THE EXPERIMENTAL ADVANCEMENTS OF BIOMARKERS IN CANCER: A REVIEW

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ABSTRACT

Cancer remains one of the leading causes of death within the world. Biomarkers are a novel approach to detect cancer in different stages of cancer including its progression and spread. They are an efficient tool for its ability for a premature indication of cancer progression. Biomarkers are selected to be easily detected, measurable across all populations, and early detection in the spread of the tumor. It can also be used to identify high-risk individuals and monitoring the risk factor associated with cancer. They can be cellular, biochemical, or molecular alterations by which both a normal and an abnormal biological process can be determined. Usually, biomarkers are a biological molecule involved in the process of biological activity and can be used to specify a certain biological process. The use of Biomarkers in cancer study is not new but the recent development in scientific advancements has led us to dive deeper into the molecular mechanism of the cancer cells. The said biomarker can be used in the form of abnormal proteins secreted in the neoplastic cells or can be a chromosomal aberration during the cell division, a genetic mutation, autoantibodies, etc. All we have to determine is the specific biomarkers and then developing a technique to monitor the biomarker in real-time to accurately determine the aspects of the disease. Although, we have discovered some of these biomarkers still the translation of these new biomarkers from discovery to viable clinical experimentations/trials has been slow.

Keywords: Cancer, Biomarkers, Neoplastic cells, Cellular, Biochemical, Molecular, Experimentations, Cell division, Autoantibodies, Chromosomal aberration

SCOPE OF THIS REVIEW

The review article focuses on a complete overview of biomarkers and their application in cancer research. This paper will provide a summarized extract from various papers dealing with several aspects of biosensor development, phases, types, and its potential in diagnosis and screening of cancer. It will provide an outline of the increasing trend of biomarkers including their limitations and future prospects. This review have taken references from published paper by individual research scientists and other cancer research institutes, to sum up, the recent and most advanced technologies, methods, and treatments that are being worked on and developed against cancer using novel biomarker tools. This manuscript helps to understand the basic knowledge of cancer biomarkers and some of its relevant research done in recent years.

INTRODUCTION

Cancer remains one of the leading causes of death within the world. They are complex diseases and are influenced by many genetic factors as well as environmental factors. Tumor formation involves uncontrolled cell growth, division, and translocation of tumor cells from one organ to

another. Neoplastic cells receive signals which are transmitted to the inside of the cell and therefore to the nucleus to activate genes related to an increase in tumor aggressiveness, angiogenesis, cell survival, or proliferation. Cancer has been extensively studied and there are vast types of malignancies. Almost all the organs present in the human body is susceptible to cancer. But among these only a few are the most frequent and have a higher rate among the cancer population like breast cancer, cervical cancer, oral cancer, melanoma cancer, etc (1).

Traditional diagnosis and screening of cancer involve diagnostic Imaging, endoscopy and Biopsy. Diagnostic Imaging techniques involve MRI, CT scan, PET scan, X-rays scan, ultrasound, and nuclear scan (2). These instrumentations are capable of detecting cancer but not for tumors that are in intricate parts of the body. These imaging techniques moreover are not feasible to all the cancer patients and are quite unable to appropriately diagnose the type and progression of the tumor. These imaging techniques are often paired with tissue biopsies or endoscopy which involves the extraction and examination of tumor cells from the individual. These methods are expensive and are not much accurate in various cases.

Biomarkers are trending research focused on identifying, extraction, and studying of markers that are associated with cancer. Usually, biomarkers are a biological molecule involved in the process of any biological activity which can be used to specify a certain biological process. These markers are selected to be easily detected, measurable across all populations, and early detection in the spread of the tumor. It can also be used to identify high-risk individuals and monitoring the risk factor associated with cancer. They can be cellular, biochemical, or molecular alterations by which both a normal and an abnormal biological process can be determined (3).

The use of biomarkers replaces these hectic and complicated tests behind and allocates an easy and safe diagnosis method which not only provides us information about the cancer progression but also the specificity of the tumor based on the individual. The detection of specific proteins, tumor cells, RNA, epigenetic causes produced by these cancer cells in individuals, and their concentration change can be used as biomarkers to detect early-stage cancer or to monitor the response to therapeutic management (4). The criteria that were established to define a good biomarker: harmless and preferably non-invasive, highly accurate (i.e., high specificity, sensitivity as well as positive and negative predictive values) for its intent and capable to improve decision-making abilities together with clinicopathological data. Following these criteria, it is unlikely that a single molecule or alteration can fulfil all requirements, and, therefore, a panel of biomarkers might provide a more reliable approach (5).

STAGES OF BIOMARKER DEVELOPMENT

Cancer biomarkers are discovered and utilized with a specific purpose in mind;
(a) early detection of cancer, (b) diagnosis, (c) screening, (d) response to anticancer therapies, and (e) cancer recurrence. Cancer cells provide the biomarker material that can lead to their own detection, which then provides the opportunity for their non-invasive detection in body fluids and tissues so as to reveal the presence of tumors or the level of tumor burden (6).
The development of a biomarker is divided into different stages and each stage corresponds to the search and utilization of the biomarker in clinical trials.
Stage 1, or the "discovery" stage, incorporates exploratory investigations to recognize viable and helpful biomarkers. It is here that the greater part of the work on disease biomarkers has been engaged. Examiners regularly investigate the gene or protein expression from tumor and

ordinary tissues to discover biomarkers that are either at an elevated rate or inhibited in neoplastic tissues. Most investigations don't advance past this first stage (7).

In stage 2, or the "validation" stage, examiners take the biomarkers revealed in stage 1 examinations and attempt to create and approve clinical measures to study these biomarkers in samples that can be acquired non-invasively. To be helpful, these tests must have the option to recognize individuals with malignant growth and those without it. Nonetheless, the subjects in these examinations have to build up tumors, and these investigations don't decide the convenience of the biomarkers for early recognition. Numerous biomarkers don't advance past this stage in light of the fact that either the agents can't create reliable, or the biomarker lacks enough sensitivity and specificity leading to rejection of the biomarker (5).

Stage 3 investigations test the limits of biomarkers to recognize preclinical trials by testing the marker against tissues gathered longitudinally from research associates. In this stage, the biomarker is estimated in samples gathered from people before their diagnosis of disease and contrasted with the biomarker level in age-coordinated controls. These examinations are normally reviewed and used as archives of tests gathered from clinically healthy people who were observed for the development of malignancy. However, if the biomarker in this stage is unable to determine the stage of the disease and detects only when the tumor is as later stages of disease then it is not suitable to be used as a biomarker for early detection (5).

Stage 4 examinations are imminent screening preliminaries to decide whether a biomarker can identify disease at the beginning phase of growth. Asymptomatic individuals are screened, and the individuals who test positive are followed up to decide whether they have the disease. These investigations give data on the test's specificity and the presence of the disease. Regardless of whether a test performs well in stage 3 and 4 examinations, it doesn't really imply that it will reduce the burden of people who have screened for cancer in the population (3).

Stage 5 investigations are huge scope in large scale population-based studies assessing not just the role of the biomarker for identification of disease but also the global impact on the general population. These investigations are intended to decide whether the screening brings about a decrease in disease prevalence and mortality and include large scale populations. These preliminaries screenings are tedious and costly, and to date, few have been attempted. These can take a very long time to finish. and unfortunately, the outcomes are at times questionable (8).

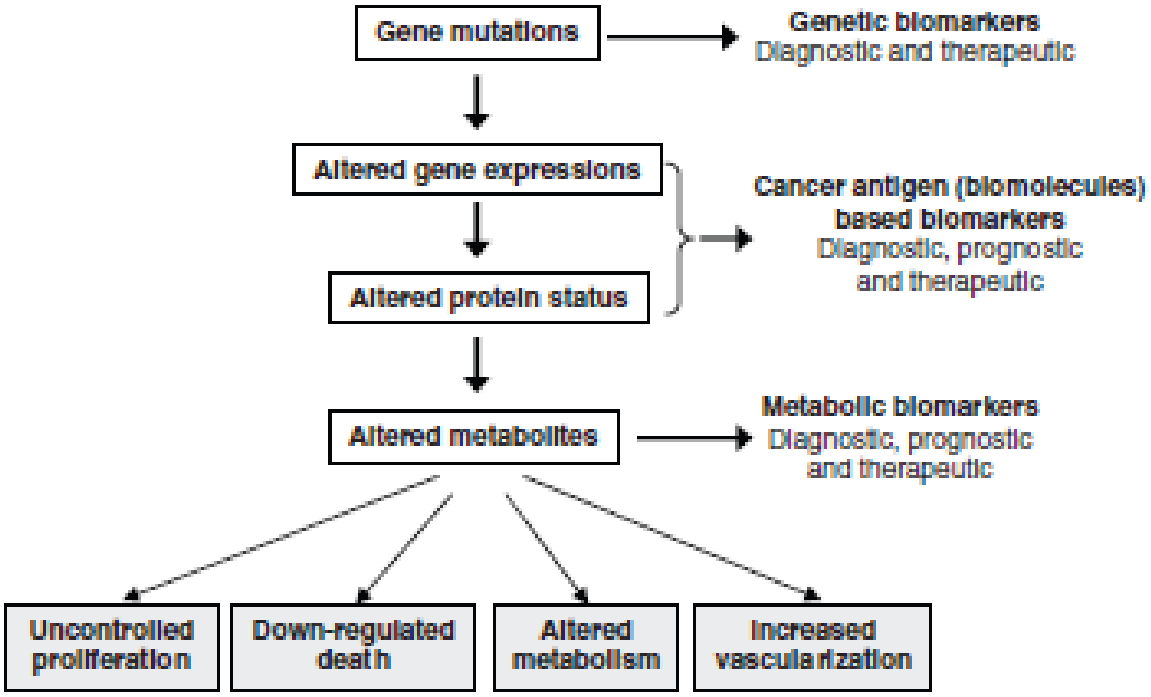

Fig. The process of carcinogenesis, showing opportunities for identifying biomarkers (8).

TYPES OF BIOMARKERS BASED ON ANALYTES

Cancer cells display a broad spectrum of genetic alterations that include gene rearrangements, point mutations, and gene amplification, resulting in disturbances in molecular pathways regulating cell growth, survival, and metastasis. When such changes manifest in the majority of patients with a selected sort of tumor, these are often used as biomarkers for detection and developing targeted therapies, besides predicting responses to various treatments. New discoveries in genomics, proteomics, and transcriptomics, along with many non-invasive imaging techniques and other technologies allow the measurement of several biomarkers (9). Currently, there is a greater understanding of the disease pathways, the protein targets, and the pharmacologic consequences of drug administration. Therefore, the application of biomarkers within the clinical practice is probably going to end in advanced knowledge resulting in a far better understanding of the disease process which will facilitate the development of simpler and disease-specific drugs with minimal undesired systemic toxicity (8).

In the context of this article, we will focus on five general areas of analytes.

1. Nucleic acid biomarkers

i) DNA

DNA sequencing is commonly used in research to identify genetic changes in candidate genes. The use of a DNA sequence of diagnostic markers is entering into clinical practice for the detection of DNA sequence variants or small insertions or deletions in genes. Somatic changes in DNA are present in tumor tissue but not present in the normal tissue from the same person. The selective advantage provided by somatic DNA sequence alterations is thought to be a critical part of the carcinogenic process resulting in changes in important growth regulatory genes (10). Oncogenic DNA sequence variants are useful diagnostic biomarkers and have provided specific molecular targets for cancer therapies, such as the mutant oncoproteins as pharmacologic targets or biomarkers of response to novel targeted agents. Large scale sequencing of numerous candidate genes is providing new biomarkers for studies of diagnosis and prognosis of the lung, germ cell, colorectal and breast cancers (8).

ii) RNA

The identification of gene expression profiles for stage or prognosis specific classification of cancer patients has become a useful tool in biomarker-driven patient management. Gene expression profiles containing a panel of mRNAs can be more effective than more standard methods of patient stratification such as histopathology biomarkers. To validate such biomarkers clinically, using fresh or archived clinical samples, the RNA preparations must have minimal degradation or alternatively use gene measurement technologies that are not overly sensitive to some reduction in the RNA size (11).
Gene expression profiling coupled with well-designed bioinformatics methods has greatly facilitated the identification of genes and pathways that regulate cellular changes critical to cancer development and molecular targets for personalized medicines. The microarray screening procedures are quite reproducible but require biological replicates or multiple samples from a particular class of sample to eventually be able to use the biomarkers for class prediction on an independent validation set. In principle, the particular changes in gene expression between classes of samples may be less informative than the pathways they impact. There are numerous software approaches to pathways analysis for a given gene expression dataset. Those that weight the impact of the particular genes provide additional guidance to the identification of shorter lists of biomarkers (10).

iii) Epigenetics

There are several normal cellular processes that are regulated in part by epigenetic modification through DNA methylation including developmental imprinting, X-chromosome inactivation, and tissue-specific gene expression. Aberrant chromatin-remodeling mechanisms such as DNA methylation and histone acetylation are epigenetic modifications that have been observed in tumor tissues leading to the inhibition of tumor suppressor gene expression. Understanding these mechanisms may provide biomarkers of response to cancer chemotherapy (12). Groups of CpG methylation events in promoters are easily detected by PCR-sequencing of bisulfite-treated DNA from cells or tumors or by methylation-specific PCR techniques which permit the interrogation of a few CpG positions. Next-generation DNA sequencing is providing whole-genome approaches to discover novel epigenetic changes at the level of DNA methylation. Aberrant promoter methylation has been found in growth-suppressive genes in human tumorigenesis (13).

In human cancers, the silencing of tumor suppressor genes through aberrant DNA methylation of a CpG island(s) in the promoters in these genes is a common epigenetic change. There is an

assortment of pathways from which genes are shown to be hypermethylated in cancer cells including DNA repair, cell cycle control, invasion, and metastasis. Higher throughput methods are being developed to identify larger panels of methylation biomarkers for disease detection and tumor progression. From such studies, panels of biomarkers for individual cancers are being developed for early detection and response to chemotherapy (9).

iv) MicroRNA

Microarray technology can provide the analysis of all known miRNAs similar to that for mRNA
profiling. Screening for miRNA expression levels is routinely performed using array technologies to obtain a miRNA profile and validation/confirmation using northern blot, RNase protection assay, or primer extension assay. Quantitative RT-PCR, in situ hybridization and serial analysis of gene expression (SAGE), has also been applied to these small RNAs (14).
There is substantial evidence for differential expression of miRNAs in a variety of cancers. MiR-15a and -16-1 are found within a cluster in chromosome 13q14, which is frequently deleted region in B cell chronic lymphocytic leukaemia The miRNAome may provide a better classifier of cancer type than the mRNA expression profile and therefore appears to be a useful technology for the diagnosis and prognosis of cancer miRNAs have been found to be repressed by epigenetic mechanisms in cancers and consistent with that observation more miRNAs are down-regulated than up-regulated in cancer when compared to normal tissues. Because of their small size, miRNAs have tremendous potential as biomarkers because they are easily quantified in normal and cancer tissues as well as body fluids (10).

v) Telomerase

Telomeres are repetitive tracts of DNA present at the end of the chromosome with sequences of TTAGGG/ AATCCC in humans that protect chromosomes from degradation and loss of essential genes. Under normal circumstances, telomeres progressively shorten in most human cells with each cycle of cell division and the length in adult human tissues is approximately half that of the new-born. Telomerase is found in nearly 90 percent of human cancers and is responsible for the indefinite growth of cancer cells; it has been a target for anticancer therapeutics that turn-off telomerase and thereby inhibits tumor growth (12). The levels of telomerase are also elevated in stem cells allowing unlimited division necessary for the repair of damaged and worn out tissues.
Most human tumors not only express telomerase but interestingly also have very short telomeres. Telomerase is one of the best markers for human cancer, associated with only malignant tumors and not the benign lesions making it a diagnostic marker as well as an ideal target for chemotherapy. In cancer cells, telomerase is found throughout the cell, implying that the telomerase-shuttling system is impaired. Identification and manipulation of proteins normally involved in telomerase transfer could convince be useful targets for anti-telomerase therapies (9).

2. Protein biomarkers

Protein biomarkers are often identified in basic science studies of cancer cells as overexpressed proteins. Given the proven ability of most manufacturers of clinical laboratory tests to adapt a protein-based immunoassay on to a standard clinical platform, the expectation of a rapid translation of protein discoveries to a clinical test should be quite rapid and efficient. The

difficulty in establishing a clinical test is often at the level of developing antibody pairs for sandwich immunoassays when multiple protein analytes must be assayed in a body fluid such as a serum, plasma, or urine (15). Cancer-specific alterations in proteins may occur at the level of protein abundance or post-translational protein modification such as glycosylation or phosphorylation. If the protein being developed as a biomarker is present in a body fluid but only the post-translational modification is cancer-specific, then antibodies to these specific changes represent a formidable challenge for the development of the antibody pairs. In addition, the issue of matrix complexity is formidable (15). The concentration range of plasma proteins comprises about nine orders of magnitude. Given that the abundant plasma proteins may be functionally related to the disease processes, depleting abundant plasma proteins may cause the unintentional but direct removal of some important protein analytes or indirectly remove key biomarker proteins that were associated with the abundant protein thus intentionally removing them from the serum or plasma (11). Proteomic changes in cancer can be discovered by a combination of two-dimensional gel electrophoresis for the separation of the proteins with a variety of potential methods for their visualization such as direct radioactive labelling, covalent attachment of fluorescent tags, and silver staining. Mass spectrometric analysis can then be used for the sequence identity of each protein spot. Some protein biomarkers are given:

i) Cancer autoantibodies

Antibodies in the serum of cancer patients are induced by the humoral immune response to overexpressed or mutant tumor proteins. Autoantibodies to MUC1 have been found in the sera of women with benign breast disease, as well as invasive breast cancer at an early and advanced stage. MUC1 autoantibodies also are found in patients with other cancers also indicating a lack of their specificity. Directly screening for autoantigens can be performed to discover the autoantibodies using the serologic identification of antigens by recombinant expression (SEREX) technology. This method has resulted in the discovery of numerous antigens for breast cancer. Several modifications have been introduced to improve SEREX technology.

The variation involves established tumor cell lines instead of fresh tumor specimens as a source of cDNA, thus avoiding contaminating tumor specimens with normal cell RNAs so that they won't be included in cDNA preparation. Also, this avoids cDNA cloning of immunoglobulin sequences expressed by tumor-infiltrating B cells giving rise to false positives in the library screening (16).

ii) Antibody microarrays

Detection of protein having multianalyte property has been done by using highly parallel immunoassays using ELISAs. One strategy is to utilize previously validated antibody pairs from sandwich ELISA tests and multiplex the spotted capture antibodies on a surface and then label the detection antibodies for the parallelized assay. The protein analytes can be measured in serum, plasma, or urine for non-invasive biomarker assays or in liquefied extracts of tumor tissue for evaluation of prognostic biomarkers or potential therapeutic targets. Another strategy is to choose candidate over-expressed proteins from other studies and develop pairs of antibodies suitable for antibody microarrays (10).

iii) Human chorionic gonadotrophin (HCG)

It is a hormone produced normally by the placenta, whose level is elevated in the blood of patients with certain types of testicular and ovarian cancers (germ cell tumors) and choriocarcinoma. The presence of increased serum levels of hCG and its metabolites is generally considered to be a sign of poor prognosis and it has been suggested that βhCG might directly modify the expansion of cancer, resulting in a worse outcome. The clinical use of free βhCG as a tumour marker has been limited to a little number of patients due to brief half-life and rapid renal clearance (17).

iv) Heat shock proteins (HSPs)

Heat Shock Protein's expression is tailored for particular stress response, with the accumulation of denatured proteins as the proximal signal for its induction. They are overexpressed during a wide selection of human cancers and are implicated in tumor cell proliferation, differentiation, invasion, metastasis, death, and recognition by the system . the circulating levels of Hsp and anti-Hsp antibodies in cancer patients may be useful in tumor diagnosis. Several Hsp are implicated in the prognosis of specific cancers; most notably Hsp27, whose expression is associated with poor prognosis in gastric, liver, and prostate carcinoma, and osteosarcomas (8).

v) p53

The p53 gene is one of the tumor suppressor genes that normally prevent uncontrolled multiplication of abnormal cells and experimental findings from the last two decades have established a crucial role for wild-type p53 in intrinsic tumor suppression. During malignant transformation, p53 or p53-pathway related molecules are disabled most often and a mutant form of p53 may not only negate the wild type p53 function but plays an additional role in tumor progression165. Mutated p53 gene is a major cause of cancer accounting for nearly 50 percent of the human tumors. Although p53 is not a typical cancer-specific antigen, its central role in the control of cell growth and apoptosis and frequent mutations in tumors make p53 a unique target for cancer therapy (8).

3. Metabolites and metabolomics

There appears to be greater than 2000 metabolites that are accessible using current technologies which results in a smaller range of potential molecular targets than genomics, transcriptomics or proteomics. Metabolic patterns and biomarker classifiers for tumor staging and stratification have been developed for breast cancer, prostate, and renal cell carcinoma. The changes in the metabolic profile of adenine nucleotide profiles provide a rich source for biomarker discovery (10).

i) Glycolysis

Enhanced glucose dependency is one among the prominent characteristics of most malignant tumors and correlates well with resistance to radio- and chemotherapy. Recent observations in many human tumor cell lines with varying degrees of glycolysis (endogenous and induced) have shown an inverse relationship between the rate of glycolysis (glucose usage and lactate production) and the manifestation of damage induced by radiation and chemotherapeutic drugs.

Clinical trials in patients with malignant brain tumors (glioblastoma multiforme) using a hypo fractionated radiotherapy protocol combined with 2-Deoxy-glucose have been very encouraging (18).

Mechanisms underlying these fundamental alterations in metabolism during carcinogenesis include mutations within the mitochondrial DNA leading to functional impairment, oncogenic transformation linked upregulation of glycolysis, enhanced expression of metabolic enzymes, and adaptation to the hypoxic tumor micro-milieu in case of solid tumors (9).

4. Cytogenetic and cytokinetic markers

Structural and numerical aberrations within the chromosomes are classical markers of cancer because the association between chromosomal aberrations and neoplastic transformation has been well established. Clinical staging has been noted in many tumors. Somatic mutations (in reporter genes, oncogenes, and tumor suppressor genes) are promising biomarkers for cancer risk as these can capture genetic events that are related to malignant transformation. There is growing evidence that specific polymorphism in certain genes is associated with cancer risk (16).

Enhanced cell proliferation is one of the foremost important hallmarks of cancer, which is straightforward to spot employing a number of histological, biochemical, and flow cytometric analyses. Identification of S-phase cells and analysis of a variety of other antigenic determinants of proliferation (PCNA, Ki67 NOR, etc.) studied employing a sort of cell biology techniques have also been used as complementary markers. Mini chromosome maintenance genes also produce proteins that have been proposed as useful biomarkers for cancer; with high levels of organic phenomenon indicating poor prognosis. All these cell-cycle regulated proteins are found among the genes associated with proliferation in tumors (19).

5. Cells as biomarker

In advanced stages of tumors, cells start appearing in the bloodstream where it can be easily monitored. Advanced clinical practice in certain malignancy has effectively used tumor and immune cells where it served as a good biomarker of prognosis (8).

i) Circulating tumor cells

It is a simple yet powerful biomarker in the field of oncology. The presence of circulating tumor cells (CTCs) has been shown to predict survival in patients with metastatic breast cancer multiple times. CTCs provide an early, reliable indication of disease progression and survival for patients on systemic therapy for metastatic carcinoma. Elevated CTCs at any time during therapy is a harbinger of progression, while the elimination of CTCs indicates the effectiveness of the therapy. CTCs are not only potential surrogate endpoints in oncology clinical trials but may guide the selection of patients into the trials. CTCs have been shown to be superior to standard tumor markers in predicting prognosis. The available evidence clearly suggests that CTCs can be used as an early predictor of treatment efficacy and extremely useful in sparing patients from futile therapy early in the course of their treatment (8).

ii) T-regulatory cells

It is becoming increasingly clear that regulatory T (T-regs) cells are equally important in inducing and maintaining peripheral self-tolerance and thus preventing immune pathologies. studies in cancer patients suggest that increased T-regs activity may be associated with poor immune responses to tumor antigens and contribute to immune dysfunction resulting in tumor growth. High numbers of T-regs have been found in lung, pancreatic, breast, liver, and skin cancer patients, either in the blood or in the tumor itself (16). However, levels of T-regs are also elevated in certain infectious diseases. it appears that although t-regs may serve as a surrogate immune marker of cancer progression (and perhaps prognosis), it seems to be more useful as a predictor of response to therapies. T-regs and more importantly its intracellular marker should be explored as a biomarker for human tumors enabling better decisions in inpatient care, as well as prepare the field for novel therapeutic agents (20).

iii) Cancer stem cells (CSCs)

In recent years, the cancer somatic cell model of tumorigenesis has received increasing attention. This model postulates that tumors are driven and maintained by a minority subpopulation of cells that have the capacity to self-renew and to get the more differentiated progeny which makes up the bulk of a tumour56. The former population has been termed cancer stem cells (CSCs), tumorigenic cancer cells, or tumor-initiating cells, by various investigators, to indicate that only these can give rise to new tumors when transplanted into an immuno-deficient animal (19).

Table. Cancer biomarkers for diagnosis and prognosis of the disease

Biomarker	Tumour	Application	Sample type/ Method of detection
Cancer antigen (biomolecules) based biomarkers:			
Prostate specific antigen (PSA)	Prostate cancer	Diagnostic and prognostic	Serum/ Immunoassay
Alpha-foetoprotein (AFP)	Hepatocellular carcinomas (HCC)	Diagnostic and prognostic	Serum/ Immunoassay
Cancer antigen 125 (CA125)	Ovarian cancers Fallopian tube cancer	Diagnostic and prognostic	Serum/ Immunoassay
Cancer antigen 15-3 (CA15-3)	Breast cancer	Diagnostic and prognostic	Serum/ ELISA, Lymph node/ IHC, Bone marrow/ IHC
Cancer antigen 19-9 (CA 19-9)	Pancreatic cancer Bladder cancer	Diagnostic and prognostic	Serum/ ELISA Urine/ ELISA
BRCA-1, BRCA-2	Breast cancer	Diagnostic	Tumour samples/ RT-PCR
Carcinoembryonic antigen (CEA)	Colorectal cancer	Diagnostic and prognostic	Serum/ ELISA
Human chorionic gonadotrophin (hCG)	Germ cell tumours (ovarian and testicular)	Diagnostic	Serum/ ELISA
Thyroglobulin (Tg)	Papillary and follicular thyroid cancer	Diagnostic and prognostic	Serum/ ELISA or IHC with TPO Ab
Heat shock proteins (HSPs) Hsp27; Hsp70	Gastric, prostate carcinoma, osteosarcomas, uterine, cervical, and bladder carcinoma	Diagnostic and prognostic	Serum/ ELISA
TGFβ	Malignant tumours	Diagnostic and prognostic	Serum / ELISA
Metabolic biomarker:			
Glucose metabolism	All cancers, general	Daignostic, prognostic and therapeutic	Imaging/ FDG-PET scan
Genetic biomarkers:			
Genetic translocations *viz.* Philadelphia chromosome, Bcl2 and other gene translocation fusion products	AML, ALL, CML, MDS and Burkitt's lymphoma	Diagnostic	Bone marrow or peripheral blood/ FISH
APC gene	Adenocarcinoma, squamous cell carcinoma of the stomach, pancreas, thyroid and ovary	Diagnostic and prognostic	Blood, Tumour sample/ RFLP of chromosome 5q21-22, Methylation status of APC gene
Cells as biomarker:			
Circulating tumour cells (CTCs)	Metastatic breast cancer, *etc.*	Diagnostic and prognostic	Blood/ Immunocytometry
Cancer stem cells (CSCs)	AML, melanoma, brain tumour, breast cancer, prostate cancer	Diagnostic, prognostic and therapeutic	Tumour sample/ Immunocytometry

Figure 1.Some cancer biomarkers for diagnosis and prognosis of the disease (8).

RECENT TRENDS IN BIOMARKER RESEARCH

1. Long noncoding RNAs (lncRNA) are used as biomarkers in cancer. Various types of lncRNA have been identified as potential markers. Wang et al., (2015) recently classified these lncRNAs according to their location in genome, mechanism, and functioning. The biggest reason that makes lncRNAs suitable as cancer diagnostic and prognostic biomarkers is their high stability while circulating in body fluids, especially when included in exosomes or apoptotic bodies (21). This property of lncRNA detection of circulating cancer-associated lncRNAs in body fluids might be utilized in the assessment of cancers at distinguishing tumor patients from healthy people at early stages with both high sensitivity and specificity (21).

MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1) represents a promising diagnostic biomarker detectable in blood, to effectively screen lung cancer identified by Wang et al., (2015). One study has shown downregulation of MALAT1 in blood samples of lung cancer patients which were contrary to MALAT1 levels in lung cancer tissues, where it was significantly upregulated (22).

UCA1 lncRNA has been identified as a possible biomarker for bladder cancer. Due to its relatively high overall specificity, it's a high potential to discriminate between the bladder/urothelial cancer and other cancer types, or other diseases associated with the urinary tract (sensitivity 80.9%; specificity 91.8%). UCA1s are often detected in urine samples of bladder cancer patients, mostly within the cellular sediments. Wang et al., (2006) (23).

2. Direct assessment of Programmed death-ligand (PD-L1) expression on tumor cells is a logical biomarker for the prediction of treatment response to anti-PD-1 or anti-PD-L1 checkpoint therapies. PD-L1 expression by immunohistochemistry as a predictive biomarker has been evaluated in many clinical trials as done by Guan et al., (2017) (24).

Carbognin et al., (2015) reported a clinical response in 239 of 702 patients (34%) with PD-L1-positive cancers, however, 154 of 773 patients (20%) with PD-L1-negative cancers also showed an objective response to those checkpoint blockade drugs. carcinoma. PD-L1 expression is useful but not an ideal biomarker because of its poor sensitivity and specificity (25).

3. Chang et al. described Prostate-specific antigen (PSA) as one of the best-known prognostic biomarkers. This biomarker has been widely used to screen men for prostate cancer. Serum PSA levels can be used before radical prostatectomy to predict outcomes, including tumor volume, a grade of disease, and biochemical progression (14).

Martin et al.,(2012) and Sardana et al.,(2012) reported Prostate cancer antigen 3 (PCA3), a non-coding RNA that is highly expressed in 95% of Prostate cancer, presenting a 66-fold upregulation compared with matched normal tissues, is the most promising and recent this biomarker to become commercially available (PROGENSA™) (12).

Immunohistochemical markers (IHC) including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) are classically used for breast tumors sub-typing as described by Fulford et al., (2006) (12). Experiments testing these markers have been routinely carried out in pathology laboratories, with staining and evaluation protocols well established worldwide. ER is the most vital and prevalent biomarker for carcinoma classification. It was first identified within the 1960s and utilized in carcinoma clinical management. PR positive tumors comprise 65% to 75% breast cancers and several studies have suggested its clinical implications in the classification of such tumors. Dai et al., (2016) (17).

4. Phosphorylated derivatives of phosphatidylinositol, PI, the phosphoinositides, PIPs, are unique among glycerophospholipids. The inositol headgroup of PI can be reversibly phosphorylated by kinases and phosphatases which lead to the generation of seven naturally known isomers. These lipids are formed by phosphatidylinositol 3-kinases (PI3Ks) which are among the most promising drug targets in oncology (26). Hyperactivation of PI3K signaling plays a crucial role in the development of several different neoplasms including breast, prostate, colon, thyroid, and ovarian cancers. PI3K has also been shown to be mutated in various cancers which lead to constitutive activation of kinase activity as stated by Fernandis et al., (2009) (26).

Some tumor regulating functions might be controlled through interactions of sphingolipids with proteins. Targets of ceramide include protein phosphatases and kinases that regulate important signaling pathways in cancer, Hannun et al., (2008). Recently, one of the best-characterized ceramide-transfer proteins, (CERT), was implicated in cancer biology (27).

5. DNA hypomethylation is an early molecular event in carcinogenesis. Whether methylation measured in peripheral blood mononuclear cells (PBMCs) DNA may be a clinically reliable biomarker for early detection of cancer risk assessment is to be established. Friso et al., (2013) (28).

It was discovered that genomic PBMCs-DNA methylation may be a useful epigenetic biomarker for early detection and cancer risk estimation. Results: Cancer subjects had significantly lower PBMCs-DNA methylation than controls [4.39 (95% confidence intervals (CI), 4.25–4.53)]. A DNA methylation threshold of 4.74% clearly categorized patients with cancer from controls in order that those with DNA methylation but 4.74% showed an increased prevalence of cancer than those with higher levels (28).

6. Recent blood-based miRNA profiling studies, reporting their presence in serum and plasma, have generated the concept that circulating miRNAs hold much potential as novel noninvasive biomarkers for cancer and other disease processes. Heneghan et al., (2010), experimented and found the first report of circulating miRNAs in breast cancer patients and the results demonstrated that cancer-associated miRNAs in the blood can potentially serve as novel noninvasive biomarkers for breast cancer (17).

7. Ralhan et al., (2007) identified a panel of proteins by multidimensional LC-MS/MS that were differentially expressed when head and neck cancer patient samples were compared with the controls. Using this approach, a panel of three cancer protein markers that are potentially modified on their serine residues were identified that achieved remarkable sensitivity of 0.92 (18).

Patz et al., (2007), studied by combining two-dimensional difference gel electrophoresis (2D-DIGE) and Matrix-assisted laser desorption/ionization (MALDI-TOF) analysis, A panel of four proteins were able to discriminate healthy controls from patients who had lung cancer. This panel of four biomarkers included carcinoembryonic antigen, retinol-binding protein, α-1-antitrypsin, and squamous cell carcinoma antigen (29).

In a study by Wu et al., (2012), a glycoprotein binding lectin array was used to identify glycosylation changes in the sera of patients with benign diseases compared with those with stage III ovarian cancer. The studies revealed four glycoproteins, including corticosteroid-binding globulin (CBG), serum amyloid p component (SAP), complement factor B (CFAB), and histidine-rich glycoprotein (HRG) to differentiate ovarian cancer patients from benign diseases and healthy controls (29).

8. One of the most extensively studied tumor-associated autoantibodies (TAAs) is p53, a tumor suppressor protein. Autoantibodies to p53 in cancer were first reported in 1982, and since then there have been numerous reports confirming and extending this finding. The types of cellular proteins that induce autoantibody responses are quite varied and include oncogene products such as HER-2/neu, onconeural antigens, differentiation-antigens such as tyrosinase and cancer/testis antigens and anti-apoptotic proteins such as surviving, LEDGF, etc. A highly informative study showed that in lung tumors containing several types of p53 gene mutations, including missense, stop codon and frameshift mutations, only the missense p53 mutations, with overexpression of a protein that altered function and increased protein stability, correlated with autoantibody production. Certain cancer patients have an immune system capable of sensing these aberrant tumor-associated proteins as foreign antigens and have the capability to respond by producing autoantibodies. Thus, tumor-associated autoantibodies might be regarded as reporters

identifying aberrant de novo or dysregulated cellular mechanisms in tumorigenesis. Liu et al., (2010) (16).

9. For the analysis of tumor biopsies, a quantitative or semi-quantitative methodology is essential for robust DNA methylation analysis. Less sensitive methodologies may be used, as long as their dynamic range is able to span the potential range of tumor purities. Coolen et al., (2007), describes MassARRAY® methodology allows (semi) quantitative methylation detection determined from fragment masses after base-specific cleavage utilizing MALDI-TOF mass spectrometry (16). Methylation levels are determined as an average for a single CpG dinucleotide, or for multiple CpG dinucleotides across all amplicons generated during PCR amplification, while the analytical sensitivity is similar to that of bisulfite pyrosequencing (30).

Finally, amplicon-based approaches using massively parallel sequencing have been successfully applied for methylation analysis of selected loci in leukaemia and lymphoma by Taylor et al., (2001), as well as for breast cancer by Korshunova et al., (2018). This methodology offers the opportunity to analyze multiple DNA methylation biomarkers in parallel, making this approach relatively cost-effective (31).

10. The dysfunctional mitochondria have been long proposed to play a major role in tumorigenesis. Mutations In mitochondrial DNA especially the Mt DNA 4,977 bp deletion has been found in patients of various types of cancer. In order to comprehend the Mt DNA 4,977 bp deletion status in various cancer types meta-analysis was done by Hezhongrong et al.,(2013) which suggests that in the Mt DNA 4,977 bp deletion is found mostly in cancerous tissue and thus has the potential to be a biomarker for cancer occurrence in the tissue, but at the same time being selected against in various types of carcinoma tissues (32).

11. Research has shown that the frequency of chromosomal aberrations (CAs), but not of sister chromatid exchanges (SCEs), predicts cancer risk. Chromosome-type chromosomal aberrations may have a more pronounced predictive value than chromatid-type CAs. CA frequency appears to predict cancers at various sites, although there seems to be a specific association with gastrointestinal cancers. SCE frequency does not appear to have cancer predictive value, at least partly due to uncontrollable technical variation (13). Numerous genetic polymorphisms of xenobiotic metabolism, DNA repair, and folate metabolism affect the extent of CAs and might collectively contribute to the cancer predictivity of CAs. Additional factors that will influence the association between CAs and cancer include, e.g., exposure to genotoxic carcinogens and internal generation of genotoxic species. Norppa et al., (2006) described the association between CA level and cancer is seen at the group level, an association probably also exists for the individual, although it's not known if an individual approach could be feasible (13).

12. Cui et al., (2003) examined colon tissue biopsies and blood samples from 172 colonoscopy patients for loss of imprinting (LOI) in the insulin-like growth factor II (IGF2) gene. LOI is an epigenetic phenomenon in which certain genes are generally silenced during embryonic development through the addition of methyl groups. Cui and colleagues show that LOI of the IGF2 gene is associated with a family history of developing CRC and with a personal history of colon adenomas and CRC. They used a hypothesis-driven approach in which a molecule like IGF2, thought to be involved in the biology of cancer, provides the target for which a marker is developed (11).

13. Kalluri et al., (2006), studied the subsequent tumor development and progression and found that the stroma consists of the non-malignant cells of a tumor, the vasculature, and its cells, the activated fibroblasts, macrophages, and another immune. It is well known that ECM-producing cells are activated in cancer and this leads to a phenomenon known as tumor desmoplasia, which appears to be important for tumor progression (19).

The cancer cell and stroma both modulate the ECM of a tumor by secreting tumor-associated proteases, which subsequently break down proteins of the ECM such as collagens, proteoglycans, etc. This remodelling also releases substances sequestered in the ECM, such as vascular endothelial growth factor (VEGF), which further influences tumor progression. Additionally, many cleavage products from ECM proteins have properties that affect tumor progression. A well-known example of this is the anti-angiogenic activity of endostatin, tumstatin, canstatin, arresten and hexastatin—all substances cleaved from the basement membrane (BM) proteins types XVIII and IV collagens during tumor growth. Sund et al., (2009) (19).

14. A novel core technology was developed by Wei et al., (2014) involving an electric field-induced release and measurement (EFIRM), which can detect the epidermal growth factor receptor (EGFR) mutations directly in bodily fluids, including saliva. This approach is an electrochemical method based on immobilized nucleic acid probes for capturing mutated sequences and applying electric fields to facilitate the hybridization process (33).

Lau et al., (2013) provide the mechanistic and biological rationales, why the biomarkers of pancreatic cancer can be appearing in saliva. They investigated the role of pancreatic cancer-derived exosomes in salivary biomarker development by constructing a pancreatic cancer mouse model (15). Their results showed that the salivary biomarker development was disrupted by inhibiting the biogenesis of pancreatic cancer-derived exosomes (33).

LIMITATIONS AND CHALLENGES

The development of biomarkers has been established as a complicated study because of the several complications that arise due to various factors associated with the selected biomarker. The process in devising a new ideal biomarker includes a better understanding of biological heterogeneity, including host/tumor heterogeneity; analytical factors, such as interference and analytical sensitivity; clinical-pathological factors, such as current histopathologic standards; and health service and market factors (34). Technological advancements in other areas like genomics, proteomics, and bioinformatics have recently grown exponentially, helping greatly in the discovery of new biomarkers for cancer research and detection by unveiling the possible mechanism behind the progression of disease and identification of key growth regulators of the tumor (4). Yet in the past few years, only a handful of successful ideal biomarkers have been put to clinical use. Furthermore, the validation studies which are still in the experimentation stages using newly identified biomarkers in clinical trials are still to be evaluated (7).

There are a number of issues arising, such as the development of new analytical methods for cancer screening and problems such as selecting a panel of markers and how many of the markers should it contain for the specific and sensitive diagnosis of various malignancies (34). The discovered biomarkers and the ones that are in continued testing stages have a number of drawbacks. For DNA and RNA biomarkers, a whole lot of genes and their associated proteins have to be taken into consideration among which only those responsible and ideal enough are

taken as a biomarker (21). This takes a very long time and is lucrative requiring immense instrumentation and clinical studies. Moreover, in the RNA studies, due to the less stability and short lifespan of RNA particles, most of it proves to be an unreliable source of a biomarker. There are difficulties in a laboratory developing standards for implementation in a clinical setting studying the epigenetic biomarker, particularly as a major direction of this field is the early detection of cancer using methylation of circulating tumor DNA in plasma and other bodily fluids (33). Many of the sensors isolated lack specificity and are unable to perform during clinical trials. In most of the cases, a panel of biomarkers is selected as an indicative tool for cancer. In such cases, any deviation or error in the quantification of the data may lead to an improper assessment of patients (19).

Biomarkers, unlike pharmaceuticals, do not have clinical endpoints and should not be evaluated as such. If the endpoint for accurate detection of disease has not been determined, then the identification of molecular changes with sensitive assays could identify patients at risk of developing cancer, but might not be suitable for early detection. Thus, only prospective testing in patients at risk of cancer will identify the critical threshold for the accurate detection of early tumor development (35). The future of biomarkers in the clinical application requires collaboration among the traditional constituencies: the government, the pharmaceutical and biotechnology industries, and academies. There are challenges in leveraging and sharing these constituencies' unique research resources. The comparison between normal and cancer protein profiles requires tissue or bodily fluids. These tissues and fluids are precious to the biomarker scientist as they might retrospectively hold the outcome of the assays they seek to demonstrate have clinical significance (33).

CONCLUSION

Discovery and clinical use of new biomarkers are required to assume a noteworthy role in reshaping cancer research and industry, significantly impacting the discovery and treatment of numerous malignancies along with other ailments. Clinical oncology is ready to enter another period in which cancer identification, diagnosis, and treatment will be guided progressively by the molecular traits of the individual patient, obtained from various sources viz., tumor tissue, host cells/tissues that impact tumor and body liquids (6). The resultant panel of biomarkers won't just assist the recognition and analysis, but also additionally answer key inquiries regarding the biologic behavior of tumors, protection from treatment, and its effect resulting in a more specified treatment for a particular individual. The fate of cancer treatment lies in the utilization of biomarkers that offer the possibility to recognize and treat disease years before it is either obvious or indicative. Investigating the effectiveness of such markers that don't require the tumor tissue to distinguish them, however, are emitted by cancer growth cells into the circulation system will likewise be the possibility for population-based screening. Contemporary as well as upcoming genomic and proteomic technologies are quite promising in identifying new biomarkers, which can significantly enhance the efficacy of cancer management by facilitating the individualization of therapy targeting the patient-specific molecular lesions and also by providing tools for predicting/monitoring of therapeutic response (37). Although the current understanding of signalling pathways has identified specific targets for developing newer drugs and therapeutic strategies, a comprehensive understanding of how the complex signalling networks function in intact cells is still required, to evolve strategies based on the genetic alterations in individual cancers.

Future difficulties in the biomarkers utilizing genomic and proteomic innovation incorporate the advancement of complex algorithms to deal with the synchronous investigation of numerous

parameters (maybe up to thousand even) to help the determination of the ideal biomarkers rather than a single parameter. Further, issues in regards to quality examination and instrumentation techniques and systems additionally should be created for utilizing these markers with unwavering quality and reproducibility. Complete comprehension of the significance of each biomarker will be imperative to productively analyze the disease and give appropriate therapeutics alternatives likely to cure the malignancy without the follow up of any side effects.

The National Cancer Institute's Early Detection Research Network (EDRN) has started a creative, investigative initiative to improve strategies for distinguishing biomarkers of neoplastic cells. The EDRN is a consortium of 32 establishments to interface the discovery of biomarkers to the subsequent stages in the process of developing early detection steps (1). The EDRN has three fundamental parts: Biomarkers Developmental Laboratories, Biomarkers Validation Laboratories, and Clinical/Epidemiology Centres. The Biomarkers Developmental Laboratories concentrates into the determination of novel biomarkers or the refinement of existing ones; the Biomarker Validation Laboratories fill in as an EDRN asset for clinical and lab approval of biomarkers to incorporate technological changes, the normalization of measurement strategies and refinement; and the Clinical/Epidemiology Centres direct clinical and epidemiological exploration on the use of biomarkers (1).

Although ELISA and PCR remain as the gold standards for protein and nucleic acid assays in clinical diagnosis, each of them still has shortfalls for advanced diagnostic applications (36). Hence, continuous efforts have been devoted to further optimize these standard methods or to find new and better techniques for the measurement of cancer biomarkers. Advances in technology and research in genomics, proteomics, bioinformatics, and nanotechnology have improved exponentially, and numerous new biomarkers are being found (10). These new innovations will eventually prompt these as a valuable assessment tool for early detection of cancer. These have great potential in enriching the cancer diagnosis as well as new personalized treatments for various cancers. Within the discovery of novel ideal biomarkers lies the key to a better future in cancer science.

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