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MOLECULAR ANALYSIS OF KEY PROTEIN PATTERN FOR THE TREATMENT OF TRIPLE NEGATIVE BREAST CANCER (TNBC)

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ABSTRACT
Triple negative breast cancers (TNBC), are extremely heterogeneous diseases. The available breast cancer treatment options currently focus on receptors that are generally expressed in normal cancer. However, these receptors are not expressed in triple negative breast cancer cells. Absences of estrogen receptor (ER), progesterone receptor (PR), as well as human epidermal growth factor receptor-2 (HER-2), many of the current treatment options are ineffective. According to the World Health Organization's global status report on non-communicable diseases, cancer accounted for 8.2 million deaths in 2012 out of a total of 56 million deaths worldwide. This is 10.7% of the total 38 million deaths due to non-communicable diseases. Interestingly, the majority of cancer deaths occurred in high-income countries. Breast cancer is the second highest cause of death in high income countries. Adjuvant chemotherapy is the only available treatment option for TNBC at present. New targeted treatments have been focused on the usage of specific antibodies or specific pathway inhibitors that target cancer cells. These targeted drug delivery systems use specific antibodies conjugated carriers deliver to the cancer cell in order to minimise toxicity. The TNBC cells express many other protein receptors and there is no single protein that can cover the entire TNBC spectrum. Many possible targets can be utilised in the development of targeted therapy options. The protein receptors includes EGFR, Cytokeratin receptors, C-Kit, Androgen Receptors, BRCA-1/BRCA-2, Cadherins, P16INK4, P53, PI3K Pathway Inhibitors, TOP2A and others, such as alpha B-Crystallin and Chk1.The purpose of this review article is to analyse the expression patterns of TNBC cells and to identify the key proteins that will offer better coverage for the TNBC treatment.

Keywords: Triple-negative breast cancer; epidermal growth factor receptor; Cytokeratin; protein receptors.

INTRODUCTION

Triple-negative breast cancer (TNBC) is characterized by the absence of ER (estrogen receptor alpha) and PR (progesterone receptor) expression and overexpression of HER-2. TNBC occurs more frequently in younger and premenopausal women and to identifying the more effective treatment most of the medical researchers were involving in it [1].

Breast cancer is the most heterogeneous family of diseases and it's classified into 5 subtypes based on the presence and absence of certain nuclear or surface receptors such as ER, PR and HER-2. The subtypes are as follows: luminal A-like (ER and/or PR +ve, HER-2 -ve & Ki-67 low, luminal B-like HER-2-ve ("ER and/or PR +ve, HER-2 -ve & Ki-67 High"), luminal B-like HER 2+ve ("ER and/or PR +ve, HER-2 over expressed or amplified & Any Ki-67"), HER-2+ve Non-luminal (HER-2 over-expressed or amplified, ER and PR -ve) and Basal-like/triple negative (Ductal) (ER-ve, PR-ve and HER-2 -ve, EGFR+ve and/or CK 5/6 +ve) [2,3].

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Although triple negative breast cancers and basal like cancers are often used interchangeably, but they are not identical and not all basal like cancers are TNBC and vice versa. The most recently available information suggests that there are many similarities as well as differences between basal like cancers and TNBCs notably. However, most TNBCs exhibit basal-like phenotype and most basal like cancers are TNBC. Though, 8 to 29 % of TNBCs are of non-basal types [4].

TNBC has been reported to comprise 6 to 60% of cases, whereas studies on large populations have reported TNBC prevalence to be 13 to 21%. Ethnic variation has been shown to affect the reported TNBC incidence rates in various studies. A review by K.M. McNamara et al. reported that the incidence of TNBC was 7 to 39% in the Korean population, 20

to 80% in the African American population, 4 to 32% in the Caucasian population, 6 to 24% in the Japanese population and 15 to 37% in the ethnic Han Chinese population [5]. Similar variations have also been reported in South East Asian population studies (Table 1).

Due to the absence of hormonal receptors, traditional hormone therapy or drugs targeting HER-2 are not useful treatments for TNBC [6]. However, many studies have been reported the benefits of adjuvant chemotherapy. Neoadjuvant therapy is helpful for 30% of TNBC patients, but the overall five-year survival rate is lower compared to other types of breast cancers. This is primarily due to the residual disease remaining after chemotherapy [7]. Neoadjuvant chemotherapy with agents such as anthracyclines and taxanes has shown better chemosensitivity and high response rates for these tumours. However, higher recurrence and death rates have been reported for TNBC. It has also been reported that TNBC patients have benefited from a combination therapy of Paclitaxel, Adriamycin and Cyclophosphamide compared to ER+ve cancer patients.

In addition to neoadjuent chemotherapy, there are several targeted therapies under development. These include PARP inhibitors, EGFR inhibitors, multi-tyrosine kinase inhibitors and anti-angiogenic agents. However, many of them could not be validated in subsequent clinical trials, were only effective for certain sub-types, and demonstrated higher toxicity. Due to this reason, these treatment options have not yet reached patients as promising therapy options.

Table 1: Heterogeneity in TNBC Population

Country/Race	TNBC Incidence
Indian	12 - 25%
Chinese	16 – 20 %
Han Chinese	15 – 37 %
Malaysian	12 – 12.4 %
Singaporean	10 – 17 %
Korean	7 – 39 %
African American	20 – 80%
Caucasian	4 – 32 %
Japanese	6 to 24 %

OPPORTUNITIES FOR NEW TREATMENT OPTIONS OF TNBC

Cancer cells express many molecular or receptors that assist in cancer cell growth, progression and metastasis. Receptors within the cell receive extracellular chemical signals, which triggers cellular changes. Targeted cancer therapies use substances that can block the growth and metastasis by interfering with molecular targets. Unlike standard chemotherapies, which are cytotoxic and affect both normal and cancer cells, targeted therapies specifically act on their molecular targets and are cytostatic in nature. However, the development of the ideal targeted therapy requires the identification of the proper targets that will have a critical impact on cancer cell growth and survival [8]. To better understand TNBC and to develop superior and more targeted therapies, it is important to identify the potential candidates (receptors) that are expressed in TNBC cells.

Potential candidates:

Many possible targets can be utilised in the development of targeted therapy. These are include EGFR, Cytokeratin receptors, C-Kit, Androgen Receptors, BRCA-1/BRCA-2, Cadherins, P16INK4, P53, PI3K Pathway Inhibitors, TOP2A and others, such as alpha B-Crystallin and Chk1.

Epidermal growth factor receptor (EGFR or HER1):

EGFR is a 170 kDa glycoprotein in the transmembrane tyrosine kinase receptor family. The HER1 gene is located on 7q12 and is important for cell growth, migration and protection against apoptosis-facilitated activation of intracellular pathways. HER1 expression has been identified in 40% of all breast cancers and 80% of TNBCs [9]. Recent studies suggested that targeting EGFR is a good therapeutic target candidate, and identified as a better biomarker than androgen receptor and breast cancer susceptibility gene 1 (BRCA1).

Cytokeratin receptors:

Cytokeratin's are proteins that form intermediate filaments called keratin. Cytokeratin's are generally classified into acidic and basic types. Basic

cytokeratin's are CK1 through CK8, and acidic cytokeratin's are CK9 through CK20.

TNBBC is very heterogeneous, and the high percentage of cases with positive expression may be due to accidental inclusion of similar expression patterns. Therefore, it is important to further study the specificity of 34 TNBBC is very heterogeneous.

The available studies for cytokeratin 5/6 have reported varying results, ranging from 6% to 72% of TNBC cases with positive expression. This can be explained based on the core definition of basal-type breast cancers. TNBBC is currently identified using a five-marker method, and it is characterized by ER-PR-HER2–negative expression and positive expression of either epidermal growth factor receptor (EGFR) or cytokeratin 5/6 (CK5/6) [10]. Other studies considered that additional basal markers, including CK14 & CK17. However, the five marker method remains the gold standard for the classification of basal-type TNBCs. From this definition, basal-type TNBC may express either EGFR and/or CK 5/6. Depending on the population and genetic behaviour, the presence of these markers may vary. Therefore, it is important to further compare the significance of these two markers for the expression of EGFR and CK5/6 in TNBCs.

Mast Cell growth factor receptor (C-KIT or CD117):

CD117, or C-Kit, is a proto-oncogene that translates a tyrosine kinase receptor for Mast Cell growth factor. Mast Cell growth factor belongs to the tyrosine kinase receptor subclass 3 family and is a 145 kDa transmembrane glycoprotein [11]. Studies on the Asian population, as reported by Kanapathy Pillai et al. found that C-Kit expression, in combination with EGFR and CK5/6, was strongly associated with TNBC. A report showed that 61% sensitivity and 88% specificity of CD117 as a potential marker. A similar study by Shams and Shams [12] noted that 75% of the test cases expressed C-Kit. Therefore, C-Kit could also be a potential target in the development of TNBC treatment options.

Androgen Receptors (AR):

Androgen receptor belongs to the steroid nuclear receptor family that includes oestrogen and progesterone receptors and expressed in 50 to 100 % of breast cancers. Recent studies have demonstrated that androgen receptor expression plays a vital role in the biological and clinical behaviour of breast cancers, and the manipulation of its expression may have therapeutic value.

Meta-analysis by Zhang, Fang et al. showed that androgen receptor expression is more significant in the Non-TNBC group rather than the TNBC group. Data show that 23% of cases in the TNBC group expressed AR expression, compared to 75% of cases in the Non-TNBC group. This is also consistent with a study by Sherri Z. Millis et al. [13] that concluded that androgen expression in TNBC is much less prevalent (17%) than human epidermal growth factor receptor 2 (HER2)-positive breast cancers (79%). Although AR expression is not specific to TNBC, there are several studies showing its usefulness in targeting non-basal type of TNBC [14].

BRCA 1 & BRCA 2:

Approximately, 10% of breast cancers are due to an inherited mutation in the BRCA1 or BRCA2 genes. There is a strong association between the TNBBC subtype and BRCA phenotypes [15]. BRCA1-associated breast cancers are a cluster of basal-like cancers and its similarity in gene expression profiles. The tumours that occur due to BRCA mutation are generally sensitive and resistant to many cytotoxic agents. Furthermore, the responses to cytotoxic agents are unpredictable. A study by Manxiu Li et al. determined that a deleterious somatic BRCA1 mutation (3.9%) occurs in only a small subset of TNBC patients, and these patients are likely to respond to neoadjuvant chemotherapy [16]. Relatively, 75% of TNBCs express a mutated BRCA1 gene. In a normal BRCA-viable cell, double strand breaks (DSBs) are repaired by homologous recombination (HomR), but in a cancer cell with a disrupted BRCA pathway, DSB can cause chromosome instability, cell cycle inhibition and cell death [17]. Poly (ADP-ribose) polymerases (PARPs) are required to repair DNA single strand breaks (SSBs) by detecting SSBs and recruiting the BER multiprotein complex to the damaged DNA site. PARP inhibitors have been useful in disrupting the BRCA pathway. Third generation PARP inhibitors are now in clinical trials for their application in the treatment of TNBC. Np63, which is a p63/p51 isoform, is essential for stem cell maintenance. It can induce inactivity and control the BRCA1 pathway in ER+ luminal breast cancer, but it is not an effective target of other breast cancer types [18].

Cadherins:

Cadherins are proteins with key roles in mediating cancers. They comprise a family of transmembrane glycoproteins that are vital for embryonic formation and development and tissue/organ development. The

cadherin family consists of E-cadherin, N-cadherin and P-cadherin [19].

N-cadherin is generally expressed in neural tissues and is abnormally expressed in some epithelial cancers. E-cadherin and P-cadherin play a major role in cell–cell adhesion of epithelial tissues. They also play crucial roles in various developmental processes and in sustaining adult tissue integrity and maintenance. It is now known that changes to these two molecules occur during tumour progression of most carcinomas. Changes in the E- and P-cadherin encoding genes (CDH1 and CDH3) or variations in their protein expression result in tissue disorder, cellular de-distinction, increased tumour aggressiveness and metastasis [20].P-Cadherin expression increases the aggressiveness of epithelial breast cancers [21]. P-Cadherin expression is associated with TNBC, particularly TNBBC-types of breast cancers.

The current data suggest that the absence of ERα signaling is responsible for the ectopic P-Cadherin overexpression and for initiating breast cancer cell growth and spreading. Together with basal cytokeratin's and EGFR, TNBC cells express a high proportion of P-Cadherin. The significance of P-Cadherin expression has been reported for many cancers, including breast cancers.

p53:

p53 is one of the regularly mutated genes among the 20,000 genes in the mammalian genome. It is a 53 kDa tumour suppressor that regulates the response to cellular stresses. The p53 protein prevents cell cycle completion if the cell is suffering from any form of damage, as in the case of a tumour cell. p53 is mutated in at least 50% of human malignancies. To target mutated p53, a predictive biomarker is required. EGFR overexpression is common in basal-type TNBC, and this overexpression is caused by p53 transformation. Almost 82% of basal-like breast cancers express p53 compared to 13% in the luminal A subgroup. This transformation also causes ineffectiveness of anti-EGFR treatments, resulting in the aggressiveness of TNBC. EGFR overexpression is caused by p63, which is a member of the p53 family. A total of 44 to 64% of TNBCs carry a mutation in p53 [22].

p53 status can be used to subdivide TNBC into two subgroups with distinct treatment outcomes. Based on recent studies, p53 has been targeted in treatment using multiple strategies, including blocking the interface between mutated p53 and p63 proteins, correcting the mutations in the p53 gene, blocking the link between EGFR and CSF 1-R and by using statins to decrease the dependence of basal-like breast cancer cells carrying p53 mutations on mevalonate pathways [23].

P16INK4:

P16INK4 is a 16 kilodalton protein produced by the tumour suppresser gene INK4a. This tumour controlling protein acts as a CDK4 and CDK6 inhibitor. Similar to p53, it prevents cancer progression by interfering in the cell cycle through inhibition of the cyclin-dependent kinase CDK-4. These D-type cyclin-dependent kinases initiate the phosphorylation of the retinoblastoma tumour suppresser protein RB. P16INK4a overexpression is strongly associated with the aggressiveness of TNBC. Jessica, Venetia, Yan Peng [24] further studied this association and demonstrated that 75% of TNBC cases expressed p16. Their findings suggest that p16 expression may be a useful marker with high prognostic value. Studies also suggest a strong association between increased P16INK4a expression and high p53 expression, as well as loss of Rb1 tumour suppressor gene expression.

PI3K Pathway Inhibitors:

PI3K pathway has been found to play a major part in metabolism, growth, and survival of cancer cells in many human cancers, including breast cancer. PI3K pathway signaling can be triggered by G protein-coupled receptors (GPCRs) and also through cell surface receptor tyrosine kinases (RTK). Phosphatidylinositol triphosphate (PIP3) which is formed from PI3K phosphorylates phosphatidylinositol diphosphate (PIP2) mediates the activation of oncogene AKT [25]. Over activity of PI3K/ AKT is detected in many breast cancers including TNBC By dephosphorylating PIP3 to PIP2, PI3K pathway is down-regulated by tumor suppressor gene phosphatase and tensin homolog (PTEN).

A lipid phosphatases like Inositol polyphosphate 4-phosphatase type II (INPP4B) can change PIP2 to PIP and thus can act as tumor suppressor. The mechanistic target of rapamycin (mTOR) can stimulate translation of protein, growth, angiogenesis and reproduction. By the inhibition of mTOR mediated PI3K signalling pathway, we can potentially prevent cellular growth and can initiate cancer cell death [26]. Clinical studies for drugs such as rapamycin and its analogs temsirolimus, everolimus, and deforolimus, have already started for their effectiveness in TNBC treatment.

Recent studies using breast cancer cell lines show that statin is a promising drug for the treatment of TNBC through PI3K pathway by the loss of PTEN and activation of AKT. These studies also show that TNBC cells with mutated PTEN shows high response to simvastatin than TNBC cells with wild type PTEN. Therefore, a combination of AKT inhibitor in combination with statins may be a promising treatment option for TNBC.

Topoisomerase II Alpha (TOP2A):

TNBC type is associated with TOP2A expression and the gene located on 17q21e22, encoding topoisomerase II alpha, and a target for anthracycline therapy. Amplification of TOP2A gene is deliberated as a treatment response predictor for anthracycline treatment for TNBC [27]. About 1.3 to 10 % of TNBC cases show amplification of TOP2A gene. TOP2A amplification can bring good response to anthracyclines whereas its deletion may bring resistance Therefore, it is evident that TOP2Aexpression is important for anthracycline therapy, but it has no benefits as a target for new therapy.

CONCLUSION

TNBC is one of the heterogeneous types of breast cancer and the treatments are ineffective by lacks of expression critical proteins. We hope this review could be expressed some other proteins that can be used to explore targeted therapy options for TNBC.

CONFLICT OF INTEREST

The authors declare that there are no conflicts interests.

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