Triple Negative Breast Cancer (TNBC): Possible Biomarkers and Treatments

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ABSTRACT

Triple negative breast cancer (TNBC) is one of the breast cancer subtypes, which account for around 15-20% of overall breast cancer cases. Women with Hispanic or African-American descent are having higher risk factor to develop this type of breast cancer, especially in their premenopausal period (under 40). This highly aggressive and poor prognosis type of breast cancer does not have several molecular receptors (estrogen receptor, progesterone receptor, and HER2 receptor). The absence of those receptors leads to its insensitivity to hormonal therapies, resulting in limited choice of therapy. Numerous studies have been conducted to discover other potential therapies. The clinical characteristic and other information regarding triple negative breast cancer will be discussed along with several potential prognostic biomarkers and treatments for this cancer.

Keywords: BRCA1-like, EpCAM, ITB1, prognostic biomarker, triple negative breast cancer

ABSTRAK

Triple negative breast cancer (TNBC) merupakan salah satu subtipe kanker payudara, yang mencapai 15-20 % kasus kanker payudara secara keseluruhan. Wanita keturunan Hispanik atau Afrika - Amerika lebih berisiko, terutama dalam periode pre-menopause (di bawah 40 tahun). Kanker jenis ini sangat agresif dan tidak memiliki beberapa reseptor molekul (reseptor estrogen, reseptor progesteron, dan reseptor HER2). Tidak adanya reseptor mengarah pada ketidakpekaan tumor untuk terapi hormonal, mengakibatkan pilihan terapi terbatas dibandingkan dengan jenis kanker payudara lainnya. Sejumlah penelitian telah dilakukan untuk menemukan terapi potensial lain. Karakteristik klinis dan informasi lain mengenai kanker payudara *triple* negatif akan dibahas bersama beberapa *biomarker* prognostik potensial dan pengobatan untuk kanker ini. **Dias Rima Sutiono, Widad Aghnia Shalannandia. *Triple Negative Breast Cancer* (TNBC): Potensi *Biomarker* and Terapi**

Kata kunci: *Biomarker* prognostis, BRCA1, EpCAM, ITB1, *triple negative breast cancer* (TNBC),

INTRODUCTION

Breast cancer is a heterogeneous diseases which also account as one of the most common women cancer in the world, regardless race or ethnicity.[1] Statistical study in 2012 stated that around 1.7 million women were newly diagnosed this cancer, representing 25% cases of all cancers in women as well as 12% of all new cancer cases in the world.[2] Triple-negative breast cancer (TNBC) is the subtype of breast cancer which lack of estrogen receptor (ER), progesterone receptors (PR), and also human epidermal growth factor receptor 2 (HER2). This subtype of breast cancer, which account for around 15%-20% of overall cases, has a poor prognosis yet very aggressive behavior.[3] The absence of cell receptors on TNBC tumors make the patients could not take the advantages of hormonal therapy such as tamoxifen and aromatase inhibitor; and also HER2 receptor

direct targeting therapy such as lapatinib and trasazumab.[4] Hence, the treatment of TNBC are commonly the combination of surgery, chemotherapy, and radiation. Many studies have focused on discovering another effective treatment for TNBC, including study of potential prognostic biomarkers of TNBC tumors.

Clinical Characteristic, Pathology, and Risk Factor of TNBC

The triple-negative type of breast cancer is known to have a great diversification of its subtype and also its histological pattern. Numerous studies had also shown a very poor clinical outcome of this type of cancer.[5,6] However, the TNBC tumors also appear to have a larger morphological size compared to non-TNBC tumor with higher node positivity rates.[7] The diverseness of this subtype of cancer also followed by a high grade of

tumor and positive lymph node numbers, not to mention that most of metastasis happen during the first three years after being diagnosed.[5,6] Moreover, the survival rates of patients with TNBC are around five-year lower compares to the patient who suffer another type of breast cancer, regardless of the more susceptible TNBC tumor in chemotherapy.[5]

TNBC can be characterized by its histological types of tumor. The ductal TNBC, which account for around 82% of histologic type of TNBC tumor, has a relatively high five-year survival rate, which is around 62%.[5] Other histologic type of TNBC tumor have better five-year survival rate than the ductal TNBC; including medullary, apocrine, and neuroendocrine histological type that have approximately 100% rate of five-year survival, while the rate was lower in papillary histologic type (approximately 50%).[5] In some

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cases, TNBC tumors do not show histological type, example for this case is medullary and metaplastic carcinoma of TNBC. The metaplastic carcinoma can be characterized by the differentiation into squamous epithelium containing mesenchymal components, while the medullary carcinomas are an invasive carcinomas with pushing border of invasion characteristic that infiltrate dense peripheral lymphoid tissue.[5,8]

This aggressive behavior type is more likely presents in women with Hispanic or African-American descent. Recent study also stated that approximately 39% of all breast cancer patients with African-American origin are actually having TNBC type.[8,4] The Carolina Breast Cancer Study also showed the higher prevalence of TNBC tumors, which majority are categorized as basal-like tumors, in premenopausal African-American women compared to the African-American women in postmenopausal period and non-African-American women.[8] Further epidemiologic study also showed that in comparison with luminal A tumors or ER and PR-positive with HER2-negative tumors, basal-like tumors are more prone to occur on women with higher body mass index (BMI), higher parity, menarche at younger age, have full-term pregnancy in younger age, and particularly in pre-menopausal period.[8] Women who applied some methods to suppress their lactation also have higher risk to develop basal-like tumors.[8]

The higher tendency of lymphocytic infiltration of TNBC made it common to have early visceral metastasis at the time of diagnosis. TNBC patients also have tendency to develop brain metastases with prevalence of approximately 15%, which also contribute to the shortened lower survival rates.[4,8] In addition, TNBC patients also have a higher rate of distant recurrence despite having a higher initial response rate on the neoadjuvant chemotherapy.[4]

POTENTIAL TREATMENT FOR TNBC
Mutation of BRCA1 Tumor Suppressor in TNBC and BRCA1-like Tumors
BRCA1 tumor suppressor gene expressed a protein which hold essential role in cell growth and development, which includes transcriptional regulation, DNA repairs, ubiquination, and also act as cell cycle checkpoint control.[8] In addition, the BRCA1

is also essential in DNA double-stranded repair which is responsible on maintaining DNA stability.[4] The mutation of BRCA1 tumor suppressor gene tend to lead to the development of basal-like breast cancer,[8] some investigation also showed around 10-15% of overall TNBC cases have mutation on that particular tumor suppressor gene.[6] Moreover, in those cases, the tumors were commonly high-grade, consistent with other study findings.[5,6] Those tumors with non-functional BRCA1 are highly prone to mutation and loss of its heterozygote characteristic which leads to genomic instability.[6] Virtually, those tumors have a high tendency on gaining and losing genomic DNA, thus have numerous copy number aberrations (CNAs), which patterns are used to identify other non-BRCA1-mutated tumors with similar patterns of BRCA1-mutated tumors' CNAs.[6] That group of tumors that have the similar CNAs pattern as BRCA1-mutated tumors are known as BRCA1-like tumors.[6]

BRCA1-like tumors are actually is a larger group compared to the BRCA1-mutated tumors.[6] The prevalence of BRCA1-like tumors on TNBC cases are relatively higher, approximately 68% of overall TNBC cases.[9] Some studies also found that TNBC tumors have significantly high TP53 mutation frequency, frequently correlated with BRCA1 associated breast cancer.[6] The frequency of TP53 mutation was found slightly lower in TN breast cancer (approximately 54%) compared to overall basal-like breast cancer (80-90%).[6] The recent study indicates that the high frequency of TP53 mutation in BRCA1-like tumors might cause the tumor to be more susceptible to combination of Chk1 inhibition with irinotecan: DNA-damage inducing agent which treated TN xenograft experiment with favorable result.[6]

Further study also identified that the interaction between chemotherapy treatment and the status of BRCA1-like did not show significant correlation, thus suggest the high possibility of using BRCA1-like status as a prognostic marker of TNBC.[6] The study have also determined the possibility of susceptibility of BRCA1-like TNBC to DNA repair and cell cycle pathway targeting therapies.[6]

Integrin Beta 1 (ITB1) Expression in TNBC
Integrin beta 1 or ITB1 has been reported to be highly expressed in many types of breast

cancer, including lung cancer, ovarian cancer, prostate cancer, colon cancer, and even in TNBC.[10] The level of ITB1 was also found higher in more severe stage of cancer correlated to the lower survival in invasive breast cancer.[10] Study showed that the knockdown of ITB1 resulted in the inhibition of TNBC tumor migrations and invasions.[10] The inhibition of TNBC tumor migration and invasion were assumed to occur by regulating Ca^{2+} influx via SOC channel; the knockdown of ITB1 leads to reduction of Ca^{2+} influx through SOC channel.[10] In this case, calcium ions are important regulator of cell migration; therefore, the lessen influx of calcium ions resulting in the inhibition of TNBC tumors migration and invasion.[10] That finding provides a possibility of using ITB1 as target therapy of prognostic biomarker to help treat TNBC.

Epithelial-mesenchymal Transition Genes Expression in TNBC
The mechanism of epithelial-mesenchymal transition is essential on tumor progression, which also support the invasion of tumor and metastasis.[3] Some reports stated that expression of EMT markers are more frequently occur in basal-like TNBC compared to other subtype of TNBC.[3] However, recent study also found that gene expression that related to EMT markers are also more frequent in specific subtype of TNBC, including the messenchymal-like (ML) and messenchymal stem-like (MSL).[3] Thus, there is high possibility of potential therapeutic target or prognosis biomarker among those EMT markers in TNBC.

Recent study showed that 86.7% of the TNBC patient in batch study expressed multiple EMT-related marker by which the loss of E-cadherin and expression of vimentin were found in more than half of the TNBC cases.[3] In the same batch, the expression of CD146 on TNBCs is around 60%, while the ZEB1 expression was not significant, only 16%.[3] Until now, most studies of EMT-related markers are limited to the expression of vimentin and loss of E-cadherin investigation. Vimentin expression is usually associated with poor prognosis of TNBC, while the loss of E-cadherin in TNBC patient are assosiacted with lower survival rates compared to patients who have E-cadherin.[3] However, there is no correlation between both vimentin and E-cadherin with the prognosis of TNBC.[3]

Another common EMT-related marker expressed by TNBC patient are CD146 with 60% prevalence.[3] This marker was also identified to hold important role in inducing epithelial-messenchymal transition in breast cancer cases which also found to be associated in expression of other mesenchymal markers and the loss of E-cadherin.[3] Nevertheless, the expression of CD146, again, are not related to the TNBC clinical outcome progression.[3]

The least common EMT-related marker, only expressed on 16% of TNBC cases, is ZEB1 or zinc finger E-box binding homeobox; which is actually one of the main EMT inducer in many types of cancer.[3] The expression of ZEB1 can influence by down-regulating the expression of E-cadherin as well as induce EMT in breast cancers.[3] On the other hand, more recent study reported that the prevalence of expression of ZEB1 in that batch are significantly higher, approximately 44%, with high correlation with vascular invasion, higher grade, and larger tumor class.[3] Those findings showed that ZEB1 actually do not associate with the EMT progression but more likely related to the poor clinical outcome of TNBC. Therefore, ZEB1 can be regarded as one of potential prognostic marker of TNBC.

Epithelial Cell Adhesion Molecule (EpCAM) Targeted by Gramzyme B-based Cytolytic Fusion Protein

The epithelial cell adhesion molecule of EpCAM is a membrane glycoprotein that holds a role in maintaining the undifferentiated stage of embryonic stem cells in human.[11] EpCAM and glycosylation actually expressed on the surface of normal epithelial tissue. However, the EpCAM expression and glycosylation level are significantly higher in cancer cells, specifically on both TNBC cells and tumor initiating cells (TICs), compared to the normal cells.[11] Anti-EpCAM antibodies that were used in several clinical trials showed that the affinity of corresponding antibodies has important function on their therapeutic effect. In one of the study, anti-EpCAM(scFv) in a novel CFP (cytolytic fusion protein) showed its efficacy in eradicating various carcinoma cell lines.[11] Furthermore, another experiment showed that the same anti-EpCAM(scFv) are not lethal on those cells that express low level of EpCAM.[11] The recent study by Amourny and her colleagues also showed that the fusion of anti-EpCAM(scFv) with the human granzyme B could retain the scFv and granzyme B protease activity's tumor-selective binding activity.[11] Therefore, the anti-EpCAM can be considered as one of the possible treatment of TNBC with stable therapeutic fusion protein structure that can specifically differentiate the cancer cells.

CONCLUSION

TNBC is one of subtype of breast cancer with aggressive behavior and poor prognosis or clinical outcome. The absence of cell receptors on this type of cancer cause ineffectiveness of various hormonal therapy that can be performed on other type of breast cancers; leaving limited option of treatment including surgery and chemotherapy. Many studies have focused on discovering other possible prognostic biomarker and other possible therapy for this cancer, such as BRCA1-like, ZEB1, EpCAM, and DNA repair and cell cycle pathway targeting therapy. However, further study of those potential treatments of TNBC is needed.

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