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Emerging Diagnostic and Prognostic Biomarkers of Triple Negative Breast Cancer

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Abstract

Triple negative breast cancer is one the most frequently diagnosed and major causes of cancer deaths among young women. Generally, mammography is used to detect breast cancer at an early stage. Magnetic resonance imaging (MRI) in addition to mammography is recommended for annual screening. Breast ultrasound is sometimes used to evaluate abnormal findings. However, these methods increase the likelihood of false-positive results. TNBC cells express or release some molecules into the blood stream and other body fluids at quite low level and their levels increase with cancer progression. The present review is aimed to update the currently available conventional and emerging biomarkers, and presenting the most effective biomarkers for early diagnosis and prognosis of TNBC.

Keywords: Breast Cancer; Mammography; MRI; Ultrasound; Biomarkers

Introduction

Breast cancer is the most common cancer and major cause of cancer related deaths among women [1]. Worldwide, over 1.3 million cases of breast cancer are diagnosed, and annually more than 4.5 lakhs women die from breast cancer. In the developed countries the mortality rate of breast cancer has declined since 1990 [2] due to early detection, screening and improved adjuvant therapy [3,4]. Unfortunately, breast cancer morbidity among women remains an alarming concern in developing countries and accounts for 15% of all cancer deaths [2]. However, the survival rate of breast cancer patients can be enhanced by early detection of cancer through effective prognostic markers [5].

Clinically, breast cancer has distinct subtypes, which need unique prognostic markers for therapeutic implications. Majority of breast cancers (70-80%) are estrogen (ER) or progesterone receptors (PR) positive. Approximately, 15–20% of the breast cancers over express human epidermal growth factor receptor (HER2) protein and/or amplified HER2 gene. Around half of breast cancers co-express hormone receptors [6]. The remaining 10–15% of breast cancers are negative for ER, PR and HER2, defined as triple negative breast cancer (TNBC) [7]. The breast cancer patients are routinely evaluated in terms of expression of estrogen receptor (ER), progesterone receptor (PR), and amplification of HER-2/Neu [8]. Mammography is the primary imaging modality for breast cancer screening and diagnosis [9]. Women at high risk of breast cancer are recommended for annual screening using magnetic resonance imaging (MRI) after 30 years [10]. Breast ultrasound is sometimes used to detect abnormal findings from a screening or diagnostic mammogram or physical exam [11].

TNBCs have high histopathological grades and high risk of invasion with early metastases and short survival. It is associated with early recurrence of disease and poor outcome. TNBC is clinically diagnosed based on immuno histo chemistry (IHC) [12]. Even though ultrasound and mammographic methods can reveal the smooth borders of TNBC tumors, they do not always efficiently image necrosis and fibrosis, which are typical of TNBC [13]. Research on biomarker investigation for early detection, prognosis and the prediction of treatment responses in TNBC is rapidly expanding. However, effective biomarkers for use in routine clinical practice, and detection and management of TNBCs are not yet reported [14]. For early diagnosis of TNBC, there is a demanding need for specific biomarkers. Main objective of this review is to update the currently available conventional and emerging biomarkers, and presenting the most effective biomarkers for early detection prognosis of TNBC. TNBC is characterized by the marked expression of certain biomarkers. The presence of these molecules is not restricted to TNBC but show increased expression in this subgroup.

The following are the important biomarkers in TNBC.

Estrogen and progesterone receptors (ER and PR)

Breast cancers with different clinical, pathological, and molecular characteristics express ER and PR. Etiology of breast cancers negative for ER and PR are independent of hormone

exposure [15]. Estrogen mediates its functions through two specific intracellular receptors, the ERα and the ERβ, which acts as hormone-dependent transcriptional regulators. The ER pathway plays a critical role in the patho physiology of human breast cancer. Over expression of the PR serves as a functional assay because it indicates that the ER pathway is intact, even if the tumor is reported as ER-negative. Breast cancer cells tested negative for hormone receptors can contribute valuable information for treatment plan.

Human epidermal growth factor receptors (HER)

The most frequently implicated receptors and growth factors in TNBC are members of the EGFR subfamily of tyrosine kinase receptors. The type-1 subfamily includes HER-2, HER-3 and HER-4. All receptors possess a large glycosylated extracellular ligand-binding domain, a single hydrophobic transmembrane domain, and a cytoplasmic tyrosine kinase domain. HER-2, also known as c-erbB-2 or neu, is a proto-oncogene that encodes a 185-kDa tyrosine kinase glycoprotein. It is structurally related to epidermal growth factor receptor (EGFR), encoded by EGFR/HER2 oncogene located on chromosome 17q21. Amplification of HER-2 gene plays an important role in the pathogenesis of breast cancer. Around 60% of ductal carcinoma in situ and 20-30% of infiltrating breast carcinomas are reported to over express the HER-2 protein [15]. The presence or absence of HER2 is an essential step in the diagnosis [16].

Breast cancer susceptibility genes (BRCA1 and BRCA2)

The BRCA genes are tumor-suppressor genes and the loss of wild-type allele has been observed in tumors of heterozygous carriers. Approximately 80% of familial breast cancers are associates with breast cancer susceptibility genes, BRCA1 and BRCA2. Germ line mutations in the BRCA1 and BRCA2 genes have found to be associated with up to 15% of TNBC. TNBC accounts for 70% of breast tumors arising in BRCA1 mutation carriers and 16-23% of breast tumors in BRCA2 carriers [17]. These are high-penetrance breast cancer susceptibility genes [18]. BRCA1 gene is located on the long arm of the 17th chromosome, 17q21. This gene encodes a protein related to many nuclear processes including transcription, chromatin remodelling, gene silencing and DNA repair. BRCA2 gene is located on the long arm of the 13th chromosome at position 13q 12.3. Most of cancers caused by hereditary mutations involve BRCA1 and the close homologous BRCA2 gene. BRCA2 protein play important role in different cellular processes, including activation and transcriptional regulation, repair of DNA damage, cellular proliferation, and differentiation. Women carrying mutations in BRCA1 or BRCA2 genes are at high risk of breast cancer development [19]. Therefore, young women with a high-grade TNBC and no family history of cancer should undergo BRCA genetic testing [20].

Carbohydrate 15-3 and carcinoembryonic antigen (CA 15-3 and CEA)

Cancer antigen 15-3 (CA 15-3) and carcinoembryonic antigen (CEA), are often used to follow up care of breast cancer and provide important clues to the clinicians about disease progression in metastatic and recurrent breast cancer. CA 15-3 along with CEA is a relevant tumor marker for breast cancer [21]. CEA concentrations greater than 7.5μg/l are associated with high probability of subclinical metastases. Prognosis of patients having CEA levels at normal range at the time of diagnosis is significantly better than those with elevated CEA levels. Monitoring of breast cancer patients after surgical treatment using only this marker is insufficient. However, simultaneous use of both serum markers (CA 15-3 and CEA) allows the early diagnosis of metastasis in up to 60-80% of patients with breast cancer [22].

Human mammaglobin (hMAG)

Mammaglobin, known for its mammary tissue specificity, has been considered a promising diagnostic marker in breast cancer for almost 10 years. Human MAG is belongs to the uteroglobin/Clara cell protein family of small epithelial secretory proteins, the secretoglobins. This serological marker in breast cancer appears to reside in the sequences and framework of the hMAG-lipophilin complex. Watson and Fleming reported that mammaglobin expression was 10-fold more in 91% of the breast cancer cases, independent of stage and histological type. Mammaglobin was detected at high levels in primary breast cancers. This is not detectable in non-breast tumors or present at low levels in healthy breast tissue, thus making it a suitable candidate for diagnosis of breast cancer. MAG-A positive expression by IHC staining was found in approximately 90% of invasive ductal carcinoma and in 80% of intraductal carcinoma. Hence, the usefulness of hMAG in diagnosis stems from its abundant detection in breast tumors, the low existence in tissues and tumors other than breast, and its efficiency in detecting residual disease and predicting recurrence.

Proliferating cell nuclear antigen (PCNA)

PNCA is a non-histone nuclear protein, forming homotrimeric ring encircling DNA double helix. It acts as a molecular platform to recruit proteins involved in DNA synthesis, cell-cycle control, DNA damage response and repair. This protein is correlate with mitotic activity and tumor grade. Its role in signal transduction has an important impact on growth regulation of breast cancer cells and also associates with poor overall survival. Based on these characteristic, Zhao et al. [23] suggested PCNA as a marker for TNBC [23].

Ki 67 antigen

It is a nuclear antigen found in the proliferative phases of the cell cycle but not in the resting phase. Ki-67 score is most frequently measured on histological sections by IHC methodology and used to evaluate invasive carcinomas. According to St. Gallen consensus, the proliferative index is considered low or negative, when there is 14% or less stained nuclei and positive or high, when there is more than 14% of stained nuclei. Patients, over expressed Ki67 in more than 50% of the cells are at high risk of developing recurrent disease. The prognostic and predictive value of ki-67 was evaluated and considered as a prognostic factor for therapeutic decision [24]. Ki67 levels was significantly high in TNBC (80%) compared to other histologic types. Moreover, its expression has direct correlation with tumor size and grade in TNBC patients. High expression levels of Ki67 (> 35% staining) was linked with an increased risk of death

[25]. Ki-67 can be used for further classification of TNBC into two subtypes with different response and prognosis [26]. In TNBC patients, accumulation of Ki67 was associated with a higher pCR to chemotherapy. Its expression was also used for further subdivision of TNBC into two subtypes where only 26.7% of TNBC patients showed lower Ki67 expression

Growth factors (EGF, HGF, IGF, VEGF and TGF-β)

The role of growth factors has been extensively analyzed both in cancer risk and tumor progression. A negative correlation was found between insulin-like growth factor 1 (IGF1) and severity of breast cancer. The IGF1 and EGF serve as a stimuli for metastasis, and have been implicated in the development and progression of human breast carcinoma. The hepatocyte growth factor (HGF) is considered as a progression and aggressiveness marker of breast cancer. This marker could potentially be used as an additional tool for the differentiation between benign and malignant tumor. Stromal VEGF-A expression is a valuable prognostic indicator of breast cancer-specific and disease free survival at diagnosis. Over expression of TGF-β in both tumor and stromal tissue facilitate the development of metastasis. According to Sheen-Chen et al. high TGF-β1 level in serum has been associated with advanced stages of breast cancer [27,28].

MYC

The MYC proto-oncogene family comprises of c-myc, N-myc, L-myc. Extensive research has been performed to illuminate role of this group of genes in context of several biological processes. This gene has an important role in carcinogenesis and tumor replication, growth, metabolism, differentiation, and apoptosis. Amplification of MYC has been reported in breast cancer as well as in many other cancers. Several converging studies have suggested that MYC may play an important function in breast cancer. A recent literature reported that TNBC tumors exhibit elevated MYC expression and increased activity of the MYC pathway. They showed that CDK inhibition effectively induced tumor regression, indicating that aggressive breast tumors with elevated MYC expression are uniquely sensitive to CDK inhibitors. Thus, expression of MYC is significantly different in breast cancer patients and healthy controls. Regardless of lack of evidence for the prognostic significance of MYC amplification, it could represent a clinically useful predictive parameter in TNBC [29].

Urokinase-type plasminogen activator (uPA)

It is a serine protease that plays an important role in the invasion and metastasis process through degradation of the extracellular matrix. High levels of tissue uPA and its inhibitors (plasminogen activator inhibitor (PAL-1, PAL-2) has been correlated with poor outcome in TNBC. Patients whose primary tumors had low levels of uPA and PAL-1 have low risk and with elevated levels have high risk [29].

Lipid rafts

Caveolae are special molecules found in the majority of mammalian cells and invaginated microdomains of the plasma membrane. They serve as a membrane organizing centres. The expression of CAV1 and CAV2 was reported to has association with histological grade and hormone receptors status in basal-like breast cancer subtype [19]. This provide evidence that these proteins can be potential markers.

Tetraspanin CD151

CD151 is a member of the mammalian tetraspanin super family, involved in fertilization, infectious processes and tumour progression [30,31]. CD151 contributes to integrin-dependent cell adhesion and motility by interacting with laminin-binding integrins [31]. According to Yang et al. [31], CD151 expression was elevated in breast cancer, even further up regulated in high-grade and oestrogen-negative subtype, basal-like breast cancer [32]. Moreover, it was also demonstrated that deletion of CD151 decreased the integrin-mediated cell migration, spreading, invasion and signalling of basal-like breast mammary cells [33]. Therefore, CD151 may have potential role for diagnosis of breast cancer.

Exosomes

Exosomes are membrane-structured nano-vesicles playing role in cell-cell communications and regulating diverse biological processes in TNBC. Tumor-derived exosomes accumulate in blood and enrich with selective repertoire of signaling proteins, miRNAs and mRNAs. Researchers have reported surface markers that distinguish tumour-derived exosomes from normal one, which provides the specificity of exosome in diagnosis. Probing exosomes is emerging as a new paradigm for non-invasive diagnosis and monitoring of treatment response. The studies on exosomes have been started since several decades, however the utilization of exosomes in the diagnosis is a relatively new field [34]. Recently, researchers have found some molecules in exosomes with great potential to utilize as a platform for personalized diagnostics. Some of these associated with TNBC prognosis also exploited as biomarkers of personalized diagnostics [35].

Micro RNAs (miR)

miRs are approximately 22-nucleotide non-coding RNAs that are thought to regulate gene expression through sequence-specific base-pairing with target mRNAs [35]. Initially, microRNAs are discovered in *Caenorhabditis elegans* as control of developmental stages [36-38]. Studies over decade have shown that miRNAs are deregulated in human breast cancer [39]. Another study reported that miRNAs were differentially expressed in breast tumor and correlate with HER2 and estrogen receptor (ER) status [40]. miRNAs can modulate oncogenic or tumor suppressor pathways, including p53, c-MYC, RAS and BCR-ABL. However, expression of miRNAs themselves can be regulated by oncogenes or tumor suppressors. Wu et al. detected more than 800 miRNAs in the circulation of breast cancer patients [41]. miR-375 and miR-122 exhibited strong correlation with clinical outcome, including neoadjuvant chemotherapy response and metastatic relapse. Wang et al. [42] demonstrated that miR-122 act as a tumor suppressor and plays an important role in inhibiting tumorigenesis [42]. TNBC patients with elevated expression of miR-497 have better prognosis, and this marker may turn out to be a new prognostic marker. Mir-373 and miR-520c stimulate cancer cell migration and invasion in

vitro and in vivo. Many studies have demonstrated the potential of miRNAs, as regulators, and they may serve as novel diagnostic and prognostic candidates and potential therapeutic targets.

Conclusion

TNBC is the most poorly understood and is refractory to current targeted therapies. It is a cause of significant breast cancer mortality because of very few treatment options. Biomarker may be useful as prognostic or predictive indicators as well as suggest possible targets for novel therapies. Though a specific biomarker for TNBC has not yet found but assessing certain parameters like exosomes, CD151, uPA, BRCA1 & 2, Ki76 and ER, PR, HER can give an indication of TNBC. However, a negative value or normal values doesn't rule out the possibilities for the breast cancer, so as for detecting early breast cancer, some unconventional markers needed to be assayed such as micro RNAs. Though, these markers still under research can give a proper indication of TNBC.

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