Adeeb Shehzad *Editor*

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Preface

Despite the recent decline in cancer incidence rates, long-term mortality rates remain unchanged. One of the most important factors for increased survival of cancer is detection at an early stage. Clinical assays that detect the early events of cancer through the use of molecular signatures or biomarkers offer an opportunity to intervene and prevent cancer progression. Molecular signatures of the phenotype of a cell that aids in early cancer detection and risk assessment will likely play an important role in screening and early detection. Although new information and technologies are clearly the driving force in biomarker discovery, translating new findings into clinical application remains a major challenge.

Tumor development is a complex process requiring coordinated interactions between numerous proteins, signaling pathways, and cell types. As a result of extensive studies on the molecular pathogenesis of cancer, several novel regulatory pathways and networks have been identified. The steps in these pathways have delineated a number of unique events in cells, marked by morphological and histological changes and altered expression of genes and proteins. During the transformation of a normal cell into a cancer cell, the cell signature changes, and these changes become unique signals of their presence and inherent features. By reading these signals accurately, we can improve the early detection and diagnosis of individual cancers. After decades of using basic research in an attempt to unravel the underlying cellular and molecular mechanisms of cancer, the scientific community has uncovered novel candidate targets for the early detection of cancer. By the time a tumor is detected, several molecular changes have already occurred. Diagnostic assays to detect these changes using BIOMARKERS have considerable potential for early detection.

Discovering cancer biomarkers is a relatively easy process based on the number of papers published every year on this subject. However, translating these discoveries into useful clinical assays will be very difficult. To date, fewer than 25 cancer biomarkers have been approved by the US Food and Drug Administration (FDA) and most of these are for monitoring the response to therapy. In the field of biomarkers, much of the biomarker research remains "stuck" at the discovery phase. A number of explanations have been given for the lack of cancer biomarkers being moved into clinical use. These include the high-performance standards needed to make a biomarker clinically useful, the complex biology of tumors, a flawed discovery process, lack of validation or a validation process that is cumbersome

and expensive, regulatory requirements, and an academic system that does not reward translational research.

Biomarker research requires a knowledge-driven environment in which investigators generate, contribute, manage, and analyze data available from a variety of sources and technological platforms. The goal is a continuous feedback loop to accelerate the translation of data into knowledge. Collaboration, data sharing, data integration and standards are integral to achieving this goal. Only by seamlessly structuring and integrating data sources will the complex and underlying causes and outcomes of cancers be revealed, and effective prevention, early detection, and personalized treatments be realized. There is a general consensus that if markers from the early stages of the tumor may be identified, then treatment is likely to be more successful.

Screening tools are needed that exhibit the combined requirements of high SENSITIVITY and high SPECIFICITY for early-stage cancers which are widely accepted, affordable, and safe. Significant improvement in our basic understanding of the biology of cancer initiation and progression has shown that oncogenes and tumor suppressor gene mutations can be identified in bodily fluids that drain from an organ affected by the tumor. In this book, a number of chapters discuss the various aspects of biomarkers, from discovery to development to validation to clinical utility and clinical use. Clinical utility refers to the ability to make clinical decisions and improve outcomes.

The chapters in the book are organized to shed lights on biomarker discovery, development, and validation. We also highlight clinical needs in early detection, the natural history of the disease and associated evidence in support of biomarker research, an overview of the current state of the art in biomarker research, current progress toward bringing these biomarkers into clinical use, and the future of biomarker-based screening and early detection for the respective organ types. This book also addresses the use of nanotechnology in the current screening strategies and discusses deficiencies with the present practice and identifies clinical needs that may benefit from biomarker-based approaches.

It is hoped that readers will appreciate the complexities of biomarker research, especially for detection and screening, and be inspired or inspire others to take the challenging tasks of biomarker discovery, development, and validation. In the era of precision medicine, biomarkers have an important role to play by only not identifying disease-specific molecular changes, but also in identifying targets for precision treatments. Early detection and precision medicine are inseparable approaches and will jointly inform the future course of actions in the clinical management of diseases. The use of biomarkers (for instance in triaging patients who are likely to benefit and who will not benefit from subsequent diagnostic workups) will not only benefit clinical management but will also be an important part of health economics in which the cost to the society and medical care will be optimized, reduced, and improved.

Islamabad, Pakistan Adeeb Shehzad

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Editor and Contributors

About the Editor

Adeeb Shehzad is currently serving as Assistant Professor in the Department of Biomedical Engineering and Sciences at the School of Mechanical and Manufacturing Engineering, National University Sciences and Technology, Islamabad, Pakistan. He completed his MS (2008–2010) and PhD (2010–2014) from the School of Life Sciences at Kyungpook National University, Daegu, South Korea. He has earlier served as a Research Professor in the School of Life Sciences at Kyungpook National University, Daegu, South Korea (2014–2015) and Visiting Professor at Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman bin Faisal University (IAU), Dammam, Saudi Arabia (2019–2021). His research interest is mainly oriented to the screening and development of plant-isolated compounds as anticancer agents, as well as the in vitro and in vivo studies of synthetic drugs on modulation of different signaling pathways in cancers. He has been conferred with various scholarships, notably Kyungpook Honor Scholarship and Higher Education Commission awards for MS and PhD studies. He is also serving as a referee for well-known international research journals. He has more than 10 years of teaching experience in Cancer Biology, Pharmacology, and Biotechnology.

He has also published more than 70 research articles in peer-reviewed international journals and authored or coauthored numerous book chapters. He is a member of many international scientific societies and organizations, importantly Korean Society of Molecular and Cellular Biology, South Korea, honorary member of Drug Regulatory Authority of Pakistan (DRAP), and Pakistan Pharmacist Association, Pakistan.

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Introduction to Cancer Biomarkers

1

Musawira Iftikhar, Aroosa Younis, Young Sup Lee, and Adeeb Shehzad

Abstract

The global increase in cancer rates and mortality warrants the identification of biomarkers that are mechanism-and-disease-specific for the detection, diagnosis, disease progression, and development of new regimes for treatment. Cancer biomarkers are biologically active molecules including proteins (enzymes or receptors), nucleic acids (coding and noncoding RNAs), immunoglobulins, or shorter chains of amino acids or peptides. A biomarker can also be used for the detection of modifications in gene expression or protein activity and epigenetic changes or productions of stimuli-induced antibodies by either tumor or healthy cells under normal or pathological conditions. These biomarkers carry a unique and identifiable molecular structure, such as extent and activities of the genome, polypeptides, or epigenetic alterations in circulatory fluids (whole blood, serum, or plasma), excretory fluids (stool, urine, sputum, or milk), and tissues, providing great potential for early diagnosis, monitoring, and selecting a suitable drug for patients with cancer. This chapter underpins the recent findings of cancer biomarkers concerning their expression pattern, molecular and biochemical characterization, diagnostic and therapeutic utilization, and translation into the clinics for the therapeutic intervention of patients with cancer. Several studies have

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reported various prognostic and predictive cancer biomarkers, although few have been commercialized. However, large multicenter validation studies are required to elucidate their effectiveness and role in translation to the cancer clinics for development into personalized medicines for the management of patients with cancer. Finally, we discuss the potential role of nanotechnology in the development and validation of future prospective cancer biomarkers. In this chapter, we summarize the processes for discovery and development of important diagnostic biomarkers with clinical utility, informing clinical monitoring to improve outcomes of patients with cancer.

Keywords

Cancer · Biomarkers · Molecular markers · Prognosis · Diagnosis · Proteomics

1.1 Introduction

In recent years, great effort has been made in the early detection, typing, and staging of cancer, which consequently has impacted patient management, duration, and selection of treatment. Traditionally, Chinese, Ayurvedic, and Egyptians have used a variety of substances to identify and differentiate between breast cancer and mastitis as well as markers for severe and acute malignancies. The first fluid test and examination were reported by Bence-Jones in 1847, wherein he identified a cancer-associated immunoglobulin G (IgG) protein, which is light chain antibody protein produced by the patients of multiple myeloma and can be detected in the urine of patients due to the heat denaturation and coagulation properties of IgG. In 1986, Sinclair et al. also identified and confirmed that patients of multiple myeloma have Bence-Jones protein in their sera (Dimopoulos et al. 2007). There is evidence that the Federal Drug Authority (FDA) approved and validated the use of Bence-Jones protein for the detection and confirmation of various cancers including multiple myeloma and leukemia. In 1867, Michael Foster evaluated the pathology and physiology of the pancreas and observed an increase in the levels of urinary amylase in patients indicated that pancreatic cancer originated from the ductal cells and not from the acinar cells (Shehzad et al. 2021). The use of polyclonal antibodies by Yalow and Berson in 1950 allowed the use of cancer biomarkers and caused a major shift in the diagnostic strategy and evaluation for precise cancers (Shehzad et al. 2021). In 1965, Joseph Gold reported the use of carcinoembryonic antigen (CEA) for the detection of colon cancer as it was usually only expressed in fetal tissue (Poole 2016). In the early 1970s, a serum diagnostic test had been developed and was commercially available for the detection of various cancers, such as the CEA immunoassay additional biomarkers that were developed in 1975 were monoclonal antibodies and in 1982, CA 19-9, CA 15-3, and CA-125 were developed for colorectal cancer (CRC), breast, and ovarian cancer, respectively. These markers are used in routine practice as reliable indicators in patients with cancer (Hou et al. 2012). Moreover, many studies have successfully translated the use of cancer

biomarkers into clinical use. The electrophoretic peaks of urine and monoclonal serum are used for myeloma detection, human chorionic gonadotropin and α-fetoprotein is used for germ cell cancers, and alkaline phosphatase is used in bone tumors and prostate-specific antigen for the detection of prostate cancer (Kalof et al. 2010; Shehzad et al. 2018a, b). Despite these recent advances in molecular techniques and the discovery of various cancer biomarkers, very few have been approved by the FDA for diagnostic and therapeutic purposes in the clinic. High-throughput genomic and proteomic studies are urgently required for the identification and understanding of the putative functions of cancer biomarkers for their diagnostic and therapeutic applications in the management of patients with cancer (Shehzad et al. 2020).

There has been a dramatic increase in the incidence and mortality rates of cancer in recent years, and cancer has been declared a major health problem. According to the World Health Organization, cancer is expected to be the leading cause of death, with an estimated 10 million deaths and 18.1 million incidences globally in 2020 (Ferlay et al. 2020; Shehzad et al. 2019). Extensive knowledge of the underlying mechanisms of cancer development, its early detection and identification using cancer biomarkers, suitable treatment with proper regimes, and patient monitoring with follow-up measures could certainly decrease cancer incidence and burden around the globe (Arbyn et al. 2020). The definition of biomarkers according to the National Cancer Institute is a biological molecule or a substance that is present in bodily fluid, tissues, or blood, which indicates a conventional, aberrant, or pathophysiological process of any specific disease, such as cancer. Generally, biomarkers can differentiate between healthy and infected tissue at the early stages of the disease or can indicate the risk of developing, or the presence of, disease. The number of biomarkers being discovered is growing continuously, and a wide range of biomarkers can include polypeptides (enzyme or receptor), RNA (coding and noncoding RNAs), antibodies, and peptides concentrations Shehzad (2021). Biomarkers are present and detected in the bloodstream (whole blood, serum, or plasma), secretary fluid (stool, urine, sputum, or milk), or tissue-derived, which need special biochemical techniques for its evaluation, such as biopsy and special imaging including mass spectroscopy of low-molecular-weight plasma peptides for surface-enhanced laser desorption/ionization to characterize the ovarian tumor cells and can be removed from the surrounding healthy cell populations (Kohler et al. 2011; Shehzad et al. 2013d). Bioinformatics studies and peptide assays describe markers that differentiate between tumor and nontumor patients, independent of the insight of disease the patterns represent. Cancer biomarkers can be indicators of modifications or alterations present in gene or protein expression, epigenetic changes, or dysregulated metabolism. Some biomarkers may be inherited from ancestors, and their mutation in sequence can be detected in both germline and tumor-derived DNA (AlDubayan et al. 2018).

The term cancer biomarker refers to a biological substance that is produced in response to an infection, which can be used as an indicator to differentiate between the normal process or carcinogenic processes at different cellular stages; tumor-associated antigens are one such biomarker that is a clinically significant marker for

the detection of tumor (Davis et al. 2019). Cancer biomarkers are efficient diagnostic tools to calculate risk, screen, and detection of cancer, and they can differentiate between benign and malignant tumors and predict and monitor the status and overall outcome of therapeutic intervention and patient management in a clinical setting (Kalinke et al. 2020; Shehzad et al. 2017). In line with this, many processes are required for approval, validation, and translation of biomarkers into the clinical setting for their use in the early detection and treatment of cancer. In this chapter, we detail the various processes required for the identification and validation of important diagnostic biomarkers that have clinical use and inform the clinical monitoring for improved outcomes for patients with cancer.

1.1.1 Clinical Pathology of Cancer and Biomarkers

Cancer is a broad term that refers to a group of diseases identified by an abnormal increase in the proliferation of cells. Cancer arises and progresses due to the accumulation of genetic and epigenetic alterations in the cell, which ultimately leads to disruption in the cell cycle and enhances the role of regulatory proteins triggering the proliferation of cells and reducing the function of proteins that typically prevent cell proliferation (Shehzad et al. 2016, 2017). Successive mutations in oncogenes and truncation or deletion in the coding sequences of tumor suppressor genes contribute to the formation of cancer. In particular, cancer is caused by two types of genomic mutations: chromosomal instability (CIN) and microsatellite instability (MIS) (Shehzad et al. 2013a). CIN is characterized by abnormal chromosomal segregation and accumulation of abnormal DNA content, whereas MIS results in the loss of gene function for proteins that are normally involved in the repair of DNA during DNA replication in dividing cells (Shehzad et al. 2013b). There is compelling evidence that suggests that epigenetic changes that occur due to methylation and/or acetylation of the promoter genes, and histone modifications, can also trigger the development of cancer through condensation and alteration of the chromatin (Shehzad et al. 2014a). Generally, tumors begin and extend with abnormal proliferation of cells (hyperplasia) to form an uneven morphology and acquire an adequate supply of nutrients and oxygen through the growth of new blood vessels (angiogenesis). Consequently, it can reach secondary sites where it can develop new tumors (metastasis). Cancer development is also mediated by the overexpression of survival genes, growth factors, antiapoptotic genes, and those genes participating in drug resistance (Shehzad et al. 2013c). Globally, cancer is a devastating public health burden, and its early detection and identification can decrease its morbidity and mortality rates. Genetic alterations of cancer cells and dysfunction of the signal transduction networks of various proteins, enzymes, receptors, and growth factors misregulate cellular processes and cell-to-cell communications, which can be detected by the release or expression of biomarkers in several patients with cancer. Therefore, a thorough understanding of these biomarkers may provide early assessment in multiple cancers, including type and stage of cancer, the differentiation between a benign and malignant tumor, determining prognosis and predictive factors

for patients with cancer, monitoring the recurrence and drug resistance, and response to therapeutic interventions within clinical settings (Lánczky et al. 2016). A thorough understanding of the underlying molecular mechanisms, cellular processes, and dysregulated signaling networks of cancer tumors, as well as advancing high-throughput and biochemical analysis may identify cancer biomarkers and determine their clinical use in patients with cancer. The development of advanced assays is required for the comparison of the therapeutic efficacy of biomarkers with cancer pathology, followed by their association with other cellular markers for early detection, disease nature, and therapeutic intervention for particular cancers. A significant factor for oncology research is the development of precise and standardized tools for measuring the type and stage of cancer against different currently known biomarkers (Shehzad et al. 2014b). A deep understanding of normal variations, preneoplastic status, grade of neoplasm, and stage will allow cancer biomarkers to be useful for the treatment and prevention of cancer, as well as for better management of patients with cancer.

1.2 Serum, Biological Fluid, and Tissue Cancer Biomarkers

The release of CB in tumors cells, blood cells, or other bodily fluids can allow the understanding of the underlying mechanisms of carcinogenesis. CB molecules are detected during the initiation, proliferation, progression, or metastasis of cancer. CB molecules can be present in biological fluid as a result of three possible mechanisms: epigenetic changes, amplification of a gene product, or overexpression of a gene. In ovarian cancer, human epididymal secretory protein 4 (HE4) has been identified as a biomarker that is overexpressed and can be detected in serum (Al-Amodi and Kamel 2016).

Understanding carcinogenic components can help elucidate the formation and secretion of CB in blood, bodily fluids, and infected cells and understand their progressive increase during malignant growth, progression, and metastasis. The increase in CB expression levels in various samples can be identified in three ways. First is the overexpression of the biomarker in the sample as a result of an increase in the epigenetic modifications, such as methylation of DNA, which results in the presence of CB, such as HE4 in ovarian malignancy. HE4 overexpression in ovarian carcinoma can be easily identified in serum (Harmsma et al. 2013). HE4 overexpression is also present in endometrial, breast, and bronchial adenocarcinoma (Harmsma et al. 2013; Mahmoud et al. 2014). The second indication is the increase in serum biomarkers. One such example of a serum biomarker includes alpha-fetoprotein (AFP), a single-peptide oncofetal protein, utilized for patients suffering from hepatocellular carcinoma. Another serum biomarker is HER2-neu, a cell layer surface-bound tyrosine kinase, which is increased in the serum of patients with breast cancer, as a result of it undergoing proteolysis (Jain 2017). The FDA specifies that HER2-neu should be evaluated for the presence of breast cancer metastasis. The third indication evaluates the presence of invasive cancer cells and angiogenesis, and prostate-specific antigen (PSA) can be utilized in this manner in patients with

prostate cancer (Osborne et al. 2011). An elevation in PSA levels can occur due to a misshaped cellular layer of the prostatic cells and/or lymphangiogenesis (Shehzad et al. 2013a). The clinical utilization of CB, particularly circling proteins, is progressing due to an improvement in the "omics" discoveries of disease biomarkers present in bodily fluids that are allowing the development of novel and sensitive diagnostic tools for the early development of malignant growth. Of significance are those malignant growths that are not currently effectively diagnosed, such as nasopharyngeal, ovarian, and pancreatic diseases (Thurnham and Northrop-Clewes 2016; Shehzad et al. 2013b). CB can be distinguishable among the malignant tissues, cells in the lymph system, bone marrow, or circulating cancer cells (Comen et al. 2018). CB can be detected in natural bodily fluids including, serum, plain liquid, pleural liquid, or urine, which are all noninvasive samples for analyzing. Cerebrospinal fluid is suitable for the cerebrum and CNS malignancy. Urine is among the most promising samples for identifying bladder malignancy, as well as patient observation (Dube et al. 2019). Likewise, prostate disease antigen 3 (PCA3) is another promising biomarker for the diagnosis and development of malignancy in the prostate (Grambergs et al. 2019). Stool samples are utilized for colorectal malignancy, and areola suction liquid, ductal lavage, and pimple liquid are different liquid types for breast cancer that can be utilized for evaluation of CB (Grambergs et al. 2019).

1.3 Clinical Applications and Performance Indications of Cancer Biomarkers

The clinical usefulness of CBs was previously limited as an effective tool for patient diagnosis and treatment. The fifth International Conference arranged on the topic of Human Tumor Markers clearly defined the definition of tumor markers (Gyani et al. 2014). In 1988, in Stockholm, Sweden, biochemical tumor markers were defined as substances that were formed in cancer cells and discharged in body liquids where their quantification can be obtained through nonobtrusive examinations. The correlation between marker levels and dynamic tumor mass, and tumor markers are useful for the management of malignancy patients (Rizza et al. 2018; Shehzad et al. 2012). Markers, accessible in most disease cases, are a significant tool for the prediction of patient outcomes, although they are by and by inappropriate for screening. Serosymptomatic estimations of markers should accentuate relative patterns rather than be utilized as outright cutoff levels. However, CB can be utilized additionally for the screening of all-inclusive communities, progression risk, differential determination, and clinical arranging of patients with cancer (Abuawad et al. 2021). Moreover, CB can be utilized to understand the tumor risk and indicate the progression and clinical endpoint (Wiley 2020). Those biomarkers that are commonly used in clinical practice include PSA, AFP, CA125, and CEA. Among all those serum biomarkers, PSA is utilized to evaluate the risk and development of prostate disease. Disease antigen CA-125 is a biomarker of ovarian malignancy, although it has low sensitivity and specificity. CEA is another biomarker that is elevated in patients with

colorectal, breast, lung, or pancreatic malignant growth (Garg et al. 2021). Other promising biomarkers include survivin and HER2-neu (Lianidou et al. 2014). A significant development would be to progress a reliable CB test that allows the detection of patients with cancer and tailor treatment options based on their CB expression levels to improve patient outcomes.

1.3.1 Sensitivity and Specificity for the Evaluation of the Accuracy of CB

Tumors release CBs and can therefore be identified in body liquids, discharges, or cancer tissues and cells (Garcia-Cao et al. 2012). CB can be identified in circulatory fluids, such as blood, plasma, or serum, as well as in discharges, such as sputum, urine, or CSF. Therefore, CB evaluation can be conducted in a noninvasive way. The assessment of malignant growth biomarkers present in cells or tissues requires different methods, such as tissue biopsy, which is a more intrusive method than analysis of serum biomarkers. Hereditary biomarkers can be identified in the DNA of tumor tissue, buccal mucosa cells, or blood (Joshi et al. 2019). Assessment of the symptomatic estimation of any biomarker is typically performed concerning the sensitivity and specificity of that particular biomarker. Specificity indicates the ability of that biomarker to distinguish between healthy and diseased patients, whereas sensitivity alludes to the capacity of that test to identify a particular biomarker in those patients with cancer (Garcia-Cao et al. 2012). At complete biomarker cutoff levels, the biomarker values may be over the positive cutoff range, however not all of those patients who fall above the positive cutoff value are diseased patients and are therefore known as false positives. Therefore, sensitivity is determined by the proportion of all positive cases over the positive cutoff value, against the absolute number of genuine positive cases; sensitivity is the genuine positive rate (TPR) (Aada and Tiwari 2019). Essentially, by applying a similar cutoff level for similar tests, few people with ordinary outcomes underneath the cutoff values are typical (genuine adverse), and not every one of them is diseased (false antagonistic). Subsequently, the genuine negative rate or specificity can be identified as the proportion of all negatives identified underneath the cutoff value compared with the number of genuine negative patients (Black 2018). Assuming that the sensitivity of a given CB is 100%, this implies that it will identify all disease patients, and if another CB has 90% sensitivity, it will only be able to identify 90% of patients with a malignant growth (genuine positives) but will miss 10% of malignant patients (false negatives) (Wu 2020). Therefore, sensitivity and specificity could be applied across all conceivable cutoff limits and both are equally identified with one another (Naqi et al. 2019).

1.3.2 Receiver Operating Characteristic (ROC) Curve Examination

The investigation of various sensitivities and specificities at varying limits would be useful to develop the precision of diagnostic tests. During World War II, the ROC curve was presented by the British to identify exact radar indicators and later utilized for assessing radiological tests (Bleibel et al. 2006). ROC curve can be utilized to evaluate a biomarker by plotting its sensitivity along the *Y*-axis and its specificity or false-positive rate (FPR) along the *X*-axis to evaluate its diagnostic ability in separating healthy and unhealthy subjects (Duev et al. 2019). ROC curve has been broadly utilized for evaluating the precision of a diagnostic test and can provide important data on its use. Such data that the ROC curve provides includes the area under the curve (AUC) and indicates the normal sensitivity levels for all conceivable specificity values and includes the entire area under the whole ROC curve (Lin et al. 2018; Ponnibala et al. 2021). The AUC will have values in the range of one to zero as the estimations of *X* and *Y* presumably also have values going from zero to one. The closer the estimated value of AUC is to 1, then the better the clinical execution of that test where it can distinguish between positive and negative cases (Zheng 2018). The test with a greater AUC value is generally of better execution (Liu et al. 2014), whereas comparing the two tests if both AUC regions are equivalent indicates the same diagnostic ability of the two tests (Isomaa et al. 2013).

1.3.3 Ideal Biomarkers

Biomarkers that fall outside of the cutoff points will result in patients being identified as a false-negative; however, this will diminish the number of false positives, which indicates the specificity of the biomarker (Ceccarini et al. 2015). Likewise, if the cutoff point is low, it will have good sensitivity but low specificity, as there are fewer false-negative subjects but more false-positive subjects. In line with this, sets of sensitivities and specificities may portray the preciseness of the biomarker and its capacity to segregate between healthy and unhealthy (Ceccarini et al. 2015). If the cutoff of the sensitivity is 100% or less, then we must consider the implications of what that value means for the patient (Ray et al. 2010). The cutoff values will predict whether that patient undergoes further examinations between tests or not (Ballman 2015). An ideal biomarker should be able to differentiate between tumor and benign cases and aggressive tumors from nonaggressive ones, and it must be of high specificity and sensitivity. Moreover, the isolation of that biomarker must be noninvasive and economical (Hayes 2015). The ideal biomarker needs to require as little hands-on approach as possible in its evaluation, and the CB needs to satisfy the following listed general properties to be an ideal candidate. Currently, no biomarker meets these prerequisites; however, these models should be considered for the identification of a diagnostic biomarker (Andre et al. 2011).

- High clinical sensitivity: identified in all patients with a particular disease (100% TPR).

- High clinical specificity: low false-negative rate (100% true negative).
- Organ- or tissue-specific.
- Proportionality to tumor weight or volume: quantitative proportionately to tumor volume or illness movement.
- Shorter half-life: rapidly reflecting early changes in tumor progression for legitimate observation of treatment.
- Present (assuming any) in the serum of healthy people and patients with infection at low levels.
- Clear metastasis segregation.
- Exists in measures, such as quantitative, normalized, reproductive, and approved measures.
- Inexpensive to obtain and analyze.
- Obtained in a noninvasive way—identified in serum, body fluids, or effectively open tissue.

1.4 Clinical Uses and Limitations of Cancer Biomarkers

Tumor biomarkers or CB are usually either proteins or glycoproteins, which are presumed not to be directly involved in carcinogenesis or advancement of the disease but rather a by-product of the cellular dysregulation (Pan et al. 2015). Low subatomic weight and small particles or markers of nucleic acids, lipid metabolites, proteins, and peptides are all promising biomarkers and have recently been evaluated for their use. The use of CB relies upon various elements including their synthetic nature, proposed systems for their delivery, and applications. Six years ago, Mishra and Varma proposed the characterization and use of CB in a clinical setting (Kuspinar and Mayo 2014). They grouped CB into recognition biomarkers such as atoms of RNA, diagnostic biomarkers such as proteins, and visualization biomarkers such as glycol biomarkers. The clinical application of CB is for the early identification, confirmation of the symptoms, evaluation of the predicted therapeutic response, and disease monitoring (Kuspinar and Mayo 2014; Ward 2019). CBs can also be used to indicate those patients at high risk of progression and those that would benefit from early interventions (Ward et al. 2015). Biomarkers can indicate aggressiveness, oxidative pressure, single-nucleotide polymorphisms, and other mutations (Ward 2019). As confirmed by the FDA, CBs can have clinical significance in a range of disease types.

1.4.1 Screening/Early Identification

Screening is characterized as the orderly utilization of a test to recognize those subjects who are at adequate danger of developing a particular problem to profit by additional examination or direct preventive activity, among people who have not looked for clinical consideration by virtue of side effects of that issue (Lorincz 2016). In asymptomatic patients, earlier therapy interventions have a much more

favorable outcome compared with interventions at a later stage where the tumor is progressing. Data suggests that there was a fall in the 5-year survival rate in patients with breast cancer from ~90% in patients with early localized breast cancer to ~60% in local metastasis, and 30% in distant metastasize (Zhao et al. 2018). Screening for CB would allow the identification of malignant growth at the early or asymptomatic stage and will result in an increment in the survival rate. A screening test should have high specificity to limit the false positives as much as possible (Sicsic and Franc 2014; Shehzad et al. 2011). Having high specificity reduces the FPR and ultimately reduces the need for unneeded and further intrusive diagnostic techniques. An ideal screening program must be noninvasive, economical, and unquestionably leads to an evident decrease in mortality rates and subsequently increases the survival rate. As a rule, all screening programs are coordinated for malignant growths, and further treatment and follow-up are required (Sicsic and Franc 2014; Zhao et al. 2018). One restriction with screening for CBs is that they may not be elevated until later in disease progression. However, there are not many biomarkers that are used as screening biomarkers, such as AFP, which is used in the screening of hepatocellular malignant growth in those patients who are considered high risk. PSA is used for screening for the development of prostate disease, CA125 in detecting ovarian malignant growth, and fecal occult blood testing (FOBT) for the screening of colorectal tumors (CRC), and vanillyl mandelic corrosive (VMA) for the screening of neuroblastoma in infants (Prensner et al. 2012). The approval of PSA as a screening biomarker for prostate malignant growth has been informed by the FDA; however, levels of PSA can increase in patients without cancer as a result of benign prostatic hyperplasia and prostatitis, resulting in false positives (Prensner et al. 2012). The use of PSA screening for reducing the mortality rate is controversial (Amaro et al. 2014).

1.4.2 Identifying/Differential Determination

A diagnostic biomarker can be used for at risk patients as well as for those already symptomatic. An ideal diagnostic biomarker must have similar attributes as those needed for screening. The vast majority of established biomarkers used for screening can also be used as diagnostic markers, such as PSA, which is a very well-established model. PSA, together with a digital rectal examination, is the most generally utilized diagnostic tool for prostate malignancy (Baker 2009). The constraints for symptomatic accessible biomarkers include low symptomatic sensitivity and specificity; however, diagnostic biomarkers need to have high sensitivity to be a suitable diagnostic biomarker (Baker 2019; Shehzad et al. 2010). For instance, the Bence-Jones protein in urine is one of the most established symptomatic markers of various myelomas (Rundle et al. 2012). In any case, some CB are valuable in confirming analysis, frequently used with a suite of different markers, particularly to distinguish metastatic tumors alongside other clinical imaging instruments (Simon 2011). Utilization of a series of CB to improve sensitivity and specificity of diagnosis has been conducted for the analysis of specific malignant

growths (Huang et al. 2017a, b). It has been reported that a panel of four biomarkers comprising leptin, osteopontin, prolactin, and insulin-like development factor 2 altogether have a sensitivity and specificity of 95% for the detection of ovarian malignant growth (Bergman et al. 2013). Furthermore, two other biomarkers, when used with the aforementioned biomarkers, CA125 and macrophage inhibitory factor, will increase the sensitivity from 95% to 99.4% for the detection of ovarian cancer (Munksgaard and Blaakaer 2012). Different studies have aimed to improve the detection sensitivity and specificity using a mix of CA125 with ultrasonography for the diagnosis of ovarian malignant growth (Bell et al. 2013).

1.4.3 Prognosis/Estimation

Prognosis is the likelihood of cure or the outcome for any patient. A prognostic marker is obtained from a patient at diagnosis to indicate the disease stage and likelihood of favorable outcome independent of treatment. In the interim, a predictive biomarker allows the understanding of the need for various treatment types; subsequently, a present biomarker is fundamental for customizing treatments (Griffiths et al. 2002). The extent of the CB levels increases usually reflects the weight or mass of the tumor; subsequently, high CB levels generally can indicate a poor prognosis. As indicators of tumor stage, CB can also indicate the arranging framework for malignant growth or tumor-node-metastasis (TNM) characterization for tumor development. For instance, tumors in testicular germ cells that have elevated levels of a CB, such as AFP, LDH, and HCG-β, may indicate the aggressive nature of the disease with a poor prognosis. Therefore, such biomarkers can be used for the staging of the TNM framework set up with a site-specific factor, called a prognostic factor. LDH alone has been used for staging lymphoma tumors (Dalle Grave 2012). Nevertheless, the precision of the marker in determining tumor stage is poor. Estrogen receptor (ER) is a prognostic and predictive tissue biomarker, which has been utilized for deciding on which patients will respond to hormonal treatment. Along these lines, patients with ER-positive tumors will generally respond favorably with ER modulators or aromatase inhibitors, independent of the stage of the breast cancer. ER is considered as a prognostic marker because when tumors are ER-negative, it indicates a poor prognosis, and ER-positive patients will have a better prognosis (Osborne et al. 2011). In a similar setting, high levels of HER2 in the serum of patients with breast cancer have a poor prognosis. Treatment for HER-2 positive breast malignant growth patients includes trastuzumab (Herceptin), which is a recombinant monoclonal immunizer. Herceptin has been used in females suffering from metastatic breast malignant growth that overexpresses HER2 and is the first-line chemotherapy used in patients. KRAS is a biomarker that is used for colorectal malignancy, and patients with substantial changes in KRAS have a poor response against epidermal development factor receptor (EGFR)-mediated treatments (Hartig 2011; Stolow 2020).

1.4.4 Therapeutic Monitoring/Follow-Up/Evidence of Metastasis or Recurrence

Clinically valuable biomarkers normally vary as per the tumor status, size, or burden changes, and incremental changes of CB can be associated with the advancement of the diseases or indications of remission. CB levels tend not to change with a stable tumor. The recurrence of a disease can be identified biochemically by monitoring any elevation in the levels of CB in asymptomatic patients and prior to clinical assessment. Continuous monitoring of the patient during and posttreatment will indicate the successfulness of the treatment provided based on the levels of CB. However, increasing CB values from the basal level indicates the reappearance of the disease. Numerous CB can be used to monitor the treatment, identification of recurrence, or metastasis; for example, CEA in colorectal cancer, cancer antigen 125 (CA 125) in ovarian tumors, and PSA for prostatic tumors, respectively (Al-Amodi and Kamel 2016).

In a few patients wherein the treatment strategies are not working, they will encounter escalating levels of CB, and depending on patient prognosis, the elective treatment should be stopped. Assessment of CB as a screening and predictive biomarker should be diagnostically accurate and specific to tailor viable treatments, continue the beneficial treatments, or early termination/substitution of ineffective treatment for those cancers. Carbohydrate antigen 19-9 (CA19-9) is a CB that is used in pancreatic CRC (Mystkowska 2015). The FDA endorsed the use of CA19-9 in 2002 as a biomarker of pancreatic cancer. However, it is not recommended to be used as a screening biomarker (Mystkowska 2015; Wang et al. 2011). Monitoring biomarkers have been broadly utilized in clinical practice, although when used as a detection biomarker, there are issues such as poor accuracy of the results (Ishiura et al. 2019).

1.5 Uses of CB in Malignant Cancers

Cancer is a global problem, and The American Cancer Society has stated that cancer is one of the primary causes of death, and causes one in four deaths in the United States. According to The American Cancer Society, it has been estimated that there was a total of 1,898,160 (970,250 men and 927,910 women) new cancer cases in 2021, and 608,570 deaths (319,420 men and 289,150 women) in the United States. A recent report from the International Agency for Research on Cancer, the GLOBOCAN recognized that the types of tumor among men were lung, prostate, colorectal, liver, and urinary bladder; whereas breast malignancy, liver, lungs, and ovarian diseases were the most widely found in females worldwide. In the most recent decade, there has been a large increase in the cost of medical care, combined with the restricted viability of single malignant treatment therapies, which has indicated the importance of biomarkers. CB is a compelling apparatus in clinical practices for the management of patients with cancer. Therefore, currently used or future CB may be used for the risk assessment of disease, screening among

asymptomatic individuals, confirming detection, differentially segregating between benign and metastatic tumors, calculating the expected outcome or prognosis, and evaluating the effectiveness of treatment (Hirschberg et al. 2016).

1.5.1 Breast Malignant Growth

Breast cancer is the most common malignancy among females and is the leading cancer cause of death worldwide. Its prevalence is increasing compared with other types of cancers in females (Westen and Morrison 2001). Therefore, it is important to use all accessible tools for its early diagnosis and appropriate management of patients with cancer. Clinically, the pathophysiology of breast cancer includes irregular lump formation, areola release, skin, or phenotypic changes. The screening instructions defined by The American Cancer Society indicate that women over the age of 40 years should have a mammogram and a clinical breast test either yearly or every other year (Heru 2013). Detection of breast cancer usually relies on patient discovery; nonetheless, the use of CB in breast malignancy is used to indicate prognosis, observing treatment success, and for follow-up. In particular, CB do not have a particular use for early analysis or diagnosis (Krishna and Rajabhushnam 2019). The evaluation of ER and progesterone receptors in the tissue can be used for the detection of breast cancer as indicated by the European Society of Medical Oncology. They can also be used for predicting the response to hormone therapy in the early stages of breast cancer and to manage advanced breast cancer disease (Krishnavenia et al. 2020). Another prognostic marker is HER-2, generally used in identifying patients with early or metastatic breast malignancy, and is used to identify patients who require therapy with trastuzumab (Herceptin) (Zebari et al. 2019) or tamoxifen treatment at the early stage of breast cancer development (De and Biswas 2020). Identifying those patients at a high risk and therefore should be included in a screening program utilizes the genetic mutations of *BRCA1* or *BRCA2* genes, which are present in up to 5% of breast malignant cases. Because of their high vulnerability to breast and ovarian cancer development, it is unequivocally suggested that females with BRCA1 or BRCA2 mutations should be routinely screened (Ceccarini et al. 2015; De and Biswas 2020). It is suggested that low levels of urokinase plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) correlate to a decreased risk of breast cancer recurrence and demonstrated to be a self-regulating prognostic element of a recently analyzed lymph node-negative breast cancer (Gowthaman 2019). The use of serum biomarkers is the most appropriate for monitoring during the treatment process, and to a lesser degree, they also function as prognostic markers, such as CA15.3, CEA, and BR (Wu et al. 2014). They are used in combination with different radiological and clinical applications to monitor chemotherapy progress in breast cancer cases. The rise in serum levels of these biomarkers may indicate a recurrence or cancer development (Fu and Li 2016).

1.5.2 Prostate Cancer

Prostate malignant growth (PC) is a common disease in men and is the leading cause of cancer-related death (Mohler et al. 2010). The PSA test has dramatically improved screening and diagnosis of prostate malignancy, and since its implementation, PSA has resulted in an increase in the number of detected cases at an early stage, thereby decreasing the mortality rate of prostate malignancy (Ilic et al. 2013). Numerous studies have improved upon the sensitivity of PSA as an asymptomatic marker by using PSA and its isoforms (pro-PSA) and comparing the percentage rate of pro-PSA with PSA; this may separate benign and malignant prostatic tumor presence in patients with PSA values from 4 to 10 μg/L (Mottet et al. 2014). Other novel and promising biomarkers under investigation include human kallikrein type 2, prostate cancer-specific antigen 3 (PSA 3), and prostate stem cell antigen (Andriole et al. 2012). Evaluating PCA 3 in urine samples may also be used for the detection of identifying prostate malignancy. Increased levels of metalloproteinase 2 and 9 (MMP-2 and MMP-9), members of the protease family, are associated with prostate malignant growth (Djulbegovic et al. 2010). MMPs have been investigated as biomarkers for therapeutic assessment and monitoring in prostate malignancy (Markt et al. 2015).

1.5.3 Ovarian Malignancy

A large proportion of patients with ovarian cancer present late in tumor growth and have usually progressed to stage III or IV, meaning poor survival rates. Therefore, there is an urgent need for a sensitive and specific diagnostic biomarker (Moore et al. 2011). CA 125 is the CB most often used for ovarian cancer. It is used as a screening biomarker for women with a family history of the disease or who are at high risk of developing an ovarian disease. CA125 is used in conjunction with vaginal ultrasonography as a reliable, diagnostic biomarker (Aziz et al. 2020). CA125 has additionally been used as a diagnostic biomarker, which diminishes with chemotherapy or medical procedures. Monitoring of CA125 14 days prior to the beginning of any therapeutic intervention and subsequent follow-up required for the continual monitoring of the disease are needed (Irungu 2016). Other biomarkers are currently under investigation for use as a biomarker of ovarian disease including kallikreins (Deo et al. 2019), hCG, interleukin-6 (IL-6), prostasin, TPA, lysophosphatidic corrosive, plasminogen activator inhibitor-1(PAI-1), Her-2/neu, tumor-related inhibin, CEA, and trypsin inhibitor (Anderson 2010; Deo et al. 2019; Huang et al. 2011).

1.5.4 Colorectal Malignant Growth

CRC is the third most common cancer worldwide. According to The American Cancer Society, a total of 149,500 new cases and 52,980 deaths related to colorectal cancer are predicted to occur in the United States in 2021 (Siegel et al. 2021). For

colorectal carcinoma, the most common site for malignancy is the rectum with 38% of all cases developing here, followed by sigmoid accounting for 29% of the cases (Chalya et al. 2013). The screening program for CRC should be provided to all asymptomatic people over the age of 50 years (Wolf et al. 2018), according to the National Academy of Clinical Biochemistry (NACB). Several screening techniques are available as previously described. Fecal occult blood test (FOBT) is the most widely used stool CB (Westwood et al. 2017). Testing for blood in the stool involves either identifying globin, which is a part of the blood (hemoglobin), using a fecal immunochemical test or the guaiac test that estimates pseudoperoxidase action of the heme portion of hemoglobin. CEA was brought into clinical practice in 1965 (Bevan and Rutter 2018), and it is generally used as an all-inclusive or non-organ, non-tissue-specific tumor marker. CEA is not used in the screening of CRC as it has low sensitivity, and there is a low prevalence of CRC among asymptomatic individuals. Nonetheless, CEA is a proficient prognostic and treatment monitoring biomarker (Spada et al. 2014). CEA testing is recommended at the start of treatment and subsequently every 1–3 months during the therapeutic routine; it is also used for deciding on treatment options for metastatic CRC cases (Brenner and Tao 2013). CA19-9 has been used as a prognostic marker to monitor CRC after surgical resection and as a monitoring marker for therapeutic interventions (De Wijkerslooth et al. 2012). CA242 and the tissue inhibitor of metalloproteinase type 1 (TIMP-1) are two more CBs that are under investigation, and both have been used in addition to CEA for the detection of patients with colorectal cancer (Chiu et al. 2013).

1.6 New Biomarkers/Approval/Advancements

In the last 20 years, the FDA has only approved a few of the large number of malignancy biomarkers identified for detecting, monitoring, or indicating the recurrence of cancer (Frisoni et al. 2017). The biomarkers need to have significant use in the clinical setting to enhance the outcome of patients with cancer, as well as have potential in diagnosis and therapeutics (Javitt et al. 2020). At first, CB needs to identify individuals with the disease and then used in follow-up to plan for treatment. In line with this, numerous biomarkers do not meet these ideal characteristics in the clinical settings as they are either unable to precisely indicate the therapeutic strategies, or their sensitivity and specificity are not as high as required (Wallentin et al. 2014). In reality, the proportion of effective clinical interpretations of biomarkers was low (0.1%) (Allinson 2018). Studies have shown that CB development is a multistep process, which starts with identifying the biomarker, developing a measuring approach for assessment, evaluating its clinical potential for beginners, normalizing the test, and finally approving the biomarker for clinical use (Bonora et al. 2019). In line with this, a well-established and organized stage model for the advancement of the assessment and interpretation of biomarkers for a clinical setting is warranted. This model is in agreement with other models commonly used in medication advancement procedures and comprises preclinical exploratory examinations, clinical measures and accuracy, large-scale longitudinal repository

studies, advancement in screening assessments, and finally malignant growth control considerations. In addition, the biomarker must be evaluated for its clinical use for providing state-of-the-art patient management, as well as support the therapeutic benefits of cancer therapy in patients (Fiserova et al. 2020).

1.6.1 Challenges for the Investigation of Novel Biomarkers

Improvements in biomarkers for the screening of malignancies, early detection, and observation of the treatment are hindered by biological and monetary difficulties. Most of the available diagnostic methods used are generally for detecting late-stage or fully developed malignant growth. However, premalignant or early neoplasm is amenable to surgery and prevention. Therefore, screening tests could recognize malignancy at the preclinical stage, although they may not identify micrometastasis, thereby restricting the advantage of early recognition and treatment (Goel et al. 2021). It is also challenging that in many organs, such as the prostate and colon, paraneoplastic injuries are much more typical than malignant cancers (Mathur et al. 2020). Therefore, screening techniques simply detect early cancers and do not necessarily dissect the underlying mechanisms and malignant behaviors of the tumors (Sureshkumar et al. 2020). The advancement of CB must consider the heterogeneity of the tumor; it contains numerous naturally interconnecting networks with various responses to therapeutic interventions. The improvement of biomarkers may be hampered by this heterogeneity, and as a result, the development of biomarkers using genomic and proteomic methods may be used to cautiously address these heterogeneity concerns (Fuchs and Buhmann 2011). Definite and extensive information on cancer at the molecular level has developed drastically in the last 20 years and has brought about critical improvement in the description of human tumors, which has been shifted toward the advancement of focused treatments, allowing customized treatment options (Shi et al. 2018). Hence, it is proposed that the development of profoundly ground-breaking technologies, such as genomics, epigenomic, transcriptomic, proteomic, and metabolomic studies, can advance scientific discovery and optimization with high accuracy over currently available biomarkers (Stewart et al. 2017). The aforementioned technologies have increased the number of potential biomarkers, such as DNA, RNA, and other protein biomolecules, that have potential in the clinical setting (Zoppi 2020). The previous idea of a single biomarker has recently been substituted with the discovery of multibiomarkers of genes or proteins, raising the question of whether heterogeneous and multifactorial malignant growth may have a single distinct mark (Myal et al. 2010).

1.6.2 Genomic Advancements

Genomic advancements have been used broadly for the interpretation of tumors at the submolecular level, which subsequently has given a better understanding of

malignancy and may provide researchers the essential tools to develop drugs that could target specific molecules (Jin et al. 2020). The US National Cancer Institute defined customized medication as a type of medication that utilizes data about an individual's phenotype and genotype, proteins, and assessment tools to prevent, diagnose, analyze, and treat the particular disease (Fancello et al. 2019). Genomic changes that might be related to malignant growth include gene modification and amplification, transformation, chromosomal mutations, and atypical methylation. These genomic changes are characterized by DNA double-strand breaks, transcription errors in mRNA or microRNA, translation of proteins, or the regulation of various metabolites at the cellular level. Genotyping changes can be assessed using genome sequencing innovations or microarrays (Hebbring 2019). Mutation screening may be evaluated via a sequencing procedure, whereas DNA microarrays and PCR can be used to assess DNA microarrays and DNA expression levels (Fancello et al. 2019). Genomic microarrays are an exceptionally ground-breaking and sensitive method; they can predict the clinical behavior of tumors (Fancello et al. 2019). For biomarker discovery and implementation, genomics has been widely used. The accessibility of biological strategies allows the oncologist to have an insight deep into the underlying mechanism of cancer progression, in terms of planning for the synthesis of biological medication with a better understanding of pharmacogenomics. In this way, biomarkers allow the impact of hereditary variation, providing new techniques for treating patients at a personalized level. Customized medicine is the product of such investigations (Logotheti et al. 2019).

1.6.3 Epigenomics

Epigenetics refers to the heritable changes in the expression level of DNA rather than the changes that normally occur in the sequence of DNA. Epigenetic changes include DNA methylation, histone alterations, and noncoding RNAs. These changes are present extensively in various human malignancies, and their regulation may be associated with early disease improvement. In this manner, they are a useful source of potential markers with expansive applications in diagnostics (Bernstein et al. 2010). Methylated DNA is a distinct stable epigenetic mechanism that is associated with tumor growth and progression. Furthermore, the primary advantage of detecting methylation is due to the inherent stability of DNA. Methylated genes in tumors can be quantified to diagnose disease (Horvath and Raj 2018). The assessment of DNA methylation can be conducted using a range of techniques using various types of biological material, such as tissue, plasma, serum, sputum, and urine for a variety of cancers (Kelley and Fishel 2016). The techniques for evaluating DNA methylation have improved considerably over time. An epigenetic change including bisulfate transformation of DNA, followed by PCR amplification, allows the evaluation of gene-specific methylation, which uses probes and primers specifically designed for the detection of methylated DNA (Olejniczak et al. 2010). The identification of DNA methylation areas is now possible because of this breakthrough. These areas may be converted into algorithms of ones and zeros, resulting

in a sophisticated activity that depicts hereditary changes in each cell or tissue, independent of whether it is functioning normally or abnormally. A large genome-wide screening effort has investigated over 200 such biomarkers for various human malignancies for DNA methylation biomarkers in fluids, tissue, and blood (Mockenhaupt 2015).

1.6.4 Proteomics

The proteomics-based methodology is one of the most dynamic and innovative tools for the identification of various diseases with a wide range to confirm, complement, or provide more detailed information compared with other high-throughput approaches, such as genomic, transcriptomic, and epigenetics. Notwithstanding genomic expression profiling, it is a profoundly solid technique for malignancy characterization and diagnosis (Sallam 2015). Studies that focus on differential expression levels of mRNA have been very descriptive and normally do not tend to correspond to the level or activity of functional proteins. Proteomic technologies that correspond to the protein expression profiles with cancer are essential for a detailed and comprehensive understanding of the malignant growth mechanism. Besides, focusing on a specific protein pathway associated with tumorigenesis presents a valuable approach in malignant growth treatment, as proteins mediate their effect through specific pathways rather than functioning separately (Janjua et al. 2020; Liang et al. 2012). Macromolecules including proteins are exceptionally unique and very dynamic regulatory bodies. It is well known that proteins are modulated by the regulation of several processes including posttranslational modification, proteolytic degradation, and interaction with a complex network (Huang et al. 2017a, b).

Proteomics studies are associated with the whole structure and expression of a protein in biological fluids, in cells and tissues, an organelle, or the whole body. In this way, knowing the concentration of proteomic profiles of different proteins obtained from biological samples of patients with cancer can provide an in-depth understanding of tumor pathogenesis, observation, the outcome of malignancy treatment, and management of patients with cancer. Several different proteins are associated or released by the tumor cells into the biological fluids, due to the activation complex networking of inflammation, cell death, and necrosis (Batta et al. 2012). Cellular events taking place during the abnormal cell cycle or cell death change the expression levels of proteins. Abrupt signal transduction and expression of proteins can be detected and easily correlated with healthy individuals for early diagnosis of patients with cancer. Secreted proteins are investigated by assessment of secretory pathways utilizing secretomic approaches and have advanced the understanding and clinical application of cancer biomarkers. Proteomic advancements can also be used for improving gene annotations based on proteogenomic data. Cross-examination and comparative analysis of the genome and the proteome encourage the discovery of posttranslational changes and proteolytic episodes (Batta et al. 2012; Indovina et al. 2011).

1.6.5 Metabolomics

A cancer biomarker can be a secreted metabolite, which is released by tumor cells, during metabolic pathways, or biological processes, and can be used for the analysis of cancer and monitor patient response toward treatments (Johnson et al. 2012). Although the key tumor markers are proteins, they can be sorted as cellular, biochemical, physiological, or anatomical. These markers might be used for the diagnosis (to distinguish between the stages of cancer), prognosis (survey lethality), and assessment of the patient's response to cancer therapy. The markers can be detected in body fluids (blood, urine, serum, stool, saliva), or tissues samples or biopsies of the cancer. In addition, it has been demonstrated recently that cancer volatile organic compounds markers can be detected in the breath (Beger 2013). In any case, recognition of these markers is a mechanistic process, and metabolomics is one of the omics innovations and is a better representation of the phenotypic changes in patients with cancer (Liesenfeld et al. 2013). Investigating the cancer metabolome is an effective and powerful method to study the phenotypic changes, which are tumor-associated. Screening biomarkers by selecting a variety of analytical procedures have been emphasized (Armitage and Ciborowski 2017). It is believed that the pattern of several metabolites is more accurate and precise and indicative of cancer status than a single metabolite. Metabolomic approaches allow the detection of a variety of metabolites in a single examination. The foremost tools utilized for metabolome investigation are mass spectrometry and nuclear magnetic resonance spectroscopy (Wang et al. 2016).

1.7 Conclusion

Biomarkers for various cancers play a significant role in the field of oncology and clinical practice for the risk assessment, screening, and diagnosis, especially when coordinated with other diagnostic techniques to determine prognosis and the response to treatment. Cancer biomarkers can facilitate a deep understanding of the development of cancer. Clinicians and scientists need a thorough understanding of the underlying molecular aspects, clinical use, and reliability of biomarkers to determine if they are suitable for patient use, or whether additional testing is required before incorporation into routine clinical practices. The challenges and future potential biomarkers will play a significant role in advancing personalized medication when combined with current therapeutics and diagnostics.

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Technologies for Identification and Validation of Cancer Biomarkers

2

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Abstract

With the recent advances in genomic profiling, high-throughput technologies and improved treatment strategies based on personalized medcines, biomarkers have emerged with an important role in the early detection and clinical management of cancer patients. Genetic-based biochemical analysis has developed to examine specific molecular pathways with abnormal expression of regulatory proteins and has been evaluated as potential predictive biomarkers for therapeutic decision in various cancer treatments. Genome-based prognostic biomarkers can measure and detect the risk of developing cancer in various tissues or, alternatively, assess the progression of cancer following clinical staging or potential response to the available therapeutic strategies. The development of novel cancer

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biomarkers for clinical utilization including diagnosis, prognosis, and drug therapy response is hindered by various challenges including scientific validation, regulatory, and legislation for the efficient translation to the clinics. This chapter underpins the different stages of biomarker development, identification and validation of cancer biomarkers, and successful implementation in the cancer management. With challenges, time is no far when biomarkers will shape the future of personalized medicine and cancer therapy.

Keywords

Cancer · Biomarker · Identification · Prognosis · Validation · Response

2.1 Cancer Biomarkers

Cancer is a cluster of diseases, responsible for the death of about nine million individuals and almost one-sixth of global mortality. The rapidly increasing number of cancer cases has been greatly affecting the health sector. The study forecasts that over the next 20 years the cases may increase by 70%. This disease burden can be reduced effectively by the application of cancer biomarker for predictive measures, early detection, and appropriate therapy followed by routine checkup. The US Food and Drug and Administration (FDA) define biomarker in the following context "Any biological molecule that can be used as diagnostic indicator to measure the risk and presence of disease" (Ilyin et al. 2004; World Health Organization 2017). It can be enzyme, cell, gene, protein, nucleic acids which can be detected in blood, urine, tissues, and body fluid, etc. Cancer biomarkers (CB) are biological substances secreted by tumors or other cells, that can be utilized as an indicative tool to detect, prognose and diagnose cancer and can be used to distinguish the subpopulation of patients' response to a therapy (Goossens et al. 2015; Rhea and Molinaro 2011).

2.2 Types of Cancer Biomarkers

Cancer biomarkers can be categorized into the following classes based on their usage:

2.2.1 Screening Biomarkers

Screening biomarkers are the first type of cancer biomarkers that can be utilized for early detection of cancer: it is used to identify those individuals that are at danger of developing a specific disease or to detect a disease when the individuals having it are asymptomatic which is different from the diagnosis of symptomatic individuals. This results in increased survival rate and reduces other complications and morbidity (Weigelt et al. 2005). Example of screening biomarkers includes APF which is used

in screening for hepatocellular cancer in high-risk individuals, CA125, in screening for ovarian cancer, for prostate cancer PSA is used as screening biomarker and in screening for colorectal cancers, fecal occult blood testing (FOBT) is used (Duffy 2015).

2.2.2 Predictive Biomarkers

Predictive biomarker, another type of cancer biomarker used to detect/predict the response of cancer cells to specific therapy or drug, i.e., the HER2 activation in breast cancer in response to trastuzumab or the prediction of mutated KRAS activation resistance to EGFR inhibitor cetuximab in colorectal cancer (Cameron et al. 2017; Romond et al. 2005; Slamon et al. 2001; Van Cutsem et al. 2009).

2.2.3 Prognostic Biomarkers

Prognostic biomarkers can be used to provide information regarding the disease recurrence or progression, but not linked directly with therapeutic interventions, i.e., 21-gene recurrence score in breast cancer, used to predict the cancer recurrence in tamoxifen-treated node-negative breast cancer (Paik et al. 2004).

2.2.4 Diagnostic Biomarkers

Diagnostic biomarkers, another type of cancer biomarker utilized to detect the presence or absence of a particular disease in a patient. Stool cancer DNA in colorectal cancer surveillance is used as diagnostic biomarker lately (Imperiale et al. 2014).

2.2.5 Monitoring Biomarkers

The biomarkers used for the monitoring or prediction of cancer recurrence post therapy is known as Monitoring biomarkers. The level of these biomarkers increase above the basal level in cancer recurrence can be predicted biochemically prior to any clinical or radiological evidence, i.e., carbohydrate antigen CA19-9, used as monitoring biomarker in pancreatic cancer and is FDA approved since 2002 (Bast et al. 2001; Koprowski et al. 1979; Rosty and Goggins 2002; Sharma 2009).

2.3 Discovery of CBMs

The discovery of cancer biomarkers employs numerous routes that includes the coverage of several disciplines ranging from high-throughput data initiation to generation of big-data and utilization of machines learning algorithms to the validation of biomarkers in different preclinical and clinical trials. These comprehensive steps involved in the cancer biomarker discovery has depicted in the Fig. 2.1.

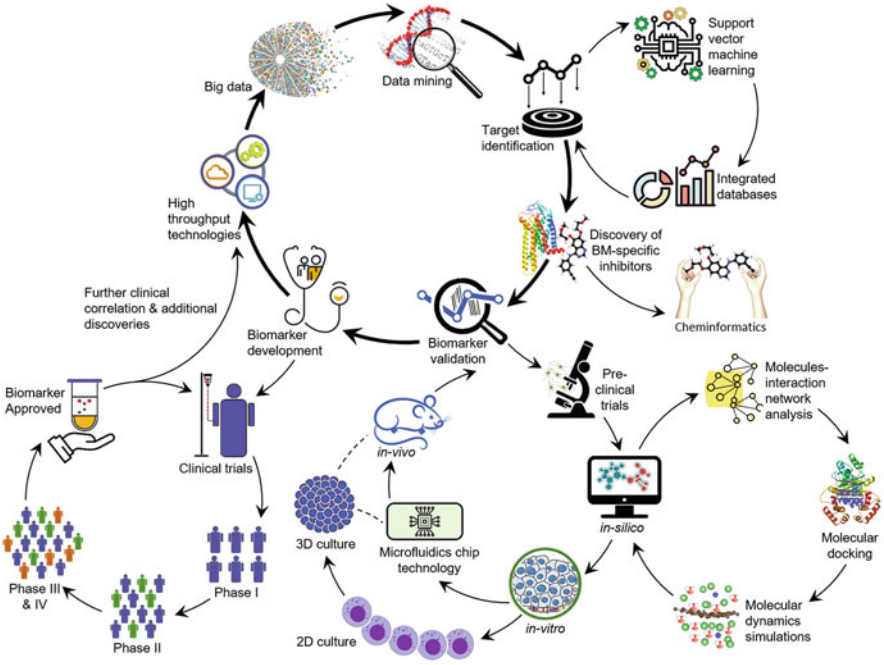

Fig. 2.1 Depiction of numerous technologies for identification and validation of cancer biomarkers. High-throughput technologies have generated huge bulk of big-data that is being deciphered by data mining for the generation of meaningful information. This data-mining results in the identification of novel targets that goes on becoming a cancer biomarker following different approaches such as support vector machine learning and analysis of integrated databases. In the meantime, the potential biomarker-specific inhibitors' hunt also begins that employ different computed techniques such as cheminformatics to identify potential functional groups for having binding affinity with the identified biomarkers. The potential cancer biomarker is being validated in preclinical studies employing in silico, in vitro, microfluidics and in vivo approaches. This leads to the developmental validation of cancer biomarker in human population following its comprehensive journey in clinical trials, with ultimate success of biomarker approval for cancer clinics

2.3.1 Preclinical Studies

2.3.1.1 In-Silico Studies

The integration, evaluation, and analysis of gene banks from huge databases present in gene expression profiling repositories can be done through sets of tools termed as "Bioplat (biomarker platform)". The core purpose of user-friendly Bioplat software is to aid in early diagnosis and prognosis of cancer patients by means of functional genomic data. Along with "in-silico identification" of new cancer biomarkers it is also helpful in extracting data from gene repositories as well as gene expression analysis.

Bioplat plays a significant role in edition of gene and creation of biomarkers with the help of identifiers in the embedded database, named Gene name, Entrez, Ensembl and Probe IDs. Additionally, Bioplat can also integrate gene data by means of online available resources including DAVID (Database for Annotation, Visualization and Integrated Discovery), STRING (Search Tool for the Retrieval of Interacting Genes/Proteins), Enrichr, Expression Atlas, RNA-seq Atlas and Gene Cards.

The gene signature optimization process is the prominent step in the Bioplat software development. The significant processes of Bioplat comprises of "blind search" and "particle swarm optimization (PSO)" helps in hitting the right optimum gene in less time (Butti et al. 2014).

However, another study encompasses some other approaches for in-silico identification of cancer biomarkers includes Panther, UniProtKB, NetOGlyc, NetNGlyc, Oncomine, and Cytoscape (Azevedo et al. 2018).

2.3.1.2 In Vitro

The use tissue culture paved a promising path towards the discovery of cancer biomarkers. The tissue cultures are rich in tumor cell lines and hence, wide spectrum of candidate biomarkers (Minamida et al. 2011). The limitation in the accessibility of patient tissue sample leads towards the transition to use tumor cell lines as second option for the discovery of potential biomarker.

The major ingredient of Conditioned media (CM) is secretory proteins that plays the major role in the identification of biomarkers with greater efficacy (Xu et al. 2010).

The traditional 2D (two-dimensional cell cultures) are replaced by 3D (three-dimensional cell culture) for the exclusive representation of homeostasis during in vitro analysis. The 3D cultures resemble tissue engineering models which helps in the understanding of gene expression and molecular mutated pathways of cancer (Lenas et al. 2009; Martin et al. 2008).

Among several techniques for better understanding of biomarkers, mass spectrometry has got the central focus. Through minimal number of sample, mass spectrometry has the significance to calculate accurate molecular mass with precision (Boja and Rodriguez 2012). Two broad categories of mass spectrometry for the identification of biomarkers are gel-based (2-DE and 2D-DIGE) and gel-free (SILAC, iTRAQ) techniques (Leong et al. 2012).

Additionally, gel-free techniques are also emerged as promising technique for the discovery of biomarkers. In tissue culture-model system, Stable isotope labelling by amino acids in cell culture (SILAC), that includes the integration of amino acid within stable isotope nuclei are now considered as method of choice. iTRAQ (Isobaric tags for relative and absolute quantitation) can also be used as alternate method (Mann 2006).

2.3.1.3 Microfluidics Chip Technology

Microfluidic chip technology utilizes an approach that can control fluids on a microscale, thus manipulating the cell-culture-related parameters in a comprehensive way to mimic the microenvironment of a malignant tumor in vivo (Xu et al. 2016). The microfluidic chip has strongly emerged as a biochip that can assimilate numerous fields, including cell biology, oncology, pathology, physiology, biophysics, biomechanics, bio-printing, motorized design, and so forth (Chaudhuri et al. 2016; Rosenbluth et al. 2008). In the recent decades, the application of biochip technology has displayed remarkable potential in the field of cancer treatment. A number of science validation techniques such as 2D and 3D cell and tissue cultures, spheroids and tissue organoid cultures can be performed on microfluidic biochips (Vadivelu et al. 2017). Moreover, cancer patients' derived cell lines and tissues can also be cultured on microfluidic biochips in a observable, controllable, manageable, and a high-throughput fashion that will significantly advance the progress of personalized medicine (Mulholland et al. 2018).

The novel biomarker and drug development consist of a number of major practices, including drug discovery, validations via preclinical trials and clinical developmental trials. Since the initial progress in 1990s, microfluidic biochip technology has been employed in multiple research disciplines including single cell analysis, medicinal synthesis, proteomics, tissue engineering, libraries screening, and medical diagnosis (Yu et al. 2014). Such platforms deliver novel understandings of biological mechanisms and endow the effective and rapid generation of novel data analysis. The microfluidics biochip revolution escalated due to the numerous effective applications offered by system size shrinking, while in the meantime providing high-throughput analysis, improved sensitivity, enhanced analytical potential, multiplexing abilities, and utilizes less volume of reagents, as well as its portable and easily fabricated (Boobphahom et al. 2020). This ultimately results in the development of economical in vitro models for lead compounds' identifications that can steadfastly predict the effectiveness, cytotoxicity, and pharmacokinetics of test compounds in humans, as well as for novel library screening analyses.

2.3.1.4 In Vivo

With the emergence of biomarkers discovery from in vivo mouse models, the extraction of plasma from genetically modified mouse model can be an attractive approach (Hingorani et al. 2003). Extraction of plasma from mice during stages of pancreatic tumor development, followed by proteomic approaches helps in marking the protein alterations (Aguirre et al. 2003).

Through comparative analysis technique, the noticeable similarity in expression of candidate biomarkers in human and mouse models were observed. To mark out differences in the protein concentrations, different samples are labeled with Cy dyes, IPAS (intact-protein analysis system) is done to indicate the protein differences. On the other hand, mass spectrometry can be helpful to highlight the gaps in protein bands (Wang et al. 2005).

Another sera comparison between the mouse model having human A549 lung adenocarcinoma cells with the control mouse group. The result showed very prominent quantitative and qualitative alterations in "expression of protein" between two groups. The key investigation revolves around the fact that differences in protein expression due to acute-phase inflammatory protein responses or antibody-mediated immune responses. Through histopathological staining techniques, it can be concluded that protein alterations are due to secondary changes in host origin and are not related to tumor cell derived proteins (Subramaniam et al. 2013).

2.3.2 Clinical Studies

2.3.2.1 CBMs Already in Clinics?

The EPGR (epidermal growth factor receptor) family member named as HER2 (ERBB2) is used as molecular biomarker in clinical settings. The amplification and overexpression of HER2 shows considerable responses against monoclonal antibodies, e.g., trastuzumab and pertuzumab. Among 20% of breast cancer patients, the phase 3 trails reflect the appreciable results of anti-HER 2 therapy along with better survival rates (Piccart-Gebhart et al. 2005; Romond et al. 2005).

Presently, ten HER 2 assays have been approved as companion diagnostic devices by FDA as well as approval of three HER2 assays (nucleic-acid based tests) are done by the Center for Devices and Radiological Health. However, other categories of biomarkers in clinics are BCR-ABL in chronic myeloid leukemia, KRAS mutations in colorectal cancer and multiple mutations in non-small cell lung cancer (NSCLC) (Kalavar and Philip 2019) (Table 2.1).

2.3.2.2 CBMs Clinical Trials

To replace the invasive cancer biomarkers, significant efforts are done to introduce predictive biomarkers. They are majorly based on single protein or gene and are mostly in phase II or III trials for evaluation and validation along with therapeutic targets (Tables 2.2 and 2.3).

Table 2.1 List of FDA-approved protein tumor markers presently utilized in clinical practice adapted from (Füzéry et al. 2013)

Biomarker	Clinical use	Year approved or cleared for the first time	Specimen	Methodology	Type of submission	Type of cancer
Pro2PSA	Differentiating between cancer and benign disease	2012	Serum	Immunoassay	PMA	Prostate
Free PSA	Differentiating between cancer and benign conditions	1997	Serum	Immunoassay	PMA	Prostate
p63 protein	Assist with the differential diagnosis	2005	FFPE tissue	Immunohistochemistry	510(k)	Prostate
ROMA (HE4 + CA-125)	Prediction of cancer	2011	Serum	Immunoassay	510(k)	Ovarian
OVA1 (multiple proteins)	Prediction of cancer	2009	Serum	Immunoassay	510(k)	Ovarian
HE4	Tracking recurrence of disease progression	2008	Serum	Immunoassay	510(k)	Ovarian
CA-125	Keeping track of the disease's course and how well it's responding to treatment	1997	Serum, plasma	Immunoassay	510(k)	Ovarian
Fibrin/fibrinogen degradation product (DR-70)	Keeping track of the disease's progression	2008	Serum	Immunoassay	510(k)	Colorectal
c-Kit	Detecting malignancies and assisting in the selection of patients	2004	FFPE tissue	Immunohistochemistry	PMA	Gastrointestinal stromal tumors
CA19-9	Keeping track of the disease's progress	2002	Serum, plasma	Immunoassay	510(k)	Pancreatic
Estrogen receptor (ER)	Prognosis and treatment response	1999	FFPE tissue	Immunohistochemistry	510(k)	Breast
Progesterone receptor (PR)	Prognosis and treatment response	1999	FFPE tissue	Immunohistochemistry	510(k)	Breast

CA27.29	Keeping track of the disease's reaction to treatment	1997	Serum	Immunoassay	510(k)	Breast
CA15-3	Keeping track of the disease's reaction to treatment	1997	Serum, plasma	Immunoassay	510(k)	Breast
Circulating tumor cells (EpCAM, CD45, cytokeratins 8, 18+, 19+)	Predicting cancer's progression and prognosis	2005	Whole blood	Immunomagnetic capture, immune-fluorescence	510(k)	Breast
HER-2/neu	Assessment in the context of therapy	1998	FFPE tissue	Immunohistochemistry	PMA	Breast
AFP-L3%	Risk assessment for disease development	2005	Serum	HPLC, microfluidic capillary electrophoresis	510(k)	Hepatocellular

Table 2.2 List of CBMs in Phase 1–Phase 4 clinical trials adapted from (Goossens et al. 2015)

Organ	Cancer	Biomarker	Associated drug
Breast	Breast	BRCA1/2	Olaparib
		HER2 (Tumor is negative, but CTCs are positive)	Lapatinib
		TOP2A (in people who have HER2 overexpression)	Anthracycline-based neoadjuvant chemotherapy
		CTCs that are HER2 positive	Trastuzumab—Emtansine
Gastrointestinal	Colorectal	BRAF	LGX818, BYL719
		RAS (type of mutation)	FOLFOXIRI and bevacizumab
		Biomarkers that are new (unspecified)	Cetuximab
	Esophago-gastric	HER2	Trastuzumab and afatinib
Hematological	Cutaneous and peripheral T-cell lymphomas	GATA-3	MLN9708
Head and neck	Squamous cell carcinoma	HER and KRAS	HM781-36B
Lung	NSCLC	BRAF V600E	Trametinib, dabrafenib
		ROS1	Crizotinib
Skin	Melanoma	BRAF V600E/K	Binimetinib, trametinib

2.4 Technologies That Lead to CBMs Discovery

2.4.1 Genomics (Nuclear and Mitochondrial CBMs)

2.4.1.1 Next-Generation Sequencing (DNA and RNA seq)

Genomic alterations are under study for most major tumors using sequencing techniques (Brooks 2012). Maxam Gilbert and Sanger laid the basis for next-generation sequencing through their cleavage method and dideoxy synthesis respectively (Maxam and Gilbert 1980; Sanger and Coulson 1975; Sanger et al. 1977). Next-generation sequencing, deep or massively parallel sequencing can sequence an entire genome in a single day which is extremely fast in comparison to Sanger sequencing which took almost 10 years to sequence human genome (Behjati and Tarpey 2013). Short-read whole genome sequencing and barcode linked read sequencing are novel approaches that can be used to resolve genomic rearrangements which can lead to tumorigenesis (Cunha 2017).

Table 2.3 List of ongoing clinical trials for CBMs adapted from (Kirwan et al. 2015)

Marker	Full name	Cancer type	Detection type	Clinical applications
PSA, or Pro2PSA	Prostate-specific antigen	Prostate	Concentrations of proteins	Screening, differentiating between cancer and benign disease
AFP	α-Fetoprotein	Liver	Protein concentrations and fucosylation of the core (for AFP-L3)	Diagnosis, staging, recurrence detection, and therapy monitoring
OVA1 test (multiple proteins)	Apoliprotein A1 + prealbumin + transferrin (down), B-2 Microglobulin + CA 125II (up)	Ovarian	Concentrations of proteins	Prediction
HE4	Human epididymis protein 4	Ovarian	Concentrations of proteins	Detecting recurrence, monitoring therapy
CA125	Cancer antigen 125	Ovarian	Concentrations of proteins	Detecting recurrence, monitoring therapy
ROMA test	HE4 + CA125	Ovarian	Concentrations of proteins	Prediction
CA19-9	Carbohydrate antigen 19-9	Ovarian, pancreatic	SLe[a] on mucin glycoproteins and gangliosides	Monitoring therapy
hCG	Human chorionic gonadotropin	Ovarian, testicular	Concentrations of proteins	Diagnosis, staging, recurrence detection, and therapy monitoring
CA15-3	Cancer antigen 15-3	Breast	Sialylated O-linked oligosaccharide on MUC1	Monitoring therapy
CA27-29	Cancer antigen 27-29	Breast	MUC1 protein levels	Monitoring therapy
HER2/neu	Human epidermal growth factor receptor 2	Breast	Concentrations of proteins	Therapy choice
CEA	Carcinoembryonic antigen	Breast, lung, pancreatic, gastric and colon	Concentrations of proteins	Detecting recurrence, monitoring therapy
Tg	Thyroglobulin	Thyroid	Concentrations of proteins	Monitoring therapy

2.4.1.2 Microarrays: Gene Expression Profiling

Microarray is basically an arrangement of nucleic acids attached to a solid surface and it can be used to detect expression of different nucleic acids (DNA, mRNA, miRNA, circRNA, etc.). Recently, circulator RNAs microarray was used to discover novel circulating biomarkers for diagnosis of gastric cancer.

2.4.1.3 Genome-Wide Association Studies

Genome-wide association studies or GWAS is used to identify linkage between genotype and phenotype and it can be used to associate a genetic variant with a particular disease (Tam et al. 2019). This approach has proved to be effective in particular with respect to breast cancer, where it has been used to associate many risk factors and biomarkers to this particular disease (Walsh et al. 2016).

2.4.2 Proteomics (Cytoplasmic and Membrane CBMs)

2.4.2.1 Western Blotting

Western blotting is an important procedure for the immunodetection of proteins particularly less abundant proteins after electrophoresis (Kurien and Scofield 2006). Diagnostic and therapeutic biomarkers for hepatocellular carcinoma, ovarian cancer, and breast cancer were discovered using western blotting (Cho 2007).

2.4.2.2 FACS

Fluorescence-activated cell sorting or FACS is a technique which is utilized to sort, detect, and count fluorescently labelled cells. Recently, a better technology has been devised, intelligent image-activated cell sorting (iLACS), which is a machine intelligence technology and has the capacity to analyze fluorescence-intensity profiles as well as multidimensional images of the cells and hence can sort cells and their components more efficiently (Isozaki et al. 2019).

2.4.2.3 MALDI-TOF

MALDI-TOF or matrix-assisted laser desorption/ionization-time of flight is an inexpensive technique which can be used with mass spectrometry to analyze protein composition of a tissue and it has been proven valuable in discovering novel biomarkers of gastrointestinal cancer, cancer of respiratory system, breast cancer, ovarian, and has the potential of discovering many more valuable biomarkers in other types of cancer (Rodrigo et al. 2014).

2.4.3 Bioinformatics (Predictive/Deduced CBMs)

2.4.3.1 Molecular Docking

Molecular docking is a tool which can be used to analyze interaction between two molecules (Morris and Lim-Wilby 2008) and hence can show us whether two molecules are likely to interact in in vivo conditions or not. Many tools are available

online to perform molecular docking, of which one is HADDOCK 2.4 (High ambiguity driven protein–protein docking), it uses information of already identified or predicted protein interfaces in ambiguous interaction restraints and dock proteins accordingly (Van Zundert et al. 2016) and is different from ab-initio methods.

2.4.3.2 Simulations

Simulations or molecular dynamics (MD) simulations is a basic tool for evaluating biomolecules and biomolecules interactions that were generated through in-silico approach (Hansson et al. 2002). For MD simulation, many software and servers are also available, for example, CABS-flex 2.0 which is an online server for quick modeling of protein structural flexibility (Kuriata et al. 2018) and GROMACS which is a software to simulate Newtonian equation of motions on particles (Van Der Spoel et al. 2005).

2.4.3.3 Molecules-Interaction Network Analysis

TargetScan and STRING are just an example of servers that can be used to visualize interaction of miRNAs with their targets and proteins with proteins respectively (Agarwal et al. 2015; Szklarczyk et al. 2019). These interactions can be used to analyze and predict biomarkers.

2.4.3.4 Support Vector Machine Learning

The support vector machine (SVM) learning, which is a supervised learning method, utilizes a collection of labeled training data to generate input–output mapping functions (Wang 2005), or in simple words has the advance ability to classify things through its learning abilities. It is a powerful classification tool that can be used to discover new biomarkers (Huang et al. 2018). ISOWN is a program based on this approach (Kalatskaya et al. 2017).

2.4.3.5 Integrated Databases

The Cancer Genomic Atlas (TCGA) dataset contains molecular characteristics of 33 different types of over 20,000 cancer and matched normal samples. TCGA and other similar databases are used by ISOWN. OncoMX is also a database more focused on biomarkers which consists of literature from different databases such as EDRN, Bgee, BioXpress, Reactome, and BioMuta (Singleton and Mazumder 2019).

2.4.4 Metabolomics

To detect cancer, predict response to different therapies and predict or monitor cancer recurrence, metabolites released as a byproduct by any metabolic pathway or during tumor growth can be used as a cancer biomarker. During cancer occurrence and development, specific metabolites expression changes due to which they can be used as biomarkers for cancer (Cardoso et al. 2018; Haukaas et al. 2017; Winter et al. 2003; Zaimenko et al. 2017). These biomarkers can be detected in circulatory fluids

like blood and CSF, excretory fluids like urine, saliva and by the tissues itself (Cavaco et al. 2018; Hadi et al. 2017; Harvie et al. 2016; Jagannathan and Sharma 2017). The exploration of the cancer metabolome appears to be an effective approach to analyze the phenotypic variations connected with tumor proliferation because metabolome is a strong representative of phenotype compared with genome, transcriptome and proteome (Holmes et al. 2008). Metabolite markers are different from traditional biomarkers (e.g., biochemical indices) and rely on various analytical techniques with includes nuclear magnetic resonance spectroscopy and mass spectrometry. Various metabolite markers have been identified until now. One of them thoroughly studied is 2-hydroxyglutarate (2-HG) which is being identified in many types of cancer which includes breast cancer, renal cancer, papillary thyroid carcinoma, and AML and is a product of IDH1 and IDH2 mutation (Borger et al. 2014; Dang et al. 2009; Fathi et al. 2014; Kanaan et al. 2014; Montrose et al. 2012; Rakheja et al. 2011; Shim et al. 2014; Wang et al. 2013).

2.4.5 Epigenetics Biomarkers

Heritable changes occurring at the molecular level in the cell are primarily due to alterations in the nucleotide sequence, as deciphered clearly by the human genome project. However further analysis has now led scientists to discover the importance of the other components of the human genome that can alter how phenotypes are expressed. These includes the epigenetic mechanisms like DNA methylation and histone modifications as well as the role of non-coding RNA.

These changes maybe because of external (environmental effects) or internal mutations by controlling trigger zones on the DNA, i.e., repressor proteins. These epigenetic factors have been identified to play a major role in various malignancies and thus maybe used as potential biomarkers for tumor identification, progression, and recovery (Kamińska et al. 2019). Bisulfite sequencing is a valuable technique to analyze DNA cytosine methylation. After bisulfite treatment of the sample, PCR amplification is performed which converts unmethylated cytosines into thymine (Xi and Li 2009).

Therefore, whatever the genetic sequence the final phenotypic expression depends on how the mutations are translated and hence the term epimutation. Epimutations is heritable and is associated with repression of genetic activity in somatic and in some cases germ cells.

The Human Epigenome Project (HEP) has evolved and expanded to add data to the ENCODE database (Encyclopedia of DNA elements) and the Cancer Genome Atlas (TCGA) with 212 cell culture lines. Covalent modifications of DNA or its histones (chromatin) play central role in epigenetic inheritance. This section shall investigate epigenetic markers in the field of oncology as under:

2.4.5.1 DNA Methylation: Aberrations

Both hyper and hypomethylation of promoters can silence important tumor suppressor genes. Since its first discovery in 1983 there has been immense progress in

developing in vitro diagnostic (IVD) assays for cancer screening and progress. DNA methylation is important in reprogramming the predetermined genetic makeup. Post fertilization there is loss of the original methylation from the paternal side and some from the maternal, erasing epigenetic memory of the parents and then later on re-methylation introduces a phenotype very specific and tailored to the new individual or offspring (Bradbury 2003). The two major known regions for methylation to occur are the promotor region and the CpG-rich region (cytosine residues) converting cytosine to 5-methylctosine. They silence the non-coding promoter sites and attract methyl-CpG-binding domain proteins (MBD).

2.4.5.2 Histone Posttranslational Modifications

Histones are made up of amino acids and once the amino acids are changed, the shape is modified and thus a new lineage-specific transcription is continued after cell division. Modification of histone by methylation and acetylation lead to euchromatin whereas, phosphorylation and deacetylation, heterochromatin that is condensed and inactive. Global histone acetylation modifications are potential markers of tumor recurrence with a better prognosis as compared to global methylation.

Thus based on these, patient can be classified into two subtypes, but as it is more dangerous minute modifications such as Lys16 and Lys20 hypomethylation is considered characteristic of human tumor cells (Shain and Pollack 2013), for example breast cancer with these modifications has a worse prognosis (Elsheikh et al. 2009). The presence of isoforms of histone also upsurge the tendency of cancer as in overexpression of H2A.Z in prostate and bladder tumors (Monteiro et al. 2014). Increased levels of circulating histones because of cancerous cell death or vigorous release are an indication of tumor progression and are a non-invasive biomarker to predict tumor response to chemotherapy as well. Upregulation of H3Cit histone have been documented in predicting short-term mortality (Thålin et al. 2018).

2.4.5.3 Chromatin Spatial Modifications

One of the chromatin remodeling complex, the Switch/Sucrose Non-Fermentable (SWI/SNF) is mutated in a wide range of cancers from ovarian, gastric to pancreatic (Shain and Pollack 2013).

2.4.5.4 MicroRNAs

These are non-coding RNAs that regulate various biological functions and each miRNA targets approximately 200 or so messenger RNAs (mRNAs), thus inhibiting translation. These miRNAs are regulated by either CpG islands or histone modifications. miRNAs act as biomarkers from both tumor tissue and body fluids like blood, CSF, urine, and saliva. Thus, the study of circulatory miRNAs in liquid biopsy's samples delivers encouraging biomarkers' platforms for non-invasive-based diagnosis in many human cancers. The detailed role of miRNAs as prognostic, predictive, and diagnostic factor is give in Table 2.4.

Table 2.4 The predictive, prognostic, and diagnostic role of different epigenetic markers in the field of oncology

Epigenetic biomarkers	Prognostic	Predictive	Invasive/non-invasive diagnostics	Biological material	Target cancer
Methylation					
MLH1 hypomethylation	+	–	Invasive	FFPE	Colorectal cancer (Jass 2007; Weisenberger et al. 2006)
MGMT hypermethylation	+	+	Invasive	FFPE	Glioblastoma (Wick et al. 2012)
IDH1 p.R132H mutation and *MGMT* hypermethylation	+	–	Invasive	FFPE	Glioblastoma (Roszkowski et al. 2016)
RB1 hypermethylation	+	–	Invasive	FFPE	Retinoblastoma (Livide et al. 2012; Ohtani-Fujita et al. 1997)
GSTP1, *RASSF1*, *APC* methylation status	+	–	Invasive	FFPE	Prostate cancer (Partin et al. 2014; Stewart et al. 2013)
SEPT9	+	–	Non-invasive	Blood	Colorectal cancer (Mikeska and Craig 2014; Wang et al. 2018)
					Lung cancer (Powrózek et al. 2014)
MGMT-STP27	–	+	Invasive	FFPE	Oligodendrogliomas and oligoastrocytomas (van den Bent et al. 2013)
ESR1	–	+	Non-invasive	Blood	Breast cancer (Mastoraki et al. 2018)
ZNF331	+	–	Invasive	FFPE	Colorectal cancer (Vedeld et al. 2018)
SALL1	–	+	Invasive	FFPE	Head and neck cancer (Misawa et al. 2018)
Histone modifications					
H3Cit	+	–	Non-invasive	Blood	Advanced cancers (Thålin et al. 2018)
cf-nucleosome epitope combination	+	–	Non-invasive	Blood	Colorectal cancer (Rahier et al. 2017)
H3K4me3 and Wdr82 expression	+	–	Non-invasive	Blood	Colorectal cancer (Liu et al. 2018)
Chromatin conformation					
ARID1 (*ARID1A* and *ARIDB*)	+	–	Non-invasive	Blood	Neuroblastoma tumors (Sausen et al. 2013)
Methylation status of *CTCF* locus	+	–	Invasive	FFPE	Colorectal cancer (Liu et al. 2017)

SMARCA4/BRG1	–	+	Invasive	Frozen tissue	Non-small cell lung cancer (Bell et al. 2016)
miRNA					
miR-21	+	+	Invasive/non-invasive	FFPE/ blood	Multiple types of cancers (Jansen et al. 2005; Larrea et al. 2016; Leupold et al. 2007; Resnick et al. 2009; Ryu et al. 2011)
miR-30d, *miR-21*	+	–	Invasive	FFPE	Non-small cell lung cancer (Czubak et al. 2015)
miR-31-3p	+	+	Invasive	FFPE	Colorectal cancer (Manceau et al. 2014)
miR-106a, *miR125a-5p*, *miR-129-3p*, *miR-205*, *miR-21*, *miR-29b*, *miR-375*, *miR-7*	+	–	Invasive	FFPE	Non-small cell lung cancer (Gilad et al. 2012)
miR-29a, *miR-92a*	+	–	Non-invasive	Blood	Colorectal cancer (Huang et al. 2010)
miR-506, *miR-4316*	+	–	Non-invasive	Blood	Colorectal cancer (Krawczyk et al. 2017)
miR-126, *miR-145*, *miR-210*, *miR-205-5p*	+	–	Non-invasive	Blood	Non-small cell lung cancer (Leng et al. 2017)
miR-149-3p, *miR-150-5p*, *miR-193a-3p*	+	–	Non-invasive	Blood	Melanoma (Fogli et al. 2017)
miR-200 family, *miR-17* family	–	+	Non-invasive	Blood	Prostate cancer (Lin et al. 2014)
miR-17, *miR-155*	+	–	Invasive	FFPE	Non-small cell lung cancer (Czubak et al. 2015)

2.4.6 Microbiomics Biomarkers

Omics technologies are promising contributors towards the discovery of biomarkers. The path towards the development of personalized medicines is paved by the discovery of relevant biomarkers under the umbrella of omics technologies (Quezada et al. 2017).

The microbial communities resides over and inside human body consisting of bacteria, viruses, fungi and archaea. They are termed as "microbiota/microflora" and encoded genes are called "microbiome" (Schwabe and Jobin 2013). Maintenance of homeostasis and shielding effect against pathogen are highlighted roles of microbiomes (Shreiner et al. 2015).

In 2007, Human microbiome project (HMP) brought the importance of microbiome in limelight through bioinformatics approaches. The major outline was to manipulate the components of microbiome to trigger immunity responses against deadly diseases (Clemente et al. 2012).

However, the disturbances or alterations in microbiome are directly proportional in triggering different cancer. Even a single alteration in microbiota can lead to drastic consequences (Bultman 2014). A continuous evolving microbiome has been recognized as playing a crucial role in carcinogenesis at a molecular level. One of the penalties in coexisting with these bacteria, fungi and viruses is the potential silent hazardous effect on human health. Thus, elaborating the taxonomy of theses microbes and understanding their basic mechanisms can we shed a light on the role they play not only in disease development but also in reversing these to become therapeutic agents and diagnostic tools (Singh et al. 2015).

Different composition of microbiota in multiple organs in human reflects the variability of inflammation responses and carcinogenesis in different body parts. Additionally interpersonal alterations of microbiome compositions at various location within the same organ can also lead towards cancer (Huttenhower et al. 2012).

The susceptibility of cancers also varies with the presence or percentage of microbiome in multiple organs. The higher densities of microbiome in large intestine are indicators of higher risk of cancer compared to small intestine (Breitbart et al. 2008; O'Hara and Shanahan 2006).

The variety of microbiome along with metabolites are present in body fluids, i.e., blood, saliva, urine, and cervicovaginal discharge is a promising factor in proving microbiome as novel as well as non-invasive cancer biomarkers (Farrell et al. 2012). For example, in non-small lung cancer, the higher percentage of hippuric acid metabolite was marked in PD-1 blockade therapy responders as compare to non-responders. Therefore, hippuric acid can act as "combinatorial biomarker" for the screening of patients for cancer immunotherapy and others are directed towards different therapies (Hatae et al. 2020).

The advent of next-generation sequencing technology has permitted us to further explore the inter-relationship of the disease, host, and microbe triad especially so in the gut microbiomes elaborating their role in cancer via direct or even immunological mechanisms. Any imbalance of these factors or dysbiosis is then linked with a plethora of diseases, including cancers and so these microbiomes may in future be

used as markers for cancer diagnostic. This has led to a rapid expansion of the study of DNA of microbes or microbiomics (Feng et al. 2020).

Though many studies have identified these pathogens in different cancers it is still not clear whether these are a cause or effect of these cancers. Do these proliferate under the influence of the tumor cells or lead to the growth and progression of these cancers? In either case identifying and using these as markers may help track the prognosis of disease or even be possible routes for targeted therapies.

There are however many challenges because of the complexity of the technologies involved for example, in case of gut microbiota, whether the sample is from stool versus biopsy samples, correctly defining the genes and finally understanding the source of microbial genes because of this being a very young field (Cong and Zhang 2018). To overcome the insufficient biomass as well as contamination and variability of kits, repetition is the best possible way to validate and substantiate the findings across labs and microbiomes.

The most studied microbiome is the gut microbiome and it has shown in some cases that treatment with simple antibiotics can lead to reversal of tumors like Helicobacter pylori-induced gastric mucosa-associated lymphoid tissue (MALT) and lymphoma using lansoprazole 30 mg, amoxicillin 1 g and clarithromycin 500 mg (PREVPAC) (Stolte et al. 2002). By creating enzymatically active protein toxins, directly inducing host cell DNA damage or interfering with critical host cell signaling pathways of cell proliferation, apoptosis, and inflammation, certain bacterial species can have a pro-tumoral effect (Fiorentini et al. 2020).

The mechanisms that the carcinogenic microbes employs are shown in Table 2.5 (Goodman and Gardner 2018).

2.4.7 Cancer Imaging Technologies

Imaging technologies are used commonly to detect and categorize cancer. Imaging is performed widely to stage cancer, to monitor cancer therapy, to detect disease recurrence, or for surveillance purposes (Dregely et al. 2018).

In oncology, Image Biomarkers (IBs) that are used commonly include clinical TNM (tumor, node, metastasis) stage, objective response, and left ventricular ejection fraction. Beside these other biomarkers that are used extensively in cancer research and drug development are MRI, CT, PET, and ultrasonography biomarkers (O'Connor et al. 2017). In the diagnosis, staging and treatment of cancers, the imaging modalities range from radiological X-rays, computed tomography (CT) and magnetic resonance imaging (MRI) to ultrasound (US) and radioactive single-photon emission computed tomography (SPECT), positron emission tomography (PET), and optical imaging. Imaging in cancer is still poor despite advances in other aspects of diagnostic radiology unless tumor-to-background ratio improves by 2–4 times with increase efficiency in sensitivity and contrast agent targeting (Frangioni 2008).

Table 2.5 Description of carcinogenic microbes' mechanisms

Hallmark	Microbes	MOA	Source cited
Tumor-promoting inflammation	Gram-negative strain broadly	TLR2 and TLR4 mediated overexpression of innate inflammation	Garrett (2015), Zitvogel et al. (2016)
		Initiation of IL-17/23 pathway cytokines	
	Clostridium species	Production of DCA from bile and induction of IL-1β and IL-6	Yoshimoto et al. (2013)
	Toxigenic *Bacteroides fragilis*	Induction of IL-17 cytokines and Th17 skewing	Wu et al. (2009)
	Fusobacterium	Increase in IL-6, IL-8, IL-18 and NF-κB signaling	Kostic et al. (2013), Rubinstein et al. (2013)
	Helicobacter	Increase in IL-1B, IL-8 and NF-κB signaling through CagA	Peek and Blaser (2002)
	Propionobacterium acnes	IL-6, IL-8 and IFN-γ induction	Fehri et al. (2011)
Avoiding immune destruction	*Helicobacter*	Induction of Tregs	Peek and Blaser (2002)
		Upregulation of epithelial PD-L1	
		GGT and VacA inhibition of T-cell proliferation	
	Fusobacterium	Induction of immunosuppressive MDSCs	Gur et al. (2015), Kostic et al. (2013)
		Fap2 inhibition of TIGIT	
Sustaining proliferative signaling	*Fusobacterium nucleatum*	E-cadherin binding and upregulation of β-catenin signaling	Rubinstein et al. (2013)
	P. acnes	Increased proliferation and upregulation of COX-2	Fehri et al. (2011)
	Helicobacter	Reduction of p27 in epithelium, increase in gastrin	Peek and Blaser (2002)
Genome instability and mutation	*Escherichia coli*	PKS producing calobactin that induces double-strand breaks	Arthur et al. (2012)
	Helicobacter	ROS production in response to infection that damages host cells	Peek and Blaser (2002)

For several cancers, MRI is now the main imaging evaluation method and plays a key role in management decisions. It is the initial imaging tool for prostate cancer and myeloma diagnosis; for rectal, cervical, and endometrial cancer staging; and for hepatocellular cancer response evaluation. A variety of MRI biomarkers are already identified or are well on their way to being established for oncology evaluation in clinical practice. These MRI biomarkers include BI-RADS (Breast Imaging Reporting and Data System), PI-RADS (Prostate Imaging Reporting and Data

System), and LI-RADS (Liver Imaging Reporting and Data System), to diagnose breast, prostate, and hepatocellular cancers, respectively (Dregely et al. 2018).

PET (Positron Emission Technology) scans are used for the detection of cancer and also for the examination of the effects of cancer therapy. It is used to identify localized biochemical changes at the site of cancer. PET scans show only the location of a molecular marker, they do not provide anatomical information. It is a diagnostic test that requires the acquisition of physiological images that are dependent on positron detection. Positrons are tiny particles which emit from a radioactive substance when administered to the patient (Scaros and Fisler 2005).

Patient receives an injection of radioactive tracers that contain a type of sugar attached to a radioactive isotope. When cancer cells take up the sugar and attached isotope, positively charged, low-energy radiations known as positrons emit. The electrons in the cancer cells react with the positrons and result in the production of gamma rays. These gamma rays are then detected by the PET machine, which transforms this information to the form of a picture.

For example, ^{18}F-FDG is a commonly used tracer to detect cancer in clinical oncology. FDG-PET is very useful in the diagnosis, staging, and monitoring cancer therapies, particularly Hodgkin lymphoma (Zaucha et al. 2019).

The newer and improved versions of these modalities include PET radiotracers using Gallium 68, and hyperpolarization MRI using Carbon 13 pyruvate will be needed to increase sensitivity in diagnosis of cancers. The specificity may be increased by using cancer-specific targeting ligands like immunoglobulin (Fass 2008).

Cancer treatment using image-guided chemotherapy by MRI, optical tomography using radioisotopes for neoadjuvant therapies are now changing our approach to cancer treatment (Table 2.6).

2.5 Emerging Technologies

2.5.1 Circulatory Cancer Biomarkers

2.5.1.1 Circulating Tumor Cells

It is possible to find circulating tumor cells (CTCs) in the peripheral blood of patients with metastatic cancer. Recently, with the advent of technologies that are sufficiently sensitive to detect very rare cells, research to enhance the detection of CTCs has increased considerably. The development of such tools has empowered research into defining the clinical implications of CTCs and has revealed that the levels of CTCs in patients' blood shows a relationship with prognostic outcomes and is a clinically significant biomarker for patients' prognosis with metastatic prostate, colon and breast cancers. Several studies have shown that CTC tracking can be used to assess patient responses to therapy and to track genetic and phenotypic tumor changes in real time (Preedy and Patel 2015).

Because of the correspondence with traditional tumor tissue's biopsy, the word "liquid biopsy" for measuring the concentration of CTCs in blood was introduced

Table 2.6 New imaging technologies for different cancers types and their advantages and disadvantages

Clinical problem	Cancer/ discipline	New technology	Advantage	Disadvantage	Reference
Screening	Breast	Dedicated CT-scan	High resolution and increased sensitivity	Ionizing radiation (same as 2-view mammogram)	Boone et al. (2006), Boone and Lindfors (2006)
		PEM	High sensitivity, moderate to high resolution	Requires IV injection of radiotracer	Weinberg (2006)
		DCE-MRI	High resolution and specificity	Requires IV injection of lanthanide chelate	Dougherty et al. (2007)
		Diffuse optical tomography (spectroscopy)	Non-contrast analysis, safe, 3D, quantitative, high specificity, able to combine with MRI	Low resolution, low to moderate sensitivity	Carpenter et al. (2007), Hsiang et al. (2005), Nioka et al. (1994)
		Optical (photon scattering)	Fast, inexpensive, safe	Surface imaging only, interference from blood	Georgakoudi and Van Dam (2003), Kim et al. (2006), Lovat et al. (2006)
		Optical (multi-wavelength spectroscopy)	Fast, inexpensive, safe	Surface imaging only, interference from blood	Perelman (2006)
		Optical (autofluorescence spectroscopy)	Fast, inexpensive, safe	Surface imaging only, interference from blood	Orfanoudaki et al. (2005)
		Optical (polarization spectroscopy)	Fast, inexpensive, safe	Surface imaging only, interference from blood	Huh et al. (2004)
		Optical coherence tomography	Fast, high resolution, safe	Limited depth to ≈2 mm, endogenous contrast only	Bouma et al. (2000), Peter et al. (2005)
		Exogenous fluorescence	High sensitivity and specificity	Requires IV injection of contrast agent	DaCosta et al. (2005), Kennedy et al. (1996), Wallace et al. (2006)

	Virtual colonoscopy	CT-based	Non-invasive, relatively fast	Uses ionizing radiation, difficulty with flat lesions and small polyps	Alencar et al. (2007)
		MRI-based	Non-invasive, relatively fast	Difficulty with flat lesions and small polyps	Pickhardt and Kim (2007)
Staging	PET	Replacements for SPECT radiotracers	Higher sensitivity and resolution	Desired half-life not always available	Ajaj and Goyen (2007)
		Time-of-flight detection	Twofold higher resolution or sensitivity	Limited availability	Cherry (2006), Gabriel et al. (2007)
	MRI	Hyperpolarization	High sensitivity possible, in vivo tracking of molecule metabolism	Relatively short relaxation times of agents tested to date	Golman et al. (2006), Surti et al. (2007)
		PARACEST	Higher sensitivity than traditional lanthanide imaging	Sensitivity not yet adequate for receptor-based imaging	Jonischkeit et al. (2006)
	All	Low-molecular weight targeting ligands	Rapid biodistribution and clearance	Tumor contact time often inadequate	Handl et al. (2004), Humblet et al. (2006), Kelloff et al. (2005), Mammen et al. (1998), Misra et al. (2007), Vinogradov et al. (2007)
		Signal amplification/ background reduction (optical)	Improved SBR	Requires endocytosis and pH-dependent activation	Graff et al. (2004)
Treatment	Chemotherapy	Image-guided treatment (^{18}FDG-PET)	Highly sensitive	Expensive, not all tumors FDG-avid, difficult to quantify log kill	Kenmoku et al. (2007), Lordick et al. (2007), Nahmias et al. (2007)

(continued)

Table 2.6 (continued)

Clinical problem	Cancer/ discipline	New technology	Advantage	Disadvantage	Reference
		Image-guided treatment (^{99m}Tc-Annexin V)	Moderately sensitive	Presently unavailable, difficult to quantify log kill	Kartachova et al. (2004), Kelloff et al. (2007), Rottey et al. (2006)
		Image-guided treatment (DCE-MRI)	No ionizing radiation	Requires intravenous injection of lanthanide chelate, difficult to quantify log kill	Kartachova et al. (2007)
		Image-guided treatment (optical spectroscopy)	No ionizing radiation, fast, safe, quantitative, high sensitivity and specificity	Low resolution, moderate sensitivity, difficult to quantify log kill	Chou et al. (2007)
	Radiotherapy	Ion beam-induced PET and PET/CT	Near real-time feedback on dose delivery	Requires specialized and expensive infrastructure, difficult mathematical modeling	Cerussi et al. (2007)
	Surgery	Optical (reflectance NIR fluorescence)	Fast, real-time, high sensitivity and specificity	Poor depth penetration (≈1–3 mm)	De Grand and Frangioni (2003), Figueiredo et al. (2006), Frangioni (2003), Horowitz et al. (2006), Ke et al. (2003), Li et al. (2006), Nishio et al. (2006), Tanaka et al. (2006)
		Optical (tomographic NIR fluorescence)	Depth penetration up to several cm, quantitative, high specificity	Requires separate acquisition and reconstruction, low to moderate resolution, low to moderate sensitivity	

(Alix-Panabières and Pantel 2013). In comparison to tissue biopsy, the liquid biopsy offers numerous advantages, for example, efficient and simple pulling out of liquid sample from patients, cheaper and least painful procedure and low risk for patients suffering because of its nominal invasiveness. This does not only deliver the prospect for improved understanding of the underlying biological mechanisms such as cells' spreading and metastasis, but also to utilize these types of circulatory cells as biomarkers for the detection, analysis, and treatment of complete cancer more efficiently and successfully. Nevertheless, due to the exceptionally low levels of CTCs in blood and mostly the missing of cancer-specific biomarkers, their detection still poses a major challenge and holds some limitations upon their significance in cancer diagnosis. Liquid biopsy has many advantages as compared to tissue biopsy such as low cost, rapid extraction, and minimal invasiveness. This not only helps in the better understanding of cancer biology but also helps in the use of these cells as biomarkers to more effectively diagnose and analyse cancer.

Racila and colleagues described a major scientific breakthrough in 1998 to identify the extremely rare Circulating Tumor Cells (CTCs) (Racila et al. 1998). They used antibodies designed against epithelial cell adhesion molecules (EpCAM) joined with ferrofluids. These were combined with flow cytometry that they performed as immunomagnetic CTCs enrichment. This method was used for the origination of the CellSearch® (CS) system that is currently being used frequently and is the lone CTCs detection method approved by the US-FDA (Marcuello et al. 2019).

For detecting CTC in the peripheral blood of cancer patients, several in vitro approaches have been reported. However, currently used in vitro techniques, they have limitations such as less yield and sensitivity. An innovative in vivo CTC isolation product, the GILUPI CellCollector® can isolate CTC directly from the circulating blood. It intends to increase the yield while capturing CTC and has been approved with a Conformité Européenne (CE) mark, for application in solid cancers and by the China Food and Drug Administration for breast cancer. This new strategy has been found to have high capture rates for advanced stage lung cancer and can even detect CTC in ground glass nodule patients as well (He et al. 2020).

2.5.1.2 Circulatory DNA/RNA

The circulatory fluids such as the blood samples carries small quantities of circulatory tumor DNA/RNA (ctDNA/ctRNA) released from the primary and metastatic tumors cells along with the cell-free DNA (cfDNA) from non-malignant cells, primarily hematopoietic cells. ctDNA can provide a more detailed description of the range of mutations that could be found in the tumor of a patient as compared to single tissue biopsy. ctDNA can provide a potential for minimally invasive disease course monitoring and residual disease evaluation following surgery (Marcuello et al. 2019).

2.5.1.3 miRNA

MicroRNAs (miRNAs or miR-) are endogenous single stranded non-coding RNAs that can post-transcriptionally control the expression of hundreds of target genes.

There are two main mechanisms by which they can negatively regulate gene expression, firstly through binding to the 3′-untranslated regions (3′-UTRs) of target mRNAs, thus inhibiting the translation. Secondly, by binding effective complementarily to messenger RNA sequences, consequently resulting to their degradation (Luo et al. 2013; Yang et al. 2015). On the other hand, there is also some data present that miRNAs can also trigger translation of target mRNAs (Vasudevan et al. 2007).

The initial association between human cancer and miRNA was revealed in 2002 (Calin et al. 2002). MiRNAs can be present alone or in combination with other proteins in the circulation. In addition, they are able to be released directly into extracellular fluids and can also be carried with the help of microvesicles (O'Brien et al. 2018). In 2008, Chim et al. found placental miRNAs in maternal plasma, making it first principal research on miRNAs in biological liquids (Chim et al. 2008). Subsequently many studies were conducted for characterization of miRNAs in fluids as biomarkers.

MiRNAs possess many distinctive features that makes them as ultimately non-invasive cancer biomarkers. Cancer-specific miRNAs are extra stable and resistant to storage, their sequences are conserved throughout different species, they can be identified by cutting-edge technologies in small amounts of samples with high specificity and reproducibility, and are found in many biological fluids (e.g., blood, breast milk, amniotic fluid, saliva, feces, tears, urine) that makes their detection easy and minimal-invasive (Mitchell et al. 2008).

2.5.1.4 Exosomes

In both natural and pathological conditions, exosomes are released by cells. These exosomes carry nucleic acids and proteins which are the indicators of the pathophysiological conditions and hence can be used as biomarkers in clinical diagnostics. Tumor cells release exosomes which contain tumor-specific RNAs that can serve as potential biomarkers for cancer diagnosis. Exosomes include several proteins, including common membrane and cytosolic proteins, as well as origin-specific protein subsets that represent cell functions and conditions (Roldán Herrero 2021).

For example, exosomes are highly enriched with tetraspanins, a family of scaffolding membrane proteins. The exosomal marker CD63 is also a member of the tetraspanin family. In 2009, Logozzi and colleagues revealed that plasma CD63+ exosomes were significantly higher in patients with melanoma relative to healthy controls (Logozzi et al. 2009). All of these circulatory cancer biomarkers and their promising role in cancer research are depicted in Fig. 2.2.

2.5.2 Drug Repurposing

Repurposing or repositioning involves drugs of which the mechanism of actions is completely or partially understood. Clinical repositioning studies may also take benefit of this information and provide predictive biomarkers from initial phase development or trials. These biomarkers are frequently established among

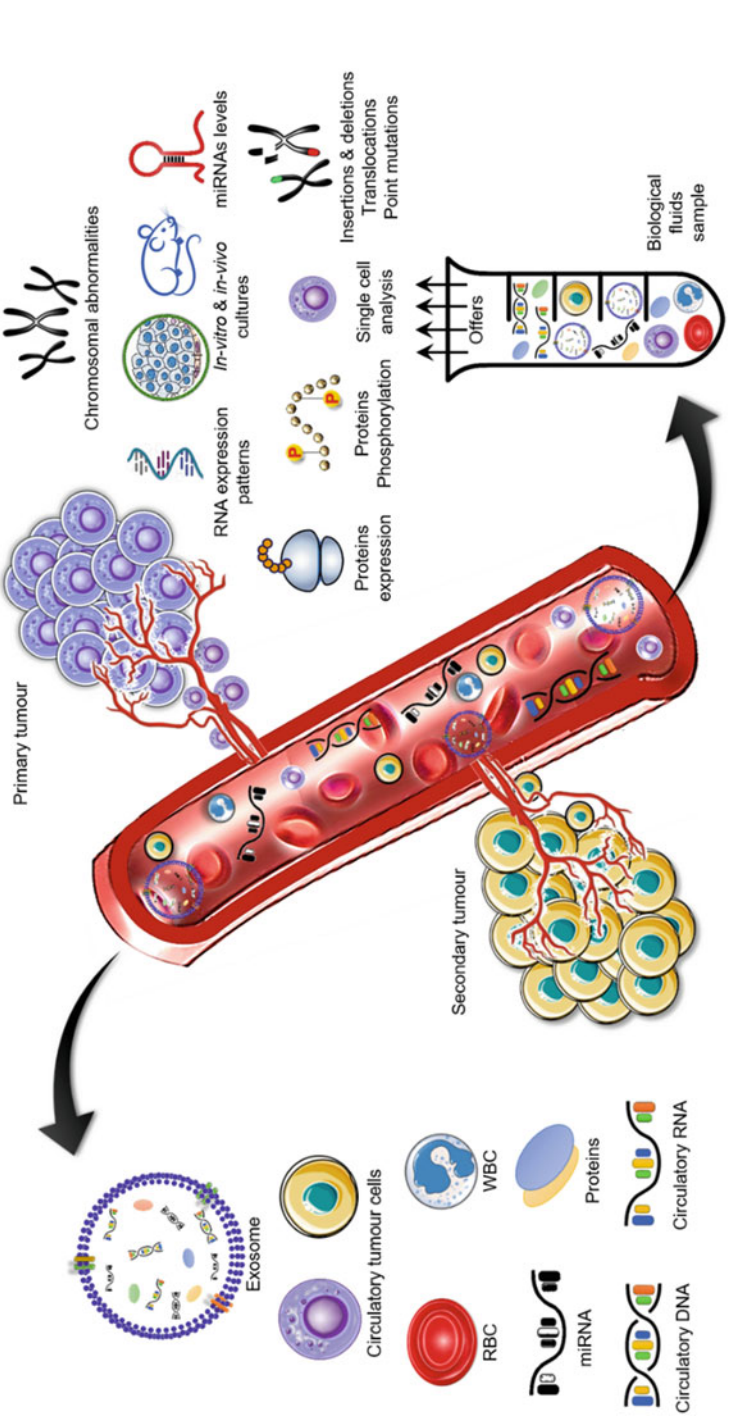

Fig. 2.2 Depiction of circulatory cancer biomarkers in liquid biopsies and its wide range applications in understanding cancer genomics and proteomics' instabilities. The biological fluids from cancer patients contain large number of circulatory biomarkers that includes exosomes, circulatory tumor cells from primary origin or metastatic site, blood cells, different types of micro RNAs, circulatory proteins such as cell surface receptors, enzymes and signaling molecules, and cells' free circulatory DNA and RNA released from the tumor site. These circulatory cancer biomarkers offers numerous diagnostic, prognostic and therapeutic applications by analyzing the cancer cells' chromosomal abnormalities, single cell analysis, RNA expression profile, types and levels of miRNAs, proteins expression and its phosphorylation, in vitro and in vivo cultures assays, genes amplifications, insertions and deletions, the segments translocations and other different types of genetic mutations

molecules, which are recognized to be involved in sensitivity or resistance to the test compound. In early drug agent testing, the use of predictive biomarkers may upsurge the treatment efficacy of the testing agent in question by raising the efficacy of the test agent in the favorable population of the selected biomarker. In the same way, drug-induced cytotoxicity in the unfavorable population of the selected biomarker can be avoided as these clinical trials-involved participants will not be exposed to the test agent/drug (Stenvang et al. 2013).

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Biomarkers for Cancer Drug Development

3

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Abstract

Cancer biomarkers, which are indicators of cancer cells in the body, can help with diagnosis, prognosis, and treatment effectiveness as well as recurrence prediction. We discuss recent advances in cancer therapeutics, as well as the use of biomarkers and anticancer drug advancements. The discovery and application of biomarkers will boost oncology drug development efficiency. Preclinical trials or basic research are often used to identify potential clinical biomarkers of drug efficacy. The approval rate for oncology drugs is poor, and most of the drugs that did not receive approval were in late stages of development. In addition to that, attrition rates are high. Biomarkers have been shown to increase response rates, progression-free survival rates, and overall survival rates in drug growth. As a result, the biomarker-based approach seems to be linked to more active drug programmes, with a shorter time frame and a higher chance of success. The next wave of advancements in cancer therapy will be guided by the search for novel biomarkers, innovative designs, and delivery methods for targeted agents.

Keywords

Cancer · Biomarkers · Survival · Growth · Cells death · Diagnosis · Therapy

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3.1 Introduction

Cancer is an important health problem that is the world's second leading cause of death. According to last year's annual statistics of World Health Organization (WHO), cancer patients and deaths from cancer were estimated to be 18.1 million and 9.6 million, respectively. Similarly, one in every six women and one in every five men will develop cancer at some point in their lives. Furthermore, one out of every eleven women and one out of every eight men will die as a result of it. The number of patients that were diagnosed from cancer within 5 years was reported to be 34.8 million. The prevalence and mortality rate of cancer is higher in Asia and Africa compared to other areas. In most countries, WHO recognizes poor prognosis and limitations as key factors preventing timely access to diagnostic and therapeutic agents (Bray et al. 2018). In future there will be globally predicted experience of new cancer cases and death (Fouad and Aanei 2017). However, further improvement of research in cancer diagnosis and therapy may help in health care management of many types of cancers (Kourou et al. 2020). Likewise, understanding mechanisms of cancer at the molecular and cellular levels can help in early diagnosis and precise prediction of treatment (Zangooei and Habibi 2017). Furthermore, effective subtyping of cancer patients into clinically relevant subtypes will aid in the development of fair and evidence-based diagnosis and therapy (Wu et al. 2020).

3.2 Cancer Therapy

There are many cancer-fighting strategies ranging from chemoprevention (strategy to block or slow the onset of premalignant cancers with relatively non-toxic chemical substances to chemotherapy, radiotherapy, and ultimately surgical oncology) (Zanni et al. 2015). Previously, major efforts have been made to classify cancers based on biomarker expression profiles, including mRNAs and protein. (Kim et al. 2021). The acquisition of tumor resistance to chemotherapy is found in nearly all cases, greatly reduces its effectiveness, and remains a major challenge for advanced cancer clinical management. Genetic alterations, improvements in the pH of the tumor microenvironment, activation of survival signalling pathways, increased drug efflux by ABC transporter proteins, or the proliferation and development of tumor cell subpopulations that are inherently immune can all lead to multidrug resistance (Dlugosz and Janecka 2016).

There is a rapidly rising interest in developing molecularly focused therapeutics that block or activate unique signalling pathways of tumor cells in order to enhance cancer treatment outcomes. The US Food and Drug Administration (FDA) has licenced more than 80 molecularly targeted oncology medications to treat multiple human malignancies over the last two decades. These targeted therapies include small molecules and monoclonal antibodies aimed at blocking specific pathways that drive carcinogenesis and tumor development. They have different mechanisms of action: inducing cancer cell programmed cell death (apoptosis), blocking particular enzymes and receptors of the growth factor involved in the proliferation of cancer

cells, or altering the role of proteins that control gene expression and other cellular functions. Among these therapeutic targets, the signalling components of human epidermal growth factor receptor 2 (HER2), epithelial growth factor receptor (EGFR) and programmed death receptor-1 (PD-1) have contributed to the effective development of cancer therapy powered by molecular markers. These targeted therapies are promising to enhance patient outcomes by focusing on particular oncogenic proteins, rather than interacting with all rapidly dividing cells. Therapeutic targets are most likely existent in some but not all tumor cells due to the great heterogeneity that occurs in cancers, both between and within patients (Wu et al. 2010).

Accordingly, prescient biomarkers are expected to help distinguish subset populaces that are well on the way to encounter a positive or horrible impact from these mediations (Nalejska et al. 2014). In clinical settings, an approved prescient biomarker is assessed utilizing in vitro buddy indicative gadgets (IVDs) which give data vital for the protected and compelling utilization of a relating restorative item (Lee and Shen 2015).

Over the last few decades, there has been an explosion in anticancer drug discovery studies, ranging from novel general cytotoxic agents that target malignant features (such as accelerated proliferation) to the development of more focused compounds including kinase-targeted small molecules that target addictive oncogenes specifically (Hoelder et al. 2012).

Given the vigorous nature of this discovery campaign, and the production and non-development of thousands of drugs, only 5 percent of leading drug candidates end up progressing through the health center. Indeed, our capacity to foresee patient outcomes before reaching clinical trial remains a major constraint on drug discovery and clinical performance. The best preclinical model will be relatively inexpensive, suitable for high-throughput screening and, most significantly, match the biology of human tumors as accurately as possible (Dhandapani and Goldman 2017). The accurate detection of biomarkers that influences the efficacy of a potential therapeutic aids in improving sensitivity, which in turn improves overall survival rates (Jin et al. 2019). Precision medicine or customized cancer treatment involves adjusting antitumor therapy to patients' specific clinical characteristics, tumor molecular profiles and related microenvironments in order to treat cancer more efficiently and with a reduced amount of toxicity (Massard et al. 2017). While molecular and immunotherapy agents have revolutionized cancer care over the past decade, only a small percentage of patients react, whereas individuals who react ultimately develop progressive disease and gain drug resistance. There is therefore a vital need for robust biomarkers to be identified and validated that can predict resistance or sensitivity to such treatments. Key insights into the dynamic biology of cancer growth and progression have been provided by the incorporation of cancer genomic profiling into clinical practice. However, the implementation of such innovations has also unintentionally introduced many data analysis-related problems, containing the problems associated with the detection of driver changes, the target classification of various levels of evidence, and the selection of rational rehabilitations for patients with appropriate drugs at the accurate time at the person level (Johnson et al. 2015).

In oncology, where drugs are expensive, life expectancy is limited, and the risk of drug toxicity is always high, there is an urgent need to recognize and treat the patients who are most likely to receive assistance from a given drug..

3.3 Biomarkers

World Health Organisation defines biomarker as any constituent, design, or succession that can be measured in the body or its product and impact or foresee the occurrence of result or infection (Singh et al. 2020). The utilization of delicate and explicit biomarkers for sickness determination, forecast, and checking, is an alluring option in contrast to a significant number of the current strategies being used. The presence and levels of certain tissue-inferred atomic markers can help recognize subtypes in heterogeneous sicknesses, for example, malignant growth (Duffy et al. 2017). Cancer biomarkers are either delivered by the tumor or by the body in light of the tumor (Fig. 3.1).

3.3.1 Biomarkers Discovery

3.3.2 Cancer Biomarkers Classification

Biomarkers of cancer can be classified into the subsequent classes dependent on their use. Predictive biomarkers predict reaction to explicit remedial mediations, for example, positive/enactment of HER2 that forecasts reaction to trastuzumab in

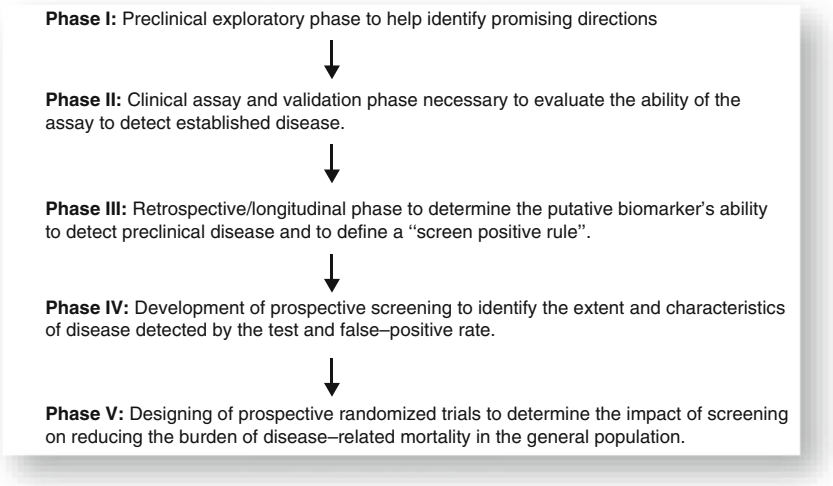

Fig. 3.1 Flowchart of various phases in the biomarker discovery (Kumar et al. 2006)

breast malignant growth (Goossens et al. 2015). Similarly, KRAS-enacting transformations anticipate protection from EGFR inhibitors, for example, cetuximab in colorectal malignant growth Van Cutsem et al. 2009). Prognostic biomarker, then again, may not be straightforwardly connected to or trigger explicit helpful choices, yet intend to advise doctors with respect to the danger of clinical results, for example, malignant growth repeat or illness movement later on. An illustration to a prognostic disease biomarker is the 21-quality repeat score which was prescient of breast malignant growth repeat and in general endurance in hub negative, tamoxifen-treated breast malignant growth (Paik et al. 2004). Diagnostic biomarker is another class of biomarker, utilized to distinguish whether a patient has a particular infection condition (Imperiale et al. 2014). Customized disease treatment coordinates analytic biomarkers, prognostic biomarkers, prescient biomarkers, pharmacokinetic, pharmacodynamic, pharmacogenomic biomarkers and substitute biomarkers show in Fig. 3.2. (Ileana Dumbrava et al. 2018).

3.4 Biomarkers in Drug Development

Drug improvement in every helpful zone, including oncology, has entered a basic period driven by the business need of drug organizations to make sure about key expansions in profitability, reducing the time and cost needed for dispatching new medications. Low efficiency in medication improvement creates costs that are progressively dreadful in the present drugs market including the expenses of late-stage drug steady loss in stage 2 or 3 for lacking adequacy; the expenses of neglecting to distinguish preclinically those mixes having unmanageable dangers; and the chance expenses of ending mixes in preclinical advancement for security issues in light of the fact that accessible logical data is deficient to help sound danger evaluation and the executives in the canter. Cancer drugs' improvement has been advancing enormously all through the past 50 years. Appreciation of sub-atomic variations and different pathways which lead to the last occasion of harmful cells and its outcomes has prompted a cycle called customized or accuracy oncology. Biomarkers have arisen as demonstrative or prognostic devices, yet in addition as prescient devices of reaction to medicines. By and large, the biomarker is a proxy for particular focuses to cancer treatments (Lara Gongora et al. 2020).

Industry at present is occupied with a huge activity to create powerful viability and well-being biomarkers that can be utilized with regards to new medication improvement techniques to altogether diminish these expenses by supporting sound "go-on" choices to end unsatisfactory mixes at the soonest conceivable stage and educated "go" choices and danger the executive's methodologies to keep up great medications being developed (Floyd and Mcshane 2004).

Drug improvement dependent on biomarker evaluation is an arising and as of now settled field of study. Focal points of this technique, for example, viability and less ideal opportunity for the endorsement of the medication, have been talked about. There are a few difficulties with regards to biomarkers and medication advancement. A high steady loss rate is one of these difficulties, particularly in late phases of the

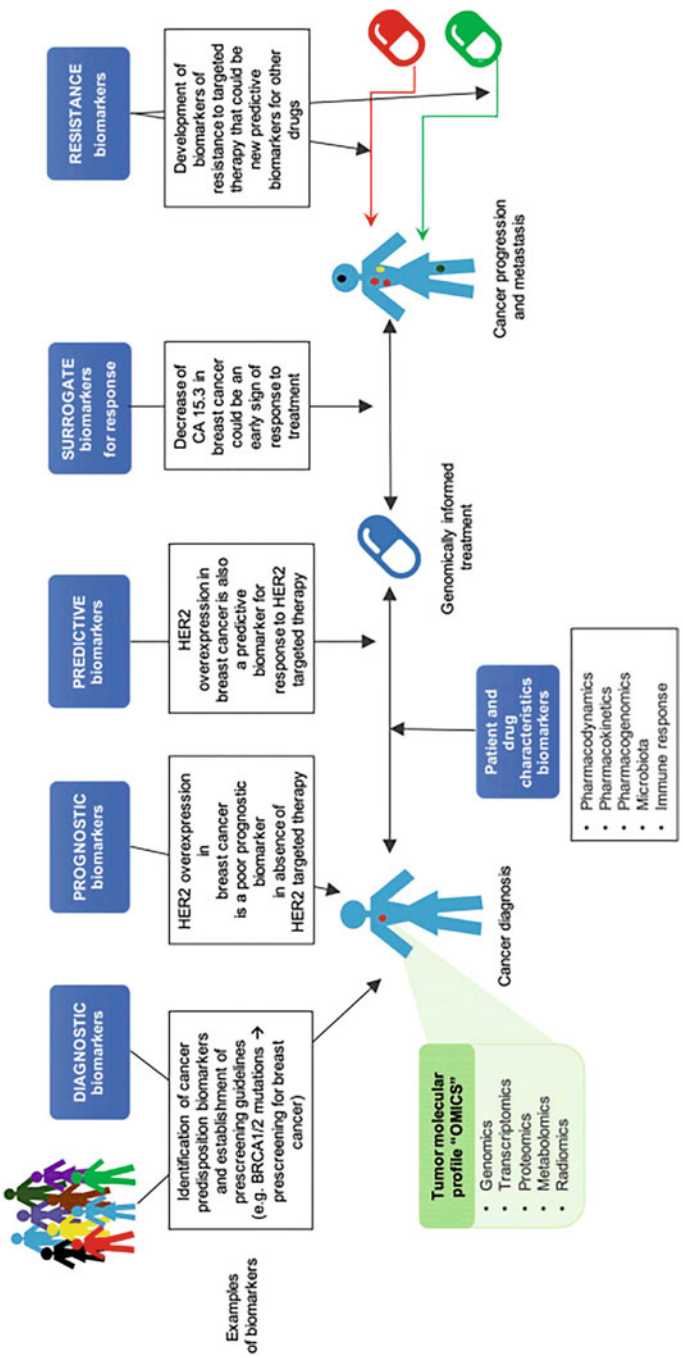

Fig. 3.2 Types of biomarkers in the multistep drug development process

clinical turn of events. Exploring with recognizable proof of biomarkers should zero the potential for treatment. Likewise, substitute endpoints must be approved which can further improve the medication advancement technique. As an outcome, expenses can be reduced with the identification of early medication viability (Smith et al. 2014).

The significant difficulties in tumor growth drug improvement are separate reactions, adequacy, and harmful results. The drug business, drug strategy producers, and executives are continually searching for novel pharmacogenomic or pharmacoproteomic examinations that may recognize likely biomarkers to help take care of these issues. Without a doubt, the atomic objective of most helpful specialists stays obscure. This has prompted costly turn of events and creation of malignant growth drugs in light of an absence of data on targets, which can be utilized to test the adequacy of therapeutics. Novel approaches are expected to recognize individualized patient advantages of treatments, limit the danger of harmfulness, and decrease the expense of treatment. The essential test is which kind of biomarker to use across the wide range of illness measures. Phenotypic articulation markers (RNA/protein) shift among cell types and change after some time and show distinctive posttranscriptional or posttranslational adjustments. Notwithstanding, proteins are bountiful, effectively open, and show guarantee for estimating results and contemplating changes in infection state. Another test in portraying biomarkers is the multifaceted nature of the articulation profile of likely markers in generous conditions near the sickness aggregates (Bensalah et al. 2007).

3.5 Biomarkers in Cancer Treatment

The significant difficulties in cancer drug improvement are separate reactions, viability, and harmful results. The drug business, drug strategy producers, and overseers are continually searching for novel pharmacogenomic or pharmocoproteomic examine that may recognize likely biomarkers to help take care of these issues. In reality, the atomic objective of most helpful specialists stays obscure. This has prompted a costly turn of events and the creation of disease drugs due to an absence of data on targets, which can be utilized to test the viability of therapeutics. Novel approaches are expected to distinguish individualized patient advantages of treatments, limit the danger of harmfulness, and diminish the expense of treatment. The essential test is which sort of biomarker to use across the wide range of illness measures. Phenotypic articulation markers (RNA/protein) shift among cell types and change over the long haul and show distinctive posttranscriptional or posttranslational adjustments. Nonetheless, proteins are bountiful, effectively open, and show a guarantee for estimating results and contemplating changes in the sickness state. Another test in describing biomarkers is the unpredictability of the articulation profile of likely markers in generous conditions near the sickness aggregates (Bensalah et al. 2007).

Presently, cancer therapy is turning into an undeniably customized clinical intercession. There are numerous prescriptions which follow up on a particular

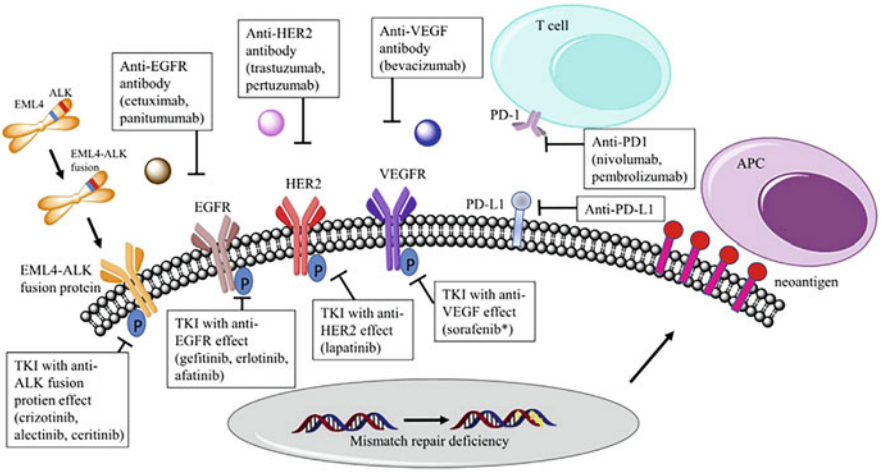

Fig. 3.3 The biomarkers and responding medications in colorectal, breast and lung cancers (Kuo et al. 2019)

objective to repress the development of malignancy cells, and the utilization of these focused on treatments might be led by the occurrence of biomarkers. The normal biomarkers for malignant growth treatment are introduced in Fig. 3.3. For patients having breast malignant growth, endocrine treatment is advantageous for patients with ER/PR articulation, while a HER2 inhibitor ought to be utilized for patients with HER2 articulation. In like manner, in CRC (Colorectal malignancy) patients, an EGFR inhibitor ought to be thought of, if tolerant have EGFR articulation without RAS transformation (Douillard et al. 2013). For patients with NSCLC (Non-little cell cellular breakdown in the lungs), different TKIs and immunotherapy are the primary line medicines against sickness with EGFR change, ALK revamp, ROS1 reworking, or customized passing ligand 1 (PD-L1) expression (Reck et al. 2016). The investigation led by Kuo et al. (2019) expressed that in Taiwan, the recurrence of EGFR transformation and ALK improvement in patient with NSCLC are 55.7% and 9.8%, respectively that biomarkers are analyzed before settling on a therapy choice. For instance, EGFR articulation test for CRC conclusion is repaid by the NHI, and some other biomarker tests, for example, those for EGFR transformation, RAS change, and PD-L1 articulation, are typically supported by drug organizations. Afterward the illness is portrayed for explicit biomarkers articulation, doctors will settle on an educated choice on most accurate cure for patients. All in all, these focused on treatments furnish treatment benefits in patients with a particular biomarker. The utilization of biomarkers for malignancy treatment choice in Taiwan is reliable with NCCN and Pan-Asian rules (Kuo et al. 2019).

Meta-analysis of stage one contemplates distributed in a 3-year time frame was incorporated in an aggregate of 13,203 patients. The creators showed that the malignancy treatment on biomarker-based procedure was related with a developed

reaction rate (30.6% vs. 4.9% $p < 0.001$) and a more drawn out middle PFS (5.7 vs. 2.95 months, $p < 0.001$) the techniques that did not have the utilization of a biomarker. Besides, customized arms utilizing a DNA biomarker (genomic modification) had a higher reaction rate than those with a protein biomarker (42% vs. 22.4% $p < 0.001$). All things considered, the biomarker procedure was still minority among the examinations 16% (58 of 351 arms). The middle treatment mortality was not measurably unique among biomarker-driven methodology as opposed to not, guaranteeing security of the system. Endurance couldn't be gotten to, as it was not revealed in most of the examinations. Another fascinating result was that 8 of 9 anomaly preliminaries with reaction rates exceeding 60% was customized, underscoring the significance of the biomarkers to the recognize treatments that will address blockbusters in oncology (Lara Gongora et al. 2020).

In the area of cancer research and treatment, the idea of exact medication—anticipation and management procedures that consider singular changeability—depends on the improvement of substantial biomarkers grilling key distorted pathways conceivably targetable with focused on immunologic treatments (Collins and Varmus 2015). In spite of the fact that biomarkers, for example, prostate-explicit antigen (PSA), have been known and utilized for quite a long time to endeavor to direct prognostic and remedial choices, the new upheaval in sub-atomic science, with the ascent of high-throughput sequencing and expanded sub-atomic portrayal of tumor tissue has prompted an outstanding expansion in endeavours to quantify and target distorted pathways at the sub-atomic level. By and by, there has been a huge hole between different beginning reports of biomarkers, regularly with symptomatic execution that can't be duplicated in later examinations, and full clinical usage and approval of the biomarkers because of issues in investigation, configuration, test stages, and accessibility of examples for biomarker advancement (Tzoulaki et al. 2011). By the by, with the new development of exceptionally specific sub-atomic focused on specialists and high-throughput genomic portrayal advances, hearty and very much approved malignancy biomarkers are progressively required. For example, over 90% of oncological medications that enter clinical advancement won't arrive at market endorsement because of disappointment of clinical preliminaries to exhibit helpful advantage, adding to expensive and slow malignancy drug improvement (Paul et al. 2010). As recognized by FDA, the sensible utilization of biomarkers is relied upon to assume a significant part in limiting danger of clinical preliminary disappointment by enhancing the preliminary populaces with explicit atomic subtypes reacting better to tried treatments. (Goossens et al. 2015).

TAA (Tumor-Associated Antigens) or disease biomarker are significant targets for malignant growth treatments. Neutralizer-based specialists focusing on malignant growth biomarkers incorporate monoclonal antibodies radiolabelled MoAbs, bispecific T-cell engagers (BiTEs), and counter acting agent drug forms (ADCs). In the past few years, illusory antigen receptor-designed T cells (CAR - T) has become a significant discovery in malignancy immunotherapy (June et al. 2018).

3.6 Cancer Biomarkers Currently Available in Clinic

Overexpression/intensification of HER2 (ERBB2), a member of the epidermal growth factor receptor (EGFR) family, predicts reaction to monoclonal antibodies such as trastuzumab and pertuzumab in breast cancer, is an example of a molecular biomarker in use (Goossens et al. 2015). In critical stage III breast cancer preliminaries, it was discovered that subjects with HER2 overexpression (roughly 20% of patients) infected with anti-HER2 therapy had increased infection-free and overall stamina (Goossens et al. 20152015). HER2 overexpression is comparatively prescient of reaction to trastuzumab in esophago-gastric adenocarcinoma (Bang et al. 2010). Other most important prescient biomarkers, remembering BCR-ABL for ongoing myeloid leukemia and KRAS changes in colorectal disease and various transformations in non-little cell cellular breakdown in the lungs (NSCLC). Regardless of this, numerous other prognostic biomarkers are accessible over the LDT pathway (Koscielny 2010). Another quality articulation-based test, Oncotype Dx breast Cancer Assay estimates 21 qualities foreseeing bosom malignant growth repeat in ladies with hub negative or hub positive, ER-positive, HER2-negative obtrusive bosom disease (Mamounas et al. 2010). Analytic biomarkers are perhaps the most assorted classes of biomarkers going from examines produced for malignancy screening to indicative tests surveying movement of a known disease. One ongoing illustration of a symptomatic biomarker is Cologuard, a multigene DNA (KRAS transformations, unusual NDRG4 and BMP3 methylation) stool test joined with fecal immunochemistry intended to screen for colorectal disease in people at normal danger of colorectal malignancy (Imperiale et al. 2014). These urging results prompted the endorsement of this test by the FDA in August 2014. As of late, there has additionally been expanded interest in growing insignificantly intrusive symptomatic tumor biomarkers, utilizing the estimation of flowing DNA or microRNA. For example, another innovation named malignancy customized profiling by profound sequencing (CAPP-Seq) has been tried on flowing tumor DNA in patients with non-little cell cellular breakdown in the lungs (Newman et al. 2014).

Biomarkers approved in a particular kind of disease are going through disclosure and approval in different malignancies (for example BRAF transformations or HER2 overexpression) hidden in certain common oncogenic drivers and less common tumors are additionally profiting by the fast advancements in the field (Rutgers et al. 2013).

3.6.1 FZR1 as a Probable Biomarker for NACT in Breast Cancer

In the field of bosom disease treatment, an exhaustive treatment methodology is built up that included a medical procedure, chemotherapy, radiotherapy, endocrine treatment and atomic focused on therapy (Gass et al. 2018). Careful therapy is as yet the main methodology for bosom malignant growth. The idea of bosom moderating and the advancement of bosom rationing a medical procedure is quite possibly the most noteworthy accomplishments of disease treatment. Right now, Neoadjuvant

chemotherapy (NACT) adds to bosom saving malignancy treatment that chemotherapy medications are conveyed before a medical procedure to shrivel the tumor. NACT is decreasing the tumor size by preceding a medical procedure causes less harm to encompassing tissue and separate the edge of the tumor from solid tissue. NACT is broadly utilized in breast cancer treatment to downstage privately progressed (inoperable) sickness and make it operable, especially for huge tumors (Mougalian et al. 2015). FZR1 is a biomarker of breast cancer NACT dependent on the transcriptomic information examination and the atomic component examination. FZR1 is associated with the guideline of the strength and transcriptional movement of tumor silencer p53. The capacity of FZR1 is to weak the cell apoptosis and cell cycle capture by chemotherapy drug acceptance. The approval with a companion of clinical patient examples showed that the declaration of FZR1 can be a biomarker for the adequacy of NACT. The assessment was performed by the IHC and the evaluation of optical thickness score, which is achievable and appropriate for the medical application (Liu et al. 2020).

FZR1 is a possible NACT biomarker in breast cancer, and it interacts with the apoptosis-inducing guideline of chemotherapy drugs.

These outcomes propose that FZR1 influences bosom malignant growth cell protection from chemotherapeutic specialists by controlling cell cycle capture. It is accounted for that FZR1 restrains BRAF oncogenic capacities through both APC-subordinate proteolysis and APC free interruption of BRAF dimers. FZR1 is considered as a tumor silencer that are adversely controlled by the enactment of the MEK/ERK oncogenic flagging cascade (Wan et al. 2017). The proof demonstrated that FZR1 associated with PRL-3 to manage the movement of colorectal malignancy by controlling the soundness of AURKA (Zhang and Wang 2019). These results are consistent with our clinical findings that the outflow of FZR1 is linked to the guess and resilience of patients with bosom disease. FZR1 has been identified as a tumor suppressor and an oncoprotein in a variety of diseases. Deficiency of FZR1 adds to the improvement of chemotherapy safe clones in mouse and human B cell intense leukemia. FZR1 represses the replicative pressure and p53-subordinate cell passing in neural forebears. Due to all these facts, this information is reliable that FZR1 balanced apoptosis through p53 strength. The FZR1 could be the biomarker that can be used to demonstrate the efficacy of NACT in apoptosis and cell cycle arrest, that FZR1 could contribute to recognition of drug for chemotherapy and apoptosis. Our findings suggest that FZR1 may be a possible biomarker for NACT in breast cancer (Liu et al. 2020).

3.7 Biomarkers for Preclinical Modelling

3.7.1 Screening Apoptosis

Though preclinical cancer models have their own set of biological challenges, screening drugs necessitates a concerted effort to identify specific biomarkers that indicate anticancer viability. Novel phases have been designed as a strategy to

enhance the biomarkers predictive of clinical response or resistance. ChemoINTEL (unique name MICK Assay), for example, measures the in vitro apoptotic response of a patient's tumor to chemotherapy drugs by continuously measuring multiple biochemical and morphologic apoptotic markers within single cells over a 48-h cell culture period. ChemoINTEL is a new classification of chemo affectability test based on medication-induced apoptosis in cell lines rather than the traditional phenotypic markers that have been used for a long time (Bosserman et al. 2012).

3.7.2 CD20, CD22, CD30, and CD79b as Lymphoid Malignancy Targets

MoAbs against CD20 have been broadly utilized for lymphoid malignancies (Marcus et al. 2017). ADCs are progressively utilized as chemoimmunotherapy. Four new ADCs have been endorsed for the treatment of lymphoid malignancies: brentuximab vedotin focusing on CD30, inotuzumab ozogamicin and moxetumomab pasudotox is focusing on CD22 and polatuzumab vedotin focusing on CD79b (Kantarjian et al. 2016). More biomarkers are being focused with ADCs or CAR-T cells. These biomarkers incorporate CD25, CD37, CD56, CD70, CD74, and CD138 (Yu and Liu 2019).

3.7.3 CD33, CD123 and CLL-1 as Focuses for Myeloid Malignancies

Gemtuzumab ozogamicin (GO) is an ADC against CD33 that is generally communicated on myeloid cells. GO has been affirmed for recently analyzed just as hard-headed/backslid (RR) intense myeloid leukemia (AML). GO might be utilized as a solitary specialist or in blend with chemotherapy regimens. What's more, a few novel ADCs focusing on CD33 are under clinical turn of events. These incorporate vadastuximab talirine (SGN-CD33A), IMGN779, and AVE9633 (huMy9–6-DM4). ADCs focusing on CD123, for example, IMGN632 and SGNCD123A, are being tried in clinical preliminaries. Further advancement of SGN-123A was anyway ended because of well-being concerns. Nibble and ADCs focusing on CLL-1 are at present going through preclinical or early clinical examinations for AML. CLL-1-directed CAR-T cells are in clinical preliminaries for AML treatment (Liu 2019).

3.7.4 Biomarkers for Strong Tumor Immunotherapy

CD133-focused in on CAR-T cells have been utilized for strong tumors counting cholangiocarcinoma (Glumac and LeBeau 2018). Mesothelin-zeroed in on CAR-T cells have been represented in mesothelioma, cell breakdown in the lungs, chest threatening development, gastric sickness and pancreatic danger (Kelly et al. 2012). Lymphocyte receptor-engineered T cells against AFP and MAGE-A1 have been spoken to immunotherapy of resistant tumors (Zhang and Wang 2019).

3.7.5 Tyrosine Kinase Biomarkers as Targets of Small Molecule Inhibitors

Inhibitors of BCR-ABL, JAK2, FLT3, Bruton tyrosine kinase have prompted a change in perspective in the administration of leukemia. TKIs focusing on an assortment of tyrosine kinase oncoproteins, for example, VEGFR, EGFR, FGFR, RET, HER2, MET, MEK have particularly changed the helpful scene of such tumors as non-little cell cellular breakdown in the lungs, bosom malignant growth, bladder disease, liver malignancy, and renal cell carcinoma (Liu 2019).

Biomarkers of numerous non-tyrosine kinase oncoproteins are significant targets additionally for disease treatment. Inhibitors of BCL-2, isocitrate dehydrogenases (IDH1 and IDH2), PI3 kinase, BRAF, mTOR, PARP and CDK have immensely extended the armamentarium against an assortment of disease types, for example, leukemia, lymphoma, melanoma, bosom malignancy, and ovarian malignancy (Kopetz et al. 2019).

3.7.6 Designing Biomarkers Through Systems Biology for Cancer Treatment

CD33 could be a myeloid marker and the target of the GO ADC in AML (Hoseini et al. 2018). In any case, off-tumor harm levels due to clarification of CD33 in ordinary hematopoietic cells restrain the clinical applications. Right when CD33 quality was taken out from the human hematopoietic stem and ancestor cells (HSPC), CD33-zeroing in on CAR-T cells expressly slaughtered AML cells without myelotoxicity in receivers migrated with CD33-invalid HSPCs. Clinically, the similar philosophy using structures science to plan HSPCs has been attempted in a HIV+ lenient with outstandingly resolute exceptional lymphoblastic leukemia (ALL) (Xu et al. 2019). For the present circumstance, CCR-5 of a totally HLA facilitated allogeneic common benefactor HSPCs were taken out with CRISPR advancement. The benefactor HSPCs with eliminated CCR-5 were migrated into the HIV+ lenient. The ALL went into complete decrease with creativity of CCR-5 negative hematopoiesis. The ALL went into total diminish with imagination of CCR-5 negative hematopoiesis. This framework utilizing system biology and arranging opens up a few other seasons of fabricated hurt express biomarkers for centered affliction treatment. Journey for new biomarkers and novel plans similar as transport methodologies, for instance, nanotechnology of centered experts are driving the accompanying surge of advances in threatening development treatment (Liu 2019).

T-PLL (T-cell prolymphocytic leukemia) is a helpless predictive illness with exceptionally restricted alternatives of productive treatments. Maximum patients are headstrong to chemotherapies and regardless of high reaction rates after alemtuzumab, basically all patients backslide. Along these lines, there is a neglected clinical requirement for novel treatments in T-PLL. As the CCR7 chemokine receptor is a particle communicated in a wide scope of malignancies and applicable

in numerous tumor measures, the current examination tended to the biologic job of this receptor in T-PLL. In addition, we clarified the rebellious action interceded by a foe of CCR7 monoclonal neutralizer (mAb) and surveyed whether its foe of tumor development would warrant progression towards clinical applications in T-PLL. Our results appear that CCR7 could be a prognostic biomarker for the most part of continuance in T-PLL patients and a valuable receptor locked in with the development, assault, and continuance of leukemic cells. Focusing on CCR7 with a mAb controlled ligand-intervened hailing pathways and affects tumor cell executing in fundamental cases, and planning antibodies against CCR7 also significantly effective in T-cell leukemia xenograft models. Together, these disclosures make CCR7 an engaging molecule for novel mAb-based remedial applications in T-PLL, a sickness where late medicine screen endeavours and considers tending to unused blends have focused in on chemotherapy or small molecules (Cuesta-Mateos et al. 2020).

3.8 Challenges of Biomarkers in Medical Revelation

The difficulties of biomarkers in medication disclosure and improvement might be measured at 3 unique points: (1) distinguishing the correct objective of the medication such as biomarker; (2) approval of the biomarker investigation being referred (3) advancement of coordinated biomarker-driven medicines (Fig. 3.4). Pushing ahead, unmistakably these biomarker difficulties will be dependent upon constant mechanical enhancements. For instance, as DNA sequencing improves and turns out to be more savvy, entire genome sequencing could be regularly used to recognize uncommon however exceptionally penetrant germline changes that might actually prompt atomic screening programs and early discovery of applicable tumors. (Ileana Dumbrava et al. 2018) (Fig. 3.4).

3.9 Future Recommendation

In the upcoming years, cooperation among clinicians, researchers and administrative organizations is substitute for a fruitful medication improvement model. To start with, the improvement of explicit, clinically practised and systematically approved biomarkers is principal. A critical test is to diminish costs, when the track is provoking progressively more express biomarkers, limited to a small portion of patients. Second, the improvement of high viability medicines with insignificant harmfulness stays a test. Third, old style plan of clinical investigations sequencing ought to be returned to, to advance the expenses, which right now are impractical, and to improve the period from medication improvement to patients' entrance. Eventually, the administrative offices should accept Manuscript Information Classification: General invigorate drug advancement and depend on exceptional projects to quicken the endorsement and admittance to promising medications.

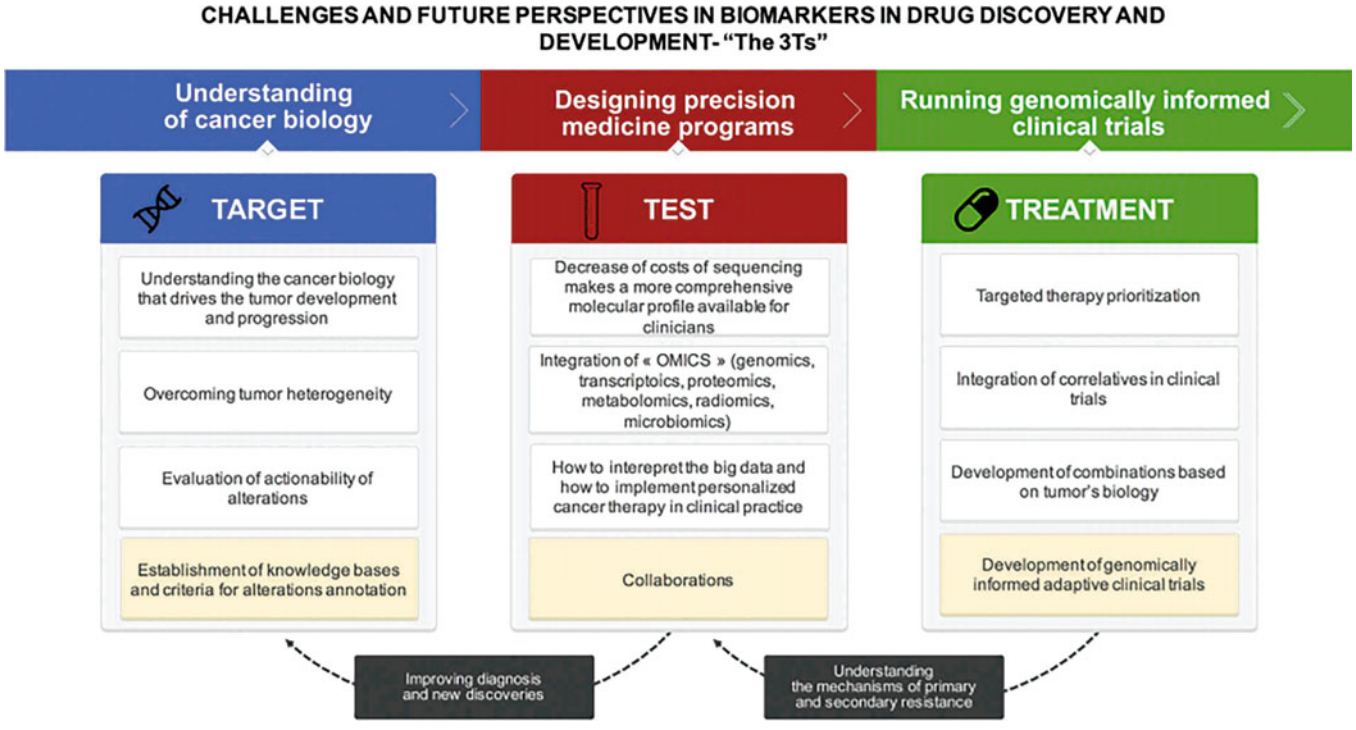

Fig. 3.4 Challenges and future perspectives in biomarkers in drug discovery and development. Adapted from Ileana Dumbrava et al. (2018)

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Clinical Proteomics: Diagnostics and Prognostic Markers of Cancer

4

Saima Zafar, Aniqa Saeed, and Saadia Zahid

Abstract

In the recent years, through the introduction of high-throughput technologies to innumerable fields of medicine, investigation of big cohorts of data is no longer a problem in the advanced research laboratories. However, designing of coherent study, obtaining of high-quality data under optimal conditions, and persuasive elucidation are critical features in ensuring the advancement of decent discipline available of these current technologies. The emerging field of proteomics have transformed the practices in the field of cancer biomarkers.

Currently, the application of rigorous biomarker identification and validation is an emerging arena. These studies emphasize on the multicenter studies homogenous procedures for high-throughput targeted MS assays. Furthermore, advances in MS sensitivity are accessing toward novel tumor-specific proteoforms comprising posttranslational modifications and variants devising from genomic anomalies. Moreover, proteomic data complementing the genomic and transcriptomic datasets imitates the emergent field of proteogenomics, which shows great potential to surge our understanding of cancer biology.

In this chapter, we will discuss the role of proteomics toward the diagnostic and prognostics aspects and recent improvements in MS-based clinical

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proteomics with a focus on oncology. We will deliver a detailed overview of samples types with clinical relevance, as well as, deliberation for sample preparation strategies, protein quantitation approaches, MS configurations, and data analysis pipelines currently available to researchers.

Keywords

Clinical proteomics · Targeted assay · Mass spectrometry · Cancer proteome · Diagnostics · Prognostics · Shot-gun Proteomics · Tumor pathophysiology · HUPO · Profiling

4.1 Clinical Proteomics

The successful understanding and development of human genome project (HGP) and other genomic projects has led to a stupendous and massive pathway for genomic and proteomic studies helping scientists gain a better understanding of molecular studies (Paik et al. 2008). Clinical proteomics refers to the studies of proteins and peptides, differing from genomic studies that involves studies of DNA. The qualitative and quantitative filtering of dynamic protein structures circumscribe large spectrum and preclinical diagnostic tools for cancer diseases (Apweiler et al. 2009). Eventually proteomics addresses the prerequisite of early diagnosis and the role of identified therapeutic targets towards suitable and personalized disease management and therapeutics. Progression of clinical proteomics leads to discovery of disease biomarkers that becomes cardinal desire in study of proteins to evaluate disease. However, the recent challenges are in developing exact procedures for both clinical handling and reduction of complexity and to increase detection ability of dynamic proteins and peptides that are present in minute amounts. Proteomics involves the interaction between the proteins and peptides differing from the interaction of gene expression level, that explains what genomics is, but a clear disadvantage of it is not giving the information and proper expression of the genes so the gene becomes useless and can only be started well through the proteomic studies (Fig. 4.1a) (Paik et al. 2008).

The basic concept of a disease biomarker is shifting toward a new exemplar that explains how a normal protein and peptide differs from the infective one leading toward accurate diagnosis through clinical studies (Anderson 2005). Clinical proteomics is best accessed with fresh and newly collected samples that have proper mapping of connective tissues and are morphologically consistent and easy to treat with body fluids of sufficient quantity. Clinical proteomics purposely engage proteomic methodologies, molecular structures, and vast analysis of bioinformatics to identify dynamic patterns of protein diseases that results in better assessment for disease prevention, early diagnosis, and proper selection of treatment methodologies that vary from person to person but are unique and universal for protein structures

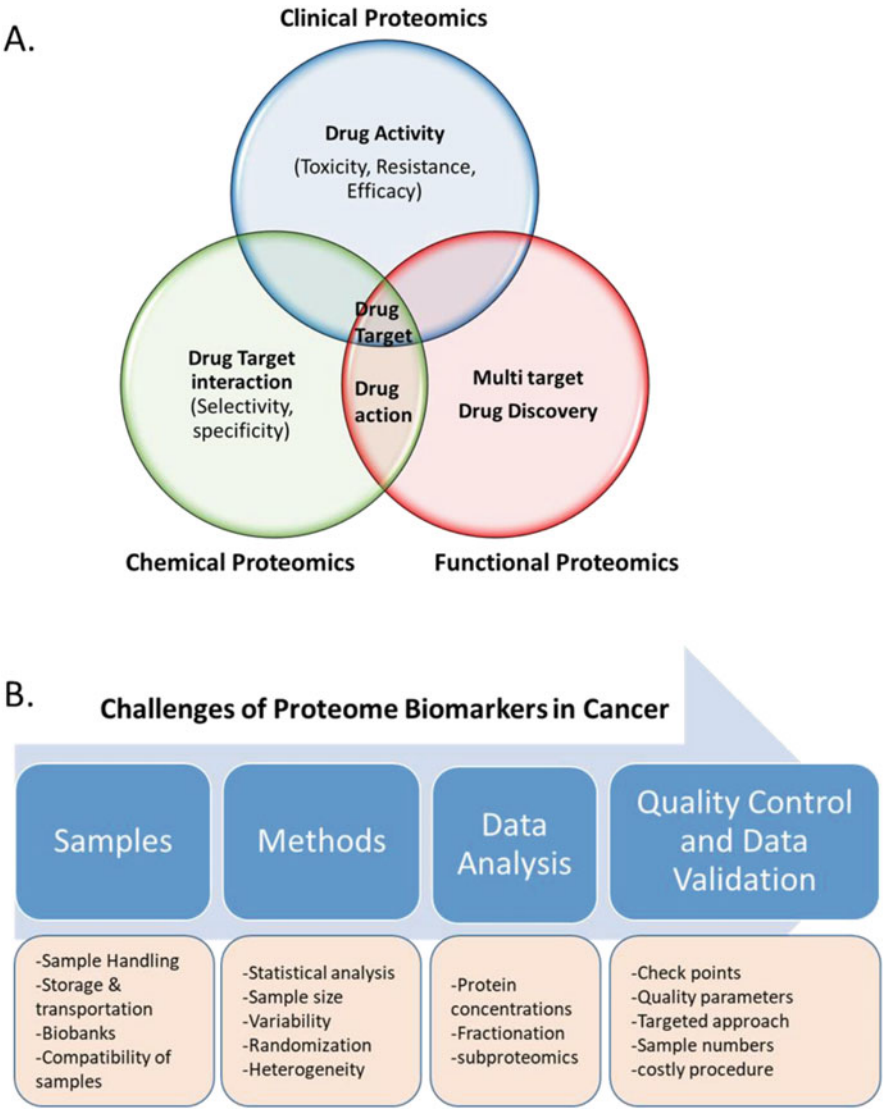

Fig. 4.1 (**a**) Main applications of proteomics, (**b**) Challenges in identification of biomarkers in proteomic studies

(Savino et al. 2012). All these steps and studies regarding clinical proteomics have helped scientists and microbiologists to focus easily on the novel diagnostic and therapeutic analysis of cancer diseases using biomarkers and dynamic protein structures for the betterment of human beings.

4.2 Goals and Need for Proteomics

Basic goals of proteomics refer to categorizing presentable dynamic proteins available in a biological system at a certain point under given reliable conditions like any drug intake, stressful environmental condition, and mutations. Advancements in mass spectrometers and MS based methods have resulted in best quantitative analysis of large number of the samples at least up to 8 in a very short time. This saves the time of the patients and gives correct analysis and diagnosis on time. What method is to be chosen depends upon the study's preference and patient's comorbidity and conditions. Some of the few important methods for proteomic studies include SILAC, iTRAQ, TMT, and Label-free methods (Rai et al. 2002).

Proteins being the major outfit of biological system gives scientists and microbiologists an accurate perception on how biological systems adapt under different conditions. The details of mutations and undergoing changes in a cell, concerning benign and malignant details of cancer cells and the differences between cells of different tissues explaining mutations in dynamic protein structures, are all elaborated during clinical proteomics (Paik et al. 2008). These details are helpful in better understanding of basic biological mechanisms and undergoing cell mutations, finding biomarkers and tumor markers that help in early diagnosis of disease and help in good prognosis after early treatment. Major need of proteomics is in proper and timely treatment using modalities like targeted therapies and other chemotherapy drugs after measurements of proteins through biomarkers (Knowles et al. 2003).

Clinical proteomics is the basic need and goal for monitoring the properties and mechanism of the whole dynamic protein structures from a cell or organism and help in determining the pathophysiology of these proteins. Proteomics enable us to gain better understanding of systemic and functional metabolism of proteins even in various physiological states and conditions like changes in cell cycle and signaling ligands. Almost all these tasks that are important for cancer diagnosis are not detected by genomics or transcriptomics and are only observable in studies of proteins (Zhang et al. 2004). A clear and major example of this is that only proteins can sense extracellular signals by detecting the exact binding protein to which the cell responds once the molecules get attached to them. Moreover, mechanisms and changes that occur when proteins bind with other complex structures including transport system and signals are not detected by genomic studies. Such systems require proteomic studies for better analysis and diagnosis of cancer (Fig. 4.1b) (Gygi et al. 1999).

Many other cellular and molecular processes involving covalent modifications and phosphorylation of proteins is a magnificent example of why proteomic studies ae important. Proteomic studies are the basic need when it comes to these processes as they are invisible and not understandable in genomics and transcriptomics (Schneider and Hall 2005). For a system to be detected by genomics, mechanism must be very fast to be covered in milliseconds while these transcription and binding processes are to slow that becomes the utmost reason of why genomics and transcriptomics fails and the need for proteomic studies became very important specially in carcinoma studies. Proteoforms, the expressed form of proteins, defining

the most important physiology and disease conditions, act as molecular actors and cellular phenotype, only accessed by proteomic details. The exemplary mechanism of regulation of a transcribed mRNA that is further translated during many important biological systems is also not assessed by genomic studies. These issues have increased the need for proteomic studies and made them important and prior goal for diagnosis of cancer studies and biomarker prognosis (Liotta et al. 2003).

4.3 Methods of Protein Measurement and Biomarker Identification

Studies and research related to protein mutations in cancer diseases have been studied for the past 70 to 80 years, but no extensive proteomic technique was well known till then. Detailed proteomic studies and extensive technologies for protein mutation and biomarker involvement for carcinomas were known since last three decades and thus have been a source of great help for cancer diagnosis and early treatment before the patient reaches extreme stages. Combination of clinical proteomics and imaging diagnostic modalities has helped better clinical presentation of protein biomarkers in different carcinomas like prostate cancer, breast carcinomas, hepatocellular carcinomas, and rectosigmoid tumors (Knowles et al. 2003).

It is important to discuss the basic methods for protein measurements and biomarkers identification here that are in best practice nowadays and have made life easier by reducing death rates of patient suffering from carcinomas.

4.3.1 Bottom-up or Shotgun Proteomics

Bottom-up or shotgun proteomics is one of the most versatile used method of protein analyzes. Basis of this type of MS-based studies involves isolation of proteins that are bisected into peptides either chemically or through enzyme involvement methods (Hoffmann et al. 2001). This involves chromatography and other separation methods that subdivides the end product mainly through reverse phase chromatography methods. Final analysis of these resultant peptides is done using MS-based methods after ionizing them through ESI, electrospray ionization (Hawkridge and Muddiman 2009).

Quantification and exact visualization of these resultant dynamic peptide structures helps in correct interpretation of disease and measurement of proteins, thus resulting in early diagnosis of carcinomas.

4.3.2 Mass Spectrometry-Based Proteomics

It is a vast and emerging technique of proteomics that helps in identification and exact quantification of protein structures along with necessary components that are essential for life. For better understanding and characterization of the proteins at

different levels including the proteome and sub-proteome levels, MS-based proteomics is used. MS-based proteomics (Hawkridge and Muddiman 2009) and used chromatographic instruments are sometimes associated with other newly advanced technologies such as separation of the ions and microchip-based proteome measurements at microscopic levels that are highly sensitive targeted techniques for cancer diagnosis. MS-based research and workshop was held in 2013 in National institute of Health (NIH) in USA that focused on these newly advanced technologies and approved them as one of the best proteomic methods for cancer diagnosis and biomarker identifications (Maes et al. 2015).

MS-based proteomics have been widely studied and used in protein analysis. After several advancements and research, this technique has been widely used for diagnosis of epigenetic cancers. This is quite simple and helpful as compared to the previous technique as it extracts the tissue or blood sample and then processes the dynamic protein structures for further evaluation. Mass spectroscopy helps in detailed study of the protein mutations the same way as genomics tells the details of the DNA through microarray images, quite advanced as compared to simple visualization of sample under microscope (Greenbaum et al. 2002). The use of MS-based proteomics is considered as one of the best advancements toward epigenetic biomarkers because of their exemplary involvements with chromatin structures and histone proteins involving histone posttranslational modifications resulting in best diagnostic and therapeutic results for cancers. Detection of proteomic biomarkers through protein microarrays, that are closely related to cancer development and prognosis has changed the perspective for cancer research studies. Metastatic carcinomas and primary tumors are easily diagnosed through MS-based techniques where all other fails to do so.

4.3.3 Polyacrylamide Gel Electrophoresis (PAGE)

This method involves extraction of proteins on the basis of their molecular masses and is frequently used in analysis of carcinomas for more than two decades. Very few advancements have been yet made on this but combining it with MS using fluorescent gels have helped diagnostic methods to become easier through proteomic studies (Gharbi et al. 2002). Proteins are separated by electrophoresis using a gel matrix resulting in migration of smaller proteins rapidly. Rate of this migration and molecular masses are one of the major factors effecting the results. Mostly PAGE-SDS technique (Maurer 1971) is used where strong protein denaturing detergents are present and binds to the protein dynamic structure in a flow. The presence of these denaturing detergents acts as reducing agents and thus bifurcates the disulfide bonds that helps in folding and unfolding of proteins and thus best analysis is made on the basis of resultant linear chains. Biomarkers and protein analysis has made diagnosis of carcinomas quite easier and faster using these MS techniques (Fig. 4.2).

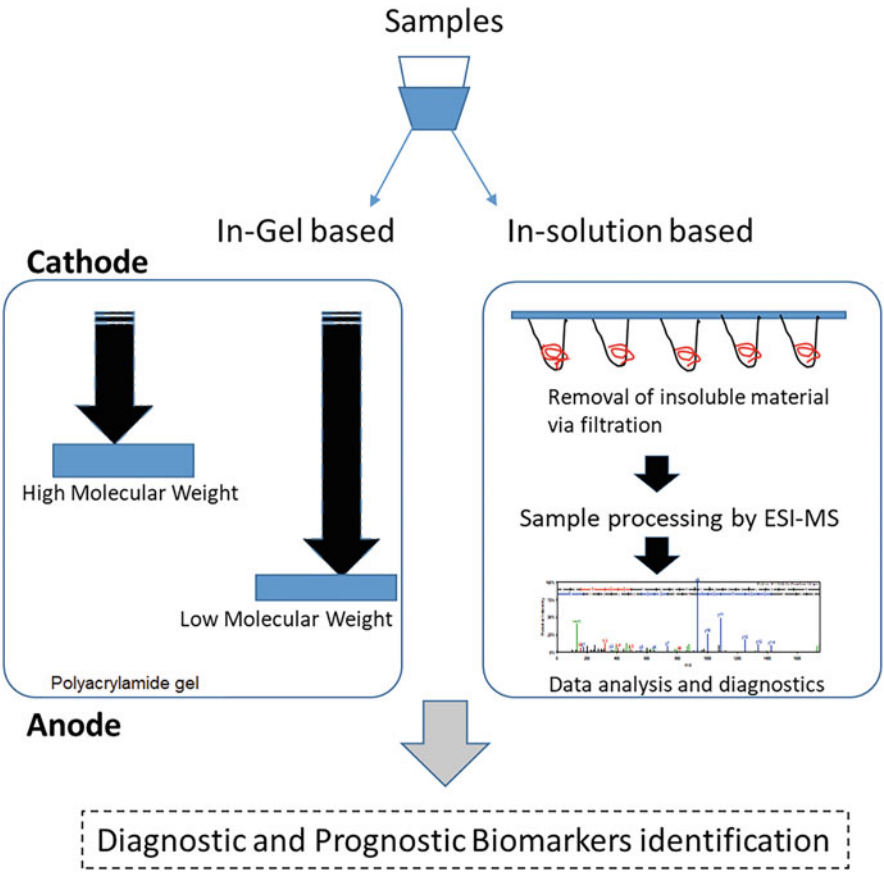

Fig. 4.2 Separation of samples (protein) on the molecular bases and ions processing's. Processing of samples (in-gel base and in-fluid base) on the polyacrylamide gels and remove the insoluble materials by filtration. The proteomic pattern of the samples is then acquired using electrospray ionization mass spectrometry and further identified and characterized as diagnostic and prognostic biomarkers of cancer

4.4 Proteomics and Cancer

Cancer being a combination of genetic and environmental abnormalities is mainly caused by uncontrolled production of cells resulting in necrosis and disrupted cellular signals (Citti et al. 2018). Large number of pathological cells accumulate and grow abnormally resulting in genomic and proteomic defects causing mutations of chromosome and DNA detected by genome structures and mutations of proteins and peptides analyzed by proteomic studies. Carcinomas are one of the most leading cause of the death majorly breast carcinomas, hepatocellular carcinoma, prostate

cancers, and rectosigmoid cancers. Mutations in protein structures that results in cancers are detected by proteomic studies (Somiari et al. 2003).

Carcinomas can easily be treated by preventive measures, early detection, and adequate therapeutic modalities varying from patient to patient. Major concern of oncologists is the early and timely diagnosis of cancer either by genomic studies or by proteomic evaluation (Petricoin et al. 2002). There is no doubt that genomic studies play an important role in cancer diagnosis but histone modifications, translations, and peptide mutations are ignored by genomic evaluation and there one needs complete study of proteomes for less false positive results and accurate staging of cancers (Haslinger et al. 2004). Genes being the basic controller of any cell behavior play an important role in cancer diagnosis but protein expressions and effectiveness explaining molecular basis of any disease are not understood by genomics. In carcinomas, almost all proteins invade, increases chances of metastases and respond better to any targeted therapies and controllers after interaction with surrounding cells (Verrills 2006). Proteins help in understanding the molecular mechanism of carcinogenesis and cell cycle network involving mitosis and meiosis thus adequately identifying the signaling network of almost all types of cancers along with its prognosis. Alterations in normal mechanism in any stage of carcinogenesis is analyzed best by proteomic studies that accurately enables oncologists and cancer biologists to better understand circumstances even in all environmental and carcinogenic changes (Apweiler et al. 2009).

Clinical proteomics concerning with cancer diagnosis is further elaborated as "Onco-proteomics" that involves protein studies in cancer mutations and interactions with proteomic alterations. This disciplinary branch of proteomics encourages better understanding of caner pathology and tumor biomarkers for best therapeutic results (Fig. 4.3a) (Sallam 2015).

One of the dormant mechanisms for the proteomic alterations in cancer is the omnipresent aneuploidy, which is known as uncontrolled and imbalanced chromosomal structures. Aneuploid cells are resultant outcomes of stressful and toxic conditions leading to defect in proteo-stasis, disturbing the equilibrium and balance of dynamic peptides and proteins.

All these details are still under research but a large number of studies have explained the relationship of defective proteo-stasis and gene mutations. Due to the abnormal increase of chromosome, any induced protein expression and genetic expression is not obligatory to be translated (Wang et al. 2004). This increases chances of protein degradation and chronic issues in folding–unfolding of proteins. It is known that many proteins and peptide structures, mainly kinases and multimeric protein complexes, need more requirements for the cellular protein folding, and thus they are more susceptive to misfolding than others.

Largely available tests for screening of cancers have low sensitivity and specificity thus differentiation of benign and malignant carcinomas is not done to the best of patient need. This leads to false positive results, (Huang et al. 2020) late diagnosis and wrong staging resulting is poor prognosis for cancer. The emergence of onco-proteomics is a source of hope for identification of biomarkers and tumor markers helping obtain best results after targeted therapies and chemotherapies for cancer

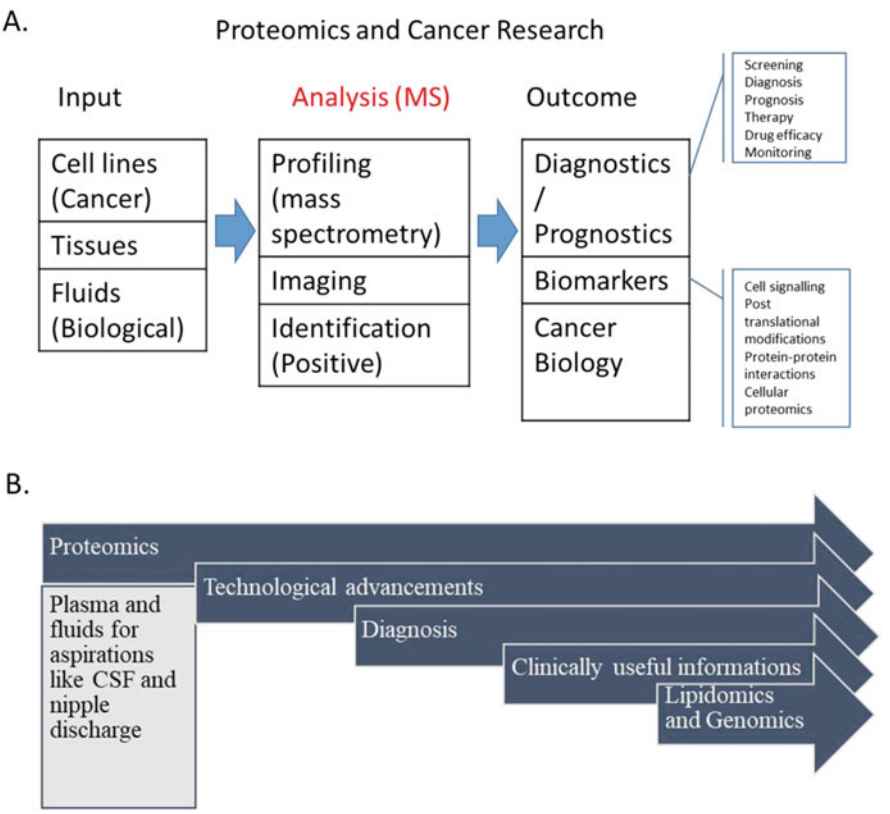

Fig. 4.3 (**a**) Proteomics in cancer research (**b**) Separation of proteins and usage of MS-based techniques for the identification of cancer biomarkers. Discovery of proteomics; Plasma and fluids (CSF and nipple discharge) for aspirations and function of dynamic proteins

patients. Best timely screening of malignancies has led to increased survival rate after ongoing treatment through accurate and timely diagnosis with the help of proteomics.

4.5 Early Diagnosis of Cancer

According to previous studies, proteomics deals with quantitative research and analysis of proteins but many recent studies have concluded the role of proteomics in structural analysis of dynamic proteins as well as leading to proper investigation of disease on time. The main goal of any disease-related research and therapeutic studies is the timely diagnosis of that disease leading to effective treatment (Hawkridge and Muddiman 2009). Proteomics play a vital role in early diagnosis of tumors giving world a new pathway in carcinogenesis.

The limitations of less sensitivity in analyzing the structures of proteins during the study of proteomics is the major hurdle and challenge for oncologists and bio-scientists. Furthermore, the proteins involved in cellular homeostasis, metabolism and structure are abundant and are present 10 thousand to 100 thousands fold greater than proteins involved in signaling networks in an individual cell leading toward great challenges in cell signaling (Yates 1998). The identification of desired biomarkers for the tumor studies in the field of proteomics have become easier specially during aspiration studies like pleural cancers, nipple aspirations in breast cancers and plasma studies due to new research in this field. Considering all fluid filled structures of human body, plasma specimens are one of the deepest fluids of human body containing large number of proteomes that help in best study and results during aspiration while treating tumor patients (Fig. 4.3b). The human plasma proteome promises the exemplary diagnosis of cancer by covering all major challenges of proteomics like early detection of cancer and prognosis of disease (Anderson 2005).

Discovery of biomarkers was initially done using different conventional methods and proteomic studies. Major methodologies for early diagnosis of tumors were, (Greenbaum et al. 2002)

- Protein distillation
- Enzyme Linked Immuno-Sorbent Assay
- Western Blot and Gel Electrophoresis

All these methodologies comprehend less specificity and sensitivity thus leading towards the prerequisite of using advanced technologies. The currently used analyzers in proteomics for biomarker identifications and early diagnosis of cancer are, (Maurer, 1971)

- 2D PAGE (Two-dimensional polyacrylamide gel electrophoresis)
- Mass spectrometry
  - MALDI-TOF (Matrix-assisted laser desorption/ionization-time of flight)
  - ESI (Electrospray Ionization)
  - SELDI-TOF (Surface enhanced laser desorption/ionization time-of-flight mass spectrometry) Many relevant studies are apprehensive with the identification of antigens or biomarkers for diagnostic, prognostic, or therapeutic use and have become supreme focus of oncologists. Genetic markers detected cytogenetically or through detection of changes in gene like mutations, are also now entering clinical practice, but some changes likely to be important in carcinogenesis, (Knowles et al. 2003) and its diagnosis such as abnormal expression of proto-oncogenes may not be associated with a detectable genetic injury (Fig. 4.2).

4.5.1 Diagnostics of Cancer and Proteomic

Cancer proteomics circumscribes the identification and quantitative analysis of many protein structures that are differentially expressed and damages the healthy tissues of human body leading to different stages of carcinomas from preneoplasia to neoplasia and necrosis resulting in malignancies (Wilson and Nock 2003).

Proteomics is of cardinal importance in the discovery of biomarkers and tumor markers because the proteome reflects both the intrinsic genetic program of the cell and the impact of its immediate environment. Expression of protein structures and peptides occur through transcription as well as posttranscriptional and translational events occurring in cells (Yeoh et al. 2002). According to previous studies, there are more than 200 posttranslational modifications that proteins could undergo that affect structure, and function of dynamic proteins. Interactions of protein–protein and nuclide–protein structures, their stability and their targeting nature, all play vital roles to a potentially large number of protein resultants from one gene. In cancer and other mutated diseases, many changes occur at protein level and convert a normal healthy cell into a mutated one with chances of necrosis. These neoplastic changes occur and result in tumor necrosis leading to carcinomas. Neoplastic cells are the resultant of protein modifications, deviant localization, and altered cell functions. Basis of proteomics is understanding these changes occurring in cell with the help of MS-based techniques (van der Merwe et al. 2007) and other proteomic technologies resulting in proper identification of biomarkers helping in early diagnosis of cancers and better prognosis of targeted treatment therapies.

Research proved that even after all proteomic and genomic efforts, some cancers metastasize rapidly even after best therapeutic interventions and therapies. One of the basic reasons behind this is late diagnosis of carcinomas leading toward less revival rate. Even then, better understanding of disease through onco-proteomic can lead to therapies that include increasing prognosis and palliation of disease (Ong et al. 2003).

4.6 Prognostics of Cancer and Proteomics

Even after the extreme advancements in cancer studies, survival rate for most cancers is not more, and the reason remains the same being late diagnosis and expensive treatment therapies. The basic goal of proteomics in cancer studies remains the same where oncologists and surgeons strive for better prognostics and palliative therapies. Where all other treatment modalities fail, the only solution left is palliation of disease to increase lifespan of human patients and help in good prognosis of disease. Estimation of prognosis of the cancer diseases and tumor patients is the basic need before the start of any treatment (Huang et al. 2020).

Many studies use RNA sequence data from the Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) after Human genome project (HGP) for evidence of good prognosis of tumor markers that help in building better prognostic models (Verrills et al. 2006). It is clear from studies that cancer is not only a

combination of genetic mutations like in DNA, but also involves modifications in protein structures along with altered metabolic levels and protein expressions. Mutations in cancer-associated genes can be manifested in defective protein structure. All known alterations can exert their detrimental impact by causing changes in stability of protein making the whole structure of protein and peptides permittable for degradation thus changing the protein's functional site and affinity that controls all major protein-protein interactions (Jessani et al. 2002).

Major genomic and proteomic alterations in cancer can be easily augmented by the recently emerging field of **"interactome profiling"** that focusses on centering of main network and provides tremendous data for protein-protein and protein–peptide interactions. Gulati and his co-workers explained this concept concluding that network related approaches have become necessary tool for interpretation of carcinomas (Sallam 2015). They explained diagnostics of carcinomas and elaborated different modalities that are used for diagnostics of tumor.

4.7 Recent Advances in Clinical Proteomics Methodologies

Presently, the large-scale total protein profiling is not a preferable approach. Instead, screening of specific subsets of protein biomarkers for cancer is the utmost applicable and significant strategy that may lead toward individualized patient-tailored therapy (Verrills 2006). Moreover, a wide array of sample types analyzed by clinical proteomics provided specific disease biomarkers. Although the importance of tissue samples cannot be marginalized, many studies also employed a noninvasive or minimally invasive sample collection manner (i.e., liquid biopsies) analyzing blood (plasma, serum) and urine to reveal novel biological insights of disease biomarkers and mechanisms. For instance, the urinary proteomics has substantially attributed for diagnostic and prognostic biomarkers for renal diseases. The exploitation of high-resolution properties of CE-MS has revealed several urinary proteins from ureteropelvic junction (UPJ) obstruction, a common clinical problem after birth (Chevalier 2004). Mass spectrometry-based approach offered a large dataset of urinary proteins associated not only with urological cancers, such as prostate, bladder, and kidney cancer but also with nonurological cancers, including breast, gastric, lung, esophageal, cervical, endometrial, colorectal cancer, and other tumors (Zhang et al. 2018).

Similarly, the plasma proteome, is also potentially suitable for comprehensive biomarker discovery for various cancers. With advent of high-throughput proteomics technology, the once expected outcome that is the development of panels of biomarkers for early detection of cancer and likelihood of the probable therapeutic response is partially achieved. However, due to complexity in plasma proteome makes it difficult to have a comprehensive dataset of targeted proteins. Perhaps combined methodological approach can overcomes these hurdles. A combination of high abundance protein ultradepletion (e.g., MARS-14 and an in-house IgY depletion columns) strategies, extensive peptide fractionation methods (SCX, SAX, High pH and SEC), and SWATH-MS (Sequential Window Acquisition of all THeoretical

Mass Spectra) unravel a subset of 5 proteins from plasma that may represent different stages of colorectal cancer (Ahn et al. 2019). Also devising an integrated quantitative proteomics pipeline combining the label-free and multiplexed-labeling (iTRAQ and TMT) in addition to targeted quantitation of proteins through multiple-reaction monitoring (MRM) and parallel-reaction monitoring (PRM) mass spectrometry is feasible for unbiased screening for plasma-based cancer biomarker discovery (Kumar et al. 2020). Moreover, SWATH-MS approach applied for tumor tissues also resulted in the quantitation of thousands of proteins with high reproducibility, providing novel insights on disease mechanism and biomarker discovery for various cancer types including soft tissue sarcomas as well (Gao et al. 2017).

Advances in mass spectrometry-based tools markedly assisted the clinical proteomics workflow such as mass cytometry (CyTOF) that enabled high-dimensional and unbiased characterization of tumor-infiltrating immune populations in cancer. Mass cytometry, also known as cytometry by time-of-flight (CyTOF), combines the high-throughput of flow cytometry and the fine resolution of mass spectrometry (Stern et al. 2018). High-dimensional single-cell proteomics analysis using CyTOF established a comprehensive dataset of immune cells and stem-like cells, providing an insight in immunotherapy decisions and prognosis for renal tumors and colon cancers (Li et al. 2020; Zhang et al. 2019). The high-field asymmetric ion mobility spectrometry (FAIMS) and trapped ion mobility spectrometry (TIMS) has further enhanced the peptide detections from routine analysis of cancer cell lines and other clinically relevant specimens from breast, pancreatic, and ovarian cancers (Hebert et al. 2018).

In addition, protein microarray technology also significantly contributed to cancer biomarker discovery. The analytical protein arrays are potentially used for accurate diagnosis and screening for high-risk individuals with pancreatic and breast cancer. Similarly, functional protein arrays also have become a popular tool for serum, plasma, and tissue protein profiling.

Overall, these methodological advancements and modifications enhance the precision and accuracy of clinical proteomics in cancer research, and making it an integral part of clinical practice both for prognostics and diagnostic determinations.

4.8 Conclusions and Future Directions

Clinical proteomics initially starts with an investment of large number of questions and need for searching right samples clinically. New biological and pathological outcomes of diseases are awaited when employ the modern proteomic approaches. Timely diagnosis and better understanding of proteomes leads to better prognostic results. Newly known and functional MS-based technologies have enabled all type of proteomic modifications even with high specific and sensitive quantitative measurement. MRM-MS is nowadays becoming a more effective introduced technology. The technological evolution and confluence of intervention and verification of

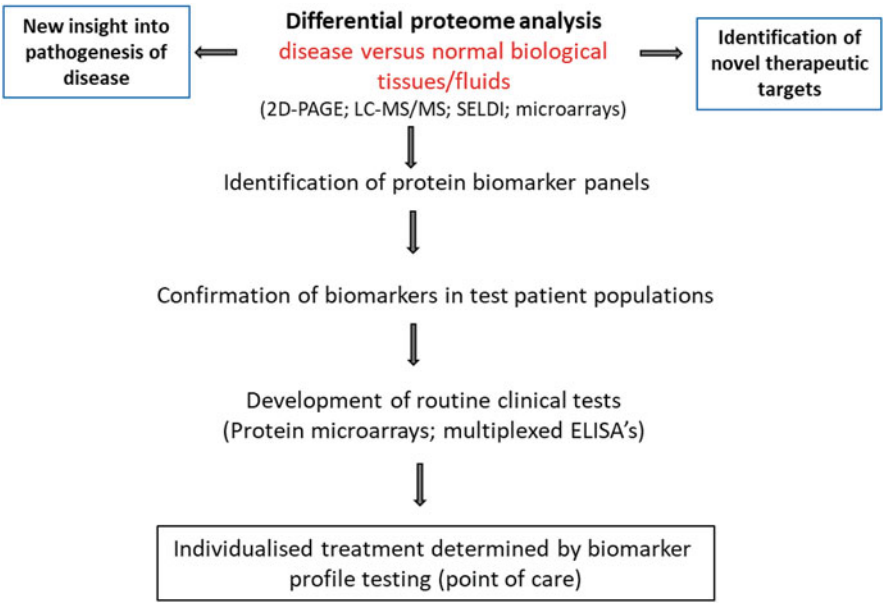

Fig. 4.4 Advancements in cancer research by proteomics

these modalities are the main reason why prognosis of tumor patients in getting better day by day.

Medical research and studies are benefited to a large extent by rapidly growing use of proteomics making diagnostic and prognostic results of cancer better with each coming era. Newly introduced therapeutic modalities and interventional techniques have shortened the span of struggle and increased the lifespan of cancer patients. The struggles of oncologists and researchers, along with the major role and support of Human Proteome Organization (HUPO), have increased the better understanding of what actually proteomics is especially in tumor studies (Fig. 4.4).

Standard proteomics and use of main proteomic technologies in identification of biomarkers, accurate diagnosis, better prognosis and immunological targeted therapies have become the main need of every cancer institute in the recent era. Moreover, the widely spreading multiplexing technological modality of protein chips should further increase the multi-planner sources for cancer treatments of such analytical tests in near future. With emerging recent technologies, proteomics has the power to impose our understanding of the molecular basis of cancer disease and to identify novel drug targeting the cells for accurate diagnosis using controllers and proteomics. Recent advancements help researchers and oncologists to better identify protein biomarkers and cancer tumor markers that will be used for better patient care in the near future.

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Microbiome as Cancer Biomarkers

5

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Abstract

Cancer is the leading cause of deaths worldwide due to their complexity, diversity, risk of reoccurrence, and limited therapeutic options, which are further hindered by the potential side effects. Moreover, the limited potential of detection tools lead to the severity of different types of cancers. An early detection definitely increases the chances of recovery and survival rates. To this end, microbiome, representing the collection of all microorganisms and their genes that live on and inside the body, is recognized as an important player in the diagnosis of different types of cancers. Different well-studied human microbiomes mainly comprising of different bacteria have potential etiological roles in carcinogenesis and/or modulating the individual response to therapies. This chapter provides the current knowledge on different healthy microbiome of different parts of the body and how it is altered during the development of different types of cancers. Specifically, it discusses the microbiome of intestine, oral, lung, vagina, gut, uterus, skin, etc. and their role as biomarkers for the detection of colorectal, pancreatic, liver, breast, lung, cervical, oral and

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oropharyngeal, and skin cancers. We also provided the current knowledge of microbiota-based therapeutics and management of different types of cancers through different therapies.

Keywords

Microbiota · Cancer biomarkers · Gastrointestinal microbiome · Skin microbiome · Cancer management

5.1 Introduction: Microbiome as Cancer Biomarkers

Microorganisms are generally perceived to be harmful to health because these are all wrongly assimilated to pathogens (Yang and Yu 2021); however, not all microorganisms are harmful and some are very useful for humans and perform different important roles in the body. Microbiome is the collection of all microorganisms, including bacteria, fungi, and viruses, living on the surface and inside the body (Temraz et al. 2019). These microorganisms reside in different parts of the body such as digestive tract, mouth, skin, and other parts. In humans, most of bacterial communities live in the intestine: the intestinal microbiome is made up of 100,000 billion microorganisms (Temraz et al. 2019). The intestinal microbiota maintains a strong mutualistic relationship with the host, which depending on the situation, will be qualified as "eubiosis" or "dysbiosis" (Song et al. 2020; Yang and Yu 2021). This amazing relationship plays an important role in the regulation of metabolism such as the chemical composition of brain, provide protection against pathogens, and regulate the immune system (Sánchez-Alcoholado et al. 2020). It seems that the intestinal microbiota is one of the major players involved in carcinogenesis (Yang and Yu 2021). This hypothesis seems to be confirmed over different studies. The result is dysbiosis of the intestinal microbiota, with abnormal overrepresentation of commensal bacterial species (Brüssow 2020). The most relevant are *Bacteroides fragilis*, *Fusobacterium nucleatum*, and *Escherichia coli* (Wieczorska et al. 2020). According to published studies, the gut microbiome plays an important role in modulating the mechanisms of response and resistance to cancer immunotherapies. Changes in the immune system brought about by the gut microbiome can be exploited to identify precise targets and bioactive compounds which could increase the response rates to cancer immunotherapies (Sánchez-Alcoholado et al. 2020; Wieczorska et al. 2020). The most frequently diagnosed cancers in the world are those of lung, breast, and colorectal cancer, while most frequent cancer-causing deaths are due to cancer of lungs, liver, and stomach (Ferlay et al. 2015). Colorectal cancer (CRC) has for several decades been an important public health concern (Temraz et al. 2019). It is one of the most common cancers worldwide, ranks third (10.2%) among different cancers with nearly 1.4 million new cases diagnosed each year and mortality approaching 50% each year (Temraz et al. 2019). Occupying the third place of the most frequent tumors in men after prostate and the second place in women after breast cancer, it is the second leading cause of

cancer-caused deaths worldwide (Temraz et al. 2019). It will therefore be possible that new biomarkers, resulting from the analysis of the microbiome, are emerging as well as therapeutic or preventive measures aimed at modulating this microbiota, the final objective of which will be to improve the care of cancer patients. One of the most promising areas of research is the development of "onco-microbiotics", or adjuvant probiotics of the anti-tumor response. The probiotics constitute a new preventive or therapeutic strategy for patients at risk for CRC. Awareness remains a redouble weapon for the prevention of CRC, because a particular diet and lifestyle modification could prevent risk of developing CRC and at the same time reducing recurrence in people with CRC. Gastric cancer kills a million people worldwide every year (Ferreira et al. 2018). Another study evaluated the intestinal microbiota by 16S rRNA gene sequencing among 116 gastric cancer and 88 healthy controls from China. The intestinal microbiota was categorized by enhancement of *Escherichia*, *Lactobacillus*, and *Klebsiella* (Qi et al. 2019). A summary of human microbiome associated with different types of cancers is given in Table 5.1.

This chapter provides a comprehensive overview of microbiome in different parts of human body and their role as cancer biomarkers. It specifically discusses the microbiome of intestine, mouth, lung, vagina, gut, uterus, and skin and their role in detecting different types of cancers such as colorectal, pancreatic, liver, breast, lung, cervical, oral and oropharyngeal, and skin. Each section in the chapter provides specific examples of microbiota and their role in detection of specific cancer type. Moreover, we provided information on the management of different types of cancers through different types of therapies.

5.1.1 Intestinal Microbiome: Biomarkers of Colorectal Cancer

5.1.1.1 Colorectal Cancer

Colon, also called the large intestine, is the terminal part of the digestive tract. It measures on average in adults 1.5 m in length and 8 cm in diameter and divided into four consecutive segments: the right colon or ascending colon, the colon transverse, the left colon or descending colon, and the sigmoid colon. The big intestine ends in the rectum. Colon cancer and rectal cancer are commonly referred to as colorectal cancer (CRC) (Raptis et al. 2015; Yang and Yu 2021). The colon and rectum contain different types of cells which can each cause a specific form of cancer. In most cases, CRCs develop from glands called Lieberkühn that line the inside of the colon and rectum walls. CRC is a malignant tumor in the lining of colon or rectum (Yang and Yu 2021). It affects all anatomical segments of large intestine, such as cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum; however, it does not affect anal canal cancer, which is a separate entity (Raptis et al. 2015).

CRC develops from the cells (healthy mucous membranes) along the lining of internal colon and rectum due to the accumulation of mutations and modifications epigenetics (Sobhani et al. 2019). In over 80% of cases, it comes from a benign tumor, called adenomatous polyp, which grows slowly and eventually becomes

Table 5.1 Human microbiome associated with different types of cancers

Cancer type	Population	Control individuals	Patients	Microbial change in patients (biomarkers)	Sample	Microbiome evaluation	References
Colorectal cancer (CRC)	Chinese	54 healthy Controls	74 CRC patients	*F. nucleatum*▲	Feces	Shotgun sequencing	Yu et al. (2017)
	Danish			*Parvimonas micra*			
	Chinese	25 healthy Controls	30 CRC patients	*Fusobacteria*▲	Intestinal lavage fluid	16S rRNA gene sequencing	Shen et al. (2020)
				Proteobacteria			
	Japanese	127 healthy controls	54 CRC (stage III/IV)	*F. nucleatum*▲	Feces	Shotgun sequencing	Yachida et al. (2019)
				Atopobium parvulum			
				Actinomyces odontolyticus			
	Italian, German and Japanese	52 healthy controls	61 CRC patients	Panel of 16 bacterial markers	Feces	Shotgun sequencing	Thomas et al. (2019)
	Chinese	92 healthy controls	74 CRC	Panel of 22 viral markers	Feces	Shotgun sequencing	Nakatsu et al. (2018)
	Chinese	204 healthy controls	184 CRC	Panel of 14 fungal markers▲	Feces	Shotgun sequencing	Coker et al. (2019)
	Japanese	60 healthy controls	158 CRC	*F. nucleatum*	Feces	Digital PCR	Suehiro et al. (2018)
	Chinese	931 healthy controls	1024 CRC	*F. nucleatum*, *Clostridium*▲	Feces	qPCR	Liang et al. (2017), Wong et al. (2017), Xie et al. (2017), Guo et al. (2018)
				Bacteroides clarus, *Bifidobacte*, *Rium*, *Faecalibacterium*			
				Prausnitzii			
	Swedish	66 healthy controls	39 CRC patients	*F. nucleatum*▲	Feces	qPCR	Eklöf et al. (2017)
				E. coli pks+			

	American	343 healthy controls	251 CRC patients	*Fusobacterium*↑ *Enterobacteriaceae*, *Lachnospiraceae*, *Porphyromonas*, *Porphyromonadaceae*, *Bacteroides*	Feces	16S rRNA gene sequencing	Zackular et al. (2014), Baxter et al. (2016a, b)
	American	23 healthy controls	21 CRC patients	*Proteobacteria*↑ *Bacteroidetes*↓	Feces	16S rRNA gene sequencing	Shen et al. (2010)
Pancreatic cancer (PC)	American	–	Patients with PDAC	*Proteobacteria*↑, *Actinobacteria*, *Fusobacteria*, *Verrumicrobia*	Pancreatic tissue	16S rRNA gene sequencing	Pushalkar et al. (2018)
		–	Mice with PDAC	*Helicobacteraceae*↑, *Bacteroidales*, *Mogibacteriacae*	Feces	qPCR	Pushalkar et al. (2018)
	Chinese	25 healthy controls	30 pancreatic head carcinoma	*Haemophilus*↑, *Porphyromonas*, *Leptotrichia*, *Fusobacterium*	Tongues coat samples	16S rRNA gene sequencing	Lu et al. (2019)
	Chinese	Ten healthy controls	Ten PC patients and 17 pancreatic disease	*Fusobacterium*↑, *Periodonticum* *Neisseria mucosa*↓	Saliva	16S rRNA gene sequencing	Sun et al. (2020)
	American	371 healthy controls	361 PAC patients	*Aggregatibacter*↑, *Actinomycetemcomi*, *Tans* *Porphyromona gingivalis*	Oral wash samples	16S rRNA gene sequencing	Fan et al. (2018)
Cervical cancer	Costarican	–	273 women with HR-HPV	*Gardnerella*↑	Cervical	Illumina MiSeq platform	Usyk et al. (2020)

(continued)

Table 5.1 (continued)

Cancer type	Population	Control individuals	Patients	Microbial change in patients (biomarkers)	Sample	Microbiome evaluation	References
	Chinese	131 Women without HPV Infection	59 patients with cervical persistent HPV infection, 139 patients with incidence HPV infection	*Prevotella bivia*↑, *Enterococcus durans*, *Porphyromonas*	Posterior vaginal fornix samples	16S rRNA gene sequencing	Chao et al. (2020)
				Uenonis			
				Lactobacillus iners↓, *Prevotella disiens*↓			
Breast cancer	American	48 control patients	48 breast cancer patients	*Clostridiaceae*↑, *Faecalibacterium*, *Ruminococcaceae*	Feces	Illumina sequencing and 16S rRNA gene sequencing	Goedert et al. (2015)
				Dorea↓			
				Lachnospiraceae↓			
Lung cancer	Chinese	65 healthy controls	42 lung cancer patients	13 biomarkers based OTU	Feces	16S rRNA gene sequencing	Zheng et al. (2020)
	Korean	–	20 lung cancer patients	*Veillonella*↑	Bronchoalveolar fluid	16S rRNA gene sequencing	Lee et al. (2016)
				Megasphaera			
	Chinese	18 healthy controls	24 lung cancer patients	*Streptoccocus*↑	Paired human lung cancer and tumor tissues	16S rRNA gene sequencing	Liu et al. (2018)
				Neisseria			
Gastric cancer	Portuguese	–		*H. pylori*↓	Gastric biopsies	16S rRNA gene sequencing	Ferreira et al. (2018)
				proteobacteria↑, *Lactobacillus*			
	Chinese	81 healthy controls	116 gastric cancer	*Lactobacillus*↑, *Escherichia*	Feces	16S rRNA gene sequencing	Qi et al. (2019)
				Klebsiella↑			

Oral and oropharyngeal cancer	American	242 healthy controls	121 patients with oral pharyn geal carcinomas	*Streptococcus*, *Actinomyces*▲, *Corynebacterium*	Oral rinse samples	16S rRNA gene sequencing	Börnigen et al. (2017)
Endometrial cancer	American	75 patients with EC	66 patients with EC and seven with atypical Hyperplasie	*Porphyromas somerae*	Hysterectomy tissue samples	qPCR	Walsh et al. (2019)
	American	–	17 patients EC, four patients with hyperplasia and ten with benign uterine	*Atopobium*▲	Fallopian, ovarian, peritoneal, and urine samples	MiSeq of 16S rDNA	Walther-António et al. (2016)
				Vaginae			
				Porphyromonas			
Prostate cancer	American	–	129 prostate cancer patients	*Streptococcus anginosus*, *Anaerococcus lactolyticus*	Urine pellet samples	16S rDNA Illumina sequencing and 16S rRNA sequencing	Shrestha et al. (2018)
				Anaerococcus obesiensis, *Actinobaculum schaalii*			
				Varibaculum cambriensem			
				Propionimicrobium lymphophilum			
Bladder cancer	Chinese	26 non-patient bladder cancer	29 patients with bladder cancer	*Actinomyces europaeus*	Urinary samples	16S rRNA gene sequencing and qPCR	Bi et al. (2019)
	Chinese	19 non-neoplastic controls	62 males patients with bladder cancer	*Micrococcus*▲, *Brachybacterium*	Mid-stream urine sample	16S rRNA gene sequencing, Illumina MiSeq sequencing	Zeng et al. (2020a)

(continued)

Table 5.1 (continued)

Cancer type	Population	Control individuals	Patients	Microbial change in patients (biomarkers)	Sample	Microbiome evaluation	References
	Chinese	18 non-neoplastic controls	62 males patients with bladder cancer	*Sphingobacterium*↑, *Acinetobacter*	Mid-stream urine samples	16S rRNA gene sequencing	Wu et al. (2018)
				Anaerococcus *Roseomonas*↓			
				Serratia			
				Proteus↑			
	American	11 healthy controls	12 males patients with bladder cancer	*Fusobacterium*	Urine samples	16S rRNA gene sequencing MiSeq. sequencing	Bučević Popović et al. (2018)
Hepatocellular carcinoma (HCC)	Chinese	56 healthy controls	30 early HCC, 45 advanced HCC	30 microbial markers including *Phylum Actinobacteria*, *Gemmiger*, *Parabacteroides*	Feces	MiSeq. sequencing	Ren et al. (2019)
	Argentinian	25 individuals without HCC	25 HCC patients	*Erysipelotrichaceae*↑, *leuconostocaceae*↓, *Fusobacterium*, *Odoribacter*	Fecal stool	16S rRNA gene sequencing and MiSeq. sequencing	Piñero et al. (2019)
				Dorea			
	Israeli	25 healthy individuals	30 HCC-cirrhosis Patients, 38 cirrhotic patients without HCC	*Clostridium*↑	Fecal stool	16S rRNA gene sequencing	Lapidot et al. (2020)
				Alphaproteobacteria↓			

	Chinese	100 healthy controls	113 HBV-related HCC patients	*Clostridium XIVa*↑ *Bacteroides*, *Lachnospiracea incertae sedis*	Feces	16S rRNA gene sequencing	Huang et al. (2020)
	Italian	20 healthy controls	21 NAFLD-related cirrhosis and HCC and 20 NAFL D-related cirrhosis without HCC	*Ruminococcaceae*↑, *Bacteroides*	Feces	16S rRNA gene sequencing	Ponziani et al. (2019)
	Chinese	15 healthy controls	21 CHB, 25 LC21 HCC patients	*Bacteroidetes*↑ *Firmicutes*↓	Feces	16S rDNA gene amplification and sequencing	Zeng et al. (2020b)

PDAC pancreatic ductal adenocarcinoma, *PC* pancreatic cancer, *PAC* pancreatic adenocarcinoma, *HPV* human papillomavirus, *HR* high-risk, *EC* endometrial cancer, NAFLD nonalcoholic fatty liver disease, *CHB* chronic hepatitis B, *LC* liver cirrhosis

cancerous (Sobhani et al. 2019). This process, called the adenoma-cancer sequence, is quite long and can extend over more than 10 years (Sobhani et al. 2019). Usually 10% of cases of CRC are hereditary while 90% are sporadic (i.e., without a family history). Genetic inheritance or mutation can induce CRC such as syndrome Lynch or hereditary nonpolyposis colon cancer (HNPCC) and polyposis familial adenomatous (PFA) (Jasperson et al. 2010). Other pathologies such as diseases inflammatory bowel disease (including Crohn's disease and colitis ulcerative) can also progress to CRC (Mattar et al. 2011). Similarly, the environmental factors, as well as risk factors such as smoking, alcoholism, sedentary lifestyle, physical inactivity, overweight, obesity, low consumption diet in fiber, excessive red meat or processed meats, would play a role important in the development of CRC (Song et al. 2020). There is a well-established link between inflammation and the development of CRC (Kang and Martin 2017).

CRC is a cancer with a good prognosis when diagnosed at an early stage: 5-year relative survival is 91% for localized stages and 70% for local stages with locoregional invasion (Young et al. 2014). In contrast, the 5-year survival is approximately 11% in metastatic situations which represent approximately 25% of patients at time of diagnosis (Young et al. 2014). CRC death rate has fallen over the past 20 years thanks to the progress made in terms of care, i.e., early diagnosis and improvement of therapeutic modalities (Zavoral 2014; Ogunwobi et al. 2020).

5.1.1.2 Relationship Between Intestinal Microbiota and Colorectal Cancer

In recent years, researchers have conducted a series of studies aiming to investigate the involvement of intestinal microbiota in the development of CRC. The CRC patients exhibit a significant increase in the density of *B. fragilis*, *E. coli*, *F. nucleatum*, *Enterococcus faecalis*, *Peptostreptococcus*, *Streptococcus gallolyticus*, *Shigella*, and *Enterococcaceae* or *Campylobacter*, and a decrease in density of *Bifidobacterium*, *Blautia*, *Faecalibacterium*, *Roseburia*, and *Clostridium* (Sánchez-Alcoholado et al. 2020). Some of these microbiota are discussed in the following sections:

5.1.1.2.1 *Bacteroides fragilis*

Bacteroides are part of the germs of the intestinal microbiota. These are rightly found in 10^9 germs per gram of stool. *Bacteroides* of the fragilis group are Gram-negative, non-sporulating, and rod-shaped bacilli belonging to the kingdom Bacteria, phylum Bacteroidetes, class and order Bacteroidales, family Bacteroidaceae, and genus *Bacteroides* (Dahmus et al. 2018). Bacteroidaceae family constitutes a very important family of anaerobic bacteria, in particular at the level of the intestinal tract. These bacteria have in common the characteristic of not using oxygen as an electron acceptor, their growth can even be inhibited in the presence of oxygen. This indicates that *Bacteroides* of *fragilis* group do not develop contact with air, but rather develop a capsule and have the particularity of fermenting glucose. A strong growth of *B. fragilis* is observed in the presence of bile (Dahmus et al. 2018). It is the most frequently isolated anaerobic bacterial species from humans during soft tissue

infections. *Bacteroides* are easily recognized by action favoring that the bile exerts on their growth. People, who consume large quantities of meat have higher concentrations of *Bacteroides* in their intestine (Madigan et al. 1997). *B. fragilis* is the most frequent opportunistic pathogen among species in the group *B. fragilis* (other species include *B. thetaiotaomicron*, *B. distasonis*, *B. vulgatus*, *B. ovatus*, *B. uniformis*, and *B. acidifaciens*) (Könönen et al. 2015). There are two types of *B. fragilis*: enterotoxigenic *B. fragilis* (ETBF) and non-toxinogenic *B. fragilis* (NTBF). The spread in the bloodstream "bacteremia" is more common with *B. fragilis* than with any other anaerobic bacteria. Infections are usually endogenous, caused by the gut's own flora of the patient (Könönen et al. 2015). These infections give rise to various manifestations, such as pleuro-pulmonary, peritoneal, gynecological, parietal, or septicaemic, and abscesses intra-abdominals. Studies have shown that ETBF is also a causative potential for acute diarrhea in children and adults. *Bacteroides* produce beta-lactamases which inactivate penicillins and cephalosporins with the exception of cephamycins. ETBF strains were first isolated from individuals with diarrhea in an uncontrolled study in 1987 (Myers et al. 1987). Due to their delicacy, they are difficult to isolate and are often overlooked. Their isolation requires proper methods of collection, transport, and culture of specimens. Treatment is complicated by three factors: slow growth, increasing resistance to antimicrobial agents, and the synergistic poly-microbial infection. Many cultures of *Bacteroides* strains form colonies with brown or black pigmentation on blood agar due to esculin hydrolysis (Balows et al. 2013).

The pathogenicity of *B. fragilis* is linked to its carbohydrate capsule, proteins of outer membrane, and production of specific enzymes, including recently recognized enterotoxin called fragilysin or bft (Sánchez-Alcoholado et al. 2020). This 20 kDa zinc-metalloprotease toxin is produced only by certain sources of *B. fragilis*, and it has three subtypes: bft-1, bft-2, and bft-3. The strains which produce this toxin are known to be ETBF. An increased prevalence of ETBF has been reported in patients with CRC compared to controls (Boleij et al. 2015; Sánchez-Alcoholado et al. 2020). In addition, the presence of ETBF is associated with the stage of the disease. This bacterium is indeed found more frequently in tumor and healthy tissue of patients at late stages than early stages of the disease (Boleij et al. 2015). Several mechanisms of action of fragilysin have been described and may explain, at least in part, the carcinogenic potential of ETBFs (Dahmus et al. 2018; Niederreiter et al. 2018). The binding of fragilysin to a receptor, yet to be identified and expressed in level of intestinal epithelial cells, induces the activation of MAPK signaling (mitogen-activated protein kinases), NFκB (nuclear factor-kappa B), STAT3 (signal transducer and activator of transcription-3), and COX-2 (cyclooxygenase-2), and leads to the expression and secretion of chemokines/cytokines such as TGFβ (transforming growth factor beta), IL8 (interleukin-8), and PGE2 (prostaglandin-E2), as well as the development of an immune response of type Th (T-helper). Fragilysin also leads to the cleavage of ecadherin, inducing the release of βcatenin in the cytoplasm, which can then pass into the nucleus and activate the transcription of genes involved in cell proliferation such as the c-myc oncogene (Dahmus et al. 2018; Niederreiter et al. 2018). The cleavage of Ecadherin also leads to decrease in the barrier function of the

colonic mucosa, which could contribute to exacerbation of inflammation (Dahmus et al. 2018). Mutagenic properties are also attributed to ETBFs, as they are capable of inducing the expression of spermine oxidase (SMO) which is responsible for production of reactive oxygen species (ROS) and causing double-strand breaks in DNA (Dahmus et al. 2018; Niederreiter et al. 2018). The ETBF strains, toxigenic producers of fragilysin, are able to induce the inflammation and colonic tumor formation in a genetic model of CRC (MinApc716 +/− mice which expressed a mutant gene encoding an Apc protein truncated at the amino acid 716 position) (Wu et al. 2009; Niederreiter et al. 2018). This induction results from activation, by fragilysin, the STAT3 transcription factor (signal transducer and activator of transcription-3) with the consequence of the establishment of colitis which is characterized by a selective Th17-type immune response (T-helper lymphocyte response-17), known to be associated with a poor prognosis in CRC (Niederreiter et al. 2018). ETBFs stimulate cell proliferation and develop resistance to apoptosis. Indeed, by binding to a receptor, yet to be identified, fragilysin induces the cleavage of Acadherin, involved in both junction and adhesion complexes in cellular and cytoplasmic sequestration of βcatenin (Wu et al. 2009; Niederreiter et al. 2018). Released in the cytoplasm, βcatenin passes into the nucleus of cells and activates the expression of genes like c-myc. Fragilysin could also participate in tumor development by promoting the survival of tumor cells. Indeed, in vitro stimulation of cells by this toxin induces an increased expression of the inhibitory protein cIAP2 (cellular inhibitor of apoptosis protein-2) and apoptosis which depends on COX-2 cyclooxygenase (Kim et al. 2008a; Dahmus et al. 2018). Fragilysin could also actively participate in causing genetic abnormalities of tumor cells. In fact, in vivo, this toxin induces colonic epithelial at cell level and DNA damage, which could lead to the accumulation of mutations (Goodwin et al. 2011).

5.1.1.2.2 *Escherichia coli*

E. coli is a commensal of human digestive tract and many warm-blooded animals (mammals and birds). It represents most of the aerobic bacterial flora of intestine (aerobic species dominant) where it is present at a rate of 10^8 germs per gram of stool (total flora: 10^{11} to 10^{12} bacteria per gram). *E. coli* is a prokaryotic organism and belongs to the kingdom Bacteria, phylum Proteobacteria, class Gamma Proteobacteria, order Enterobacteriales, and family Enterobacteriaceae (Brüssow 2020).

E. coli being the binomial name belongs to the genus *Escherichia*. This large family enterobacteriaceae enter the digestive tract during the first hours or days of child birth and constitute the most dominant aerobic intestinal flora throughout an individual's life. *E. coli* is a radio-resistant Gram-negative bacillus, rod-shaped, coccobacillary, or filamentous (in old stumps), and not sporulated. Its size varies according to the environment, but remains at a fairly short size (2–3 μm) (Brüssow 2020). *E. coli* is arguably the most studied living organism to date. Owing to its easy culturing and rapid growth (cell divides every 20 min at 37 °C in a rich environment) make it a study tool of choice. It is a bacterium which has no deaminase, which

excludes it from the genera Proteus, Morganella, and Providencia (typically ex-tribe of Proteae).

The pathogenicity of *E. coli* results from a multifactorial process. The modern technologies of biochemistry, genetics, molecular biology, and cellular microbiology have made it possible to identify and analyze the mechanisms involved in the interaction of pathogenic *E. coli* with their host. We could thus establish that certain "specialized" strains of *E. coli* are associated with various diarrhea, gastroenteritis, urinary tract infections, meningitis, septicemia, and hemolytic uremic syndrome, as well as extra-intestinal diseases both in humans and animals. Despite the diversity of conditions caused by strains of pathogenic *E. coli*, all these strains use a classic infection strategy, common to many other agents pathogens. We can distinguish it from colibacilli, which is a normal host of intestine and does not cause any illness; nevertheless, it possesses the pathogenic potential under certain circumstances (opportunistic pathogens) (Brüssow 2020). When it penetrates through the ascending urethral (contiguity) into the urinary tree, it causes three quarters urinary tract and swarming infections starting from the digestive system (cholecystitis suppurative, peritonitis, and sepsis). In addition to these extra-intestinal pathovars (ExPEC), there are several pathovars within *E. coli*, whose nomenclature is pathogenicity dependent (Brüssow 2020).

Pathogenic *E. coli* could be a cofactor in the pathogenesis of CRC (Sánchez-Alcoholado et al. 2020). In order to demonstrate the hypothesis that *E. coli* is involved in the pathogenesis of CCR, researchers conducted a study. The purpose of this study was to appear bacterial colonization of the colonic mucosa in patients screened for a CRC in noncancerous controls (diverticulosis). Given that *E. coli* represents the majority of cultivable aerobic bacteria in the colon and is positively selected for chronic inflammation, the analysis was limited to *E. coli*. Two parameters of *E. coli* were studied: *E. coli* associated with mucosa and internalized in mucosa in patients with CRC, and in patients with receiving an operation due to diverticulosis, an intestinal pathology not tumor (Brüssow 2020). The pathogenicity of *E. coli* in CRC is linked to the genotoxins produced by it. The diversity of *E. coli* is observed primarily by an organization of the species into several major phylogenetic groups (A, B1, B2, D, E, . . .) (Tenaillon et al. 2010). The strains of *E. coli* which colonize patients with CRC mainly belong to the B2 and D phylogroups, which group together mostly pathogenic *E. coli* (Tenaillon et al. 2010). These strains of *E. coli* are mostly producers of genotoxins (cyclomodulins and colibactin), which are capable of inducing DNA damage and thus disrupting the cell cycle of eukaryotic cells. The scientific community has, for the moment, mainly focused on studying the role of colibactin in CRC. A high prevalence of strains harboring the "pks" genomic island is observed, which carries genes encoding polyketide synthases (PKS) and peptide synthases non-ribosomal (PSNR) involved in the synthesis of this genotoxin (Arthur et al. 2012). Bacteria producing colibactin can induce breaks in vitro and in vivo double-stranded DNA, with the potential consequences of accumulating chromosomal aberrations and an increased frequency of genetic mutations (Cuevas-Ramos et al. 2010). Colibactin is an unstable compound that has not been purified to date. However, an intermediary in its synthesis, although unable to break DNA, is

able to alkylate DNA and induce strand bonds (Vizcaino and Crawford 2015; Niederreiter et al. 2018). Cyclomodulin CNF (cytotoxic necrotizing factor) which is a protein of approximately 115 kDa organized into three functional domains, was initially described by the researcher Caprioli and his collaborators in 1983. These proteins are grouped into three variants CNF-1, CNF-2, and CNF-3. The CNF-1 and CNF-3 are encoded by chromosomal genes while CNF-2 is encoded by a plasmid gene (Knust and Schmidt 2010; Niederreiter et al. 2018). EPEC strains have a type III secretion system required for characteristic changes of attachment and effacement that modify the cytoskeleton and apical surface of the host cells. The attachment capacity and erase is encoded on an island of pathogenicity, known as the locus enterocyte erase (LEE) that is made up of 41 open reading frames. The LEE encodes different ESP genes (EPEC-secreted protein), thus the genes espA, espB, and espD encode proteins secreted by this system which can form a translocation apparatus for the delivery of effector molecules into the host cells. The EspF protein is also encoded by the LEE via the system type III secretion (Niederreiter et al. 2018). Colibactin-producing *E. coli* induce double-stranded DNA breaks, which is at the origin of important chromosomal rearrangements, such as translocations and the formation of circular chromosomes, leading to cell cycle arrest in G2 phase (Niederreiter et al. 2018). In addition, cells infected with *E. coli* colibactin producers can leave the cell cycle and enter senescence. This is accompanied by the secretion of growth factors which support the tumor development. The CNF-1 toxin binds to the tight junctions of host cells which internalize it via endocytosis. In the cytoplasm, CNF-1 induces deamination of the glutamine residue of GTPases, enzymes bind and hydrolyze the GTP (guanine triphosphate) of the Rho family, which leads the appearance of actin stress fibers, DNA endo-reduplication, and inhibition of cytokinesis inducing multinucleation. The activation of NFκB pathway (nuclear factor-kappa B) by this toxin induces the activation of gene expression Bcl2 (B-cell lymphoma 2) and Bcl-xl (B-cell lymphoma extra-large) inducing resistance to cell apoptosis. EPEC strains are able to increase the frequency of spontaneous mutations in the host cells, notably by altering the expression of mismatch repair (MMR) and DNA repair proteins by inducting the stress oxidant and through the posttranscriptional action of a secreted bacterial effector.

5.1.1.2.3 *Fusobacterium*

Fusobacterium is a filamentous, anaerobic genus of bacteria not forming spores, narrow, with tapering ends, or pleomorphic. It is Gram-negative, identical to Bacteroides (Madigan and Martinko 2005). This strain shows irregular coloring. *Fusobacterium* belongs to the kingdom Bacteria, phylum Fusobacteria, class Fusobacteria, order *Fusobacteriales*, and family Fusobacteriaceae (Madigan and Martinko 2005). *Fusobacterium* has a potent lipopolysaccharide (LPS) which is an essential component of the cell wall of Gram-negative bacteria—it is an endotoxin. Species of the genus *Fusobacterium* are part of the normal flora of oropharynx, digestive tract, and genital tract (Könönen et al. 2015). Infections can occur after surgical or accidental trauma, edema, anoxia, tissue destruction, and animal bites (Könönen et al. 2015). *Fusobacterium* is implicated in several pathologies in

humans, especially periodontal disease, Lemierre syndrome, and skin ulcers tropical. Different species are suspected in the development of CCR.

Several virulence factors of *F. nucleatum* show potential oncogenic activity. *F. nucleatum* indeed produces a toxin which has immunosuppressive activity, FIP or FIPA (fusobacterial immunosuppressive protein). It is able to inhibit the cell cycle of T-lymphocytes during G1 phase, but its role in CRC remains to be defined (Shenker and Datar 1995; Sánchez-Alcoholado et al. 2020). The FIP protein is a cyclostatin which in its purified state has an apparent molecular mass of 90–100 kDa. It is composed of two subunits of 44 and 48 kDa. The 44 kDa subunit has a FIPA polypeptide, derived from the FipA gene, which is sufficient for mediation of the immunosuppressive activities of the host protein complex (Demuth et al. 1996; Sánchez-Alcoholado et al. 2020). *F. nucleatum* secretes an FadA adhesin (Fusobacterium adhesin A) at its membrane which by binding to ecadherin stimulates tumor growth. This causes the activation of WNT/βcatenin signaling pathway that induces expression of genes involved in cell proliferation such as the c-myc oncogenes and cyclin D1, and in inflammation such as the genes encoding cytokines pro-inflammatory IL-6 and IL-8 (Rubinstein et al. 2013). The FadA peptide expressed on the surface of *F. nucleatum* exists in two forms: intact non-secreted pre-FadA consisting of 129 amino acid residues (aa) and the secreted mature FadA (mFadA) consisting of 111 amino acids without the 18 amino acids signal sequence, with the pre-FadA anchored in the membrane internal and mFadA secreted outside of bacteria (Han et al. 2005). *F. nucleatum* is also able to inhibit the antitumor activity of NK cells (natural killer) and T-lymphocytes which infiltrate tumors by binding to its protein Fap2 (fusobacterial apoptosis protein 2) at the TIGIT receptor (T-cell immunoglobulin and ITIM domain) which is expressed on the surface of these immune cells (Gur et al. 2015). The Fap2 proteins are outer membrane proteins, which vary in size between 200 and 400 kDa, classified as type Va (T5SS) or auto-carriers (Desvaux et al. 2005). The gene fap2 codes for 3692 amino acids, resulting in a predictive molecular mass of 390 kDa (Gur et al. 2015).

Studies carried out in different countries including China, Denmark, France, and Austria in order to evaluate the potential for diagnosing CRC from fecal metagenomes on 74 patients with CRC found an associations between CRC with several species such as *F. nucleatum*, *Peptostreptococcus stomatis*, *Parvimonas micra*, and *Solobacterium* (Yu et al. 2017). Furthermore, 20 microbial gene markers were identified which differentiated CRC and control microbiomes. These studies highlighted the potential for using fecal metagenomic biomarkers for early diagnosis of CRC (Yu et al. 2017). In another study comprising 30 CRC patients and 25 healthy individuals, the pathogenic microbiota was more abundant in intestinal lavage fluid than in fecal samples revealing the association of the intestinal microbiota and CRC (Shen et al. 2020). Moreover, in 54 CRC (stage III/IV) Japanese patients with 127 healthy controls, a 55 bacterial markers were identified by using shotgun sequencing. The shotgun sequencing was used to identify 16 bacterial markers in 61 CRC including 52 healthy controls Italian, German, and Japanese patients. Furthermore, in Chinese discovery cohort: 111 CRC and 121 healthy controls, 14 fungal and 22 viral markers were identified by shotgun

sequencing study platform. In 158 CRC Japanese and 60 healthy controls, *F. nucleatum* was associated with CRC. In 1093 CRC Chinese patients, *F. nucleatum* as a ratio to *Bifidobacterium* and *Faecalibacterium prausnitzii*, *Clostridium hathewayi*, *Bacteroides clarus*, and *Clostridium symbiosum* were found as best fecal markers for CRC. Additionally, in 39 Swedish CRC and 66 healthy controls, *F. nucleatum* and *E. coli* pks^+ were detected as bacterial biomarkers. In 251 American CRC patients, bacterial markers such as *Fusobacterium*, *Lachnospiraceae*, *Porphyromonas*, *Porphyromonadaceae*, *Bacteroides*, and *Enterobacteriaceae* were detected (Wong and Yu 2019).

In conclusion, CRC is a multifactorial disease dependent on environmental factors, genetics, host, and microbiome (Fig. 5.1). The search for various promoters (protagonists) must be wide. A link between chronic inflammation and the development of CCR has already been established. The concept that there is a link between the gut microbiota and CRC is recent. The gut microbiota is potentially involved in the development of colorectal carcinoma by different mechanisms. The microbiota and the immune response system, both innate and acquired, are involved in the oncogenesis mechanism, in particular by their role in the initiation, regulation, and maintenance of chronic inflammation. This inflammation, via its mediators, considerably modifies the microbial composition, leading to dysbiosis (as opposed to eubiosis). In response to these changes in their environment, microorganisms induce complex changes in their transcriptional and metabolic responses and produce metabolites, enzymes, and toxins (enterotoxins, cyclomodulins, FIP or FIPA) which may influence the development of CRC. The microbiome must, therefore, be considered as one of the important protagonists of the chain of events culminating in cellular transformation malignant colonic epithelial cells. This section highlights a pro-carcinogenic properties of bacteria. As this is a multifactorial disease, and the development spans a long period (more than 10 years), the simple search for a correlation between the bacterial species and CRC is too limiting. We should place ourselves in the part of a complex microflora, including elements which can modify the composition of the intestinal microbiota associated with CRC such as food. It will also be necessary to understand the mechanisms of interactions between bacteria which would make up the dysbiotic microbiota associated with CRC and the cell carcinogenesis.

5.1.2 Intestinal Microbiome: Biomarkers of Pancreatic Cancer

Pancreatic cancer is the seventh leading cause of cancer death worldwide (Rawla et al. 2019b). Its symptoms usually do not appear during early stage and the majority of patients are already at an advanced stage before the cancer is diagnosed (Sun et al. 2020). The microbiome is known to influence tumor-initiating inflammation and carcinogenesis. Thus the identification of microbiome biomarkers which could serve as early diagnostic indicators could be very useful.

The microbiota of intestine and pancreas, both linked, are thought to play a major role in the development of adenocarcinoma of pancreas, one of the most fatal cancers

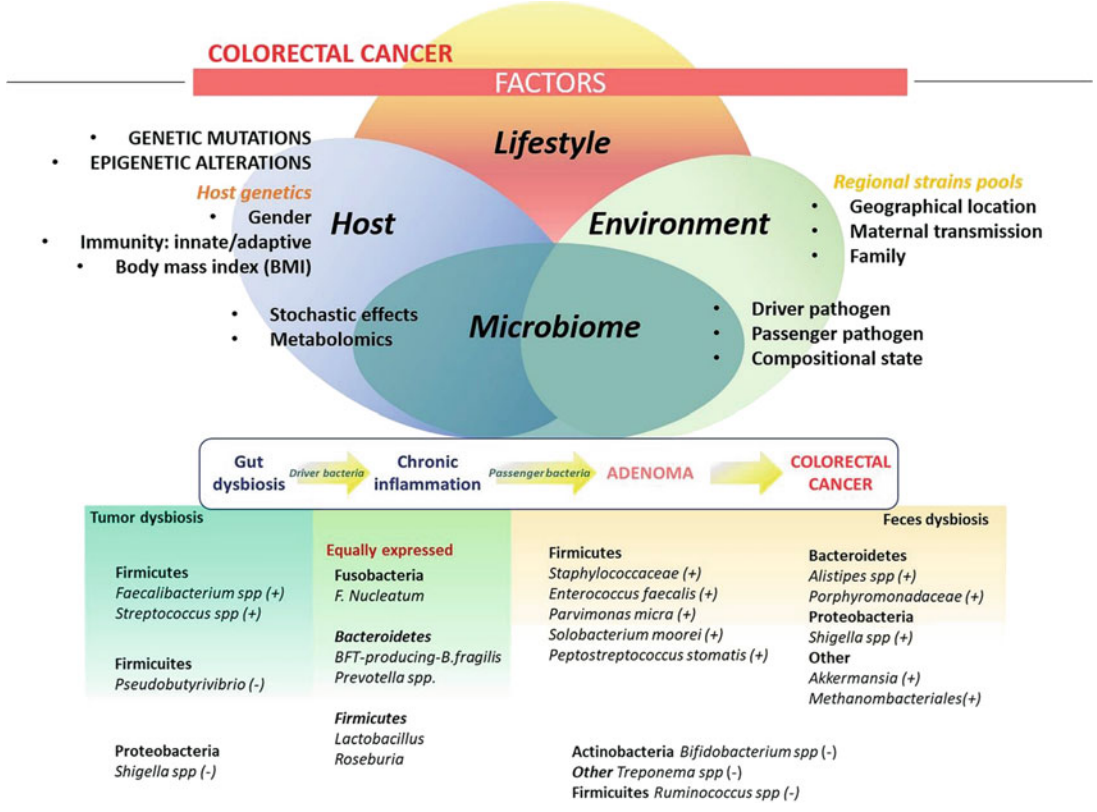

Fig. 5.1 A summary of microbiome dysbiosis in CRC. (Figure reproduced from Saus et al. 2019)

(Adamska et al. 2017; Wei et al. 2019). Pancreatic ductal adenocarcinoma (PDAC) is the most common and serious pancreas cancer, with a 5-year survival rate of only 20% after surgery followed by chemotherapy (Adamska et al. 2017; Wei et al. 2019). The gloomy statistic could be improved by taking the intestinal and pancreatic microbiomes into account in the overall therapeutic strategy of PDAC, according to the results of certain studies (Michaud and Izard 2014; Fan et al. 2018; Lu et al. 2019; Sun et al. 2020). According to these and some other studies, it appears that the pancreatic bacterial population of patients with PDAC is richer in *Proteobacteria*. In these subjects, the intestinal microbiota is also disturbed, with a more marked presence of *Actinobacteria*, *Fusobacteria*, and *Verrumicrobia* compared to the control stool samples (Pushalkar et al. 2018). These two microbiomes are also linked: for instance, a study showed the possible translocation of bacteria from the intestine to the pancreas, probably via the pancreatic duct that connects the two organs. In mice with aggressive tumors, *Helicobacteraceae*, *Bacteroidales*, and *Mogibacteriacae* were overrepresented in the gut microbiota, while *Elizabethkingia*, *Enterobacteriacae*, and *Mycoplasmatacae* were found in mice whose tumors grew more slowly (Pushalkar et al. 2018). The degree of intestinal dysbiosis could therefore serve as a marker for the disease progression (Wei et al. 2019). A schematic illustration of mechanism of microbiota affecting PDAC is shown in Fig. 5.2.

The pancreatic microbiome is found to be essential of the pathophysiological process of the disease. A study reported that certain pancreatic bacteria, in particular *Bifidobacterium pseudolongum*, inactivates the T-lymphocytes (by binding to TLRs—Toll-like receptors), protecting tumors from any immune response. However, the administration of an antibiotic cocktail to the affected mice to eliminate their pancreatic microbiota effectively slowed the progression of their tumors (Pushalkar et al. 2018). According to the authors, the preventive antibiotic treatment could be considered in certain high-risk patients with genetic susceptibilities or already with advanced lesions, as well as the use of probiotics could correct a possible intestinal dysbiosis. Another result concerning the curative aspect this time showed that the suppression of pancreatic bacteria by the same antibiotic therapy improved the effectiveness of checkpoint inhibitors. A recent form of immunotherapy aimed at removing the immune brakes on cells tumors; however, its effectiveness remains very limited in pancreatic cancer (Pushalkar et al. 2018). From diagnosis to the treatment through prevention, the targeting bacteria in the intestine and pancreas therefore seems most promising in the management of PDAC.

Although the changes in the intestinal microbiome have already been characterized to be associated with pancreatic cancer, the abrupt microbiota (microbiota dysbiosis) of the tongue layer as an indicator of the disease has not yet been well-defined. Studies carried out in China reported that the disruption in the microbial composition of the tongue layer could serve as a biomarker for early-stage pancreatic cancer. Studies found that the tongue-layer microbiomes of patients with early pancreatic stage were more diverse and composed of remarkably different bacteria, with the tongue layers of healthy people (Michaud and Izard 2014; Lu et al. 2019). In another study, a total of 37 saliva samples were collected, including ten from pancreatic cancer patients, 17 from mild pancreatic disease, and ten from

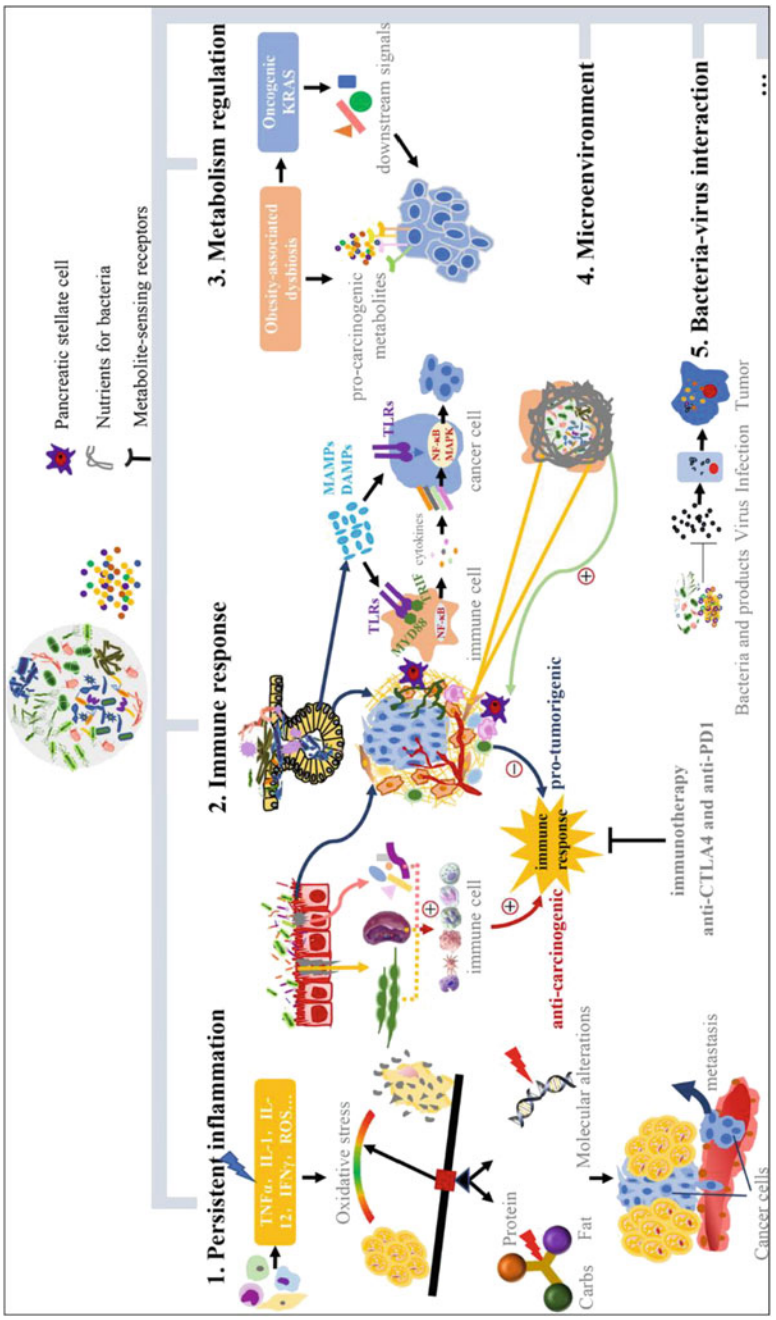

Fig. 5.2 A schematic illustration of mechanism of microbiota affecting PDAC. (Figure reproduced from Wei et al. 2019 distributed under the Creative Commons Attribution (CC BY 4.0) license)

healthy controls. A high concentration of *Fusobacterium periodonticum* and low concentration of *Neisseria mucosa* were found in patients with pancreatic and benign pancreatic cancer compared to the control subjects (Sun et al. 2020). These results confirm the potential of *N. mucosa* and *F. periodonticum* as the diagnostic biomarkers of pancreatic cancer. In addition, two prospective cohort studies selected 361 cases of pancreatic adenocarcinoma and 371 controls to study the association between the oral microbiota and the risk of pancreatic cancer from oral lavage samples. *The Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were associated with higher risk of pancreatic cancer (Fan et al. 2018). These studies provide helpful evidence that oral microbiome may play a function in the etiology of pancreatic cancer.

If an association between oral microbiome and pancreatic cancer is established in larger studies, this can possibly lead to the improvement of new microbiome-based early preventive or diagnostic tools for the disease. Although more assenting studies are needed, the study results add to the rising indication of an association between oral microbiome disturbances and pancreatic cancer. An association between microbiota dysbiosis of the tongue layer and pancreatic cancer could be linked to the immune system. The pancreatic disease can initiate immune reactions that promote the overgrowth of certain bacteria or vice versa. Research on the association between the microbiome and pancreatic cancer opens up new opportunities to develop biomarkers for identifying people at a high risk. Such studies could also lay the foundations for the development of new treatment approaches related to immunotherapies or even probiotics that could help prevent pancreatic cancer in high-risk individuals.

5.1.3 Intestinal Microbiome: Biomarkers of Liver Cancer

The intestinal microbiota is in close contact with the digestive barrier and then with the liver via the portal vein (Ma et al. 2018). Some advanced technologies of high throughput sequencing and metagenomics have revealed imbalances in the intestinal microbiome associated with cancers. Regarding liver disease, some recent studies have proposed that the intestinal microbiome is involved in the development of hepatocellular carcinoma (HCC) (Ma et al. 2018). The decrease in bacteria such as *Clostridium* family in the intestinal tract promotes the accumulation of primary bile acids (Ma et al. 2018), indicating a phenomenon which would participate in an enhanced expression of anti-tumor NKT lymphocytes at the hepatic level (Ma et al. 2018). The LPS/TLR4 system (lipopolysaccharide/Toll-like 4 receptor) and more generally, the intestinal bacterial motifs and cellular receptors (PRR type, pattern recognition receptors), play a role in the hepato-carcinogenesis (Lv et al. 2017; Rao et al. 2020). Among PRRs, the TLRs are crucial for establishing defense against microorganisms (Bakadia et al. 2020). They are able to trigger the processes of innate immunity and inflammation in response to dangerous signals of a prokaryotic or eukaryotic nature (Bakadia et al. 2020). TLRs recognize PAMPs (pathogens-associated molecular pattern) from microorganisms and DAMPs (damages-

associated molecular patterns) from damaged tissues (Roh and Sohn 2018). After interaction with PAMPs or DAMPs, the TLRs induce the secretion of IL-6 (interleukin-6) and TNFα (tumor necrosis factor-α) as proinflammatory cytokines via an intracellular molecular activation cascade involving, among others, Myd88 (myeloid differentiation primary response 88) and IRAK (IL1-receptor-associated serine kinase), which leads to the activation of transcription factors such as nuclear factor κB (NFκB) and activator protein 1 (AP1), as well as the activation of factors triggering the adaptive immune response (Jain et al. 2014; Bakadia et al. 2020).

HCC ranks fifth among cancers and is the third leading cause of cancer death worldwide (Ren et al. 2019). HCCs is a genetically heterogeneous tumor that develops in 75–85% of cases from cirrhosis, most often of alcoholic or viral origin. The repetition of inflammatory episodes, the origin of which can be viral (hepatitis B and C virus), alcoholic and metabolic, and the development of associated chronic hepatopathies, such as steatohepatitis and cirrhosis, are major causes of hepatic carcinogenesis (Lv et al. 2017; Rao et al. 2020). The successive cycles of aggression of liver which precede the onset of HCC lead to real remodeling of the organ (regenerative liver nodules). The latter is molecularly associated with profound alterations in the signaling pathways regulating the cell proliferation, death, and migration/invasion, and angiogenesis (Lv et al. 2017; Rao et al. 2020). It is also well-known that cirrhosis is frequently accompanied by an alteration in the integrity of the intestinal barrier associated with an increased susceptibility of cirrhotic patients to bacterial infections originating in the digestive tract. The translocation of bacterial products, such as lipopolysaccharide (LPS, component of the wall of Gram-negative bacteria), by the portal venous system thus contributes to the perpetuation of inflammatory lesions of the liver (Yu and Schwabe 2017). A study has shown that the activation of LPS/TLR4 pathway promotes hepatocyte proliferation via the secretion of TNFα and IL-6 by Kupffer cells (resident macrophages of liver), and increases the resistance of hepatocytes to oxidative stress and apoptosis resulting in the accelerated development of HCC in an experimental model chemo-induced by diethylnitrosamine (Yu et al. 2010). In the experimental model used in this study, the depletion of circulating LPS by antibiotic therapy or the interruption of the LPS/TLR4 activation pathway through the inactivation of the gene encoding TLR4 inhibited the initiation and progression of HCC (Yu et al. 2010). Another study showed a potential upregulation of anti-tumor immunity via an action of the gut microbiota. This study investigated the impact of the reduction of commensal bacteria on the evolution of levels of primary and secondary bile acids, and therefore of the immune response. Primary acids promote the accumulation of natural killer T-lymphocytes (NKT), which produce anti-tumor interferons-gamma, while secondary acids have the opposite action (Ma et al. 2018). After administration of a cocktail of antibiotics aimed at destroying the intestinal commensal bacteria in mice suffering from primary or metastatic hepatic carcinoma, the researchers evaluated the effect on the tumor growth kinetics, and concluded that the decrease in species belonging to the genus *Clostridium* caused an increase in the number of NKT cells regardless of the mouse genus, lineage, and tumor type. The correlation was confirmed after colonization by a bacterium known to metabolize primary bile acids into secondary

acids—*Clostridium scindens*—in mice with altered microbiota. This renormalization caused a decrease in the number of NKT cells, and therefore lifted the inhibition of tumor growth (Ma et al. 2018).

More studies (Piñero et al. 2019; Ponziani et al. 2019; Ren et al. 2019; Huang et al. 2020; Lapidot et al. 2020; Zeng et al. 2020b) suggest a relationship between the gut microbiome, their metabolites, and the hepatic immune response; a relationship that might herald novel therapeutic methods in HCC, a main source of death in oncology. Additionally, the results of some studies make it possible to stratify the patients with HCC according to their metagenome, or even to identify particular risky metagenomic profiles, which could then benefit from a treatment modulating the gut microbiota of the antibiotic, prebiotic, or probiotic type.

5.1.4 Intestinal Microbiome: Biomarker of Breast Cancer

A relationship between intestinal dysbiosis and breast cancer has been suggested by a number of clinical studies, where the researchers have emphasized the use of antibiotics, which influence the intestinal microflora with an increased risk of breast cancer (Velicer 2004; Friedman et al. 2006; Tamim et al. 2008). However, to date, there is very little data available on the precise contribution of the gut microbiota in the development of breast cancer. This section summarizes the experiments resulting from animal models and clinical studies that have established the link between the intestinal flora and breast cancer, as well as the hypotheses explaining this relationship.

The intestinal microbiota has an essential functional role and interferes in particular with the exogenous and endogenous metabolites such as estrogen. Estrogens are recognized as a causative factor in the etiology of breast cancer, and play an important role in the initiation and promotion of breast tumor (Russo and Russo 2006; Yue et al. 2010). A high level of endogenous or circulating estrogen are directly associated with an increased risk of breast cancer in postmenopausal women (Lewis et al. 2005; Sampson et al. 2017). Estrogens and their metabolites undergo a second metabolic step in liver, where these are conjugated. These are then excreted in the intestinal tract or through the urinary tract, in conjugated forms. It has been widely described that the human gut microbiota can contribute to the metabolism of estrogen in humans. The authors in a study defined the term "estroboloma" as "the set of enteric bacterial genes whose expression products are capable of metabolizing estrogen" (Plottel and Blaser 2011). Among these metabolic reactions, the most documented activities are the β-glucuronidase and β-gluconidase enzymatic activities expressed by intestinal bacteria. As previously described, these enzymes are known to participate in the deconjugation of xenobiotics, drugs, but also steroid hormones such as estrogen. These can eliminate the glucuronic acid part (for β-glucuronidase) or the glucose part (for β-gluconidase) from the conjugated estrogens, and therefore promote the reabsorption of their free forms through the enterohepatic circulation. Therefore, a bacterial community enriched with bacteria possessing these enzymatic activities, including the β-glucuronidase activity, could

lead to greater reabsorption of free estrogen, and thus influencing the development of estrogen-dependent neoplasia (Kwa et al. 2016). Bacteria possessing β-glucuronidase activity are distributed among the *Enterobacteriaceae* family (in particular the *E. coli*), in two dominant clusters *Clostridium leptum* and *Clostridium coccoides*, such as the genera *Roseburia*, *Faecalibacterium*, *Clostridium*, and *Ruminococcus* (Jefferson et al. 1986; Dabek et al. 2008). β-glucosidase activity is represented by many members of *Firmicutes*, some species of *Bifidobacterium*, and the species *Bacteroides thetaiotaomicron* (phylum Bacteroidetes) (Dabek et al. 2008). In 2012, Flores and co-workers demonstrated that the β-glucuronidase activity of intestinal microbiota is negatively correlated with the level of total estrogen (conjugated and deconjugated) in fecal samples, in a study comprised of 29 individuals (seven postmenopausal women and 22 men). They also showed that the richness and diversity of fecal bacteria positively influenced the excretion of total estrogen in urine (Flores et al. 2012). In addition, according to a study in 60 postmenopausal women, the diversity of the gut microbiota was positively associated with an increased ratio of estrogen metabolites to estrogen in urine (Fuhrman et al. 2014). Elevated levels of urinary estrogen metabolites are associated with the decreased plasma estrogen concentrations, and thus leading to a reduced risk of hormone-dependent breast cancer (Moore et al. 2016). Studies, therefore, suggest that the risk of developing breast cancer may be greater in women with a gut microbiome favorable to estrogen deconjugation or with a low gut bacterial diversity.

5.1.5 Intestinal and Lung Microbiome: Biomarkers of Lung Cancer

The Human Microbiome Project, launched in 2007 by the United States National Institute of Health (NIH), studied various organs, including digestive tract, urogenital tract, skin, mouth, and nose (NIH HMP Working Group et al. 2009). When the study of the microbiota took off, the lung was not viewed with interest. But the vision of the pulmonary microbiota has changed recently, passing from that taught in medicine ("A healthy lung is a sterile lung"), to that of an organ occupied by microorganisms, most of which, in normal conditions, are good for its health (Faner et al. 2017).

Lung cancer, often diagnosed at an advanced stage, is deadly. An earlier diagnosis would greatly improve care and the chances of survival. What if, as with many diseases including other cancers, the intestinal dysbiosis is a sign of lung cancer (Liu et al. 2020a). In a study, the intestinal microbiota of 42 patients with early stage lung cancer of three different types (only three patients with metastases) as well as that of 65 healthy controls were analyzed through sequencing of 16S RNA. The intestinal dysbiosis was observed in patients with lung cancer compared to the controls, which showed increased presence of species belonging to the genera *Ruminococcus* and to the families *Lachnospiraceae* and *Enterobacteriaceae*, among others. Thus, the composition of the microbiota could change with the development of lung cancer. Finally, the composition of the intestinal microbiota specifically indicated the stage

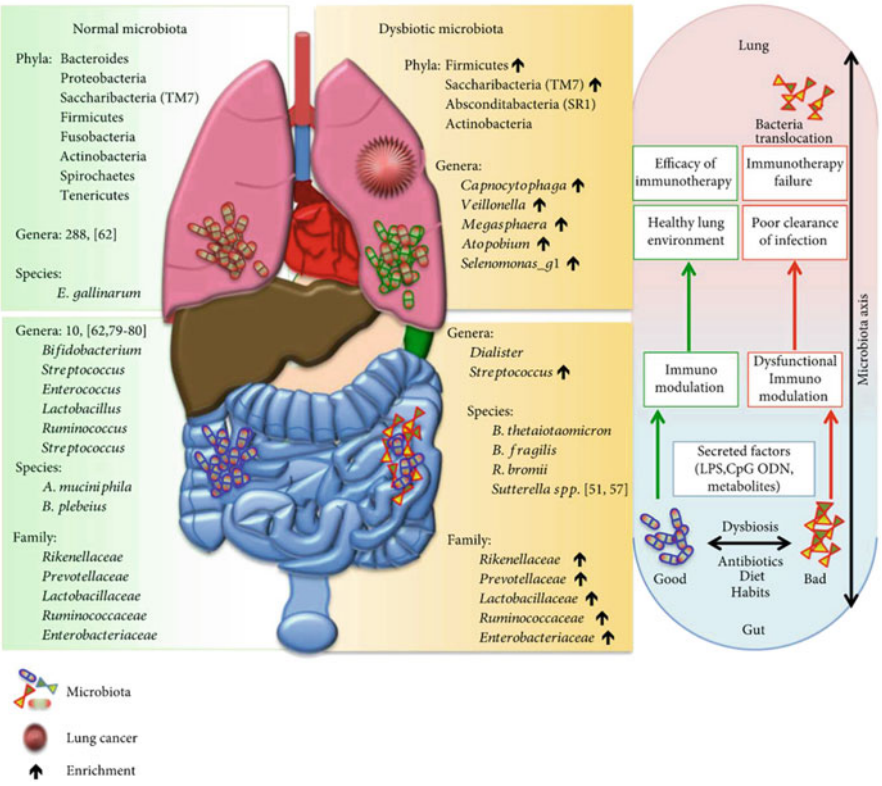

Fig. 5.3 A classification of normal and dysbiotic microbiota in lungs and the microbiota axis between lungs and gut/intestine. (Figure reproduced from Carbone et al. 2019 distributed under the Creative Commons Attribution (CC BY 4.0) license)

of the tumor: certain bacteria were only present in patients with metastases (Zheng et al. 2020). In order to provide a noninvasive diagnostic tool for early detection of lung cancer, 13 biomarkers, based on operational taxonomic unit (OTU), have been identified. Together, these can accurately predict (97.6%) the presence of lung cancer. This model was confirmed in a second cohort (34 patients and 40 controls): its predictive power remains high (76.4%), although lower than that of the initial cohort. From this model, it was possible to construct a "patient discrimination index", easy to use in clinical practice (weighted score) to identify patients with early lung cancer. Its predictive power in the initial cohort (92.4%) is also found to be greater than that measured in the validation cohort (67.7%). Larger cohorts could improve the model and its predictive power (Zheng et al. 2020).

The analysis of lung microbiota (Fig. 5.3) reveals characteristic signatures of various respiratory symptoms, making it a diagnostic, prognostic, and a follow-up tool, in step with the developing precision medicine (Mao et al. 2018; Xu et al. 2020b). In lungs, the airways are poorly described compared to other microbial

deposits (Laguna et al. 2016). This is due to the difficulty of access by invasive samples (bronchoscopy or bronchoalveolar lavage). The other difficulty comes from the oropharyngeal contamination, which must be overcome to make the observation reliable. Encountered by the increase in patients with chronic respiratory diseases (more than 300 million people according to WHO), different research teams are interested in studying the pulmonary microbiota, betting that it could be the key to progress for diagnosis and cares (Sharifi et al. 2019). If the human intestine contains around 100 trillion bacteria, the bacterial lung biomass is a million times smaller (100,000 bacteria per cm^3 of bronchoalveolar lavage) (Wouters 2019; Liu et al. 2020a). Despite the low density, this microbiota is distinguished by its exceptional biodiversity (Xu et al. 2020b), due to different origins of bacteria. Lung is an ideal playground (approximately 75–100 m^2 of alveolar surface in adults) (Fröhlich et al. 2016) for bacteria, which mainly come from the oral sphere but also from the inhaled air (100,000 microorganisms per liter of inhaled air) and the digestive tract (by micro-aspiration) (Wouters 2019). The combined action of mucus and cilia of the respiratory mucosa, to which are added the cough and the local antimicrobial defenses, limit the pulmonary bacterial colonization, with a microbiota theater of an incessant ballet where some bacteria settle and others leave by the muco-ciliary escalator (Xu et al. 2020b; Liu et al. 2020a). Local lung conditions select the microorganisms best suited to this ecosystem (Xu et al. 2020b; Liu et al. 2020a). Colonization of airways begins early in life, influenced by the method of delivery (Xu et al. 2020b). The maturation of this microbiota would be fundamental for lung health, with three roles including barrier against pathogenic bacteria, education of the local immune system, and modeling of the lung architecture. It is likely that other roles will be identified, particularly in relation to the regulation of metabolic pathways (Xu et al. 2020b). The pulmonary microbiota results from a dynamic balance, whose disturbances accumulated since childhood could play a role in the onset or aggravation of diseases such as asthma, chronic obstructive pulmonary disease, or genetic diseases such as cystic fibrosis, which all result in a profound change in the pulmonary microbiota and especially a decrease in commensal anaerobes. Thus, for asthma, one hypothesis is that a modification of this microbiota during infancy could modify the local inflammatory response, favoring its occurrence (Loverdos et al. 2019).

Some recent studies show that the gut microbiota may affect lung health through cross communication with lungs (Xu et al. 2020b). This axis of communication is carried out via the passage of microbial metabolites (short-chain fatty acids), endotoxins, and cytokines in the bloodstream connecting the intestinal and pulmonary epithelia (Mao et al. 2018; Xu et al. 2020b). The intestinal dysbiosis could thus lead to acute or chronic lung diseases, such as asthma, tuberculosis, and lung cancer. Conversely, some patients with lung disorders such as tuberculosis, asthma, and chronic obstructive pulmonary disease (COPD) also have intestinal symptoms, which clearly indicates interference between the intestine and the lungs (Mao et al. 2018; Xu et al. 2020b). Overall, intestinal homeostasis could affect pulmonary homeostasis and vice versa.

5.1.6 Intestinal and Gastric Microbiome: Biomarkers of Gastric Cancer

Gastric cancer, the third leading cause of cancer death, has a poor prognosis because it is often diagnosed at an advanced stage and therefore difficult to treat (Li et al. 2017; Gantuya et al. 2019; Ye et al. 2019). The highest death rates from stomach cancer are seen in East Asia as well as in Eastern Europe and countries in Central and South America (Wroblewski et al. 2010). The identification of obtaining a biomarker capable of early detection of this cancer is essential to reduce the number of deaths (Li et al. 2017). *Helicobacter pylori* is a pathogenic bacterium that colonizes the stomachs of almost half of the world's population (Gantuya et al. 2019). Its infection is acquired during childhood and lasts for decades (Pacifico et al. 2010; Kotilea et al. 2018). It remains asymptomatic in most people, but in some cases the infection will progress to gastric cancer (Pacifico et al. 2010; Kotilea et al. 2018). Today, it is believed that *H. pylori* is responsible for around 90% of gastric cancers worldwide, resulting in an estimated number of deaths of around 800,000 persons per year (Moss 2017; Rawla and Barsouk 2019). The sequence of events triggered by the bacterial infection that will ultimately lead to gastric cancer is starting to be deciphered, with the instability of DNA of the infected cells at the heart of the mechanism (Liu et al. 2020b). Indeed, previous studies have shown that *H. pylori* causes DNA breaks and disrupts its DNA repair system by promoting the accumulation of mutations that can target p53, a protein called the "guardian of the genome" (Li et al. 2016b; Williams and Schumacher 2016). The p53 protein is essential for the proper functioning of the cell, because it allows—in the event of significant damage in the genome—to temporarily stop the cell cycle, time necessary to repair the DNA (Williams and Schumacher 2016). An inactivation of p53 therefore promotes genome instability and the transformation of a normal cell into a cancer cell (Williams and Schumacher 2016). It is important to understand the cell transformation induced by *H. pylori* that promotes cancer development, in order to define a marker of susceptibility. This would allow early management of patients and thus could prevent the development of gastric cancer.

H. pylori infection of the stomach is acquired during childhood and lasts for decades or even the entire life of the infected person (Gantuya et al. 2019). This develops a strong local and humoral inflammatory response that gradually settles into chronicity. In most people, chronic gastritis progresses without further consequence and remains asymptomatic (Gantuya et al. 2019). A small proportion of patients (about 10% of those infected) develops ulcer over time and in 1–3% of cases stomach cancer; gastric cancer (adenocarcinoma), or gastric MALT lymphoma (Rawla and Barsouk 2019). However, the data accumulated in recent years show that these two clinical pictures are mutually exclusive, and that the evolution towards one or the other of the pathologies is a function of the genetic predispositions of the host of environmental factors (food in particular), and bacterial properties. The evolution of *H. pylori* infection to ulcerative disease is associated with a predominantly antral gastritis (lower part of the stomach) and an acid hyper-secretion which leads to colonization and inflammation of the duodenum, site of duodenal ulcer

(which accounts for 95% of ulcer disease) (Stewart et al. 2020). The progression of the infection to gastric atrophy and then gastric cancer is usually associated with pangastritis (gastritis of the upper and lower parts of the stomach) (Raza and Bhatt 2020). It is generally observed in patients with acid hyposecretion and more particularly affects populations over 50 years of age (Osefo et al. 2009). Thus, *H. pylori* has the sad privilege of having been recognized as the first, and still today the only bacteria directly involved in the genesis of cancer (Wroblewski et al. 2010). The correlation between *H. pylori* infection and adenocarcinoma of the stomach is clearly demonstrated. *H. pylori* causes chronic inflammation of the gastric mucosa (gastritis) which can lead to a process of carcinogenesis. However, it appears that a number of cancers are independent of *H. pylori* infection. These results were provided by the work of Portuguese researchers, who studied and characterized the gastric bacterial flora of 54 patients with stomach cancer before comparing it with that of 81 people with chronic gastritis. The composition of the gastric microbiota of the two types of patients is different. Patients with adenocarcinoma present with gastric dysbiosis characterized by the decreased microbial diversity, enrichment in *Proteobactria*, *Lactobacillus*, *Clostridium*, and *Rhodococcus* and a lower proportion of *H. pylori*. This imbalance causes an increase in the enzymatic activity of nitrites and nitrate reductases, which participate in the metabolism of nitrogen by producing, for example, carcinogenic nitrogen compounds (Ferreira et al. 2018). This discovery is the first evidence of a gastric dysbiosis (potentially genotoxic) associated with stomach cancer different from that seen in chronic gastritis. It provides new elements of the direct involvement of the gastric microbiota in the occurrence of stomach cancer.

5.2 Oral Microbiome: Biomarker of Oral and Oropharyngeal Cancers

The human oral microbiota is the set of microorganisms present in the mouth of human beings (Aas et al. 2005). The oral microbiota can be normal or pathological. It is still poorly understood because many oral bacteria cannot be cultivated (Socransky et al. 1963). It is made up of several hundred to thousands of different species of microorganisms (Schwiertz 2016), 700 species having been discovered by screening (Paster et al. 2006). It is mainly its free (planktonic) forms that have been studied, but it is mainly made up of colonial forms consisting of organized biofilms (Schwiertz 2016). Each individual is home to 100–200 of these 700 species. The oral microbiota, mainly made up of bacteria, has developed strategies of resistance and perception of its environment allowing it to a large extent to evade the immune system, or even to modify the host for the benefit of the microbe (for example dental caries). Oral microbiota is more or less diversified according to age and individuals, and it contributes to the smell of the breath and can have systemic effects on the health of the whole organism and even on the intellectual capacities (Bik et al. 2010). This microbiotic heritage is partly acquired from the mother and the father at birth or in infancy (Alaluusua et al. 1991). It could be positively or negatively modified by

brushing teeth, dental care (Anwar et al. 1992; Auschill et al. 2002), diet (sugar in particular), and ingestion of antibiotic products (alcohol, natural antibiotics, certain drugs, chlorinated water, etc.) with consequences that are still poorly understood (Arweiler et al. 2014) which can contribute to the appearance of phenomena of microbial resistance to antibiotics. A part of oral microbiota could be transgenerational (Attar 2016). The oral environment (temperature, humidity, pH, constant presence of saliva, and nutrients and mucous residues) selects adapted populations (and sometimes pathogens) of microorganisms (Faran Ali and Tanwir 2012; Kilian et al. 2016).

In a healthy young or adult person consuming a healthy diet, the resident mouth microbes adhere to the mucous membranes, teeth (including enamel) (Al-Ahmad et al. 2009), some artificial implants (if present in mouth) (Fürst et al. 2007; Al-Ahmad et al. 2010), and gums to resist leaching out by saliva, but are subsequently mostly destroyed by passage through the stomach (through the action of secreted of hydrochloric acid) (Faran Ali and Tanwir 2012; Wang et al. 2014; Kilian et al. 2016). The salivary flow (Watanabe and Dawes 1990) and locoregional conditions inside the mouth vary (Cruchley et al. 1989), and these largely depend on the time of day and personal habit, for example the individual sleeps with open or closed mouth. From childhood to old age, the respective surfaces of different areas of oral cavity (as well as their quality) including those of teeth evolve over the course of life (Kerr et al. 1991) by interacting with the oral microbiota, according to factors that science explore (Busscher and van der Mei 1997). Through the larynx, many of these bacteria migrate to the respiratory tract where mucus is responsible for repelling them. A part of this microbiota is involved or co-involved in the production of factors (like γδ T or γδT17 cells) which promote autoimmune diseases such as psoriasis, arthritis, as well as cancer of colon, lungs, and breast (Fleming et al. 2017).

Tobacco consumption and poor oral hygiene are the two major risk factors for oral or throat cancer associated with the depletion of oral microbiota. What differences does the latter then mark compared to that of a nonsmoker and concerned about his hygiene? To answer this question, a German–American research team analyzed the composition, diversity, and functions of microorganisms in the oral cavity of 121 patients with oral-pharyngeal carcinomas and 242 healthy individuals (Börnigen et al. 2017). The main risk factors for developing oral cancer (tobacco, alcohol, oral hygiene, and papillomavirus infection) were assessed in 363 volunteer participants. The microbial composition of the oral cavity was then detailed in each individual in order to identify the main differences between the two populations. While all of the cancer risk factors are not accompanied by significant changes in the microbiota, smoking and poor dental health are a source of significant deterioration of the oral ecosystem. This deterioration is accompanied in particular by a decrease in microbial diversity and disruption of certain essential functions of the microbiota (metabolism of sugars, transport of metals, organic compounds, etc.) (Börnigen et al. 2017). Above all, the study highlighted the major impact of total tooth loss. The disappearance of a privileged "habitat" drastically reduced the microbial diversity and their associated functions, which are essential for maintaining a healthy mouth. Directly linked to the occurrence of oral and pharyngeal cancers, the absence of teeth

could therefore also increase the risk by modifying the local microbiota. While there is currently no certainty regarding this eventuality, the results of the study allow at least to confirm that the presence of certain risk factors for oral cancer alters the composition and functions of the oral microbiota (Börnigen et al. 2017).

5.3 Vaginal Microbiome: Biomarker of Cervical Cancer

Vaginal microbiome is comprised of a plethora of bacteria, with the most abundant representation by *Lactobacillus* (Chee et al. 2020). Modern methods have been used to characterize the healthy vaginal microbiome and to distinguish it between the different healthy profiles that keep vaginal homeostasis in check (Chase et al. 2015). It is well-established that a balanced microbiome in the vaginal region is pivotal in avoiding infections of the genital tract; however, recent evidences show that it may even influence the development of malignant changes in the cervix and other constituents of the reproductive system (Chase et al. 2015; Champer et al. 2018). Exploring the association between the vaginal microbiome and gynecological malignancies is a developing and exciting area of research, as several studies that have been published recently proposed that such a relationship may exist (Chase et al. 2015; Champer et al. 2018). The general hypothesis is that the vaginal bacteria play a major role in the tumor microenvironment.

Evidences for how the vaginal microbiome influence the human papillomavirus (HPV)-induced cervical cancer is mounting (Ramchander and Crosbie 2018). The HPV is the most common sexually transmitted infectious agent. While most infected women clear it quickly, only a small fraction develops a persistent infection with a high risk of developing precancerous lesions and then cervical cancer (Usyk et al. 2020). The cervico-vaginal microbiota (CVM) has also been shown to be involved in the prevalence of the disease. However, its influence on the elimination of infection or, conversely, on the progression to moderate to severe dysplasia is not yet known. The presence of *Gardnerella* in the cervico-vaginal microbiota of women with a high-risk oncogenic papillomavirus would be an indicator of an increase in microbial diversity and a sign of progression to precancerous lesions (Usyk et al. 2020). A study hypothesized that the vaginal dysbiosis promotes the progression of oncogenic HPV infection to precancerous lesions. The study specified that biomarkers exist within CVM and make it possible to identify patients at risk. In future, if other studies confirm the central role of CVM in the progression of the disease, therapeutic strategies could be considered to prevent the progression of the infection by modulating CVM (Chao et al. 2020). Moreover, a study showed that the microbiome in women with HPV demonstrates a greater diversity of substance from bacteria, particularly *Gardnerella vaginalis* and *Gasseri lactobacillus*, which was corroborated by another study which found higher diets of HPV in women with generally lower levels of *Lactobacillus* and higher microbiome diversity (Ramchander and Crosbie 2018). In addition, HPV elimination rates (and hence the risk of malignant transformation) are also influenced by the vaginal microbiome composition, and a bacterial genus that has repeatedly been linked to stagnant HPV

clearance was Atopobium. Additionally, vaginal infection with *Chlamydia trachomatis* appears to predispose women to HPV infection simply by altering the vaginal microbiome (Mitra et al. 2016; Kyrgiou et al. 2017). Some studies have tried to elucidate certain putative substance which alone can act. For example, *Lactobacillus crispatus* has been associated with healthy women, while *Lactobacillus iners* alone have been found in those with cervical cancer—or with HPV (particularly in those patients with high grades of cervical intraepithelial neoplasia) (Mitra et al. 2016). A disruption of the vaginal microbiome may also be an indirect risk factor for the development of endometrial and ovarian cancer (Kyrgiou et al. 2017). Recent studies have shown that the ovaries, fallopian tubes, and uterus are characterized by unique microbial profiles, and that differences in their composition may be related to certain malignant conditions (Chase et al. 2015; Champer et al. 2018; Xu et al. 2020a).

In conclusion, the notion that the vaginal microbiome may retain a secret of cervical carcinogenesis is intriguing, and may represent a comprehensive different perspective on the optimal prevention and treatment of this frequent malignant process. Nevertheless, supplementary studies which may support this association are desirable, and thus there is simply not sufficient indication for the connection between the vaginal microbiome and gynecological cancers.

5.4 Gut Microbiome: Markers for Modulation of Immune System and Breast Cancer

It is widely accepted that inflammation is an essential part of pathogenesis and progression of breast cancer. Neutrophil dysregulation is a hallmark of breast cancer development, and a high ratio of neutrophils to lymphocytes predicts a poor clinical prognosis in patients with breast cancer (Ethier et al. 2017). Moreover, neutrophils and lymphocytes could be modulated by the intestinal microbiota and inflammation. In genetically engineered female mice with a predilection for developing breast cancer, studies have shown that orogastric infection with *Helicobacter hepaticus* upregulates the inflammatory cells associated with tumor, including neutrophils. In particular, systemic neutrophil depletion inhibits the formation of mammary tumors induced by *H. hepaticus*. Furthermore, CD8 + T lymphocytes are recognized as the most powerful immune cells, which are capable of eliminating foreign antigens and mammary tumor cells (Gritzapis et al. 2008). Patients with a higher level of CD8 + T cells infiltrating their breast tumors are associated with better survival (Wang et al. 2011). The gut microbiota plays an important role in conditioning systemic CD8 + T cells to modulate other peripheral immune cells, such as plasmacytoid dendritic cells and iNKT (invariant nature killer cells) (Wu and Wu 2012). In contrast to bacteria with carcinogenic potential, there are also beneficial bacteria which can inhibit carcinogenesis in extraintestinal tissues. The oral administration of probiotic bacteria, such as *Lactobacillus reuteri* to mice has been reported to reduce the production of systemic inflammatory cytokines and the accumulation of neutrophils, and to activate CD4 + CD25 + lymphocytes, thereby reducing the risk of breast cancer in

mice (Lakritz et al. 2015). These data demonstrate that host immune responses to intestinal bacteria could cause or inhibit cancer progression in extraintestinal tissues, such as mammary glands.

Recently, Goedert and co-workers showed that the gut microbiota of breast cancer patients had less diversity compared to the controls. In addition, the patients had higher levels of *Clostridiaceae*, *Faecalibacterium*, and *Ruminococcaceae* (phylum Firmicutes) and lower levels of *Dorea* and *Lachnospiraceae* (phylum Firmicutes) (Goedert et al. 2015). In order to validate the relationship between the gut microbiota and breast cancer, some studies have been conducted to investigate the changes in microbial composition in patients with breast cancer depending on their pathological characteristics. At the same time, differences in the intestinal microbial profile between the patients and healthy women were analyzed. First, a significant reduction in gut microbiota diversity was found in breast cancer patients compared to the healthy women, which was in agreement with the results presented by Goedert and co-workers. It is accepted that a low bacterial richness and diversity are found in subjects with more marked overall adiposity, insulin resistance, and hyperlipidemia (Le Chatelier et al. 2013), which are associated with an increase in the occurrence of breast cancer (Gunter et al. 2015). In addition, the diversity of gut microbiota in postmenopausal women is positively associated with the increased ratio of estrogen/estrogen metabolites in urine (Fuhrman et al. 2014). The elevated levels of urinary estrogen metabolites are associated with a decreased plasma estrogen concentrations and thus reducing the risk of hormone-dependent breast cancer (Moore et al. 2016). In a study, it was observed that the abundance and relative proportion of certain bacterial groups, such as *C. coccoides* cluster, *C. leptum* cluster, *F. prausnitzii*, and *Blautia* sp. group were higher in patients with severe clinical stage and a higher histopronostic grade. In agreement with these results, a second study revealed that the gut microbiota of patients is enriched in *C. coccoides* cluster, *C. leptum* cluster, *F. prausnitzii*, and *Blautia* sp. Thus most of the bacteria expressing identified β-glucuronidase and β-glucosidase activities belong to two clusters, *C. leptum* and *C. coccoides*, such as the genera *Clostridium*, *Faecalibacterium*, *Ruminococcus*, and *Roseburia* (Dabek et al. 2008). The bacterial enzymes can catalyze the hydrolysis of the conjugated form of estrogen, thereby promoting the reabsorption of their free forms into the enterohepatic circulation. Thus, the intestinal microbiota enriched in *C. coccoides* and *C. leptum* cluster could contribute to the occurrence of breast cancer in these patients. The genus *Bifidobacterium* has been recognized as one of the probiotics which exerts numerous beneficial effects for the host (Hemarajata and Versalovic 2013). Kim et al. showed that oral intake of the probiotic *Bifidobacterium* reduced β-glucuronidase and β-glucosidase enzymatic activities in rats (Kim et al. 2008b). The probiotic *Bifidobacterium* has also shown its beneficial effect on the immune response against the growth of melanoma in several mouse models (Sivan et al. 2015). Concerning the genus *Coprococcus*, a low abundance of these bacteria has been found in patients with colorectal cancer in autistic children (Kang et al. 2013) and in subjects infected with HIV (McHardy et al. 2013). In addition, the abundance of *Coprococcus* was reduced in mice exposed to social disruption stress, and is negatively correlated with

circulating levels of pro-inflammatory stressor-induced cytokines (Bailey et al. 2011). This indicates that some bacterial populations, such as the genera *Bifidobacterium* and *Coprococcus*, may be beneficial in healthy women for the primary prevention of breast cancer. In summary, these studies show that the intestinal bacterial profiles differ between patients with breast cancer, depending on their clinical situation, but also between patients and healthy women. Their findings raise the question of whether gut bacteria could be used for interventions aimed at preventing the onset of this disease. Future studies on large populations, supplemented by the determination of the concentrations of estrogen and estrogen metabolites in different matrices (serum, urine, feces), as well as analysis of diet should be performed to clarify the relationship between gut microbiota and breast cancer.

Breast cancer is considered to be one of the world's top public health priorities (Ghoncheh et al. 2016). The diagnosis of breast cancer is based on clinical examination, in combination with breast imaging, and confirmed by pathological evaluation of the tumor (Li et al. 2016a). The management of breast cancer consists of excision of the tumor, radiotherapy, or drug treatments (Moo et al. 2018). Differences in the composition of the gut microbiota influence plasma estrogen levels. Thus, a high diversity of the intestinal microbiota would reduce plasma estrogen levels and consequently reduce the risk of developing hormone-dependent breast cancer. In addition, it should be noted that the composition of gut microbiota is interconnected with other preestablished risk factors for breast cancer such as obesity and eating habits, alcohol, age, all leading to intestinal dysbiosis (Hullar et al. 2014; Hills et al. 2019; Sampsell et al. 2020). Thus, the study of the intestinal microbiota would make it possible to assess the risk of the occurrence of breast cancer and would help to develop strategies for preventing this type of cancer.

5.5 Uterine Microbiome: Biomarkers of Endometrial Cancer

Endometrial cancer—or cancer of the uterus (not to be confused with cervix cancer)—is one of the most common gynecologic cancers after breast cancer, and the most common of the female reproductive system (Kato et al. 2021). Indeed, the endometrium is the covering inside the lining of the uterus, which is part of uterus where the embryo develops during pregnancy (Kelleher et al. 2019). Diagnosed at an early stage, the prognosis for the endometrial cancer is quite good (Matteson et al. 2018). Unfortunately, due to the lack of characteristic symptoms, the average age of patients at diagnosis is 68 years (Matteson et al. 2018). Interest in the microbiome of the genital tract has grown in recent years. Studies of vaginal and placental microbiomes have shown the associations between these microbiomes and obstetric outcomes and it is plausible that the uterine microbiome is also associated with these findings (Molina et al. 2020). Since infection is a major cause of preterm birth, many studies over a decade have focused on identifying microorganisms remaining in the uterus and recording any association with pregnancy outcomes (Agrawal and Hirsch 2012). Current knowledge about the uterine microbiome has been greatly improved

by the availability of molecular techniques in the field of microbiology, which has led to the discovery of various bacterial taxa which had not been previously described (Verstraelen et al. 2016). The vaginal microbiota, the microorganisms inhabiting in the vagina, could facilitate screening for endometrial cancer. The predominant taxa in endometrial cancer are *Treponema*, *Porphyromonas*, *Anaerotruncus*, *Bacteroides*, *Acinetobacter*, *Anaerostipes*, *Ruminococcus*, *Comamonadaceae*, *Peptoniphilus*, *Escherichia*, *Cloacibacterium*, *Pseudomonas*, *Arthrospira*, *Dialister*, and *Atopobium* (Walther-António et al. 2016; Walsh et al. 2019; Winters et al. 2019).

Different studies identified a microbiome signature associated with endometrial cancer, which is in part favored by postmenopause (Walther-António et al. 2016; Walsh et al. 2019). These studies were aimed to understand how risk factors for endometrial cancer alter the microbiome of the reproductive system and the risk of endometrial cancer. The studies analyzed samples taken from the female reproductive system (vagina, cervix, ovaries, and fallopian tubes) from patients who had the endometrial cancer or hyperplasia and compared them with samples taken from patients with benign uterine conditions (Walther-António et al. 2016; Walsh et al. 2019). As reported, the high sequencing of next throughput DNA recovery was used to identify microbiota present, which showed that several taxa were more abundant in patients with endometrial cancer and hyperplasia with patients who have had mild conditions (Walther-António et al. 2016; Walsh et al. 2019). In particular, with the presence of the *Atopobium vaginae* and *Porphyromonas somera*, a high vaginal pH was significantly associated with the presence of endometrial cancer. These microbes have previously been shown to be associated with other pathologies and their findings suggest a role for the microbiome in the cause and progression of this cancer that needs more research (Walther-António et al. 2016; Walsh et al. 2019). Another study comprising women suffering hysterectomy for endometrial tumors and identified *Pseudomonas*, *Comamonadaceae*, *Escherichia*, *Acinetobacter*, and *Cloacibacterium* as principal taxa (Winters et al. 2019).

5.6 Cutaneous Microbiome: Biomarkers of Skin Cancer

Although it has long been known that skin is colonized by commensal microbes, their identification has traditionally been based on methods of culturing and counting living colonies (Sender et al. 2016). This method of identifying bacterial species is biased towards bacteria that can easily grow (Gill et al. 2006). The advent of molecular techniques, which allows the sequencing of gene encoding 16S ribosomal RNA specific for prokaryotic cells and sufficiently variable for phylogenetic analysis, has revealed a greater diversity of species in the microbiota that lives with its host (Grice et al. 2009). Indeed, more than 1000 bacterial species have been identified which collectively constitute the human microbiota (Grice et al. 2009; Grice and Segre 2011). While the classic skin microbiota includes bacteria such as *Staphylococcus epidermidis*, *Staphylococcus hominis* (common commensal bacteria), *Streptococcus mitis*, *Propionibacterium acnes*, *Corynebacterium* spp., *Acinetobacter*

johnsoni (common commensal bacteria), and *Staphylococcus warneri* (infrequent commensal bacteria), new techniques based on 16S ribosomal RNA have revealed that skin contains more than 300 commensal bacterial subspecies (Grice et al. 2009; Grice and Segre 2011). Another discovery made possible by the new identification methods is the variability of the microbiota between different anatomical areas of the skin: different skin areas harbor different bacterial communities. The level of diversity depends on temperature, humidity, and lipid content of the skin (Nakatsuji et al. 2013). Three main skin microenvironments can be defined: oily areas (face, inside part of ears, back of scalp, upper torso, and back), wet areas (inside of nostrils, armpits, between the fingers, cubital fossa, popliteal fossa, inguinal folds, intergluteal fold, arch, and navel), and dry areas (arms, palms, and buttocks) (Nakatsuji et al. 2013). By comparing the bacterial species found in these different skin areas, it was shown that *Propionibacteria* predominate in fatty areas, *Corynebacteria* and *Staphylococci* are abundant in wet areas, while a mixed population is found in dry areas, among which β-proteobacteria are the most abundant (Grice et al. 2009). Although the skin microbiota found in each skin area appears to be stable over time for a given person, the relative frequencies of these bacterial species can vary greatly between individuals (Grice et al. 2009). This inter-individual variability of the skin microbiome raises many questions about its acquisition (environmental, role of genetics) and about its role in the development of skin diseases. The commensal bacteria that partly make up the skin microbiota are present on the surface of the skin, in the epidermis, and also in deeper layers such as the dermis. They protect the host against colonization by pathogenic bacteria by competing for nutrients and secreting bacteriocins. They also induce the expression of IL-17 by T-cells and antimicrobial peptides (AMP) by keratinocytes, leading to the development of protective immunity against the risk of infection (Macpherson and Harris 2004). The increase or reduction in bacterial diversity, called dysbiosis, promotes the emergence of pathogenic bacteria and a disruption of immune responses which can be the cause of the development of certain skin diseases such as acne, atopic dermatitis, hidradenitis suppurativa, or maybe even psoriasis and cancer (Seite and Bieber 2015).

There are two main types of skin cancer: carcinoma and melanoma. Carcinomas are the most common skin cancers (Liu-Smith et al. 2017). They generally occur after the age of 50, on exposed areas of the body (face, neck, shoulders, forearms, and legs, etc.) (Liu-Smith et al. 2017). They are most often due to excessive and chronic sun exposure. Carcinomas are easily curable in the majority of cases. However, some of them, called "squamous cell carcinomas", can lead to distant lesions (metastases) if not removed in time (Liu-Smith et al. 2017). Cutaneous melanoma is much rarer than carcinoma but it is the most serious of the skin cancers, because of its "high metastatic potential", that is to say of its ability to spread rapidly to other parts of the body (Liu-Smith et al. 2017). A good balance of the skin microbiome protects against skin cancer. Furthermore, a study showed that strains of *S. epidermidis*, which are frequently present on normal skin, produce, alongside antimicrobial peptides, a molecule (6-*N*-hydroxyaminopurine) (6-HAP) which has a role in providing protection against cancerous growth (Nakatsuji et al. 2018). In

culture, 6-HAP inhibits the proliferation of cancer cell lines, without affecting normal keratinocytes. Injecting 6-HAP into the mice inhibits the growth of experimental melanomas. In addition, mice whose skin is colonized by *S. epidermidis* producing 6-HAP are more resistant to UV-induced carcinogenesis than those whose skin is colonized with strains which do not produce 6-HAP (Nakatsuji et al. 2018). This research confirms the importance of the composition of the skin microbiome in the antitumor immune response. The anticancer activity of nucleotide bases such as adenine analogue 6-HAP has long been known, and the digestive microbiome is also known to play a role in controlling tumor growth (Nakatsuji et al. 2018). We can expect new discoveries regarding the anticancer functions of bacteria in the microbiome, and possibly the development of anticancer drugs derived from metabolites of the bacteria.

5.7 Gastrointestinal and Urinary Microbiota: Biomarkers of Prostate Cancer

The urinary microbiota of people with prostate cancer harbors pathogens which could contribute to the onset and development of this cancer. This is the conclusion of a US study, the most comprehensive to date, on the links between urinary microbiota and prostate cancer. Recent work has shown that the bacterial infections and chronic prostatic inflammation promote the development of cancer by prompting the identification of species likely to be involved in the pathophysiology of the disease. Thus, the existence of a urinary microbiota could promote repeated exposure of the prostate to various opportunistic bacteria in the proximity of the urethra. To explore this lead, researchers analyzed the composition of urinary microbiota of 129 people with prostate cancer before a prostate biopsy. Among them, 63 presented a benign tumor, 61 a malignant tumor, and five others an initially benign tumor which progressed to cancer. The results indicate that the urinary microbiota of all participants were substantially equivalent, composed of about 60 species dominated by the genus *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Lactobacillus*, or *Gardnerella*. However, the presence of a group of bacteria containing *Streptococcus anginosus*, *Anaerococcus lactolyticus*, *Anaerococcus obesiensis*, *Actinobaculum schaalii*, *Varibaculum cambriense*, and *Propionimicrobium lymphophilum* was more often associated with cancer (70.8% of cases). However, all these species are involved in urogenital infections including prostatitis. Researchers have also identified more pathogens such as *Ureaplasma parvum* or *Ureaplasma urealyticum* in cancer and G. vaginalis in moderate to severe chronic inflammation of the prostate. These data do not allow to conclude on the specific role of certain bacteria in this cancer but shed light on the composition of the urinary microbiota in patients with prostate cancer. They also suggest the presence of pro-inflammatory and uropathogenic bacteria in patients with prostate cancer (Shrestha et al. 2018).

Researchers have shown how the gut microbiota interacts with an oral drug used in prostate cancer, indicating an important influence of certain bacteria in response to

the treatment. Conventional therapies designed to deprive the body of androgens, which are responsible for the growth of prostate cancer, are not always effective. Abiraterone acetate is considered here, and unlike other treatments, it is taken orally. As this reagent is poorly absorbed, a significant portion is excreted in the stool which interact with the intestinal microbiota. Several studies have demonstrated the role of the intestinal microbiota in the development and progression of certain cancers, as well as in the effectiveness of treatments. However, knowledge of the involvement of the gut microbiota in prostate cancer remains limited. Researchers therefore sought to demonstrate how AA, which is very effective in this type of cancer resistant to conventional therapies, impacts the intestinal microbiota, and whether this could act on the response to treatment. To do this, they examined the composition of the gut microbiota by sequencing 16S rRNA from 68 patients with prostate cancer and divided them into three groups: patients not receiving treatment ($n = 33$), patients receiving conventional therapy ($n = 21$), and patients receiving conventional therapy + AA ($n = 14$). The androgen deprivation by conventional therapy alone or in addition to AA led to a significant reduction in *Corynebacterium*, pro-inflammatory bacteria that metabolize androgens like testosterone, compared to the control group. The intake of AA induced a significant enrichment of *Akkermansia muciniphila*, accompanied by an increase in the production of vitamin K2, known for its antitumor properties. These results were confirmed in an intestinal model, thus ruling out the possibility of immune involvement. These investigations reveal that AA is metabolized by intestinal bacteria. The components resulting from this degradation would have a selective impact on the intestinal microbiota characterized by the growth of *A. muciniphila*. This species, known for its health benefits and anti-inflammatory properties, is believed to play a key role in response to treatment, according to the authors. Previous work had also demonstrated its beneficial role in response to treatments of certain immunotherapies. This study highlights the key role of the intestinal microbiota in response to an anticancer treatment taken orally, through the mechanisms that still need to be clarified (Daisley et al. 2020). Exploring the drug–microbiota interactions could further improve the treatment outcomes for many diseases.

5.8 Urinary Microbiome: Biomarkers of Bladder Cancer

Urinary microbiota is located in the bladder. As a result, the urine produced by the body is loaded with microorganisms (Andolfi et al. 2020). The number of urinary bacteria remains heterogeneous from one individual to another, and thus this microbiota is different (Andolfi et al. 2020). A major difference is observed according to the sex: the microbiota of women and men are divergent (Ackerman and Chai 2019; Andolfi et al. 2020). In women, the urinary microbiota is marked by a certain proximity to the vaginal microbiota, largely dominated by the bacteria of genus *Lactobacillus*, *Gardnerella*, and *Streptococcus* (Ackerman and Chai 2019; Pohl et al. 2020). Thus the urinary microbiota and the vaginal microbiota have several points in common, but remain very different from the intestinal microbiota

(Ackerman and Chai 2019; Pohl et al. 2020). Most often, the urinary microbiota is less important than the vaginal microbiota. A study has shown that the presence of certain *Lactobacillus* could be associated with the urinary incontinence (Govender et al. 2019). Likewise, another study linked the presence of two bacterial species with overactive bladder problems (Siddiqui et al. 2014). In men, such links between the urinary disorders and the makeup of the urobiome has not been found. The presence of urinary microbiota would be closely related to the method of urine collection (Hourigan et al. 2020). In men with benign prostatic hyperplasia, a study found a possible link between the frequency of bacteria in urine and the severity of urinary symptoms (Lepor 2004). While the urinary microbiota differs by gender, several studies suggest that urobiome may play a role in the development of urinary disorders (Aragón et al. 2018). The data indicate that the urinary microbiota evolves with age: the diversity of this microbiota decreases with age (Liu et al. 2017).

Researchers have also looked at the urinary microbiota of patients with bladder cancer. One such study showed that the urobiome of 29 patients with bladder cancer contained more *Actinomyces europaeus* than the urobiome of 26 noncancer patients (Bi et al. 2019). An another study showed that the urobiome of 62 males patients with non-muscle invasive bladder cancer contains more bacteria, including *Micrococcus* and *Brachybacterium* after undergoing transurethral resection of bladder tumor than the urobiome of 19 non-neoplastic controls (Zeng et al. 2020a). A study including 31 male patients with bladder cancer showed increased strains of *Sphingobacterium*, *Acinetobacter*, and *Anaerococcus* and decrease of *Roseomonas*, *Serratia*, and *Proteus*, compared to the 18 non-neoplastic controls. The increase of *Bacteroides*, *Herbaspirillum*, and *Porphyrobacter* was observed in cancer patients with a high risk of recurrence suggesting that these bacteria could be considered as biomarkers (Wu et al. 2018). Furthermore, a study comprised of 12 male patients with bladder cancer exhibited more *Fusobacterium* than 11 healthy. Next, 42 bladder cancer tissues selected as independent sample had 11 *F. nucleatum* sequences detected by PCR (Bučević Popović et al. 2018). A summary of occurrence of microbiota in healthy and non-muscle invasive bladder cancer is shown in Fig. 5.4.

Despite its recent discovery, the investigations into the urinary microbiota revealed its importance in the development of various urinary disorders, but also of certain pathologies such as benign prostatic hypertrophy or bladder cancer. Other studies may confirm the importance of the urinary microbiota.

5.9 Conclusions

Different parts of digestive tract as well as other parts like mouth, skin, and others in human contain a variety of microbial community. These microbial communities are beneficial in performing several physiological functions such as digestion, homeostasis, and metabolism in gut and perform protection functions. Most importantly, these microbiota are serve as biomarkers for detection of different types of cancers. These biomarkers predict the risk and prognosis. Among the different microbiota, the intestinal microbiota represents a considerable biomass with many physiological

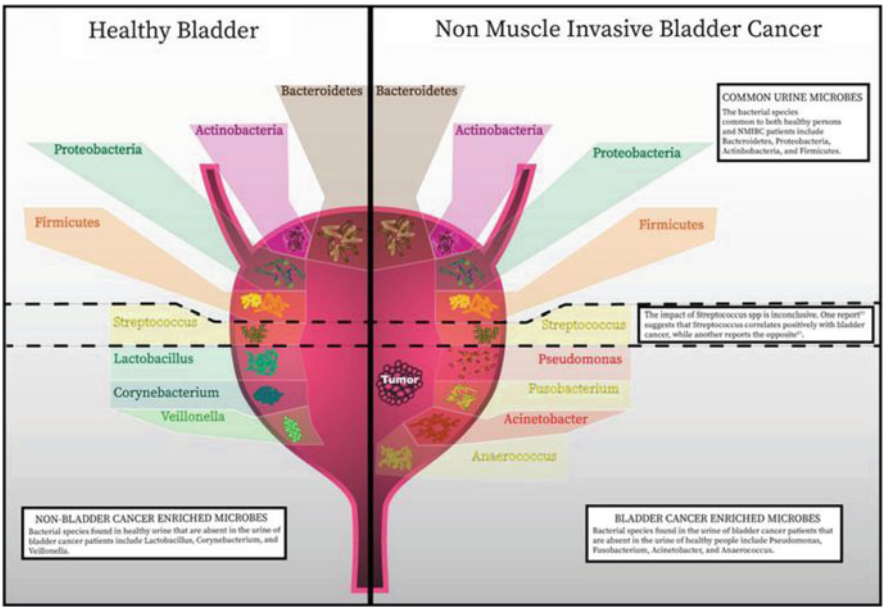

Fig. 5.4 A summary of occurrence of microbiota in healthy and non-muscle invasive bladder cancer. (Figure reproduced from Andolfi et al. 2020 distributed under the Creative Commons Attribution (CC BY 4.0) license)

functions essential for the host, thus representing an extremely complex ecosystem. Each individual has their own intestinal microbiota which is therefore unique in terms of quality and quantity. The intestinal microbiota has local and systematic effects on the development of different types of cancers.

The extensive research carried out on different microbiota has led to the emergence of knowledge and establishing links between them, which could ultimately lead to open up therapeutic options. For instance, a knowledge of the pulmonary microbiota and its links with the intestinal microbiota opens up therapeutic potential through the use of probiotics on different respiratory sites. Thus, the pulmonary and digestive administration of a cocktail of *Lactobacilli* before infection with the dreaded pyocyanic *Bacillus* seems protective, whether in cystic fibrosis or nosocomial pneumonia. Moreover, deciphering the lung microbiota will also enable the discovery of next-generation probiotics and tackle common diseases like asthma from an entirely new perspective. Similarly, a deep understanding of the interaction of immune cells with different microorganisms would clarify the difference between the commensal and pathogenic bacteria which could influence the immunotherapeutic treatment. It is highly desirable to develop novel probiotics which could be used in combination with chemo- and immuno-therapies for treating different types of cancers.

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Predictive Biomarkers for Anticancer Drugs

6

Nosheen Fatima Rana and Tahreem Tanweer

Abstract

The adverse effects of anticancer drugs, the acquired drug resistance, and tumor heterogeneity among cancer patients limit the effective clinical management of advanced malignancies. To overcome these challenges, predictive biomarkers emerged as an indispensable tool to aid medical oncologists in identifying cancer patients who may respond to several anticancer therapies, hence increasing the risk-to-benefit ratio. This chapter will offer a brief overview of predictive anticancer biomarkers, their characteristics, and brief details about the tools and techniques in practice for their identification. This chapter will also discuss the validated and also commonly researched predictive anticancer biomarkers for anticancer drugs against different cancer types. Finally, the challenges in identification and commercialization of the predictive biomarkers for anticancer drugs will be discussed.

Keywords

Anticancer drugs · Drug resistance · Predictive biomarkers · Techniques · Identification · Treatment

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6.1 Introduction

Cancer diagnosis and treatment are nowadays mainly dependent on morphological features determined by histological assessments. While this approach results in a confident diagnosis and prognosis in most cases, molecular characterization of the tumor tissue can effectively determine tumor grade, it's aggressiveness and also predicts possible outcomes for various existing medications. Therefore, throughout the decision-making process, molecular characterization might be an indispensable tool for clinicians (Buonaguro et al. 2014). A biomarker is an attribute that can be objectively evaluated and used to define the normal or pathological condition of an organism through studying biomolecules like protein, peptide, DNA, RNA, as well as chemical modifications of these biomolecules (Institute of Medicine 2012). Yet, it should be noted that the term "biomarkers" has evolved in the last decade. The World Health Organization (WHO) offered an extremely broad definition that "a biomarker is any substance or structure, that can be measured in the body or its products can influence or predict the incidence of outcome or disease" (Lassere 2008; World Health Organization 2001). Based on the clinical value, the cancer biomarker can measure the likelihood of developing cancer inside a certain tissue or measure the chances of its advancement or probable response to therapy. Cancer biomarkers are increasingly associated with the deregulation of several molecular pathways and cancer pathogenesis to enable the use of several therapeutics or intervention methods and supply important information in guiding clinical decision-making. With the remarkable increase in the scope of omics examination of clinical biospecimens following the traditional course of the biomarker deployment, the conceptual model of cancer biomarker discovery has also been transformed (Institute of Medicine 2012).

Cancer biomarkers are categorized into many groups based on their application. Predictive biomarkers are cancer biomarkers that predict response to certain treatment modalities, like HER2 positivity in breast cancer, predicting response to the trastuzumab. Likewise, in colorectal cancer, KRAS-activating mutations predicted resistance to EGFR inhibitors like cetuximab (Goossens et al. 2015). Such predictive biomarkers are quite often classified as biological markers that are assessed using tissue or bodily fluids. They are identified by using different techniques, such as through genomics and proteomics. They can also be identified through radiographic approaches such as dynamic contrast-enhanced imaging to create imaging markers (DCE-MRI) and standard Response Evaluation Criteria in Solid Tumors (RECIST). They are routinely utilized during patient follow-up in multiple clinical trials or routine practice (Mankoff et al. 2014; El Bairi et al. 2019). In evaluating the clinical and substitute endpoints, such biomarkers offer great potential for assessing the effectiveness and safety of therapies tried on cancer victims in a timely, efficient, as well as pharmacoeconomic manner (El Bairi et al. 2019).

The earliest research on predictive biomarkers yielded significant results for cutting-edge directed cancer therapies. Some of the validated predictive biomarkers produced as a result include expression of PD-L1 to predict response to pembrolizumab (Merck Sharp Dohme, Keytruda®), the status of BRAF to predict

response to nivolumab (Bristol Myers Squibb, Opdivo®) as well as vemurafenib (Hoffmann La Roche, Zelboraf®) and dabrafenib (Novartis Pharms Corp, Tafinlar®). Other validated predictive biomarkers include RAS expression to predict response to cetuximab (Erbitux®), panitumumab (Vectibix®, Amgen), and Imclone; PDGFR status to predict response to imatinib (Novartis; Gleevec®), as well as BRCA expression for PARP inhibitors. These predictive biomarkers for anticancer drugs are currently widely used in the routine clinical practice of oncologists. Consequently, logical use of such biomarkers is appropriate to realize targetable disease features and has allowed the personalized medical care of cancer patients (El Bairi et al. 2019).

This chapter will introduce predictive anticancer biomarkers and discuss tools and techniques in practice for thier identification such as different clinical trial designs, molecular imaging, and computational and statistical techniques. This chapter will also discuss some validated and commonly researched predictive anticancer biomarkers for anticancer drugs against different cancer types, e.g., circulating tumor cells (CTCs), mutations and polymorphisms, methylation, and miRNA expression. Finally, the challenges in identifying and commercialization of the predictive biomarkers for anticancer drugs will be discussed.

6.2 Introduction to Predictive Biomarkers

As the name indicates, the predictive biomarkers disclose details about the likelihood of achieving a response to anticancer therapy. Therefore, they aid the oncologists in therapeutic decision-making process (Nalejska et al. 2014). They could also give information that can assist patients in evading the toxicity of conventional (systemic) medications and determine their eligibility for targeted therapy. Most of the predictive biomarkers have been discovered for prostate, lungs, and breast cancer. These cancer types are presently the most prevalent cancer forms throughout the general population (Nalejska et al. 2014).

Understanding a patient's cancer molecular characteristics can lead to the personalization of drug therapies with a better probability of effectiveness. Patients with specific kinase domain mutations upon EGFR, for instance, may not give a response to EGFR targeted therapy like erlotinib (Fig. 6.1a). This also has the added benefit of limiting a patient's exposure to toxic side effects from a drug from which they may not even have benefitted (Goossens et al. 2015).

The Predictive markers were also reported for their ability to predict the aggressiveness of a certain patient's cancer, the anticipated course of the disease, or even the prospect of response to the cancer therapy. Therapeutic predictive markers also show the prospect of a patient's response to a specific treatment, as mentioned earlier. They help the patient and the oncologist decide a therapy against cancer that is almost certain to be successful for that patient's cancer type. Different predictive biomarkers have dual capacities; they exhibit prognostic as well as predictive characteristics. ER expression in breast cancer is one such example. It is a prognostic as well as a predictive marker. Compared to non-ER-expressing

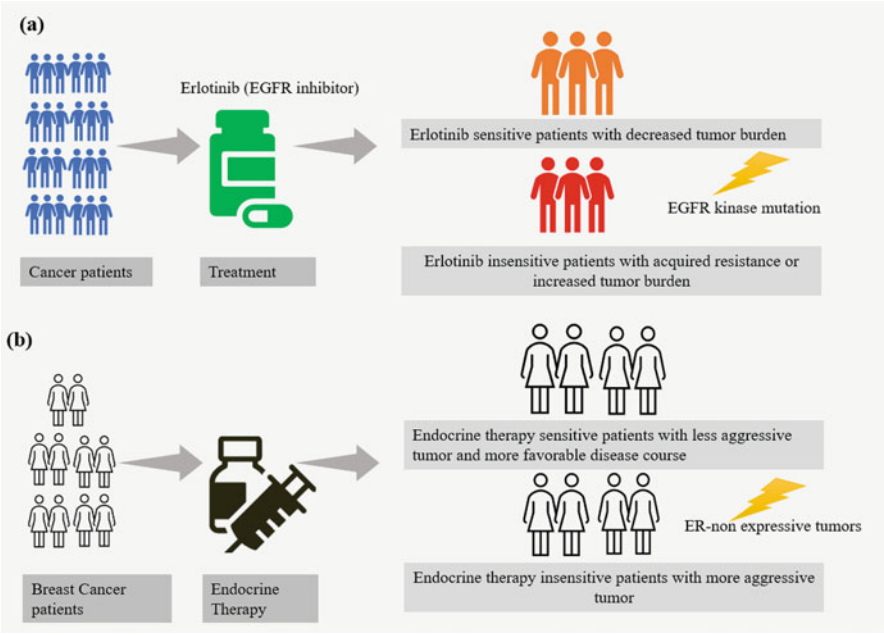

Fig. 6.1 (**a**) EGFR expression predicts response to erlotinib (**b**) ER expression predicts response to endocrine therapy in breast cancer patients

cancers, cancer with ER expression is less aggressive and shows a better clinical course.

Furthermore, an absence of ER expression envisages that breast cancer will not respond to endocrine therapy (Fig. 6.1b). Recently, multigene testing like Oncotype DX (Genomic Health Inc.) also aided in predicting tumor aggressiveness and response to the therapy. These tests are frequently utilized to assess the necessity for receiving aggressive treatment, for instance, adjuvant chemotherapy (Mankoff et al. 2014).

Generally, the development of predictive cancer biomarkers follows a multistep development process which includes (1) identification of biomarker, (2) development of assay, (3) clinical application, (4) validation of the assay, (5) clinical testing employing large prospective trial, and lastly, (6) approvals from the regulatory authority and then integration into the clinical practice guidelines and marketing. This sector has profited from some well-known international recommendations, notably the REMARK guidelines, which were established to tackle the consistent utilization of reporting in biomarker research studies (El Bairi et al. 2019; Sauerbrei et al. 2018; Altman et al. 2012). Positive results from randomized controlled trials led to introducing effective anticancer drugs into clinical practice. Therefore, the identification of biomarkers has become a chief topic in cancer research since biomarker research is expanding in tandem with the development of novel anticancer treatments like targeted medicines, immune checkpoint inhibitors, and all those

obtained from natural sources including plants and aquatic organisms (El Bairi et al. 2019).

6.3 Need for Predictive Biomarkers

Assistance and support required to develop predictive biomarkers mainly comes from various sources, including regulatory, commercial, and clinical sides. These considerations apply to several newly discovered cancer drugs which target specific molecular abnormalities and are often successful against a fraction of the cancer patients, often in the 10–20% range (Sawyers 2004). This suggests that around $45 billion of the yearly expenditures on cancer treatments (approximated $60 billion in 2010) will be spent on medicines with low benefits without patient selection. As a result, there is a convincing reason for developing companion diagnostics that can evaluate biomarkers, thereby identifying the responsive subset of patients. This enhances therapy's affordability, which works in parallel with improved clinical utility and safer drugs. Several chemotherapy regimes may result in fatality rates ranging from 0.5% to 2.0%, whereas 30–40% have grade 3/4 toxic effects, resulting in a considerable morbidity burden, particularly if a considerable proportion of this group does not benefit from the treatment. Targeted therapies could be hazardous in the same way. Bevacizumab, for instance, has substantial adverse effects such as gastrointestinal, cardiovascular, and renal damage (Badgwell et al. 2008; Segal and Saltz 2009) The minimization of unnecessary treatment and undesirable effects would be a key aspect of predictive biomarker-driven cancer treatment.

Due to evolving pharmacoeconomic environment and rising medication costs, regulatory authorities have emphasized the necessity of predictive biomarkers and the importance of predictive biomarker testing on patients' and providers' budgets. Both the FDA and European Medicines Agency are urging drug manufacturers to explore predictive biomarkers as the companion diagnosis, which would be widely expected to become communal guidelines and general practices (Goodsaid and Papaluca 2010). Generally, there is a rising demand for prediction tools to help identify and treat individuals with responsive diseases (La Thangue and Kerr 2011).

6.4 Identification of Predictive Biomarkers for Anticancer Drugs

The evidence for using predictive biomarkers as companion diagnostics to aid in the clinical use of cancer therapies is growing. To date, only a few biomarkers have got to the clinic and achieved the value of companion diagnostics. These biomarkers are being revealed mostly by the retrospective assessment of clinical trial data and random ad hoc genetic testing. Before drug development, historical information of mutations and related molecular heterogeneity has been rarely used as an integrated factor of the future clinical trial design. Nowadays, the issue that integrates existing methodologies is to position predictive biomarkers in a methodical prospect-driven

approach, permitting drug development to advance in parallel with the allied biomarker, thus opening a new, better hypothesis-based method to develop personalized cancer therapy (La Thangue and Kerr 2011).

For identifying predictive biomarkers, various high-throughput technologies in use include DNA sequencing at large-scale, microarray-based transcript profiling, proteomics, and single-nucleotide polymorphism (SNP) analysis (Nalejska et al. 2014; Schilsky 2010).

These approaches are responsible for generating correlative data, which is further utilized to identify the genes and proteins associated with the cancer type or response to the treatment. However, these approaches are very challenging to understand the processes involved in cancer and the treatment response. A clinical study was conducted to understand response to the treatment by a potential anticancer drug; it showed a response rate of around 20% and improved survival of 3 months in an unselected group of patients. However, the mean survival rate of the population compared to this group (12.6 months vs. 12 months) could not reflect a significant difference. Therefore it was not adequate to justify the clinical activity of this drug. In another study, a population of cancer patients was evaluated for a predictive biomarker. The stratified subset of the population, with 70% PPV for response and followed by treatment with the new cancer drug, reported an average survival rate of 14.1 months. This improved survival rate was better when compared with the unselected group, therefore requiring further clinical study (Sawyers 2004). As a solution, research has concentrated on establishing platforms that enable the identification of functionally relevant biomarkers, which can then be justified in the new drug's mechanism of cancer cell killing and utilized to assist and enhance its clinical development (La Thangue and Kerr 2011).

6.5 Tools and Techniques for Predictive Biomarker Identification

6.5.1 Clinical Trial Designs and Analysis Techniques

During the last decade, a variety of biomarker-based design methods have been proposed to study therapies in potentially heterogeneous patient subpopulations. There are several levels to which these can be classified.

Clinical trials for selective therapies are broadly classified into different phases. The first type is the phase 1 trial that involves simultaneous investigation of the predictive biomarker and the treatment in two groups, i.e., the healthy and the tumor group. Once the investigation is completed, the assay is validated, followed by carefully selecting the appropriate biomarker positivity levels. The second type is the phase 2 trials that involve searching and validating a marker-based population. At this stage, the efficacy of the targeted therapy is more promising compared to the phase 1 trial. In phase 3 trials, a typical population-based randomized treatment comparison is carried out, based on the benefits achieved from the earlier phase 2 studies (Mandrekar and Sargent 2014; Renfro et al. 2016). The predictive marker-

based trial designs are categorized as retrospective and prospective study designs. In the retrospective trial design, the marker and the treatment outcome relationship are assessed after the trial's completion. Whereas, in future trial designs, the predictive biomarkers are formally included in the design considerations. Future trial designs are usually required to assess the clinical validity of the predictive biomarker. Another classification of the predictive biomarker trial designs is strictly based on statistical methodology, i.e., the frequentist or "classical" designs versus the Bayesian designs. These designs have variations in the procedures for carrying out the testing of hypothesis, decision-making, and the use of preceding (or historical) knowledge (Renfro et al. 2016) (See Fig. 6.2).

For the last decade, substantial progress has been made in understanding the molecular basis of cancer and clinical trial design methods to meet biomarker-based goals. Future studies in all fields are needed to carry out fully customized cancer management plans for standardized care. There is still a need for an adaptive design paradigm within a clinical trial methodology that can prospectively identify both combination or continuous biomarkers that predict treatment response and clinically validate certain markers along with their thresholds or classifications, usually achieved in different studies ad hoc basis. Additionally, when spontaneously continuous or mixed biomarkers or signatures are used, then there is a necessity for more robust methods and better techniques for the selection of biomarker threshold (i.e., cataloging the patients as a marker-"positive" versus marker-"negative"). As advancements in clinical testing and trial methods are made, we must keep the viewpoint of particular patients in mind. Participation in biomarker-based clinical trials becomes a more rational and ethical choice (Meric-Bernstam et al. 2015). In this age of personalized and selective medicine, rigorous review and implementation of novel design techniques, both for early and final phase trials, would be needed to ensure the suitable clinical validity of specific experiments and therapies (Renfro et al. 2016).

6.5.2 In Situ Hybridization and Immunohisto Chemistry Techniques

In Situ Hybridization (ISH) and Immunohisto chemistry (IHC) are morphology-based techniques that directly explore the cancer cells (Schildhaus 2019).

IHC detects changes of protein expression qualitatively or as a semiquantitative measurement. The reliability of IHC-based predictive biomarkers depends on pre-analytical factors, selection of suitable antibodies, staining procedures, and an assessment of staining. Specific reading and scoring approaches for different tumor entities make evaluations complex, especially for evolving biomarkers in the context of immuno-oncology treatment (Schildhaus 2019).

ISH assays are capable of detecting gene amplifications, large deletions, and gene fusions. Definitions of amplifications are gene and entity-specific. Activating rearrangements frequently involve genes encoding receptor tyrosine kinases, which tyrosine kinase inhibitors can address (Schildhaus 2019).

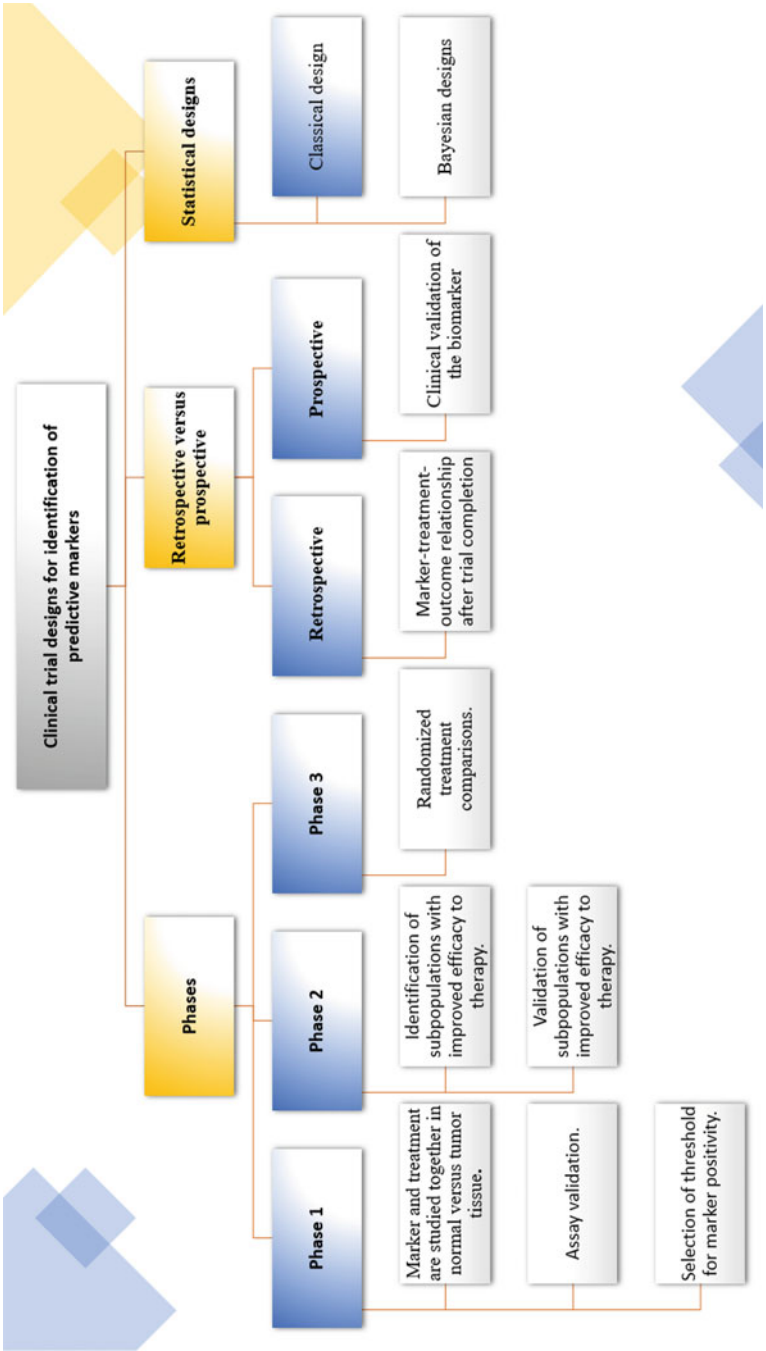

Fig. 6.2 Classification of clinical trial designs for identification of predictive biomarkers for anticancer drugs

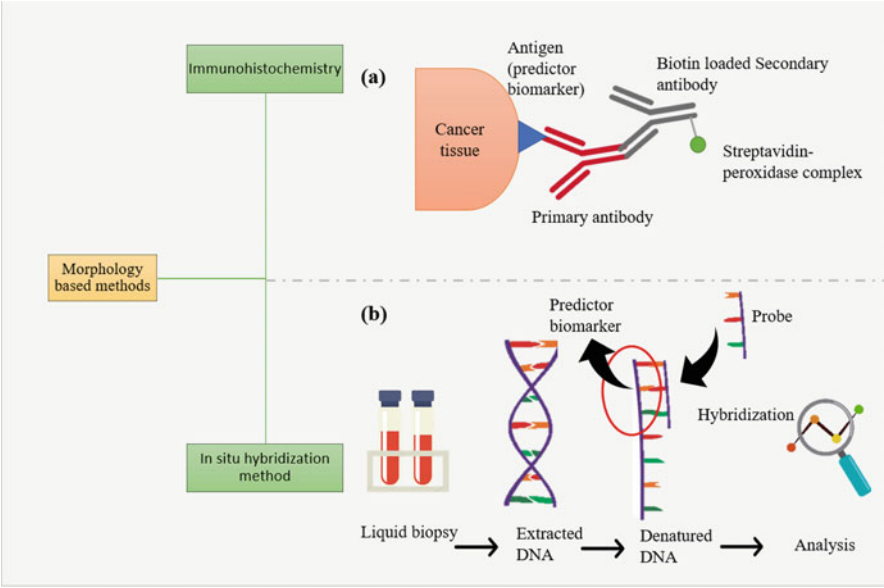

Fig. 6.3 Morphology based methods for identification of predictive biomarkers for anticancer drugs (**a**) Immunohistochemistry (**b**) In situ hybridization

Most of the currently applied predictive biomarkers are based on either IHC or ISH. The number of assays are steadily growing, great efforts are needed to achieve and maintain the highest level of reliability. Future developments will introduce multiplexing IHC and ISH (Schildhaus 2019) (Fig. 6.3).

6.5.3 PCR-Based Technologies and Multiplexed Gene Analysis

Polymerase chain reaction (PCR) has become an invaluable tool for assessing the presence and type of nucleic acids in tissues and body fluids. It is the enzymatic synthesis and amplification of particular DNA sequences in vitro. It can replicate a single molecule of DNA or RNA into billions of duplicates in a matter of hours. This enables mutation tracking for the management of any cancer, which is particularly crucial in targeted therapies. Novel applications include analysis of blood for circulating DNA for tumor-associated mutations. RNA analysis has been extensively used for the quantification of gene expression. This forms the basis of multiple gene expression assays, including multigene panels developed for prognostic and predictive purposes (Gökmen-Polar 2019).

6.5.4 Microarray Technology

Microarrays allow for the simultaneous measurement of expression levels of multiple genes inside a sample. The Microarrays have also been widely used in screening, profiling, and quantifying genome-wide gene expression profiles. They are crucial in identifying and also validating the predictive cancer biomarkers. In cancer diagnostics as well as biomarker research, gene expression arrays, single nucleotide polymorphism (SNP) arrays, as well as comparative genomic hybridization (CGH) arrays are indeed the primary approaches for identifying gene expression patterns, copy number variability, and sequence polymorphisms (Palanisamy 2019).

6.5.5 Massive Parallel Sequencing

Cancer research is advancing at an unprecedented rate, notably in identifying and characterizing clinical features and biomarkers that would be used to predict treatment response. The purpose of cancer therapy is to tailor the right treatment to the appropriate patient. To that aim, some clinical studies are now utilizing patient stratification by "Massive Parallel Sequencing" (MPS), widely known as "Next-Generation Sequencing" (NGS), to detect clinically responsive targets in real-time.

The omics upheaval gives significant, meaningful insight further into origins, processes of disorders, drug responses, roles of genetic and environmental factors in cancer predisposition and developed resistance. It lays the groundwork for precision medicine, which concentrates on factors unique to a particular patient to give individualized care; details about patients' genes, proteins, and environment are utilized to prevent, diagnose, and customize medical care to an individual patient. Omics data and NGS use are currently transforming the way of cancer patients' treatment. Many different forms of cancer are presently being treated using targeted medicines that take the benefit of the information about an individual's unique cancer cells.

We must also consider the multidimensional nature of cancer biomarkers while developing predictive indicators. They will necessitate a systems biology approach involving the integration of omics platform data in order to build innovative probabilistic models for early diagnosis, prediction, prognosis, and prevention. They will ultimately be based on existing tumor profiling, translating into optimal therapy that will improve patients' overall survival and quality of life (Abramovitz et al. 2019).

6.5.6 Flow Cytometry

Modern biology heavily relies on optics-based instruments to grasp the intrinsic functional and structural features of cells and biomolecules. Flow cytometry itself is such a technique that allows the biomarkers detected by imaging technology to be measured quickly. Flow cytometry is distinguished by its ability to rapidly resolve, count, and sort distinct populations of cells or organelles, dependent on their

expression of markers. Laser flow cytometry is widely used in fundamental and clinical testing, diagnostics, and disease control. Flow cytometry is used for a variety of purposes, including immunophenotyping of surface and intracellular markers, genome prediction, cell cycle analysis, antigen quantitation, membrane potential, ploidy analysis, calcium influx, pH, chromosome analysis, fluorescent reporter proteins, and so on (Tembhare et al. 2019). They have also been extensively used to identify predictive cancer biomarkers (Tembhare et al. 2019).

6.5.7 Molecular Imaging

The PET imaging of an ER expression, which is most often conducted using the ^{18}F-fluoroestradiol, is an ultimate illustration of molecular imaging through a predictive biomarker. The absorption of ^{18}F-fluoroestradiol has been shown to be associated with tissue-based ER expression assays (Linden and Dehdashti 2013; Mankoff et al. 2014). ^{18}F-fluoroestradiol PET, including tissue-based ER assays, predicts endocrine responsiveness of breast cancer, and notably, a lack of the ^{18}F-fluoroestradiol uptake positively predicted no response. Early researches on other ER-expressed cancers, like endometrial cancer, suggests a possible predictive role (Tsujikawa et al. 2009; Mankoff et al. 2014). There is currently an NCI-held investigational new drug application (IND) for the ^{18}F-fluoroestradiol to fund clinical trials. Thanks to the sustenance of a National Cancer Institute (NCI) Cancer Imaging Program, a limited prospective phase 2 trial has been completed successfully (Peterson et al. 2014; Mankoff et al. 2014). This approach can be useful in clinical trials for the novel endocrine therapeutics in breast cancer, particularly in helping the patients whose tumor expresses the therapeutic target (ER); nevertheless, more rigorous, highly controlled, as well as properly powered experiments are required to assess the negative predictive value of no ^{18}F-fluoroestradiol uptake versus the fine uptake thresholds. Multicenter trials together under NCI IND have been planned to meet this need. Many other molecular imaging methods for evaluating the territorial expression of cancer therapeutics' targets, such as progesterone receptor, HER2, or epidermal growth factor receptor, are also emerging (Kenny et al. 2011; Mankoff et al. 2014).

6.5.8 Digital and Computational Pathology

Digital pathology is also at the forefront of tissue analytics, offering a suite of new advanced methods for biomarker testing, interpretation, detection, and translation. For several years, the benefits of digital image analytics in tissue science have been recognized. Recent developments in high-resolution whole slide scanning, image analytics, web-enabled multisites collaboration, imaging information, and machine learning, on the other hand, are now allowing researchers to boost quantitative biomarkers' development and transform the clinical translation and discovery of

lucrative diagnostics for accurate therapeutics for the first time (Hamilton et al. 2019).

6.5.9 Bioinformatics and Biostatistical Tools

Biomarkers are now an essential part of modern medicine. Biomarker identification, clinical confirmation, and biomarker acceptance in clinical practice all present new problems in bioinformatics and biostatistics. With the increasing availability of high-throughput technologies, the medical research challenge is identifying individual biomarkers or biomarker signatures that predict treatment or therapy outcomes. Various bioinformatic and biostatistical tools and methods are used to study predictive biomarker discovery and biomarkers testing in clinical trials. For reporting and processing issues, using biomarkers in clinical routine (including the bioinformatics and machine learning method) required omics data, clinical trials, and validation (Perera-Bel et al. 2019).

6.6 Cancer Biomarkers for Predicting the Response Toward the Treatment

6.6.1 Circulating Tumor Cells (CTCs)

Circulating cell-free DNA (cfDNA), which can be extracted from serum or plasma through noninvasive techniques, has been identified as an appealing biomarker for cancer patients to evaluate therapeutic response, identify drug tolerance, and predict the clinical outcome (De Mattos-Arruda and Caldas 2016; Carpinetti et al. 2015). Tumor cells have been shown in experiments to release genomic DNA into blood, then circulating DNA may reflect tumor strain and biologic characteristics (Jiang et al. 2015; Xia et al. 2015; Chen et al. 2020).

Elevated levels of cfDNA were found to be strongly associated with low survival in Non-small cell lung cancer (NSCLC) patients. Furthermore, our findings suggest that cfDNA could be a capable predictor of reaction to EGFR-TKIs in NSCLC patients (Ai et al. 2016).

Circulating cell-free DNA (cfDNA) derived from blood serum or plasma by a noninvasive technique has also been suggested as an alternative and appealing tool for detecting early lung cancer, assisting in developing a tailored care plan, and estimating patient treatment response (Carpinetti et al. 2015; De Mattos-Arruda and Caldas 2016). cfDNA has shown to be feasible and predictive for cancer patients (Newman et al. 2014; Zhou et al. 2012). Furthermore, cfDNA is more appropriate for a general diagnostic test for cancer patients and even more relevant for clinicians in monitoring cancer dynamics, unlike biopsy. In the case of NSCLC, several experiments have looked into the success of cfDNA in cancer diagnosis and prognosis (Wang et al. 2014; Zhang et al. 2013), and the findings differ for a variety of reasons. Thus, a systematic meta-analysis was performed to analyze the

diagnostic accuracy of cfDNA for cancer diagnosis and EGFR and KRAS mutation and to govern the predictive role of cfDNA in NSCLC patients with a low prognosis (Chen et al. 2020).

The CTC count analysis before, during, and then after treatments at various periods allows for the estimation of treatment outcome. About the fact that the number of isolated CTCs from the patient samples is very small, some researchers have investigated new techniques for extremely efficient enrichment of CTCs required to conduct the profiling of gene expression. The molecular characterization of CTCs will aid in predicting response to therapy. Reduced mammaglobin 1 (MGB1) mRNA levels in CTCs obtained from the patients of breast cancer (metastatic), for instance, may better predict response to therapy (Nalejska et al. 2014).

6.6.2 Mutations and Polymorphisms

Some of the early episodes in colorectal carcinogenesis involve the somatic mutations inside the KRAS gene. In 1988, the first finding demonstrated a link between KRAS mutations and this tumor type's growth (Vogelstein et al. 1988). Codons 12 and 13 are the most often mutated, whereas codons 61 as well as 146 are the least often mutated (Loupakis et al. 2009). The status of mutations in codons 12 and 13 of the KRAS gene is a gold standard predictive biomarker for determining whether patients with advanced CRC are eligible for targeted treatment with monoclonal antibodies targeted to the extracellular domain of the EGFR (Lewandowska et al. 2012). EGFR promotes colorectal cancer (CRC) cells (through signaling pathways including MAPK, JAK/STAT, pik3). The growth of tumor cells is reduced due to therapy, while the rate of apoptosis increases. The absence of codon 12 and 13 mutations in the KRAS gene hence presents a significant predictive value. However, it must be remembered that patients with high-level EGFR expression without mutations in 12 and 13 codons of the KRAS gene may have a worse response to therapy when the occurrence of somatic mutations in 61 or 146 codons of KRAS genes or the somatic mutation of V600E in BRAF gene in their tumor cells has been determined (Lewandowska et al. 2013). The occurrence of both V600E mutations inside the BRAF gene as well as Codon 61 mutations inside the KRAS gene, over 10% are determined by CRC mutation analyses (Lewandowska et al. 2013). Therefore, rapid molecular biology diagnostic assays for both the KRAS gene (12, 13, and 61 codons) and BRAF gene (V600E) while using the very sensitive PCR method is still appropriate, in particular when DNA is to be collected from low-density body tissue with the limited detection for carcinoma pattern (Lewandowska et al. 2013). Notwithstanding the alterations in the KRAS gene, PIK3CA gene mutations and deletions inside the PTEN gene might also reduce the response to monoclonal metastatic CRC antibody treatment (Sartore-Bianchi et al. 2009). The evaluation of mutations throughout the BRAF, PIK3, and PTEN genes may also reflect targeted treatment in the future.

Thymidylate synthase (TS) is an important molecular target for several chemotherapeutics, including 5-fluorouracil, since it is a crucial enzyme for synthesizing the DNA (5-FU). Resistance to 5-FU is linked to TS overexpression. In comparison to just two copies of the tandem repeats (TSER*2) in a TS promoter region, the three copies of tandem repeats (TSER*3) presence promote higher TS expression. Furthermore, individuals with the TSER*2/TSER*2 or TSER*2/TSER*3 genotype had a greater response to 5-FU treatment. Compared to individuals with TSER*3 homozygotes, these patients had a high OS (overall survival) rate. It is imperative to notice that in patients having metastatic CRC, an overexpression of TS (due to many gene copies) leads to failure of 5-FU treatment and deteriorates the OS rates (mCRC) (Jiang et al. 2012).

The 5-FU catabolism is regulated by dihydropyrimidine dehydrogenase (DPD), a pharmacogenetic biomarker validated by the FDA. 5-FU has significant toxicity in individuals with DPD deficiencies, leading to death in some cases (Gross et al. 2008). The G>A transition at the donor splice site (IVS14 + 1G>A) (that consequently leads to the skipping of exon 14) is the most important mutation resulting in the reduction of enzymatic activity by the DPD protein (Raida et al. 2001). Patients can be classified with a high-low risk of grade 3 or 4 toxicity, while 5-FU therapy is based on genetic analysis of the DPD polymorphism (IVS14 + 1G>A). In other investigations, individuals with grade 4 neutropenia were found to be homozygous or heterozygous for an IVS14 + 1G>A gene in 50% of cases (Van Kuilenburg et al. 2002).

The investigation of tumor's microsatellite instability (MSI) can also give prognostic and predictive information. The findings of a single-factor study including grade 2 and 3 CRC patients demonstrated that fluorouracil medication was more beneficial in patients having stable microsatellites (MSS) or the low-frequency MSI than in colorectal cancer patients having high-frequency MSI (MSI+). Later on, fluorouracil did not alleviate cancer and even worsened the condition (Ribic et al. 2003).

The use of topoisomerase I inhibitor, i.e., camptothecin-11 (CPT-11), targets MSI + tumor cells with mutated genes that play a role in mismatch system repair (MMR), has the opposite effects (Bras-Gonçalves et al. 2000). However, the MSI status' prognostic significance is still under investigation, and currently, it is not widely employed in cancer therapy.

The evaluation of mutations in an EGFR kinase domain may be employed as the predictive biomarkers in NSCLC patients. Somatic mutations in exons 19 or 21 have been associated with tumor susceptibility to TKIs (tyrosine kinase inhibitors) like erlotinib and gefitinib medications. In the instance of deletion in exon 19, a median survival rate was greater than in the case of a point mutation L858R in exon 21 (Riely et al. 2006).

In a Polish population, activating mutations of the EGFR genes were observed in about 12% of adenocarcinoma patients during the assessment of deletions in exon 19 (Krawczyk et al. 2012), and a substitution of L858R was observed in 13% of adenocarcinoma patients while scrutinizing the 29 mutations in exon 18, 19, 20 and 21. TKI-susceptible NSCLC cells might develop resistance with time.

Unfortunately, NSCLC cells susceptible to TKIs may acquire resistance over time. Apart from alterations that make the tumor sensitive to TKIs, a somatic mutation T790M in an EGFR kinase domain of exon 20 has been found in about half of all patients of lung adenocarcinoma (Pao et al. 2005). At position 790, the substitution of threonine for methionine enhances the affinity of ATP (adenosine triphosphate), which is the fundamental mechanism of drug resistance development (Yun et al. 2008).

Furthermore, irrespective of EGFR gene's T790M mutation, MET gene amplification may be a significant source of drug resistance in 20% of cases. The stimulation of the ERBB signaling pathway may result in acquired resistance. The H820 cell line's ERBB signaling is largely reliant on the activity of MET, according to studies using XL880 molecules (MET kinase inhibitors) and small interfering RNA (siRNA) that suppresses MET expression. The findings support the possibility of low molecular weight inhibitors in lung adenocarcinoma patients resistant to EGFR inhibitors yet have multiple copies of a MET gene (Bean et al. 2007). Soda et al. discovered a minor inversion in the p region of chromosome 2 in NSCLC patients for the first time in 2007, resulting in the fusion gene EML4-ALK. The inversion was discovered in 6.7% of the patients in the study, which comprised 75 Japanese subjects (Soda et al. 2007). A lower incidence rate of 4.9% was seen in research with bigger groups ($n = 266$) (Wong et al. 2009).

It's important to notice that EML4-AKL mutation occurs in various histological types of NSCLC in nonsmokers, and it's not always associated with the mutations in the KRAS and EGFR genes (Wong et al. 2009). Within that group of patients, translocation is the most important factor in tumor development. Hundreds of adenocarcinoma tumors appeared in both lungs after a few weeks of birth in transgenic mice harboring the differentially expressed EML4-ALK fusion gene as the model organism. This prompted the researchers to examine further the oncogene's dominant involvement in lung carcinogenesis (Soda et al. 2008). Following studies of MET and ALK inhibitors, the crizotinib was swiftly introduced in a market (2011), while a predictive evaluation of a fusion gene EML4-ALK, employing fluorescence in situ hybridization (FISH) for paraffin-embedded material or the reverse transcriptase PCR (RT-PCR) for cytological content (Soda et al. 2012), is critical for determining the eligibility for the treatment of the patient by this selective kinase inhibitor.

The presence of BCR-ABL (the crucial fusion gene for targeted treatment) in chronic myeloid leukemia patients is another illustration of a fusion gene as a predictive biomarker (CML). The newly formed chimeric protein, i.e., tyrosine kinase BCR-ABL, is enabled by a translocation among the chromosomes 9 and 22 (Druker et al. 2006); however, for more than 10 years, the predictive analysis of the presence of that protein has permitted the assessment of patients' candidacy for imatinib treatment.

The evaluation of somatic mutation in codon 600 of a BRAF gene in advanced melanoma patients is another intriguing predictive biomarker. The only medicine recognized by the FDA for this condition was dacarbazine (DTIC) for several years, which had a very low response rate of about 10% (Chapman et al. 1999). The

discovery of the most prevalent mutation in a BRAF gene (V600E) and the inclusion of vemurafenib (a powerful inhibitor) in treatment marked a significant milestone in this type of cancer treatment. BRAF protein belongs to the raf/mil family of the serine-threonine kinases that control the MAPk and ERK signaling pathways, which regulate cell proliferation. Many tumor types have somatic mutations (missense) within BRAF gene (V600E/K/D/R/M). Malignant melanoma, on the other hand, has the greatest incidence rate (66%), with the V600E mutation accounting for the majority of cases (c. 1799T>A) (Davies et al. 2002).

In untreated and inoperable individuals (having stage III or IV), melanoma expresses the mutation V600E. The phase III clinical trial findings showed that vemurafenib reduced the risk of mortality by 63% and the risk of tumorigenesis by 74% compared to dacarbazine. Patients treated with vemurafenib responded to therapy in 48% of cases, compared to just 5% of patients treated with dacarbazine. Patients with the V600K and V600D mutations were also included in the research group. A significant number of patients (40%) having the mutation V600K well responded to vemurafenib treatment (Chapman et al. 2011); therefore, it appears critical to identify not only the prevalent mutation, V600E, but also much more known activating mutations, using the real-time PCR or pyrosequencing (Spittle et al. 2007).

Unfortunately, with CRC and NSCLC, developing melanomas resistance to the abovementioned inhibitors is becoming manifest. The CRAF protein was more active in drug-resistant clones produced from BRAF V600E M14 melanoma cell lines treated with an inhibitor AZ628. The CRAF protein may obtain a higher predictive value in evaluating the BRAF inhibitor therapeutic effectiveness following these findings. The possible predictive value of geldanamycin (tumor cells with a high degree of CRAF expression appeared vulnerable to this medicine) has also been investigated (Montagut et al. 2008). Further molecular studies in personalized medicines showed somatic point mutation in a MEK1 gene (in BRAF V600E A375 melanoma cell lines), which contributes to the resistance toward the MEK inhibitor (AZD6244) (Emery et al. 2009), also a somatic mutation in a KIT oncogene, which is found 21% in mucosal melanomas, 11% in acral melanomas, and 16.7% in melanomas linked with chronic damage induced by the sun. Furthermore, mutations in the KIT gene have been found in imatinib-resistant melanomas (Curtin et al. 2006), making the gene a prospective therapeutic target that has been investigated for almost a decade.

Breast cancer is considered the most prevalent malignant tumor in women, and researchers have been looking for biomarkers to predict its prognosis for more than a decade. Somatic mutations and polymorphisms are both included in the list of predictive biomarkers below.

Tamoxifen is known as a common drug in the treatment of breast cancer. The CYP2D6*10/*10 (and CYP2D6*5/*10) polymorphism is linked to the endoxifen's lower concentration (tamoxifen's active metabolite) and *N*-desmethyl tamoxifen's (NDM) higher concentration. This suggests that NDM accumulation in the plasma is a straight result of damage to NDM metabolism into endoxifen (Lim et al. 2011). The link between CYP2D6*10/*10 and low amounts of tamoxifen's primary active

metabolites (such as 4-hydroxytamoxifen and endoxifen), as well as the possibility of a connection between these findings and a lower response to tamoxifen in the treated female patients, has previously been discussed (Lim et al. 2007). This hypothesis appears to be supported by certain research. For the first time, Xu et al. (2008) looked and examined this association in a Chinese female population. Compared to the wt C/C homozygotes' control group, individuals who were homozygous for the CYP2D6*10 variation T/T had a significantly lower concentration of 4-hydroxytamoxifen. Following that, the impact of this polymorphism on chemotherapy was investigated. Patients with the CYP2D6*10 T/T genotype exhibited worse clinical outcomes than C/C and C/T genotypes, as predicted. Importantly, there was no influence of this genetic change on survival among non-treated women (Xu et al. 2008). In contrast, no statistically substantial correlations were seen among the alleles CYP2D6*1, *4, *5, *9, *10, *41, and *UM and OS or BCSS (breast cancer-specific survival) in 3155 patients medicated with tamoxifen and 3485 non-medicated patients over 7 years in a study that looked at the alleles CYP2D6*1, *4, *5, *9, *10, and *41 (Abraham et al. 2010). A similar lack of connection was seen in the female breast cancer patients from japan having tamoxifen as adjuvant treatment and possessing the genotype *CYP2D*6*10 (Toyama et al. 2009).

Similarly, HER2 gene amplification analysis is the most common genetic test performed in cancer diagnostics to determine patients' eligibility for lapatinib treatment or trastuzumab. Trastuzumab treatment, however, does not always provide the anticipated response in patients. According to studies done with the NIH3T3 andMCF-7 tumor cell lines, the isoform HER16, which is associated with Src kinase, improves its metastatic and carcinogenic potentials, may be linked to the aforementioned drug resistance. One of the recommended solutions in such conditions is to utilize suitable inhibitors to segregate the HER and kinase pathways. In the defined experiment, the dasatinib TKI use ensued the inhibition of the Src kinase (Mitra et al. 2009).

6.6.3 Methylation

The most well-studied epigenetic change in cancer is abnormal DNA methylation (Baylin 2005). In eukaryotic cells, abnormal hypermethylation of promoters can result in the silence of essential genes, such as tumor suppressor genes, and eventually illness. The reverse mechanism can also influence cancer growth. Hypomethylation of normally methylated genes, such as oncogenes, can upregulate the expression (Søes et al. 2014; Kamińska et al. 2019). DNA hypomethylation was the first DNA methylation anomaly to be documented in human cancer (Feinberg and Vogelstein 1983). Despite the original data published in 1983 and subsequent work, epigenetics has received little attention in investigating the molecular pathways that contribute to cancer (Curtin et al. 2011; Kamińska et al. 2019). Instead, the loss of heterozygosity (LOH) and DNA mutation were emphasized; Toyota et al. suggested the CpG Island Methylator Phenotype (CIMP) as another

cancer pathway in 1999. For the first time, they employed CIMP to characterize the clinical and pathological aspects of colorectal cancer (CRC). The MINT31, MINT27, MINT25, MINT17, MINT12, MINT2, MINT1, THBS1, MLH1, and CDKN2A (p16) genes were observed methylated in tumor tissue in this pioneering work (Weiss et al. 2017). We have now devised methylation in vitro diagnostic (IVD) techniques for tissue and blood, over 20 years after the Toyota et al. (1999) findings (Curtin et al. 2011). They've also been used successfully in the clinical context for prognosis, prediction, and cancer screening (Weiss et al. 2017; Kamińska et al. 2019).

A DNA mismatch repair gene i.e., MLH1, was one of the gene listed in the first methylation mapping in 1999. Microsatellite instability is instigated by epigenetic silencing of a MLH1 gene by hypermethylation of its promoter (MSI). MLH1 hypermethylation has been discovered in 13% of sporadic colorectal cancer, and a BRAF c.1799T>A, p.Val600Glu mutation has been discovered often in the DNA of tumor (Weisenberger et al. 2006). Lynch syndrome (one of the most frequent causes of hereditary CRC) similarly causes MSI and loss of MLS1 (Rustgi 2007); however, it is linked with the mutations in the MMR genes. Genetic evaluation of constitutional mutations in the MMR genes is used to diagnose Lynch syndrome fully. The two-level screening test is used to distinguish nonheritable CRC from Lynch syndrome. The first tier consists of MMR gene expression analysis and MSI testing. When MMR expression is lost and MSI is positive, constitutional mutations in MSH6, MLH1, MSH2, EPCAM, or PMS2 are studied. Alternatively, the BRAF V600E mutation and the MLH1 methylation level can be determined. To confirm Lynch syndrome, constitutional MLH1 epimutations testing is suggested (Giardiello et al. 2014). Pérez-Carbonell et al. (2010) found that methylation examination of MLH1 can enhance patient selection for the genetic testing of Lynch syndrome, lowering the cost of discovering a mutation by nearly half. Methylation of MLH1 may be determined by the MS-MLPA (methylation-specific multiplex ligation-dependent probe amplification) (Castillejo et al. 2015), and pyrosequencing (Newton et al. 2014; Kamińska et al. 2019) in certain laboratories.

In glioblastoma, clinical studies have shown that O6-methylguanine-DNA methyltransferase (O6-me–MGMT) is a helpful predictive and prognostic marker (Wick et al. 2012; Cabrini et al. 2015). MGMT is one of the DNA repair genes that help O6-meG eliminate cytotoxic and mutagenic alkyl groups. MGMT protects cells from damage by preventing DNA alkylation, which causes mutations (Coulondre and Miller 1977; Esteller et al. 1999). Temozolomide damages alkyl DNA, which results in cell death. Because cancer cells' DNA repair processes are disrupted, its harmful impact is higher against rapidly proliferating cancer cells in comparison of normal cells (Chakravarti et al. 2006). As a result, cells with hypermethylated MGMT had a stronger response to temozolomide medication (Cabrini et al. 2015). MGMT methylation has been observed in 40% of tumors in glioma and CRC and 25% of tumors in head and neck carcinoma, lymphomas, and non-small cell lung carcinomas (NSCLCs). The assessment of MGMT methylation (which is a significant step in the treatment algorithm and delivers valuable insights regarding the temozolomide's response) is one of the diagnostic recommendations for glioma. In

addition, the methylation status of MGMT in conjunction with IDH1 mutations serves as a predictive biomarker. The Glioma Patients having the IDH1 p.R132H mutation and MGMT hypermethylation have a better prognosis (Roszkowski et al. 2016; Kamińska et al. 2019).

There are a variety of commercial tests accessible to determine the methylation level of MGMT, including (1) methylation-specific polymerase chain reaction (PCR): Predict the MDx Glioblastoma (MDx Health); (2) real-time PCR: MGMT Methylation Detection Kit (EntroGen); (3) MS-MLPA: SALSA MS-MLPA probe mix ME011 MMR genes (MRC-Holland); and (4) PY (Qiagen) (Kamińska et al. 2019).

The RB1 gene is mostly linked to retinoblastoma, instigated by losing the functioning of RB1. An LOH or RB1 mutation is linked to the absence of expression of this gene in retinoblastoma and other malignancies, like malignant neuroendocrine lung carcinoma and bladder carcinomas. Nonetheless, methylation of RB1 can cause its expression to be silenced in some situations (Greger et al. 1989) It is reported that, in addition to the LOH and mutations, RB1 methylation must be evaluated for complete molecular diagnosis of retinoblastoma. According to Ohtani-Fujita et al. (1997), RB1 gene hypermethylation is usually acquired and accounts for around 9% of sporadic tumors. There are currently assays on the market for determining the RB1 promoter methylation level, based on the MS-MLPA approach (Livide et al. 2012; Kamińska et al. 2019).

GSTP1, RASSF1, and APC are tumor suppressor genes that are frequently methylated in prostate cancers and are thus used as cancer biomarkers. ConfirmMDx®, a commercially available test, uses a subset of these genes (MDxHealth). Patients having the prostate biopsy with negative result might be better stratified with this test. It makes use of the epigenetic fields effect, which is predicated on the idea that normal cells around cancer foci might carry DNA methylation alterations. Methylation Analysis to Locate Occult Cancer (MATLOC) and Detection of Cancer Using Methylated Events in Negative Tissue (DOCUMENT) are two independent studies that confirmed ConfirmMDxpredictive®'s value, with 68% sensitivity, 64%specificity, and 90% negative predictive value (Stewart et al. 2013). Furthermore, it was shown that using the methylation-based biomarkers RASSF1, APC and GSTP1 ensued in a decrease of up to 64% in the number of unnecessary repeated biopsies (Stewart et al. 2013; Kamińska et al. 2019).

6.6.4 Gene and miRNA Expression

MicroRNAs (miRNAs) are 22-nucleotide noncoding RNA molecules that control post transcriptional gene expression by the degradation or inhibition of their target mRNA. Biogenesis of miRNAs is a dynamic process that starts in the nucleus and moves to the cytoplasm (Kashyap et al. 2018). MiRNAs epigenetically control the 60% of mammalian genes which are involved in the diverse pathological and physiological diseases (Kashyap et al. 2018). MiRNAs, which are considered as cell-free miRNAs, may be found both within and outside of cells in body fluids like

the blood, saliva, serum, plasma, urine, and pancreatic juice (Kashyap et al. 2018; Sohel 2016). According to reports, 10% of all known miRNAs may be detected in bodily fluids, however, at varying quantities (Turchinovich et al. 2013). Serum/plasma is the greatest option for the analysis of miRNA expression compared to numerous other bodily fluids (Williams et al. 2013). The reason for this is that it is easy to extract and contains a larger volume of miRNA, making it possible to perform safe and exact global cell-free miRNA quantification (Max et al. 2018). According to many studies, cancer patients had greater amounts of unique miRNAs in their blood than healthy persons (Moldovan et al. 2014; Cortez et al. 2011; Filipow and Laczmanski 2019; Kosaka et al. 2010; Hetta et al. 2019). Furthermore, several studies have demonstrated the importance of miRNA (cell-free) in the prognosis and diagnosis of cancer (Chonggao et al. 2018; Kodahl et al. 2014). NSCLC was identified by scrutinizing the change in miRNA expression in the blood of patient and was associated with prognosis and overall survival (Yu et al. 2016). Cell-free miRNAs with dysregulated expression can be utilized to identify the CRC (colorectal cancer) from healthy controls, according to Zhang et al. (2013). The miRNA levels in the plasma of ovarian and prostate cancer patients have been evaluated and associated with disease progression (Kashyap et al. 2018). For cervical cancer identification, the blood miRNAs screening may be a novel testing technique in health care, as per a systemic study (Pardini et al. 2018). In vivo and in vitro studies have demonstrated that cell-free miR-373 and 520c are the indicators for metastasis in breast and prostate cancer (Eichelser et al. 2014; Huang et al. 2008). Similarly, increased blood levels of miR-221 have been associated to tumor metastasis in individuals with renal, lung, and pancreatic cancer (Li et al. 2018; Teixeira et al. 2014). Several studies have also shown a link between cell-free miRNA expression assessment and tumor progression, proliferation, and therapeutic response in breast cancer (Li et al. 2015; Al-Khanbashi et al. 2016).

6.7 Challenges in Identification and Discovery of Predictive Biomarkers

Identification of predictive biomarkers is a challenging process. One of the challenges associated with identifying a predictive biomarker for immune checkpoint blockage therapy is the lack of systematic technique for collecting tissue before, during, and after the therapy. There is a lack of clinical models available for the assessment of these biomarkers. One of the other issues with these markers is that a single marker cannot be suitable for patients due to the complexity of the immune system of every individual (Lei et al. 2021).

The reality is that developing new predictive biomarkers for personalized therapy is a tedious and challenging process fraught with failure. This should be unsurprising to any researcher since it reflects the complexities of pharmacological development process. This is a method with a high failure rate and high cost. It necessitates a multistep validation procedure during which a large supply of candidates comes, but candidates are usually rejected at each level. Adopting comparable procedures and

criteria to the one used in drug discovery will lead to a higher number of clinically significant biomarkers. Biomarker development researchers must acquire knowledge from the drug development process since it offers useful schema and aids in preventing much more costly errors on the route to enhanced patient care (Hewitt et al. 2007).

6.8 Conclusion

The modern age of precision cancer medicine has begun, with the groundbreaking possibility of personalizing almost every new or current cancer drug. Predictive response-specific biomarkers can now be identified and tested in the laboratory, thanks to the incredibly efficient and reliable technologies that are currently available. Following that, the hypothesis would be tested in the scope of clinical disease. Biomarkers discovered by robust experimental research and validated in targeted, well-designed clinical trials would make for more successful clinical growth, with a lower drug-attrition rate as a result. The benefits to cancer patients would be immense due to the approval of much more effective and less toxic treatments. It is now possible to associate biomarker expression with disease development, define a biomarker "code," and continuously customize therapy to optimize patient gain. In effect, the long-desired dream of treating cancer as a chronic illness, with clinical decisions driven by an insightful predictive biomarker "code," has actually become a possibility. A large-scale concerted campaign to provide biomarkers that advise on drug responsiveness, which are then implemented in the cancer center as comprehensive companion diagnostics, provides us with a rare and unparalleled ability to deliver customized cancer therapy on an ongoing and appropriate basis.

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Biomarkers in Cancer Survival and Drug Resistance

7

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Abstract

Cancer survival and resistance biomarkers are biological molecules which are distributed in different tissues or fluids of the body, they can be considered as indications of abnormal processes pointing toward cancer cell growth and drug resistance. These biomarkers have attracted great attention in all aspects of cancers like screening for primary cancer, patient assessment, estimation of disease risk, differentiating benign tumors from malignant one, prediction, prognosis determination in cancer-diagnosed individuals, and assessing the disease status by either investigating the progression or unveiling drug response to treatment. Currently, numerous biomarkers have been explored on the basis of their applications and molecular changes, among them, drug resistance and treatment response biomarkers which consist of predictive and prognostic markers have important role in drugs selection and improving patient's survival rate. Among the various biomarkers, higher expression of specific microRNAs (miRNAs) have been detected as potential lead biomarkers, which predicts the survival rate in several types of human cancer and drug resistance. Tumors are highly adaptable and the activation of survival signaling pathways and the inactivation of downstream death signaling pathways can also lead to drug resistance and cancer cell growth. Similarly, epigenetic changes and the effect of cancer cells in microenvironment have also been recognized as vital players to drug resistance. Moreover, failure of treatment strategies has been ascribed to the presence of cancer stem cells, which are highly resistant to different treatment approaches. Furthermore, the increasingly recognized molecular and genetic

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heterogeneity that is present in many tumors is another major problem that can contribute substantially to resistance and cancer survival.

Keywords

Cancer · Cell survival · Biomarkers · Drug resistance · Prognosis · MicroRNAs

7.1 Introduction

A cancer biomarker is a chemical that can be used to detect the presence of cancers in a person, these chemicals are produced by certain body reactions to the existence of malignancy or other complications, which could help in different conditions such as tumor prediction and identification, epidemiology, genetic, epigenetic, proteomic, glycemic, and imaging. Such indicators should ideally be tested by not involving the introduction of instruments in the body for obtaining body fluids such as urine and blood (Calzone 2012; Herceg and Hainaut 2007; Mishra and Verma 2010). There are numerous obstacles while taking biomarker analysis into the therapeutic setting, a variety of indicators have been recognized, for example AFP (Hepatic cancer), BCR-ABL (Chronic Cancer of Blood), BRCA1/BRCA2 (Breast/Ovarian Carcinoma), BRAF V600E (Lymphoma/bowel Carcinoma), CA-125 (Ovarian Tumor), CA19.9 (Cancer of Pancreas), CEA (Colon Cancer), EGFR (Non-small-cell lung cancer), HER-2 (Breast Cancer), KIT (Stromal Cancer of Gastrointestinal tract), PSA (Prostate-Specific Antigen) (Glandular Cancer), S100 (Carcinoma), and a number of other genes have been identified and already been used in patient care (Rhea and Molinaro 2011; Ludwig and Weinstein 2005). Similarly, mutant proteins which have been derived from an existing tumor discovered by Selected Reaction Monitoring (SRM) method is the topmost indicator of malignant tumors, hence most of the malignancies been estimated as 40% of the total cases could be successfully treated if early diagnosis is made (Wang et al. 2011).

Even though numerous effective biomarkers have been discovered so far, but the advances in biogenetics and bioinformatics promises to unveil potential biomarkers that can alter molecular biology's application to human disease. Owing to the specific link of genetic alteration in tumor cells, biomarkers are leading the way in cancer research. As a result, DNA-based biomarkers are already being used in everyday patient management which are highlighting the value of proper diagnostic testing. Furthermore, cancer treatment has disclosed the disease complications, which could be thoroughly investigated using proper biomarkers, disease categories, disease development, and the multistep procedure of tumor therapy. Similarly, risk evaluation, diagnosis for initial phase illness, identification and localization of disease, disease classification and diagnosis, and screening for disease recurrence for those who are no longer in treatment, these are all different scenarios for cancer diagnosis based on cancer biomarkers (Arm 2017).

7.2 Role and Uses of Biomarkers in Cancer Cell Survival

7.2.1 Risk Assessment

Cancer biomarkers, particularly those linked to hereditary abnormalities or epigenetic modifications provide a quantifiable technique to predict when people are prone to certain types of cancers. Predominant cancer biomarkers include mutations in the nucleic acids KRAS, tumor protein TP53, epidermal growth factor receptor, protein-tyrosine kinase, esophageal, hepatic, and pancreatic carcinoma. Moreover, methylation of cancer inhibitor proteins such as p16, cyclin-dependent kinase inhibitor 2B, p14ARF for brain tumor, hypermethylation of MYOD1, CDH1, and CDH13 for ovarian cancer, hypermethylation of p16, p14, and RB1 for oral carcinoma are examples of potentially predictive biomarkers (Verma and Manne 2006).

7.2.2 Diagnosis

Cancer biomarkers can also be used to narrow down cancer diagnosis process, this is especially true when it comes to determining whether cancers are elementary or secondary. To determine this differentiation, scientists can compare the nucleic acid changes identified in cells from the main cancer location to those present in cells from the metastatic tumor area. If the changes are the same, the metastatic cancer is considered secondary; if the changes are not similar, then the cancer is considered a separate elementary cancer (Lapin et al. 2018). Furthermore, after the death of tumor cells several markers still present in the blood and other body fluids in such a way that the patients have significant quantities of circulating tumor DNA (ctDNA). These markers can be seen in body fluids like sputum, and blood of patients (Li et al. 2019). In view of the great biological homogeneity of cancer, the prospect of discovering a useful indicator for early-stage cancer detection has been underway (Dragani et al. 2020).

7.2.3 Prognosis and Treatment Predictions

Apart from disease diagnosis, biomarkers are also used in tumor therapy and investigation of different stages, which are performed after an individual has been discovered with a tumor. Similarly, biomarkers are also used to determine the stages of malignancy along with its possibility of reacting to a particular therapy. This is partly related to the fact that cancers displaying specific biomarkers may respond to treatments based on the presence or expression of that targeted biomarker. Higher concentration of metallopeptidase inhibitor 1 (TIMP1) has been linked to more combative forms of plasma cell malignancy which is a typical example of predictive biomarkers (Terpos et al. 2010), breast cancer patients with higher expression of estrogen receptors (ER) or progesterone receptors (PR) have a greater overall survival rate (Harris et al. 2007). Particularly, amplification of the HER2/neu gene

which indicates that breast cancer possibly reacts to Herceptin therapy and alteration in exon 11 of the proto-oncogene c-KIT, which indicates that a gastrointestinal stromal tumor (GIST) will possibly react to imatinib treatment and EGFR1 tyrosine kinase zone mutations, this biomarker indicates that whether or not a patient's squamous cell carcinoma will react to gefitinib or erlotinib therapy (Herbst et al. 2005; Lynch et al. 2004).

7.2.4 Pharmacodynamics and Pharmacokinetics

Biomarkers which play their role in malignancy can also be used in diagnosis and to figure out what kind of treatment is best for a specific individual who is suffering from cancer (Sawyers 2008). Some patients alter the molecular arrangement of medications due to variances in their genetic makeup, meanwhile reduced drug metabolism can sometimes lead to harmful situations when large quantities of the medication accumulate in the body. Meanwhile, screening of biomarkers can help with drug-dose recommendations particularly in cancer therapies, such as enzyme thiopurine methyltransferase is encoded by a gene thiopurine methyl-transferase (TPMP) that alter metabolism of certain anticancer drugs and could be an important biomarker in this area (Karas-Kuzelicki and Mlinaric-Rascan 2009). Hence, patients with thiopurine methyl-transferase gene modification are incapable to digest higher doses of mercaptopurine which is a medication used in the treatment of blood cancer, the reduced metabolism of mentioned anticancer drug results in deadly reduction in leukocytes count which leads to several complication. To address the affiliated side effects, it is advised that individuals with thiopurine methyl-transferase gene modification should be given small doses of mercaptopurine (Relling et al. 1999).

7.2.5 Treatment Response Monitoring

Cancer biomarkers can also be used to track how effectively a treatment protocol is working. In this regard, a lot of investigations have been done to know the potential biomarkers having ability to significantly reduce patient care costs, as image-based diagnostics like computed tomography scans and magnetic resonance imaging which are currently used for observing cancer levels are quite expensive (Schneider et al. 2012). Similarly, the protein biomarkers like S100-beta have received a lot of attention as a biomarker for evaluating the response of melanotic carcinoma. Melanin producing cells which are responsible for black color in our skin also produce large levels of these S100-beta protein in such melanomas which are proportional to the number of cancer cells. As a result, lowered amount of calcium-binding protein (S100-beta) in the body fluid of such people is linked to good treatment response (Harpio and Einarsson 2004). Similarly, apoptotic tumor cells can produce biological compounds such as cytochrome c, nucleosomes, cleaved cytokeratin-18, and E-cadherin which have been reported in additional laboratory studies. Apart from these biomarkers other macromolecules have been detected which freely

circulate in body fluids during cancer treatment, suggesting that they could be used as clinical indicators to monitor the ongoing treatment (Relling et al. 1999).

7.2.6 Recurrence

Malignancy biomarkers may potentially aid in cancer recurrence prediction and surveillance. To estimate the recurrence of breast cancer, a specific type of assay such as Oncotype DX breast cancer assay is used to know about the recurrence of such cancers. This test is usually recommended for the patient with initial-stage (Stage I or II) invasive breast cancer who are taking hormones for their treatment. The tumor cells are assessed using biopsy and a cluster of 21 genes are examined. These test's results are expressed as a recurrence score, which reflects the likelihood of recurrence in next 10 years (Lamond et al. 2013; Biroschak et al. 2013).

7.2.7 Developing Drug Targets

Despite its widespread use in cancer therapy, the application of these biomarkers has been extended to anticancer drug discovery and development. Researchers have discovered the Philadelphia chromosome, which is genetic abnormality in chromosome 9 and 22, that is widely common in the chronic myelogenous leukemia patients. Mutation in these chromosomes has led to the formation of BCR-ABL gene, also known as fusion gene. For a long time, the BCR-ABL gene was merely considered as a biomarker to classify various types of leukemia, meanwhile Imatinib was developed as a potent medicine that efficiently blocked this protein and dramatically reduced the generation of cells with the Philadelphia chromosome (Moen et al. 2007; Lemonick and Park 2001).

7.2.8 Surrogate Endpoints

Surrogate endpoints is another interesting area of biomarkers usage, where biomarkers are utilized as substitute to replace the effects of medicine on over whole cancer cells growth. Especially, using verified biomarkers would eliminate the need for cancer biopsies and lengthy and costly clinical investigations to evaluate whether a current medicine was effective. Currently, quality of treatment which reflects efficacy of medicines is assessed by observing that whether the disease progression slows down in humans which could also extend the life span. On the other hand, potential biomarkers substitutes could unveil about the pharmacokinetic and pharmacodynamic properties of the proposed drugs and save a lot of time, energy, and money by eliminating unsuccessful drugs from the development before clinical investigation (Cohen and Khuri 2003).

Circulating tumor cells (CTCs) and circulating miRNAs are the two promising biomarkers that have attracted great interest as substitute markers (Lu et al. 2013;

Balic et al. 2013; Madhavan et al. 2012; Redova et al. 2013). These biomarkers have direct relationship with the number of cancer cells present in the blood, so in this way they can serve as a substitute indicator for cancer growth and survival. Currently, several obstacles still exist in the detection and monitoring of these circulating tumor cells and miRNAs however, latest technologies and advanced research is needed that could solve these issues (Joosse and Pantel 2013; Hou et al. 2013; Dhondt et al. 2019). All cancer biomarkers do not have to be cancer-specific, some of the biomarkers detected in the blood could be used to assess abnormal proliferation of cells, which are detected by performing blood tests. Having regular blood tests, cancer biomarkers could be easily detected and diagnosed at early stages which could be a nice strategy to properly treat the specific disease at early stage and stop its spread at the right time.

Different types of cancers have nonspecific determinant in the form of neutrophil-to-lymphocyte ratio. This ratio examines the functionality of important components of immune system, both of these components; neutrophil and lymphocytes are important players in inflammatory reactions, hence higher concentrations of these markers have been reported in malignant cancers. Furthermore, the basic fibroblast growth factor (bFGF) is a cell proliferation protein, this protein is very active in the presence of cancerous cells which undergoes rapid multiplication which results in rapid proliferation and cancer cells survival. Interestingly, recent studies have reported that anti-bFGF antibodies are promising agents to treat different types of rapidly growing cancers. In addition, cell proliferation and growth are also increased by insulin-like growth factors (IGF-R), so it is predicted that they could have a possible role in preventing apoptosis, or programmed cell death (Yu and Rohan 2000).

7.3 Drug Resistance

Cancer drug resistance is a condition in which a tumor becomes resistant to the chemotherapeutics that further leads to cancer cells' survival and ultimately death of that individual. This resistance against the anticancer medicines comes from several factors such as increased efflux of targeted medicines, genetic modification, and other diverse cellular and biological processes (Wang et al. 2019). Drug resistance refers to a medication's reduced effectiveness in treating a disease or condition, this word is used in the context of pathogens or tumors that have "acquired" resistance (Alfarouk et al. 2015; Davies and Davies 2010). Because of heterogeneity, malignancies tend to become increasingly heterogeneous throughout the time, the tumor mass may have a variety of cells with different biomolecular impressions and altered responses to the ongoing treatment. This heterogeneity could lead to an uneven dispensation of genetically dissimilar cancer-cell subgroup across and inside disease sites (spatial heterogeneity), and time-related fluctuations of genomic makeup of cancer cells (temporal heterogeneity). Because tumor heterogeneity fuels resistance against treatment, so a precise estimation of cancer heterogeneity is critical for the evolution of successful treatment. Multiregional sequencing,

single-cell sequencing, autopsy sample analysis, and liquid biopsy analysis are all new technologies that have the ability to analyze the complex clonal architecture of malignancies (Dagogo-Jack and Shaw 2018).

7.3.1 Types of Drug Resistance

7.3.1.1 Intrinsic and Acquired Drug Resistance

The drug resistance during cancer treatment can be classified as either intrinsic or acquired one depending on when the concerned resistance develops. Particularly, the intrinsic resistance is present even prior to medication therapy and has no relationship with cancer therapy, whereas acquired resistance develops after treatment that could be due to the drugs used during treatment, both types of resistances prevail in 50% patients each, who are suffering from different types of cancer resistance (Lippert et al. 2008; Kelderman et al. 2014; Wang et al. 2019).

7.3.1.2 Intrinsic Resistance

Intrinsic resistance is defined as the inborn resistance that exists prior to the use of anticancer medicines and the drugs have no role in imitation or development of intrinsic resistance. This type of resistance causes decrease in the potency of the targeted anticancer drugs, which could be either caused by: (1) preexisting genetic mutations which can lead to decreased drug response such as in the case of triple breast cancer resistance is developed against the targeted drugs, (2) tumor heterogeneity which include preexisting unresponsive subpopulations like cancer stem cells are chosen based on drug therapy. Intrinsic resistance is caused by a variety of factors: (1) preexisting or inherent genetic mutations in most of the cases causing cancer cells to be less sensitive to both chemotherapy as well as target medicines, e.g., triple negative breast cancer cells, (2) Heterogeneity of cancers which include medication treatment option preexisting unresponsive subpopulations, such as cancer stem cells, resulting in decline in later stages of treatment strategy, (3) stimulation of intrinsic mechanisms involved in environmental toxin defense (such as anticancer drugs). Because of genetic mutations in genes involved in proliferation of cancer cells and/or program cell death, in cancer cells intrinsic drug resistance may be present before therapy. For example, HER2 overexpression has been linked to a poorer result of cisplatin treatment in patients with gastric carcinoma (Wang et al. 2019; Huang et al. 2016). The increased expression of the HER2 gene results in increased level of snail which is a transcription factor causing a morphological shift similar to the epithelial-mesenchymal transmission (EMT), making neoplastic cells to be more resistant. Moreover, individuals who were snail/HER2 double positive had a poorer survival rate than those who were just single positive or double negative genetically.

Snail and Slug transcriptional repressors were discovered to be involved in EMT as well as resistance to apoptosis induced by a self-renewal mechanism and p53 (Kurrey et al. 2009; Wang et al. 2019). Cancer stem cells (CSCs) become much resistant to chemotherapies as well to radiations as a result of the second and third

activities. Resistant cells were also discovered to be more mesenchymal in nature (Witta et al. 2006; Sayan et al. 2009; Wang et al. 2019). Intrinsic drug resistance is linked to EMT and CSCs by these concurrent alterations.

Relapse after chemotherapy may also be caused by preexisting resistant subpopulations in malignancies. The occurrence of intra-tumoral genetic variability in primary cancers predates clinical intervention, according to a growing body of research (Burrell et al. 2013; Turner and Reis-Filho 2012; Kreso and Dick 2014). As majority of tumor cells are responsive to the medicine as a result patients would initially respond to treatment. However, after pharmacological therapy, resistant subclones would multiply and induce recurrence (Kuczynski et al. 2013; Greaves and Maley 2012). Because the tumor shrinks first after treatment and the resistance appears to be gained as a result of therapy, intrinsic drug resistance is sometimes confused with acquired resistance. CSCs are a type of cancer cells that have the ability to self-renew and differentiate, and they play a key role in tumor genesis and progression (Frank et al. 2010). They've been linked to chemotherapeutic drug resistance in a variety of cancers including glioblastoma, gastric carcinoma and leukemia (Viale et al. 2009). To combat medication resistance, it may be necessary to use a combination of therapies that target both CSCs as well as majority of tumor cells. Intrinsic mechanism activation, which is utilized as a shield against environmental contaminants, together with anticancer treatments, can diminish a drug's therapeutic effects. The ATP binding cassette or shortly ABC, transporter-mediated drug efflux and the glutathione (GSH)/glutathione *S*-transferase system, which operate to minimize cellular drug aggregation and detoxify cancer cells treated with drugs are two examples of these defensive mechanisms (Gillet et al. 2012; Traverso et al. 2013).

7.3.1.3 Acquired Resistance

Acquired resistance is defined as a steady decrease in a drug's anticancer activity after treatment, that can be caused by a variety of factors, including: (1) Activation of a second proto-oncogene which transforms into a new emerging driver gene, (2) Mutations in drug target or changes in its expression and (3) alterations in the tumor microenvironment (TME) after treatment. When new mutations are generated in target proteins or their level of expression changes, cancer cells can develop resistance to targeted treatments. The mutation of threonine 315 to isoleucine (T315I) in the BCR-ABL kinase domain is an excellent example of secondary mutations within the target kinase. The BCR-ABL which is a target of imatinib, a tyrosine kinase inhibitor (TKI), is routinely practiced in the treatment of chronic myelogenous leukemia, however about 20–30% of individuals will develop post-treatment resistance or recurrence (Quintás-Cardama et al. 2009). A mutation point T315I in the tyrosine kinase protein fusion is one cause of resistance (Quintás-Cardama et al. 2009; Jabbour et al. 2013; Kimura et al. 2014). The removal of hydrogen bond required for imatinib to bind to the ATP-binding region of BCR-ABL, occurs when threonine 315 is changed to isoleucine, resulting in dramatically reduced therapeutic efficacy.

Drug resistance can also be achieved as a result of TME dynamic changes during treatment. Crosstalk lies between tumor cells and their tumor microenvironment throughout malignancy and the development of resistance. The interaction is aided by exosomes generated by cancer and stromal cells. Researchers discovered that cancer cells and tumor associated macrophages in the TME communicated through exosomes are generated by cancer cells and carry specific miRNAs. NBL cells secrete exosomic miR-21 in cisplatin-treated neuroblastoma (NBL) tumors, which causes TAMs to create exosomic miR-155, which silences the TERF1 gene in NBL cells. Reduced expression of the TERF1 protein, which inhibits telomerase resulting in enhanced activity of telomerase and chemotherapeutic resistance. As a result, drug resistance may be facilitated via exosomic miRNA exchange in TME between stromal and tumor cells.

The resistance mechanisms (intrinsic and acquired) outlined above might coexist throughout the growth and treatment of tumor. Acquired drug resistance can have completely distinct mechanisms than intrinsic drug resistance. It could also be the result of a selective growth of innate drug resistance. The extent of intrinsic drug resistance defines a cancer cell's sensitivity to a specific medication. To eliminate any preexisting drug resistance, genomic as well as other biochemical investigations ought to be undertaken prior to the creation of drug therapy plan. Following the development of acquired drug resistance, therapeutic strategies must be changed consequently. One important goal of medication therapy must be to successfully inhibit or slow down the tumor growth while avoiding the development of acquired, or at the very least unmanageable acquired drug resistance. Ideal pharmacological therapeutic strategy must consider preventing or delaying acquired/adaptive drug resistance (Wang et al. 2019; Holohan et al. 2013). Some cancers can quickly become resistant to targeted treatment. For drug researchers, the goal is to predict these consequences and reduce the hazards by selecting new drug targets for patients who do not respond to targeted drug therapy.

7.3.2 Role of MiRNAs in CRC Drug Resistance Regulation

Reduced intracellular drug accumulation, enhanced DNA damage repair, reduction in apoptosis, altered oncogenes expression and tumor suppressors, among other factors, can all contribute to drug resistance. MiRNAs are closely implicated in these processes and so affect the drug resistance developments, including resistance to 5-FU, oxaliplatin, and EGFR-targeted treatment, the latter of which is extensively researched. Autophagy is expected to increase chemoresistance by boosting cellular energy generation, making it a key mechanism of cancer cell chemoresistance. By assisting tumor cells in surviving under metabolic and therapeutic stress, autophagy has emerged as one of the most chemotherapy resistant mechanism. In vitro and in vivo, miR-22 suppressed autophagy and induced apoptosis, increasing the susceptibility of CRC cells to 5-FU therapy. B-cell translocation gene 1 (BTG1) has been recognized as a novel target of miR-22, with the potential to reverse miR-22-induced autophagy suppression. Thus, by BTG1 posttranscriptional suppression,

miR-22 could serve as a key switch between autophagy and apoptosis to modulate 5-FU sensitivity (Zhang et al. 2015a). The stimulation of the PI3K/AKT pathway by the miR-204/HMGA2 axis regulated tumor cell resistance to 5-FU in HCT-116 and SW480 colon cancer cells. These findings suggest that the miR-204/HMGA2 axis is involved in colon cancer cells' resistance to 5-FU (Wu et al. 2016).

7.3.2.1 MicroRNAs as Drug Response Noninvasive Biomarkers in CRC

Circulating nucleic acids provide a noninvasive means of detecting cancer early, assessing prognosis, and predicting medication response. In blood, circulating miRNAs are stable and repeatable, making them interesting targets for research (Chen et al. 2008; Mitchell et al. 2008). miRNAs have recently been reported in human plasma or serum has recently been reported and has gained key attention for biomarker development (Slaby et al. 2009). We have compiled a list of miRNA biomarkers present in blood for CRC patients' responses to 5FU/oxaliplatin chemotherapy and EGFR target specific treatment.

7.4 Biomarker's Assessment Methods

Tumor biomarkers rapidly fluctuate during cancer growth or treatment, it is very important to develop invasive methods to assess drug effects. Blood collection has become a popular method for academics and physicians to measure surrogate endpoints (Twomey et al. 2017). While genotyping cancers, RNA and miRNAs can all be used, and they are being studied as prognostic biomarkers. The EGFR Mutation Test v2 is utilized to detect EGFR exon 19 deletions or exon 21 L858R replacements in NSCLC patients undergoing Tarceva medication (MacFarlane and Murphy 2010; Ma et al. 2012). When circulating miRs are combined with proven predictive biomarkers, real-time detection of emergent drug resistance is possible. miR-210, miR-125a-5p and miR-125b, in breast cancer patients, have been found to be associated with HER2 status that may be used as a less invasive predictive biomarker for HER2 targeted therapy (Twomey et al. 2017).

Other comprehensive systems-level analytics, such as full-scale proteomics, phenotypic alterations, spatio-temporal regulation of oncoproteins and the role of TME and the immune system, should be included in biomarker research and development. Monitoring PD-L1 levels in the tumor microenvironment could contribute to the development of immune and target specific therapies and serve as a secondary biomarker for emerging drug resistance (Twomey et al. 2017). Neither PFS nor OS were linked with other biomarkers (ABCB1, ABCC1, p53, cyclin E, and AKT2) (Kim et al. 2012). RAS, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), and BRAF, have all acquired prominence as chemotherapy predictors and have been used to establish criteria for combined treatment with anti-epidermal growth factor receptor drugs (Therkildsen et al. 2014). Serum levels of carcinoembryonic antigen (CEA) tumor markers and carbohydrate antigen 19-9 (CA19-9) are often used as predictors in colorectal cancer; although, the status of serum CEA and CA19-9 concentrations in metastatic colon

cancer (MCC) patients as helpful survival predictors is still uncertain. Circulating tumor cells (CTCs) are also regarded as predictive predictor, however, due to the high cost and difficult methodology, measuring CTC levels is not now widely employed. Serum levels of the tumor markers CA19-9 and CEA are the most broad and cheapest chemotherapy predictors (Therkildsen et al. 2014; Taniguchi et al. 2015).

According to Adam et al., increased blood CA19-9 levels following chemotherapy in patients with ineradicable recurring colon cancer were associated with shorter recurrence-free and overall existence (Hashizume et al. 2019; Adam et al. 2004). Sakamoto et al. found that high blood CA19-9 levels after chemotherapy reduced recurrence-free and overall survival, while high serum CEA levels after chemotherapy was a major recurrence risk factor (Sakamoto et al. 2015; Hashizume et al. 2019). In patients with colon cancer and unresectable liver metastases found that serum CEA levels of 100 ng/mL and CA19-9 levels of 100 U/L before chemotherapy were predictive markers for poor prognosis (Hashizume et al. 2019; Mitsuyama et al. 2012). miRNAs are involved in a variety of biological processes in cancer, including tumor initiation, tumor development, and drug resistance, and can operate as oncogenes or tumor suppressors (Xu et al. 2015; Fang et al. 2015; Li et al. 2015; Rossi et al. 2010; Zhang et al. 2007).

A vast number of miRNAs dysregulation have been found in CRCs, and few of them have been connected to anticancer therapy response via drug transport, drug metabolism, DNA damage response, or cell death regulation (Zheng et al. 2010). Notably, a subclass of miRNAs could be used as noninvasive biomarkers in the circulation as potential predictive indicators for medication response. Furthermore, improved RNA technology ensures miRNA-based therapies to be a unique therapeutic option for treating CRC treatment resistance (Zheng et al. 2010).

7.5 Therapy-Related Biomarkers

Acquired/adaptive resistance to treatment, which develops after chemotherapeutic drugs exposure and contributes to MDR and recurrence, is one of the most significant constraints in children ALL (chALL) treatment. The discovery of biomarkers linked to medication resistance could lead to new methods in targeted therapy and better patient outcomes. This section briefly introduces therapy-related biomarkers (Aberuyi et al. 2020). The poor treatment responses, EFS, and OS (218 BCP-ALL and 47 T-ALL) are closely associated to TP53 tumor suppressor gene mutation/deletions. Utilizing direct sequencing and multiplex ligation-dependent probe amplification in 23 matched samples, changes in the TP53 gene were found in relapsed patients after treatment (Aberuyi et al. 2020). DNA studies of paired chALL samples at relapse, diagnosis and complete remission (CR) found that several missense mutations are more common in relapsed patients following treatment as compared to those that are at diagnosis or complete remission. RGS12, LPHN1, PRMT2, CAND1, USP7, NIPSNAP1, CBX3, TULP4, COBRA1, SCARF1, SDF2, FBXO3, DPH5, NEGR1, SMEK2, NT5C2, MIER3, DOPEY1, NT5C2, and ZNF192 genes

all have mutations. RNA sequencing of ten matched cases revealed that unique somatic mutations in the NT5C2 gene are acquired after relapse but not at diagnosis. The enzymatic activity of the protein cN-II, a 5′-nucleotidase enhanced with these mutations and also caused resistance to nucleoside analogue treatments in an additional 61 relapsed individuals, according to enzyme analyses (Aberuyi et al. 2020).

According to a study of microRNA expression on paired diagnosis/relapse samples (11 B-ALL and six T-ALL patients), some of the miRNAs are expressed distinctly at various disease stages. MicroRNAs like miR-23a, miR27a and miR-223, are examples of miRNAs whose downregulation occurs at diagnosis, recovered at complete remission, and again downregulated at relapse. On the other hand, certain miRNAs, for example, miR-181a, miR-181b, miR-128b, and miR-708, during diagnosis and relapse, show overexpression. These miRNAs play important roles in leukemogenesis and medication resistance after treatment (Staal et al. 2010). Methylation study of certain genes revealed hypermethylation of O6-methylguanine-DNA methyltransferase (MGMT) and p16 in eight patients having B-ALL and one with T-ALL, both at diagnosis and relapse. On the other hand Retanoic acid receptor beta (RARB) was hypermethylated during the time of relapse that is thought to be linked to chALL progression and should be followed during the treatment (Aberuyi et al. 2020). When comparing matched diagnosis/relapse childhood BCP-ALL samples, high-throughput analysis indicated more common somatic deletions in the KZF1, VPREB1, NR3C1, and EBF1 genes in the relapse stage than at the disease beginning. Furthermore, during relapse, BCP-ALL had a considerably higher hypermethylated genome than T-ALL. Only relapsed chALLs, not those at the outset of the disease, had promotor hypermethylation, which resulted in downregulation of the tumor suppressor genes CDKN2A, PTPRO, and CSMD1. Furthermore, other genes like FANCD2, CENPM, OBSL1 and FOXM1 were exclusively expressed after relapse, not when the pediatric BCP-ALL was diagnosed (Hogan et al. 2011).

7.5.1 VEGF/VEGFR-Targeted Therapy

Angiogenesis is a process that plays a key role in tumor development and metastasis, making it a viable therapeutic option for cancer treatment (Warren et al. 1995). The vascular endothelial growth factor (VEGF) is a key pro-angiogenic factor, and inhibiting the VEGF pathway for cancer treatment, including mCRC, has been extensively used in clinical practice (Zerbini et al. 2008; Zhang et al. 2015b). The most widely used VEGF-A antibody is bevacizumab, a humanized monoclonal antibody.

7.5.2 Genetic Determinants as Susceptibility Biomarkers

The genes capable of identifying patients with high risk of specific cancer, represent another set of biomarkers. A scientist demonstrated that nonsense vs. missense Protein patched homolog 1 (PTCH1) alterations, correlated with specific protein profiles particularly in nevoid basal-cell carcinomas syndrome (NBCCS), has been designated in fibroblast conditioned media among patients with NBCCS, peculiarly during overexpression of matrix metalloproteinases 1 (MMP). Genetic elements are applicable for the progression of disease to cancer as well as for disease of pathogenic origin (Buonaguro et al. 2014; Duffy 2004, 2001).

7.6 Guidelines for Tumor Biomarkers

7.6.1 Alpha-Feto Protein

A glycoprotein (70 kDa), homologous to albumin found in serum. Due to glycosylation up to varying levels this protein exhibits micro heterogeneity. AFP is more fucosylated produced by malignancies than that produced by normal tissues. Existing assay does not variate between different forms. Mainly limited to three malignancies:

1. Hepatocellular carcinoma (HCC)
2. Germ cell tumors in testis, ovary and at some other sites
3. Hepatoblastoma (in children and extremely rare among adults) (Buonaguro et al. 2014; Duffy 2004, 2001).

7.6.2 Cancer Antigen 125 (CA125)

Mu16 is the protein detected by this antibody, physiological functions are not established yet.

Benign conditions with high levels

- Endometriosis
- Acute pancreatitis
- Cirrhosis
- Inflammatory pelvic disease
- Ascites of nonmalignant type
- Peritonitis

Malignancies with high levels
- Up to 85% of all ovarian epithelial cancer have high level of CA125 protein.

High levels in physiological states
- Pregnancy and menstruation (>100 kU/L usually) (Buonaguro et al. 2014; Duffy 2004, 2001)

7.6.3 Carcinoembryonic Antigen (CEA)

A nitrogenous substance called mucin reacting with monoclonal antibody such as 1116 NS 19-9. Biological function is considered as cell adherence. There is variation in reference range; from 0 to 37 kU/L to 0–100 kU/L. In serum, half-life is approximately 1 day, but still variation from less than 1–3 days exist.

Benign situations with high levels
- Hepatocellular jaundice (cirrhosis)
- Acute and chronic pancreatitis
- Acute cholangitis

Malignant conditions with high levels
- Colorectal carcinomas (30% approximately)
- Gastric Carcinomas (50% approximately)
- Pancreatitis, acute and chronic both

It is thought to be helpful in the diagnosis of pancreatic carcinoma (Buonaguro et al. 2014; Duffy 2004, 2001).

7.6.4 Human Chorionic Gonadotropin (hCG)

It is a Heterodimer, composed of two glycosylated alpha & beta subunits (chains). Alpha subunit chain of HCG is nearly undistinguishable to alpha chain of follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and luteinizing hormone (LH) but the beta subunit chain differs from consistent chains. Distinct 24 amino acid C-terminal extension is present. Hormone in serum is found in multiple forms (1) Intact and integral two chain of peptide and (2) Free α and β chains (3) Numerous degradation products like the β core fragment. Physiological function: to maintain and stimulate progesterone production during pregnancy in the corpus luteum. HCG Can be detected within 1 week after conception (Buonaguro et al. 2014; Duffy 2004, 2001).

7.6.5 Prostate Specific Antigen (PSA)

PSA is single chain glycoprotein of 237 amino acids with 28.4 kDa molecular weight, containing chymotrypsin-like serine protease. PSA forms complexes with α1-antichymotrypsin (PSA-ACT) (present in major quantity), α1-antitrypsin (found in trace quantity), α2-macroglobulin (not detectable by existing immunoassays), while the noncomplex free form of PSA has physiological function such as partially participate in the liquefaction of seminal fluid coagulum thus promoting the release and motility of spermatozoa (Buonaguro et al. 2014; Duffy 2004, 2001).

7.6.6 Estrogen and Progesterone receptors (ERs & PRs)

Both Estrogen receptors (ERs) and Progesterone receptors (PRs) level should be measured in all malignant breast cancers, among the two forms of ERs the ERα has more validated role, compared to ERβ. Similarly the PRs both forms dubbed PRA and PRB, both are easily detectable by immunohistochemistry assays. (Buonaguro et al. 2014; Duffy 2004, 2001).

7.6.7 Human Epididymal Secretory Protein 4 (HE_4)

Mechanisms of oncogenesis or tumorigenesis could be explained by the productivity and liberation of cancer biomarkers in cancer specific cells, blood or several body fluids. During early stage of cancer development, metastases and progression release of these molecular biomarkers get elevated. This increase of cancer biomarkers in any kind of the biological fluid can be described via three key mechanisms.

The overexpression means profuse target protein expression or genes product amplification, or augmentation of epigenetic changes, and upregulation of protein human epididymal secretory protein 4 (HE_4) are considered as key mechanisms. In ovarian cancer, protein human epididymal secretory protein 4 (HE_4) release as a result of DNA methylation. HE_4 biomarker of ovarian carcinoma shows overexpressed mechanism and can be identified in serum. Clinical evaluation of this biomarker shows that in case of endometrial, breast, and bronchial adenocarcinoma it also get overexpression. The second mechanism for elevation of cancer biomarkers could be normally applied on biological fluid serum, this includes hyper secretion of cellular proteins in serum or membrane proteins shedding. The third mechanism is angiogenesis and cell invasion, both play a critical role in cancer, each process occurs with expression of prostate-specific antigen (PSA). Generally, it is expressed by means of prostatic epithelium, however increased level of PSA takes place because of distortions of basement membrane of prostatic cell due to lymph angiogenesis and cell invasion.

Various cancer biomarkers like circulating protein targets for management of cancer has revolutionized the new era mainly with the accessibility of auspicious techniques that are used for the discovery of “omics” cancer biomarker in body

Table 7.1 Representative biomarkers in cancer survival and drug resistance

No.	Biomarker (cancerous)	Specificity (organ/cancer) type of cancer	Uses/Application
1	Prostate specific antigen	BPH/prostate	Screening, diagnosis and monitoring
2	Carbohydrate antigen 125	Ovarian	Detecting recurrence and monitoring therapy, diagnosis, prognosis,
3	Carcinoembryonic antigen	Colorectal/hepatic	Monitoring therapy, Prognosis, Detecting recurrence, Screening for hepatic metastases
4	Carbohydrate antigen 15.3	Breast carcinoma	Monitoring therapy
5	Progesterone and Estrogen receptors	Breast carcinoma	Stratification/select patients for endocrine therapy
6	HER2	Breast carcinoma	Monitoring trastuzumab therapy
7	Carbohydrate antigen 27.29	Breast carcinoma	Monitoring
8	Human chorionic gonadotropin β	Testicular carcinoma	Monitoring therapy, Detecting recurrence, Diagnosis, stages
9	Alfa-fetoprotein	Hepatocellular carcinoma	Monitoring therapy, Detecting recurrence, Diagnosis
10	Calcitonin	Thyroid carcinoma	Diagnosis and monitoring therapy
11	Thyroglobulin	Thyroid carcinoma	Monitoring
12	CA 19-9	Pancreatic carcinoma	Monitoring therapy
13	Nuclear matrix protein 22	Bladder carcinoma	Monitoring and prognosis, Screening,
14	Prostate cancer antigen 3	Prostate carcinoma	Prognostic

fluids, which may be represented as novel markers. These are extremely sensitive diagnostic techniques for the detection of early stages of cancer (Buonaguro et al. 2014; Duffy 2001) (Table 7.1).

7.7 MicroRNAs as Potential Biomarkers

The microRNAs (miRNAs) are potential biomarkers which are conserved micro noncoding RNAs, which are also known as micromanagers of gene expression. Polymorphisms in the miRNA (miR-polymorphisms) is an emerging area having promising potential to be used in the diagnosis and prognosis of different types of cancers. Recent advances in miRNA research have highlighted that deregulated miRNA genes are involved in cancer cells survival, a number of polymorphisms in different stages of pre-miRNA and miRNA binding sites have been reported to be affiliated with different types of cancers. Moreover, it has been reported that miRNA

Table 7.2 Resistance of 5-FU in CRC involving miRNAs, Upregulated: ↑; downregulated: ↓

miRNAs	Expression in resistent cells	Gene targets
miR-203	↓	TYMS
miR-218	↓	TYMS, BIRC5
miR-494	↓	DPYD
miR-27a, miR-27b	↓	DPYD
miR-21	↑	MSH2
miR-10b	↑	BIM
miR-23a	↑	APAF-1, ABCF1
miR-425-5p	↑	PDCD10
miR-139-5p	↓	BCL2
miR-200c, miR-302, miR-369	↑	MRP8
miR-519c	↓	ABCG2, HuR
miR-22	↓	BTG1
miR-204	↓	HMGA2

Table 7.3 Resistance of oxaliplatin in EGFR-targeted resistance CRC involving miRNAs

miRNAs	Therapy	Expression in resistant cells	Gene targets
miR-7	EGFR-targeted	↓	EGFR, RAF1
miR-20a	Oxaliplatin	↑	BNIP2
miR-133b	EGFR-targeted	↓	EGFR
miR-143	Oxaliplatin	↓	IGF-1R
miR-153	Oxaliplatin	↑	FOXO3a
miR-203	Oxaliplatin	↑	ATM
miR-199a-5p, miR-375	EGFR-targeted	↑	PHLPP1
miR-409-3p	Oxaliplatin	↓	Becline-1
miR-520g	Oxaliplatin	↑	P21

polymorphisms also affect the tumor characteristics in the microenvironment and cancer patient's survival. Hence, miRNAs is an important player in tumorigenesis and oncology, particularly it has wide spread usage in miRNA microarrays which has enabled the confirmation of several miRNAs as potential cancer biomarkers (George and Mittal 2010) (Tables 7.2 and 7.3).

7.8 Additional Factors Contributing to Drug Resistance and Cancer Survival

Numerous other factors, apart from the above mentioned biomarkers may also have potential role to contribute toward drug resistance and cancer survival e.g., autophagy, MDR, and regulatory genes.

7.8.1 Autophagy and Multidrug Resistance in Cancer

Drug resistance is developed after long-term cancer treatment, the kind of drug resistance which is developed against multiple drugs being used against cancer is known as multidrug resistance (MDR) that leads to tumor recurrence and cancer cell survival. Therefore, suppression of MDR is an important approach to control cancer cells survival and increase the cancer drugs effects. Similarly, autophagy, a self-degradative strategy, occurs during the treatment of MDR cancer. Autophagy has dual functions: it contributes in the development of MDR and provides shelter to the cancer cells from chemotherapeutics, on the other hand it can also kill MDR cancer cells in which apoptosis pathways are not properly working. Moreover, anticancer drugs induced autophagy could also activate apoptosis signaling pathways in MDR cells and hence reverse the MDR. Therefore, recently the autophagy-based research has attracted great attention which could suppress MDR and its related complications (Wang et al. 2019) (Table 7.4).

7.9 Challenges in Clinical Applications of Biomarkers

As biomarkers hold a crucial role at almost every level of disease, so it is important that these shall be monitored very carefully in terms of their analytical validation and clinical utility assessments prior to their use in daily clinical care. The assessment and validation of a recent biomarker is almost equal to the development and design of a new drug. Identification, authentication and launching of a novel biomarker is just as tough as developing and approving of a fresh drug. Out of 3–50% biomarkers, only 3–5% get access to clinical usage. Herein, we discuss some problems validation and application of the biomarker for clinical trials. A lot of problems are faced in the process of new drug development, including complication and biological difference to tumor response therapy which involves a complex molecular pathway with adaptive feedback and loop of cross talk. Besides the abovementioned problems, different sorts of measuring errors may be observed during the process of validation. The basic determinant which assures the efficacy of biomarkers analysis is the standard protocols application for the collection of sample and also their storage and processing. Another logistical component of the process of authentication of the biomarker data is statistical evaluation which is a tough task regarding consistency in data management, biostatistical, and bioinformatics methodologies. The statistical approach should be able to notice by chance relationship from those coming from true biological association. However, omic features (i.e., epigenetics, genomics, proteomics, etc.) and/or environmental and routine factors, may play a role in drug response. It is crucial to identify biomarkers by either with lifestyle or omic data alone, e.g., age, diet, etc., which are studied in the personalized medicine in order to get a famous phrase of pharmaceutical sciences of “right dose at the right time to the right patient.” The identification of a valid biomarker’s cohort studies is performed which involve collection of large number of samples.

Table 7.4 Resistance mechanisms of various biomarkers

Therapy (targeted)	Type of cancer	Targets	Mechanism of resistance
Bevacizumab	Colorectal cancer, NSCLC, glioblastoma and renal cell carcinoma	VEGF	Activation of alternative signaling pathways (such as IGF1R, PDGFR, FGFR or MET), Hypoxia-induced autophagy
			Induction of tumor dormancy or an increase in the cancer stem cell niche
Bortezomib	Multiple myeloma and mantle cell lymphoma	Proteasome	Mutation in the binding site of bortezomib
			Anti-apoptotic mechanism
Cetuximab	Head and neck cancer and colorectal cancer	EGFR	KRAS mutation
			EGFR-S492R mutation inhibits cetuximab binding
			Increased ERBB family signaling
Crizotinib	NSCLC	EML4-ALK	Secondary EML4-ALK mutations or rearrangement
			COT-mediated MAPK reactivation
			CD74-ROS1 rearrangement
Dasatinib	ALL and CMIL	BCR-ABL1	T315 mutation in ABL1
Gefitinib	NSCLC	EGFR	EGFR kinase domain mutations (for example, T790M)
			Epithelial-mesenchymal transition, Epigenetic mechanisms
			Increased ERBB family signaling or MET amplification
Imatinib	CML, ALL and GIST	BCR-ABL1, KIT and PDGFRa	Mutation of the target (for example, T315 in ABL1, T670I in KIT and T674I in PDGFRα) Elevated MDR1 expression
Nilotinib	CML	BCR-ABL1	BCR-ABL1 upregulation
			T315 mutation in ABL1
Trastuzumab	ERBB2-positive breast cancer	ERBB2	PTEN loss, Truncation of ERBB2, Activating mutations of PIK3CA
			Activation of alternative signaling pathways (such as IGF1 and ERBB3)
Vemurafenib	Melanoma	BRAF-V600E	Elevated BRAF-V600E expression
			Acquired mutation in KRAS, NRAS or MEK1
			Activation or EGFR, IGF1R and PDGFR β pathways

In order to find effective and reliable biomarkers, cohort studies should be applied assessing huge quantity of sample groups. At this point of view, the limited count of children involving in research studies is the main challenge to identify novel

biomarkers as there is painful or invasive operations which are involved for gathering of data for sample collection. Age-matching control finding of samples is another difficulty, which is crucial to observe values of normal reference. Besides, a small number of participants with children malignancies are not ready to participate in the trail based interventions.

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Biomarkers in Tumor Recurrence and Metastasis

8

Dilawar Khan and Mudassir Khan

Abstract

Biomolecules that are produced or secreted by tumor cells themselves or in response to tumor by other cells of body are cancer biomarkers that further define cancer at molecular level. Biomarkers can be specific cells, genes, products of genes, hormones, molecules, enzymes found in blood, urine, or tissues. Every cancer has its unique signatures in terms of biomolecules/cancer biomarkers which give precise and identifiable characteristics to that cancer. Cancer biomarkers hold specific biochemical and molecular characteristics with roles in diagnosis, screening of clinical complications, and response to therapy or treatment stratification for patients. Besides these roles, cancer biomarkers play very essential role in metastasis and recurrence/relapse of cancer as well. The focus of this chapter is to highlight cancer metastasis and recurrence with a particular focus on the role of key biomarkers involved in metastasis and recurrence.

Keywords

Biomarkers · Hormones · Metastasis · Recurrence · Diagnosis · Screening · Clinical complications

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8.1 Introduction

Cancer is the second leading cause of death and is a major public concern. In 2020, approximately 10 million deaths were reported, 19.3 million new cases of cancer were diagnosed according to GLOBOCAN (Sung et al. 2021), and this number increases every year (Cho 2007). Cancer control and its timely diagnosis, treatment, appropriate follow-up, and comprehensive use of predictable measures (cancer biomarkers) are certainly effective tools that help to reduce the burden of cancer. US Food and Drug Administration (FDA) defined biomarkers as "Any measurable diagnostic indicator that is used to assess the risk or presence of diseases" or they can be defined as "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention".

Cancer biomarkers are tools for cancer screening/early detection, diagnostic, prognostic, or can also be used to predict patients overall outcome. Recent technological advances in DNA and RNA sequencing as well as proteomics have enabled identification of predictive, diagnostic and prognostic biomarkers, also it improved more precise clinical management of cancer patients (Ilyin et al. 2004; Ansari and Andersson 2021). Even patients subpopulations can be identified by cancer biomarkers who are most likely to react to specific treatment (Goossens et al. 2015).

Metastasis describes the spread of cancer cells to distant organs and surrounding tissues from the primary tumor (Tarin 2011). During metastasis cascade, metabolism is adapted either dynamically or selectively by metastasizing cells (Bergers and Fendt 2021). Tumor cells interact with host cells and extracellular matrix during metastasis (Valastyan and Weinberg 2011).Tissue destructive macrometastases disrupt the function of organ and are also a major source of morbidity and lethality for almost all solid tumor types (Gupta and Massagué 2006; Hanahan and Weinberg 2011; Dillekås et al. 2019). Variety of cellular processes are involved with different molecular programs that regulate migration, survival, and proliferation of cancer cells during metastasis (Stracke and Liotta 1992; Nguyen and Massagué 2007; Lambert et al. 2017). Molecular changes such as proteolytic and adhesion enzyme expression as well as cellular processes influenced usually by cytoskeleton dynamics are the processes that are required by cells for migration (Tahtamouni et al. 2019). In addition, cell lines grown in culture fail to recapitulate metastatic behavior in in vivo. Although different cancers share the same process of metastatic progression, the diversity of molecular programs across (and within) cancer types necessitate the analysis of cancer-type-specific molecular and cellular programs that contribute to metastasis (Ko et al. 2021).

The stroma that surrounds the tumor, as well as the immune system effectors, hormones, blood platelets, and other humoral factors, make up the tumor microenvironment (Goubran et al. 2014).Tumor metastasis is also associated with changes in tumor microenvironment. For example, immune response to cancer cells and surgical removal of the tumor tissue can stimulate tumor recurrence and growth of the metastatic tumor in distant parts of the body (Nishikawa 2008).

The primary tumor releases a significant number of disseminated tumor cells, but only a small number of cells survive as viable seeds (Deyell et al. 2021). Primary tumor cells metastasize before diagnosis of primary cancer and can remain for years in patients after treatment. These cells have a special biology that enables them to avoid immune surveillance, form tumors, and colonize metastatic microenvironments (Ganesh and Massagué 2021). These dormant malignant cells remain undetectable, asymptomatic, occult for long time and can awaken after decades which leads to relapse/recurrence (Friberg and Nystrom 2015; Phan and Croucher 2020). Tumor dormancy and distant metastatic relapse regulation are influenced by a number of molecular factors (Werner et al. 2021). Dormant cells have self-renewal capacity, potential to escape from different treatment options, and detected more frequently in different types of cancer (Müller et al. 2005); prostate cancer (Pound et al. 1999; Amling et al. 2000), B–cell lymphoma (Davis et al. 1998; Press et al. 2001), breast cancer (Meng et al. 2004), and melanoma (Faries et al. 2013; Callaway and Briggs 1989). A subpopulation of primary tumor cells like cancer stem cells (CSCs) have been discovered that play critical role in tumor progression, proliferation, relapse (Castelli et al. 2021) and are resistant to current therapeutics, can metastasize, escape immune system, may lead to dormancy, or may be left after primary tumor excision in case of solid cancers. The role of CSCs in cancer recurrence can be viewed from a variety of angles. The development of CSCs, which retains a community of tumor cells where recurrence occurs, causes resistance to chemotherapy and radiation (Dave et al. 2012). CSCs role in cancer recurrence can be viewed from a number of perspectives. Resistant to chemotherapy and radiation, cancer cell growth is the source of a collection of tumor cells in which recurrence occurs. The development of cancer stem cell model usually helps to explain many unresolved questions, including why destruction of non-CSCs may involve little improvement in patient outcomes. The centrality of CSCs in cancer has recently been discovered, providing a conceptual basis for researchers to overcome ambiguous mechanisms in cancer pathophysiology and care also it provided new insights and objectives to oncology (Chang 2016).

Tumor metastasis also occurs “silently” during the clinical treatment process, according to a significant number of case studies. Successful cancer metastasis inhibition is difficult due to the heterogeneity of the surface of metastatic cancer cells (Hirota et al. 1996; Chen et al. 2019), for example,surface antigens are different in metastasis of the same melanoma at different locations (Zeltina and Bowden 2017; Staalsoe et al. 2004). The second reason is that most molecular chemodrugs are ineffective against metastatic tumors like in metastatic prostate cancer, resistance was observed against docetaxel–prednisone (Seruga et al. 2011).

Different therapies for different forms of cancer have been approved by the FDA. Personalized medicines can be designed for the treatment of tumor metastasis, but the problem is detection and identification of the specific biomarkers which is related to therapeutic response of tumor. Metastasis-related biomarkers of cancer patients can help identify spread, preferred metastatic sites, and probability of recurrence (Brinton et al. 2012). Profile of biomarkers vary a lot with original cancer type, metastatic site, and metastasis histotypes. In breast cancer metastasis of human,

established receptors like epidermal growth factor receptor 2 (EGFR2), progesterone receptor (PR), and estrogen receptors (ERs) are essential biomarkers to predict response to anti-HER2 and endocrine therapies in decision-making process of clinical settings (Martinez-Perez et al. 2019). Different biological components linked to tumor metastasis processes and signaling pathways are targeted by treatment methods have also been explored by researchers that include PIK3CA mutation, mTOR, PI3K, CDK4/6, BRCA1/2 germline mutation, ERBB2 mutation, positive programmed death ligand-1 (PD-L1), a high level of tumor-infiltrating lymphocytes (TILs), etc. (Dieci et al. 2020). To effectively diagnose and inhibit malignant tumor metastasis is still a major challenge and hurdle in current clinical cancer treatment (Obradovic et al. 2019; Lu and Kang 2019).

Metastasis and recurrence both are major problems in the treatment of cancer (Kong et al. 2021). Treatment failure, as well as tumor recurrence or relapse, is believed to be caused by inability of traditional therapies to completely kill all infiltrative tumor cells. In a variety of tumors CSCs which is a small subset of cancer cells, have been suggested and believed to be responsible for cancer genesis, tumor formation, metastasis, drug resistance, recurrence, escape from immune system, and ability to self-renew (Visvader and Lindeman 2008; Chen et al. 2013), and results in tumor metastasis and recurrence after treatment (Lei et al. 2021). The role of CSCs in cancer recurrence can be viewed from a variety of angles. Growth of CSCs is the reason of accumulation of tumor cells which generally leads to recurrence; this is the cause of resistance to chemotherapy and radiation (Dave et al. 2012). Existing therapies failed to offer long-lasting therapeutic responses in patients (Mehlen 2006). Despite all advances in cancer treatment, the key target for any anti-cancer strategy is tumor metastasis and recurrence (Hanahan and Weinberg 2011). A major therapeutic goal is eradicating cancer cells, that initiate metastasis and contribute to tumor growth, survival, and treatment resistance (Mimeault and Batra 2010).

8.2 Cancer Metastasis and Recurrence

After surgery of cancer patients, most common reasons of death includes metastasis and recurrence. Recurrence can be distant, regional and local, different factors like adjuvant therapy, surgical stress and trauma, and cancer malignancies determine the incidence of recurrence, severity and type (Horowitz et al. 2015). Anesthetic techniques can be a possible factor during surgery in contributing recurrence and relapse of cancer. During cancer patient's surgical treatment shorter recurrent free life has been reported in patients who received inhalational anesthetics in comparison to intravenous anesthetic propofol (Wigmore et al. 2016). The ability to detect disease early can have the greatest impact on mortality, because if any advanced treatment is possible for the patient, mortality rate can be decreased. It is very critical to prognosticate, diagnose the stage, and control treatment, as well as detect cancer recurrence, the ability to diagnose the disease early could have the largest impact on mortality. Improving detection methods for early-stage cancer in asymptomatic population is a particular challenge at present. Cancer is a persistent disease, genetic

reports show that many cancers last a decade or more before significant clinical signs appear (Yachida and Iacobuzio-Donahue 2013; Brown and Palmer 2009).

The pattern of growth is a serious consequence of patient's outcomes, almost without exception, cancers that have been diagnosed earlier and have confined organs are treated with acceptable surgical excision combined with a range of adjuvant therapies, such as radiation or chemotherapy. Although metastasis is the cause of the majority of cancer deaths, it is also the most mysterious aspect of disease (Chaffer and Weinberg 2011).

8.2.1 Cancer Metastasis

When genetically labile cancer cells adapt to a microtissue environment far from the underlying tumor, this is known as metastasis. This process includes cancer cells as well as substances that are useful for stromal tumor properties and the recruitment of traits that can regulate metastatic cell invasion (Gupta and Massagué 2006).

8.2.1.1 Mechanism of Metastatic Cascade

There are three major processes in the metastatic cascade:

8.2.1.1.1 Invasion

It occurs as tumor cells gain the ability to infiltrate neighboring tissues, passing through the extracellular matrix, and basement membrane (Yousefi et al. 2021; Reuten et al. 2021).

8.2.1.1.2 Intravasation

It involves the penetration of the lymphatic or vascular system by the motile tumor cells (Zavyalova et al. 2019; Majidpoor and Mortezaee 2021).

8.2.1.1.3 Extravasation

It involves the journey of metastatic cancer cells through the circulatory system at a secondary site to infiltrate the extracellular matrix and vascular basement membrane (Miles et al. 2008) (Fig. 8.1).

8.2.1.2 Changes in Extracellular Matrix (ECM)

To become independent of the main tumor mass and invade the surrounding stroma, metastatic cancer cells must undergo the loss of cell–cell adhesion and changes in cell–matrix interactions (Poturnajova et al. 2021; Niland and Eble 2021). These changes include the secretion of heparinases and matrix metalloproteinases for basement membrane and ECM degradation, as well as suppression/expression of proteins involved in motility and migration. Many metastatic biomarkers have been associated with the dysregulation of the ECM to promote metastasis. Within the clinic, the tight junction transmembrane protein Claudin-7 is utilized as a prognostic biomarker in the analysis of invasive ductal breast cancer and is being explored as a cancer biomarker to distinguish chromophobe renal cell carcinoma from other renal

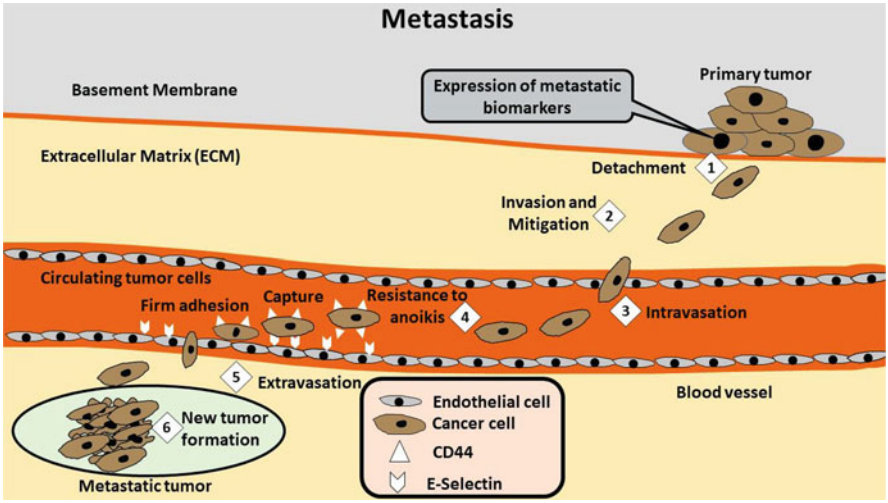

Fig. 8.1 Cancer cell metastasis

tumor subtypes (Bernardi et al. 2012; Rashed et al. 2021). A change in extracellular matrix metabolism may be involved in a variety of pathways and circulating TIMP-1 or MMP-9 levels could be excellent markers (Hansson et al. 2011).

Claudin-1 expression is used as a prognostic biomarker and potential drug target for colorectal cancer and lung adenocarcinoma cases (Chao et al. 2009; Zuo et al. 2020). Vimentin and E-Cadherin are known as prognostic biomarkers for non-small-cell lung cancer treated with erlotinib and colorectal cancer (Richardson et al. 2012; Niknami et al. 2020).

8.2.1.3 Epithelial to Mesenchymal Transition

The epithelial to mesenchymal transition (EMT) is a multi-stage mechanism wherein epithelial cells weaken their cell adhesion and polarity and become invasive metastatic cells. Wnt, TGF, and Notch are among the signaling pathways and growth factors that control this transition. These signaling pathways show crosstalk that facilitates EMT and subsequent cell invasion also Notch signatures and pathway is involved in metastasis and progression of cancer (Li et al. 2020a, b; Misiorek et al. 2021). Expression of vimentin, APC, and β-catenin may be markers of malignant transformation (Galichon and Hertig 2011). A variety of additional factors have been shown to contribute to EMT, such as hypoxia, metabolic stressors, matrix stiffness, and epigenetic and post-translational modulators. The precise contribution of each of these factors to EMT remains unknown and may vary according to each specific cancer (Fares et al. 2020). Clinically, a variety of EMT biomarkers are used in the diagnosis and prognosis of different cancer types. EMT is characterized by downregulation of E-cadherin and a connection between E-cadherin is lost, EMT has been established in a variety of cancer types (Martin et al. 2013; Nader et al. 2020). Overexpression of MUC1, MUC4, and MUC16 are all utilized in the

diagnostic and prognostic assessment of cancers, including breast, pancreatic, acute myeloid leukemia and ovarian cancer (Vergara et al. 2016; Abdelhady et al. 2020).

8.2.1.4 Angiogenesis and Lymphomagenesis

Local diffusion for removal of waste products from site of tumor and transfer of nutrients will only be sufficient for very small tumors, so the tumor must initiate angiogenesis otherwise it will fail to grow. Detached tumor cells may enter the circulatory system via blood vessels in the tumor's vicinity and spread to secondary sites. Imbalances in angiogenic and lymphangiogenic processes are thus, frequently involved in cancer and lend several biomarkers that indicate cancer progression and metastasis (Kilvaer et al. 2015), other metastatic biomarkers are given in Table 8.1.

Vascular endothelial growth factor (VEGF) has been a key therapeutic target in the development of anti-angiogenic therapies to treat a range of cancers.

Table 8.1 Biomarkers of cancer metastasis

Cancer type	Biomarker	References
Lung cancer	CA-125 and CEA	Clevers et al. (2021)
	TRAcP-5b	Yao et al. (2011)
	Angiopoietin-2	Dong et al. (2018)
	NTx	Tamiya et al. (2013)
Breast cancer	Oxidative stress-responsive kinase 1 (OSR1)	Li et al. (2020a, b)
	CAPG, GIPC1	Westbrook et al. (2016)
	Itgbl1	Li et al. (2015a, b)
	DOCK-4	Westbrook et al. (2019)
	nPAK4	Li et al. (2019)
	LPC1, PRDX4	Tiedemann et al. (2019)
	PRL-PRLR	Shemanko (2016), Sutherland et al. (2016).
	Osteopontin	Shevde et al. (2010), Rodrigues et al. (2007)
	CTX	Lipton et al. (2011)
	P1NP	Brown et al. (2018)
Adenocarcinoma of gallbladder	Nectin-2 and DDX3	Miao et al. (2013)
Colorectal cancer	Circulating exosomal miRNAs	Alves dos Santos et al. (2021)
	ECA39	Yoshikawa et al. (2006)
Prostate cancer	P1NP	Koopmans et al. (2007)
Pancreatic cancer	Long-non-coding RNAs (MALAT-1)	Cheng et al. (2018), Huang et al. (2016)
Renal cell carcinoma	C-reactive protein (CRP)	Casamassima et al. (2005)
Cervical cancer	Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)	Guo et al. (2010)

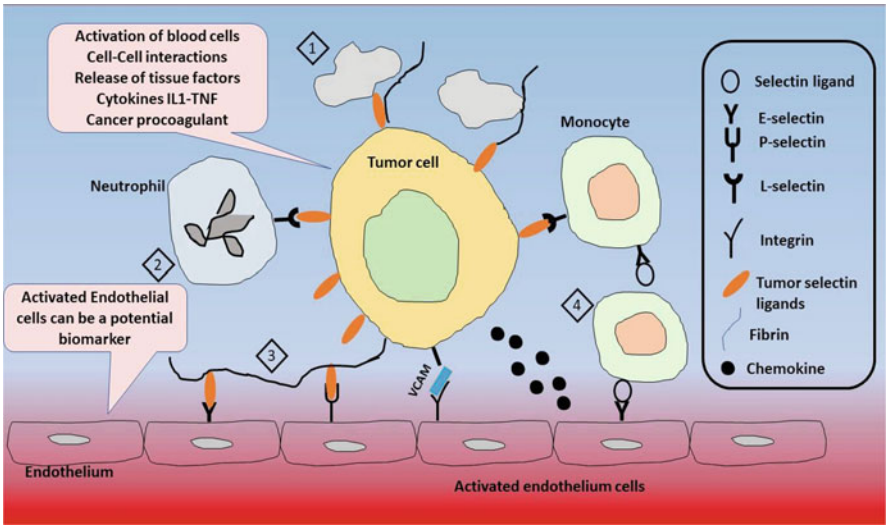

Fig. 8.2 Mechanism of endothelial cells activation via tumor cell in metastasis. (1) The aggregation of platelet tumor cells is normally mediated by p-selectin and integrins via fibrinogen and fibrin. (2) The aggregation of neutrophils, which encourage tumor cell proliferation, improves the survival of circulating tumor cells. (3) P and E selectin mediate tumor cell contact with the endothelium, and integrins facilitate tumor cell firm adhesion, as seen with VCAM-1 on tumor cells. (4) Monocytes promote endothelial cell–tumor interactions and help to initiate tumor cell extravasation. (5) Specific biomarkers in metastasis. Modified from Läubli and Borsig (2019).

Bevacizumab is a VEGF neutralizing antibody that has been approved for use in a number of cancers, including non-squamous non-small-cell lung cancer and colorectal cancer, as well as epithelial ovarian cancer (Sato and Itamochi 2012; Syed 2020). Lymphatic metastases are common, with a number of cancers first metastasizing to regional lymph nodes. Biomarkers for lymph angiogenesis and metastatic spread are being investigated, with VEGF-C and VEGF-D and VEGFR3 emerging as biomarkers for breast cancer, colorectal cancer, and non-small-cell lung cancer (Al-Rawi and Jiang 2011; Albrecht and Christofori 2011; Huang et al. 2021) (Fig. 8.2).

8.3 Biomarkers Related to Cancer Metastasis

The spread of cancer from the primary tumor to other tissues and organs is known as metastasis. This mechanism is a key factor in determining cancer morbidity and a defining feature of cancer malignancy (Gupta and Massagué 2006; Ganesh and Massagué 2021). ETNK2 overexpression in the GC promotes hepatic metastasis, likely by disrupting p53–Bcl-2-associated apoptosis. Expression of ETNK2 may be used as a therapeutic target biomarker and to predict recurrence of hepatic cancer (Miwa et al. 2021).

The prediction of tumor metastatic potential is important and could allow for personalized therapy at an early stage to better treat cancers (Parol et al. 2021; Singh et al. 2021). Accurate biomarkers of metastasis thus, represent an enormous advance in the potential cancer treatment. The use of these cancer biomarkers in the clinic could help detect initial stages of metastasis, preferred sites of cancer metastasis, and the probability of cancer recurrence (Pavlou et al. 2013; Lokshin et al. 2021), early cancer detection using miRNA biomarkers and their use as new diagnostic tools (Galvão-Lima et al. 2021).

To escape circulation and establish a metastatic tumor involves the synthesis of a number of conditions and events, such as traversing the endothelial wall and trans endothelial migration and angiogenesis. Consequently, many of the biomarkers of extravasation reflect earlier metastatic processes, such as VEGF, chemokines and cytokines, and ECM components such as heparinase and matrix metalloproteinases (Martin et al. 2013; Winkler et al. 2020). Additionally, the tumor microenvironment plays a key role in metastasis and offers a number of biomarkers for clinical utility.

8.3.1 Prostate Cancer

Prostate cancer is the most common cause of death in men all over the world (Velonas et al. 2013). As current prognostication and detection methods are limited in precision, diagnosis and treatment remain the most complex clinical challenges (Bidarra et al. 2019). Therefore, clinically significant molecular biomarkers for prostate cancer diagnosis are in fierce competition, serum measurements show that ICTP and PINP are useful in the timely identification of skeletal metastases in prostate cancer patients, also other various biomarkers have been reported for metastasis (Koopmans et al. 2007).

8.3.2 Breast Cancer

Breast cancer is uncontrolled growth of breast cells. It has different types which depend on the type of breast cells involved that turn to cancer (Aroef et al. 2020). They include lobular breast cancer, inflammatory breast cancer, mucinous breast cancer, mixed tumor breast cancer, and ductal breast cancer. Breast cancer is often diagnosed in men also (Alimkhodjaeva et al. 2020). Overexpression of osteopontin is related to cancer metastasis and has been found in melanoma, ovarian cancer, stomach cancer, colorectal cancer, lung cancer, and breast cancer (Westbrook et al. 2016). The presence of epithelial cells in the bone marrow, circulating tumor cells, HER2/ErbB2, PR, ER, and uPA/PAI1 are all potential prognostic biomarkers for breast cancer metastasis (Van De Vijver et al. 2002; Lorusso and Rüegg 2012), nucleobindin-2 (Zeng et al. 2017) and E-cadherin (Fry et al. 2013).

8.3.3 Lung Cancer

Lung cancer is the most common cancer and one of the leading causes of death worldwide. Late diagnosis is a major hindrance in lung cancer prognosis (Hoseok and Cho 2015). CircRNAs (circular RNAs) are continuously being shown to play a key role in lung cancer cell invasion, migration, and proliferation (Di et al. 2019). Lung cancer biomarkers, especially those that predict metastatic risk, are of great importance. Just a few biomarkers, such as epigenetic changes and DNA mutations can necessitate tissue from the risk site; others, including micro RNAs (miRNAs) and serum proteins, are less invasive, but may not be lung-specific (Subramaniam et al. 2013).

8.3.4 Colorectal Cancer

Colorectal cancer is a form of cancer that affects specifically the colon and rectum. Colorectal cancer is reported as the second leading cause of death in the world (Hampton et al. 2021; Bach et al. 2021). ECA39 is an influential biomarker in colorectal cancer for distant metastasis (Yoshikawa et al. 2006).

8.4 Cancer Recurrence

After the recovery period, when cancer restores, it is known as recurrence or relapse. Cancer recurrence occurs when some of your cancer cells persist despite your best efforts to get rid of cancer, or because cancer cells have metastasized to your organs at the time of resection or had circulated via vascular and lymphatic systems (Kinoshita and Goto 2021).

These cells may be in the place where cancer started at first or can be in another part of body, still it is named after the part of the body from which it originated. These cancer cells may have been dormant for a long time, but eventually begin to grow, leading to cancer recurrence. It can happen weeks, months, or even years after the first or primary cancer treatment. Hematogenous and loco-regional recurrence require careful monitoring (Sugiyama et al. 2012). Cancer recurrence can be detected biochemically with an increased level of cancer biomarkers before any radiological or clinical evidence is appeared. Cancer biomarker can be a threatening sign of cancer relapse earlier by 3–12 months prior to diagnosis. Many cancer biomarkers can be used for detection of cancer metastasis, recurrence, or monitoring therapy, i.e., in prostate cancer PSA, Ovarian Cancer Antigen 125 (CA125), and in colorectal cancer CEA (Bast et al. 2001).

8.4.1 Mechanism of Cancer Recurrence

Cancer recurrence is one among the most commonly known cancer treatment failure. This biological phenomenon can be caused by incomplete tumor eradication after radiotherapy and chemotherapy. Tumors are heterogeneous and CSCs population can be found in the tumor. Tumor cells are destroyed by radiotherapy and chemotherapy, but CSCs are resistant to these treatments and can live for years after being exposed to them.Therapeutic resistance raises the risk of recurrence and tumor re-formation also, meanwhile tumor cell metastasizes and spread into other body tissues. Tumor relapse is much less likely when radiotherapeutic and chemotherapeutic interventions are directed at CSCs (Esmatabadi et al. 2016; Spring et al. 2020)

8.5 Biomarkers Related to Cancer Recurrence

Individual's survival can be improved with early diagnosis because survival is inversely associated with the stage of the disease (Chang et al. 2011). Cancer biomarkers are used to quantify tumor burden or calculate clinical benefit, harm, lack of benefit or damage (Goossens et al. 2015; Levenson 2004).

Cancer biomarkers should detect cancer early or at a stage when there are no symptoms, this will improve safety and reduce morbidity and complications of cancer patients. To reduce the number of false positives, screening tests must be highly specific. Ideal screening programs should be low-cost and noninvasive and they must result in a clear reduction in mortality and morbidity, as well as an improvement in survival rate. Generally, screening programs are aimed at cancers that are highly prevalent, as well as further treatment and follow-up are necessary (Duffy 2001), other biomarkers for recurrence are enlisted in Table 8.2. Limiting factors for cancer are low specificity and diagnostic sensitivity of many of the biomarkers which are in use currently that act as screening markers and then later on in cancer treatment if it escalates. However, some biomarkers are being used as screening biomarkers for hepatocellular cancer in high-risk individuals, such as AFP (Xu et al. 2021), in screening of prostate cancer PSA (Clift et al. 2021), in ovarian cancer CA125 (Zhang et al. 2021), in newborns for screening of neuroblastoma vanillylmandelic acid (VMA) (Duffy 2015), and for colorectal cancers screening fecal occult blood testing (FOBT) (Lee et al. 2011). FDA approved PSA as biomarker for screening of prostate cancer, however, on individuals with inflammatory or benign conditions false positive elevated levels of PSA can be found like prostatitis and benign prostatic hyperplasia (Catalona et al. 1991). The role of PSA screening in lowering mortality is still a source of debate (Schroder et al. 2009; Andriole et al. 2009).

Table 8.2 Cancer biomarkers in recurrence

Cancer type		Biomarker in cancer recurrence	References
Papillary thyroid cancer		MiR-9 and miR-21	Sondermann et al. (2015)
Breast cancer		Long non-coding RNAs (IncRNAs)	Zhou et al. (2016)
		CA15-3	Keshaviah et al. (2007)
		Ki-67	Nishimura et al. (2010)
		ACLY	Chen et al. (2020)
Colorectal cancer		miRNA-29c	Yang et al. (2013)
		ExosomalmiRNA	Matsumura et al. (2015)
		TMEM240	Chang et al. (2020)
		miR-34a-5p	Gao et al. (2015)
		Carcinoembryonic antigen (CEA)	Tan et al. (2009)
		miRNA-148a	Tsai et al. (2013)
Lung cancer	Small cell lung cancer	Long-non-coding RNA *HOTAIR*	Ono et al. (2014)
		Exosomal miRNAs	Munagala et al. (2016) .
		Histone macroH2A	Sporn et al. (2009)
	Non-small cell or small cell lung cancer	miRNA-451a	Kanaoka et al. (2018)
		miRNA-486	Li et al. 2015a, b
		Collagen XXIII	Spivey et al. (2010)
	Adenocarcinoma	CA 19-9 and CA 125	Isaksson et al. (2017) .
		Anterior gradient homolog 2 (AGR2)	Hanada et al. (2012)
Hepatocellular carcinoma		Mortalin (HSPA9)	Yi et al. (2008)
		miRNAs	Sugimachi et al. (2015)
		NDRG1	Cheng et al. (2011)
		Cytokeratin 19 (CK19)	Uenishi et al. (2003)
		Osteopontin (OPN) mRNA	Pan et al. (2003)
		Alpha-fetoprotein (AFP)	dos Santos Schraiber et al. (2016)
		MCM6	Liu et al. (2018)
		DCP, GP73	Yamamoto et al. (2010)
Ovarian cancer		circSETDB1	Wang et al. (2019)
		Human epididymis protein 4 (HE4)	Plotti et al. (2012)
		CA125	Murakami et al. (2006)
		KLK6 and KLK13	White et al. (2009)
		KLK7	Tamir et al. (2014)
Bladder cancer		Nuclear matrix protein 22	Shariat et al. (2005)

(continued)

Table 8.2 (continued)

Cancer type	Biomarker in cancer recurrence	References
	Cystatin B	Feldman et al. (2009)
Cervical cancer	SCC-Ag and hsCRP	Hoogendam et al. (2013)
Pancreatic cancer	CD44	Hsu et al. (2018)
Gastric cancer	miRNA27a	Huang et al. (2014)
	miRNA-203	Imaoka et al. (2016)
Acute myeloid leukemia	miR-15a/16-1	Gao et al. (2011)
Acute lymphoblastic leukemia	miR-135a	Diaz-Beya et al. (2014)
	miR-708	Han et al. (2011)
	LINC00152	Bárcenas-López et al. (2020)
Multiple myeloma	Circulating exosomal circMYC	Luo and Gui (2020)
ITD+ leukemia	IL-10, IL-1β, TNF- α	Rickmann et al. (2013)

8.5.1 Breast Cancer

It is reported in a study that ACLY is an independent and potential biomarker for the prediction of recurrence in breast cancer patients (Chen et al. 2020).

When a patient's primary breast cancer relapses then immunohistochemistry is often used to guide treatment adjustments. Confirmatory tissue sampling and detection of HER2, PR, or ER switches that change clinical care for one in every six patients must be part of relapsed breast cancer management (Thompson et al. 2010)

8.5.2 Prostate Cancer

Despite early prostate cancer treatment, most men experience an increase in PSA. Although some of those men may undergo a metastatic or local recurrence, requiring further treatment, some will show no evidence of progression of the disease (Nakagawa et al. 2008). Prostate cancer studies over the years developed metabolite profiling to classify prognostic, diagnostic and predictive biomarkers (Kelly et al. 2016). Additional biomarkers of prostate cancer aggressiveness are being sought by urologists. Higher risk of prostate cancer recurrence is linked with increased level of insulin in serum (Lehrer et al. 2002).

8.5.3 Leukemia

The optimal cure for acute myeloid leukemia (AML), which is a heterogeneous disorder and cancer of white blood cells of myeloid origin, depends on molecular markers and cytogenetic risk factors. MicroRNAs (miRNAs) have been linked to a

greater risk of AML relapse in many studies, a study reported that low levels of miR-135a and miR-409-3p being linked to a greater risk of AML patients relapse (Diaz-Beya et al. 2014).

In the last decade, evidence of the role of long-non-coding RNAs (lncRNAs) in leukemogenesis has emerged. These genes have been suggested as diagnostic and/or prognostic biomarkers in children with acute lymphoblastic leukemia (ALL). In the last decade, evidence of the role of long-non-coding RNAs (lncRNAs) in leukemogenesis has emerged.Early relapse and mortality in patients, *LINC01013* and *LINC00152* were among the most differentially expressed genes (Bárcenas-López et al. 2020).

8.6 Applications of Cancer Biomarkers in Most Common Cancers

Cancer has been identified as leading cause of death for several years; in 2012, an estimated 8.2 million cancer patients died around the world (Bray et al. 2012). According to the GLOBOCAN worldwide, incidence of cancer and mortality rate reported in 2015 by the International Agency for Research on Cancer (IARC), breast cancer, liver cancer, and ovarian cancer is among the most prevalent cancers in women worldwide (Ferlay et al. 2015). Cancer biomarkers can be used for the assessment of cancer risk for screening in the asymptomatic community, distinguishing benign from malignant tumors, prognosis, and following treatments (Henry and Hayes 2012).

8.6.1 Breast Cancer

Breast cancer is the most common malignancy in women and the leading cause of death worldwide; its incidence rate is rising at an alarming rate at the moment (Pisani et al. 2002). Early intervention and proper case management is an important way to control cancer. The most common clinical signs of breast cancer are nipple discharge, lump in breast, and prominent change in skin or nipples. According to the American Cancer Society's screening recommendations, women above the age of 40 can get a mammogram and a clinical breast test once a year or every other year (Sabatino et al. 2015). Cancer biomarkers role in breast cancer treatment is primarily very helpful with prognosis, monitoring of treatment, and follow-up; in particular, does not seem to be very useful for early detection. (Ludwig and Weinstein 2005). European Society of Medical Oncology has proposed testing for ER and PR for diagnosed breast cancers to predict response to advanced breast cancer cases (Mirza et al. 2002; Duffy 2006) "HER-2" is an additional prognosis marker which is also most effective for targeting early or metastatic breast cancer patients in Trastuzumab therapy (Ross et al. 2003); or estimate resistance of tamoxifen treatment in early stages of breast cancer (Duffy 2005). For the treatment of breast cancer, screening scheme should be introduced and discovery of "BRCA1 or BRCA2 gene

mutations", 5% of cases of breast cancer. Because of high vulnerability to breast and ovarian cancer, it should be advised to females for regular cancer screening to observe BRCA1 or BRCA2 mutations (Vietri et al. 2012). Low levels of "urokinase plasminogen activator (uPA)" and "plasminogen activator-1 (PAI-1)" have been confirmed to link with a decreased risk of relapse of breast cancer (Harbeck et al. 2001). During therapy, serum biomarkers are used which normally aided in postoperative surveillance, and CBs included in that group include "CA15.3, CEA, and BR 27-29" (Molina et al. 1998) used for conjugation with radiological and therapeutic evaluation instruments to track chemotherapy in advanced breast cancer cases. The elevation of serum levels of markers may propose for recurrence or disease progression (Sparano 2006).

8.6.2 Prostate Cancer

Prostate cancer is the most common cancer in men and the leading cause of death (Pisani et al. 2002). Strong evidence suggests that the "PSA test" transfigured screening and detection of prostate cancer in earlier stages and also helps in lowering prostate cancer mortality (Bjartell 2013). Many experiments later showed that using "PSA subtractions and isoforms [-2] (proPSA)" improved the sensitivity of PSA as a diagnostic marker significantly. Patients with PSA levels range from 4 to 10 g/L, these fractions can aid in the differentiation of benign and malignant prostatic tumors (Gao et al. 2003). "Human kallikrein type 2", "prostate cancer antigen 3 (PSA3)", and "prostate stem cell antigen" are the biomarkers under investigation. The PCA3 urine assay has the potential to improve prostate cancer diagnostic accuracy (Haese et al. 2005). Increased amounts of the protease family "members metalloproteinase 2 and 9" (MMP-2 and MMP-9) have been linked to prostate cancer diagnosis (Moses et al. 1998). MMPs have been investigated as clinical screening biomarkers of prostate cancer (Morgia et al. 2005).

8.6.3 Ovarian Cancer

Mostly patients are diagnosed late with epithelial ovarian cancer stages III and IV at the time of diagnosis, ovarian cancer diagnostic biomarkers must be responsive and specific (Coticchia et al. 2008). CA 125 is one of the most commonly used and well-known CBs. CA125 has been used in combination with vaginal ultrasound as a diagnostic biomarker for females having a family history of ovarian cancer (Duffy et al. 2005). CA125 is often used as a screening biomarker, as its level drops after beginning chemotherapy or surgery, which coincides with a positive reaction. Follow-ups at frequent intervals should be strongly recommended 2–3 weeks before starting clinical intervention (Morgan et al. 2008). Other biomarkers have been studied extensively in the monitoring of ovarian cancer and its prognosis; however, more research is required to validate their exact function. "Kallikreins, osteopontin, Her-2/neu, tumor-associated inhibin, CEA, trypsin inhibitor, hCG, interleukin-6

(IL-6), prostasin, TPA, lysophosphatidic acid, and plasminogen activator inhibitor-1 are all included in this panel (PAI-1)" (Coppola et al. 2004).

8.7 Conclusion

Cancer biomarkers have a vital role in cancer for screening, risk assessment, diagnosis, response to treatment, prognosis, and recurrence or relapse. It is important for researchers and clinicians to have thorough understanding of clinical utility, molecular aspects, and biomarker's reliability to know in which state biomarkers are important in clinical use for better evaluation and patient care. Biomarkers can facilitate the diagnosis and therapy of patients and can have vital role in personalized medicine. Despite many efforts to identify the risk factor, the possibility of recurrence is unlikely to be eliminated. However, there is an urgent need to develop or identify biomarkers which show us patient-related factors and can be used to predict cancer in advance. Cancer biomarkers can be used for novel targeted and personalized treatments as it investigates patient therapeutic profile and characteristics.

8.8 Future Perspectives

The drawbacks of the clinical use of these new markers, at the detection level, have been reduced to the rapid use of markers for validation and clinical practice, owing to the increased detection of potential biomarkers. To ensure cost-effectiveness, oncology practice will be tracked for the next decades. Biomarkers that can diagnose cancer, predict cancer outcomes, and affect treatment choices would be crucial in evaluating the efficacy of clinical cancer treatment. It necessitates a method that is simple, efficient, safe and dependable. No biomarker is likely to be accurate enough to affect treatment decisions when it comes to predicting disease outcomes. As a result, cancer diagnosis in the future can depend on small plaques of 6–10 markers that provide an accurate molecular measure indicating the probability of metastatic involvement and the need for rapid systemic care. Advanced technologies will aid in the development of several new biomarkers for a specific disease at the same time. Today, with microchip technology, 2D gels, and mass spectrometers are not readily available in the clinical setting and require well-equipped laboratories and skilled personnel. To advance biomarker detection in clinical practice, simple, fast, and responsive microbe-based protein chips, unlabeled detection systems and antibody-based chip systems have been developed. We are getting closer to a time when biomarkers can be used to help track and control cancer diagnosis, progression and care. A key aim for the future of cancer is to develop simple test kits that correctly and efficiently diagnose cancer and can be used in clinics or on potential patients.

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Biomarkers for Cancer Immunotherapy

9

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Abstract

Management of advanced-stage cancers of various types has revolutionized with the introduction of immune checkpoint inhibitors (ICIs) in 2010. However, achievement of optimum benefit is limited to a small number of patients only. Identification of these responsive patients prior to administration of ICIs gives rise to the critical need for predictive biomarkers for immunotherapeutic agents. In this chapter, we have focused on the current status of famous biomarkers along with their predictive utility in various types of cancers. PD-L1, mutational burden, TILs, neutrophil/lymphocyte ratio, LDH, miRNA, and microbiota have been discussed in detail. Multiple studies exemplifying their usefulness have been

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presented. It is pertinent to mention that optimization of response biomarkers precedes the optimization of ICIs. For this purpose, tumors and their microenvironment must be characterized for selection of specific biomarkers to predict ICI response. Further studies and deeper insights are required to eliminate all the drawbacks associated with the predictive precision of the biomarkers for the successful design of individualized immunotherapy regimens.

Keywords

Biomarkers · PD-L1 · Lymphocytes · Tumor mutational burden · Microbiota · miRNA · Lactate dehydrogenase · Pembrolizumab

9.1 Introduction

Managing cancer via drugs or agents affecting immune system has changed the landscape of anti-neoplastic therapies. Extensive research in the recent years has made it clear that modulation of immune system is effective in the elimination of distant metastatic tumors. Cancer immunotherapy has recently shown promising outcomes with respect to their safety andeffectiveness profiles, making it a strong candidate besides radiotherapy and chemotherapy (Callahan et al. 2016; Hanahan and Weinberg 2011). Although much needs to be done pertaining to the assessment and standardization of their manufacturing with respect to environment & sustainability, current priority is given to the evaluation of pharmacological and clinical benefits (Ahsan et al. 2020; Capela et al. 2019). Research in this field has resulted in the introduction of more than 2000 immunotherapeutic agents for cancer (Tang et al. 2018; Walk et al. 2020).

Induction of lymphocytes to generate vigorous immune response in the tumor environment has proven to be effective in various cancer patients (Suzuki et al. 2017; Sheikh et al. 2013; Torphy et al. 2018). A major disadvantage associated with positive clinical outcome is that cancer immunotherapy has been found beneficial in only 20% of cancer patients onassessment through clinical trials. Selectivity of clinical response is the biggest disadvantage of immuno-oncological therapies. Moreover, adverse drug reactions, wasted financial cost, burden on private, and government drug procuring facilities are additional disadvantages. Other sets of reasons for unsuccessful development of an ideal predictive biomarker for immunotherapy, include tumor heterogeneity, individualized host–immune responses, distinct molecular, pathological, and genetic changes in the tumors. Pre-assessment of outcome is necessary to determine benefit-to-risk ratio. (McKean et al. 2020).

Selection of patients for a specific immunotherapy drug to achieve optimum clinical care and targeted therapy at minimal cost necessitates the development and use of efficacy biomarkers of cancer immunotherapy. Therefore, the prerequisite for effective personalized cancer immunotherapy is the development of biomarkers that act as response predictors of specific immunotherapies (Kourie and Klastersky 2016). These would also enable the clinicians to understand the complex mechanism

of cancer immune cycle and the stages at which relevant biomarkers affect and predict the outcome of immunotherapy (Schumacher et al. 2015). Personalized analysis and interpretation of biomarker testing will eventually be required for selection of individualized regimens (McKean et al. 2020). Herein, we discuss the potential response biomarkers in cancer immunotherapy.

9.2 Programmed Death-Ligand 1 (PD-L1)

Use of this endogenous molecule as a biomarker was first determined during the study of PD-1 inhibitors in 2010 (Yarchoan et al. 2019). This biomarker, present on T cells and tumor cells, has become the most popular and widely used standard prior to commencement of PD inhibitors as mode of cancer immunotherapy (Brahmer et al. 2010; Patel and Kurzrock 2015). It is the ligand of PD-1, one of the important receptors on T lymphocytes involved in controlling tumor-immune responses. The ligand causes decreased proliferation of T cells and also helps in escape of tumor from immune responsiveness. Antibody against the PD-1 receptor to inhibit its activation via PD-L1 will result in making the tumor cells susceptible to intense immune response (Fig. 9.1) (Shindo et al. 2015).

A study reported the results of earlier clinical trials with an anti-PD-1 antibody against carcinoma of various organs such as kidneys, lungs, colon, and prostate. 36% of patients expressing PD-L1 showed anti-neoplastic response to the administered antibody, while patients not expressing PD-L1, did not show any anticancer effects (Topalian et al. 2012). Another study reported the association of PD-L1 biomarker with efficacy of pembrolizumab (anti-PD-1). The trial revealed that patients with non-small-cell lung cancer (NSCLC) respond to the antibody when PD-L1 expression was high. Greater and better response was directly related to better disease outcome and survival (Garon et al. 2015; Topalian et al. 2012). PD-L1 expression, determined via tumor proportion score, showed that there is direct relationship of PD-L1 with efficacy of pembrolizumab. Hence, PD-1 inhibitors can prove to be

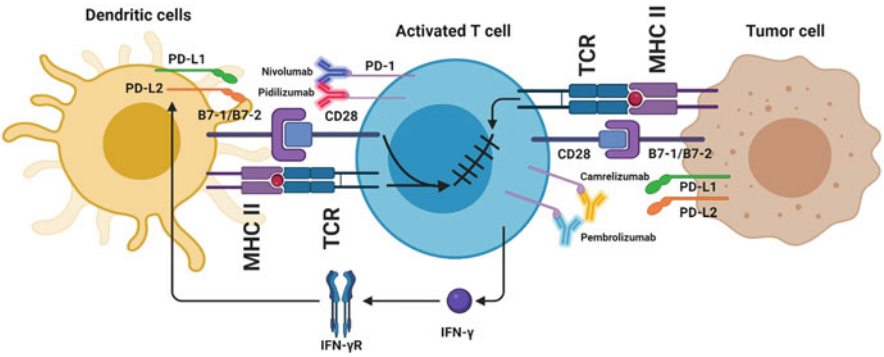

Fig. 9.1 Mechanism of action of anti-PD-1 agents. TCR T-cell receptor; MHC II Major histocompatibility complex II; CD28 cluster of differentiation 28

good candidates for cancer immunotherapy and its effectiveness can be determined by prior determination of PD-L1 expression (Allgäuer et al. 2018; Balar et al. 2017; Herbst et al. 2016; Reck et al. 2016). Correlation of PD-L1 expression with immunotherapy was also found in patients with Hodgkin's lymphoma. A response of 87% to immunotherapy was observed along with higher expression of biomarker in the patients (Ansell et al. 2015). In addition to its expression in tumor cells, it has also been reported that degree of PD-L1 expression on TILs is also a representation of patient suitability to immune checkpoint inhibition therapy (Herbst et al. 2014; Zou et al. 2016).

PD-L1 testing is carried out by immunohistochemical analysis. The ratio of PD-L1+ tumor and immune cells to the total tumor population is measured. This procedure is considered prerequisite before recommendation of anti-PD-1 agents for the management of advanced head and neck cancer, esophageal cell carcinoma (second-line treatment recommendation),and adenocarcinoma of stomach and esophageal junction (as third-line therapy recommendation). Versatility of PD-L1 as response biomarker has been verified by many other studies showing improvement in clinical severity and increase in overall survival of patients (McKean et al. 2020).

9.3 Tumor Mutational Burden, Mismatch Repair Deficiency, and Neoantigens

Tumor mutation burden (TMB), defined as the total number of nonsynonymous somatic mutations within protein encoding area of tumor cell genome (Allgäuer et al. 2018). These mutations in tumor genome express to produce "neoantigens" which are specific to tumor cells only. These antigens are expressed on cell surface of the tumor cells followed by their immune recognition by lymphocytes (Schumacher and Schreiber 2015).

A total of 2–5 base pairs sequences occurring repeatedly between coding and noncoding regions of gene bear the deficiency of DNA mismatch repair, making them prone to develop instability, and mutations. The unstable microsatellites phenotype has been associated with greater neoantigen production, making the tumor more responsive to immunotherapy (Gupta and Heinen 2019).

It has been reported that greater mutational burden causes greater immune responsiveness. Patients with melanoma when treated with ipilimumab showed greater responsiveness, progress, and survival to therapy when TMB was higher (Snyder et al. 2014). One study presented similar results in another group of individuals to whom pembrolizumab was administered for NSCLC. Alexandrov et al. have reported the results of their studies on TMB that tumors with abundant mutational load (TMB) are more likely to respond to cancer immunotherapy, because multiple mutations generate neoantigens causing activation of immune system (Alexandrov et al. 2013).

With the innovation of liquid biopsy, estimation of TMB in circulating tumors has been reformed. One research group studied the effect of atezolizumab in NSCLC

patients. The intensity of response to drug was compared with proportion of TMB estimated (by liquid biopsy) to find if the correlation exists between objective response and TMB. Researchers found that patients with higher TMB showed greater and better pharmacological response to immune checkpoint inhibitors (ICIs) therapy than those with lower TMB (Gandara et al. 2018). In its continuation, tumors with faulty and deficient DNA repair mechanisms producing neoantigens respond strongly to immunotherapy than those tumors with efficient DNA repair mechanisms and lesser neoantigens (Chang et al. 2018). Recently, a study reported variable response of immunotherapy in cancer patients with high TMB. The variability of results was attributed to the nature and significance of genomic mutations and their effect in inducing the robust immune response against the tumor cells. Authors also suggested immunoediting as one of the escape mechanisms from host's immune system. This might generate immune-resistant tumors against which anti-PD-1 agents would not be optimally effective. In NSCLC, it has been reported that greater expression of neoantigens in the initial stages of malignant transformation makes the tumor more responsive to immunotherapy. Hence, neoantigens are also considered predictive response biomarkers of cancer immunotherapy (Jamal-Hanjani et al. 2017; McGranahan et al. 2017). Hugo et al. 2016 have also reported that BRCA2 mutations cause defects in DNA repair. Loss-of-function mutations in this gene have, therefore, been associated with better response outcomes when treated with anti-PD-1 drugs (Hugo et al. 2016; Pérez-Ruiz et al. 2020).

TMB has been positively correlated in many tumors as response biomarker for ICIs. In one study, the ICI objective response rate in various types of tumors and its subtypes was observed and correlated to TMB measurement (Steuer and Ramalingam 2018). Analysis showed that TMB measurements were correctly representing the response to ICIs (direct proportionality) in all tumor types. Desmoplastic melanoma is one of the tumors having highest TMB. Likewise, this cancer type also shows very good response to ICIs (Boussemart et al. 2019). Similar trend of TMB was reported with improved overall survival with ICI therapy in 151 patients having various types of cancer in advanced stage. TMB was assayed by next-generation sequencing (Goodman et al. 2017).

In a recently published study by Valero et al., it was demonstrated that the relation between TMB and patient survival is significant from treatment perspective. They concluded that higher TMB will result in greater improvement and survival from cancer only when treated with immune checkpoint inhibitors (Valero et al. 2021).

9.3.1 Concerns About TMB

Despite substantial advantages of TMB, various concerns need to be addressed to generalize its use as preferable response biomarker. Of prime importance is the absence of standard assay method for TMB determination. Multiple tests based on different principles and criteria are being used. One study has demonstrated that TMB is a better predictive biomarker in younger age cancer patients (Wu et al.

2020). Additionally, various cutoff values have been suggested for TMB which makes it more difficult to assent on a single universally accepted value (McKean et al. 2020).

9.4 Tumor-Infiltrating Lymphocytes (TILs)

Human immune system responds to cancerous cells via various lymphocytes, such as cytotoxic T cells and natural killer (NK) cells. The number of these cells in the tumor microenvironment is directly related to overall survival rate and response to ICIs. All such cell types inside the tumors are classified as tumor-infiltrating lymphocytes (TILs)(Uryvaev et al. 2018). These lymphocytes are present in tumor environment after infiltration from systemic circulation under the influence of immune system. They have been studied for their predictive and prognostic potential of immunotherapy in various types of cancers (Rosenberg et al. 1988). Likewise, they have shown to be good efficacy predictors of ICIs.

Tumor with robust immunogenicity involves infiltration of CD8+ and CD4+ T cells in tumor microenvironment. Such tumors are termed as immune-inflamed tumors (Chen and Mellman 2017). Tumor assessment for immune infiltrates is carried out by immunohistochemical staining (Clark Jr et al. 1989; Elder et al. 1985; Hendry et al. 2017).

Immune-inflamed tumors have been associated with better response and overall survival (OS) with ICIs, suggesting lymphocyte infiltration as predictive biomarker directly related to the ICI therapy in various advanced cancers (Herbst et al. 2014; McKean et al. 2020; Tumeh et al. 2014).

In the tumor environment, activated T cells release cytokines such as interferon-gamma (IFN- γ) that causes upregulation of PD-L1 receptors (Ikeda et al. 2002). It has also been suggested that defective IFN- γsignaling causes reduction in response to ICI therapy (Garcia-Diaz et al. 2017; Stühler et al. 2019). This biomarker has recently gained importance to help recruit patients with greatest response rates for a specific anti-PD-1 therapy. In breast cancer patients, stromal TILs density (determined by H&E staining) have also positively correlated to response of immunotherapy (Gonzalez-Ericsson et al. 2020; Hendry et al. 2017). Several studies have demonstrated the correlation of CD8/CD4 ratio of TILs as efficacy predictor of anti-PD-1 regimen (Bald et al. 2014; Garon et al. 2015; Ribas et al. 2015; Robert et al. 2015). One study was designed to determine the role of CD4+ and CD8+ TIL as response biomarkers of anti-PD-1 therapy in NSCLC (26 patients) and malignant melanoma (30 patients). The researchers found interesting association of CD8+/CD4+ ratio to treatment response. In both cases, their results demonstrated that the TIL ratio greater than 2 produces better response to anti-PD-1 treatment. Other studies conducted in smaller number of NSCLC patients were also suggestive of better response to anti-PD-1 agents due to combined increased in PD-L1 expression and TILs (Herbst et al. 2014; Herbst et al. 2016; Reck et al. 2016; Uryvaev et al. 2018). One study reported that cytotoxic T cell density in microenvironment of metastatic melanoma is proportional to the overall response rate of specific

immunotherapy drugs (Tumeh et al. 2014). Another study reported a strong proportionality of CTL (expressing CTLA-4) density as response predictor of immunotherapy (Daud et al. 2016; Loo et al. 2017). Similarly, increased TIL density in bladder carcinoma were indicators of high response rate and improved survival with anti-cancer immunotherapy agents (Sharma et al. 2007).

9.4.1 Effect of Chemokines on TILs

Th1 chemokines have been found in tumor microenvironment and have been found to affect Tcell density in tumor. Therefore, these chemical cytokines have indirect effect on the patient response to immunotherapy by affecting lymphocyte concentration in tumor (Galon et al. 2006; Kryczek et al. 2009; Zhang et al. 2003). Resistance to immune responses by tumors is also believed to be produced via regulation of chemokine expression and signaling mechanisms. This might also reflect the failure of immunotherapy to manage these cancers. One such mechanism has been reported in mouse melanoma model where β-catenin negatively regulates CCL4. CCL4 is responsible for migration of dendritic cells that leads to T cell activation. Tumor halts lymphocytes activation by negative regulation of chemokines (Spranger et al. 2015). In other models of cancers (ovarian and colon), negative regulation of CXL9 and CXL10 cause T cell inactivation and reduce the lymphocyte migration, infiltration, and density. Epigenetic pathways found to support tumor progression and reduce anti-tumor immune responses, by affecting the expression of chemokines, including polycomb repressive complex 2 (PRC2) and DNA methylation (Nagarsheth et al. 2016; Peng et al. 2015).

9.5 Neutrophil–Lymphocyte Ratio (NLR)

Advancement in knowledge of various pathways and parameters involved in managing cancer has led us to discover the potential of NLR as predictive biomarker of cancer immunotherapy. Many studies have shown NLR to be a very useful parameter for judging inflammatory status of tumor and its surroundings (Chua et al. 2011). Basic advantages of this parameter, include its simplicity, ease to collect sample, and conduct test (Park and Lopes 2019).

Effect of NLR on tumor microenvironment is mediated by the release of cytokines which regulate downstream expression pathways of various genes that lead can aggravate or alleviate the tumor microenvironment (Fig. 9.2). A study revealed that good prognosis of cancer is linked to high lymphocyte and low neutrophil counts. Patients treated with nivolumab for melanoma having high absolute lymphocytic count (>1000 μL) and low absolute neutrophil count of equal to or less than 4000 μL was positively associated with patient OS (Nakamura et al. 2016). Higher lymphocyte count observed in peripheral blood sample has shown positive correlation to immunotherapy with pembrolizumab and ipilimumab (Martens et al. 2016; Park and Lopes 2019; Weide et al. 2016). This ratio of

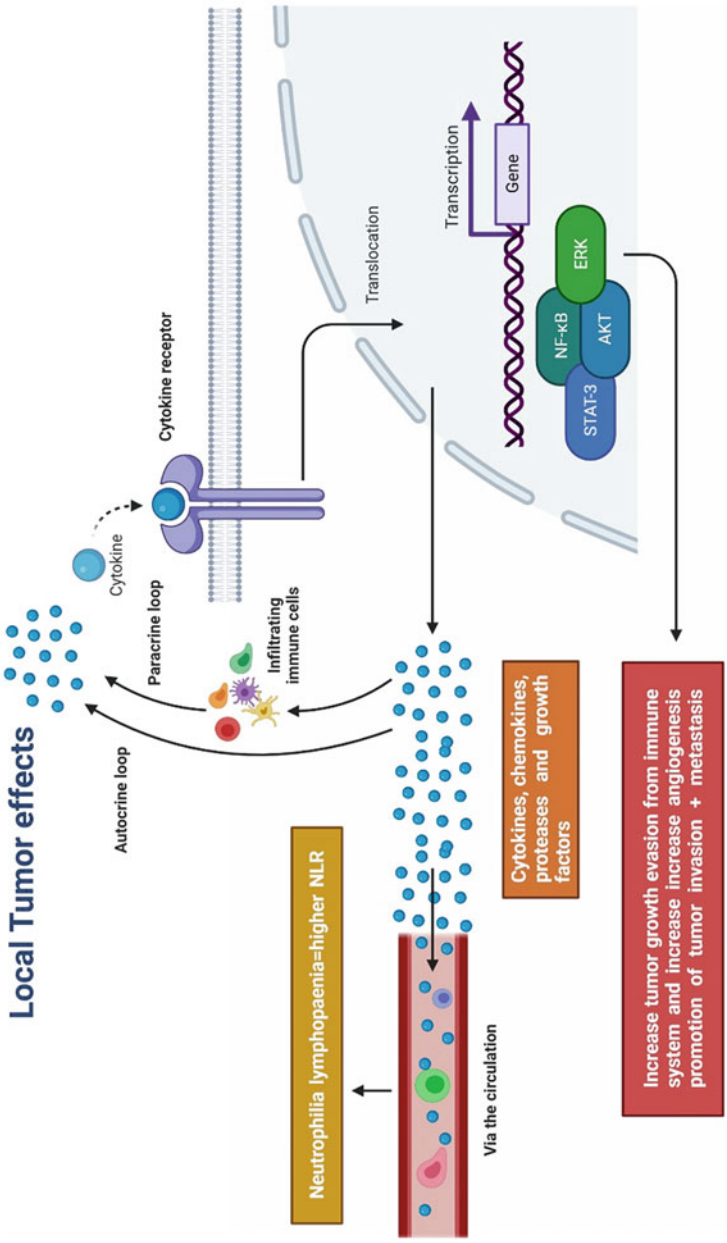

Fig. 9.2 Consequences of low lymphocyte count (high NLR) resulting in cytokine-mediated initiation of various pathways aggravating tumor severity

neutrophil-to-lymphocyte is an index of robust systemic immune inflammatory response (Zahorec 2001). Higher ratio is indicative of feeble immune response to tumors and hence, less responsive to cancer immunotherapy agents in advanced stages (Bilen et al. 2019; Maymani et al. 2018; Shindo et al. 2017b). It has been suggested that tumors with lymphocyte count less than 15% of normal limit should not be dealt with immunotherapy because of lesser chance of any possible improvement in cancerous condition or clinical manifestation of the disease. Furthermore, it has also been reported that NLR greater than 3 is suggestive of poor prognosis in patients with colorectal cancer (CRC) receiving vaccine (Hazama et al. 2014).

Another related ratio is called derived NLR (dNLR) which includes monocytes and granulocyte count while predicting the responsiveness of a certain tumor to immunotherapy (Proctor et al. 2012). A relatively large number of patients with melanoma treated with ipilimumab for the same were checked for dNLR. It was observed that lower ratio (less than 3) correlated with better therapeutic response and survival rates. Higher ratio corresponded with increased risk of fatality and disease progression (Ferrucci et al. 2016). These results show that dNLR can be used as predictive and prognostic agent in cancer patients to check suitability of immunotherapeutic agents.

9.6 Lactate Dehydrogenase (LDH)

LDH is the last enzyme in carbohydrate metabolism. It is part of glycolysis where it is responsible for interconversion of lactic acid and pyruvic acid (Talaiezadeh et al. 2015). Due to Warburg phenomenon, LDH is increased in cancer patients representing greater utilization of glycolysis (Wong et al. 2013). It has inverse relationship with the patient's prognosis. In a study on 230 patients with melanoma who were treated with an anti-PD-1 agent ipilimumab, the overall survival of patients was higher (10 months) when baseline LDH values were less whereas, the overall survival was 2.9 months when LDH value was two-fold higher than upper limit of normal value (Kelderman et al. 2014). Other studies exploring the correlation between LDH and melanoma patient survival reported similar results with pembrolizumab and nivolumab used as anti-PD-1 agents (Diem et al. 2016; Weide et al. 2016; Wagner et al. 2018). Other studies were also conducted to evaluate LDH levels with death rate in lung cancer patients. The studies concluded that higher LDH levels are associated with higher death rate. Moreover, administration of immune checkpoint inhibitors did not improve the survival of patients with elevated LDH concentration (Inomata et al. 2020; Ichiki et al. 2019; Oya et al. 2017). Similar results were shown in patients with esophageal cancer and high LDH, representing reduced improvement in clinical symptoms when treated with camrelizumab (Simeone et al. 2014).

9.7 MicroRNA (miRNA)

miRNA comprises 18–25 base pairs that are of noncoding nature. They affect gene expression post-transcriptional level. Although miRNA does not translate into a protein, they regulate the transcription of many mRNAs (Shindo et al. 2017a; Zaharie et al. 2015). Alterations in the expression of miRNA change the behavior of tumor. These changes are sometimes favorable making the tumor more responsive to anti-cancer regimen, while in other cases tumor becomes more resistant to anti-cancer therapies. Hence, it is reported to be a prognostic and predictive biomarker for various types of tumors (Iorio et al. 2005; Nagao et al. 2012; Schetter et al. 2008).

In advanced CRC patients, better OS was observed in those having lower expression of miRNA-6826 and miRNA-6875 in systemic circulation (Kijima et al. 2017). This indicates that miRNAs are involved in strengthening tumor resistance to immune system. Hence, downregulation of these miRNA can increase the vulnerability of tumors to immune system and agents potentiating immune system (Halvorsen et al. 2018). Similarly, seven circulating miRNAs (215-5p, 411-3p, 493-5p, 494-3p, 495-3p, 548j-5p, and -93-3p) were also found to be associated with better survival and response rates to nivolumab when treated for NSCLC (Halvorsen et al. 2018). Therefore, miRNAs have versatile functions, some being tumor suppressors while others acting as tumor inducers (Li et al. 2016). MRX34, a liposomal miRNA-34a was the first miRNA developed to act against cancer (Beg et al. 2017). In murine model, when administered as adjuvant to radiotherapy, miRNA-34a inhibited PD-L1 expression and enhanced TILs (Cortez et al. 2016). In NSCLC, miRNA-200 is an inhibitor of PD-L1 expression. It increases lymphocyte infiltration, in tumor microenvironment, from systemic circulation. Moreover, it has also been shown to retard metastatic potential of tumor (Romano and Kwong 2018).

9.8 Microbiome

Intestinal flora affects personal health and host's immune system (Picardo et al. 2019; Wang and Jia 2016). Intestinal microflora and cancer coincide in newer but unexplored avenues of cancer biology. Complete elucidation of their relationship and interdependence has not been done so far. Anti-cancer treatment modality such as cyclophosphamide (Viaud et al. 2013) and PD-L1 inhibitors (Sivan et al. 2015) affect the intestinal microbiota. Abundance of *Akkermansia muciniphila* in patients' stools has been associated with better overall response rates to anti-PD-1 therapy in renal cell carcinoma and NSCLC (Routy et al. 2018). It has also been reported that bacterial transplant into murine cancer model improved the response rate to anti-PD-1 therapy. The effect was thought to be mediated by IL-2 causing migration and recruitment of CCR9+, CXCR3+, and CD4+ T cells to generate robust immune response against tumor. Similarly, Bacteroides fragilis has been correlated with the efficacy of CTLA-4 inhibitors (Vétizou et al. 2015). Therefore, cancer patients

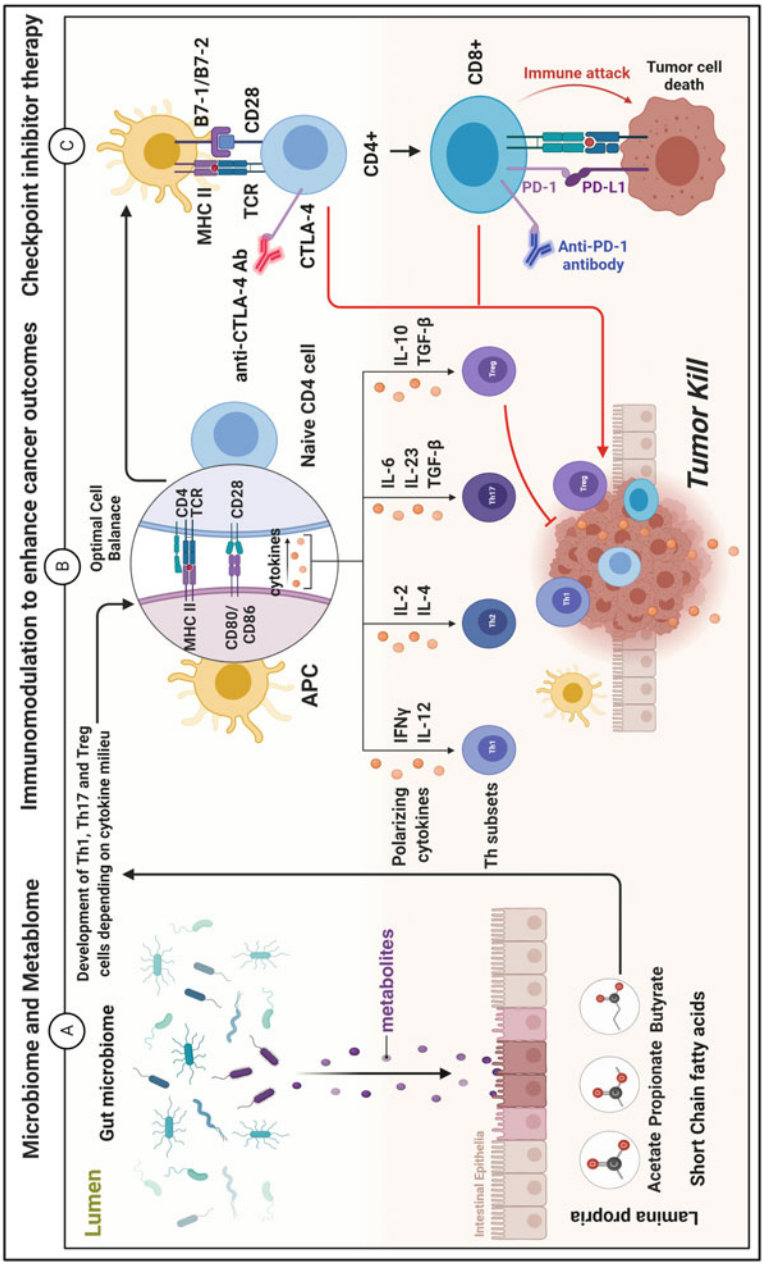

Fig. 9.3 Effects of short-chain fatty acids produced by gut microbiota on the lymphocyte differentiation and tumor microenvironment

receiving any antibiotic regimen which can harm intestinal microflora can indirectly lead to less responsiveness of main anti-cancer therapy. The outcome worsens when the antibiotic has broad or extended anti-microbial spectrum (Bunney et al. 2017; Pérez-Ruiz et al. 2020).

Fusobacterium nucleatum has been associated with upregulation of oncogenic genes, thereby helping in CRC development (Abed et al. 2016; Rubinstein et al. 2013; Tahara et al. 2014; Yamaoka et al. 2018). A study in cancer patients of Japanese origin revealed that the *F. nucleatum* is associated with poor clinical outcomes and less responsiveness to anti-cancer therapies (Abed et al. 2016). Another study revealed that skin cancer patients who showed optimum response to anti-PD-1 agents had abundant number of bacteria of family Ruminococcaceae (Gopalakrishnan et al. 2018).

Metabolic compounds produced by gut microbiota have been considered as response predictors of cancer immunotherapeutic agents (Fig. 9.3). Short-chain fatty acids produced as bacterial metabolites, from carbohydrate fibers, induce lymphocyte differentiation. They have also been reported to promote effector T cell activity. Quantification of these fecal/microbial metabolic products can be linked with magnitude of response to immune checkpoint inhibitors (Malczewski et al. 2020).

9.9 Conclusion

Profiling of multiple response biomarkers is in dire need of successful anti-neoplastic immunotherapies. Methods for assay of the biomarkers need to be improved with greater accuracy and better sensitivity. Continuous investment of efforts is required to develop methodologies that minimize variations in assay to avoid false-positive/-negative outcomes. Success of cancer immunotherapy in improving quality of life lies in the development of accurate response biomarkers for patient selection.

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Role of Biomarkers in Personalized Medicine

10

Salman Ul Islam, Muhammad Bilal Ahmed, Haseeb Ahsan, and Young Sup Lee

Abstract

Biomarkers are a key tool in medicine, especially in the domain of personalized medicine. They are valuable for the early detection, prognosis, and diagnosis of disease as well as for the prediction of treatment response. They enable us to select appropriate individuals for treatment with personalized medicine and provide the right medication to the right patient. At present, the development of individually targeted patient therapy remains the key objective of the medical world. The achievement of this goal needs advances in biomarker discovery and the development of therapeutic strategies that can be optimized for individual drug and dose selection. This chapter discusses strategies for the use of biomarkers and their impact on drug development. Further, it highlights the establishment of enabling technologies involved in pursuing the goal of personalized medicine. It is important that regulatory agencies, clinicians, and scientists establish collaborations to address the challenges surrounding this field.

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These challenges include enhancing approaches for the development of biomarkers, minimizing the cost of drug development, and delving into the contribution of next-generation sequencing tests in drug development.

Keywords

Biomarkers · Personalized medicine · Cancer · Screening · Diagnosis · Prognostication

10.1 Introduction

The Food and Drug Administration (FDA) has defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological and pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Food 2014). A biomarker is simply an indicator of an alteration in normal physiology. A biomarker can be any distinct alteration of DNA, RNA, or protein. Among other applications, biomarkers are used as tools for the early detection of cancer and the development of individualized treatment (Patel 2014; Ogunwobi et al. 2020; Pellino et al. 2018).

Biomarkers can be divided into various groups based on their biology, measurement, and purpose (Grecchi et al. 2012). Several categories of biomarkers defined by the FDA (Group 2016) and the European Medicines Agency (Barcikowska 2018) have been reviewed in an article by Karen D Davis and coworkers (Davis et al. 2020). With respect to biology, molecular, physiological, or morphological characteristics can be used as biomarkers. Currently, scientists working with translational and personalized medicine prefer molecular markers. However, physiological and morphological markers still play important roles in clinical assessment (Banin Hirata et al. 2014). The generation of objective measurements is a crucial characteristic of a biomarker, so that assay results are obtained with little or no dependence on the subjective decisions of the observer. Biomarker tests can produce quantitative, semiquantitative, or qualitative results. Biomarkers can be further subgrouped into drug response or diagnostic markers. Several other types can be defined based upon their specific applications, such as disease monitoring and surveillance, prognosis, diagnosis, safety/toxicology assessment, pharmacodynamic analyses, and stratification (Landeck et al. 2016).

10.2 Discovery and Validation of Biomarkers

The process of biomarker development is a systemized and directed task, starting from recognition of the need for a biomarker followed by candidate biomarker discovery, initial identification, and preliminary proof-of-concept investigations. During this process, the degree of validation evidence supporting the use of the biomarker exhibits the prime importance, which reaches to the highest level whenever intended purpose of the biomarker enters clinical practice (Food and Drug

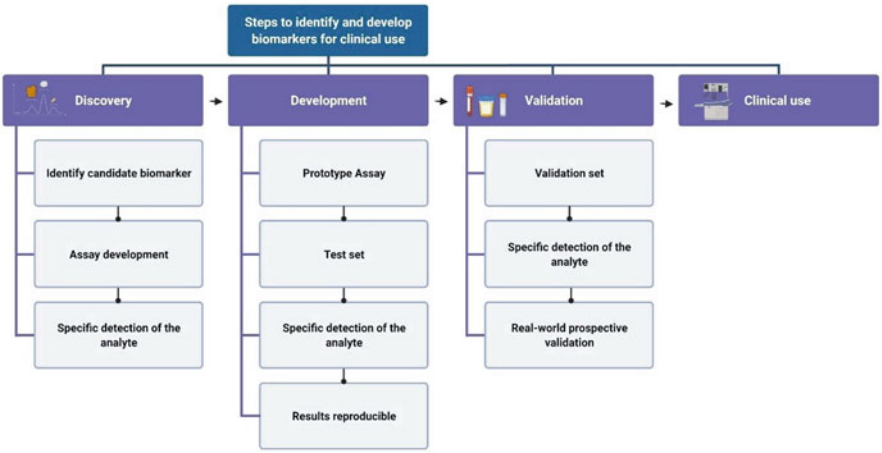

Fig. 10.1 Key steps for identifying and developing biomarkers for clinical use

Administration 2018) (Fig. 10.1). The biomarker development process may also include investigations concerned with the verification of reliability and accuracy of the detection method. Moreover, this process also encompasses the analysis of the connection between the biomarkers and the clinical outcomes.

Various levels of validation are required after the biomarker identification. Regarding analytical validation, it involves testing of the performance of the assay or detection technology in a way that is feasible for the purpose of the biomarker. Precision, dynamic range, and sensitivity of the detection method remain the noteworthy variables assessed during the analytical validation process. The clinical validation step is based on the assessment of the specificity and sensitivity of the biomarkers for identifying, measuring, or predicting the clinical outcome. Of note, specificity is linked with the rate of true negative findings, whereas sensitivity means the rate of true positive findings. In the biomarker validation process, the degree of evidence, required to provide the necessary confidence, is dependent upon context of use. The required degree of validation evidence is going to be increased, requiring further multisite validation data, as the context of use moves from research use to accepted utility in clinical trials/practice.

10.3 Biomarker's Role in Early Detection and Diagnosis

Identification of disease-based biomarkers is a crucial step of research supporting diagnosis and predicting prognosis in almost all types of human disorders. Biomarkers help establish guidelines for screening, response to treatment, and monitoring of disease progression. In the following sections, we use examples of certain critical biomarkers identified through various molecular biological

techniques for diffuse large B-cell lymphoma (DLBCL), which provide insights into disease mechanisms and pathogenesis.

10.3.1 B-Cell Lymphoma 2

B-cell lymphoma 2 (BCL-2), an oncogenic biomarker, is located on the mitochondrial outer membrane (Tilly et al. 2015). BCL-2 induces cell survival and inhibits apoptosis. BAX and BAK, pro-apoptotic proteins belonging to BCL-2 family, stimulate the release of cytochrome *c* from mitochondria, trigger the apoptotic signaling cascade, and are blocked by BCL-2 itself (Siddiqui et al. 2015). Considerable research has shown that BCL-2 chromosomal translocation t(14;18) occurs in DLBCL, resulting in elevation of BCL-2 levels as well as BCL-2-mediated resistance to the apoptotic cascade (Tilly et al. 2015; Akyurek et al. 2012) (Fig. 10.2). The presence of the BCL-2 chromosomal translocation t(14;18) has been observed in 20–30% of DLBCL cases, and is often associated with GCB-DLBCL-like variants (Akkaya et al. 2016). When this translocation is present, the cells become immortalized because of an elevated expression of BCL-2. High BCL-2 expression in DLBCL results in poor prognosis and shortened life span (Adams et al. 2019; Kawamoto et al. 2016). The inclusion of rituximab to standard chemotherapy helps to overcome the impact of BCL-2 on adverse prognosis (Chiappella et al. 2017; Frei et al. 2013). The prognosis remains consistently poor in "double hit lymphoma" (DHL), in which a BCL-2 translocation goes along with a translocation of MYC [t(8;14) for MYC, and t(14;18) for BCL-2] (Kawamoto et al. 2016). Patients representing lymphoma cells coexpressing BCL-2 and MYC showed a good response to ABT-737 (specific inhibitor of BCL-2), suggesting a key role for BCL-2 in DHL (Mason et al. 2008; Li et al. 2019).

10.3.2 B-Cell Lymphoma 6

Chromosomal translocations and mutations result in B-cell lymphoma 6 (BCL-6) being deregulated. Mice, which were engineered to constitutively express BCL-6, developed DLBCL in germinal center (GC) B cells (Baron et al. 2004; Cattoretti et al. 2005). Due to mutations in the BCL-6 locus, BCL-6 appears to be constitutively active in individuals with active B-cell lymphomas (Ye 2000; Cerchietti et al. 2010). Aberrant blockage of the BCL-6 repressive function causes genetic instability, which ultimately leads to neoplastic transformation (Aquino et al. 2014; Shustik et al. 2010). BCL-6 has also been shown to autoregulate its own transcription, and indirectly increases the expression of several genes, which then induce GC reactions (Basso et al. 2012). B lymphocyte-induced maturation protein 1 (BLIMP1) displaying a zinc finger domain (PRDM1)/B participates in the terminal differentiation of GC B cells to plasma cells, and is one of the protein directly regulated by BCL-6 (Alkodsi et al. 2019; Pasqualucci et al. 2006). PRDM1 appears to specifically inactivate ABC-DLBCL. The deregulation of BCL-6 and inactivation of PRDM1/

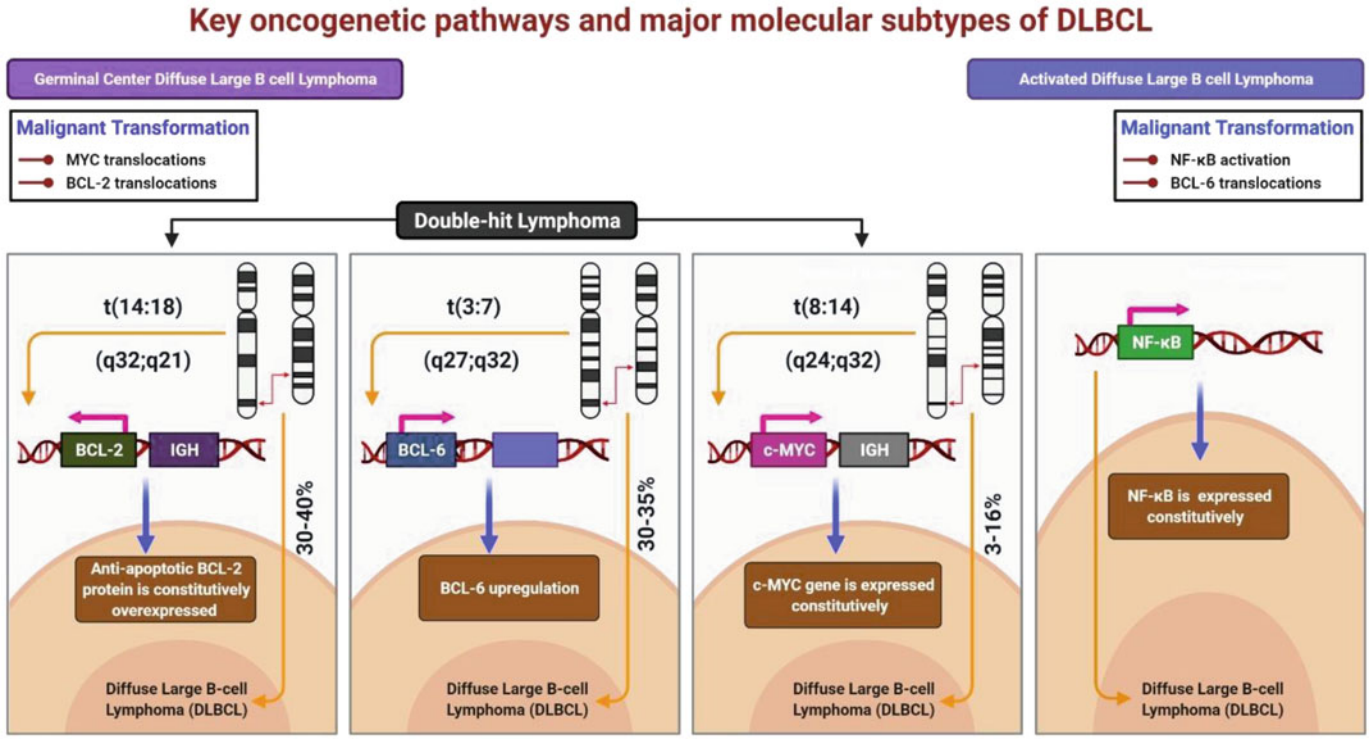

Fig. 10.2 DLBCL can develop from diverse oncogenetic alterations in B cells. Somatic hypermutations, gene amplification, and translocation of genetic material are the main oncogenic pathways involved in the development of DLBCL. The two important subtypes of DLBCL, the germinal center, and the activated type, have been described

BLIMP1 indicates the existence of alternative pathogenetic pathways causing inhibition of postGC differentiation; subsequently promoting lymphomagenesis (Pasqualucci et al. 2006; Vrzalikova et al. 2011; Wagner et al. 2011). The BCL-6 translocation and hypermutation at chromosome 3q27 with t(3;7) (q27;p12) was shown in 30–35% cases of DLBCL (Shustik et al. 2010), and frequent somatic mutations also occur in this chromosomal region. BCL-6 rearrangement is linked with poor outcomes in patients receiving rituximab, cyclophosphamide, vincristine, doxorubicin, and prednisone (R-CHOP) (Shustik et al. 2010). Barrans and colleagues' study found that individuals with poor prognosis had BCL-6 rearrangements as well as MYC translocation and BCL-2 deregulation. This study demonstrates that the rearrangement of BCL-6 rarely appears as a sole genetic disorder in DLBCL (Barrans et al. 2010).

10.3.3 Nuclear Factor Kappa-B

Nuclear factor kappa-B (NF-κB) is one of the family of inducible transcription factors that is responsible for regulating multiple genes involved in a range of immune and inflammatory responses. NF-κB can modulate biological processes, such as stress responses, inflammation, B cell development, and lymphoid organogenesis (Hayden and Ghosh 2011). The activation of NF-κB is essential for growth and survival of various types of cancer cells. Lymphoid malignancies evade apoptosis by the constitutive activation of NF-κB signaling (Park and Hong 2016; Hoesel and Schmid 2013). Both the canonical and alternative NF-κB pathways get activated in DLBCL (Compagno et al. 2009; Davis et al. 2010; Nagel et al. 2014; Zhang et al. 2015). Activated B-cell (ABC) DLBCLs show classical NF-κB activation, as they have the potential for rapid phosphorylation and show frequent nuclear translocation of p50/p65 heterodimers, while showing minor nuclear translocation of p50/c-rel heterodimers (Davis et al. 2001). RelA/p65 and p50 are the major subunits of NF-κB participating in the classical NF-κB pathway, and nuclear translocation of RelA/p65 is significantly linked with poor survival in individuals with early stage DLBCL (Zhang et al. 2016). Multiple receptors, including CD40, BCR, and B-cell-activating factor, stimulate the NF-kB pathway in B cells (Hoesel and Schmid 2013; Ying et al. 2013; Young et al. 2015). The NF-κB activation is believed to be a hallmark of ABC–DLBCL (Camicia et al. 2015). Compared to GCB–DLBCL, a greater number of NF-κB-regulated genes appear in ABC–DLBCL. Hence, ABC-DLBCL lines are highly sensitive to the blockage of NF-κB. Mutations in CARD11 (a part of the CBM), stimulate the activity of NF-κB in ABC–DLBCL (Jiang and Lin 2012; Zachos et al. 2005).

10.3.4 MYC

MYC is a key regulator of cell proliferation and metabolism. Many oncogenic pathways stimulate MYC leading to malignant transformation (Miller et al. 2012).

The recombination of MYC with other genes has been observed in 3–16% of DLBCL cases (Akyurek et al. 2012; Montero et al. 2018). The frequently occurring t(8;14) (q24;q32) translocation involves MYC rearrangement in GCB–DLBCL, resulting in its upregulation (Akyurek et al. 2012; Kawamoto et al. 2016; Akkaya et al. 2016). In DLBCL, the fusion of MYC and Ig is known to result in the upregulation of MYC expression. A meta-analysis revealed that rituximab treatment did not overcome the consequences of MYC translocations (Zhou et al. 2014). In DLBCL patients who received R-CHOP therapy, MYC served as a prognostic factor, although these findings need further investigation (Akyurek et al. 2012). The presence of an n-MYC rearrangement in DLBCL patients is often linked with poor outcome (Chastain and Duncavage 2015; Logothetis 2014).

10.4 Role of Biomarkers in the Early Detection of Colorectal Cancer (CRC)

In order to enhance survival outcomes in individuals with asymptomatic CRC, early diagnosis is crucial. The sensitivity of CRC detection utilizing current FIT testing (100 ng/mL) was 73.8% compared with 92.3% for a stool-based DNA test (Stiell et al. 2003). The sensitivity of FIT testing for analyzing advanced precancerous lesions remained at 23.8% compared with 42.4% with stool DNA assays (Stiell et al. 2003). These parameters indicate the shortcomings of current diagnostic testing and indicate the difficulty of establishing reliable markers for the early detection of CRC. Ongoing noninvasive screening of stools is not sufficiently efficient and sensitive for detecting precancerous lesions with any confidence and may miss notable numbers of early CRC cases. It is, therefore, necessary to maintain a low threshold at which patients undergo the more invasive colonoscopy, and to use novel, advanced tools for identifying early CRC.

Prognostic biomarkers, including early recurrence and mortality rates, can be used to predict the progression of CRC (Patel et al. 2019; Pellino et al. 2018). A good example of the use of prognostic biomarkers is the use of KRAS, a member of the RAS proto-oncogene family of GTPases. Mutations in KRAS lead to an increased risk of recurrent metastatic CRC (Tsuchida et al. 2016; Margonis et al. 2015; Tie et al. 2011). Mutations in the BRAF are linked with decreased survival, encompassing progression-free survival, and up to 50% worse overall survival compared to wildtype BRAF (Guo et al. 2015; Venderbosch et al. 2014; Yokota et al. 2011) (Fig. 10.3). In the novel field of radiogenomics, prognostic sensitivity can be increased by the use of a combination of radiological and genetic features, which attain a higher sensitivity than can be achieved by either of these modalities in isolation (Badic et al. 2019; Horvat et al. 2019). A high-molecular-weight glycoprotein, carcinoembryonic antigen (CEA), has been successfully used as a biomarker in the detection of early recurrence in postoperative patients, although it exhibited despite low specificity and sensitivity (Chao and Gibbs 2009; Koulis et al. 2020). Investigators are hopeful that prognostic markers will change the thresholds at which

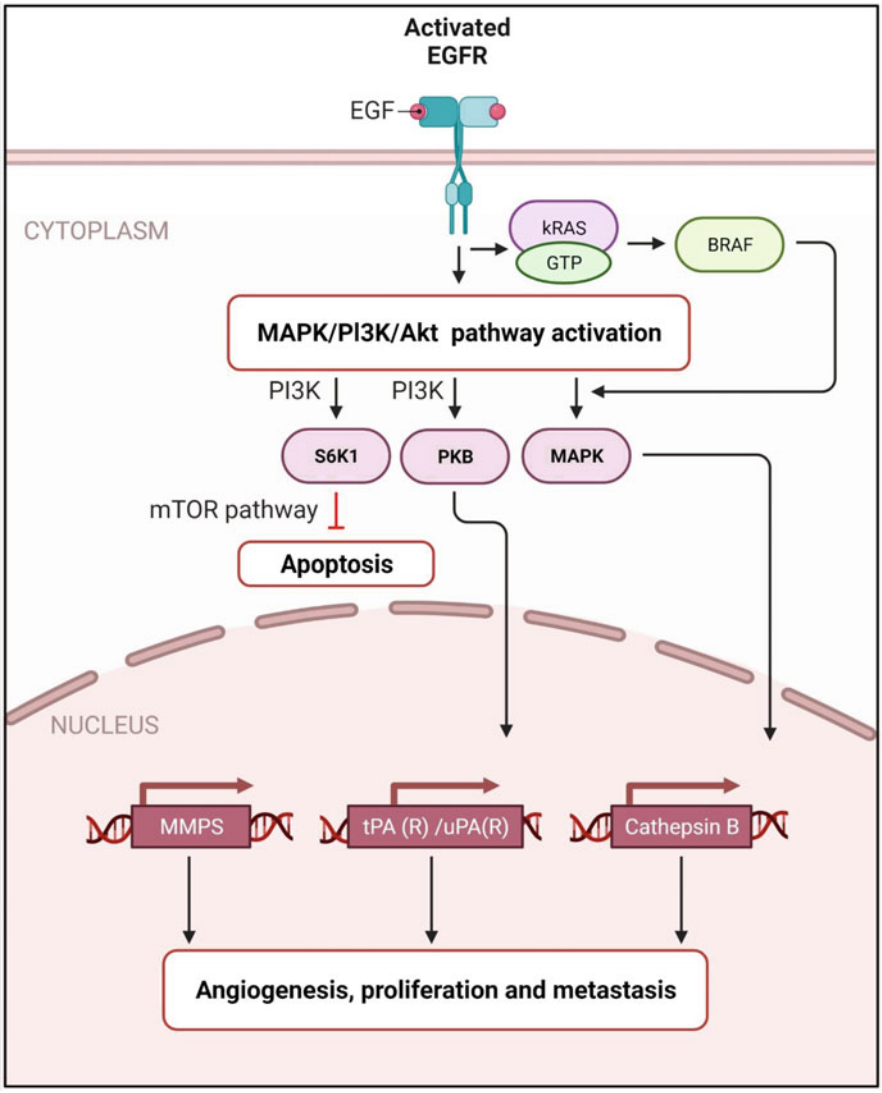

Fig. 10.3 Intracellular signals for CRC manifestation via EGFR. Activation of EGFR results in a change from GDP- to-GTP form of the KRAS, leading to increased concentrations of BRAF to the plasma membrane. BRAF activation leads to the stimulation of MAPK signaling pathway, which subsequently regulates proteins involved in angiogenesis, proliferation, and metastasis

individuals are given more potent therapy, provide further insights into recurrent disease, and improve the chances of early detection and intervention.

Predictive biomarkers can be used to tailor individual therapies according to molecular subtype. Mutations in KRAS are linked with poor responses to therapy with anti-EGFR receptor agents, including panitumumab and cetuximab (Karapetis

et al. 2008; Amado et al. 2008). Compared to the 4% decrease in KRAS mutants, a 16% increase in overall response rate was observed in patients with KRAS wildtype who received cetuximab and FOLFIRI. A topoisomerase inhibitor, irinotecan, utilized as part of a FOLFIRI strategy, is metabolized by diphosphate-glucuronosyltransferase 1A (UGT1A). Homozygosity for the UGT1A1*28 allele is linked with dose-dependent toxicity compared to the UGT1A1*1 genotype (Palomaki et al. 2009). Dihydropyrimidine dehydrogenase (DPD) metabolizes more than 80% of 5-FU (Koulis et al. 2020). DPYD*13 and DYPD*2A variants, however, contribute to increased toxicity of the treatment, and there is evidence that a reduction in 5-FU dose by 25–50% reduces its toxicity (Amstutz et al. 2018). These strategies may improve response to treatment, and decrease the toxicity resulting from ineffective interventions. They can also assist in making the adjustment of drug doses, to produce maximum benefit from a specific regimen. Although several biomarkers are currently under investigation, there is a clear need for more, and more effective, biomarkers. To date, only NRAS, KRAS, MSI, and BRAF status are recommended by national guidelines, for use in following CRC therapy response and predicting outcomes (Shinagawa et al. 2018).

10.5 Potential Biomarkers in Skin Cancer

Biomarkers have been extensively studied, and their use is well established, in skin cancer. Prognostic biomarkers are the most important type of biomarker in skin cancer. Tumor thickness is believed to be one of the most important and oldest prognostic biomarkers in skin cancer. The expression nuclear cell proliferation factor Ki-67 is another important example of a biomarker being used clinically (Gimotty et al. 2005). In ulcerated melanomas, there is close correlation between survival and CD2 count and number of tumor-infiltrating lymphocytes (de Moll et al. 2015). The presence of tumor marker protein S100 beta in blood is utilized to assess disease progression in skin cancer (Forschner et al. 2010). By the use of highly sensitive assays, KIT D816V can be detected in peripheral blood leucocytes from most patients with systemic mastocytosis, and is considered as a major step in early diagnosis of the disease (Arock et al. 2015). Active nuclear I kappa-B kinase is correlated with the risk of metastasis of cutaneous squamous cell carcinoma (Toll et al. 2015).

10.6 Biomarkers for Asthma

Asthma is a highly heterogeneous disease with several underlying mechanisms; different subsets or clinical phenotypes respond differently to standard therapy (Seys et al. 2019; Kuruvilla et al. 2019). Biomarkers have been validated for Type 2 asthma (Diamant et al. 2019). Sputum eosinophils or blood eosinophil counts, FeNO, and serum specific IgE have all been identified as important clinically applicable biomarkers (Alving et al. 2020; Diamant et al. 2019). The biomarkers

reflect different features of Type 2 inflammatory signaling, although some overlapping of Type 2 biomarkers can occur within individuals (Diamant et al. 2019). These biomarkers, along with specific clinical characteristics, have led to current guidelines using algorithms adapted to their use, which are hoped to be of value in predicting responses to therapies, and can be utilized to monitor subsequent therapeutic responses (Holguin et al. 2020; Agache et al. 2021). There are some confounders of the existing biomarkers. Fractional exhaled nitrous oxide (FeNO) has been shown to be correlated with dietary nitrate intake, smoking, virus infections, and bronchoconstriction, whereas systemic corticosteroids and parasites have been reported to be the most common culprits for circadian variation in blood eosinophils (Diamant et al. 2010). Oxidative stress is caused by an excess of reactive oxygen and nitrogen species. Investigators have reported multiple direct or indirect markers of oxidative stress, including glutathione disulfide, malondialdehyde, bromotyrosine, thiobarbituric acid, and isoprostane in plasma, urine, BAL fluids, and sputum of individuals with asthma. The levels of these markers were linked with the severity and clinical output of the disease (Comhair et al. 2000; Comhair and Erzurum 2002). The collection of exhaled breath condensate is another noninvasive analytical approach, which allows direct measurements of H_2O_2, pH changes, and numerous indirect by-products of oxidation, such as ethane and 8-isoprostane (Aldakheel et al. 2016; Thomas et al. 2013). The detection of high levels of urinary bromotyrosine represents another important noninvasive biomarker of oxidative stress for clinical use in patients with asthma (McDowell and Heaney 2020; Sze et al. 2020).

10.7 Significance of Biomarker Strategies in Drug Development

The significance of personalized strategies has been tested in phase I, II, and III clinical trials. A meta-analysis of phase I trials published over a 3-year period included 13,203 patients. It was found that, compared to approaches that did not utilize a biomarker, the biomarker-based cancer therapeutic strategies produced a longer median progression-free survival (PFS) time, and an improved response rate (Schwaederle et al. 2016). Phase II clinical trials were also reviewed in a meta-analysis of single-agent studies published over a 3-year period. Here also, the biomarker-based approach gave a higher median response rate, longer PFS, and better overall survival. Nonpersonalized targeted approaches had poorer outcomes than personalized, targeted strategies. The personalized strategies proved to be safer, and resulted in a lower treatment-induced death rate (Schwaederle et al. 2015) (Fig. 10.4). These investigations suggest that personalized therapy produces better outcomes, and may improve the effectiveness of cancer therapies during all phases of drug development (Schwaederle et al. 2015, 2016). The clinical utility of personalized medicine has, therefore, been established, at least for some biomarkers, but the cost/benefit ratio of targeted therapy is still a subject of debate (Aitken et al. 2018). Higher treatment costs may be ascribable to a longer treatment time due to enhanced survival instead of higher monthly drug costs (Chawla et al. 2018). The financial return from newly launched personalized drugs comes at a higher initial

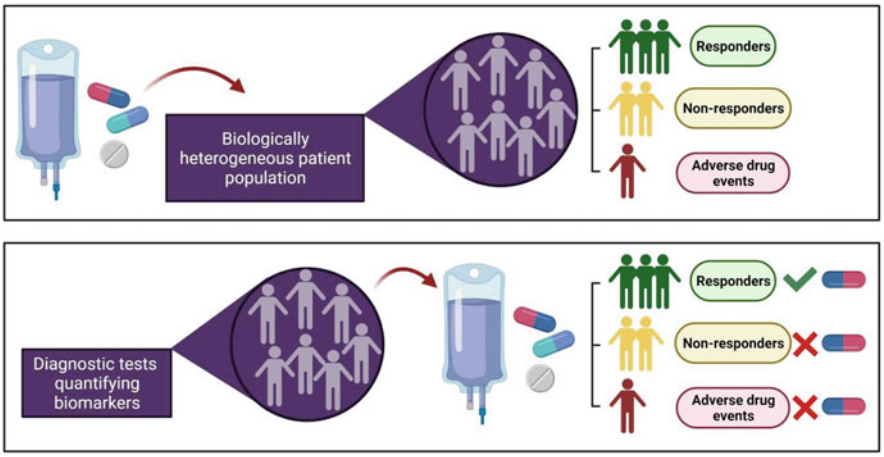

Fig. 10.4 General idea of personalized medicine. Biological variability can result in different outcomes (beneficial or harmful) for a population of patients (upper panel). Prediction of biomarker-based treatment response helps select appropriate patients for treatment and avoids the high risk of adverse drug incidents (lower panel)

investment, and use is prolonged due to better efficacies. It is expected that advanced technologies like artificial intelligence will influence cancer treatment and costs in the future (Mak and Pichika 2019).

10.8 Personalized Medicine in Conventional Therapeutic Approaches

New advances in the understanding of the underlying mechanisms of cancer have opened a new horizon of personalized medicine. One example is that of Bacillus Calmette–Guérin (BCG) vaccine therapy in non-muscle-invasive bladder cancer (NMIBC). During the year 1976, Morales and colleagues introduced the idea of utilizing BCG as a therapeutic and preventive approach in NMIBC (Moss and Kadmon 1991). BCG antigens provoke an immune response which attacks tumor cells, resulting in an anti-neoplastic effect when instilled during therapy (Saad et al. 2017). However, BCG treatment failed in many bladder cancer patients, and nearly 40% of them experienced recurrence (Alhunaidi and Zlotta 2019; Zlotta et al. 2009; Ślusarczyk et al. 2019; Lima et al. 2013). The ability to identify patients unlikely to respond to treatment would save time and hence, avoid progression of disease. Many investigations have been carried out to identify biomarkers which could be used to predict patient response to BCG. Good biomarkers would help physicians to effectively select candidates, and put poor responders on an alternative therapeutic strategy (Kamat et al. 2016). Patients with mutations in the AT-rich interaction domain 1A (ARID1A) were highly prone to recurrence of NMIBC after BCG therapy. This study suggested that the screening of BCG candidates could give

useful insights into patient prognoses (Pietzak et al. 2017). However, further investigations are needed to demonstrate the functionality of ARID1A as a reliable biomarker for BCG therapy. Researchers have also struggled to establish the predictive value of the tumor suppressor protein p53, for response of BCG in bladder cancer (PAGES et al. 1998; Berggren et al. 2001). p53 mutation was not found for predicting clinical response, but was utilized to predict cancer prognosis (Du et al. 2016; Malats et al. 2005). Cell adhesion molecules like sialyl-Tn (STn) and sialyl-6-T (s6T), which play roles in cell–cell adhesion and immune responses, were also included in trials for BCG response (Pinho et al. 2007). STn, alone or in combination with s6T, appeared to be linked with lower recurrence rates after BCG instillation, although the underlying mechanism remains poorly understood (Lima et al. 2013; Severino et al. 2017). Researchers also studied ezrin, a cell adhesion molecule, during BCG response, and found that the loss of ezrin was correlated with reduced survival (Palou et al. 2009; Andersson et al. 2014). During an investigation into BCG nonresponders versus responders, Kates et al. found that programmed death ligand-1 (PD-L1) appeared in nearly 25% and 4% of BCG nonresponders and responders, respectively. This study suggested that PD-L1 can be involved in the NIMBC-induced resistance to BCG therapy (Kates et al. 2020). As recent investigations lack standardization regarding response measurement criteria, study validation techniques, and cutoff points, further intensive and qualitative investigations are required to find a single biomarker which could be used to predict patients response to BCG therapy (Kamat et al. 2018).

10.9 Personalized Medicine in Novel Therapeutic Strategies

The existence of fibroblast growth factor receptor (FGFR3) mutations, fusions, and amplifications have been found in numerous tumors, including bladder cancer (Nogova et al. 2017). It has been shown that FGFR3 appears in bladder cancer preferentially in low-grade NMIBC, which indicates that FGFR3 may serve as a crucial marker for disease severity and management (Akanksha and Sandhya 2019). Researchers have utilized different techniques, such as the development of monoclonal antibodies and selective tyrosine kinase inhibitors, to interfere with FGFR3 signaling (Paul and Mukhopadhyay 2004; Qing et al. 2009). B701, a fully humanized immunoglobulin, resulted in significantly improved survival when included to novel PD-1 inhibitors or traditional chemotherapeutic agents (Holash et al. 2016). MFGR1877S, an antibody targeting the FGFR3 receptor, and LY3076226, a FGFR3 antibody conjugated to a cytotoxic drug (DM4), have shown promising results, and are currently in Phase I clinical trials (Qing et al. 2009; Surguladze et al. 2019). Investigators have also attempted to influence FGFR3 signaling at a more distal point utilizing tyrosine kinase inhibitors (TKIs). Pazopanib, a potent TKI, has shown partial responses in 7 out of 21 patients in a Phase II clinical testing (Necchi et al. 2012). During Phase II clinical trials, pazopanib monotherapy in individuals with advanced urothelial cancer (UC), showed partial response in seven patients and stable disease in 14 out of 41 patients

(Necchi et al. 2012). Although pazopanib showed encouraging results, two other TKIs, brivanib, and dovitinib, failed to show a strong response (Milowsky et al. 2014; Hahn et al. 2017; Ratain et al. 2011). It has been noted that drug molecules showing a more specific effect on the tyrosine kinase domain of FGFR produce more optimistic results. For example, erdafitinib, a small molecule inhibitor of FGFR approved for treating advanced or metastatic UC and marketed under the name Balversa, which harbors FGFR2/3 alterations, gave a response rate of up to 40%, although 37% of the responses were partial. However, the response rate was almost 60% among patients who previously received immunotherapy (Loriot et al. 2019). A few other TKIs, like infigratinib, AZD4547, and pemigatinib, are currently under trial (Marandino et al. 2019; Jones et al. 2016; Merz et al. 2021).

Boosting host immunity by blocking inhibitory receptors is another strategy extensively used in bladder cancer (Khalil et al. 2016). The primary signal for the stimulation of T cell is the recognition of antigens by the T-cell receptors (TCRs) presented by APCs via MHC. A second signal for T cell activation involves the binding of T cell CD28 with CD80/86 on APCs. The two most common immunomodulatory molecules, CTLA-4 and PD-L1, inhibit this interaction. CTLA-4 plays its inhibitory role in blocking the secondary signal by competing for CD80 and CD86 binding (Collins et al. 2002; Parry et al. 2005). PD-L1 blocks downstream TCR signaling and results in the inhibition of T cell responses (Sage et al. 2018). Strategies which block the inhibitory effects of these molecules would allow the immune system to attack the tumor more aggressively.

10.10 Bioengineering and Personalized Medicine

The concept of medicine is diverging from the "one size fits all" mentality. It usually occurs that patients having same disease respond differently to drugs. Therefore, now is the time to deeply understand this response and provide patients with individual treatment. Biomaterial engineers, specifically, can play a crucial role in making personalized medication a reality. Biomaterials can present different effects on cell growth and survival, and it is highly recommended that they should be screened via high-throughput approaches for a given application. For example, a dextran–dendrimer composite was shown to work as an adhesive differently in colon cancer than in colitis, which involves the same organ with a different environment (Artzi et al. 2009; Oliva et al. 2015). These studies suggest that the use of biomaterials cannot be generalized, and they must be designed according to the organ environment. The appropriateness of biomaterials for certain organs, tissues, or cells can be determined using a combination of small and large animal models (Vegas et al. 2016a, b; Lind et al. 2017). To avoid the use of living models, different extracellular matrix formulations can also be utilized to observe the effects of biomaterials on cell differentiation, proliferation, and apoptosis (Beachley et al. 2015). Optimal biomaterial formulations produced via novel engineering platforms can enhance personalized biocompatibility and therapeutic outcomes.

There is an emerging idea of "organ-on-a-chip platforms" for individualized drug-screening investigations. Scientists have developed a microfluidics-based model of human intestine, in which they recreated the complex gut microenvironment. This model paved the way for monitoring the interactions between the immune cells, gut microbiome, and bacteria. It also opened the way for the observation of the pharmacokinetics, absorption, and metabolism of drugs (Bein et al. 2018; Prantil-Baun et al. 2018). Drug pharmacokinetics, absorption, and metabolism potentials may also be used for designing personalized therapeutic strategies. Accurate and timely detection of treatment response is needed for accurate personalized treatment; the latter includes parameters, such as appropriate drug selection and dosing regimens. The commonly used techniques for acquiring these parameters, include urine and serum analyses, or imaging modalities, such as X-rays, MRI, CT, and ultrasound, which could be narrow in terms of testing frequency. It has been demonstrated that wearables and other novel technologies can help in overcoming the problem of infrequent measurements, which would thereby improve the design of personalized therapeutic strategies (Blicharz et al. 2018). Over time, new breakthroughs in personalized medicine have been introduced, encompassing the application of nondrug-based strategies like digital therapies, to cope with conditions such as cognitive impairment, mental health, and substance abuse (Kee et al. 2019; Davis et al. 2018; Cho and Lee 2019) (Fig. 10.5).

A common feature of these strategies is their ability to utilize only a subject's own data to direct only their own care. This approach has been exemplified regarding artificial intelligence-driven drug dosing and engineered cell therapy. Another advantage in the connection of personalized medicine and engineering is the parallel adjustment of intervention and diagnosis for ongoing therapy optimization.

10.11 Personalized Cell Therapy and Drug Delivery

A major advancement in personalized cancer treatment is the approval of chimeric antigen receptor T-cell (CAR-T) immunotherapy. Tisagenlecleucel (Kymriah, Novartis) was the first approved CAR-T therapy, which is being used for treating patients with acute lymphoblastic leukemia. During this treatment, T cells are removed from patient, reprogrammed, and expanded in a processing facility and are finally introduced to the patient (Prasad 2018). Axicabtagene ciloleucel (Yescarta, KITE Pharma/Gilead Sciences) has recently been approved for the treatment of aggressive non-Hodgkin's lymphomas (Roberts et al. 2018; Mullard 2017). Scientists worldwide are working to broaden the indications that are managed utilizing CAR-T. The approval of CAR-T remains an ideal shift for the FDA towards efficacious and safe living cell therapies. It has been demonstrated recently that nonviral approaches, like sleeping beauty transposition, can improve the scalability of CAR-T for broader deployment (Monjezi et al. 2017). This technique is based on the use of simple DNA minicircles for inserting CAR genes, which effectively reduces the risk of genotoxicity and mutagenesis associated with viral modalities (Fig. 10.6). It also reduces the cost of CAR-T engineering and minimizes the

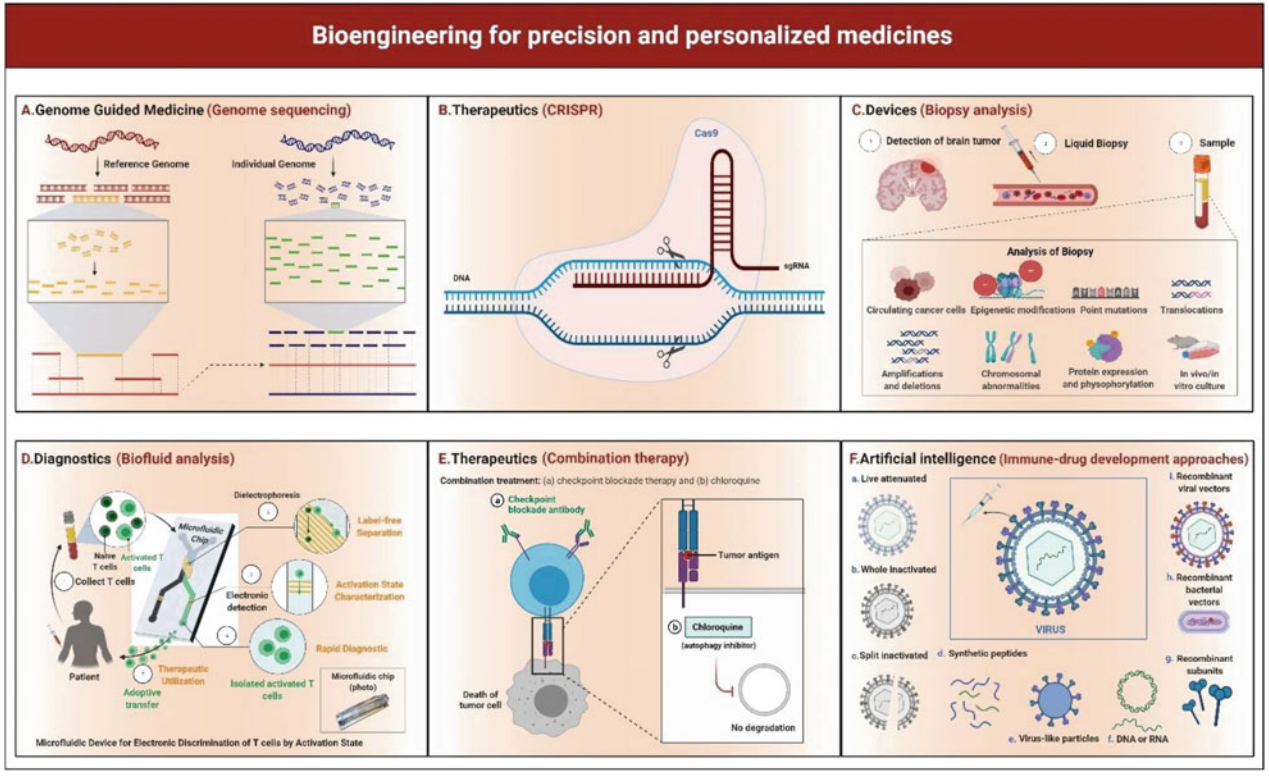

Fig. 10.5 From genome-guided medicine to CRISPR, multiple advanced and highly accurate technological platforms bridge engineering and personalized medicine together, improving clinical outcomes. Moreover, striking improvement in personalized medicine approaches may be realized by linking wearable technologies, artificial intelligence, and other engineering platforms

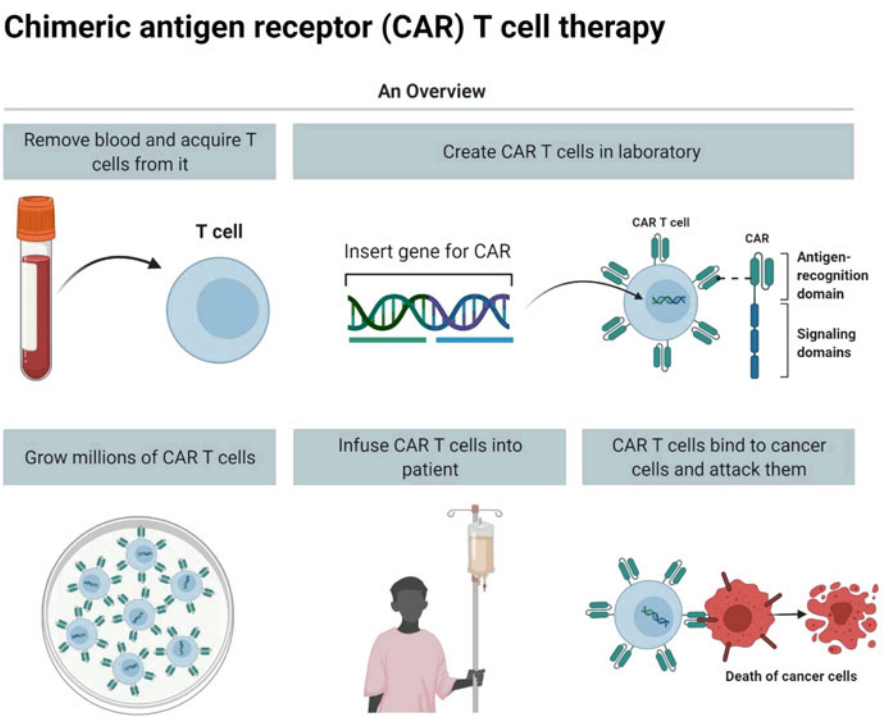

Fig. 10.6 How does engineered T cell therapy work? Enough blood is obtained from patients to collect T cells from it. T cells are purified and modified by viral vector transfection to express specific CARs/TCRs on their surface. Following amplification and quality control, engineered CAR-containing T cells are infused into the patient body to improve antitumor ability

regulatory hurdles faced. "Off-the-shelf" cell therapy, which does not need autologous T cells, is used by advanced engineering approaches for improving CAR-T manufacturing (Sadelain et al. 2017; Cooper et al. 2018; Ruella and Kenderian 2017). Zinc-finger nuclease technology, a novel tool composed of engineered DNA-binding proteins, facilitating targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations, is being utilized for modifying both allogeneic and autologous cell therapies. This technology is expected to broaden the chances of off-the-shelf CAR-T manufacturing, and significantly decrease the treatment duration (Dolgin 2018).

It is now possible to reprogram induced pluripotent stem cells, obtained from a patient, to a desired cell like a brain cell, a specialized kidney cell, or a beta cell, which can be introduced into the body and will carry out their specific functions (Vegas et al. 2016b; Peruzzotti-Jametti et al. 2018; Ma et al. 2018). Mitochondrial replacement therapy (MRT) is an additional example of cell therapy implemented in the United Kingdom. Mutations in mitochondrial DNA (mtDNA), referred to as mitochondrial disease, can also be maternally transferred to the offspring, and leads to severe disorders, such as deafness, epilepsy, optic neuropathy, and diabetes

mellitus. In MRT, the healthy nucleus from a maternal egg with malfunctioning mitochondria is transferred to a healthy egg, including the donor mitochondria, without a nucleus, which can result in a fertilized egg containing nuclear DNA from two parents and mtDNA from a donor, thus eliminating the genetic disorder in children (Saxena et al. 2018).

Another particularly relevant area is the cellular engineering. Scientists have designed synthetic cells for the early detection of malignancy and diabetes (Danino et al. 2015; Courbet et al. 2015). With the application of synthetic cells and biosensors, scientists become able to detect early disease markers and deliver therapeutic entities to improve symptoms. This is a major goal of personalized medicine, wherein the cell is an autonomous therapy and sensor, delivering therapeutic entities without physician or patient intervention. During an investigation, chronic and acute psoriasis was observed by a population of synthetic cells implanted in mice, an approach which provided a new opportunity for personalized medicine (Schukur et al. 2015).

Delivering therapy in a personalized fashion is also a promising approach. Personalized biomaterial-mediated controlled release or 3D printing technologies are being introduced to serve as cornerstones for improving drug delivery. A study reported the tailored release profiles from 3D-printed tablets, enabling the customization of the temporal administration of the drug (Sun and Soh 2015). A technique termed stamped assembly of polymer layers (SEAL) has been successfully utilized to produce drug-loaded 3D microstructures with temporal drug release control (McHugh et al. 2017). Specific responses of individuals to combination therapy are often monitored by unique dosing profiles. Hence, these microstructures and tablets can be a feasible drug delivery platform for personalized medication.

10.12 Concluding Remarks and Future Directions

Many drugs show efficacy only in a subgroup of patients (Laserna-Mendieta et al. 2020; Wang et al. 2021). Therefore, the "hit-or-miss" utilization of these drugs is costly, ineffective, and puts patients at risk. Drug development is expensive and the number of FDA-approved drugs per billion US dollars of spending decreases by half every 9 years. The cost of launching a new drug exceeds one billion euro, and this exorbitant price raises concerns (Scannell et al. 2012). The era of personalized and precision medicine is expected to solve this problem. In this era, patients will no longer be restricted to dose escalation-defined administration protocols and target-based drug selection.

High failure rates are a major cause of the high research as well as development costs involved in discovery of drugs. Less than 1% of drug development projects launched result in the approval of a new drug. It has frequently been observed that, after several years of significant investments, drugs fail late in the clinical trials. It is alarming that the traditionally low success rates for new drug development projects in Phase II clinical trials decreased even further from 28% to 18%. In the past few years, insufficient efficacy has remained the most frequent reason for failure

(Arrowsmith 2011). Personalized medicine can help to address this challenge. Smaller sized clinical investigations conducted using biomarker-based stratification can show better results. It is recommended, even in clinical trials lacking a stratification strategy, to include biomarker candidates. It would also be supportive to acquire patients' informed consent, to enable retrospective assays conducted later. If retrospective assays produce favorable outcomes in biomarker candidates, these findings need to be verified in another prospective clinical study. Although such a protocol may increase the cost of the initial study, it could rescue a project. To support findings related to dosage, pharmacodynamic biomarkers are also recommended to be added more rigorously in clinical studies. This approach will have the benefit of increasing tolerance and establishing recommended dosage. Moreover, this approach could provide important experience in case of study failure. In this case, the pharmacodynamic biomarker represents a full-target engagement, showing that the target is not relevant to the disorder. When a biomarker engages the target insufficiently, it can indicate that the compound, rather than the efficacy of the molecular target, is the cause of the problem.

Scientists are of the opinion that future medical products can be introduced as a "double pack": the drug molecule and the diagnostic assay to identify the feasibility of the patient for this approach. This approach requires the creation of consortia, for closer collaboration between the pharmaceutical and the diagnostic industry (Salter and Holland 2014). Various models are in use for various categories of biomarkers, and many more are needed for different stages of the drug discovery and development process (Asadullah et al. 2015; Lessl et al. 2011; Dorsch et al. 2015). Studies have shown major inconsistencies between the number of biomarkers identified and the number reported ($\leq$150,000) and the few ($\leq$100) entered in clinic trials (Poste 2011). The reproducibility of publications is also an important issue (Prinz et al. 2011). High transparency and more coordinated efforts are needed for biomarker discovery, development, and validation, involving collaborations between academia and the diagnostics and pharmaceutical industry, since this process requires significant resources and complementary skills (Landis et al. 2012; Asadullah et al. 2015).

The Biomarkers Consortium (URL), the predictive safety testing consortium (URL), and the Coalition Against Major Diseases (URL) are noteworthy examples of successful consortia in the biomarker area, and it is expected that the number of collaborations would increase in future (Wholley 2014; Stephenson and Sauer 2014).

Scientists are expecting changes in the number of biomarkers tested. Currently, only one biomarker is used to guide a treatment protocol, whereas future molecular diagnostics may result in the simultaneous comprehensive profiling of several markers. This approach reflects a movement from the use of a single marker to a signature, which would allow us to choose the most suitable and potent therapeutic combination for each patient. Although in the discovery of biomarkers, panels of markers are frequently already measured, much remains to be done in validating candidates' biomarkers. Further improvements in precision and personalized medicine in the present population are required for a successful transfer of validated biomarkers and personalized medicine platforms into the clinical setting. Several

technology-linked validation challenges associated with ethics, healthcare economics, and data privacy need to be addressed (Reddy et al. 2020; Cohen 2019; Dinh-Le et al. 2019; Lee et al. 2019). Biomedical engineering, which is currently playing a key role in breakthroughs, is expected to eventually improve the human condition in an individualized fashion.

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Development of Novel Cancer Biomarkers for Diagnosis and Prognosis

11

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Abstract

In the past few decades, extensive research has been carried out to find novel biomarkers for various cancers. Cancer biomarkers play an important role in the screening, diagnosis, and posttreatment prognosis of patients in all the stages of the disease. The utilization of biomarkers is multifaceted; from screening healthy patients for risk assessment to timely diagnosis, accurate staging, patient stratification into risk groups, determining prognosis, and continued surveillance. Biomarkers play an indispensable role in both diagnosing and managing cancer treatment. Recent advancements in the field of precision medicine have led to the discovery of novel diagnostic and prognostic cancer biomarkers. Different protein-based, RNA, DNA, miRNA, and SNP-based biomarkers have been identified and developed as accurate, noninvasive, and cost-effective alternative towards managing different neoplastic diseases. The rapid progression in the field of diagnostic and prognostic biomarkers has led to the development of companion diagnostics and targeted therapies for the treatment of cancer patients, which has resulted in improved diagnosis and preventing unnecessary chemotherapy along with the associated toxicities.

Keywords

Biomarkers · Noninvasive · Prognosis · Risk · Diagnosis · Cancer

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11.1 Introduction

In the past few decades, extensive research has been carried out to find novel biomarkers for various cancers. Cancer biomarkers play an important role in the screening, diagnosis, and posttreatment prognosis of patients in all stages of the disease (Sanhueza and Kohli 2018). The utilization of biomarkers is multifaceted; from screening healthy patients for risk assessment to timely diagnosis, accurate staging, patient stratification into risk groups, determining prognosis and continued surveillance (Fig. 11.1).

Researchers are keen to develop cancer biomarkers that can be quantified easily. There has been a global interest in developing specific and highly sensitive markers which may provide reliable and reproducible results. This can be achieved by the development of novel noninvasive and cost-effective approaches. Different protein-based, RNA-, DNA-, miRNA-, and SNPs-based biomarkers are developed and identified as a diagnostic and prognostic marker by utilizing biological samples of prostate cancer patients such as urine and prostate tissue, non-neoplastic tissue, blood, etc. (Fig. 11.2). Some of these biomarkers are approved by the FDA, while others are still under investigation (Sanhueza and Kohli 2018). In this chapter, novel biomarkers for various cancer types such as breast cancer, ovarian cancer, prostate cancer, lung cancer, leukemia, lymphoma, etc., are discussed in detail.

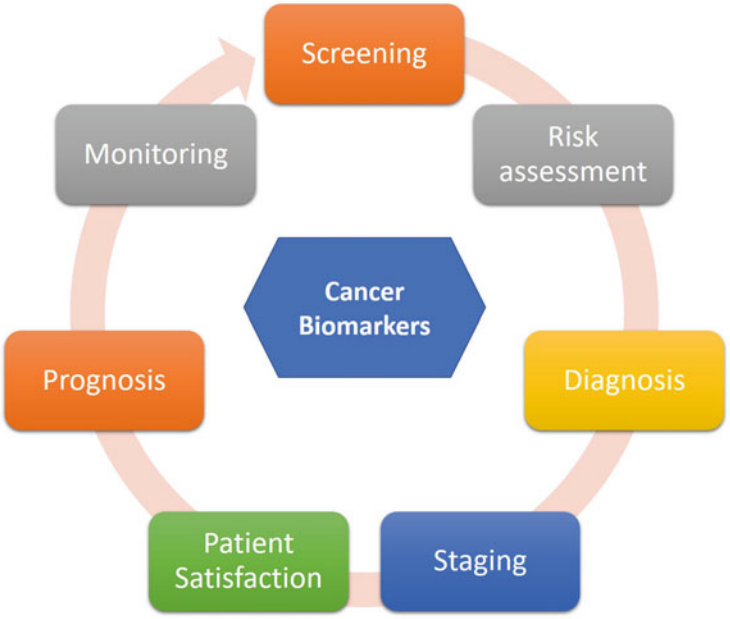

Fig. 11.1 Uses of the cancer biomarkers

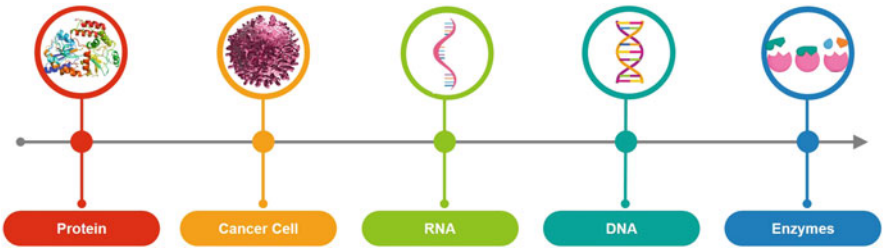

Fig. 11.2 Development of cancer biomarkers

11.2 Novel Biomarkers for Diagnosis and Prognosis of Prostate Cancer

Prostate cancer is one of the most common types of cancer in males. It often progresses slowly and stays limited to the prostate gland. Some kinds of prostate cancer are slow-growing and treatable, while others are advanced and can be fatal. An early diagnosis of prostate cancer in a patient increases the chances of successful treatment (Litwin and Tan 2017).

11.2.1 Serum Prostate-Specific Antigen (PSA)

Serum prostate-specific antigen (PSA), the serine protease member of the kallikrein family, is currently widely used as a prostate cancer biomarker (Tchetgen and Oesterling 1997). It has been broadly used as a marker in almost all prostate cancer stages, from initial screening to prognosis after receiving the therapy. It has also been used in clinical practice to monitor cancer recurrence and evaluate drug efficacy (Sanhueza and Kohli 2018; Tchetgen and Oesterling 1997). Unfortunately, the use of PSA is limited due to its poor specificity. It is not able to differentiate the indolent tumor from aggressive tumors. Therefore, it is now considered a suboptimal tool for screening, diagnostic, and prognostic purposes (Tchetgen and Oesterling 1997). Research has been carried out to improve the screening, diagnostic, and prognostic capacity of the PSA by modifying the PSA testing. It has been reported that a group of multiple kallikrein biomarkers such as total PSA, intact-PSA, free PSA, and Hk2 improves the diagnostic capacity of PSA as a prostate cancer biomarkers (Lazzeri et al. 2013; Darson et al. 1999; Benchikh et al. 2010). Research has now been focused on developing novel biomarkers that belong to different classes such as DNA-based, RNA-based, cancer cell type, SNPs, proteins, etc. Some of the recently developed biomarkers for prostate cancer are discussed here.

11.2.2 Field DNA Methylation

An important biomarker for prostate cancer is field DNA methylation, that is observed around the prostate cancer foci. It can be considered a cell senescence marker (Desotelle et al. 2013). Recently, a DNA field methylation-based novel assay was developed, which was named ProCUrE. It demonstrated enhanced prostate cancer diagnosis and patient identification in patients with clinically significant disease. This assay was based on two novel biomarkers, i.e., HOXD3 and GSTP1 (Zhao et al. 2018).

11.2.3 mtDNA Deletion

mtDNA deletions is another field-effect-based biomarker observed around the prostate cancer foci and can be an important diagnostic marker for prostate cancer. mtDNA deletions are made from the damage of the genomic segment that affects multiple genes and therefore becomes the cause of cancer in humans (Robinson et al. 2010). A 3.4 kb mtDNA deletion extracted from blood plasma has been reported to predict prostate cancer accurately. It has been reported to correlate well with the image-guided biopsy outcome in men's first biopsy setting in the PSA' grey zone. It is considered a novel biomarker that provides novel information to evaluate risk (Creed et al. 2017).

11.2.4 Alpha-Methylacyl Coenzyme a Racemase (AMACR)

Alpha-methylacyl coenzyme A racemase (AMACR) DNA is another DNA-based biomarker that codes for mitochondrial and peroxisome enzymes. It is a prostate tissue-based biomarker and was reported for its diagnostic capacity. However, it has not been approved by FDA yet (Sanhueza and Kohli 2018). The Germline BRCA2 mutations are another set of novel prostate cancer biomarkers found in both organ-confined and advanced prostate cancer patients (Eeles and Raghallaigh 2018). CpGs are novel DNA-based biomarkers for prostate cancer reported to discriminate the patients based on the metastatic lethal stage. CpGs were reported to be present on five genes, i.e., FHAD1, KLHL8, ALKBH5, PI15, ATP11A (Zhao et al. 2016).

11.2.5 Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs)-based biomarkers are also investigated as an addition to the group of DNA-based biomarkers. They consist of a set of biomarkers that are not very effective for diagnosis and prognostic purposes but can effectively inform about the risk of developing prostate cancer (Sanhueza and Kohli 2018). Recently, ancestry-informative markers (AIMs) based on SNPs were investigated that provided information about the risk factors contributing to prostate

cancer. These SNPs can detect allelic differences between different populations and are reported to classify genetic drivers of developing prostate cancer accurately. However, they have the limitation not to provide how much genetic heterogeneity is present between the populations (Mitchell and Williams 2019). If AIMs are incorporated in the risk prediction models, they can effectively improve prostate cancer detection and risk stratification in different populations (Mitchell and Williams 2019).

11.2.6 ERG

The novel RNA-based prostate cancer biomarkers are also an important area of cancer biomarker discovery. ERG is an important RNA-biomarker, with the most frequently found molecular aberrations in prostate cancer. However, it has the limitation of low sensitivity (Sanhueza and Kohli 2018).

11.2.7 PCA3

Another important RNA-based biomarker that is PCA3 has been reported to predict pathologically insignificant prostate cancer. This noncoding RNA was reported for its diagnostic capacity due to its high specificity (Sanhueza and Kohli 2018). It is not expressed in the normal prostate tissue; however, it is highly expressed in prostate cancer (Sanhueza and Kohli 2018). The urinary PC3-A levels are reported to be elevated in case of elevated PSA levels in patients who were previously found negative for prostate cancer. Its sensitivity is 69% compared to the 27% sensitivity observed for PSA. There is no cutoff value reported for the urinary PCA-3 levels (Sanhueza and Kohli 2018). However, it cannot predict aggressive prostate cancer (Sanhueza and Kohli 2018). Besides having a diagnostic capacity, urinary levels of post-DRE PCA3 were found to have a high probability of finding GS levels $\geq$7 and higher prostate tumor stage in surgical pathology (Sanhueza and Kohli 2018). These two biomarkers were recently combined for the detection of prostate cancer. They were found to preserve 95% sensitivity for detecting aggressive prostate cancer. Furthermore, it also improved the specificity from 18% to 39% (Sanda et al. 2017).

11.2.8 SAP30L-AS1 and SChLAP1

SAP30L-AS1 and SChLAP1 are another set of diagnostic markers that were recently reported to distinguish prostate cancer. These two lncRNAs, when combined separately with PSA, developed a moderate discriminating ability (Wang et al. 2018a, b).

11.2.9 Multiple Truncated AR Variants (AR-Vs)

Multiple truncated AR variants (AR-Vs) are RNA-based prognostic biomarkers that were reported for their predictive capacity. They were expressed in castrate-resistant prostate cancers (CRPC) (Cao et al. 2016). It has been reported that AR-V7 can be used as a biomarker to look for the response rate in patients receiving therapy. They are detected in tumor cells of patients in which the Androgen-depriving therapies such as abiraterone and androgen-blocking agents seem to be futile. These patients had a 0% response rate and shorter survival times (Sun and Abdollah 2015). Other novel AR-Vs (T878A or L702H) were observed to be related to worse progression-free and overall survival (O'Reilly 2019). In chemotherapy-naïve patients, AR-Vs amplifications are found to be associated with lower response rates to treatment with abiraterone and enzalutamide.

11.2.10 miRNAs

miRNAs-based biomarkers have been developed to monitor tumor growth, disease progression, and metastasis. They can monitor cancer cell activities and predict prostate cancer progression (Munteanu et al. 2020). They are found to be circulating in urine, serum, and plasma.

Several downregulated miRNAs were observed in the urine samples of prostate cancer patients. A novel diagnostic model consisting of three-miRNA that is (miR-222-3p*miR-24-3p/miR-30c-5p) was reported as a diagnostic marker that distinguishes BPH from prostate cancer patients (Fredsøe et al. 2018). Recently, a study identified a set of seven mRNAs (miR-451a, miR-148a, miR-144-3p, miR-3195, miR-512-5p) with prostate cancer predictive ability (Chaudhry et al. 2020). The list of miRNAs-based biomarkers for prostate cancer is long. A meta-analysis that included 37 miRNAs reported overexpression of 15 while under the suppression of 22 miRNAs in prostate cancer (Pashaei et al. 2017). Among them, only the following microRNAs were correlated with prostate cancer: miR-1, miR-133b, miR-449a, miR-137, miR-370, miR-221, miR-449b, miR-125a-5p, miR-199a-3p, miR-301b, miR-340, miR-361, miR-363, miR-98. Among these, MiR-1, miR-133B, miR-449B, and miR-221 exhibited significant utility in predicting prostate cancer after having radical prostatectomy (Munteanu et al. 2020). Furthermore, another three-miRNA-based model (miR-125b-5p*let-7a-5p/miR-151-5p) with prognostic ability was also recently developed to predict the time for biochemical recurrence after receiving the radical prostatectomy (Fredsøe et al. 2018).

11.2.11 Circulating Tumor Cells (CTCs)

Circulating tumor cells (CTCs) shed from primary tumors and metastatic sites and possess a half-life of around 1–2 h. Apart from the biomarkers mentioned above,

circulating tumor cells (CTCs) are FDA-cleared markers, showing increased CTC counts in castration-resistant prostate cancer. They are associated with worse prognosis in several phase III trials in CRPC patients (Kohli et al. 2017). They are reported to have low sensitivity/yield in blood, notably in early cancer stages, and limited capacity to monitor early-stage prostate cancer (Riaz et al. 2018; Ghosh et al. 2019).

11.2.12 Exosomes

Exosomes are extracellular vesicles bearing a diameter of 30–150 nm. They are naturally produced from almost all mammalian cells by fusing multiple vesicular bodies with the plasma membrane. Exosomes have recently gained growing attention due to their release from the outward budding plasma membrane (Kim et al. 2018). They also recently emerged as a potential source of noninvasive biomarkers for prostate cancer. These are the nanovesicles that carry molecules from the cancer cells and can therefore be detected in biofluids (Skotland et al. 2017). Flotillin 2 and PARK7 is a set of exosomes that gave signals at specific thresholds in combination, and they gave 68% sensitivity and 93% specificity (Wang et al. 2017c).

Exosomal biomarkers have been reported to have the ability to detect prostate cancer and distinguish between indolent and malignant tumors with higher PPV. Recurrence and treatment responses were also observed to be predicted through these biomarkers (Wang et al. 2017a, b, c; Arancio et al. 2017; Ghosh et al. 2019).

11.3 Novel Biomarkers for Diagnosis and Prognosis of Ovarian Cancer

Ovarian carcinomas-related morbidity and mortality ranks considerably higher than other gynecological malignancies. Its early detection is difficult due to the lack of precise and accurate screening methods and absence of physical symptoms.

11.3.1 CA 125

The classic “gold standard” tumor biomarker in ovarian cancer is CA 125. It is a glycoprotein with a high molecular weight and has a sensitivity of 50–60% with an overall specificity of 90% in postmenopausal women diagnosed with early-stage ovarian cancer. The expression of CA 125 is enhanced above normal level in about 90% of patients diagnosed with epithelial cell ovarian cancer (Rein et al. 2011; Delaney et al. 2020; Colaković et al. 2000). CA 125 is the only biomarker currently used widely in cancer therapy (Rein et al. 2011). CA 125 can be used as a potential biomarker for the early detection of ovarian cancer, as suggested by the literature (McIntosh et al. 2004). Moreover, CA 125 is also helpful in determining chemotherapy responses, differentiating malignant pelvic tumors and benign tumors, and

detecting recurrence. A decrease in CA 125 is considered a favorable sign during chemotherapy, and its level is also important in assessing disease stabilization (Bast et al. 1998, 2005; Guppy and Rustin 2002).

11.3.2 Circulating Fetal Protein Alpha-Fetoprotein (RECAF)

A study conducted by Tcherkassova et al. reported novel RECAF (Receptor for circulating Fetal Protein Alpha-Fetoprotein), an oncofetal antigen biomarker in conjunction with CA 125 for the early detection of ovarian cancer among healthy women. It was noticed that the addition of RECAF to CA 125 increased the sensitivity of detecting ovarian cancer to about 83% as compared to when CA 125 is used alone (Chu and Rubin 2006). Thus, due to the relatively low sensitivity of CA 125 when used alone, adding it with different biomarkers can create a panel of multiple biomarkers with increased efficacy (Jacobs et al. 2011; Menon et al. 2009a, b).

11.3.3 Human Epididymis Protein (HE4)

Human epididymis protein (HE4), having a molecular weight of 25 kDa, coded by the gene "WFD2," is a protein that belongs to a family of "four-disulfide core" that consists of various groups of small proteins which are heat-stable and acid-resistant made up of different functional groups. After being produced by the epithelial ovarian cancer cells circulating in the blood, this protein can be detected using enzyme immunoassay (Moore et al. 2012). The use of HE4 for diagnosing and monitoring women with epithelial ovarian cancer was approved by the Food and Drug Administration (FDA) in 2009. Scientists have reported the overexpression of HE4 in Epithelial Ovarian Cancer (EOC) but not in other types of ovarian cancers (Montagnana et al. 2011). Physicians always requested CA 125 combined with identifying serum HE4 levels for the early detection of ovarian cancers as CA 125 is found to be elevated in various benign lesions and other diseases. Early detection of ovarian cancers based on HE4 has an overall sensitivity of about 90% and a specificity of 72.9%. However, the combined use of CA 125 and HE4 certainly improves identifying different benign and malignant tumors (Moore et al. 2008). In ovarian cancer, the overexpression of HE4 leads to the irritation of Human Epidermal Growth Factor Response (EGRF). It induces MAPK signaling, contributing to inducing tumor cell growth, migration, and adhesion (Lu et al. 2012). The normal reference range of serum HE4 is <140 pmol/L. HE4 levels are found to be raised in conditions like pregnancy, aging, and post-menopausal women.

Food and Drug Authority (FDA) approved the use of ROMA (Risk of Malignancy Algorithm) for measuring the levels of CA 125 and HE4 for diagnosing the epithelial ovarian cancers in women with post-menopausal status and are presented with pelvic masses. The sensitivity (90.7%) and specificity (93.1%) of this test are higher than CA 125 alone. ROMA score of greater than and equal to 1.31 reflects a

high risk of ovarian cancer in premenopausal women. However, ovarian malignancy in post-menopausal women is considered by a ROMA score of greater than and equal to 2.71 (Moore et al. 2009; Bast et al. 1981).

11.3.4 Mesothelin

Mesothelin is a glycoprotein having a molecular weight of 40 kDa, which is expressed on the surface of mesothelial cells. The serum and urine levels of mesothelin are found elevated in some cancers, including mesothelial cell carcinoma, ovarian cancer pancreatic cancer (Hassan et al. 2005; Hassan and Ho 2008). Studies reported on ovarian cancer have demonstrated the interaction of CA 125 with mesothelin on the surface of cancer cells that mediate cell attachment (Massova et al. 1998; Schorge et al. 2010). Overexpression of mesothelin in mesothelial cell carcinoma and ovarian cancer triggers the MAPK, PI3K, and NF-κB signaling pathways (Hilliard 2018). Sensitivity (60%) and specificity (98%) of mesothelin alone are less as compared to when used in combination with CA 125 (McIntosh et al. 2004). Urine assay of mesothelin is found to be more effective as compared to serum assay. However, the amount of mesothelin in both assays can be found by Enzyme-Linked Immunosorbent Assay (ELISA) (Badgwell et al. 2007). Factors that affect mesothelin's expression level include age, smoking, and BMI (Lowe et al. 2008).

11.3.5 Kallikrein-Related Peptidases (KLKs)

Kallikrein-related peptidases (KLKs) are a group of serine proteases having a molecular weight of 30 kDa with proteolytic activity. Fifteen related serine proteases play various roles in the human body encoded by a cluster of genes on the 19q13 chromosome. Research on SKOV3 epithelial ovarian cancers indicated that some kallikrein-related peptidases, including KLK4, KLK7, and KLK6, play certain vital roles in ovarian cancers and are found to be overexpressed. The sensitivity of KLKs in the early detection of ovarian cancers is low when used alone, but sensitivity (72%) and specificity (90%) have been reported by the combined use of CA 125 and KLK 42. ELISA measures kLKs serum levels, and levels of more than 4.4 mg/L indicate patients with poor prognosis (El Sherbini et al. 2011).

11.3.6 Osteopontin

Osteopontin is associated with the invasion and metastasis of tumor cells. It is a secreted, adhesive, extracellular glycoprotein synthesized by osteoblasts and vascular endothelial cells whose function is linked with immunity and bone remodeling. Osteopontin has a sensitivity of 83.3% in detecting ovarian cancers. However, the

increased sensitivity is reported by the combined use of osteopontin with CA 125 (Nakae et al. 2006).

11.3.7 ApoA1

ApoA1 belongs to the family of highly dense lipoproteins. Reduced levels of ApoA1 are found to be reported in Ovarian Cancers. The mechanism through which the levels of ApoA1 are reduced is unclear; however, this reduction is made in the serum. Research conducted in the past reported that the reduction in the levels of ApoA1 in ovarian cancers is linked with the destruction of cellular bio-membranes. ApoA1s serve as a potential biomarker having a sensitivity of 93.9% and specificity of 95% in detecting OvCa when used in combination with CA 125 (Gadomska et al. 2005; Su et al. 2010).

11.3.8 Vascular Cell Adhesion Molecules 1 (VCAM-1)

Vascular cell adhesion molecules 1 (VCAM-1) is a receptor located on the surface of endothelial cells and mesothelial cells. It overexpresses in the mesothelium layer of ovaries of women diagnosed with ovarian cancer. VCAM-1is responsible for the stimulation of cancerous cells to move to the peritoneal cavity. It has a sensitivity of 86% in detecting early-stage OvCa and a sensitivity of 93%, and a specificity of 98% in detecting the last stages of OvCa when combined with other biomarkers (Slack-Davis et al. 2009; Yurkovetsky et al. 2010).

Different types of cancer, including ovarian cancer, are attributed to the mutations in the tumor suppressor genes and genes responsible for the cell cycle, leading to uncontrolled growth, survival, and tumor cells' metastasis. Genetic biomarkers including BRCA1, P53, and KRAS aid in early detection by identifying the disease-subtype, stage, and prognosis and serve as a source of selecting effective treatments and therapies. In ovarian cancer, genes that mutate normally include the following:

11.3.9 BRCA1

BRCA1 is a gene located on chromosome 17q12-21, which has a vital role in family-related ovarian cancer. The function of this gene is to help in genome repair. Different studies reported the hyper-methylation of BRCA1 in ovarian cancers and tumor cells. Owing to this hyper-methylation, a 12–16% reduction in expression occurs, and this hyper-methylation is associated with diminished protein concentrations of RNA and BRCA1, specifically in epithelial cells ovarian cancer. The poor prognosis of OvCa is also linked with hyper-methylation (Miki et al. 1994; Baldwin et al. 2000; Wilcox et al. 2005; Strathdee et al. 2001).

11.3.10 P53

P53 is a tumor suppressor gene that is involved in maintaining the cell cycle and apoptosis. As reported by studies, 50% of ovarian cancers occur due to mutations in P53. It serves as a potential genetic biomarker to identify the metastatic potential of ovarian cancer and differentiate between epithelial cells, ovarian tumors, and other types of ovarian cancers. In all stages of ovarian tumors, mutations of P53 are found present (Milner et al. 1993).

KRAS is a GTPase belonging to the family of RAS protein. It is an early player in various signaling pathways. In normal tissue signaling, KRAS serves a vital role; however, a mutated version of the KRAS gene is a significant step in causing and developing cancers (Tsuchida et al. 1982). About 25% of cancers are reported to have mutated KRAS. In type I epithelial ovarian cancers, mutations are reported in almost 40% of patients (Nodin et al. 2013).

EGFR, known as Endothelial Growth Factor Receptor, is a tyrosine kinase receptor that plays an important role in normal cell function. Mutations in this receptor are responsible for changing normal cells' phenotype into tumor cells (Huang and Harari 1999). In 70% of ovarian cancers, mutations in EGFR are reported. Mutations in the receptor cause overexpression of endothelial growth factors leading to the AKT signaling pathway. Aggressive forms of ovarian cancer's poor prognosis are attributed to the EGFR/AKT pathway's overexpression (Siwak et al. 2010; Zeineldin et al. 2010).

11.3.11 MicroRNAs

MicroRNAs (miRNAs) are made up of 21–24 nucleotides and are noncoding RNA types. Its function is to act in the posttranscriptional regulation of gene expression (Iorio et al. 2007). miRNAs that have been investigated to be used as a biomarker in detecting ovarian cancer include miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, and miR-214 (Taylor and Gercel-Taylor 2008; Szajnik et al. 2013).

11.4 Novel Biomarkers for Diagnosis and Prognosis of Lungs Cancer

Lung cancer is among the top causes for cancer-related mortality in both men and women in Western nations, accounting for 30% of cancer-related deaths in the United States consistently (Granville and Dennis 2005).

The rate of spread of lung cancer is manifolds higher, as compared to that of prostate cancer among men and about double of that in breast cancer among women. Smoking has been attributed as one of the main causes, with the hazard in smokers found to be ten times higher than in nonsmokers. Lung cancer is broadly categorized as non-small cell lung cancer (NSCLC), making up around 85% of cases. Small cell

lung cancer (SCLC) contributes to 15% of cases and includes a few histological sorts, like adenocarcinoma, large cell carcinoma, and squamous cell carcinoma (Lehtiö and De Petris 2010).

Lung cancer is considered a heterogeneous disease involving subtypes with distinct pathologic and clinical characteristics (Fujimoto and Wistuba 2014). It is important to distinguish between these histologic subtypes of non-small cell lung carcinoma (NSCLC), specifically adenocarcinoma, squamous cell carcinoma, and large cell lung carcinoma to determine the best course of therapy and provide reliable information regarding the disease prognosis (Kerr et al. 2014).

There have been significant breakthroughs in the recent years in developing biomarkers for lung cancer that would allow the molecular categorization of different cancer subtypes, leading to customization of anti-cancer therapy (Mok 2011).

The progression in molecular profiling and targeted treatment has also given rise to a renewed interest in the characterization of NSCLC into major subtypes like adenocarcinoma, squamous cell carcinoma, and large cell lung carcinoma (Travis et al. 2015).

Lung cancers are typically analyzed and diagnosed by transthoracic core needle biopsy and fine-needle aspiration (FNA), transbronchial needle aspiration, endobronchial ultrasound-guided transbronchial needle aspiration, and endoscopic ultrasound-guided FNA (Travis et al. 2013).

Proteomics may offer a significant advantage over genomics since protein biomarkers offer more reliable information regarding a disease considering proteins, and not transcripts, are the actual players (Stroncek et al. 2005).

Since a persistent dynamic inflammation state describes cancer, the cancer microenvironment often contains infiltrating inflammatory cells and pro-inflammatory cytokines. Acute phase reactant proteins (APRPs) are released in response to the aggravated inflammation. The relationship between the altering APRP levels and progression of neoplastic disease has been established previously, but the latest proteomics studies showed that APRP adjustments are diverse in specific tumor types (Pang et al. 2010). Hence, APRPs can likely be utilized biomarkers for categorizing the different cancer types. Among APRPs, the haptoglobin (Hp) β chain, serum amyloid A (SAA) (Sung et al. 2011), and apolipoprotein A-1 (Apo A-1) (Maciel et al. 2005) proteins have demonstrated considerable potential as diagnostic markers for lung cancer.

A similar study has shown that the haptoglobin levels are three times higher in the blood samples of lung cancer patients as compared to healthy controls. The Hp levels also provide remarkable specificity when distinguishing between lung cancer patients and those suffering from other respiratory diseases such as pulmonary fibrosis, tuberculosis as well as bronchial asthma where the levels are similar to those in normal population. Furthermore, the Hp levels in other types of cancers such as breast cancer and hepatocellular carcinoma, are also found to be significantly lower than that in lung cancer (Kang et al. 2011).

11.4.1 SAA Proteins

SAA proteins are apolipoproteins that are involved in various key processes like cell–cell communication, cholesterol transport in the liver, induction of immune cells to the site of inflammation, and the breakdown extracellular matrix (Uhlar and Whitehead 1999).

The SAA1 and SAA2 of the SAA protein family have been recently investigated as diagnostic markers for lung cancer. The expression levels of the proteins from blood and tissue samples of lung cancer patients were analyzed using LCMS/MS, ELISA, and immunohistochemistry examinations. It was revealed that the SAA1 and SAA2 levels in the samples from lung cancer patients were significantly higher than the samples from healthy volunteers as well as from patients suffering from other types of cancer (Sung et al. 2011).

In another study comparing the adenocarcinoma patients with healthy controls, the upregulation of SAA1 and SAA2 in lung cancer was found to be associated with reduced levels of Apo A-1, an APRP responsible for removal of endogenous cholesterol from tissues (Maciel et al. 2005). In a more recent study, the proteome of serum and pleural effusions was compared between NSCLC patients and non-malignant lung disease using 'two-dimensional difference gel electrophoresis' (2D-DIGE). Considerably higher levels of biomarkers were found in pleural effusion and serum of cancer patients, although the level in pleural effusion was higher than that in serum (Rodríguez-Piñeiro et al. 2010).

11.4.2 Epidermal Growth Factor Receptor (EGFR)

The EGFR (Epidermal Growth Factor Receptor) is a tyrosine kinase receptor belonging to the family of ERBB. At the short arm of chromosome no. 7, the gene for EGFR is located at the twelfth position. Overexpression of EGFR is reported in nearly 62% of NSCLCs associated with poor prognosis (Sharma et al. 2007). In the US, around 10% of patients diagnosed with lung adenocarcinomas and 30–50% in Asia are found to have developed cancer due to the mutations in the EGFR gene (Sharma et al. 2007). Tyrosine Kinase Inhibitors (TKIs), including gefitinib and afatinib, due to their high response rates of 55–78%, are established as standard treatment for patients diagnosed with lung adenocarcinomas due to EGFR mutations 6. For identifying EGFR mutations, techniques like gene sequencing and polymerase chain reaction (PCR) are used (Fujimoto and Wistuba 2014).

11.4.3 Anaplastic Lymphoma Kinase (ALK)

Anaplastic lymphoma kinase (ALK) is also a tyrosine kinase family belonging to the insulin receptor superfamily. On the short arm of chromosome 2, the gene for ALK is located at 23rd position (Zhao et al. 2015). Mutations of the ALK gene are reported in a subset of NSCLC tumors which harbor a fusion of both ALK with

echinoderm microtubule-associated protein-like 4 (EML4) (Fujimoto and Wistuba 2014). 3.7–7% of NSCLCs have been associated with EML4-ALK fusion and are found to be more prevalent in adenocarcinomas reported in young patients who have never smoked. Patients with verified EML4-ALK fusion have reported high response rates (57–74%) after treatment with ALK inhibitors such as crizotinib (Solomon et al. 2014).

11.4.4 Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS)

KRAS is an oncogene located on the long arm of chromosome 12 at 12.1 positions (McBride et al. 1983). It belongs to the RAS family of membrane-associated G-proteins (Edkins et al. 2006). In 25–30% of patients with NSCLC, KRAS mutations are reported specifically in adenocarcinomas of solid pattern, which are found more in white people than Asians (Dogan et al. 2012). KRAS mutations lead to unfavorable outcomes and are considered a negative predictor of chemotherapy's response (Ying et al. 2015; Macerelli et al. 2014). Additionally, it is also linked with the development of the "second-time primary tumor". It is also considered a predictor of resistance in patients with NSCLC who are administered targeted EGFR-TKIs therapy (Macerelli et al. 2014).

11.4.5 Receptor Tyrosine Kinase (ROS1)

Receptor tyrosine kinase (ROS1), a proto-oncogene, is a gene from the insulin receptor family's tyrosine kinase receptor (Bergethon et al. 2012). It is located on the long arm of the 6th chromosome at position 22. ROS1 rearrangements are found in 1–2% of patients with NSCLC (Yoshida et al. 2013). This rearrangement is commonly reported in young females who have never smoked, with a diagnosis of adenocarcinomas (Bergethon et al. 2012; Yoshida et al. 2013). Response rates of 80% are recorded in advance NSCLC patients with ROS1 mutations given crizotinib treatment (Bergethon et al. 2012). Assessment of ROS1 rearrangement is an expensive and laborious technique. Since this type of cancer is rare, screening by IHC is considered a tool for identifying patients suitable for ROS1-targeted therapy (Popescu et al. 1989).

11.4.6 The Human Epidermal Growth Factor Receptor 2 (HER2)

The human epidermal growth factor receptor 2 (HER2) gene is localized on chromosome 17 at position 12 that encodes a transmembrane member of the tyrosine kinase epidermal growth factor receptors, which are usually expressed less in all epithelial cells in the tissues of the normal fetus and normal. These receptors are essential in the proliferation and survival of cancer. Increased or over-expression of HER2 mRNA is associated with the HER2 gene amplification, which is responsible

for triggering carcinogenesis by uncontrolled proliferation due to self-sufficiency in growth signals, enhanced invasive and metastasis processes (Yarden and Pines 2012; Oh et al. 1999). Seven to 34.9% of NSCLCs have been reported to have overexpression HER2 associated with a poor prognosis in patients with these tumors (Ricciardi et al. 2014). Various studies reinforced this proposal of screening patients with lung adenocarcinomas for HER2 mutations to select patients who could benefit from targeted HER2 therapies such as afatinib and trastuzumab. The response rate in NSCLC patients diagnosed with HER2 mutations is recorded as 50% (Mazières et al. 2013).

RET proto-oncogene that encodes for a tyrosine kinase receptor is located on chromosome 10 at position 11.2 51. Rearrangement of RET was initially recorded in papillary thyroid carcinomas (Knowles et al. 2006). Around 1–2% of NSCLC are found to have RET rearrangements which typically occurs in young patients who have never smoked in adenocarcinomas with poorly differentiated solid characteristics (Lipson et al. 2012). The standard assay used for diagnosing RET is FISH. PCR is considered insufficient for identifying RET rearrangements' various isoforms (Lipson et al. 2012).

11.4.7 Phosphatidylinositol-4,5-Bisphosphate 3-Kinase, Catalytic Subunit α (PIK3CA)

Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α (PIK3CA) codes 1 of 2 phosphoinositide 3-kinase (PI3 K) subunits (German et al. 2013) and is a cancer gene. It is considered the most common mutated gene and KRAS in human cancers (Samuels and Velculescu 2004). These mutations are found in 1–4% of patients with NSCLC (Yamamoto et al. 2008; Fujimoto and Wistuba 2014).

11.4.8 The Neurotrophic Receptor Tyrosine Kinase 1 (NTRK1)

The neurotrophic receptor tyrosine kinase 1 (NTRK1) proto-oncogene, also known as a tropomyosin-related kinase (TRK) A, belongs to tyrosine kinase. It is located on 1q21-22 chromosome 117. In lung cancer, rearrangement in the NTRK1 gene is reported in 3% of patients diagnosed with adenocarcinomas that harbor NTRK2 fusions (Vaishnavi et al. 2013). Early phase 1 studies such as entrectinib and LOXO-101 have shown good results in patients diagnosed with solid tumors with NTRL1 fusions (Passiglia et al. 2016).

11.4.9 The Fibroblast Growth Factor Receptor (FGFR)

The fibroblast growth factor receptor (FGFR) is a gene located on the eighth chromosome at position 12 responsible for encoding TKR belonging to the FGFR family (Jiang et al. 2015). In lung cancer, FGFR1 gene amplification is reported

higher in carcinomas of squamous cells (20%) as compared to adenocarcinomas which is found amplificated in only 3% of the cases and is more commonly found in current smokers as compared to the people who smoked and never smoked (Jiang et al. 2015; Weiss et al. 2010).

11.4.10 The Discoidin Domain Receptor Tyrosine Kinase Two Genes (DDR2)

The discoidin domain receptor tyrosine kinase two genes (DDR2) encodes for a TRK receptor. It is located on the long arm of chromosome 1 at 23.3 positions. In lung cancer, DDR2 is found to be reported in 3–4% of carcinomas in lung's squamous cells (Hammerman et al. 2011) as compared to the adenocarcinomas (0.5) (Ding et al. 2008) and are only found to be present in smokers (An et al. 2012).

11.5 Novel Biomarkers for Diagnosis and Prognosis of Breast Cancer

Biomarkers play an indispensable role in both diagnosing and managing cancer. Over the last few decades, advancement in precision medicine has led to the discovery of cancer biomarkers. This discovery and advancement in diagnostic and prognostic biomarkers have led to the delivery of targeted therapies to the patients, which has spared patients from getting overtreatment and has significantly reduced the side effects of the diagnosis and treatment of cancer (Meehan et al. 2020).

In women, breast cancer is considered the most common cancer whose incidence rates increase day by day (Jemal et al. 2010). Currently, biomarkers play a crucial role in managing patients with breast cancer, specifically when deciding the nature of systematic therapy administered to the patient. In 2005, guidelines were published by the European Group on Tumor Markers (EGTM) on the use of biomarkers for the treatment of breast cancer (Molina et al. 2005). However, thenceforth, many new developments are made and reported, specifically tissue-based biomarkers.

The use of biomarkers in cancer is limited to providing additional information about various clinical factors. Still, it is also linked to providing more treatments to the patients with a better and controlled benefit–risk balance (Polley et al. 2013). Analysis using biomarkers in breast cancer is being used as a routine practice. Initially, it began with testing the expression of hormone receptors in guiding therapy using tamoxifen. However, a revolution in the biomarker field started with the succeeding inclusion of targeted therapies against HER2 (Human Epidermal Growth Receptor 2) (Albanell et al. 2009).

Breast cancer accounts for about one-third cancer cases in women, and the overall health burden worldwide due to breast cancer is reported as 10% (Jemal et al. 2010). At the beginning of this century, with the introduction of mammographic screening techniques, early detection of breast cancer has further led to identifying cases, thus

increasing the incidence rates. In developing countries, the incidence rates of breast cancers are also increasing rapidly, which depicts that in the next decade, a major disease burden in both developing and developed countries would be contributed by breast cancer (Cedolini et al. 2014; Driul et al. 2013). Improvements and the use of adjuvant chemotherapy and endocrine therapy have significantly reduced mortality due to breast cancer to about 50%. However, increasing incidence rates of breast cancer has led to the debates of patients with overtreatment, which has contributed to the increase in family and social burden and has also resulted in causing unnecessary side effects and harm to the patient (Esserman et al. 2013; Katz and Morrow 2013). Thus, research is being conducted worldwide, which is focused on targeted drug delivery with fewer side effects. In this regard, many new biomarkers have been studied and introduced to serve both as an important prognostic tool that enables determining whether the cancer is progressing slowly or aggressively and as a therapeutic agent (Veer et al. 2002; Wang et al. 2005).

Breast cancers mainly consist of a heterogeneous group of tumors with an extensive spectrum of morphologically and molecularly special subtypes, ensuing in special biological behaviors, presentation, and prognosis. Along with the ailment level and the affected person's performance, recognizing the molecular pattern of tumors is fundamental in identifying patients who will benefit from a certain and more specific treatment type. In the standard care of all breast cancer patients (primary, recurrent, and metastatic), the use of estrogen receptor (ER), the human epidermal growth factor receptor (HER2), the progesterone receptor (PR), and the Mib1/Ki-67 proliferation index are the molecular markers that are firmly established and are considered the most important ones (Naoi and Noguchi 2016; Harris et al. 2016).

Human breast tumors rely on sex hormones for their growth as their origin is from breast tissue, which is responsive to endogenous hormones (Zumoff et al. 1975). In 1896, it was noticed that bilateral oophorectomy could significantly reduce the progression of breast cancer, especially in fertile age (Stockwell 1983). This has led to the acceptance of endocrine therapy as a standard treatment for patients with the barest cancer. However, the positive response rate was recorded in only one-third of patients.

In the early 1960s, the existence of estrogen receptors was supposed due to the concentration of radiolabeled estrogens in the target areas, which could be considered as a predictive factor in reporting positive, the responsive need of breast cancer to oophorectomy (Jensen and Jordan 2003; McGuire 1975). About 60% Estrogen Receptor (ER) positive tumors and 8% ER-negative tumors responded to the endocrine therapy. However, the small proportion of ER-negative patients who responded to the endocrine therapy may be due to faulty receptor assay results.

11.5.1 Human Epidermal Growth Receptor (HER2)

This successful identification of the estrogen receptor has proven both a therapeutic target for the treatment and prevention of breast cancer. It has also been recognized

as a selective molecular model for all succeeding efforts in designing targeted oncological therapies. Thus, the estrogen and progesterone receptors, along with the Human Epidermal Growth Receptor (HER2), represent the most fundamental biomarkers in the standard care of all patients with breast cancer. Their assessment was also critical in evaluating every diagnosed breast cancer.

Among women younger than 50 years of age, about two-third of invasive breast cancers are expressed by hormone receptors, and around 80% of women are found to be older than 50 years of age (Anderson et al. 2002). For the evaluation of breast cancers, measuring and recognizing hormone receptors is a routine practice that represents a potential predictive factor in determining the given hormone therapy's responsiveness. Increasing levels of both estrogen and progesterone receptors correlate with a better and more precise response, decrease chances of failure and long survival rate (Buzdar et al. 2004; Ravdin et al. 1992).

11.5.2 ER Expression

The expression of hormone receptor also represents an important favorable factor with prime prognostic properties, recognized as an important growth marker rather than metastatic potential. Specifically, the prognosis of patients with ER+/PR+ tumors is better than patients with ER+/PR− tumors, who have a better prognosis than patients with ER/PR tumors (Bardou et al. 2003). Prognostic indicators, including old age, a lower fraction of dividing cells, and low grading, lower genetic mutation, are significantly associated with ER expression (Anderson et al. 2002; Elledge et al. 1993; Wenger et al. 1993; Nadji et al. 2005).

The recurrence rates of breast cancer patients with ER-positive tumors can be halved with adjuvant hormone therapy (Early Breast Cancer Trialists' Collaborative Group 2005). Moreover, due to its limited and reduced side effects, patients with comorbidities and elderly patients can also be administered with high success rates. In some patients with metastatic disease, its response can last for years. Compared to the stage-I patients with ER positive tumors who receive no systematic therapy, the recurrence rate in 5 years is 5–10% lower than those breast cancer patients who have ER-negative tumors. On the contrary, ER-negative tumors respond better to cytotoxic chemotherapy than hormone therapy (Fisher et al. 1988). The human epidermal growth factor receptor 2 (HER2) gene is localized on chromosome 17 that encodes a transmembrane member of the tyrosine kinase epidermal growth factor receptors, which are usually expressed less in all epithelial cells in the tissues of the normal fetus and normal. These receptors are essential in the proliferation and survival of cancer. Increased or overexpression of HER2 mRNA is associated with the HER2 gene amplification responsible for triggering carcinogenesis by uncontrolled proliferation due to self-sufficiency in growth signals, enhanced invasive and metastasis processes (Yarden and Pines 2012; Oh et al. 1999). The HER2 gene results are amplified in the certain breast, ovarian, bladder, endometrial (Saffari et al. 1995), salivary gland (Press et al. 1994) and gastric cancer (Park et al. 1989).

The most popular humanized monoclonal antibody against human endothelial growth factor receptor (HER2), Trastuzumab, has significantly improved response rates; time is taken for progression and survival when used alone or when added to chemotherapy in both early-stage breast cancer patients and metastatic (Slamon et al. 2011). Among the other HER2-targeted medicines which include lapatinib (tyrosine kinase inhibitor), pertuzumab (the monoclonal antibody), adotrastuzumab emtansine T-DM1 (the antibody-drug conjugate) have improved outcomes in HER2-positive metastatic breast cancers.

11.5.3 Mib1/Ki-67

Mib1/Ki-67 is a proliferation index used both as a prognostic and predictive marker, though due to the lack of standardization, its widespread use is limited (Colozza et al. 2005; Harris et al. 2007). This proliferation biomarker is a significant predictor for the responsiveness to both endocrine therapy and chemotherapy (Dowsett et al. 2017; Fasching et al. 2011). Moreover, the decrease in Mib1/Ki-67 in posttreatment women given neoadjuvant therapies is a powerful, independent predictor depicting better clinical outcomes (Ellis et al. 2011). Retinoic acid receptor α (RARA) is considered a potential biomarker for tamoxifen resistance (Niederreither and Dollé 2008). About one-third of breast cancer patients with ER-alpha positive receptors (ERα) experience relapse when treated with tamoxifen (Early Breast Cancer Trialists' Collaborative Group 1988). Anti-tumor properties of Retinoic Acid Receptor α can be attributed to the interaction of the receptor with ERα receptors and their combined binding sites (Hua et al. 2009). Cells resistant to tamoxifen were reported to have high levels of RARA (Johansson et al. 2013). Breast cancer patients with ERα positive tumors diagnosed with a high internal protein level of RARA administered with tamoxifen displayed RFS (recurrence-free survival) compared to patients diagnosed with low levels of RARA (Johansson et al. 2013).

11.5.4 Osteopontin

Osteopontin is associated with the invasion and metastasis of tumor cells. It is a secreted, adhesion, extracellular protein (Senger et al. 1979; Brown et al. 1994). An experiment was conducted by Pang et al. (2013), where he examined the clinical and pathological effects of osteopontin, E-cadherin, and β-catenin adhesion molecules and reported higher levels of all the above-mentioned adhesion molecules in patients suffering from the breast when compared with the normal. Metastasis of lymph nodes was associated with the expression of higher levels of osteopontin-c. Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) is a human multifunctional regulatory protein that is overexpressed in cancer cells (Kuroki et al. 1992; Blumenthal et al. 2007). Its expression in atypical ductal hyperplasia serves a potential role in the development of breast cancer. Breast

cancers that are invasive and treatment-resistant are also associated with CEACAM6 (Poola et al. 2006).

11.5.5 Phosphatidylinositol-4,5-Bisphosphate 3-Kinase, Catalytic Subunit α (PIK3CA)

Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α (PIK3CA) codes 1 of 2 phosphoinositide 3-kinase (PI3 K) subunits (German et al. 2013) and is a cancer gene. It is present in 20–40% of breast cancer patients (Young et al. 2015). Cizkova et al. (2013) has identified 17 tumors with PIK3CA mutations among 80 breast cancer patients with positive HER2 treated and administered with trastuzumab for 1 year. Breast cancer patients showing wild type.

PIK3CA showed an improved disease-free survival (DFS) as compared to the patients possessing PIK3CA mutations. The prognosis of HER2 positive breast cancer patients treated with trastuzumab was poor than for the patients possessing wild-type PIK3CA. Therefore, patients with HER2-positive tumors are only required to detect PIK3CA mutations.

11.5.6 Tissue Inhibitor of Metalloproteinases-1 (TIMP-1)

Tissue inhibitor of metalloproteinases-1 (TIMP-1) has shown efficacy in protecting cells from apoptosis. The first chemotherapy drug of choice for patients diagnosed with lymph node metastasis is Paclitaxel (Hortobagyi and Holmes 1996; Olayide et al. 2015). Currently, no biomarkers are available that could predict the susceptibility to chemotherapy (Chromek et al. 2004). An epidemiolocal study conducted previously has reported an association between TIMP-1 and responsiveness to cyclophosphamide/methotrexate/5-fluorouracil and anthracycline-based chemotherapy regimens. Higher levels reduced would be the responsiveness to various therapies (Schrohl et al. 2006).

11.5.7 Ferritin Light Chain (FTL)

Ferritin light chain (FTL) is an iron-binding protein. In chordates, apoferritin has two types, including light and heavy chain, which are assembled by 24 subunits. The ratio between heavy chains and ferritin light chains depends on the type of tissue and conditions (Arosio et al. 1976). The rise in ferritin protein from tissues affected with cancer shows a correlation between these two. Ricolleau et al. (Zeidan and Townsend 2008) calculated a ferritin light chain cut-off level in tumors as 2.4 by studying the utilization of FTL (a prognostic marker) in breast cancer patients with a positive lymph node metastasis. The metastasis-free survival rate was recorded low in high FTL patients (Jézéquel et al. 2012).

11.5.8 Urokinase-Type Plasminogen Activator (uPA)

Urokinase-type plasminogen activator (uPA) is a tumor-associated proteolytic factor. In contrast, plasminogen activator type 1 inhibitor (PAI-1) plays a potential role in invasion and metastasis of tumor 41, cell signaling and adhesion, migration, and cell-proliferation (Duffy et al. 2014). C-reactive protein (CRP) is used as a marker of acute inflammatory reactions and serves as an important prognostic marker in breast cancer (Allin and Nordestgaard 2011; Pradhan et al. 2018). Chromosome 17 centromere enumeration probe (Ch17CEP) is the second most dense chromosome that codes for several genes, including BRCA1 and HER2, which play an important role in breast cancer and repairing genes TP53, RAD51C, and RAD52B (Zody et al. 2006).

11.5.9 Soluble Human Epidermal Growth Factor Receptor 2 (sHER2)

Soluble human epidermal growth factor receptor 2 (sHER2) is a protein released from the intracellular transmembrane and extracellular domains 51. sHER2 is considered an important biomarker in breast cancer patients with HER2 positive at all stages. 53. In early-stage breast cancer, HER2 positive patients sHER2 is found as a suitable relapse prognostic biomarker and survival determination in relapse patients (Moreno-Aspitia et al. 2013).

11.5.10 Mitotic Arrest Deficient Like 1 (MAD1L1)

Mitotic arrest deficient like 1 (MAD1L1) is considered a checkpoint gene linked with chromosomal instability. Several different types of cancer, including colon and lung cancer, have been reported with abnormalities in MAD1L1 (Foijer et al. 2008). A study conducted has revealed that the expression of nuclear MAD1L1 seems to have increased the treatment resistance and has affected the prognosis of breast cancer, showing that breast cancer patients with positive MAD1L1 was not susceptible to treatment with Taxol (Sun et al. 2013). Methylation of paired-like homeodomain 2 (PITX2P2), including C-phosphate-G islands, is located within the gene regulatory site and linked with gene expression suppression. The introduction of a methyl group (methylation) in the DNA—nucleotides of this gene is commonly recorded as an early event succeeding the onset of cancer (Baylin and Herman 2000; Jones 1996; Herman and Baylin 2003). Patterns of methylation are specific to the tumor's subtypes, including breast cancer (Frühwald 2003; Laird 2003; Absmaier et al. 2018). Data analyzed from several studies reported that methylation of the DNA of PITX2 was associated with an increased risk of relapse in metastasis of lymph nodes positive, hormone-receptor-positive breast cancer patients taking adjuvant tamoxifen therapy (Qian et al. 2017; Bibikova et al. 2006).

11.6 Novel Biomarkers for Diagnosis and Prognosis of Leukemia

Leukemia is a set of progressive hematological malignancies originating in stem cells of bone marrow that contributes to a large proportion of cancer deaths. Novel biomarkers have managed to revolutionize the clinician's approach towards prognostication and therapy.

11.6.1 CD123

A recent study revealed that the biomarker CD123, interleukin-3 receptor alpha (IL-3Rα), has significant expression in Leukemic stem cells (LSCs), but not in the normal hematopoietic stem cells (HSCs), and is associated with better treatment response, as well as minimum residual disease detection (MRD) and prognosis. Moreover, CD123 is a significant marker for detection and targeted therapy of LSCs for relapsed and refractory leukemia. Anti-CD123 targeted therapies in preclinical testing and clinical evaluation validate the effectiveness of anti-CD123 neutralizing antibody drugs, CD3 and CD123 bi-specific antibodies, dual affinity retargeting (DART), and anti-CD123 chimeric antigen receptor-modified T-cell (CAR-T) treatment therapies in the process of development (Shi et al. 2019).

11.6.2 Tumor Necrosis Factor Receptor 2 (sTNFR2)

A research group from University of the Ryukyus (Nishihara, Japan) has successfully identified a novel biomarker for diagnosing acute T-cell leukemia (ATL). ATL is an adult T-cell neoplasm that is linked to human T-cell leukemia virus type-1 (HTLV-1). Elevated levels of HTLV-1 carriers contribute to higher prevalence rates of ATL in Japanese population. To identify a novel biomarker that helps in the early detection of ATL, scientists identified a higher expression of soluble tumor necrosis factor receptor 2 (sTNFR2). There is a need to study further the importance of high STNFR2 levels in ATL onset prediction and examine the method by which tumor necrosis factor 2 is cast off from the cell surface, as a possible therapeutic route (Guerrero et al. 2020).

11.6.3 NPM1 and FLT3 Mutation

Novel biomarkers based on the nucleophosmin (NPM1) and FLT3 mutation help decide individual treatment regimens for the patients of acute myeloid leukemia. Similarly, BCR-ABL genetic relocation has worked as a novel biomarker for diagnosis of chronic myelogenous leukemia (CML). Imatinib is a small molecule inhibitor of ABL and has served as a modern era therapeutic regimen for CML,

effectively shifting the trend away from allogeneic stem cell transplant (Hsueh et al. 2013).

11.6.4 BCR-ABL Tyrosine Kinase Inhibitor (TKI)

Several proteins that act as promoters of cancers, can be utilized as biomarkers so that effective targeted anti-cancer agents can be developed against them, such as BCR-ABL tyrosine kinase inhibitor (TKI), imatinib. These TKIs have helped form the groundwork for targeted oral therapy for various types of leukemia. Inhibitors of BCR-ABL, FLT3, JAK2, Bruton tyrosine kinase have set a novel example of success for leukemia treatment (Liu 2019).

Certain novel biomarkers, comprising of non-tyrosine kinase oncoproteins, have been developed for targeted therapeutic action. For example, inhibitors of biomarker BCL-2, isocitrate dehydrogenases (IDH1 and IDH2), PI3 kinase, BRAF, mTOR, PARP, and CDK are currently under research to target many cancer types, including leukemia (Liu 2019).

Gemtuzumab ozogamicin (GO) is an "antibody-drug conjugate" (ADC) targeting the CD33 receptor that is commonly expressed on myeloid cells. GO has been authorized for use in both newly diagnosed and refractory/relapsed (RR) acute myeloid leukemia (AML). GO has been found effective when used alone and also in combination with other chemotherapeutics. Several novel ADCs targeting CD33 are being investigated for clinical efficacy. Some of the more promising ones include vadastuximab talirine (SGN-CD33A), IMGN779, and AVE9633 (huMy9–6-DM4) (Liu 2019).

ADCs targeting the CD123, such as IMGN632 and SGN-CD123A, have also undergone early phase trials. However, further development of SGN-123A was halted due to safety concerns (Liu 2019).

BiTE and ADCs targeting CLL-1 are currently being investigated in preclinical or early clinical trials for effectiveness against AML. CAR-T cells that target CLL-1, are further tested in clinical trials for AML therapy (Liu 2019).

11.6.5 MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNAs that block or degrade the target mRNAs in the posttranscriptional phase. Several studies have demonstrated the role of miRNA dysregulation in the initiation, invasion, proliferation, and metastasis of cancer. It was also shown that secretory miRNA levels in the blood and other body fluids have a significant correlation with cancer progression, therapeutic outcome, and overall survival. The presence of miR92-a in the plasma of acute myeloid leukemia and acute lymphoblastic leukemia patients may serve as a novel biomarker for leukemia. In contrast, the miR-638 is present in healthy individuals and can be used as a control during miRNA quantification. The ratio of miR-638 and miR-92a in plasma is significantly associated with early detection of acute leukemias.

Similarly, miR-150, miR-195, miR-29, and miR-222 have been identified as potential biomarkers for CLL. Thus, secretory miRNAs hold a great potential as powerful and noninvasive cancer biomarkers. Further research is required to verify different approaches for the detection of miRNA detection in body fluids to aid early cancer diagnosis and therapeutic efficacy prediction (Zhang et al. 2012).

11.6.6 Exosomes

Exosomes are extracellular vesicles (EVs) made up of characteristic lipid bilayer, which may contain lipids, proteins, DNA, messenger RNA (mRNA), and non-coding RNAs. Recent studies have indicated that the lipid membrane of exosome acts as a protection for nucleic acids. Almost all types of cells in the human body release exosomes into biological fluids. In cancer, the exosomes derived from tumor cells contain miRNAs with deregulated expression that leads to metastasis and treatment resistance. Owing to the extensive presence of exosomes in various body fluids and the increased stability of miRNAs inside exosomes, the exosomal miRNAs can serve as a new class of biomarkers for minimally invasive and timely detection of cancer (Salehi and Sharifi 2018).

11.7 Novel Biomarkers for Diagnosis and Prognosis of Lymphoma

Classical Hodgkin lymphoma (cHL) is one of the most common types of lymphoma in the developed countries. Advancement in the therapeutic options for cHL have resulted in improved outcomes leading to cure rates as high as 80%. Despite this progress, a certain subset of patients still face unresolved clinical issues due to relapsed or refractory disease and treatment-induced toxicity. The inclusion of targeted drugs such as PD-1 blockers and the CD30 antibody-drug conjugate such as brentuximab vedotin has expanded the available treatment options for cHL patients. It has also highlighted the urgent need for identification of biomarkers that will provide guidance for treatment selection, improve drug utilization, and reduce drug-related toxicity. The characteristic biology of cHL is made up of tumor microenvironment consisting of both tumor cells as well as non-malignant immune cells. This environment provides a variety of potential biomarkers related directly to the tumor cells, crosstalk in the tumor microenvironment or host immune response. The following section discusses the use of prognostic models based on gene expression and circulating tumor DNA as novel biomarkers for lymphoma (Guerrero et al. 2020; Aoki and Steidl 2018).

11.7.1 Imaging-Based Biomarkers

The functional imaging assessment using '[18F]-fluoro-2-deoxy-D-glucose' (FDG) positron emission tomography (PET)/computed tomography (CT) has shown promising results in evaluating the chemosensitivity and disease outcome in cHL (Wiedmann et al. 1999; Gallamini et al. 2007).

11.7.2 Peripheral Blood-Based Biomarkers

Peripheral blood is an ideal, easy-to-access, and minimally invasive and biomarker source. Various malignant cell-based, immune response-based markers, and TME-based biomarkers, for instance, neutrophil-to-lymphocyte ratio, serum thymus, lymphocyte-to-monocyte ratio, galectin 1, activation regulated chemokine (TARC), micro-RNAs, serum CD30, and serum CD163, have been reported as a potential tool as a prediction outcome using the peripheral blood that is taken at the time of diagnosis ("pretreatment") or during the treatment ("interim") in cHL. (Jones et al. 2013, 2014; Plattel et al. 2016). Captivatingly, serum TARC was reported to correlate with the disease status and may be predictive for the PET/CT outcomes in cHL, which indicates that the serum TARC has a strong potential for monitoring tumor dynamics (Farina et al. 2014). These biomarkers reflect TME or the immune system's biology, for instance, the infiltrating lymphocytes and tumor-related macrophage; though, more detailed understanding of the multifaceted ecosystem of TME of the cHL-related lymphatic tissue may not be possible. Another significant limitation of such biomarkers is the lack of sufficient data to determine the optimal cutoff values for each marker (Farina et al. 2014).

11.7.3 Circulating Tumor DNA (ctDNA) Biomarkers

The circulating tumor DNA (ctDNA), collected from the peripheral blood, has been studied extensively as a tool enabling the dynamic monitoring of tumor biology (Kurtz et al. 2015; Roschewski et al. 2015). The ctDNA comprises DNA fragments that are released from the apoptotic and necrotic cancer cells. These can be detected and then quantified by the use of next-generation sequencing. The tumor-specific immunoglobulin gene segments have also been identified in the peripheral blood of cHL patients (Oki et al. 2015).

Even though the recent research in the field of cHL has established multiple biomarker candidates, there is still a gap in identification of biomarkers that can provide information regarding disease relapse. The existing candidates need improvement in validity and reproducibility for use in patients with refractory or relapsed disease.

11.7.4 Identification of Biomarkers Using Gene Expression Profiling

Gene expression profiling is a novel technique employing recent technological advances to examine sample resources generated during routine diagnostic procedures, such as formalin-fixed and paraffin-embedded biopsies. Due to the absence of the HRS (Hodgkin Reed-Sternberg) cells in the whole-tissue biopsies of cHL, analysis of the whole-tissue sections shows the structure of the tumor microenvironment in the tissues with cHL involvement. For this reason, a growing number of studies aimed at identifying biomarkers through gene expression profiling, are focused on studying the TME.

One of these studies established the significant association between the macrophage count and clinical outcomes in treatment naïve cHL patients. These findings were successfully extended by applying the NanoString platform in a follow-up study. This resulted in development of a prognostic model to predict the overall survival of cHL patients with advanced disease that received ABVD chemotherapy regimen. The model involved 23 genes, including ones that reflected a macrophage signature and TH1 response, cytotoxic T cells, cytokines, and natural killer cells. It aimed to incorporate the intricate biology of the tumor–host interactions in the TME. This model, however, failed at providing accurate prediction of patient outcomes in the S0816 and RATHL trials, which can be linked to the response-adapted treatment strategy used in these trials (Burton et al. 2017).

11.8 Novel Biomarkers for Diagnosis and Prognosis of Adenocarcinoma of the Upper Digestive Tract

Adenocarcinoma involving the upper digestive tract (UDT), which includes the gastro-esophageal junction (GEJ), distal esophagus, and stomach, is a serious disease that impacts over 20,000 people every year (Mohammadi et al. 2021). Since early detection and therapeutic selection are essential for disease management and treatment, identifying effective biomarkers can significantly improve the prognosis (Calanzani et al. 2021). The lack of predictive biomarkers, responsive to conventional chemotherapy and radiotherapy, and selective therapy, is currently one of the major challenges in the treatment. In the context of UDT adenocarcinoma, several new trackable proteins have been investigated (Mohammadi et al. 2021).

11.8.1 Mammalian Target of Rapamycin (mTOR)

Multiple inputs involved in cellular growth stimulate the mammalian target of rapamycin, that is a serine/threonine kinase. Phase II trials in severely pretreated gastric cancer-focused at mTOR inhibition as a monotherapy. Both trials revealed moderate tumor responses (Doi et al. 2010; Yoon et al. 2012a, b). Large levels of phosphorylated S6 protein (the mTOR downstream target) were associated with a better response to this therapy (Yoon et al. 2012a, b). GRANITE −1, the subsequent

phase III randomized trial, observed that mTOR inhibition individually promoted progression-free survival but not overall survival (Kanagavel et al. 2015). This approach can reliably be paired with conventional therapy but needs further investigation.

11.8.2 c-Met

The c-Met gene, which is responsible for coding the hepatocyte growth factor receptor, is another subject in UDT adenocarcinoma research. MET inhibition has shown some persistent responses in patients with c-MET amplification. It is not frequently expressed in UDT adenocarcinoma (Lennerz et al. 2011). However, in an unselected gastric cancer population, Shah and colleagues observed no c-MET repression effect as monotherapy (Shah et al. 2013). On the other hand, Cecchi and colleagues reported that inhibiting c-MET in conjunction with chemotherapy has a substantial advantage (Cecchi et al. 2012). This influence was most noticeable in patients with c-MET stimulation, emphasizing the significance of proper patient screening for investigational interventions.

11.8.3 Vascular Endothelial Growth Factor (VEGF)

One of the driving factors of tumor angiogenesis is VEGF-A. When VEGF binds to several VEGF receptors (VEGFRs 1, 2, and 3), several signaling cascades associated with angiogenesis are activated. Inhibition of VEGF signaling has been studied in several solid tumors (Arnold et al. 2020). There is insufficient retrospective evidence in UDT adenocarcinoma to indicate that VEGF overexpression is associated with cisplatin sensitivity (Boku et al. 2007). Still, VEGF signaling has mainly been studied in this disease as an underlying factor for intervention. VEGF targeting in conjunction with standard treatment is reliable and effective in early phase II trials (Chen et al. 2017; Shah et al. 2006). Despite these preliminary observations, the AVAGAST trial showed that adding bevacizumab to therapy in the first-line setting has almost no advantage (Sawaki et al. 2018). Higher serum levels of VEGF-A, and low tumor neuropilin (a receptor that binds to VEGF-A), are correlated with improved outcomes in a subset study of this trial, emphasizing patient significance selection for this kind of trial (Butters et al. 2019). Keeping in view the discussed conditions, VEGF could be the potent marker for targeting the UDT adenocarcinoma.

11.8.4 Human Epidermal Growth Factor Receptor 2 (HER2) and Epidermal Growth Factor Receptor (EGFR)

HER2 and EGFR are three-domain tyrosine kinases found at the cell membrane that are closely related. Unlike EGFR, which has various recognized activating ligands

like EGF and TGF, HER2 has none. These two proteins can form homo- and heterodimers, resulting in the activation of various signaling pathways. Several agents that target EGFR and HER2 have been used in clinical trials. The most glaring example is the addition of trastuzumab (a monoclonal antibody that inhibits Her2 mediated signaling), which dramatically increased survival in HER2 (Sardesai et al. 2020). Studies on HER2 overexpression in UDT adenocarcinoma are contradictory. HER2 overexpression is associated with better survival in patients only treated with surgery in one comprehensive study (Heidarpour et al. 2020). Other reports have come to a different conclusion (Chan et al. 2012). HER2 heterogeneity may be a factor, as patients with substantial heterogeneity in their HER2-positive UDT adenocarcinoma have poorer outcomes (Yoon et al. 2012a, b). In patients treated with adjuvant chemotherapy or chemoradiation, Her2 positivity does not significantly alter response or outcome (Jácome et al. 2015). Since up to 20% of patients with UDT adenocarcinoma overexpress Her2, targeting this receptor has been studied and studied (Phillips et al. 2013).

11.8.5 Germline Alterations (Single Nucleotide Polymorphisms (SNPs))

The need for tumor tissue makes it difficult to develop the potential predictive markers of UDT adenocarcinoma responsiveness. Obtaining adequate pre-therapeutic cancerous tissues for evaluation is difficult, time-consuming, and tumor heterogeneity can skew the findings (Gerlinger et al. 2012). The expression of several candidate biomarkers can be influenced by the medication, making post-therapy markers unhelpful for evaluating response. As a result, germline genetic alterations, i.e., single nucleotide polymorphisms (SNPs), have been evaluated as potential predictive biomarkers for UDT adenocarcinoma. This method has the advantages of being relatively noninvasive (blood specimen can be used) and cost-effective. Several studies have focused on evaluating the association between therapeutic responses and SNPs in various genes (Khorasani et al. 2021; Takahashi et al. 2013; Yoon et al. 2011). SNPs in the kt/mammalian target of rapamycin (mTOR), X-ray repair complementing protein 1 (XRCC1), epidermal growth factor receptor (EGFR), p53, and cyclin D1 genes have all been related to therapeutic responses. However, utilizing SNPs to determine therapeutic responsiveness has the limitation of needing a greater patient cohort and validation sets to draw clinically useful conclusions (Khorasani et al. 2021; Huang et al. 2017a, b; Stocker et al. 2009; Takahashi et al. 2013).

11.8.6 Chemotherapy-Associated Metabolism Genes

5-Fluorouracil is a widely used chemotherapeutic drug in UDT adenocarcinoma (5-FU). This uracil analog is rapidly transferred into cancer cells, where dihydropyrimidine dehydrogenase converts it to uracil. It has been proposed that

evaluating these enzymes' expression in tumors may provide information about the 5-FU response. In UDT adenocarcinoma treatment, i.e., 5-FU-based chemotherapy alone or in conjunction with radiation, higher thymidylate synthase (TS) expression has been associated with a poor therapeutic response and outcome (Harpole et al. 2001; Huang et al. 2017a, b; Smid et al. 2016). However, this observation is not conclusive, as at least one study found no relation between TS levels and outcome after treatment with a 5-FU. Dihydropyrimidine dehydrogenase (DPD), methylenetetrahydrofolate reductase (MTHFR), thymidine phosphorylase (TP), orotate phosphoribosyltransferase (OPRT) are all 5-FU-related enzymes that have been studied in the framework of predicting responsiveness to 5-FU-containing regimens (Miyazaki et al. 2010; Wang et al. 2016).

11.8.7 NF-κB

Chronic inflammatory mechanisms, regulated by NF-κB, are known to be involved in invasive UDT adenocarcinoma progression (Yang et al. 2012). This protein is overexpressed in UDT adenocarcinoma and associated with chemo- and radioresistance in vitro (Li and Sethi 2010). Multiple studies have been done to evaluate its involvement in predicting therapy response due to these findings. Izzo and colleagues focused on pretreatment NF-κB expression in 43 patients with UDT adenocarcinoma who were part of a prospective study (Gambhir et al. 2015). They discovered that elevated NF- κB expression levels before treatment were linked to a poor response to docetaxel-based chemoradiation and poor survival. Some reports have corroborated this conclusion.

Furthermore, it has been shown that chemoradiation can activate NF-B, and this effect is linked to poor survival (Izzo et al. 2009). Other studies also indicated that chemoradiation-induced NF-B downregulation is linked to enhanced therapeutic response (Duggan et al. 2018). This phenomenon tends to be specifically predictive of chemoradiation response rather than generally prognostic. At least one group has associated NF-B overexpression with a better prognosis in UDT adenocarcinoma patients that were only treated with surgery (Huang et al. 2016a, b).

11.8.8 Excision Repair Cross-complementing 1 (ERCC1)

The development of cross-linked DNA adducts by cisplatin and other platinum compounds is the primary process by which these substances exert their cytotoxic effects. Nucleotide excision repair, a complex mechanism involving the identification of impaired DNA strands, excision of the DNA-adduct, and "filling in" of the excised strand, is thus used to repair these DNA lesions. ERCC1, required for the impaired DNA strand's cleavage, is a key protein in this process. Several studies have investigated the relationship between low levels of ERCC1 expression and responses to platinum-based chemotherapy. Decreased levels of ERCC expression are related to higher response rates and longer survival. Pretreatment ERCC1

expression has been associated with responses to the platinum-based chemotherapy and patient results in UDT adenocarcinoma in various retrospective and prospective studies (Chen et al. 2013a, b; Fareed et al. 2010; Jinjia et al. 2019; Zhuo et al. 2020).

Furthermore, patients with elevated ERCC1 expression levels in their tumors have poor responses to platinum-based chemoradiation (Warnecke-Eberz et al. 2004). This finding, however, is not universal, as two retrospective studies in UDT adenocarcinoma found no relation between ERCC1 expression and responses to platinum-based chemotherapy (Langer et al. 2005, 2010). As a result, ERCC1 expression is rarely used in clinical decision-making for the treatment of UDT.

11.8.9 ATP-Binding Cassette Transporters (ABC Transporters)

Efflux of the drug from the cell before reaching therapeutic concentration is one of the highly investigated chemotherapeutic resistance methods. ABC transporters execute this process as they move the drugs out of the cell in an ATP-dependent manner (Ge et al. 2018). Chemotherapeutic resistance has been linked to several proteins in the ABC superfamily. Overexpression of Multidrug Resistance Protein 1 (*p*-glycoprotein, MDR-1) and multidrug resistance-associated protein-1 (MRP-1) in UDT adenocarcinoma has been linked to poorer chemotherapy response (Langer et al. 2010; Shi and Gao 2016). In UDT adenocarcinoma, MDR-1 overexpression is attributed to a weak response to concurrent chemoradiation (Fujishima et al. 2017). Also, chemotherapy treatment has been shown to suppress MRP-1 expression in UDT adenocarcinoma (Blank et al. 2016). Thus, these proteins are the most valuable predictive markers in reducing cancers cell resistance and combating the UDT adenocarcinoma.

11.9 Novel Biomarkers for Pancreatic Disease Treatment and Diagnosis

Pancreatic cancer is an incurable condition that often stays unmedicated. The mortality-to-incidence rates for pancreatic cancer are the largest of any solid tumor. Pancreatic cancer management includes a new, multidisciplinary approach that combines fundamental clinical research in diagnosis, identification, and therapy (Klein 2019). Multiple experiments and clinical studies have been conducted in recent years to find potential biomarkers for various pancreatic cancer forms (Khomiak et al. 2020). Serum and other body fluids, like urine and pancreatic juice, are sources of less invasive biomarker screening sources (Sahni et al. 2020).

The majority of genetic abnormalities identified in pancreatic cancer are deletions, duplications of various chromosomal loci, oncogene mutations and tumor suppressor gene mutations/deletions. These genes include; KRAS, TP53, BRCA2, MADH4/SMAD4/DPC4, and CDKN1A/p16 (Sahni et al. 2020).

11.9.1 Angiogenesis Factors

Antiangiogenic agents have been more commonly used in cancer therapy, but it is essential to discover the candidate biomarkers to assess the resistance and response. EGF (Epidermal Growth Factor), VEGF (Vascular Endothelial, Growth Factor), heparanase, thrombospondin, cathepsins are the most important angiogenic factors pancreatic cancer. Overexpression of EGF and its receptor EGFR has been associated with tumor staging, but there is currently no firm proof of overall survival (Heidemann et al. 2006). Another essential element that works on the angiogenesis mechanism is VEGF. Pancreatic ductal adenocarcinoma (PDAC) is enhanced by interacting with MMP-9, a cellular matrix remodeling component. In pancreatic cancer, therapy targeting both MMP-9 and VEGF resulted in a substantial reduction in PDAC progression and microvessel density than single target treatment (Pistol-Tanase et al. 2008).

The level of thrombospondin (TSP-1) expression has been linked with the progression of PDAC. TSP-1 protein is present in abundance in the stroma surrounding tumor cells, and its expression is found to be inversely related to the density of microvasculature (Tobita et al. 2002). The role of cathepsins in the development and progression of pancreatic cancer is still controversial.

In PDAC, the proteins cathepsin l (CTSL) and cathepsin b (CTSB) is found to be overexpressed. The role of cathepsins in local tumor invasion is inferred by an association with perineural invasion and CTSB expression level (Niedergethmann et al. 2000).

11.9.2 Gene Expression and Potential Factors

New potential factors implicated in pancreas oncogenesis are identified in studies focused on gene expression levels quantification (Sahni et al. 2020). The STAT3 transcription factor is discovered to be activated constitutively in PDAC. It is a key factor in the self-renewal of stem cells and cancer cell survival and inflammation. The tyrosine phosphorylated STAT3 levels and the gp130 receptor are highly associated. STAT3 activation in pancreatic cancer has also been related to an upregulation of the IL6/LIF-gp130 pathway (Corcoran et al. 2011). According to that study, STAT3 is necessary for the progression of precursor pancreatic lesions such as acinar-to-ductal metaplasia (ADM) and PanIN, so gp130 and phospho-STAT3 expression may be a potential biomarker.

Calcium-binding protein S100P also gained particular attention. This protein is reported highly expressive in the pancreatic precursor lesions (PanIN 2 or PanIN 3) and pancreatic tumor, thus making the way via quantification of its expression level for earlier diagnosis (Prica et al. 2016). S100P mRNA expression was found higher in the pancreatic juice of patients with pancreatic cancer and IPMN (Crnogorac-Jurcevic et al. 2013). Three members of the S100A family (S100A2, S100A4, & S100A6), on the other hand, have been associated with poor prognosis. Cell cycle

control and cell invasion are also controlled by members of the S100A family (Tanase et al. 2010).

11.9.3 ZIP3 (Zinc/Iron-Regulated Transporter-Related Protein 3)

Zinc plays a key role in cellular processes such as cell division, immune response, and free radical defense; zinc deficiency has been associated with a high risk of cancer (Sapkota and Knoell 2018; Skrajnowska and Bobrowska-Korczak 2019).

In adenocarcinoma, Costello et al. discovered a significant deficiency of zinc in the ductal and acinar epithelium compared to the normal epithelium. The reduction in zinc quantity is a feature of both pancreatic cancer and precursor lesions. ZIP3 (basilar membrane zinc uptake transporter) gene expression is present in normal ductal/acinar epithelium but absent in adenocarcinoma. The deficiency of zinc in early and advanced malignancy is determined by lowered ZIP3 expression (Costello et al. 2011).

11.9.4 Cancer Stem Cell Biomarkers

Several surface markers of pancreatic cancer cells with stem cell features have been identified. The cell surface markers CD24, CD44, and ESA, have been used to identify these cancer stem cells (Gzil et al. 2019). These human cells can self-renew and produce distinct progeny when inserted into the pancreas of immunocompromised mice, mimicking a tumor's phenotype from which they were generated. A highly tumorigenic subpopulation of cancer stem cells was isolated from PDAC patients. These CD133+ cells were capable of inducing tumor development in athymic mice. The presence of the CXCR4 receptor distinguishes this subpopulation of circulating cancer stem cells, which is involved in tumor metastasis and consequently could be good predictors in diagnosing and identifying the new ways of treatment of pancreatic cancer (Zhu and Yuan 2015).

11.9.5 Saliva Biomarkers

Recent studies have proposed that saliva may quantify particular factors to distinguish between patients with pancreatic cancer and those with mild or chronic pancreatitis. With a sensitivity of 90% and a precision of 95%, the Zhang et al. group differentiate the pancreatic cancer patients from healthy control using transcriptome profiles. The researchers discovered 12 mRNA biomarkers that are specific to pancreatic cancer patients. MBD3L2, KRAS, STIM2, MBD3L2, DMXL2ACRV1, CABLES1, DMD were found to be upregulated, while TK2, GLTSCR2, CDKL3, DPM1, TK2, TPT1 were found to be downregulated (Zhang et al. 2010).

Another group used mass spectrometry to determine metabolites in saliva and came up with a similar finding. Based on a pancreatic cancer-specific characteristic, cases of pancreatic cancer were successfully identified. Patients with breast or pancreatic cancer had higher ornithine and putrescine levels, whereas patients with oral cancer had somewhat higher levels. Pancreatic cancers have higher tryptophan levels, while arginine levels are lower in many cancers, including breast, colonic, and pancreatic cancers, which may be attributed to elevated arginine uptake by tumor tissues with enhanced arginase activity (Sugimoto et al. 2010).

11.9.6 Pancreatic Juice Biomarkers

The evaluation of protein expression profiles in pancreatic juice samples taken from a pancreatic duct can identify markers that could be used to differentiate between benign and malignant pancreatic lesions and differentiate between various phases of PDAC. Vareed et al. discovered that 56 proteins were observed to be escalated in the pancreatic juice of PDAC patients compared to controls (Schneider et al. 1984).

Increased levels of many metabolic enzymes were also discovered in the PDAC-associated secretory proteome study. Purine nucleoside phosphorylase (NP), an enzyme involved in the purine salvage pathway that is active during inflammation and neoplastic development, is the most important metabolic component (Bantia et al. 2010). NP activity is elevated in cancer sera, and NP expression, in conjunction with another component, adenosine deaminase (ADA), has been used to assess the clinical severity of different types of cancers (Kutryb-Zajac et al. 2018).

The strong correlation between NP and inflammation prompted researchers to consider this protein's expression and activity in PDAC patients, especially those with antecedent inflammatory conditions such as chronic pancreatitis and pancreatic intraepithelial neoplasia (Rebours et al. 2010). As a result of this relationship, measuring NP levels may be a valuable marker for monitoring the progression of PDAC.

11.9.7 Plasma Biomarkers

New promising biomarkers for early detection of pancreatic cancer have been published in recent papers (Balasenthil et al. 2017). Roberts et al. studied blood samples from patients with pancreatic adenocarcinoma that was either locally advanced or metastatic. The report identified one putative prognostic protein, i.e., alpha 1-antichymotrypsin (AACT), and two putative predictive proteins, complement factor H (CFH) and histidine-rich glycoprotein (HRG). Overall survival (OS) was negatively associated with AACT, while CFH had no predictive significance as a prognostic factor for OS (Longo et al. 2016). Thus, AACT may be a reliable prognostic marker in patients with advanced-stage pancreatic carcinoma.

11.9.8 Metabolomic Biomarkers

Studying the metabolome is a new method for detecting cancer signatures. There are currently not commonly used metabolomic markers in clinical practice for prognosis analysis, diagnosis, or chemotherapy response estimation. However, several studies (Di Gangi et al. 2016; Goldberg et al. 2019; Moore et al. 2019; Tumas et al. 2019) have shown distinctions in metabolic profiles in PDAC patients, patients with some other conditions, and healthy controls. Mayerle et al. aimed at the blood samples' metabolic profile from 914 patients with CP, liver cirrhosis, PDAC, healthy and non-pancreatic disease controls (Mayerle et al. 2018). The findings reveal a biomarker signature (nine metabolites plus CA 19-9) that effectively differentiated PDAC from CP.

The metabolomic profiles of cancerous tissue from 25 patients who had undergone curative resection and adjunctive gemcitabine-based therapy were investigated in another study. Lactic acid levels were noted to be elevated in the tumors of patients who had adverse health results after receiving gemcitabine. Patients with lowered lactic acid levels and higher hENT1 protein expression had a slightly longer survival time than the other groups (Phua et al. 2018). While further standardization and validity of candidate metabolic signatures are needed, metabolomics could be a successful method for developing novel diagnostic, prognostic, or predictive markers for pancreas cancer.

11.10 Novel Biomarkers for Diagnosis and Prognosis of Esophageal Cancer

Oesophageal cancer is a fatal disease with a poor prognosis and limited available treatment options. Cancer Research UK has declared it as a "cancer of unmet need" (Lavery and Turkington 2021). In developed countries, the 5-year survival rate for esophageal cancer (EC) is less than 10%, with esophageal squamous cell carcinomas (ESCC) accounting for more than 90% of cases. There are currently no appropriate adjuvant diagnostic biomarkers for ESCC to help with dysplasia diagnosis (Ishiguro et al. 2013; Taylor et al. 2013). Biomarker assessment may greatly strengthen the diagnostic accuracy by reducing the subjectivity involved with histologic dysplasia assessment (Couch et al. 2016). Identifying the suitable biomarkers for ESCC and squamous cell dysplasia that would be suitable as adjunctive use for pathological diagnosis and may provide a deep insight into molecular pathways leading to carcinogenesis (Lavery and Turkington 2021).

11.10.1 Mutations and Polymorphisms

Gene-based biomarkers play a significant part in cancer care. Mutations in the TP53 gene are the most commonly diagnosed genetic alterations in EC (Hao et al. 2013). A study demonstrated that the TP53 gene knockout rate is strongly associated with the

degree of differentiation and lymph node metastasis in EC (Niyaz et al. 2020). It may be useful for determining the clinical condition of EC patients (HUILI Zheng et al. 2016).

Other significant genetic mutations that affect esophageal tumorigenesis are single nucleotide polymorphisms (SNPs) (Wu et al. 2013). According to research that links EC and genetic polymorphisms, polymorphisms can be potential markers for evaluating the risk of EC (Li et al. 2014a, b). Renouf et al. found that esophageal adenocarcinoma (EAC) patients with p53 Pro/Pro have a shorter survival time (Renouf et al. 2013). Cescon et al. discovered that p53 Arg72Pro Pro/Pro is linked to a twofold rise in EC patients' mortality risk (Cescon et al. 2009). Several other analyses found a link between TP53 Arg72 carriers and a lower EC risk (Jafrin et al. 2020).

CYP1A1 is another studied polymorphism (Zeng et al. 2015). Several researchers investigated the relationship between the CYP1A1 polymorphism, and the EC risk have shown inconsistent effects, which can be explained by ethnic and environmental factors (Gong et al. 2014; Shen et al. 2013; Zheng and Zhao 2015). In central China, genetic polymorphisms in the CYP1A1 gene are related to a higher EC risk (Yun et al. 2014). However, after controlling for contributing causes, a study observed that no CYP1A1 genotypes and alleles are linked to the risk of EC (Shen et al. 2013). More comprehensive studies with improved designs are required to verify the relationship between CYP1A1 polymorphisms and EC vulnerability (Zheng and Zhao 2015).

11.10.2 Genomic Instability

Both chromosome instability (CIN) and microsatellite instability (MSI) are implicated in EC progression (Forghanifard et al. 2016). A small number of researchers have reported its values in EC prognosis.

CIN, in a general context, is referred to as a range of chromosome changes. Losses of chromosomes, i.e., 5q, 11p, 12q, and 17p, are common in EC and are linked to the patient's outcome. Certain essential genes are still involved in chromosome restructuring; these changes have been observed in certain genes like MYC, FHIT, etc. (Paulson et al. 2009; Xing et al. 2009).

MSI is a valuable biomarker in EC because patients with a positive MSI have specific features and prognosis. According to Matsumoto Y et al., esophageal squamous cell carcinoma patients have a comparatively high MSI-L prevalence. The degree of MSI-L is inversely related to the depth of invasion (Jiao et al. 2019). Changes in these genes trigger a cascade of mutations, which eventually contribute to the emergence of malignant phenotypes. This relation has been discovered in genes like MLH1, MSH2, BAT25, and BAT26 (Dudley et al. 2016).

11.10.3 Proteomics Biomarkers

The serum contains a large number of peptides, some of which are promising biomarkers for cancer management. An abnormal proteomics molecule levels are associated with health status, thus helpful in the prognosis of EC (Amiri-Dashatan et al. 2018).

Modern techniques used in proteomic studies have suggested several contender biomarkers in EC tissues, such as HMGB3, ANGPTL2, LSD1, and EI24.

Tissue biomarkers' detection via proteomics-based approaches accelerated the assessment and management of EC. For instance, EI24 is identified as a potential predictive biomarker by using iTRAQ and some other methods by a research group for the prognosis and management of EC (Hong et al. 2015). Recently, in discovering the new EC biomarkers, cell lines have also been used as valuable sources of proteins (Jiménez et al. 2017). However, further investigation is required due to the heterogeneity in various cell lines.

11.10.4 Epigenetic Biomarkers

LncRNAs have depicted a considerable potential as prognostic biomarkers in EC. Some reports have indicated that EC has a lncRNA profile, with particular upregulated or downregulated genes, and potentially valuable in the prognostication approach (Sugihara et al. 2015; Yao et al. 2016). Furthermore, some of them can be used to monitor EC, such as higher expression of the lncRNA SPRY4- IT1 in ESCC patients is an independent prognostic factor (Xie et al. 2014). According to Li JY et al., ESCC patients with higher lncRNA UCA1 expression have an advanced clinical stage and a worse outcome than lower expression (Kang et al. 2018). Several studies found that lncRNA HOTAIR is overexpressed in ESCC tissues compared to normal controls and that elevated expression is linked to poor ESCC patients' survival (Chen et al. 2013a, b; Ma et al. 2015). MALAT1 lncRNA expression is also associated with the prognosis of EC patients (Huang et al. 2016a). lncRNAs like lncRNA-uc002yug2, lncRNA LOC285194, lncRNA FOX CUT, lncRNA CASC9, lncRNA ZEB1-AS1[156], and lncRNA CCAT2, have been linked to EC patients' prognosis (Tang et al. 2015). According to Liu et al., an elevated level of lncRNA BANCR in plasma is associated with shorter survival, indicating that it could be used as a tumor biomarker for early detection of EC, a prospective prognostic biomarker, and a possible therapeutic target for EC patients (Liu et al. 2016b).

11.10.5 miRNA

miRNA alterations have been discovered in EC tissues and cells. So, reshaping the miRNA expression patterns can help to prevent cancers from developing malignant phenotypes. Some of these miRNAs, such as miR-27a/b, miR-335, let-7c, miR-145, miR-21, miR-133, miR-148, and others, can serve as biomarkers for predicting

prognosis (Hiyoshi and Watanabe 2015). Chen and colleagues reported the MiR-133a/b as an independent prognostic factor for ESCC patients' survival (Chen et al. 2014).

11.10.6 DNA Methylation

DNA methylation is an epigenetic modification that occurs in CpG dinucleotides at "carbon 5" of the cytosine ring (Irwin et al. 2019). Detection of methylation biomarkers in body fluids like serum and plasma is the most significant aspect of these markers. The p16 gene methylation is a typical biomarker found in body fluids. P16's methylation pattern can be observed in blood and used as a marker for early disease detection and control (Abbaszadegan et al. 2008). Other serum markers, like PTX3, and MGMT have recently been identified as valuable prognostic biomarkers (Das et al. 2014; Wang et al. 2011). SOCS-1, HLA-I, CDH13, EPB41L3, NMDAR2B, MTHFR677C > T, and other genes with altered methylation patterns have also been identified as helpful prognostic markers (Yang et al. 2019).

11.10.7 Exosomes

Liquid biopsies are advantageous over traditional tissue biopsies regarding cancer genotyping evaluation, thus making every tumor type distinct (Huang et al. 2017a, b). Liquid biopsy can also be a predictive biomarker for prognosis and therapeutic response in patients with EC.

Exosomes are small particles (ranges from 30 to 120 nm) released from cells in normal or abnormal conditions and play a significant role in intercellular interaction (Golyan et al. 2020). Exosome formation is not only an essential regulatory process during cancer progression, but it also plays a role in cancer diagnosis, treatment evaluation, and prognosis interpretation (Li et al. 2017). Exosomes played an important role in the growth of the EC (Liu et al. 2018). Studies have shown that the number of exosomes can be used as an independent prognostic marker, such as low level of exosomes demonstrates terrible effects in ESCC patients (Matsumoto et al. 2016). In ESCC patients, the expression of exosomal miR-1246 and miR-21 is linked to the tumor classification stage (Matsumoto et al. 2016). Exosome-associated DNA may also be used to identify tumor-specific genetic variations or determine the therapeutic response in esophageal adenocarcinoma (EAC) (Smith and Lam 2018). Exosomes are likely to be novel biomarkers for EAC and ESCC, but the observations mentioned above need to be validated in further animal models and major cohort clinical trials.

11.10.8 Circulating Tumor Cells (CTCs)

Clinically, disseminated tumor cells (DTCs) and circulating tumor cells (CTCs) have been linked to patient prognosis (Dasgupta et al. 2017). Wang and colleagues observed that the occurrence of CTCs in blood samples is associated with poorer disease-free survival (DFS) and progression-free survival (PFS) of EC patients as compared to CTC-negative cases (Wang et al. 2017b). The CTCs marker appears to be a promising therapeutic response marker, prognosis, recurrence risk, and treatment decisions. However, as contrasted to data on biologic markers like squamous cell carcinoma antigen (SCC) or arcinoembryonic antigen (CEA), there is also a lack of supporting data to use CTCs systematically. Furthermore, there are certain drawbacks associated with CTC identification approaches (Grover et al. 2014).

11.10.9 Circulating Tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) has recently been identified as another biomarker with clinical implications (Gabriel and Bagaria 2018). Cellular apoptosis (70–200 bp) and necrosis (200 bp–21 kbp) are the two major sources of ctDNA. The tumor cells have many chromosomal alterations compared to the normal cells, majorly depends on ctDNA and can be a novel biomarker (Leary et al. 2012). As a result, ctDNA screening may be a promising and effective approach for early cancer detection.

11.11 Novel Biomarkers for Diagnosis and Prognosis of Colorectal Cancer

Colorectal cancer (CRC) is a common type of cancer, which poses a greater morbidity and mortality rate worldwide (Rawla et al. 2019). Despite recent advances in the development of screening programs and the management of colorectal cancer patients, there are still many challenges to be addressed, ranging from prevention and early detection to determine the prognosis. The development of personalized medicine and increased survival rates could be encouraged by discovering new biomarkers to assist CRC's early detection or treatment.

An effective CRC biomarker must be easy to quantify, highly sensitive and specific, reproducible and reliable. These priorities can ideally be accomplished with a noninvasive and low-cost approach that uses readily available biological samples like urine, breath, serum, or feces (Chand et al. 2018; Choi et al. 2017; Loktionov 2020).

Colorectal cancer screening strategies lead to identifying and removing adenomatous polyps and other premalignant for decreasing CRC mortality (Alves Martins et al. 2019).

11.11.1 Caudal Type-Homeobox 2

Caudal type-homeobox 2 (CDX2) encodes a homeobox protein majorly involved in regulating cell division of the normal cell in the colon. CDX2 expression loss of might results in colorectal cancer. It is also noted that immunohistochemical finding of CDX2 protein expression could be useful in identifying the CRC. The study has revealed a high level of CDX2 expression in CRC.

Detecting CRC by evaluating CDX2 expression is also a very responsive and explicit method. CDX2 expression in CRC has been studied extensively, and its specificity and sensitivity have been observed greater than 90% (Asgari-Karchekani et al. 2020; Werling et al. 2003).

11.11.2 Cytokeratins (CKs)

Cytokeratins are keratin proteins present in an intracytoplasmic cytoskeleton of epithelial tissue. Generally, to distinguish metastatic CRC from the other tumors, tissues are stained for CK7 and CK20. CRC specimens stain positive for CK20 but negative for CK7. Ten CK20 is only found in Merkel cells and gland cells of colonic mucosa. CK7, on the other hand, is not found in the mucosa of the colon. CK7 is found in the epithelia of the bladder, mesothelium, normal lung and female genital tract (Kigasawa et al. 2017). For distinguishing the metastatic adenocarcinoma of unidentified primary origin, CK staining patterns are one of the most effective procedures. The CK7–/CK20+ pattern is a common method for diagnosing metastatic CRC. 12 A CK7–/CK20+ pattern has been documented in 65–95% of CRC cases (Bayrak et al. 2011).

11.11.3 Cadherin 17

Cadherins are cell-to-cell adhesion molecules that play a critical role in tissue structure normally. Many human disorders, including carcinomas, are linked with molecular abnormalities in cadherin expression (Lee et al. 2010). According to studies, CDH17 is represented in 96–100% of primary CRC and 100% of metastatic CRC. Some studies have shown that CDH17 is more specific and sensitive than CDX2 for detecting CRC (Bian et al. 2017; Su et al. 2008).

11.11.4 Special AT-Rich Sequence Binding Protein 2 (SATB2)

Special AT-rich sequence binding protein 2 is a part of the region binding transcription factors family. Even though the exact function of SATB2 in the GI tract is unknown (Zhang et al. 2018). Magnusson et al. discovered that SATB2 is more expressive in the epithelium of a lower GI tract, especially in the colon (Magnusson et al. 2011). The researchers examined the SATB2 expression profile of 216 cancer

samples and discovered that the maximum CRC samples had elevated SATB2 expression. SATB2 was noted to be positive in 87.8% of CRC cases when used as a single marker (Magnusson et al. 2011; Zhang et al. 2018). The addition of SATB2 to the standardized panels of CK7, CK20, and CDX2 has been studied extensively. According to Dragomir et al., the addition of SATB2 to standardized panels did not improve the specificity and sensitivity in diagnosing CRC (Dragomir et al. 2014). But according to two recent studies, SATB2 has been found as a significant marker for distinguishing the metastatic CRC from primary ovarian carcinomas (Moh et al. 2016).

11.11.5 GPA33

The *GPA33* gene codes for the membranous protein A33, a membrane-bound glycoprotein, have a homolog in an immunoglobulin superfamily (Opstelten et al. 2020). Although the role of A33 is unknown, experimental findings suggest that it could be linked with immunological processes, proliferation, and colonic mucosal repair (Pereira-Fantini et al. 2010). According to immunohistochemical studies, A33 express by epithelial cells in the colon and rectum. It is found expressed in 95% of CRC cases in humans, notably in well-differentiated tumors, suggesting that it may target CRC treatment (Murer et al. 2020). An immunohistological analysis comparing A33 and CDX2 found that A33 has equal susceptibility to CDX2 but higher specificity than CDX2 as a CRC immunomarker (Wong et al. 2017).

11.11.6 Telomerase

It is a ribonucleoprotein that regulates TTAGGG repeats to telomeres at the ends of chromosomes for reverse transcription; telomerase utilizes intrinsic RNA as a template (Jafri et al. 2016). Telomeres shorten with every cell division of normal cells. When telomeres shorten to critical lengths, a signal of DNA damage is triggered, resulting in replicative senescence (Victorelli and Passos 2017). By upregulating telomerase, cancer cells suppress DNA damage-induced inhibitory signaling pathways. It has been observed in 85–90% of all malignant tumors (Shay and Bacchetti 1997). Telomerase has emerged as a recent diagnostic biomarker in CRC. According to some studies, it is expected to have 95% sensitivity and specificity in CRC (Roig et al. 2009).

11.11.7 MicroRNA (miRNAs)

MicroRNAs are small non-coding RNAs with 18–25 base pairs that control gene expression via binding to mRNA. By functioning as oncogenes, miRNAs are known to be linked to various cancers, including CRC (Cui et al. 2019). MiRNAs have extensive blood stability than mRNA because they are not affected by the

endogenous RNase. So, miRNAs are potential noninvasive cancer biomarkers. Around 48 studies have tested their diagnostic potential for CRC (Condrat et al. 2020; Oh and Joo 2020).

11.11.8 Insulin-Like Growth Factors Binding Protein 2 (IGFBP-2)

Insulin-like growth factor ligands and IGF-1 receptors interact with a binding protein called insulin-like growth factor binding protein 2 (Ding and Wu 2018). According to many studies, elevated IGFBP-2 levels in serum are associated with colon cancer as well as other tumors (el Atiq et al. 1994). High serum and plasma IGFBP-2 levels could differentiate patients with colon polyps or CRC from healthier controls. IGFBP-2's sensitivity and specificity for early CRC and colon polyp detection are ineffective, but combining it with other biomarkers such as CEA could enhance sensitivity. Furthermore, high plasma IGFBP-2 levels are linked to larger tumor sizes, suggesting that IGFBP-2 may be used as a diagnostic and prognostic biomarker for colorectal cancer (Renehan et al. 2000).

11.11.9 Long Non-coding RNAs (lncRNAs)

Long non-coding RNAs consist of more than 200 non-translatable nucleotides. More than 150 human morbidities, including colon cancer and other cancers, are associated with lncRNAs (Wang et al. 2019). Single or panel lncRNAs have been used in 55 studies to determine their diagnostic potential for CRC (Oh and Joo 2020).

Hypoxia-inducible factor 1-alpha-antisense RNA 1 (HIF1A-AS1) level in serum was substantially higher in 151 CRC patients than 160 healthy controls, indicating that HIF1A-AS1 has a high diagnostic potential for CRC (Gong et al. 2017). A splice variant of CRNDE-h (colorectal neoplasia differentially expressed) has been shown to differentiate CRC patients from a healthy control (Liu et al. 2016a, b).

Other lncRNAs, such as ZNFX1 antisense RNA1 (ZFAS1), NEAT1, and GAS560, have shown potential as diagnostic and prognostic biomarkers for CRC (Fang et al. 2017; Peng et al. 2017).

11.11.10 Circulating Cell-Free DNA (cfDNA)

It is essentially a type of cell-free nucleic acid that enters the bloodstream after necrosis or apoptosis. CfDNA releases from apoptotic cells in healthy individuals. The DNA fragments are around 180 bp long, but cfDNA, on the other hand, is much larger fragments present in tumor cells (Fernandez-Garcia et al. 2019). As a result, quantitative study of circulating cfDNAs using the ratio of longer to shorter DNA fragments or determining cfDNA integrity number while CRC diagnosis yielded promising results. A systematic review and meta-analysis on circulating cfDNA as a

diagnostic marker for CRC were published in which the author scrutinized the 14 studies for a total of 1258 CRC patients with 803 positive controls. The sensitivity and specificity of circulating cfDNA for CRC diagnosis were 73.5% and 91.8%, respectively, indicating significant specificity for diagnosing the CRC (Wang et al. 2018a).

11.11.11 Microsatellite Instability

Microsatellites are 1–6 bp repeating DNA sequences present in both the genome's coding and non-coding regions. It is also linked with sporadic CRC (Sepulveda et al. 2017). Two mononucleotide repeats, i.e., BAT26 and BAT25 and three dinucleotide repeats, i.e., D2S123, D5S346, and D17S250, are widely used microsatellite markers (Salto-Tellez et al. 2005).

11.11.12 BRAF

RAS-RAF-MEK-ERK molecules play a major role mitogen-activated protein kinase pathway. BRAF-activating mutations are found in about 14% of localized stage and 8% of advanced CRC cases (Caputo et al. 2019). The American Society of Clinical Oncology released a recommendation for using BRAF as a molecular biomarker for CRC in 2017. BRAF p.V600 mutations account for about 90% of BRAF mutations and should be assessed for prognostic significance (Tiwari et al. 2016).

11.11.13 SMAD4

SMAD4 is mainly associated with cell cycle and cell migration (Zhao et al. 2018). According to studies, SMAD4 mutations are present in 30–40% of CRC cases. Voorneveld et al. did a meta-analysis to assess the prognostic importance of SMAD4 in patients with CRC (Voorneveld et al. 2015).

11.11.14 p53

Many studies have focused on p53 mutations and their prognostic importance in CRC patients (Russo et al. 2005). When DNA is impaired, p53 induces cell cycle arrest to restore the mutations; if it fails to restore, apoptosis is induced (Carethers and Jung 2015). The findings, on the other hand, are contradictory. According to some researches, p53 mutation/overexpression is linked to lower disease-free survival, relapse-free survival, and overall survival frequencies (Oh and Joo 2020). According to other studies, there is a lack of proof that p53 has prognostic significance for CRC (McGregor et al. 2015; Wang et al. 2017a, b, c).

11.11.15 Neutrophil-to-Lymphocyte Ratio

Lymphopenia is linked to a reduction in cell-mediated immunity, whereas neutrophilia is linked to systemic inflammation (Ménétrier-Caux et al. 2019; Soehnlein et al. 2017). Few systematic studies and meta-analyses have looked into the role of NLR in CRC prognosis. According to a review, patients with higher NLR had a significantly smaller overall survival and progression-free survival; following a treatment. Patients with an NLR <5 before treatment have a higher chance of having a 5-year overall survival and disease-free survival (Li et al. 2014a, b).

11.11.16 CEA Levels

The ASCO 2006 update the guidelines for the treatment of CRC patients supports CEA as the only marker ("2006 Update of ASCO Recommendations for the Use of Tumor Markers in Gastrointestinal Cancer," 2006). Preoperative CEA levels have been shown to have prognostic significance in several studies (Li Destri et al. 2015). According to two large-scale case studies, preoperative CEA level is strongly correlated with prognosis in CRC patients, metastasized to the liver (Fong et al. 1999; Nordlinger et al. 1996). One study focused on 2230 CRC patients and discovered that CEA levels are strongly associated with patient outcomes (Park et al. 1999).

11.12 Conclusion

The development of novel cancer biomarkers for diagnosis, prognosis, and drug therapy response prediction has benefited greatly from a deeper unraveling of the mutational landscapes in cancer initiation and progression. They have reformed the approach to patient care in various cancer types. Despite considerable advances, most cancer biomarkers for different types of cancers are still in the developmental phase. Many of them are still not approved by the FDA. Future research is expected to develop sensitive and specific diagnostic, prognostic, and predictive biomarkers that are more clinically applicable and patient-acceptable, unlike conventional biomarkers. There is a need to refine further their application for individualized management of the prospective patients. Improved biomarkers should ultimately lead to improvements in outcomes and more efficient, safe, cost-effective, and evidence-based use of health resources.

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Nanotechnology for Cancer Biomarkers

12

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Abstract

Cancer biomarkers play an essential role in early diagnosis, progression, prediction, and potential response of treatment in cancer patients. However, the traditional cancer biomarkers detection techniques lack specificity and selectivity with drawbacks, such as irreproducibility and overdiagnosis. Nanomaterials, with their unique properties, provide efficient, reliable biosensing methods with high sensitivity and selectivity. This chapter represents an overview of the development of nanomaterials-based biosensing techniques and the integration of these nanomaterials in other techniques, such as mass spectrometry (MS), Raman spectroscopy, optical detection, electrical and electrochemical detection, and lab-on-a-chip technology for detection of cancer biomarkers.

Keywords

Nanomaterials · Cancer biomarkers · Nanobiosensors

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12.1 Introduction

"A biomarker is any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" (Lassere 2008). Therefore, a cancer biomarker is the one that can predict the development and progression of cancer in a specific tissue or potential response to cancer therapy (Micheel et al. 2012). Cancer biomarkers can be classified into predictive biomarkers, prognostic biomarkers, and diagnostic biomarkers. A predictive biomarker is the one that predicts drug response, e.g., response to trastuzumab in breast cancer by measuring activation of HER2 protein (Slamon et al. 2001; Piccart-Gebhart et al. 2005; Romond et al. 2005). A prognostic biomarker can predict future development of tumor, e.g., 21-gene recurrence score that can predict survival and recurrence in node-negative tamoxifen-treated breast cancer (Paik et al. 2004). A diagnostic biomarker is used to identify the present condition of cancer, e.g., testing for cancer DNA in the stools has recently been implemented for colorectal cancer surveillance (Imperiale et al. 2014; Goossens et al. 2015).

Cancer biomarkers detection could have a significant contribution in the early diagnosis of a tumor and hence, a great impact on the successful treatment of cancer (Ye et al. 2018). However, traditional cancer screening methods, such as the pap smear for cervical cancer, immunohistochemistry, conventional cytogenetics, prostate-specific antigen (PSA) blood test for prostate cancer, and the fecal occult blood (FOB) test are not significantly successful for the precise detection of early-stage cancer biomarkers due to complication of overdiagnosis (Etzioni et al. 2002), inconsistency (Cottet et al. 2006), and insufficient sensitivity/specificity of individual markers. The detection of cancer biomarkers has also been limited by several other barriers, e.g., heterogeneity and low concentration of biomarkers in bodily fluids, the low half-life of cancer biomarkers, and complications in the analysis (Hull et al. 2014; Zhang et al. 2019). Thus, it is pivotal to develop novel technologies that are sensitive and selective enough to detect cancer biomarkers (Zhang et al. 2013). Therefore, efforts are focusing on the discovery of innovative, efficient, reliable, and authenticated detection of biomarkers for early cancer diagnosis (Patel and Ahmed 2015; Chen et al. 2015). In this regard, by employing nanotechnology, the development of nanosensor-based biomarkers amplifiers has improved assay sensitivities and specificity (Jiang et al. 2015; Kwong et al. 2013). The history of biosensors in different eras is illustrated in Fig. 12.1.

Nanotechnology defines as the formation and manipulation of materials at nanoscale levels to create products that display unique properties. It has a multidisciplinary approach that has the potential to emerge as one of the most promising fields in cancer diagnosis and treatment (Sengupta et al. 2005). The application of nanotechnology-based materials in cancer has exceptional potential for revolutionizing cancer diagnostics and therapeutics (Misra et al. 2010). The nanomaterials having at least one dimension in the nanoscale that have distinct properties as compared to bulk counterparts (Kagan et al. 2016; Weiss 2010). It provides a measurement of multiple targets simultaneously with high specificity and selectivity and can improve specific targeting of biosensors by conjugation with

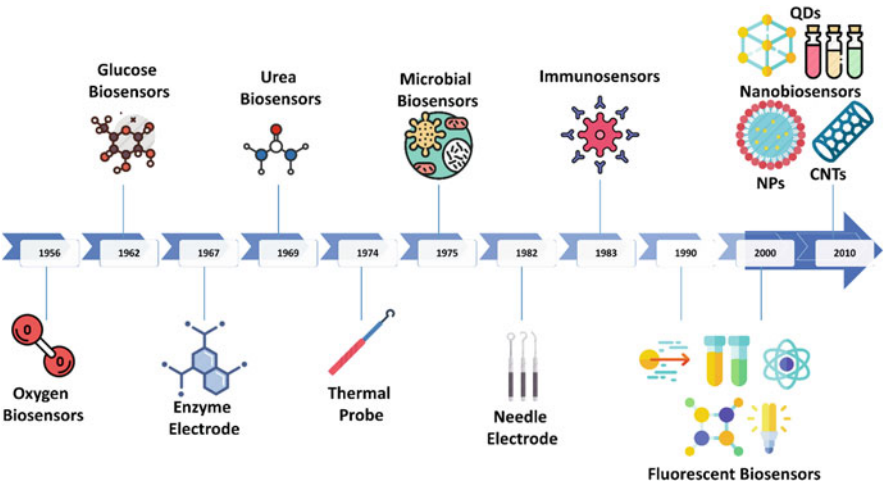

Fig. 12.1 History of biosensors. (Source: Icons from www.flaticon.com)

nanoparticles/nanomaterials (Sharifi et al. 2019). In addition, the high surface area to volume ratio of nanoparticles makes the detection of specific biomolecules a lot simpler by making biosensors more sensitive (Doria et al. 2012). Polymer dots (PDs), quantum dots (QDs), and gold nanoparticles (AuNPs) are the three most common types of nanoparticle probes used in the detection of cancer biomarkers (Harun et al. 2013).

This chapter focuses on the presentation of various nanomaterials-based platforms for the biosensing and detection of cancer biomarkers. Further, how nanotechnology is contributing to new methods in the screening of biomarkers in cancers, with an emphasis on research works published during the recent past years.

12.2 Nanomaterials-Based Biosensing Platforms

Several nanomaterials-based biosensing platforms have been used in the detection of cancer biomarkers. These platforms integrate several nanoscale components and utilize both synthesis approaches of top-down and bottom-up technologies. In this section, we will describe the most common nanomaterials platform developed for biosensing cancer biomarkers.

12.2.1 Nanoparticles

Nanoparticles (NPs)based assays, sensors, and arrays have emerged for the detection of cancer biomarkers (Malhotra et al. 2012; Zhang et al. 2013). During the past two decades, NPs have been formulated to obtain desirable biosensing applications due to their unique chemical and physical properties (Gopinath et al. 2015; Yin et al.

2015). The NPs provide the advantage of highly selective, rapid, and sensitive cancer biomarker detection methods, enabling their application in the identification of biomarkers even in trace amounts in bodily fluids, ranging from tears to urine. Different types of NPs have been developed, such as lipid and hybrid NPs (Li et al. 2014), semiconductor (Lu et al. 2015), metal (Yoo and Yeo 2016), metal oxide (Qiao et al. 2015), and polymer (Battistella and Klok 2017), for the application in cancer biomarker detection. NPs can be employed in various sensing techniques depending on their physicochemical properties. The development of nanosensor-based assays has a major challenge of differentiation of ultra-low biomarkers concentration and recognition in point-of-care (POC) devices (Gustafsson et al. 2010; Lewis et al. 2015).

12.2.2 Targeting Ligand–Conjugated NPs for Detection of Biomarkers

Cancer biomarkers are detected in tissue samples, urine, and serum through a range of targeting ligands, such as peptides and proteins (antibodies and their fragments), nucleic acids (aptamers), small molecules, or others (vitamins or carbohydrates). NPs conjugated with tumor-specific ligands having a high binding affinity to trace cancer biomarkers. They can interact with a minimal amount of cancer biomarkers and produce a detectable signal to measure them. Moreover, these NPs can also facilitate the detection of biomarkers by enhancing their secretion from cancer cells (Ye et al. 2018). Some of the examples are given below.

12.2.3 Protein-Conjugated NPs

Most of the protein-based targeting refers to antibodies and antibody fragments. Proteins as a targeting ligand have been a major focus in the field of nanotechnology. Likewise, the enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, immunoblotting, immunochromatography assays, and NP-based biomarker assays are also based on specific antibody–antigen interactions. In one such design, an antibody (Ab)-coated magnetic NP-based one-step assay was developed for the detection of prostate cancer. These Ab-coated magnetic NPs can be directly injected into blood plasma even in a very small amount. Prostate-specific antigen (PSA) concentration is efficiently detected by NPs due to high rotation frequency and biomarker-induced binding. In undiluted human blood plasma, the detection limit for PSA measurement was found to be 400–500 fmolL^{-1} in a total assay time of 14 min, and an optically probed volume of only 1 nL (Ranzoni et al. 2012).

As one of the most common cancer biomarkers, the increased amount of carcinoembryonic antigen (CEA)is associated with cancers of the liver, colon, rectum, prostate, and ovary(Duffy 2001). An electrochemiluminescence (ECL) sensor was developed through a multistep procedure to detect the threshold level of CEA(Jie et al. 2011). The fabrication of the sensor first involves the coating of

Fe_3O_4 NPs with CdSe-CdSNPs and then assembled it with gold NPs (AuNPs) on the electrode. AuNPs enhance the ECL signal by accelerating the electron transfer in the ECL reaction. Eventually, to remove unwanted nonspecific binding sites on immunosensors, it was protected by addition of bovine serum albumin (BSA). This sensor proves to be ideal for ECL immunosensing due to its display of stable and intense ECL emissions in neutral solution (Myung et al. 2002).

Metal nanoisland is a new term that describes ultrafine particles consisting of a few to several hundred metal atoms. Nickel nanoislands (NiNIs) are especially interesting due to their selective attachment to proteins. A NiNI-based biosensors technique has been described for recognition and label-free detection. Quartz crystal microbalance (QCM) surface was deposited by 5 nm NiNIs conjugated to the antibody fragments His-tagged (scFv)-F7N1N2 to detect biomarker GTPaseRhoA. GTPase RhoA is a cancer biomarker, usually overexpresses in various kind of tumors which has been chosen for this test. Results show that using NiNIs provide the possibility of anchoring the cancer biomarker to sense it (Martínez-Rivas et al. 2010).

12.2.4 Aptamer-Conjugated NPs

Aptamers are single-stranded DNA (ssDNA) or RNA sequences that can be bind to NPs as a ligand for targeting. Aptamers have low immunogenicity due to their low molecular weight and high specificity for their targets, e.g., phospholipids, peptides, ions, and bacteria even whole cells, etc. (Avci-Adali et al. 2011; Torres et al. 2017). Prostate-specific membrane antigen (PSMA) is the most common targeted cancer biomarker in this category. PSMA can be targeted by Cy5 incorporated- polymeric NPs conjugated with A10 RNA aptamer. Cy5-polylactic acid (PLA)/aptamer-NPs would not bind to PC3 cancer cell lines which do not show PSMA, while it would bind to canine prostate adenocarcinoma cells and LNCaP cells, which express PSMA. Cy5-PLA-NPs have shown excellent signals in the balb/c mice model, and also show the feature of lowering the background signals in other organs (Tong et al. 2010).

In recent years, DNAzymes (oligonucleotides that show catalytic activity) have attracted interest in the detection of cancer biomarkers. AuNPs conjugated with DNAzymes have been developed for the detection of alpha-fetoprotein (AFP) biomarker usually found in liver or germ cell cancer. An immunoassay strategy was employed by sandwiching the two types of probes. One probe was AFP monoclonal antibody conjugated magnetic microparticles and the other one was AFP polyclonal antibody/double-stranded DNA functionalized AuNPs having one complementary strand of peroxidase mimicking DNAzyme. Double-stranded DNA was rehybridized after the formation of this sandwich complex. A chromogenic reagent was added in the substrate solution that exhibited green color during the DNAzyme catalysis as DNAzyme reacted with AFP. This immunoassay identified AFP at a detection limit of 0.1ngmL-1 (Zhou et al. 2009).

In the bloodstream, circulating tumor DNA(ctDNA) fragments (approx. 100–200 base pairs) can be detected as cancer biomarkers (Schwaederle et al. 2017). These ctDNA fragments can be released from benign tumors or metastatic cancer cells and show specific genetic aberrations that can be used to predict cancer even before any symptom occurs (Mehra et al. 2008; Tan et al. 2017). A single exon in the BRCA1 gene can be detected by a fluorescent probe made up of DNA silver nanocluster (AgNC)(Hosseini et al. 2017). The detection limit was increased to 6.4 × 10-11 M under optimized conditions. AgNC produced fluorescence as they recognized the large deletion mutations in BRCA1. DNA-templated AgNC was hybridized to target DNAs which enhance the fluorescence produced by AgNC with different intensities. These differences were defined to identify BRCA1 depletion. The nanocluster sensing system provides the advantage of high fluorescence as compared to other sensing systems (Zhang et al. 2019).

12.2.5 Carbon Nanotubes (CNTs)

Carbon nanotubes (CNTs), also called buckytubes, are one of the most intensively investigated nanomaterials (Balasubramanian and Burghard 2005). CNTs are formed by rolling graphene sheets into cylindrical tubes that show a hollow structure with a nanometer-scale diameter and comparatively long length. Their excellent combination of mechanical, chemical, electrical, magnetic, and optical properties provides a promising wide range of applications, include biosensing (Biju 2014; Le Goff et al. 2011). Other compounds can be easily conjugated to the surface of CNTs for functionalization (Arkan et al. 2015). Through functionalization, CNTs can easily cross the biological barrier of a cell membrane that assists them to penetrate individual cells (Pantarotto et al. 2004). A major interest has been developed for intracellular biosensing application of CNTs due to the unique mechanism of internalization and release of CNTs from the cells (Tîlmaciu and Morris 2015).

Several types of biomarkers, such as protein receptors, enzymes, and DNA biomarkers have been detected by CNTs conjugates. These CNTs conjugates can be subdivided into electrochemical-based CNTs biosensors, optical CNTs biosensors, and immunosensors determined by their mechanism of action and target identification. A major interest has been developed for the detection of cancer biomarkers through CNTs by conjugation with targeting moieties, such as peptides, proteins, aptamers, and enzymes.

Field emission transistor-(FET)based CNTs have been developed for the detection of PSA–ACT (prostate-specific antigen complex of protease inhibitor alpha 1-antichymotrypsin) in serum blood. In this case, these FET-based CNTs have been modified with 1-pyrenebutanoic acid succinimidyl ester as the linker and 1-pyrenbutanol as the spacer. Antibodies for the attachment of PSA–ACT were then immobilized on the surface of FET-based CNTs through linkers. Linkers to spacer ratio as 1:3 on FET-based CNTs showed a detection limit of 1.0ngmL-1 of PSA–ACT complex without any pretreatment (Kim et al. 2009b).

The stacking of 20–30 nm carboxylated SWCNTs (single-walled carbon nanotubes) in upright bundles termed as CNTs forests (Rusling et al. 2009). These CNTs forest have shown a 4–ten-fold increase in sensitivity to detection of cancer biomarkers as compared to bare pyrolytic graphite surface. For the detection of IL-6 in head and neck squamous cell carcinoma (HNSCC), an ultrasensitive CNTs forest electrochemical immunosensor was developed. CNT forests were conjugated to Ab1 for IL-6 and then simultaneously combined with enzyme label HRP for a very low detection limit of $\leq$30pgmL-1. The results have shown a 16-fold improvement over ELISA. Ab2 attached to carboxylated CNTs with 106 HRP labels per 100-nm CNT gave an ultralow DL of 0.5pgmL-1 for IL-6 in 10 μL of calf serum (Malhotra et al. 2010).

12.2.6 Quantum Dots (QDs)

QDs are zero-dimensional semiconductor nanocrystals in a size regime of 2–10 nm. QDs are categorized into two subgroups based on their chemical composition, the first category is made up of elements from group III (Boron, Aluminum, and Gallium) to V (Nitrogen, Phosphorous, and Arsenic) of the periodic table, while the second category, includes elements derived from subgroup II (Zinc and Cadmium) and the main group VI (Oxygen, Sulfur, and Selenium) of the periodic group (Singh et al. 2018). QDs are characterized by a slow degradation, high molar extinction coefficient as well as high quantum yield, and high-efficiency stokes shifts (Medintz et al. 2005; Freeman and Willner 2012). They possess excellent fluorescent properties along with low photobleaching. A small change in the size of these nanocrystals can produce spans of colors ranging from large-sized QDs production of red fluorescence to small-sized QDs production of blue fluorescence. QDs emit a single wavelength when excited with an even broad wavelength range of light that shows their property of narrow emission spectrum and broad excitation range (Tan et al. 2011).

In recent years, QDs-based nanosensors have been studied for the detection of cancer biomarkers. The detection of cancer protein biomarkers is usually performed by sandwich-type assays. It consists of several components which include a capture antibody along with a secondary antibody for attachment and a secondary capture antibody (Chinen et al. 2015). This secondary antibody is stained or produces fluorescence to be visualized. Two cancer biomarkers, neuron-specific enolase (NSE) and carcinoembryonic antigen (CEA) have been detected using two QD-conjugated antibodies sandwich-type assay with each QDs-Ab detection limit of 1.0 ng/ml (Li et al. 2011a).

One of the biomarkers associated with cancer diagnosis is microRNA. miR-141, a biomarker in prostate cancer, was detected by developing a two-step QDs sensing system. The first step in developing a sensing system is to modify CdSe/ZnS QDs with Forster resonance energy transfer (FRET) quencher-functionalized nucleic acids, which are also conjugated with miR-141 recognition sequence and a telomerase primer sequence. The FRET quencher and CdSe/ZnS QDs are bonded together

through covalent bonding. A duplex-specific nuclease (DSN) was utilized to cleave the duplex formed by the hybridization of miR-141 with the probe. This cleavage results in the exposure of telomerase primer sequence and separation of the quencher unit, thus activating the fluorescence of the QDs. The second step involved the production of chemiluminescence with luminol/H_2O_2 along with stimulation of primer unit by telomerase/dNTPs for elongation and final conjugation of hemin. This sensing system was able to detect miR-141 in a serum sample and discriminated prostate cancer carriers from healthy individuals (Jou et al. 2015).

12.3 Nanotechnology-Enhanced Detection of Cancer Biomarkers

Several traditional biosensing techniques have been a part of biomarkers discovery for laboratory and clinical applications. These techniques have some limitations in detection due to biomarkers' complexity, low molecular weight, low analyte concentrations, etc. Nanotechnology provides a solution by enhancing the selectivity and sensitivity of these assay techniques using nanomaterials as illustrated in Fig. 12.2. This section will describe the nanomaterials-based enhancement of

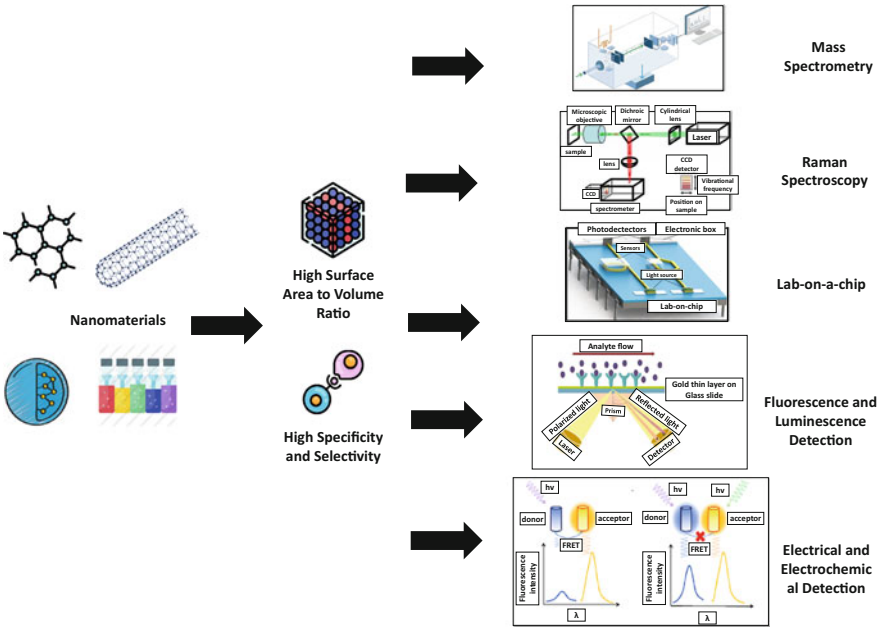

Fig. 12.2 Enhancement of conventional techniques by nanomaterials. (Source: Icons and images from hiq.linde-gas.com, stemm.info, www.flaticon.com) (Downes and Elfick 2010; Aristotelous et al. 2015; Xu et al. 2013)

conventional biosensing techniques, such as mass spectrometry (MS), Raman spectroscopy, optical detection, electrical and electrochemical detection, and lab-on-a-chip technology.

12.3.1 Lab-on-a-Chip Technology

Lab-on-a-chip (LOC) technology defines microfluidic and nanofluidic devices that are patterned on a silicon substrate. It has several components integrated, such as sensors, actuators, microchannels, pumps, mechanical elements, reaction chambers, and several other components of micro and nanoelectronics (Lin et al. 2009; Xu et al. 2012). Top-down approaches, such as photolithography or chemical etching techniques are mostly used for developing these structures. Nanowires or nanoelectrodes are also used in microfluidic channels in LOC technology for biosensing in compact devices (Kim et al. 2009a; Tian et al. 2010). This miniaturization process has a lot of benefits which include lesser power consumption, a lower limit of dead volume, lower consumption of reagents and samples, speedy analysis within a short time, high reproducibility, and high throughput that enables simultaneous samples detection. A variety of nanomaterials, such as CNTs, QDs, and polymeric and metallic nanoparticles have been incorporated in microfluidics for biosensing (Ghazani et al. 2006; Zeineldin 2013).

Gastric cancer is the second leading cause of cancer-related mortalities worldwide (Hsu et al. 2010). Early detection of biomarkers of gastric cancer can assist in preventing this multistep malignancy. An efficient microfluidic system was developed for the identification of alpha 1-antitrypsin (A1AT), a secreted biomarker, elevated in different types of malignancies including gastric cancer, and also correlated with cancer staging. Fluorescently labeled polymer NPs were utilized in the replacement of antibodies in the ELISA assay to amplify the optical detection signals in the region of near-infrared. These nanotags have low tissue fluorescence noise as a background. Finally, specific antibodies were attached to the nanotags for the detection of A1AT. Results have shown that this new sensor system is feasible for the detection of gastric cancer biomarkers as a real-time diagnostic kit (Khazanov et al. 2012).

12.3.2 Mass Spectrometry

Traditional mass spectrophotometry (MS) usually faces challenges in the detection of cancer biomarkers especially peptide cancer biomarkers due to their low molecular weight and their low concentration (Vita et al. 2005). There is also signal noise in samples due to the abundance of high-molecular weight serum proteins. Recently, nanomaterials have been applied to MS for the isolation and detection of cancer biomarkers through several strategies (Qiao et al. 2015). NPs show ligand interaction

with biomarkers and tag them in bodily fluids (Zhang et al. 2016b) as well as some nanomaterials also enhance signals in MS (Zhang et al. 2014). Matrix-assisted laser desorption/ionization-time of flight MS (MALDI-TOF-MS) has been modified with different nanomaterials for signal amplification in simultaneous multiple detections of cancer biomarkers (Li et al. 2014).

AuNPs have been reported as a signal amplifier for PSA immunoassay in inductively coupled plasma mass spectrometry (ICP–MS) by improving the size of QD tags. Deposition of gold shell around QD tags has improved the sensitivity by many folds, which enables immunoassay to detect very low PSA concentrations. The results have shown that the detection limit of the new system has six-fold improvement over conventional Mn: ZnS QD-labeled ICP–MS immunoassays (26attograms over 0.02nanograms of PSA mL^{-1}). Other than the AuNPs doped ICP–MS high sensitivity, it can also exhibit a six times better broad dynamic range to cover both PSA concentration in male and female sera. A side advantage of this amplifier is that the biomarker of interest can be changed by altering the antibody, since it is located on a secondary antibody (Garcia-Cortes et al. 2016).

12.3.3 Raman Spectroscopy

Conventional Raman spectroscopy lack detection of cancer biomarkers at very low concentration due to the low signal intensity of Raman scattering from the probe molecules (biological recognition interface and Raman reporter). Metal NPs such as AuNPs can greatly enhance the intensity (108–1014 orders of magnitude) of the Raman probe molecules due to the property of surface plasmons (Nie and Emory 1997).

Femto level detection for PSA was achieved by surface-enhanced Raman spectroscopy (SERS) with AuNPs (Grubisha et al. 2003). Due to the reason, that response of different Raman reporter molecules is different on each wavelength, multiplex detection was possible. For this procedure, different Raman reporter molecules were attached to specific antibodies on the AuNP substrate body. The most integral part of SERS is the attachment of AuNP nanotags for the detection of cancer biomarkers. As compared to QDs, these SERS nanotags never show photobleaching. Even at a low volume of 20 nL, these SERS nanotags were able to detect two hepatocellular carcinoma biomarkers (AFP and alpha-1-antitrypsin) simultaneously(Dinish et al. 2014a). The same SERS nanotags were also applied to detect three intrinsic biomarkers—EGFR, CD44, and TGFβRII in a breast cancer model (Dinish et al. 2014b). Early-stage T1 which is a biomarker of nasopharyngeal cancer was also in the detection limit of AuNP based SERS tags (Lin et al. 2014; Viswambari Devi et al. 2015). Multiplex detection of breast cancer biomarkers in a homogeneous solution using SERS-based molecular sentinel (MS) technology was demonstrated recently. Two MS nanoprobes, ERBB2-MS and KI67-MS, were

designed to respectively target critical biomarkers for breast cancer, e.g., *erbB*-2 gene and Ki-67 gene (Wang and Vo-Dinh 2009).

12.3.4 Fluorescence and Luminescence Detection

Nanomaterials can assist in effective fluorescence detection of cancer biomarkers either it's qualitative or quantitative. It can be attained in many ways; intrinsic fluorescence production of nanomaterials (Ramesh et al. 2016; Qin et al. 2017), trigging fluorescence by nanomaterials (Huang et al. 2016; Zhang et al. 2016a), Förster resonance energy transfer (FRET) (Xu et al. 2016), metal-enhanced fluorescence (MEF) (Zhu et al. 2016), or immunochromatographic fluorescence (Shen et al. 2015).

In contrast to conventional ELISA tests, the detection of PSA cancer biomarker is very quick in label-free optical biosensors. Surface plasmon resonance (SPR) provides a way for label-free detection of biomolecular interactions. Utilizing SPR and quartz crystal microbalance (QCM) a POC system for the detection of PSA–ACT in human serum was reported. A detection limit of 0.29ngmL^{-1} for PSA–ACT in 75% pure human serum was achieved by utilizing 40 nm AuNPs. The comparable results were shown by both SPR sensor and QCM sensor which indicates that both systems can be used for the detection of cancer biomarkers (Uludağ and Tothill 2010). For the detection of picograms level of PSA in serum samples**,** another AuNPs fluorescence probe was developed in which AuNPs act as a quencher**.** The probe (Ab2–RBITC–AuNPs) was composed of specific antibodies for detection, a fluorescent dye, i.e., Rhodamine B isothiocyanate (RBITC) that acts as the donor and AuNPs act as the acceptor or quencher. Due to NPs surface energy transfer effect, the RBITC fluorescence is quenched by AuNPs nearby. As the cancer biomarker, i.e., PSA attached to the mAbs, a sandwich is formed between Ab2–RBITC–AuNP probe and PSA. Finally, cystamine is added to the complex to cause displacement of RBITC from the surface of AuNPs to prevent quenching so that fluorescence measurement would be possible (Liu et al. 2013).

12.3.5 Electrical and Electrochemical Detection

Electrical and electrochemical detection of cancer biomarkers is one of the most common strategies for the development of modern biosensors fabricated through top-down or bottom-up techniques. The top-down approach has some advantages over the bottom-up approach regarding better integrated POC devices and higher yields so it is more compatible with the standard of fabrication technologies. Some reported biosensors with electrical cancer biomarkers detection are nanoporous membrane (Blundell et al. 2016; Raza et al. 2018), label or label-free flexible transistor-based sensor arrays(Shalev et al. 2013; Shehada et al. 2016),

electrochemical microfluidic chip(Xie et al. 2015), and electrochemical microspheres on the electrode(Hu et al. 2012). An electrochemical detection (Kavosi et al. 2015; Grinyte et al. 2016)is possible due to a change in conductance or resistance which is recorded as a redox reaction occurs. Nanomaterials are enhancing the promising properties of these electrical or electrochemical biosensors. Nanowires are the most studied nanomaterials in this regard due to their good biocompatibility and excellent electrical properties.

Cytokeratin-7 (CK-7), a discriminatory protein between benign and metastatic adenocarcinoma is expressed in epithelial tissues as a cancer biomarker (Tot 2002). Gold nanowires-(AuNWs) based electrochemical biosensor was developed for the sensitive detection of CK-7. The immunosensor system was developed as a sandwich-type assay in which Ab1 was attached to AuNWs and biotinylated Ab2 was attached to streptavidin-conjugated alkaline phosphatase (AP). Microelectrodes detect the electrochemical change that occurs due to the enzymatic reaction between AP and p-nitrophenyl phosphate which forms an electroactive p-nitrophenol. The results were measured as anodic peak current which is directly proportional to AP concentrations and in turn, could be correlated with the concentrations of CK-7 (Patil et al. 2008) (Table 12.1).

12.4 Conclusion

Over the recent years, a lot of efforts have been made for the development of nanomaterials (NMs)-based biosensors or probes for the detection of cancer biomarkers. These NMs-based techniques have shown significant improvement over traditional biosensing techniques with better selectivity and sensitivity. The final goal of developing these techniques is to translate them into clinics and provide benefits to the healthcare system. However, NMs-based techniques have issues with their long-term efficacy, safety, and toxicity being evaluated. On the other hand, the reproducibility of the results in different working conditions of a particular device is a concern along with standardization of robust synthesis of whole NMs-based techniques. Practically, NMs-based techniques should be cost-effective to reach the market. Point-of-care (POC) devices with the integration of nanotechnology will be more feasible in the foreseeable future for the detection of cancer biomarkers. It can be expected that nanotechnology integrated POC devices will provide the best performance and viability with the early detection of cancer biomarkers. Early detection can be helpful to the healthcare system in terms of patients' early treatment, lower risk, lower mortality, and higher quality of life. Further development can be done for the economic benefit of these techniques which will provide cheaper, efficient methods with fewer side effects and relapse.

Table 12.1 Common nanomaterials-based biosensors for detection of cancer biomarkers (Ye et al. 2018)

Nanoparticles				Biomarker detection				
Type of material	Composition	Morphology	Dimensions (nm)	Biomarker	Cancer type	Source	Sensing technique	Ref.
Metal	Au	Nanoparticle	14	Sialic acid	Breast, Liver	Model cancer cells	ICP-MS	(Zhang et al. 2016b)
	Au/Mn: ZnS	Nanoparticles with surface deposition	100–150	PSA	Prostate	Human serum	ICP-MS	(Garcia-Cortes et al. 2016)
	Au	Nanoparticle	40	fPSA, cPSA	Prostate	Spiked in FBS solution	LDI-TOF-MS	(Yoo and Yeo 2016)
	Au	Nanoparticle	10–30	Diglyceride, octadecanamide	Kidney	RCC tissue	SALDI-TOF-MS	(Nizioł et al. 2016)
	Au	Nanoparticle	60	Protein biomarkers (EGFR, HER2, CD44, CD24)	Breast	Breast tissue	SERS	(Wang et al. 2016)
	Au	Nanoparticle	100	Human IgG	Prostate	Human blood	DLS	(Zheng et al. 2015)
	Au	Nanoparticle arrays	3–5	IL-6	Breast, Cervical, Oral, Colorectal	Calf serum	Electrochemical measurement	(Jensen et al. 2011)
Metal Oxide	Fe_3O_4@SiO_2-C_{18}	Core-shell nanoparticle	10	EVOM	Breast	Urine	GC-qMS	(Qiao et al. 2015)
	NDA-Fe_3O_4	Nanoparticle	10	PSA	Prostate	Human serum	Electrochemical measurement	(Li et al. 2011b)
Semiconductor	Silicon	Nanowire-FET array	80 nm in width, 25 μm in length	CYFRA21-1, PSA	Lung, Prostate	Human serum	Electrical measurement	(Lu et al. 2015)
Hybrid	Porous-silicon gold	Nanoparticle on porous template	<30 (AuNPs)	Peptide Fragments	Colorectal	Human serum	MALDI-TOF-M	(Li et al. 2014)

(continued)

Table 12.1 (continued)

Nanoparticles				Biomarker detection				
Type of material	Composition	Morphology	Dimensions (nm)	Biomarker	Cancer type	Source	Sensing technique	Ref.
	Au-PDMS	Nanoparticle on thin film	55	PDGF-BB	Non-specific	Model cancer biomarker	Fluorescence emission	(Zhu et al. 2016)
	$NaYF_4$: Yb/Tm	Nanoparticle	16	CEA	Non-specific	Model cancer biomarker	Up conversion FRET	(Xu et al. 2016)
	Ag@Pb (II)-β-CD	Nanoparticle on MOF	20–50 (AuNPs)	PSA	Prostate	Model cancer biomarker	Electrochemiluminescence sensing	(Ma et al. 2016)
	Au-RGO	Nanoparticle on thin film	40–50 (AuNPs)	VOC	Gastric	Patient breath sample	SERS	(Chen et al. 2016)
	Liposome (DPPC,MSPC, DPSE-PEG)	Nanocapsule loaded with magnetic nanoparticles and peptides	100 (iron oxide 25 nm)	Matrix metalloproteinase	Colorectal	Mouse urine	Fluorescence emission	(Schuerle et al. 2016)
Carbon	CNT-FETs	Single-walled nanotubes	N/A	PSA-ACT	Prostate	Human serum	Electrical measurement	(Kim et al. 2009b)
	CNT-FETs	Single-walled nanotubes	5–6 (diameter)	OPN	Nonspecific	Model cancer biomarker	Electrical measurement	(Lerner et al. 2012)
	CNTs	Multiwalled nanotubes	20–35	AFP	Liver, testicles, ovaries	Human serum	Chemiluminescence measurement	(Bi et al. 2009)
	CNTs	Single-walled nanotubes	20–30	PSA	Prostate	Calf serum	Electrical measurement	(Yu et al. 2006)
Metal sulfides/ selenides	Fe_3O_4@CdSe-CDS: Au	Nanocrystals	537 nm (bandgap)	CEA	Colorectal	Model cancer biomarker	Electrochemiluminescence Sensing	(Myung et al. 2002)
	CdSe/ZnS	Quantum dots	565–665 nm (wavelength)	CEA, CA125, and Her-2/Neu	Colorectal, Ovaries, Breast	Saliva, blood	Fluorescent measurement	(Jokerst et al. 2009)
	SiO_2	QD tagged nanowires	200(diameter)	IL-10	Lungs	Model cancer biomarker	Fluorescent measurement	(Sekhar et al. 2008)

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