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REVIEW ARTICLE

Breast Cancer and various Prognostic Biomarkers for the diagnosis of the disease: A Review

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ABSTRACT:
Breast cancer starts when cells in the breast start to grow out of control. These cells often form a tumour that can be seen on an x-ray. Cancer diagnosis is currently undergoing a paradigmatic shift with the incorporation of molecular biomarkers as part of routine diagnostic panel. The molecular alterations range from those involving the DNA, RNA, microRNAs (miRNAs) and proteins. Carcinoembryonic antigen (CEA) was the first tumour marker to be used for diagnostic purposes of different human cancers (colorectal, pancreatic, breast, ovary, head and neck, bladder, kidney, and prostate) and was found to be over expressed in serum of cancer patients as compared to healthy individuals. The expression levels of miRNAs are highly tumour specific. This property of the cancer cell is being exploited in cancer diagnosis for early and accurate cancer diagnosis. Over the last few years, circulating noncoding molecules of RNA (miRNAs) are emerging as a useful class of cancer biomarkers since they are found to be aberrantly expressed in different human cancers (tissues and serum) and are characterized by unique levels of diagnostic specificity and sensitivity.

KEYWORDS: Breast cancer, biomarkers, miRNAs, CA15-3

INTRODUCTION:

Breast cancer is one where cells in the breast begin to grow out of control and it is considered as the second largest cancer.[1] These uncontrolled cells often form a tumour that can be seen on an x-ray or felt as a mass. The tumour is malignant (cancerous) if the cells can grow into (invade) and penetrate surrounding tissues or metastasize to distant areas of the body. Breast cancer commonly occurs in women, but it can also occur in men. Cells in almost any part of the body can become cancerous, and can metastasize to other areas of the body. Breast cancer originates from different parts of the breast. Most breast cancers start in the ducts that carry milk to the nipple (ductal cancers). Some begin in the glands that make breast milk (lobular cancers). There are also other types of breast cancer that are not very common.

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A small number of other cancers start in other tissues in the breast. These cancers have come to be known as sarcomas and lymphomas and are not really considered to be breast cancers. Currently, the most common approaches for treating human breast cancer include surgery, radiotherapy, hyperthermia, hormone therapy, and chemotherapy.[2]

Breast cancers can be classified by various stages, pathology, grade, and expression of estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor (Her2/neu).[3] MDA-MB-231 and MDAMB-468 are the two types of breast cancer cells that have gained interest among the investigators and researchers in medical research laboratories. MDA-MB-231 cells is characterized as ER-, PR-, and Her2/neu-negative/basal-B mammary carcinoma, while MDA-MB-468 cells is characterized as ER-, PR-, and Her2/neu-negative/basal-A mammary carcinoma [3]. MDA-MB-231 and MDA-MB-468 cells are derived from the pleural effusions of 51-year-old female patients.

MDA-MB-231 cells are derived from a Caucasian female, while MDA-MB-468 cells are derived from an African American female.[4, 5, 6]

Biomarkers and the necessity for their need:

Cancer diagnosis is currently undergoing a paradigmatic shift with the introduction of molecular biomarkers as part of routine diagnosis. The molecular aberration ranges from those involving the DNA, RNA, microRNAs (miRNAs) and proteins.[7] Every cell type has a unique molecular feature, referred to as biomarkers, which are estimable characteristics such as levels or activities (the abilities of genes or proteins to accurately perform their functions) of a multitude of genes, proteins or other molecular aspects. Biomarkers are therefore, an objective measure or estimation of normal biological processes, pathologic processes, or pharmacological responses to a therapy. This includes all the diagnostic tests, imaging technologies, and other objective measures of a person's health condition. Biomarkers are subject to active modulation, and are expected to augment our understanding of drug metabolism, drug action, efficacy, and safety. According to the National Cancer Institute, a biomarker is "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease, "(NCI) such as cancer. Biomarkers help us typically distinguish an affected patient from a person without the disease. The alterations can be due to a plethora of factors like, germline or somatic mutations, transcriptional and posttranslational modifications.

Uses of biomarkers in diagnosis:

Biomarkers can be used for patient assessments in multiple clinical settings, for estimating risk of disease, screening for occult primary cancers, differentiating benign from malignant tumours or one type of malignancy from the other, determining prognosis for patients who have been diagnosed with cancer, and monitoring the status of the disease, either to detect recurrence or to determine response or progression to therapy. Biomarkers for determining an individual's risk of developing cancer have also been identified. For example, a woman with a strong familial history of ovarian cancer can undergo genetic testing to find out if she is a carrier of a germline mutation, such as BRCA1, that will increase her risk of developing breast and/or ovarian cancer.[8] Alterations mainly in three main classes of genes *viz.*, (proto) oncogenes, tumour suppressor genes and DNA repair genes contribute to the development of cancer genotype and phenotype that resists the natural and inherent death mechanism(s) embedded in cells, coupled with dysregulation of cell proliferation events. There is increasing evidence to suggest that cancer could also be caused by 'epigenetic changes' like DNA methylation and altered patterns of histone modifications, leading to changes in chromatin condensation status thereby regulating expression of certain set of specific genes.[9, 10] Technologies to recognize and understand the characteristics of normal cells and how these become cancerous, promises to provide important insights into the etiology of cancer, that can prove to be useful for early detection, diagnosis, and treatment. Biomarkers are therefore invaluable tools for cancer detection, diagnosis, patient prognosis and treatment and therapy selection. These can also be used to localize and pin-point the tumour and determine its stage, subtype, and response to therapy. Diagnostic and prognostic biomarkers are quantifiable and measurable traits that help oncologists at the first interaction with the suspected patients. These particularly help in (*i*) identifying who is at risk, (*ii*) diagnose at an early stage, (*iii*) select the best treatment and therapeutic strategy, and (*iv*) monitor response to treatment.[11]

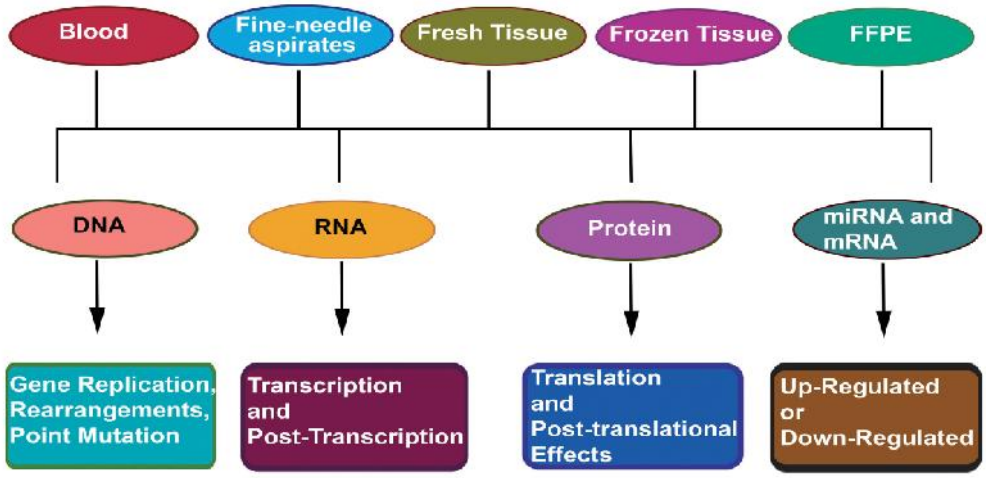

Figure 1. Schematic diagram representing routine biological specimens and their molecular alterations at the molecular diagnostic level.

Molecular alterations as part of cancer diagnosis:
Current context of the molecular aberrations that have been used for cancer diagnosis are occurring at the DNA level and include gene replication, rearrangements/translocations, point mutations/deletions or insertions.[12] At the RNA level, the changes are seen at the transcriptional and post-transcriptional modification[12, 13] stages and at the protein level, it is seen at the translational and post-translational stages as shown in Figure 1.

Biomarkers in the diagnosis of breast cancer:
CEA:
Carcinoembryonic antigen (CEA) is the first tumour marker[14] to be used for diagnostic purposes of different human cancers (colorectal, pancreas[15], breast, ovary, head and neck, bladder[16], kidney, and prostate) and was found to be over expressed in serum of cancer patients as compared to healthy individuals. Some studies showed that CEA measurement was not useful for screening or for diagnosis of early Breast cancer (BC) since it lacked sensitivity and specificity to reliably differentiate patients with early BC from those with benign disease or those with no disease. However, in case of symptomatic BC patients CEA sensitivity increases, and some scientists have suggested that CEA levels at diagnosis can be correlated with the phase of the disease.

CA15-3:
CA15-3 is the soluble form of MUC-1 protein, which is a large type I transmembrane glycoprotein. It develops to be a marker for individualizing therapy in breast cancer patients, where patients with high CA 15-3 show good response to aggressive treatments.[17] Serum CA15-3 is used as a surrogate marker of disease bulk to monitor metastatic breast cancer patients undergoing treatment and for the preclinical detection of tumour recurrence. Elevated levels of this antigen are found mainly in breast cancer which is involved in metastasis.[18] The soluble form of MUC-1 (CA15-3) was identified as a more specific marker for breast cancer with respect to CEA. But, this marker showed low sensitivity and specificity for the detection of BC, since its sensitivity is 10–15%, 20–25%, and 30–35% for stages I, II, and III, respectively. Therefore, the estimation of CA15-3 in BC patients is not recommended. When it comes to CEA, the increasing levels of CA15-3 may be useful to detect patients with advanced disease. In fact, the simultaneous positivity of both markers allows for early diagnosis of metastases in up to 60–80% of patients in the advanced disease stages.

HER-2:
The discovery of human epidermal growth factor receptor 2 (HER-2; also known as ERBB2) is considered to be a milestone in cancer research.[19, 20] After its discovery, HER-2 gene was found to be amplified (in greater numbers) in different number of epithelial cancers, and its protein over expression is associated with central tumour cell proliferation and survival pathways. HER2 is a member of a receptor family called ERBB tyrosine kinase which includes ERBB1 (EGFR), ERBB3 (HER3), and ERBB4 (HER4). The HER2 receptor is a type 1 transmembrane protein containing 1233aa with an extracellular domain comprised 630aa containing seven potential N-linked glycosylation sites, a transmembrane region of 23aa, and a cytoplasmatic portion of 580aa with a tyrosine-kinase containing domain.[20] In the last ten years, particular attention has been given to the detection of the soluble form of HER-2 in serum from BC patients. As demonstrated by several *in vitro* and *in vivo* experiments, the ectodomain of HER-2 can be cleaved proteolytically from the intact receptor and released as a soluble molecule (s-HER-2).[21, 22, 23] In disease-free healthy individuals, low concentrations of s-HER-2 can be detected in serum. However, in some BC patients, s-HER2 levels are increased with respect to the tumour load and HER- 2 status.[24] A human HER2- over expressing and PIK3CA-mutant breast cancer transgenic animal was developed and these tumors showed elevated transcripts encoding markers for EMT and the stem cell phenotype. These tumors was consistent with the claudin-low subtype and these tumors were able to produce lung metastasis and were resistant to trastuzumab alone or in combination with lapatinib or pertuzumab.[25]

Cytokeratins:
Cytokeratins (CKs) are a class of intermediate filaments primarily involved in cytoskeletal organization of epithelial cells for the fixation of the nucleus and the maintenance of cellular morphology and structure for cell protection from mechanical and non-mechanical stress.[26] Keratins have for so long now been extensively used as immuno-histochemical markers in diagnostic tumour pathology. Most cancers of glandular epithelial origin, including BC, express CK8, CK18, and CK19 as specific cancer tissue biomarkers.[27] In case of hormonally responsive BC, it has been suggested that CK18 has a regulatory role as it effectively associates with and separates the estrogen receptor-alpha (ER-α) target gene and ERαcoactivator LRP16 in the cytoplasm, thus decreasing ERα-mediated signaling and estrogen-stimulated cell cycle progression in BC cells.[28]

The miRNAs :
The expression levels of miRNAs are predominantly tumour specific. This property of the cancer cell is being exploited in cancer diagnosis for early and precise cancer diagnosis.[29] Depending on their downstream signalling effect on genes and gene products, miRNAs may either be up or down-regulated in cancers.[30] The ones which

are up-regulated in cancers are proposed to have an oncogenic potential. The classical examples of miRNAs with an oncogenic potential include miR-155, miR-17-92 and miR-21 and many others. The miR-21 has been found to be over-expressed in several malignancies.[31, 32]

Other possible biomarkers for breast cancer:

Circulating tumour cells (CTCs):

CTCs are simple yet powerful biomarkers in the field of oncology. The presence of CTCs has been shown to predict survival rates in patients with metastatic breast cancer at multiple time points throughout the course of therapy.[33] CTCs provide an early and reliable indication of disease progression and survival rates for patients on systemic therapy for metastatic breast cancer. Elevated CTCs at any time during therapy is a reliable indicator of progression.[34]

T-regulatory cells (CD4+, CD25+ and Foxp3+):

Regulatory T cells (T-regs) are important for inducing and maintaining peripheral self-tolerance and preventing immune pathologies.[35, 36] These are subpopulations of CD4 cells, characterized by high CD25 expression along with Fox P3. They are thought to play a significant role in controlling both innate and acquired immune responses.[37] Furthermore, studies in cancer patients have suggested that increased T-regs activity may be associated with poor immune responses to tumour antigens and contribute to immune dysfunction resulting in tumour 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 tumour itself.

Cancer stem cells (CSCs):

It has long been established that subpopulations of cancer cells exist within the tumours that resemble the developmental hierarchy of the normal tissue from which the tumour arose or originated. Over the past few years, the cancer stem cell model of tumourigenesis has received increasing attention. Evidence for the existence of CSCs initially came from studies of acute myelogenous leukemia (AML). Presence of CSCs have now been demonstrated in many solid tumours, including glioblastoma, medulloblastoma, breast cancer, melanoma, and prostate cancer.[38] It appears that identifying and characterizing CSCs for every possible tumour is of paramount importance and will likely lead to new therapeutic avenues.

Cancer antigens (biomolecules) based biomarkers:

The cancer proteome contains information on almost every biological process that takes place inside cancer cells, cancer tissue microenvironment, and cancer cell-host interaction.[34] Cancer cells release many proteins and other macromolecules into the extra-cellular fluid through secretion that can also serve as potential biomarkers. Some of these products end up in the bloodstream and hence serve as potential serum biomarkers.

Table 1: Cancer biomarkers for diagnosis and prognosis of breast cancer

Biomarker	Tumour	Application	Sample type/method of detection
Cancer antigen 15-3 (CA15-3)	Breast cancer	Diagnostic and prognostic	Serum/ ELISA, Lymph node/ IHC, Bone marrow/ IHC
BRCA-1, BRCA-2	Breast cancer	Diagnostic	Tumour samples/ RT-PCR
Metabolic biomarker: Glucose metabolism	All cancers, general	Daignostic, prognostic and therapeutic	Imaging/ FDG-PET scan
Cells as biomarker: Circulating tumour cells (CTCs)	Metastatic breast cancer	Diagnostic and prognostic	Blood/ Immunocytometry

Mitochondrial markers:

Mitochondria contain multiple haploid copies of their own genome (16.5 kb), including most components of transcription, translation, and protein assembly. mtDNA is present at 1000-10, 000 copies/cell, and the vast majority of these copies are identical (homoplasmic) at birth. Several mutations in the mtDNA, specifically in the D-loop region have been recently found in breast, colon, oesophageal, endometrial, head and neck, liver, kidney, leukemia, lung, melanoma, oral, prostate, and thyroid cancer.[39] The majority of these somatic mutations are homoplasmic in nature, suggesting that the mutant mtDNA plays an active role in tumour formation.[40] By virtue of their clonal nature and high copy numbers in cancer cells, mitochondrial mutations may prove to be a powerful molecular marker for non-invasive detection of cancer. It may also prove to be useful in early detection, diagnosis, and prognosis of cancer outcome and/or in monitoring response to certain preventive and interventional modalities as well as therapies.[41, 42]

Metabolic biomarker (glucose metabolism):

Increased glucose utilization is a prominent and fundamental change in many tumours irrespective of their histological origin and the nature of mutations. Mechanisms underlying this fundamental alteration in metabolism during carcinogenesis include mutations in the mitochondrial DNA resulting in functional impairment, oncogenic transformation linked upregulation of glycolysis, enhanced expression of metabolic enzymes and adaptation to the hypoxic tumour

micro-milieu in case of solid tumours.[43] Based on these observations, a bio-energetic index of the cell (BEC index) has been suggested that could be used for classification and prognosis of cancers, besides predicting the response to therapy. The extent of increase in glucose utilization measured by FDG-PET has been linked with the degree of malignancy in some of the tumours.[44]

Monitoring response to therapy in advanced BC:

The actual ASCO and ESMO guidelines do not suggest the use of tumour markers for monitoring BC patients during followup, and both confirm that they should only be used for advanced BC therapy monitoring, especially in cases where cancer lesions response to therapy are not clinically assessable. Conversely, the European Group for Tumour Markers (EGTM) in agreement with the National Academy of Clinical Biochemistry (NACB) proposes that serial evaluation of tumour markers levels is important for BC patient monitoring in order to get an early diagnosis of recurrence, since tumour markers rising often precede clinical or radiological signs of the disease. Finally, the American College of Radiology (ACR) and the European Association for Nuclear Medicine (EANM) suggested that tumour markers increasing during follow up may be an early warning that can highlight those patients needing molecular imaging investigations. CA15-3 proves to be a good serum tumour marker for those BC patients needing accurate molecular imaging investigations (PETCT) during follow up.

CONCLUSIONS AND FUTURE PERSPECTIVES:

The current routinely used serum tumour markers have limited usefulness for diagnosis and/or screening of BC due to their very low and less reliable sensitivity and specificity as well as to the fact that they can be raised also in case of some benign conditions. For example, benign breast tumour may be associated with CA15-3 rising, while other conditions such as inflammatory bowel disease, pancreatitis, and gastritis may also cause CEA elevation. Tumour marker level measurement at diagnosis may only be useful to identify those patients with advanced BC and then at risk to have liver involvement; however, it is not excluded that metastatic BC cases may present with normal serum concentrations.CA15-3 as well as other established biomarkers do not fulfil the features of an ideal biomarker especially in terms of diagnostic sensitivity and specificity. On the basis of these diagnostic gaps, many research groups are conducting studies aimed at identifying new biomarkers to diagnose BC at an early stage using minimally invasive approaches. In particular, during the last few years, circulating noncoding molecules of RNA (miRNAs) are emerging as a novel class of cancer biomarkers since they are found aberrantly expressed in different human cancers (tissues and serum) and are characterized by unprecedented levels of diagnostic specificity and sensitivity.

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