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REVIEW

Biomarkers in triple negative breast cancer: A review

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Abstract

Breast cancer is an intrinsically heterogeneous disease. In the world about 1 million cases of breast cancer are diagnosed annually and more than 170000 are triple-negative. Characteristic feature of triple negative breast cancer (TNBC) is that it lacks expression of oestrogen, progesterone and human epidermal growth factor receptor-2/neu receptors. They comprise 15%-20% of all breast cancers. We did a systematic review of PubMed and conference databases to identify studies published on biomarkers in TNBC. We included studies with biomarkers including: Epidermal growth factor receptor, vascular endothelial growth factor, c-Myc, C-kit and basal cytokeratins, Poly(ADP-ribose) polymerase-1, p53, tyrosinase kinases, m-TOR, heat and shock proteins and *TOP-2A* in TNBC. We also looked for studies published on synthetic lethality and inhibition of angiogenesis, growth, and survival pathways. TNBC is a complex disease subtype with many subclasses. Majority TNBC have a basal-like molecular phenotype by gene expression profiling. Their clinical and pathologic features overlap with hereditary *BRCA1* related breast cancers. Management of these tumours is a challenge to the clinician because of its aggressive behaviour, poor outcome, and absence of targeted therapies. As the complexity of this disease is being simplified over time new targets are also being discovered for the treatment of this disease. There are many biomarkers in TNBC being used in clinical practice. Biomarkers may be useful as prognostic or predictive indicators as well as suggest possible targets for novel therapies. Many targeted agents are being studied for treatment of TNBC.

Key words: Triple negative breast cancer; Epidermal growth factor receptor; Vascular endothelial growth factor; p53; Cyclin

Core tip: Triple negative breast cancer (TNBC) are type of breast cancer which lack of estrogen receptors, progesterone receptors and human epidermal growth factor receptor. It is a complex disease subtype with many subclasses. There are many biomarkers in TNBC used for its sub-classification. Clinically-practical assay/biomarkers that can reliably identify TNBC are

necessary. Biomarkers may be useful as prognostic or predictive indicators as well as suggest possible targets for novel therapies.

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INTRODUCTION

Breast cancer is a complex disease entity with different biological characteristics and clinical behaviour. Many clinical and pathological features have been defined to predict outcome and treatment response in breast cancer. These features include: Patient age, tumour stage, axillary lymphnode involvement, lymphovascular invasion, histologic grade, hormonal and human epidermal growth factor receptor (HER-2/neu receptor) status. In the past chemotherapy was the only systemic therapy for triple negative breast cancer (TNBC) patients. Currently lot of research is going on to further characterise TNBC with different molecular markers and find targets for therapy in order to improve its outcome. Sørlie *et al*[1] has diversified five subgroups of breast cancer by gene expression profiling (GEP) using DNA microarrays. These are luminal A, luminal B, HER-2/neu over expressing, basal like (BL) and normal like breast cancer. BL breast cancer lacks estrogen receptors (ER), progesterone receptors (PR) and HER-2/neu receptors, thus contribute to 80% of TNBC[1,2] The present review provides an insight into the different biomarkers in TNBC and its sub classification based upon the marker profile to understand molecular targets in each subtype.

TNBC

TNBC[3] are type of breast cancer which lack ER, PR and HER-2/neu receptors. It has different and poor clinical and pathological features as compared to other subtypes of breast cancer. It is usually seen in young age, advanced stage at presentation, unfavourable histopathology, grade Ⅲ, higher proliferative index, lack of tubule formation and higher rate of metastases[4-9]. It is associated with higher rate of local recurrence during 3 year after treatment and a high 5 year death rate[10]. Survival is poor after distant metastasis[11,12]. TNBC frequently affects younger patients (< 50 years) and has higher prevalence in the African-American women[13]. Patients with TNBC has inferior disease free survival (DFS) and overall survival (OS) as compared to age and grade matched controls of non-TNBC patients[11]. In TNBC metastatic rate is high to visceral organs[14,15] and lung and cerebral metastasis is more common[16-19]. Cytotoxic chemotherapy is the only treatment option[20-22].

TNBC subtypes

TNBC is a distinct breast cancer. It is classified into six groups based upon the GEP and DNA microarray. This sub-classification is not only useful in understanding the disease better but also to find molecular targets for its treatment[23].

BL-1 and BL-2: The BL-1 subtype was found to be composed rapidly dividing cells associated with increased proliferation and cell cycle checkpoint loss consistent with the increased expression of DNA damage response genes. Due to its high proliferation rate it has increased Ki67 mRNA expression and it is more responsiveness to antimitotic agents targeting cell cycle. The BL-2 subtype on the other hand displayed unique gene ontologies involving epidermal growth factor signalling as well as glycolysis and gluconeogenesis pathway. On microarray it showed a higher expression of epidermal growth factor receptor (EGFR), TP63, MET, *etc*.

Immunomodulatory subtype: Immunomodulatory (IM) is composed of immune cell responses such as immune cell and cytokine signalling, antigen presentation and processing and signalling of immune transduction pathways. Its GEP substantially overlaps with the medullary breast cancer, histologically a rare distinct form of TNBC which carry favourable prognosis despite its high grade.

Mesenchymal and mesenchymal stem like subtype: On GEP these subtypes consists of epithelial-mesenchymal (M) transition and growth factor pathways. The mesenchymal stem like subtype is also expressed by genes involved in angiogenesis including VEGFR2 and was found to be highly responsive to dasatinib [tyrosine kinase (TK) inhibitor], and mTOR inhibitors.

Luminal androgen receptor subtype: This subtype is characterised by androgen receptor (AR) signalling. It is ER negative but gene ontologies were heavily composed of hormonally regulated pathways such as steroid synthesis, porphyrin metabolism and androgen/estrogen metabolism. AR mRNA expression was nine times higher than other subtypes therefore, these lines were found to be highly sensitive to AR antagonists eg biclutamide. Patients with this subtype had decreased DFS and OS.

Basal cell and TNBC

Among TNBCs 80%-90% falls into the category of BL molecular subtype when appropriately tested for IHC cancer biomarkers and GEP but these terms are nonsynonymous and are overlapping[10,24]. At present, there is no optimal IHC panel for identification of basal like breast cancer (BLBC). Therefore TNBC, despite having above limitations is considered as a BL cancer. In a study Thike *et al*[9] with a tri-panel of cytokeratin-14 (CK-14) , EGFR and 34βE12 in TNBC reported 84% to be BL tumors with a specificity and sensitivity of 100% and 78% respectively. In BLBC over expression of ID4 leads to the deregulation of *BRCA1.* BLBCs are also

Table 1 Epidermal growth factor receptor expression in triple negative breast cancer

Ref.	Total number	No. of TNBC subjects	EGFR expression[1]
Thike *et al*[9], 2010	7048	767	30%
Patil *et al*[10], 2011	683	136	7.4%
Nielsen *et al*[24], 2004	-	21 basal like tumours	57%
Rakha *et al*[45], 2007	1726	282	37% in TNBC *vs* 15% in non-TNBC
Mehdizadeh *et al*[47], 2012	1132	103	23.3%
Rydén *et al*[48], 2010	564	48	41% TNBC *vs* 11% non-TNBC

[1]The expression is depicted as the percentage of patients expressing the marker. TNBC: Triple negative breast cancer; EGFR: Epidermal growth factor receptor.

known to have either p53 over expression or mutations in the gene[24].

In array, BLBCs are characterised by low expression of ER and HER-2 related genes, so pathologically they are usually ER-negative, PR-negative and lack HER-2 over expression[8,9] or are < 1%; < 5%; 10%; 20% immunoreactive for the above receptors[24]. They stains positive for cytokeratins (CKs) 5/6 and 17, and over express EGFR (HER1). Furthermore they show a highly aggressive GEP with low Bcl-2 but high p53 and Ki67[25-29].

BRCA AND TNBC

Genetic instability leads to cancer predisposition. Genetic mutations in the *BRCA* genes in patients predisposes them to develop many cancers such as breast, ovarian, pancreatic and prostate. *BRCA 1* plays vital role in DNA repair by homologous recombination. Inactivation of this gene due to *BRCA* mutation should trigger cell cycle arrest but this too is inhibited by p53 mutations in TNBC[30]. Lack of a functional *BRCA1/2* in cells lead to loss of repair of DNA double-strand breaks (DSB). This mechanism leads to increased risk of cancer in these patients. Histologically and transcriptionally, TNBC share similarities with *BRCA1*-linked breast cancers, which means that dysfunction of *BRCA1* is seen in TNBCs[31,32]. TNBCs are heterogeneous with respect to GEP. TNBC is associated with cancers arising in *BRCA1* mutation carrier in young women as compared to those in their late forties. Both sporadic BLBCs and *BRCA1* associated breast cancers have evidence of genomic instability. More than 80% of breast cancers in women who carry germ-line *BRCA1* mutations are TN and 10% TN breast tumors have *BRCA1* mutation. The reasons for these associations are unclear but may ultimately provide avenues for prevention as well as targeted therapy with poly(ADP-ribose) polymerase (PARP) inhibitors and chemotherapy with DNA-damaging agents such as platinum compounds[33-35].

Biomarkers in TNBC

TNBC is characterised by the marked expression of certain biomarkers. The presence of these molecules though is not restricted to TNBC but somehow show increased prevalence in this subgroup. The following are the important biomarkers in TNBC.

EGFR: EGFR is one of the members of four closely related receptors each playing an important role in tumour cell survival. The four receptors being EGFR (or ErbB-1), HER-2/neu (ErbB-2), HER-3 (ErbB-3), and HER-4 (ErbB-4)[36,37]. The inactive monomer receptor dimerizes after ligand activation followed by TK, intracellular domain of the receptor is activated by autophosphorylation, leading to cascade of intracellular events. EGFR signal cascade is important for cell proliferation, angiogenesis, metastatic spread, and the inhibition of apoptosis[38]. Most of the TNBCs express EGFR, and poses a strong therapeutic challenge[39]. Studies with different methods of gene amplification have found variable expression EGFR in metaplastic breast carcinoma, a phenotypes of BLBCs[40-42]. However, Toyama *et al*[43] with real-time polymerase chain reaction have reported high *EGFR* gene copy number in TNBCs. EGFR expression is found in 40%-50% of patients with breast cancer and in 80% of TNBC; and is estimated to substitute major proliferation pathways of breast cancer induced by activation of HER-2, ER, PR proteins which are thereby absent in TNBC[25].

In a study the authors found that 60% of patients with grade Ⅲ and > 3 lymph nodes showed EGFR expression, indicating that EGFR expression is related to aggressiveness of the disease. They also concluded that patients with EGFR expression had worse DFS, distant disease free survival (DDFS), OS and cause specific survival[44]. EGFR expression in TNBC is associated with poor response to chemotherapy[45]. Nogi *et al*[46] observed that EGFR was expressed in 24% of the TNBC patients and was related to less favourable response to chemotherapy and poorer survival and on the contrary the luminal groups where EGFR expression showed good response to chemotherapy and better survival. Recently EGFR has been defined with other markers to differentiate BL subtype from TNBC[47]. This aids in segregating TNBC into subtypes and thus defining the prognostic difference and molecular target specification between the two. Non-uniformity of expression profiles in studies shown in Table 1 is due to absence of subtype consideration or BL subtype non segregation from core TNBC. So EGFR is a biomarker in TNBC and a target for cetuximab, a TK inhibitor[48]. Many studies have evaluated its response in TNBC[48-51]. In a recent study, EGFR expression was shown as prognostic factor for DFS

Table 2 Vascular endothelial growth factor receptor expression in triple negative breast cancer

Ref.	Total number	No. of TNBC	VEGFR-2 expression[1]
Mehdizadeh *et al*[47], 2012	1132	103	93.2%
Iosifidou *et al*[62], 2009	-	73	77%
Chanana *et al*[63], 2012	70	27	54% *vs* 23%
Linderholm *et al*[67], 2008	679	87	Higher intratumour VEGF levels in TNBC
Andre *et al*[68], 2009	69	35	34%

[1]The expression is depicted as the percentage of patients expressing the marker. TNBC: Triple negative breast cancer; VEGFR: Vascular endothelial growth factor receptor; VEGF: Vascular endothelial growth factor.

not only in univariate but also in multivariate analysis[52].

Vascular endothelial growth factor: Angiogenesis is important for tumour growth and spread especially beyond a diameter of 2 mm as oxygen and nutrients cannot diffuse beyond this distance. Angiogenic signals are mediated by vascular endothelial growth factor (VEGF) to aid neovascularisation. VEGF A, B, C, D, E (viral factor) and placental growth factor is a family of six proteins. VEGF protein is found in 4 isoforms because of alternative splicing of its mRNA[53,54]. Among the different isoforms VEGF165, the 165-amino acid molecule is more common[55,56]. Its gene expression is controlled by many of stimuli such as hypoxia, nitric oxide, growth factors, oncogenes, tumour suppressor genes and HER-2[57].

It causes proliferation and maintains structural and functional integrity of cells of the endothelium. It also regulates vascular permeability and migration of endothelial stem cells from the bone marrow[58]. Neovascularisation in the tumour is also regulated by VEGF by increasing the expression of the anti-apoptotic proteins such as Bcl2, XIAP, and survivin. In its absence the endothelial cells undergo apoptosis and newly formed vessels disintegrate[59-61]. Thus neovascularisation is dependent on VEGF expression throughout tumour development. VEGF shows multiple interactions with receptor TKs, such as VEGFR-1, VEGFR-2, and VEGFR-3. The angiogenesis is initiated by VEGF binding to VEGFR-2 which triggers the specific activation of TKs followed by multiple signalling cascades resulting in the endothelial cells survival, proliferation, migration, adhesion, actin remodelling and vessels permeability[62].

VEGF expression is elevated in DCIS and invasive breast cancer. It has been also well utilised for prognosis in breast cancer[63,64]. Its quantification by IHC or immunoassay of tissue extracts has shown a significant co relation with micro vessels counts or density. High mean vascular density in breast cancer has been found to linked with more aggressive tumour behaviour and poor survival so intratumoral microvessels density is now considered as one of the important factors affecting survival[65]. According to recent studies[63,66] there was a direct co relation between serum and tissue levels of VEGF to grade Ⅲ tumours, larger tumour size, positive lymph node and negative hormone status and poor survival along with a substantial decrease in levels with chemotherapy. In TNBC higher VEGF levels are associated with shorter DFS, OS, and DDFS. Also VEGF levels have been significantly related to size of the tumour, grade and metastatic sites. In patients with higher VEGF levels disease progressed despite of therapy and such patients were associated with significantly lower progression free survival as compared to patients with lower levels. In TNBC patients it was found that VEGF level elevated from baseline to middle of the therapy significantly but showed a non significant increase from middle of the therapy to its end when patients were administered FAC[65-67]. VEGF is a target for bevacizumab in TNBC patients. Table 2 shows VEGF expression reported in different studies.

C-kit and basal cytokeratins: C-kit is a cytokine receptor present on the surface of hematopoietic stem cells and also in other cells. C-kit binds to stem cell factor and is a growth factor receptor that stimulates major cellular functions such as cell survival, proliferation, differentiation, adhesion and chemotaxis. It induces apoptosis and also increases the invasiveness of the cancer cells[68]. CKs are keratin-containing proteins of intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue. Different epithelial tissues express different CKs at the time of its terminal differentiation and the stage of development. This different CK expression helps in the classification of all epithelia. Similarly different cancers express specific CKs of that epithelium. Therefore the CK expression profile tends to remain constant when an epithelium undergoes malignant transformation.

The study of the CK profile by IHC techniques is very important for tumor pathologic classification[69]. These CKs were earlier used to distinguish malignant breast lesions from benign ones[70], but later their prognostic value was ascertained and it was seen that expression of CK-5, CK-14 and CK-17 was related to poor prognosis, high grade tumours, ER negativity, short DFS and OS[71-73]. It is expressed in BLBCs. Since BLBC and TNBC show overlapping features therefore C-kit and basal CKs along with other markers and pathological features are used for the differentiating BLBCs from

Table 3 C-kit expression in triple negative breast cancer

Ref.	Total number	No. of TNBC	C-kit expression[1]
Thike *et al*[9], 2010	7048	767	CK 5/6 in 6%, CK-14 in 48%, CK-17 in 50%, C-kit in 45%
Nielsen *et al*[24], 2004	-	21	CK 5/6 in 62% and C-kit in 29%
Kim *et al*[76], 2009	625	147	CK5/6 in 35.4% and C-kit in 11.6%
Bryan *et al*[78], 2006	66	4	75% of TNBC *vs* 29% of non-TNBC

[1]The expression is depicted as the percentage of patients expressing the marker. TNBC: Triple negative breast cancer; CK: Cytokeratin; EGFR: Epidermal growth factor receptor.

TNBC. Many studies have revealed that presence of CKs is higher in TNBC than non-TNBC and also among TNBC subgroup it is higher in the BL subclass (Table 3). BL subclass of TNBC was identified on the basis of CK and EGFR expression and when the clinicopathological features were compared between the basal and non-BL it was seen that BL subclass of TNBC were more aggressive[9,74-78].

p53: It is a tumour suppressor protein which is encoded by the *TP53* gene (the tumour suppressor gene). It is also called the "guardian of genome" as it is important cell cycle regulator[79]. It regulates cell growth, multiplication, proliferation and apoptosis, and promotes chromosomal stability. Disruption of these functions by mutation in the gene producing p53 lead to carcinogenesis. p53 is activated in response to cellular stress by many pathways that are dependent on distinct upstream regulatory kinases. First, an ataxia-telangectasia mutated proteins released in response to the DSB, second, a pathway dependent on *INK4* gene product, p14ARF activated by oncogenes, and finally, a pathway induced by chemotherapy drugs and ultraviolet light and is independent of the above two pathways[80,81].

p53 mutations are seen in 18%-25% of primary breast carcinomas (Table 4)[82]. p53 plays an important role in breast cancer prognosis. p53 over expression leads to poor response to chemotherapy[83,84]. Many studies have reported that its activation is associated with aggressive form of breast cancer and significantly decreases DFS and OS in TNBC patients[85-88]. Also co existence with HER-2 was significantly related to early relapse and death within shorter period after surgery[87]. Along with EGFR and cytokeratins it is used for segregation of a subclass, *i.e.*, basal like from core TNBC[89].

Tumours with p53 mutation are highly invasive, poorly differentiated and high grade tumours. In a study by Chae *et al*[90], p53 mutation was associated with poor response to the chemotherapy in TNBC patients. Other proteins of p53 family are p63/p73 proteins. Tumors expressing these proteins are reported to have many folds higher sensitivity to platinum based chemotherapy. p63/p73 expression is seen in one-third of patients with TNBC[91].

***TOP-2A*:** This gene encodes topoisomerase Ⅱ α and plays a crucial role in DNA transcription. This enzyme causes the temporary break of double strands of duplex DNA and rejoins them so that the strands cross through one another, therefore altering the topology of DNA. Mutation in cancer leads to depreviation of its functions and thus worsening of the situation. In TNBC or breast carcinoma the gene acts as a target for anthracycline therapy which is a topoisomerase Ⅱ inhibitor[92]. So it is a marker for the evaluation of resistance to the anthracycline therapy. A study revealed a higher expression of *TOP-2A* in 2.7% to 8.8% of TNBC patients[93]. Its over expression in TNBC leads to the decreased sensitivity towards the anthracyclines and thus decreased response[94].

Ki67: Also known as MKI67, Ki67 is a cellular marker for proliferation. Ki67 antigen is present inside the cell nucleus during interphase and during mitosis it is relocated to the surface of the chromosomes. Since it is a marker of proliferation it is found in all cells when they are in dividing phases of the cell cycle (G_1, S, G_2, and mitosis) and it is absent from cells during their resting phase (G_0). Its absence in resting cells and generalised presence in dividing cells had made it a marker of cell proliferation[95]. Proliferation is a salient feature for the spread of cancer and can be assessed by the IHC measurement of the nuclear antigen Ki67. It's over expression also correlates with levels of bromodeoxyuridine uptake and S-phase fraction, other markers of proliferation.

Ki67 expression is less in normal breast tissue (< 3%). It has been reported in many studies that Ki67 antigen and steroid-receptor are expressed in different cells in normal human breast epithelium. Ki67 was over expressed particularly in ER-negative cells and its expression in carcinoma cells was much higher[96,97]. In breast cancer high Ki67 is associated with of poor outcome although these tumours show very good clinical response to combination chemotherapy. However, its independent significance is modest and does not merit measurements in routine clinical practice. With respect to treatment response in breast cancer, Ki67 expression was found to be independent predictor of pathologic complete response (pCR), clinical complete response, OS and DDFS and locoregional recurrence. It was also seen that patients without pCR still showed a decrease in Ki67 index post therapy[98-100]. In a recent meta-analysis

Table 4 p-53 expression in triple negative breast cancer

Ref.	Total number	No. of TNBC	p-53 expression
Patil *et al*[10], 2011	683	135/683	47.8%
Nielsen *et al*[24], 2004	11	11	82%
Rakha *et al*[45], 2007	1726	282/1726	56% in TNBC *vs* 22% in non-TNBC
Chae *et al*[90], 2008	135	32/135	40.6% in TNBC *vs* 42.7% in non- TNBC
Biganzoli *et al*[89], 2011	-	(633 + 1026) from two separate sources	Divided TNBC into subclass BL which accounts for 89% of total TNBCs

TNBC: Triple negative breast cancer; BL: Basal like.

by de Azambuja *et al*[101] who retrieved DFS data from 29 studies, they concluded that high Ki67 levels was associated with poor prognosis in irrespective of nodal status and whether patients undergo treatment or not at all.

In TNBC, it was found that Ki67 levels were significantly increased in ductal TNBC compared to other histologic types (80% in TNBC *vs* 10%-30% in other types). Its expression also represented a direct co relation with tumour size and grade in TNBC patients and higher levels (> 35% staining) were linked with an increased risk of death[102,103]. In TNBC patients Ki67 accumulation was associated with a higher pCR to chemotherapy but poor RFS and OS. Its expression was also used for subdivision of TNBC into two subtypes where only 26.7% of TNBC patients showed lower Ki67 expression[104].

***PARP*:** *PARPs* are a family of cell signalling enzymes present in eukaryotes, which catalyses the poly(ADP-ribosylation) of DNA binding proteins. Till now eighteen enzymes of *PARPs* has been detected, but *PARP1* the most common isoform. *PARP1* is responsible for majority of its functions. Main function of *PARP1* is as DNA damage nick sensor. It forms polymers of ADP-ribose and nicotinamide with use of NAD^+. Activation of *PARP1* is important in tumours because of three interesting biological reasons: First, it plays a vital role in DNA repair through base excision repair pathway; second, it is capable of depleting cellular energetic pools, which results in cell dysfunction and necrosis; and third, its ability to promote the transcription of proinflammatory genes. *PARP* enzymes are involved in cellular response in inflammation, ischemia and oxidative stress. Carcinogenesis is a multistep process involving alterations in many cellular processes such as genomic stability, cell division, proliferation, growth, differentiation and cell death. *PARP1* are involved in all these cellular processes, indicating possible link between *PARP1* function and carcinognesis[105]. *PARP1* repairs DNA single strand breaks (SSB) by binding to the exposed ends of the damaged DNA strand and bring in important enzymes required for repair in SSBs[106-110]. The base excision repair pathway fails when *PARP1* is inhibited; this leads to accumulation of SSBs. In a dividing cell entering S-phase, cell division is arrested at SSBs, leading to a DSB (Figure 1). In *BRCA1* deficient cells excision repair pathway is dependent on *PARP1*, inhibition of *PARP1* leads to cell death through apoptosis[106,107]. *BRCA2* operates through excision repair pathway like *BRCA1*, mutation of this gene make the cells suceptible to *PARP* inhibitors as well[109,110]. *PARP* also plays a vital role in DNA repair as *BRCA.* Unlike *BRCA* it recognises SSBs and repairs by base excision repair pathway[105]. *PARP* inhibitors are effective in TNBC because damage to one of the arms of the DNA could not be repaired by homologous recombination due to *BRCA* mutation and *PARP* inhibition in synergism will create a state of "synthetic lethality" - a process that occurs when inactivation of individual genes have no effect but mutations in both the genes lead to death of cancer cells[107]. So *BRCA* mutation is responsible for the action of many chemotherapeutic agents in TNBC. The inhibition of *PARP1* is also known to potentiate the effect of ionizing radiation and many drugs such as DNA methylating agents, topoisomerase I inhibitors, and platinum compounds. Studies in mouse models have shown that the addition of *PARP* inhibitors with platinum compounds increases RFS and OS[35,105,107] while many of other studies on cell lines reveal that the activity of *PARP* inhibitors was increased in presence of *BRCA* mutations or dysfunction[105,108]. *PARP1* has been targeted as therapeutic option in TNBC with drugs like iniparib, olaparib etc though not found to be independently helpful but their addition to cytotoxic agents have surely brought synergism to their activity and improvement in treatment response in TNBC patients.

Heat shock protein 90: It is a cellular chaperone (proteins that assist the assembly or disassembly of other macromolecular structures) protein that mediates the post-translational modification and stabilization of a number of conformationally labile proteins, steroid receptors, cyclin-dependent kinase 4, RAF-1, AKT and other proteins that are useful for sending proliferative signals[111]. Once function of heat shock protein (HSP) 90 is blocked, its dependent proteins are broken by proteosomes. Small HSP αB-crystalline is expressed in BLBCs and is associated with shorter survival. Its' over expression is associated with neoplastic changes in mammary acini, increases cell migration and invasion in vitro. Geldanamicyn and tanespimycin both are antibiotics and inhibitors of HSP. These have shown clinical benefit in HER2-positive metastatic breast cancer[112]. The PU-H71 another HSP blocker has shown complete response in TNBC models[113].

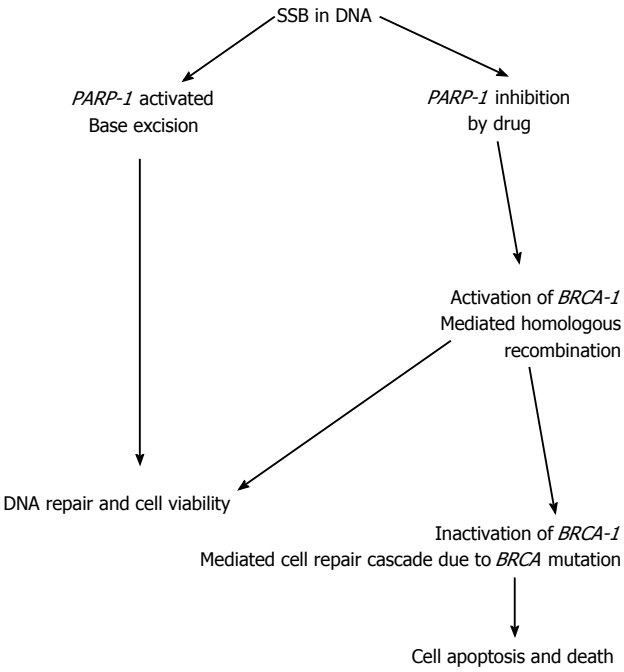

Figure 1 Mechanism of action of poly(ADP-ribose) polymerase-1 inhibitors in triple negative breast cancer. PARP-1: Poly(ADP-ribose) polymerase-1; SSB: Single strand breaks.

Cox-2: Cox is a conversion enzyme of arachidonic acid and prostaglandin. It is a 74kDa protein located in the cell endothelium, reticulum and nuclear membrane. It is expressed by stimuli such as inflammatory response and tumor promoters. In a study by Liu *et al*[114] they observed that 85% of transgenic mice with over expression of Cox developed breast cancer, suggesting the involvement of this enzyme in breast carcinogenesis. Other studies have correlated its expression with invasiveness and metastatic stimuli in breast cancer[115,116]. Approximately 40% of patient with breast cancer over expresses Cox-2. Cox-2 can also be used as a biomarker to assess response to neoadjuvant chemotherapy in breast cancer.

Lymph node status is major of prognostic significance in breast cancer patients. Studies have shown that Cox-2 expression is associated with positive lymph node involvement. So Cox-2 may have some role in lymphangiogenesis. Cox-2 expression has been also correlated to hormone receptors in breast cancer, negative hormone receptors with Cox-2 expression indicate worse prognosis. Cox-2 is correlated to HER2 through Ras/MAPK pathway and it is associated with HER2 over expression[117]. Cox-2 expression is also related to MDR-1, a multidrug resistance gene. Patients with expression of both these are least responsive to chemotherapy. So Cox-2 can be a good biomarker in breast cancer patients with its correlation with size of the tumour, number of nodes involved, hormone receptors and HER2 status[118].

TK: TKs are regulatory proteins that help in the cell growth and differentiation. These proto-oncogenes play an important role in progression and metastasis of cancer cells. They also increase sensitivity of cancer cells once the tumour has been exposed to radiation and chemotherapy through apoptosis[36]. Hence, TKs are of major interest and are subject of many active studies to look targets for therapeutic intervention in many solid tumours. HER2/neu and EGFR are also TKs receptors as discussed above. HER2/neu over-expression is seen in 20%-25% of invasive breast cancers and it is considered a poor prognostic factor. Other TKs over-expressed in carcinoma of the breast are BRK, c-Src, and EGFR[119]. Lack of expression of some of TKs such as Syk and C-kit are also linked to carcinogenesis of breast cancer. TK over-expression in women with breast cancer is have high risk of metastasis. There are many agents that target the phosphorylation of the receptor by acting at TK[120]. TK inhibitors such as imatinib, erlotinib, gefitinib and lapatinib are used for treatment of many solid tumours. Dasatinib and lapatinib are used in treatment of women with HER2/neu positive breast cancer.

Mammalian target of rapamycin: One of the pathway is commonly dysregulated in breast cancer is phosphatidylinositol 3-kinase/mammalian target of rapamycin (PI3K/mTOR). Over expression of the PI3K/mTOR is associated with poor response to treatment with hormones and trastuzumab[121]. To overcome endocrine resistance agents such as rapalogs, that efficiently block mTOR-raptor complex 1, can be used along with hormones. However, it has demonstrated variable results in hormone receptor positive metastatic breast cancer[122].

Many targets such as $\alpha V\beta 6$, cyclin E, C-kit, E-cadherin, O^6MGMT, FOXp3, β-blockers, insulin like growth factors, glycoprotein NMB and mitogen-activated protein kinase pathway needs further exploration to dissect TNBC and may possibly identify new biomarkers and targets for therapy.

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. Targeted therapy directed against many biomarkers has not shown significant improvement in outcome in TNBC, therefore it is challenging for the clinicians to deal with this distinct disease. The emphasis should be put on research for effective drugs and targets for the treatment TNBC. So, to translate the present knowledge about TNBC into oncological practice, biomarkers/molecules/GEP assays that can truly classify TNBC and can be easily translated to the clinics are necessary.

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