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Cancer Biomarkers

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Abstract

Cancer biomarkers (CB) are biomolecules produced either by the tumor cells or by other cells of the body in response to the tumor. Every cell type has its unique molecular signature and identifiable characteristics such as levels or activities of myriad of genes, proteins, or other molecular features; therefore, biomarkers can facilitate the molecular definition of cancer. Our aim was providing updated knowledge and performing detailed review about CB regarding their molecular and biochemical characterization and their clinical utility in screening, diagnosis, follow-up, or therapeutic stratification for cancer patients. Focusing on conventional, the FDA approved as well as promising future biomarkers in most common cancers. In addition, emphasizing on their prospective role may be of great value in improving the management of cancer patients. The challenge and future prospective of biomarkers, by facilitating the combination of therapeutics with diagnostics, promise to play an important role in the development of personalized medicine.

Keywords: cancer, biomarkers, molecular markers, prognosis, diagnosis, proteomics

1. Introduction

Increasing cancer burden is a major health problem; GLOBOCAN estimated nearly 8.2 million deaths and 14.1 million new cancer cases all over the world in 2012 [1] and it is expected to be 16 million new cases every year by 2020 [2]. Widespread application of existing cancer control knowledge, early detection, appropriate therapy with proper follow-up, and prediction measures through cancer biomarkers could definitely be very effective tools for the amelioration of cancer burden. Biomarkers are “Any measurable diagnostic indicator that is used to assess the risk or presence of disease” as defined by the US Food and Drugs Administration (FDA), or they would be comprehensively 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” [3]. Cancer biomarkers (CB) are biomolecules produced either by the tumor cells or by other cells of the body in response to the tumor, and CB could be used as screening/early detection tool of cancer, diagnostic, prognostic, or predictor for the overall outcome of a patient. Moreover, cancer biomarkers may identify subpopulations of patients who are most likely to respond to a given therapy [4]. Biomarkers can be genes, gene products, specific cells, molecules, enzymes, or hormones which can be detected in blood, urine, tissues, or other body fluid [5].

1.1 Historical background of cancer biomarkers

Two thousand years ago, Ancient Egyptians were the first known who try to find markers for malignancy as described in an Egyptian papyrus, they had their first attempt in distinguishing breast cancer from mastitis [6]. Use of CB in medicine then started around 170 years ago, when Sir Bence Jones described a protein in urine of multiple myeloma patients that could be identified by its special heat coagulation properties. In 1847, Bence-Jones protein was the first cancer biomarker that was discovered as a tumor-produced light chain antibody of immunoglobulin G (IgG) in multiple myeloma patients, it was excreted in urine in excess and could be identified by heat denaturation [7]. Later, in 1986, Bence-Jones protein was reported to be present also in the serum of myeloma patients [8]. Two years later, in 1988, an immunodiagnostic test was approved by the FDA for the detection of Bence-Jones protein which may aid in the diagnosis of multiple myeloma, Waldenstrom’s macroglobulinemia, leukemia, and lymphoma. In 1867, amylase was introduced by Sir Michael Foster who reported the increase levels of serum amylase in patients with cancer pancreas. He suggested urinary amylase as a biomarker for cancer pancreas. Then, after years of studying pathology and physiology of pancreas, it was realized that cancer pancreas originate from ductal cells not acinar cell; the source of amylase enzyme. Therefore, elevation of amylase enzyme may occur in large tumors impinging on acinar cells [9]. During the next 100 years, numerous studies involved other CB including hormones as chorionic gonadotropin (hCG) in choriocarcinoma and catecholamines in pheochromocytoma and neuroblastoma, and enzymes as acid phosphatase in prostate cancer, and alkaline phosphatase in bone tumors [10]. Definitely, the development of the immunoassay concept in the 1950s by Yalow and Berson has very important impact on the field of CB testing using polyclonal antibodies. Later in 1970s, CEA immunoassay was commercially available. The field of cancer biomarkers showed uprising in 1975 with the development of monoclonal antibodies and in 1982 with the development of the immune-metric (sandwich) immunoassay. This leaded to feasible expansion in the introduction of several immunoassays and new tumor antigens to be used as available tests in routine clinical practice. Recombinant antibody techniques also provided better understanding of the hypothesized structure and functions of CB. Recent molecular biology techniques were the key for discovering and realizing the putative functions of CB as tumor suppresser genes, oncogenes, nuclear proteins, and telomerase [11, 12]. Unfortunately, along all these years since the discovery Bence-Jones protein, only very few CB have been approved by the FDA as diagnostic or prognostic cancer markers in spite of being extensively studied. However, emerging

technology of omics, such as genomics and proteomics, may indeed encourage the generation and Validation of CB [10].

1.2 Cancer development and mechanisms for the production of cancer biomarkers

Cancer is a multifactorial cluster of diseases reflecting fundamental abnormality involving uncontrolled cell growth and proliferation alternating the normal cell behavior. Molecular mechanisms exhibit alterations in the expression of multiple genes mostly includes: (proto) oncogenes, tumor suppressor genes, and DNA repair genes that contribute to the development of cancer genotype and phenotype with a state of dysregulation of cell proliferation events. Cancer hallmarks hypothesis has been postulated in 2000 by Hanahan and Weinberg. They initially categorized biological mechanisms for the cancer development into six processes: proliferative signaling, avoiding growth suppression, cell death resistance (immortalization), enabling of replicative immortality, induction of angiogenesis, and finally activation of invasion and metastasis [13]. Increasing evidence suggest that cancer may be triggered also by epigenetic changes as histone modification and DNA alteration of methylation causing alterations in the condensation state of chromatin [14]. Genetic alterations of cancer cells, as point mutation, gene rearrangement or amplifications, and subsequent disturbances of cell division and proliferation will be manifested by release of biomarkers of such changes in majority of patients with a specific type of cancer. Therefore, they can be used as biomarkers for the cancer detection or predicting responses to various treatments [15–17]. Comprehensive understanding of the altered molecular mechanisms and cellular processes underlying carcinogenesis or hallmarks of cancer may link cancer biomarkers and their clinical utility in

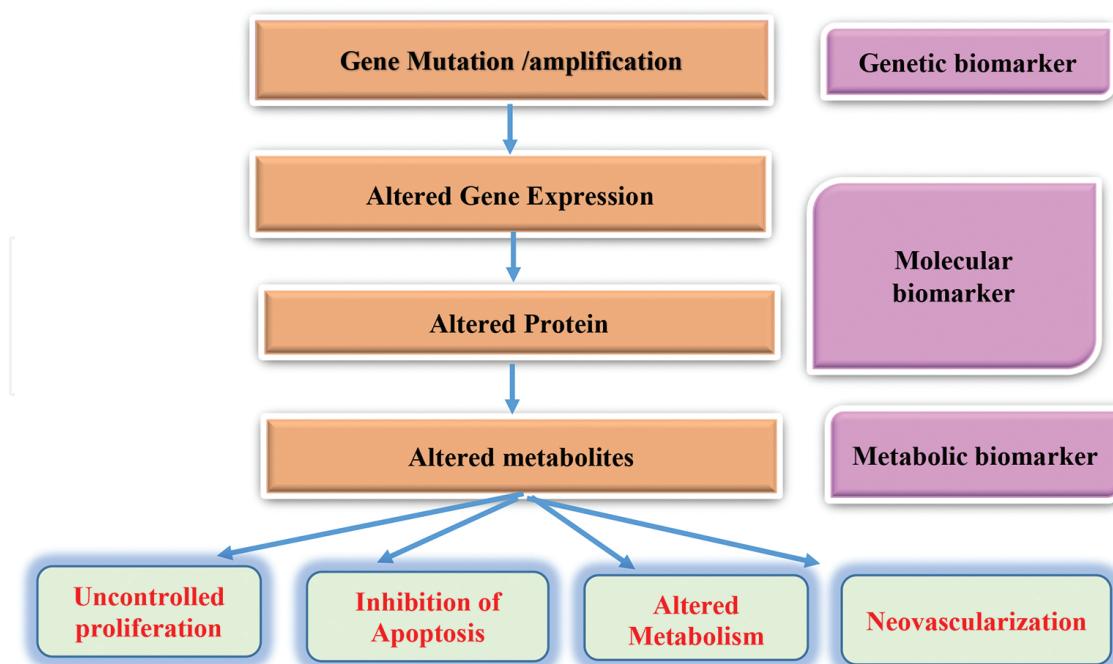


Figure 1. Identification of biomarkers in the process of carcinogenesis modified from Bhatt et al. [18].

cancer patient. Genetic, molecular, and metabolic biomarker may be identified through applying the sequential of events occurring in cancer cells from gene mutation following its effects on cellular proliferation and metabolism [18], as illustrated in **Figure 1**. One of the major challenges for oncology research is to establish the definite relationship between cancer biomarkers and cancer pathology, as well as, to detect cancer in early stage beside the development of targeted therapies targeting the exact altered gene or cellular process [16].

1.3 Serum, biological fluid, and tissue Cancer Biomarkers

Understanding mechanisms of carcinogenesis could explain the production and release of CB in cancerous cells, blood or various body fluid and hence release of those molecules and elevation during cancer initiation, development, and progression or metastasizing. Mechanisms for elevation of CB levels in any of the biological fluid could be explained by three mechanisms. The first mechanism is overexpression or amplification of gene product, or enhancement of epigenetic changes (affect gene expression) as DNA methylation with release of such CB as protein human epididymal secretory protein 4 (HE4) in ovarian cancer. HE4 is overexpressed in ovarian carcinoma and could be also detected in serum [19–21]. However, clinical evaluation of HE4 revealed that it is also overexpressed in endometrial, breast, and bronchial adenocarcinoma [22]. The second mechanism of elevation could be typically applied on serum biomarkers, which is the secretion of cellular proteins or shedding of membrane proteins. An example of such serum biomarker is alpha-fetoprotein (AFP); an oncofetal protein with altered single peptide that is elevated in circulation in patient with hepatocellular carcinoma [23] and HER2-neu, a cell membrane surface-bound tyrosine kinase, released and elevated in the serum of breast cancer patients after being cleaved by proteolysis. HER2-neu is also approved by the FDA for monitoring of metastatic cases of breast cancer [24]. The third mechanism is cell invasion and angiogenesis as occur with prostate-specific antigen (PSA). It is expressed normally by prostatic epithelium but elevation of PSA levels occurs due to distorted basement membrane of prostatic cell and lymph angiogenesis [25]. The clinical application of CB, especially circulating protein targets in cancer management, is emerging into a new era especially with the availability of promising sensitive techniques that implement the discovery of “omics” cancer biomarkers in body fluids that may represent a novel, highly sensitive diagnostic tools for the early detection of cancer. Of even much importance are hidden cancers that are not easily accessible, for example, nasopharyngeal, ovarian, and pancreatic cancers. However, there is mandatory need for validation of such biomarkers [26]. CB could be detected in cancerous cells or tissue of origin in solid tumors, bone marrow, and lymph node or as circulating cells. CB could be detected in biological body fluid such as serum, ascetic fluid, pleural fluid, or urine representing noninvasive specimens or samples. CSF fluid is a suitable candidate for brain and CNS cancer. Meanwhile, urine is one of the promising frontier for the detection of bladder cancer or for of patients’ surveillance [27]. In addition, it was postulated that prostate cancer antigen 3 (PCA3) is another promising new molecular marker for diagnosis and follow-up of cancer prostate [28]. Stool for colorectal cancer, nipple aspirate fluid, ductal lavage, and cyst fluid for breast cancer are other examples for biological fluid sources for discovery or clinical application tool for CB [29].

2. Clinical applications and performance indications of Cancer Biomarkers

More than 25 years ago, the clinical usefulness of CB was limited to be an effective tool for patient's prognosis, surveillance, and therapy monitoring. Definition of tumor markers that have been adopted by the fifth International Conference on Human Tumor Markers held in Stockholm, Sweden, in 1988 stated that "*Biochemical tumor markers are substances developed in tumor cells and secreted into body fluids in which they can be quantitated by non-invasive analyses. Because of a correlation between marker concentration and active tumor mass, tumor markers are useful in the management of cancer patients. Markers, which are available for most cancer cases, are additional, valuable tools in patient prognosis, surveillance, and therapy monitoring, whereas they are presently not applicable for screening. Sero-diagnostic measurements of markers should emphasize relative trends instead of absolute values and cut-off levels.*" However, CB have been reported to be used also for screening of general population or risk groups, for differential diagnosis, and for clinical staging or stratification of cancer patients. Additionally, CB are used to estimate tumor burden and to substitute for a clinical endpoint and/or to measure clinical benefit, harm or lack of benefit, or harm [4, 18, 30]. Among commonly utilized biomarkers in clinical practice are PSA, AFP, CA125, and CEA. PSA is one of the serum biomarker currently used consistently in primary care to assess the risk of underlying prostate cancer. Cancer antigen 125 (CA-125) can be a biomarker of ovarian cancer risk or an indicator of malignancy, but it has low sensitivity and specificity. CEA is another biomarker that is elevated in patients with colorectal, breast, lung, or pancreatic cancer [31]. A major challenge is to develop promising CB for the stratification of cancer patients not only to predict outcome or response for therapy, providing customized treatment, but also for personalized therapeutic strategies of cancer patients. Among promising biomarkers in that field is survivin and HER2-neu [32, 33].

2.1. Sensitivity and specificity for evaluation of accuracy of CB

As being released from tumor cells, or body cells in response to the tumor, CB can be detected in any of the body fluids, secretions, or tumor tissue and cells. CB can be detected in serum, plasma, or whole blood, also in whole excretions as urine, sputum, or CSF. Therefore, CB could be assessed in noninvasive and in serial manner. Evaluation of cancer biomarker in tissue or cells requires tissue biopsy or more invasive technique than serum biomarkers. CB can be detected in tissues by special techniques but in an invasive manner than serum or urine biomarkers. Genetic biomarkers could be detected in DNA derived from tumor tissue, whole blood, or buccal mucosa cells [34]. Evaluation of diagnostic value of any test or marker is usually performed with referral to the terms of sensitivity and specificity of that marker. Specificity means that ability of the marker to detect non-diseased subjects whereas sensitivity refers to the ability of that test to identify diseased subjects (patients) [35]. At definitive cutoff value, a test or biomarker may be found above that value (positive), but actually not all positives are diseased subjects. Therefore, sensitivity is calculated, as the ratio of the all positives who are found by that test, above the cutoff value to the total number of abnormals known to have the disease (true positive); simply sensitivity is the true positive rate (TPR).

Similarly, by applying the same cutoff value for the same test, some people with normal results below cutoff value are actually normal (true negative) but not all of them are not having the disease (false negative). Therefore, the true negative rate or specificity could be calculated as the ratio of the all negatives who found by the test below cutoff value to the total number of normals known not to have the disease (true negative) [36]. Therefore, a CB with 100% specificity could be used to correctly identifies all non-cancerous subjects, CB with 70% specificity could identify only 70% of the non-cancerous as being negative (true negatives), and however, 30% of non-cancerous are falsely identified positive (false positives) [37]. Supposing sensitivity of a CB is 100%, this means that it could identify all cancer patients and if another CB supposed to be with 90% sensitivity, it could detect 90% of patients with cancer (true positives) but fail to detect it only in 10% of cancer patients (false negatives). Consequently, sensitivity and specificity could be computed across all possible cutoff or threshold values and both are inversely related to each other [38].

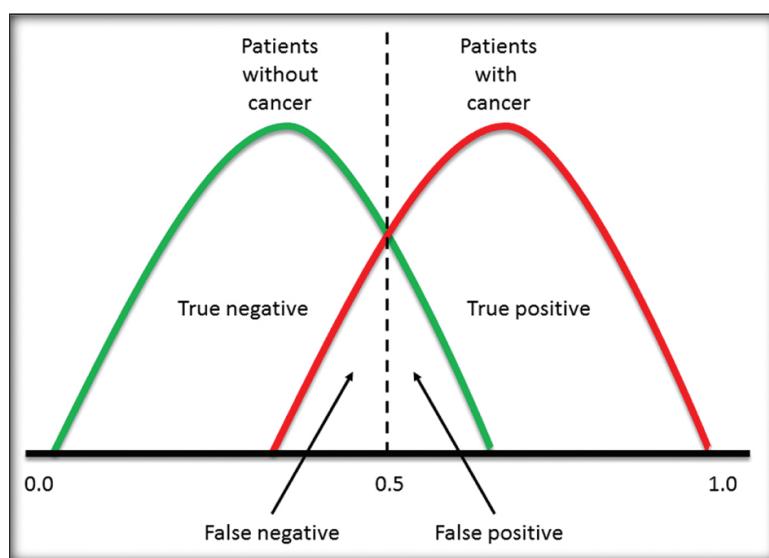


Figure 2. Cancer biomarker range of results among cancer and non-cancerous patients.

2.2. Receiver operating characteristics (ROC) curve analysis

Comparative analysis of different sensitivities and specificities at different thresholds would be very effective to judge the accuracy of diagnostic test. ROC curve was introduced by the British during World War II in order to identify accurate radar detectors and was used later in performance evaluation of radiological tests [39]. ROC curve is simply defined as performance indicator of a test or biomarker by plotting its sensitivity along the y axis and its 1-specificity or FPR (false positive rate) along the x axis to assess the diagnostic ability of such biomarker and in discrimination of the diseased from the healthy subjects [40]. ROC curves have been extensively used for evaluation of the accuracy of diagnostic tests with meaningful interpretations. Several indices could be derived from it such as the area under the curve (AUC) that determines the average of the sensitivity values for all possible specificity values and

includes whole area underneath the entire ROC curve [36]. AUC could have a range between one and zero because values of the x and y axes probably having values ranging from zero to one as well. The closer the value of AUC to one the better is the clinical performance of that test [40]. Comparing AUC areas of different tests can be used to compare their diagnostic performance as AUC is a measure of their overall performance. The test with bigger AUC value is of better overall performance. On comparison of two tests and if both AUC areas are equal, this indicates same diagnostic performance of both tests, but non-necessarily mean identical ROC curves [41]. **Figure 2** represents the CB levels among cancer and non-cancer cases, while **Figure 3** illustrates ROC curve and area under the curve.

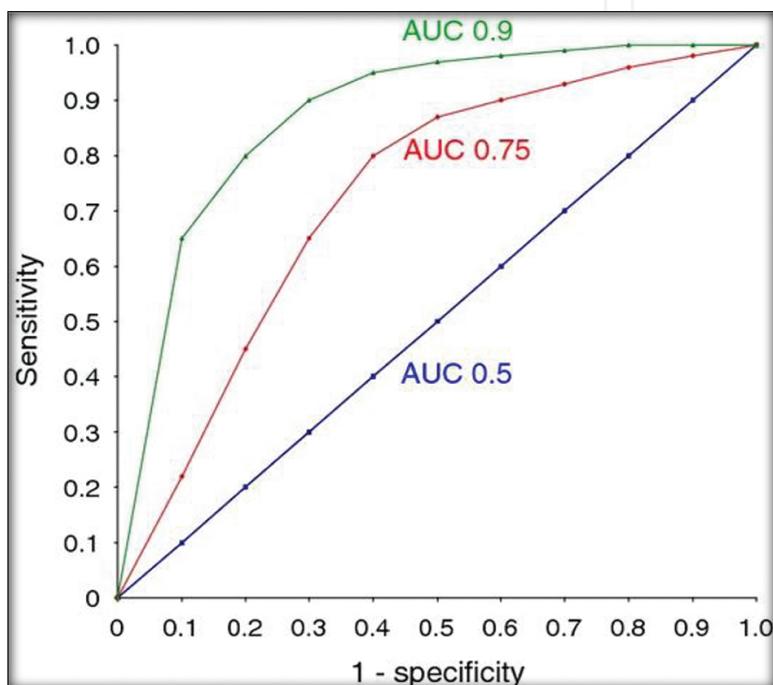


Figure 3. ROC curve analysis and comparison of area under the curve.

2.3. Ideal biomarker

Measurement of sensitivity and specificity of a biomarker at a range of cutoff values could be of an important impact for evaluation of CB as we may chose a definitive cutoff value that achieves the highest sensitivity and specificity. Increment of cutoff point will definitely lead to increase of specificity of the test or false negative patients but on the other hand, this will decrease number of false positives; this indicate a highly specific but low sensitive biomarker. Similarly, if the cutoff point is low that indicates a highly sensitive but low-specific biomarker, as there are fewer false negatives but more false positive subjects. Indeed, pairs of sensitivities and specificities may describe accuracy of the biomarker and its ability to discriminate between healthy (normal) and diseased. We can identify the threshold limit or cutoff value to a diagnostic sensitivity of 100% or less but considering the corresponding specificity for that threshold. The decision threshold must be chosen to be used in patient care, but not for

assessment of accuracy. Indeed assessment for performance at definitive point may be misleading or this may result in bias for comparison between tests [42]. Ideal biomarker must be strictly able to differentiate between cancerous from benign cases, aggressive tumors from insignificant one; it should be of high specificity and sensitivity. Furthermore, it should be a noninvasive and inexpensive [30, 43]. The characteristic features of an ideal biomarker are variable and rely to some extent on the application and classification of CB. Mostly, CB have to fulfill the following general properties to be considered ideal. Obviously, no biomarker could meet these requirements all together, but these criteria should be highly considered for selection of diagnostic biomarker [44]:

- *High clinical sensitivity:* produced by all patients with that specific cancer (100% TPR).
- *High clinical specificity:* low false negative rate (100% True negative).
- *Organ or tissue specific.*
- *Proportional to tumor burden or volume:* quantitatively proportionate to tumor volume or disease progression.
- *Short half-life:* reflecting quickly any early changes in tumor burden for proper monitoring of therapy.
- *Present (if any) at low levels in the serum of healthy individuals and those with benign disease.*
- *Sharply discriminating metastasis.*
- *Exist in quantitative, standardized, reproducible, and validated assay.*
- *Inexpensive or low costing method.*
- *Obtained in a noninvasive manner:* detected in serum, body fluids, or in easily accessible tissue.

3. Uses, clinical utility, and limitations of CB

Conventionally used tumor markers or CB may be either proteins or glycoproteins, being probably not involved in carcinogenesis or development of cancer process, rather are likely to be by-products of malignant transformation. Low molecular weight, small molecules or nucleic acids markers (as gene mutations or polymorphisms and quantitative gene expression analysis, peptides, proteins, lipids metabolites, and other small molecules are promising and recently being evaluated as potential clinically useful tumor markers, the patterns of gene expression and genetic alterations and defects may be the framework of the molecular classification of CB [11]. There are several classifications for CB depending on different aspects related to their chemical nature, proposed mechanisms for their release and applications. Six years ago, a unique classification proposed by Mishra and Verma [45] with an emphasis on clinical utility of CB. They classified CB into prediction biomarkers as DNA biomolecules,

detection biomarkers as RNA molecules, diagnostic biomarkers as protein biomarkers, and prognosis biomarkers as glyco-biomarkers. Clinical applications and uses of CB, as simply illustrated in **Figure 4** are screening and early detection, diagnostic confirmation, prognosis and prediction of therapeutic response, and monitoring disease and recurrence [46]. Another use of CB includes cancer susceptibility and risk assessment markers which include the identification of individuals who are at a high risk of developing cancer or candidates for screening programs and early preventive studies [47]. Risk or susceptibility assessment markers include markers of inflammation, oxidative stress and single-nucleotide polymorphisms (SNPs), and mutations in certain genes [48, 49]. **Table 1** illustrates most of traditional, the FDA approved, and clinically relevant CB with their uses in various cancer types.

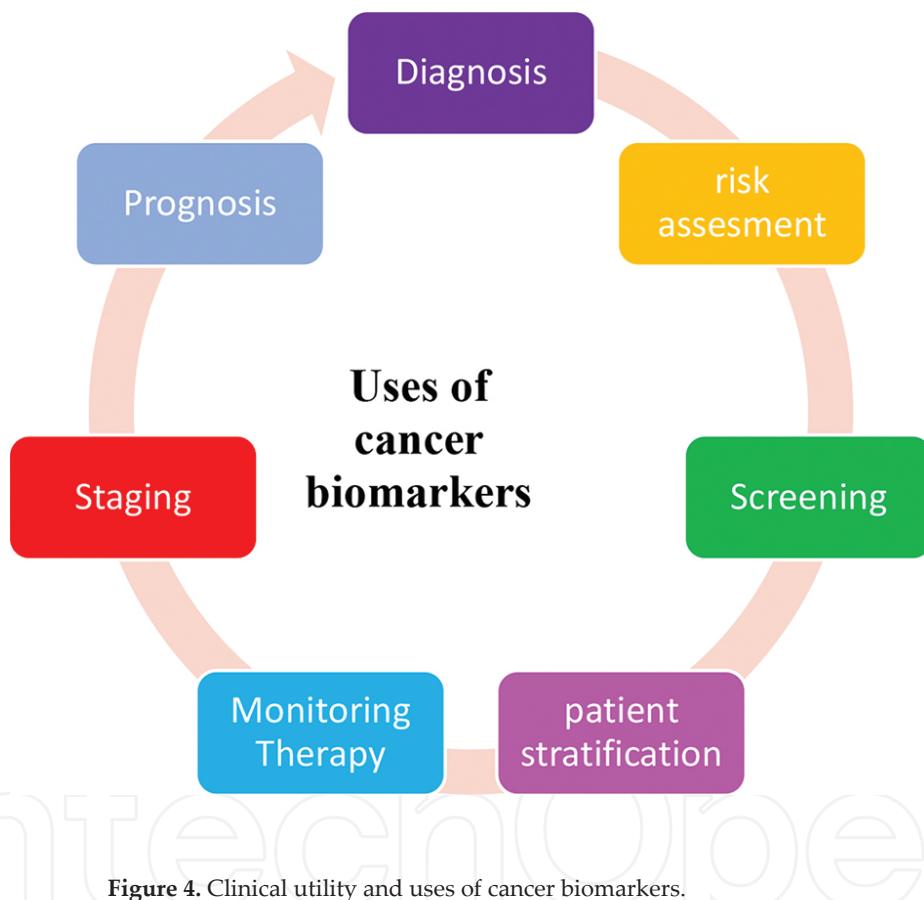


Figure 4. Clinical utility and uses of cancer biomarkers.

3.1. Screening/early detection

In 2008, Wald defined screening as “the systematic application of a test to identify subjects at sufficient risk of a specific disorder to benefit from further investigation or direct preventive action, among persons who have not sought medical attention on account of symptoms of that disorder” [50]. Earlier efficient treatment must lead to better outcome compared with the treatment available at later cancer stages or symptomatic patients. Screening aim was to detect disease when subjects are asymptomatic which differ from diagnosis of symptomatic patients. Objectives of screening and early detection of cancer were to detect cancer at curable and better

Cancer biomarker	Organ specificity/cancer type	Application/uses	References
Prostate-specific antigen (PSA)	Prostate/BPH	Screening, diagnosis and monitoring	[86, 133]
Carbohydrate antigen 125 (CA125)	Ovarian	Diagnosis, prognosis, detecting recurrence and monitoring therapy	[134]
Carcinoembryonic antigen (CEA)	Colorectal/hepatic	Monitoring therapy Prognosis Detecting recurrence Screening for hepatic metastases	[135–137]
Carbohydrate antigen 15.3 (CA 15-3)	Breast	Monitoring therapy	[69, 138]
Estrogen, progesterone receptors (ER and PgR)	Breast	Stratification/select patients for endocrine therapy	[139–141]
HER2	Breast	Monitoring trastuzumab therapy	[18, 32, 33, 142]
Carbohydrate antigen 27.29 (CA27.29)	Breast	Monitoring	[84]
Human chorionic gonadotropin-β (HCG-β)	Testicular	Diagnosis Staging Detecting recurrence	[143]
Alfa-fetoprotein	Hepatocellular carcinoma	Monitoring therapy Diagnosis Detecting recurrence	[144–146]
Calcitonin	Medullary carcinoma of thyroid	Monitoring therapy	[147, 148]
Thyroglobulin	Thyroid	Monitoring	[149]
CA 19-9	Pancreatic	Monitoring therapy	[76]
Nuclear matrix protein 22 (NMP-22)	Bladder	Screening, monitoring and prognosis	[150]
Prostate cancer antigen 3 (PCA3)	Prostate	Prognostic	[151]

Table 1. Current cancer biomarkers and uses in clinical practice.

outcome state and even before appearance of symptoms. Reports calculated a drop in the 5 years survival rate from being about 90%, in early localized breast cancer, to reach about 60% in local metastasizing and only 30% to distant metastasizing cases of breast cancer [51]. Therefore, screening CB should be able to detect cancer in an early stage or asymptomatic stage and consequently will result in increase of survival rate and decrease complications or morbidities. Screening test must be highly specific to minimize false positives as less as possible. High specificity is mandatory for screening biomarker because even a small false-positive rate could result in large number of unnecessary other invasive diagnostic procedures that may be unneeded with the associated psychological burden and excess costs. Ideal screening programs have to be noninvasive and inexpensive and definitely lead to obvious reduction in morbidity and mortality and increase in survival rate. Usually, screening programs are directed for highly prevalent cancers and further treatment and follow-up are mandatory [34]. Other limiting factors for screening biomarker are the low diagnostic sensitivity and specificity of most of the currently used biomarkers to serve as screening markers and being elevated later in the course of cancer. However, few biomarkers have been used as screening biomarkers as AFP in screening for hepatocellular cancer in high-risk subjects, PSA in screening for prostate cancer, CA125 in screening for ovarian cancer, and fecal occult blood testing (FOBT) in screening for colorectal cancers (CRC) and vanillylmandelic acid (VMA) in screening for neuroblastoma in newborns [52]. PSA was cleared by the FDA as a screening

biomarker for prostate cancer; however, false positive elevation of PSA levels can be found in individuals with benign or inflammatory conditions as benign prostatic hyperplasia and prostatitis [53]. Contribution of PSA screening in decreasing mortality is still being a matter of contraverse [54, 55].

3.2. Diagnosis/differential diagnosis

A diagnostic biomarker would be applied only for symptomatic patients in contrast to screening biomarker that would be applicable only for symptomatic individuals. Interestingly, the characteristics of an ideal diagnostic biomarker are similar to the characteristics for screening. Notably, most of well-established biomarkers for screening could be used as diagnostic markers and PSA is well-recognized example. PSA, in combination with a digital rectal examination (DRE), is the most commonly used diagnostic tool for prostate cancer [56]. Regarding encountered limitations for diagnostic biomarkers, current available cancer biomarkers are still having low diagnostic sensitivity and specificity; however, diagnostic biomarkers must be of high sensitivity in order to be a good diagnostic biomarker [57]. For example, Bence-Jones protein in urine remains one of the strongest, well-established diagnostic indicators of multiple myeloma [29]. Nevertheless, some CB have proved to be useful in confirming diagnosis, often in conjunction with a panel of other markers especially to identify primary tumor in metastatic cases with unknown primary and/or other clinical, imaging tools [58]. Use of panel of CB in order to increase sensitivity and specificity of CB in diagnosis has been used to confirm diagnosis of certain cancers. In 2005, Mor et al. [59] reported that a panel, consisting of 4 biomarkers: leptin, osteopontin, prolactin, and insulin-like growth factor 2, collectively had a sensitivity of 95% and a specificity of 95% for the detection of ovarian cancer. In another report, addition of two biomarkers to the previously studied panel included macrophage inhibitory factor and CA125, sensitivity was 95% and a specificity increase to 99.4% for the detection of ovarian cancer. Other attempts to improve diagnostic sensitivity and specificity included combination of CA125 with ultrasonography for diagnosis of ovarian cancer [60].

3.3. Prognosis/prediction

Prognosis is the probability of cure or likely outcome of any patient. A prognostic marker is a disease or patient characteristic feature at the time of diagnosis independent upon therapy; hence, prognostic marker will provide information about the natural history of the disease or the likely outcome. Meanwhile, a predictive biomarker predicts the response to different therapeutic modalities; hence, predictive biomarker is the basic concept for personalized medicine [57]. Magnitude of elevation or levels of CB usually reflects tumor burden, or mass hence higher elevation of CB level mostly reflects bad prognosis and vice versa. By reflecting the tumor burden, CB can be used in staging system for cancer or the tumor-node-metastasis (TNM) classification. For example, in testicular germ cell tumors, very high levels of a CB such as AFP, LDH, and HCG- β may indicate an aggressive cancer with poor prognosis and outcome so such biomarkers may be used for staging in TNMS system in place with a site-specific prognostic factor (S is for site-specific prognostic factors) [61]. LDH alone has been used for

staging of lymphoma as well [62]. However, the accuracy of the marker in determining tumor stage is poor. Estrogen receptor (ER) is one of the widely used prognostic and predictive tissue biomarker; as a predictive tissue biomarker, ER is used for selecting the patients likely to respond to hormonal therapy. Therefore, patients with ER positive tumors will mostly respond to selective ER modulators or aromatase inhibitors independent upon stage of breast cancer whether early or advanced [63]. ER is considered a prognostic marker as well, once ER is negative, that indicate a poor prognosis and when positive a good prognosis is likely the outcome for such patients. In spite of most of CB have some prognostic values which their specific therapeutic impact cannot be applied because of their poor predication accuracy [64]. In the same context, high serum levels of HER2 in serum of breast cancer patients correlate with poor prognosis in such patients [24]. Targeted therapy for HER-2 positive breast cancer patients, trastuzumab (Herceptin), is a recombinant monoclonal antibody against HER-2. Herceptin has been used in women with metastatic breast cancer that overexpressed HER2 and reported to increases the clinical benefit of first-line chemotherapy in those patients [65]. KRAS is a predictive biomarker for colorectal cancer, because patients with somatic mutations in KRAS have poor response to anti-epidermal growth factor receptor (EGFR)-targeting therapies [66].

3.4. Therapeutic monitoring/follow-up/evidence of metastasis or recurrence

Therapeutic monitoring may constitute the most common applications of CB markers in clinical practice [67]. Clinically useful biomarkers usually fluctuate in accordance with tumor behavior, size, or burden changes that are best elicited by increase in levels of CB with progressive disease, decrease with remission, and do not change significantly with stable disease. Kinetics of CB are more important than single measurement or elevated values [68]. Recurrence of cancer may be detected biochemically via rise in CB levels even before appearance of any clinical or radiological evidence of cancer recurrence. Continues follow-up for cancer patients during and after therapy can mirror their condition if the levels of CB were not elevated or remain at basal level, indicating successful therapy or remission. On the other hand, rising of CB level above the basal level indicates recurrence of the disease. CB can be a warning sign of recurrence earlier by 3–12 months before any other diagnostic methods. Many CB could be used for monitoring therapy or detection of recurrence or metastasis, for example, CEA in colorectal cancers, cancer antigen 125 (CA 125) in ovarian cancers, or PSA in prostatic cancer [69]. Some patients who encountered resistance to therapeutic modalities will experience increasing levels of CB, and in that case, reconsideration of alternative therapy is mandatory. Monitoring CB, as screening and diagnostic biomarker needs to be both diagnostically sensitive and specific to ensure proper assessment of effective therapy and continuation of such beneficial therapies and early discontinuation/replacement of ineffective therapy or resistant cancer to those therapies. A representing example of monitoring CB is carbohydrate antigen 19-9 (CA19-9) which has been used in pancreatic in CRC [70]. CA19-9 has been approved by the FDA in 2002 as a monitoring marker for pancreatic cancer. However, it is not recommended as a screening biomarker [71, 72]. Monitoring biomarkers have been extensively used in clinical practice with few limitations perhaps related to detectors' biomarkers of

recurrence rather than monitoring ones. Limitations of those biomarkers probably related to short lead time and poor affection to the outcome [29].

4. Applications of CB in most common cancers

Cancer is an enormous health problem all over the world, over years cancer was indicated as one of the leading causes of death among males and females; an estimated 8.2 million deaths among cancer patients occurred in 2012 worldwide [73]. Over 11 million patients are diagnosed with cancer every year, and 16 million new cases will be expected yearly by 2020 [2]. According to the latest report of the International Agency for Research on Cancer (IARC), the GLOBOCAN worldwide estimates of cancer incidence and mortality published on 2015 and the most common cancers' types among males were lung, prostate, colorectal, liver, and urinary bladder. Meanwhile, breast cancer, lung, liver, ovarian cancers were among the most common cancers in females worldwide [1]. For many years ago, few CB have been used as an effective tool in clinical practice, while also promising CB were extensively studied for their clinical utility. As previously discussed, traditionally used or promising CB may be used for risk assessment for cancer, screening among asymptomatic population, confirming diagnosis or differentially discriminate benign from malignant, prediction of outcome or prognosis, and monitoring of therapy or staging of cancer applications [58].

4.1. Breast cancer

Breast cancer is the most common malignancy among females and the first leading cause of cancer mortality worldwide; its prevalence is surprisingly increasing at a rapid rate lately [74]. Therefore, it is critical to use all available tools for early diagnosis and proper management of cases. Clinically, symptoms are mainly breast lump, nipple discharge, or skin or nipple changes. Screening guidelines by The American Cancer Society recommend that women over 40 have to perform mammography and a yearly or every other year clinical breast exam [75]. Diagnosis mainly relies on pathological examination; however, the role of CB in breast cancer is mainly helpful with prognosis, monitoring of therapy, and for follow-up. Notably, CB does not show great utility for early diagnosis [76]. Assessment of ER and progesterone receptors (PR) in tissue for newly diagnosed breast cancers has been recommended by European Society of Medical Oncology, for predicting response to hormone therapy in early and advanced breast cancer cases [63, 77, 78]. HER-2 is another prognostic marker, most useful for selecting patients with either early or metastatic breast cancer for the treatment with Trastuzumab (Herceptin) [79] or predicting resistance to tamoxifen therapy in early stage of breast cancer [63]. Determination of risk groups for the development of breast cancer, who must be included in screening program, involves the detection of genetic mutation of BRCA 1 or BRCA 2 genes, which account for up to 5% of breast cancer cases. Due to their high susceptibility to breast and ovarian cancer, it is strongly recommended that women carrying BRCA1 or BRCA2 mutations undergo routine cancer screening [80]. It was reported that low levels of urokinase plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) correlate with a reduced risk of recurrence of breast cancer and shown to be strong independent prognostic

factors of newly diagnosed lymph node-negative breast cancers [81, 82]. Serum biomarkers are mainly applicable as monitoring markers during therapy or to less extent prognostic markers and usually assisted in post-operative surveillance, and CB included under that category include CA15.3, CEA, and BR 27-29 [83, 84]. They are used in conjugation with other tools of radiological and clinical assessments to monitor chemotherapy in advanced breast cancer cases. Elevation of serum levels of these markers may indicate recurrence or progression of the disease [85].

4.2. Prostate cancer

Prostate cancer (PCa) is one of the most common cancer in men and most common causes of male cancer-related deaths [74]. Strong evidences suggested that PSA test revolutionized the prostate cancer screening and diagnosis landscape, and the introduction of PSA as a screening test has led to a sharp increase in the incidence of prostate cancer because there has been a shift to diagnosis at earlier stages, consequently reducing mortality from prostate cancer [86]. Later, many studies demonstrated significant improvement sensitivity of PSA as a diagnostic marker using a PSA subtractions and isoforms [-2] (proPSA) and its percentage derivative % proPSA (percent value relative to PSA) as these fraction may help for the discrimination between benign and malignant prostatic tumors in patients with PSA values ranging from 4 to 10 µg/L [87, 88]. Other novel and promising biomarkers under investigation include human kallikrein type 2, prostate cancer antigen 3 (PSA 3), and prostate stem cell antigen (PSCA) [89]. PCA3 urine assay has promising role in improving the accuracy of diagnosis in prostate cancer [90]. Elevated levels of metalloproteinase 2 and 9 (MMP-2 and MMP-9) members of protease family have been associated with prostate cancer diagnosis [91]. MMPs have been studied as biomarkers of therapeutic monitoring in prostate cancer [92].

4.3. Ovarian cancer

Most of the patients with epithelial ovarian cancer are diagnosed late and they have clinically advanced stage III and IV on diagnosis; therefore, ovarian cancer needs a sensitive and specific diagnostic biomarkers [93]. CA 125 is one of the most widely and conventionally used CB. It is recommended as a screening biomarker for women who have positive family history or are high risk for the development of ovarian cancer, beside CA125 has been used in conjugation with vaginal ultrasound as a well-established, diagnostic biomarker [94]. CA125 is also been used as monitoring biomarker, being decreased after starting of chemotherapy or surgery, that correlates with favorable response basal level of CA125, two weeks before starting any therapeutic intervention then follow ups and continues monitoring of its level at regular intervals are highly recommended [95]. Other biomarkers were extensively studied in monitoring of ovarian cancer and in prediction of prognosis but further studies are needed for proper confirmation of their exact role. This panel includes kallikreins (5–9), osteopontin, Her-2/neu, tumor-associated inhibin, CEA, trypsin inhibitor, hCG, interleukin-6 (IL-6), prostasin, TPA, lysophosphatidic acid, plasminogen activator inhibitor-1 (PAI-1) [95–97].

4.4. Colorectal cancer

CRC is ranked third among all cancers all over the world. An estimated one million new cases are diagnosed and half of a million cases died each year [1]. The most common site for colorectal carcinoma is the rectum encountering 38% of all cases followed by sigmoid accounting 29% of cases [98]. Screening program for CRC should be directed to all asymptomatic individuals above 50 years as recommended [99]. National Academy of Clinical Biochemistry (NACB) recommends that all subjects 50 years or older should undergo screening for colorectal cancer. Multiple screening procedures exist [100]. Fecal occult blood test (FOBT) is the most widely used CB in stool [101]. Testing for blood in the stools involves either detecting globin fraction of blood (hemoglobin) by fecal immunochemical test or the guaiac test which measures pseudo-peroxidase activity of heme fraction of hemoglobin. CEA was characterized and introduced into clinical practice in 1965 [76]. It is widely used as universal or non-organ, non-tissue-specific tumor marker. CEA is not used in screening of CRC due to its low sensitivity and specificity, beside the low prevalence of CRC among asymptomatic population; however, it is very efficient prognostic and therapy monitoring biomarker [102]. CEA estimation is recommended at the beginning of therapy then every 1–3 months all through the therapeutic regimen, it is also the marker of choice for metastatic cases of CRC [103]. CA19-9 has been used as prognostic marker, in surveillance of CRC after surgical resection and as monitoring marker for therapeutic intervention in advanced cases [104]. Other CB under investigation are CA242 and tissue inhibitor of metalloproteinases type 1(TIMP-1) and both may complement CEA in the surveillance of patients with colorectal cancer [105].

5. Discovery of new biomarkers/validation/technologies (omics)

Among hundreds of thousands of cancer biomarkers have been discovered, only few of them have been approved during the past two decades by the FDA for monitoring response, surveillance, or recurrence of cancer [106]. To be a clinically applicable and reliable biomarker, it must be of value for informing clinical decision-making to improve the patient outcome [107]. Initially, CB have to distinguish between people with cancer and those without. In fact, many biomarkers do not achieve beyond this point because the investigators are either unable to develop robust, accurate assay methods, or this biomarker lacks sufficient sensitivity and/or specificity [108]. Actually, there was very low rate (0.1%) of successful clinical translation of biomarker [109]. Developing new cancer biomarkers has been formulated in stepwise manner. About 15 years ago, Hammond and Taube proposed an approach for CB development starting from discovering the marker, developing an assay method for assessment, analyzing its clinical potential preliminarily, standardization of its assay, and finally validation of such biomarker for clinical use [110]. Structured phased model for the development evaluation, and validation of biomarkers, (shown in **Table 2**) has been proposed by Pepe et al. [111] and has been adopted and modified by others [112, 113]. This model was similar to another model commonly used in drug development strategy including five phases: preclinical exploratory studies, clinical assay and validation, retrospective longitudinal repository studies, prospective screening studies, and finally cancer control studies. Novel biomarkers must bypass an

analytical validation step concerned mainly with testing and assay methods of the biomarker (technical aspects). After that, the biomarker has to be analyzed for its clinical validity for discriminating between groups independently. Finally, candidate biomarker must be assessed for clinical utility for providing additional input for patient management or aid to provide additional information helping in decision-making for patients in order to improve patient outcome [114].

Phases	Type of studies	Outcome
Phase I	Preclinical exploration	Promising directions are explored and potential biomarkers identified
Phase II	Clinical assay and validation	Determination of the potential capacity of the biomarker to established disease
Phase III	Retrospective longitudinal	Determine how well biomarkers detect preclinical disease through retrospectively testing
Phase IV	Prospective screening	Identify the characteristics of the disease detected by the biomarker and determine the false positive rate
Phase V	Cancer control	Quantification of the role of the biomarkers in the reduction of disease burden through Phase 5 population screening

Table 2. Structured phased model for the development evaluation, and validation of biomarkers modified from Pepe et al. [111] and Paradiso et al. [113].

5.1. Challenges for discovery of novel biomarkers

Development of biomarkers for cancer screening, early detection, and monitoring of treatment has both biological and economic challenges. Most detection methods currently in use identify mostly late stage or fully developed cancer, not in the premalignant or early lesions, which are amenable to resection and cure. In spite of the fact that a screening test might detect cancer at the preclinical stage, at the same time, not applicable for follow-up so it could fail to detect micrometastasis, therefore limiting the benefit of early detection and treatment [115]. Another challenge is that in many organs, for example; prostate or colon, preneoplastic lesions are much more common than aggressive cancers [116]. This creates the question of whether any screening method should just focus on early lesions or whether it should also analyze the behavior of the tumor. Another challenge for the development of CB is the nature of the cancer as being a heterogeneous disease; it is composed of many biologically different phenotypes with different responses to intervention. The nature of its heterogeneity is found between cells of a single macroscopic cancer. This heterogeneity may complicate the development of biomarkers. Therefore, the development of biomarker by genomic and proteomic means might carefully address the heterogeneity issues [117]. Detailed and comprehensive knowledge of cancer at the cellular and molecular levels has grown dramatically and exponentially in the past two decades and has resulted in significant improvement in the characterization of human

tumors which in turn has catalyzed a shift toward the development of targeted therapies, the basic concept for personalized medicine [118]. Therefore, it has been recently postulated that the emergence of highly powerful “omics” technologies, such as genomics, epigenomics, transcriptomics, proteomics, and metabolomics [119]. Omics technologies may be the backbone toward the discovery of novel CB and/or panels, with distinct advantages over the currently used biomarkers. Omics have increased the number of potentially investigated biomarkers as DNA, RNA, or other protein biomolecules. The former concept of single biomarker discovery was replaced recently by multi-biomarkers discovery of panel of genes or proteins whereby, rising the query of whether the heterogeneous and multifactorial cancer may have single fingerprint.

5.2. Genomic technologies

Genomic technologies have been used extensively for the characterization of cancers at the molecular level hence providing better comprehensive understanding of cancer and may provide scientists the basic concepts for designing drugs that could target specific molecules or the fundamental of personalized medicine [120]. Personalized medicine has been defined by The US National Cancer Institute (NCI) as *“a form of medicine that uses information about a person’s genes, proteins, and environment to prevent, diagnose, and treat disease.”* [50]. Genomic alterations that may be associated with cancer include gene amplification, mutation, chromosomal rearrangements, and aberrant methylation. Molecular alterations are evolved in the content or sequence of DNA, its transcriptions mRNA or microRNA, the production of proteins, or the synthesis of various metabolites. Genomic alterations can be assessed through genome sequencing technologies or microarray for gene expression [29]. Mutation screening can be assessed by sequencing technique, while assessment of DNA copy numbers could be analyzed by DNA microarrays and DNA expression profile via PCR [120]. Genomic microarrays represent a highly powerful and sensitive technique; it can predict the clinical behavior of tumors [121]. Genomics has been extensively used for biomarker discovery and identification. Human genome accounts approximately 30,000 genes, the availability of omics techniques allows researchers to move another step further, which is designing and manufacturing of a biological drug with better understanding of pharmacogenomics, thus biomarkers allow the studying of the influence of genetic variation, providing new methods for treating patients on an individual basis. The outcome of such researches is known as personalized medicine [122].

5.3. Epigenomics

Epigenetics refers to heritable changes in gene expression that are not attributable to alterations in the sequence of DNA. Epigenetic changes include DNA methylation, histone modifications, and non-coding RNAs. These alterations may be present ubiquitously human malignancies and may appear in early cancer development. Therefore, they provide particularly attractive markers with broad applications in diagnostics [123]. Methylated DNA (meDNA) is a various stable carrier of epigenetic information that is directly occurred in tumor formation and

progression. In fact, the inherent stability of DNA is one of the major advantages of detecting methylation. Genes that are often methylated in tumors are termed tumor biomarkers because their methylation can be used to detect the disease. Utilization of meDNA markers is superior comparing to other types of tumor biomarkers for numerous reasons including: The analysis of DNA methylation can be achieved with a wide range of methods using different types of biological material such as tissue, plasma, serum, sputum, and urine, among others [124]. Methodology of DNA methylation measurement has progressed gradually through the years. Assessment techniques for epigenetic changes may include: The bisulphate conversion of DNA followed by PCR amplification allows gene-specific methylation analysis (methylation-specific PCR, i.e., MSP), which is based on using primers and probes specific to the corresponding methylated DNA sequence [125]. This technology makes the detection of hundreds of thousands of DNA methylation signals a reality. These signals can be digitized into a long string of ones and zeros, creating a digital phenotype that reflects genetic activity in a particular cell or tissue, that is, whether it is functioning normally or whether it is abnormal. Around 200 such biomarkers have been discovered through a large-scale genome-wide screening effort of all major human tumors for DNA methylation biomarkers in bio-specimen; tissue and serum [126].

5.4. Proteomics

Proteomics-based strategy diseases identification is considered as one of the dynamic and innovative tools that could confirm, complement, or quite often supply more elaborate information beyond that obtained by other high-throughput approaches such as genomic, transcriptomics, and epigenomics. Despite genomic expression profiling is a highly reliable method for cancer classification and prognostication [127, 128]. The function of such genes and the data interpretation in the context of functional networks require their translation into active proteins and their analysis through the power of proteomics. Moreover, although studies focusing on detecting the differential expression of mRNA have been extremely informative, they do not necessarily correlate with the functional protein concentrations. Therefore, post genomic “proteomic” projects correlating protein expression profiles to cancer are essential for a complementary and comprehensive representation of cancer biology. Moreover, targeting-specific protein pathways involved in tumorigenesis present a realistic aim in cancer treatment, as proteins exert their effects through specific pathways rather than functioning individually [120]. Macromolecules, in general, and proteins, in particular, are highly dynamic molecules. Mechanistically, proteins can be subjected to extensive functional regulation by various processes such as proteolytic degradation, posttranslational modification, involvement in complex structures, and compartmentalization. Proteomics is concerned with studying the whole protein repertoire of a defined entity in a biological fluid, an organelle, a cell, a tissue, an organ, a system, or the whole organism. Therefore, in-depth studying of proteomics profiles of various bio-specimens obtained from cancer patients is expected to increase our understanding of tumor pathogenesis, monitoring, and the identification of novel targets for cancer therapy. In a simple way, proteins may be actively secreted or released by the tumor cells as a result of necrosis or apoptosis and released into the circulation [76]. This

changes the protein profile. The difference in signal intensities may be detected by comparison with sera from normal individuals. Secretomics, a subfield of proteomics that studies secreted proteins and secretion pathways using proteomic approaches, has recently emerged as an important tool for the discovery of biomarkers. In what is now commonly referred to as proteogenomics, and proteomic technologies are further used for improving gene annotations. Parallel analysis of the genome and the proteome facilitates discovery of post-translational modifications and proteolytic events (comparative proteogenomics).

5.5. Metabolomics

A cancer biomarker can be a metabolite, secreted by tumor, metabolic pathway or process, and may be employed to diagnose cancer and predict patient response towards therapies and monitor recurrence. Though proteins are the key tumor markers that can be as diverse as molecular, biochemical, physiological, or anatomical [129]. Markers can be utilized for diagnosis (to identify early stage), prognosis (assess the lethality), and prediction (of patient's response to treatment) of cancer. The markers can be detected in body fluids (blood, urine, serum, stool, saliva), or tissues (tissue samples or biopsies of the cancer). Moreover, it has been shown recently that cancer volatile organic compounds (VOC) markers can be detected in breath [130]. However, detecting the markers is a sophisticated process and metabolomics is one of the omic technologies. Among genome, transcriptome, proteome, and metabolome, the latter is the powerful representative of the phenotype [131]. Exploring the cancer metabolome seems to be an effective way to study the phenotypic changes associated with tumor. Screening biomarkers by recruiting an array of analytical techniques has been emphasized [132]. Rather than a single metabolite, a pattern is believed to be more indicative of cancer status. Metabolomic approach makes it feasible to detect an array of metabolites in a single assay. The principal analytical tools employed for metabolome analysis are mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR).

6. Conclusion and prospective

Cancer biomarkers play an important role in the field of oncology and in clinical practice for risk assessment, screening, diagnosis integrated with other diagnostic tools and mostly for the determination of prognosis and response to treatment and/or relapse. Cancer biomarkers can also facilitate the molecular definition of cancer. It is necessary for clinicians and researchers to have a comprehensive understanding of molecular aspects, clinical utility, and reliability of biomarkers in order to determine whether and in what setting a biomarker is clinically useful for the patient care, or additional evaluation is required before integration into routine medical practice. The challenge and future prospective of biomarkers, by facilitating the combination of therapeutics with diagnostics, promise to play an important role in the development of personalized medicine.

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