeutic drug. Both active and passive targeting were used to eradicate the CSCs. Thus, the different targeting strategies can enable to wipe out the CSCs and open a new avenue in the near future for the cancer treatment.

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