

PROGNOSTIC BIOMARKERS IN TRIPLE NEGATIVE BREAST CANCER AS A POTENTIAL TARGET FOR FUTURE DRUG DISCOVERY

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ABSTRACT

Breast cancer is the most common malignancy in women globally, in which triple-negative breast cancer (TNBC) is more aggressive with poor prognosis and very less response to targeted hormone based treatment. It is a major cause of deaths among the women with breast cancer because of very few treatment options. The biomarkers could be a product of cancerous cell or molecule generated in response to cancer. It is used to understand the mechanism, prognosis, diagnosis as well as target for design and discovery of new drugs. The purpose of the study is to give a brief review on markers of TNBC.

Keywords: Breast cancer, Triple-negative breast cancer, Biomarkers, Drug discovery, Diagnosis.

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INTRODUCTION

Breast cancer is one of the most common malignant tumors among women, and it is a heterogeneous type of disease because of their therapy response. We can define its molecular subtypes by immunohistochemistry (IHC). Triple-negative breast cancer (TNBC) is one of the molecular subtypes of breast carcinoma represents by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER 2). TNBC accounts for about 12.5-15% of all breast cancers [1]. TN breast carcinoma is more frequent in younger age (<50 years) with more aggressiveness and poorer prognosis. These are more prevalent in the African-American population [2]. They need typical and differential molecular profile and distinct patterns of metastasis [3]. They metastasize commonly with lungs, central nervous system, and liver. TNBC varies 16-42% in BRCA mutation [4,5]. TNBC can be further defined by two groups, i.e., basal-like (BL) and non-BL. Although all BL tumors are not triple negative [6]. However, in TN disease, all intrinsic molecular subtypes can be identified and majority tends to BL subtype (86%, depending from the various other studies done by Researchers) [7]. Initially, on the basis of hierarchical clustering, identified four intrinsic subtypes of breast cancers (BL, HER 2 enriched, luminal, and normal breast-like), which exhibits gene expression patterns. Subsequently, luminal breast cancer leads to sub-stratified into luminal A and luminal B, which expressed significantly in the classification system for prognosis [8-10]. For TNBC, there is no standard chemotherapy till date. BL-TNBC accounts up to approximately 25-80% of all TNBC patients [11]. The above data have been taken by IHC profiling for epidermal growth factor receptor (EGFR), CK 5/6 +, ER-, HER 2 -. BL-TNBC can be further separated into two subgroups, BL1 and BL2 [12]. BL-TNBC exhibits the highest pathologic complete response (pCR) rates following chemotherapy [13]. However, various trials are going on and have been conducted in unselected patient's populations. This review explores the biological features and biomarker expression profile of TNBC. In this article, we focused to understand and classified the TNBC based on similar gene expression, biological functions, and their clinical outcomes. The purpose of this study is to revise the specific therapeutic strategies, on the basis of gene profile having different for each specific TNBC subtypes. Hence, anti-EGFR, anti-vascular endothelial growth factor (VEGF), anti-p53, and anti-ki67, the prognostic biomarkers may provide a potential treatment option for TNBC.

Selection criteria and search

We conducted a PubMed, Medline search till July 2016, using the term "TNBC," "Gene expression profiling," "EGFR," "VEGF," "ki67," "p53," and "breast cancer" followed by "AND/OR" without any date restriction (Table 1). Publications written in other than the English language were excluded. We cross-referred the information obtained to identify clinically effective TNBC groupings with similar gene expression, biological functions, and clinical outcomes.

EGFR

Human EGFR (also known as HER 1/Erb 1) and its relative three other genes human EGFR 2, 3, and 4 (HER 2/Erb 2, also known as the neu oncogene, HER 3/Erb 3, and HER4/Erb 4) plays a major role in the control of cell growth and proliferation [14,15]. The defect in these receptors like genetic abnormalities leads to major defects in cancer cells [16]. Truly EGFR is the first epidermal receptor which recognizes as an oncogene [17,18]. EGFR is a 1,70,000 membranes bound tyrosine kinase. The protein product of EGFR plays a very major role in cell proliferation, protection against apoptosis and migration in activation of intracellular mediated pathways [19]. EGFR family inhibitors lead to two major classes: Monoclonal antibody that targets the extracellular module of this receptor: And other is small molecules that target the intracellular tyrosine kinase domain. Some FDA approved antibodies, cetuximab, and panitumumab target EGFR, while pertuzumab and trastuzumab target HER 2 [20].

EGFR highly overexpressed in majority of BL TN tumors [21]. BL TN tumors have seeking attention due to recognition as a distinct entity and due to poor prognosis that the diagnosis indicates. Some of the studies demonstrated TN tumors have a good response to an adjuvant anthracycline- based chemotherapy [22]. However, various clinical trials are still going on to target the possible therapeutics for TN tumors. Targeted anti-EGFR antibodies (e.g., cetuximab) and EGFR tyrosine kinase inhibitors (e.g., gefitinib) can provide a possible therapy modality [23,24]. As the outcome, the combination of cetuximab and gefitinib, give more promising result while did not showed the appropriate response by independent agent as compared to a study done [25]. In a cohort of unselected patients, EGFR-TKIs given as monotherapy did not provide any clinical benefit. A study showed the contradictory result, as indicates the inactivation of EGFR pathway

Table 1: PubMed literature search

Term searched classified based on immunohistochemistry	Breast cancer OR
Inclusion	Triple negative breast cancer OR basal-like breast cancer AND
Limitations (exclusion)	IHC OR IHC AND
Language	Biomolecular markers OR molecular subtype OR ER -ve AND PR -ve AND
Time period	HER 2 neu -ve Humans only Abstract and full text Editorial News Case reports Animals Plants English only Till July 2016

IHC: Immunohistochemistry

Table 2: EGFR expression in TNBC (modified from Yadav et al. 2015)

Total number	TNBC (n)	EGFR expression (%)	Reference
-	653	30	Teng et al. 2011
200	198	91	Yue et al. 2015
7048	767	30	Thike et al. 2010
683	136	7.4	Patil et al. 2011
-	21 BLBC	57	Nielsen et al. 2007
1726	282	37	Rakha et al. 2007
1132	103	23.3	Mehdizadeh et al. 2102
564	48	41	Ryden et al. 2010

TNBC: Triple-negative breast cancer

Table 3: VEGF expression in TNBC

Total number	TNBC	VEGFR-2 in TNBC (%)	Reference
96	43	90.5	Abeer Bahhnassy et al. 2015
1132	103	93.2	Mehdizadeh et al. 2012
-	73	77	Iosifidou et al. 2009
70	27	54	Chanana et al. 2012
69	35	34	Andre et al. 2009
679	87	Higher VEGF level in TNBC	Linderholm et al. 2008

TNBC: Triple-negative breast cancer; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor

in TNBC by giving EGFR inhibitors [26]. Some authors demonstrated that one-third of BL TNBC develops distance metastasis in which EGFR markers were helpful in diagnosis of high risk of metastatic disease [27]. A study reported that IHC EGFR positive patients were significantly more likely to develop distance metastasis, which depicts EGFR as an important prognostic marker for distance metastasis. Whereas, in EGFR expression, there is no significant difference between TN tumors with metastasis and those without metastasis. A study showed a high frequency of EGFR expression, i.e., 91.3% in BL subtype of TN breast cancer, which suggests that this marker may be helpful to detect metastatic BL TN breast tumors and may be beneficial to the patients for treatment with anti-EGFR drugs [27]. Expressions of EGFR in TNBC from various studies has been shown in Table 2.

Ki67

Ki67 is nuclear non-histone protein and was named after its Researcher's location, as, Ki stands for the University of Kiel, Germany, and 67 refers to the number of the clone on the 96-well plate. Ki67 gene is known for its proliferating characteristics, as it is marker of proliferation. The expression of ki67 at peak in the phase of G1, S and

G2 phase of cell cycle and absent in G0 phase [28]. Ki67 may predict as pathological exemption rate in breast cancer patient who followed neoadjuvant chemotherapy, whereas increased level of ki67 following neoadjuvant chemotherapy indicates a poor prognosis [29]. Hence, ki67 has been considered to be one of the most relevant marker indicators in detecting the proliferation of the tumor cells [30]. According to a study, ki67 significantly expressed more in TNBC group when compared non-TNBC patients [31]. The expression of ki67 in TNBC is higher than that of high-grade non-TNBC, which indicates that ki67 may play a role in prognosis of TNBC [32]. A study conducted in 2011, explained that TNBC with the higher expression of ki67 was associated with more aggressive clinicopathological features despite a higher pCR rate [33]. Some research articles suggest that the expression level of ki67 is presently considered to have significant prognostic and predictive values [34,35].

Ki67 expressed significantly in a higher stage of TN tumors and high lymph node metastasis [36]. They also suggested that the patients having higher expression of ki67 showed a significantly worse overall survival (OS) time. Hence, E-cadherin and ki67 when combined might be useful prognostic markers for adjuvant chemotherapy in Stage II TNBC patients. The debate on prognosis of ki67 in TNBC is still open, although various studies have established a relation between ki67 and overall and disease-free survival. In a study, it is also suggested that the assessment of the main prognostic and predictive parameters such as ER, PgR, and HER 2 including along with ki67 should offer to the patients and their physicians a strong background on which the final therapeutic decision can be safely taken [37]. Ki67 leads to most promising yet controversial biomarker in breast cancer, which is implementing routinely in some of the pathology department but not all. Ki67 can be controversial but clinically implemented biomarker along with well-established biomarkers of ER, PR, and HER 2 are currently marketed gene expression signature [38]. In several meta-analyses, it has been shown that ki67 as a prognostic marker as well as predictive marker (in neoadjuvant therapeutics). The "optimal" cut point of expression of ki67 has been still is under a big discussion topic which leads to matter that ki67 is a continuous marker, expressing the severe variation of the proliferative rates in different cancers. Due to this variation from several research and clinical trials, it is depicted that level of ki67 is quite difficult to standardize. However although, ki67 may be clinically interpreted directly by dividing its expression into two groups, i.e., tumors with a very low or very high expression of this marker. Instead of this limitation, the assessment of proliferation for cancer cell characterization ki67 plays an important pathological role [39].

VEGF

To support tumor growth, there is a requirement of increase formation of blood vessels. Tumor angiogenesis is a multistep process requiring signaling between tumor cells and several other cell types within the internal environment of tumor cells. Due to this process, they activate

Table 4: Expression of p53 in TNBC

Total number	TNBC	p53 in TNBC	Reference
-	134	Higher than non-TNBC (%)	Nakagawa et al. 2011
683	135	47.8	Patil et al. 2011
11	11	82	Nielsen et al. 2004
135	32	40.6	Chae et al. 2008
1726	282	56	Rakha et al. 2007
-	-	82	Foulker et al. 2004

TNBC: Triple-negative breast cancer

overexpression of pro-angiogenic factors by the tumor cells, such as VEGF, referred to as the “angiogenic switch” [40]. VEGFs when binds to one receptor, it stimulates dimerization of receptor and initiates signal pathway which promotes growth and migration [41]. The tumor vascular growth can be inhibited by antiangiogenic therapy by interfering with the intracellular signaling of VEGF and VEGF receptor (VEGFR) [42-44]. A monoclonal humanized antibody was designed named as Bevacizumab and Ramucirumab to inhibit the interaction between VEGF ligands and receptors [45,46]. Expression of VEGF is controlled by many stimuli such as hypoxia, nitric oxide, HER 2, Tumor suppressor genes, growth factor, and oncogenes [47]. VEGF regulates neovascularization in tumors, by increasing the level of anti-apoptotic proteins such as Bcl2, survivin, and XIAP. The endothelial cells undergo apoptosis, and newly formed vessels disintegrate due to its absence [48,49].

Now when it comes to TNBC, some researchers suggest that VEGF is one of the promising prognostic molecular markers. The expression of VEGF is elevated in DCIS and invasive breast cancer, and it is utilized for prognosis in breast carcinoma. The quantification of VEGF by IHC or immunoassay has shown a significant correlation with density. Higher expression of vascular density in breast tumors has been correlated with more aggressive tumor behavior and poor survival. Hence, microvessel density is now considered as one of the important factors which effects survival [50]. In a study conducted in 2009, was concluded that higher VEGF expressions are associated with shorter disease-free survival (DFS), OS, and DFS in TNBC [51]. It also stated that there was a direct correlation between serum and tissue level of VEGF to Grade III tumors, larger tumor size, positive lymph node, and poor survival with a decrease in level with chemotherapy. Expressions of VEGF in TNBC from various studies has been shown in Table 3

P53

P53 is a tumor suppressor gene which is encoded by the TP53 gene. P53 plays an important role as it regulates cell growth, proliferation, multiplication and apoptosis, and promotes stability of chromosomes. Any disruption of this function due to a mutation in p53 gene is done due to cellular stress by many pathways which are dependent on upstream regulatory kinase. 18-25% of primary breast carcinoma showed p53 mutation [52]. According to a study, expression of p53 is significantly higher in TNBC comparatively than non-TNBC, which indicates in worse prognosis of TNBC [32]. Due to higher expression of p53 in TN tumors, it may be used as predictors for response to chemotherapeutic agents. The majority of breast tumors with the p53 mutation was ER negative and associated with decreased DFS [53]. Although the exact mechanism, which may involve in this association is still unclear.

In several studies, it is denoted that p53 is more favorable to response to NAC in patients with TNBC. For instance, p53 overexpression was strongly correlated with pCR rate in TNBCs [54]. The level of p53 expression in TNBC with nodal metastasis was significantly higher than non-TNBC with same metastasis [55]. Heterogeneous p53 mutations, few of proteins may not be able to produce stability, which is detectable by IHC. The positive IHC results of p53 may be due to binding with other cellular proteins which gives accumulation of p53 expression.

Studies conducted by various researchers suggest that activation of p53 gene is associated with aggressiveness of breast tumor and in TNBC patients; it significantly decreases the rate of DFS and OS [21,56-58]. It can be used for segregation of subclass, i.e., BL from core TNBC along with EGFR and cytokeratin [59]. In TNBC patients, p53 mutations are associated with poor response to chemotherapy [60]. Study conducted 2016, demonstrated that elevated p53 expression in TNBC patients, presented the worst prognosis [61]. Measurement of p53 expression may assist in making treatment decisions and in predicting response to treatment, when apply before starting neoadjuvant chemotherapy, as when p53 combines with ki67, gives great predictive accuracy[62]. The frequency of p53 mutations was found to be higher in basal breast cancer as compared to Luminal type tumors [63]. Expressions of p53 in TNBC from various studies has been shown in Table 4.

CONCLUSION

TNBC is a cause of significant breast cancer mortality because of very few treatment options. Biomarkers can be useful as prognostic or predictive indicators which also suggest possible targeted therapies. TNBC are associated with a significantly higher expression of VEGF, EGFR, ki67, and p53 as compared with non-TNBC, which indicates the poorer prognosis in TN tumors. The emphasis should be put on research for targeted therapies of TNBC. New therapeutics alternatives should be investigated for patients with this subtype of breast cancer.

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