Breast Cancer is not Just One Disease: A special focus of molecular heterogeneity of Triple Negative Breast Cancer

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ABSTRACT

In oncology, it is not "One Size" fits all. After decades of research, a new hypothesis is recently dominating the field of oncology which is "tumors are not alike". breast cancer (BC) is a perfect example of such heterogeneous tumors. Triple Negative Breast Cancer (TNBC) is one of the most aggressive subtypes of BC. It is complex & heterogeneous in nature; there is no available targeted therapy, as it lacks expression of any receptors (ER, PR & HER2) which render it resists any hormonal therapy used in treatment of other subtypes of BC. That is why this type of cancer needs especial kind of therapy or even combinational therapies. In this review, we focus on the TNBC patients and the currently available treatment protocols for those patients. Also, a special focus on the altered molecular pathways in TNBC tumors and their naturally derived and synthetic molecular weapons has been specifically tackled in this review.

Introduction

Breast Cancer

Breast Cancer (BC) is a complex heterogeneous disease which is molecularly sub-classified into several subtypes as shown in Table 1 [1]. It is one of the most leading causes of women death worldwide especially in developing countries. It is the most common cancer worldwide and the 2nd cause of cancer-related deaths in American woman after lung cancer. According to WHO, about 2.1 million annually are newly diagnosed with BC and 167.000 deaths has been reported in 2018 [2]. This represents 15% of all cancer deaths among women. Most BC tumors originate in the breast tissue itself. This either takes place in glands for milk production (lobules) and which is called "Lobular Carcinoma"; or in ducts which is called "Ductal Carcinoma". Identification of the molecular subtype of each patient makes a great difference; it is not only important in evaluating the disease prognosis, but also, in selecting the optimum treatment regimen. Consequently, the maximum patients' response rates and overall survivals are obtained [3].

Table 1: Molecular Subtypes of Breast Cancer Patients.

Breast Cancer Subtype	Molecular Profile
Luminal A	ER$^+$ and/or PR$^+$, HER-2$^-$, and low Ki-67 (<14%)
Luminal B	ER$^+$ and/or PR$^+$ and HER-2$^+$ (luminal-HER2 group)
HER-2 Enriched	ER$^-$, PR$^-$, and HER-2$^+$
Triple Negative Breast Cancer	ER$^-$, PR$^-$ and HER-2$^-$

Triple Negative Breast Cancer (TNBC)

TNBC comprises a heterogeneous group of tumors that is considered one of the most aggressive BC subtypes with the poorest prognosis compared to other BC subtypes [4]. Therefore, TNBC will be the focus of our review. The absence of hormonal receptors, as well as, HER-2 receptors renders it as an unresponsive tumor to hormonal therapies or therapies targeting HER2 protein. Almost 15-25% of BC women are diagnosed with TNBC. Its relapse rates greatly differ from other BC subtypes as TNBC relapse rates

are the highest in the first 3-5 years. Nonetheless, it drops sharply afterwards; it even may become below that of hormone positive subtypes (Luminal A/B). TNBC is usually classified as a "basal– type" cancer; however, many studies have been done for more deep classification of TNBC using gene expression profiling as shown in Table 2 [5-8].

Table 2: Triple Negative Breast Cancer Classification.

References	Subtypes of TNBC	Type of Classification
[5]	Seven distinct subtypes, six are stable : Basal like1(BL1), Basal like 2 (BL2), Immunomodulatory (IM), Mesenchymal (M), Mesenchymal Stem Like (MSL) & Luminal Androgen Receptor (LAR) besides one Unstable subtype (UNS)	**Lehmann study**
[6]	He applies nonnegative matrix factorization & define Four subtypes of **TNBC:** Basal like immune active, basal like immune suppressed, Mesenchymal & Luminal AR	**Burstein et al. study**
[7]	Using data collected from clinical & gene expression profiles of 494 **TNBC** breast tumor samples, the study shows four subtypes of **TNBC:** Luminal-Androgen receptor (LAR), Basal, Claudin-low & Claudin-high. Further classification for Luminal subtype was done (based on its immunology status) into Luminal-immune positive [with better prognosis] & Luminal-immune negative. This classification enables researchers to predict tumor response to immune therapy	**Guillermo Prado-Vazquez**
[8]	Study classifies **TNBC** microenvironment into three clusters: **Immune desert cluster:** low cell infiltration, cannot attract immune cells & show "MYC" amplification. **Innate immune –inactivated:** has inactive innate immune cells & nonimmune stromal cell filtration. It has low tumor Ag presentation (which may lead to immune escape) & has a mutations in PI3K-AKT signaling pathway. **Immune inflamed:** rich in innate & adaptive immune cells infiltration with high level of immune check point molecules which render it the most affected cluster with immunotherapy strategies.	**Yi Xiao**

Conventional Therapeutic Approaches for TNBC Patients

Chemotherapy still represents the mainstay treatment for TNBC patients as a result of lacking receptors that can be treated by targeted therapy [9]. Neo-adjuvant chemotherapy is used as the first line of treatment for TNBC patients, especially for cases where surgery is contraindicated [10]. Nonetheless, this treatment approach gives higher pathological complete response (PCR) in TNBC patients rather than in HR+ BC patients [11]. It has been found that the PCR rate in basal-like1 subtype is greater than in basal-like 2 & LAR receptor subtypes. Furthermore, TNBC patients have lower than three year progression free survival (PFS) and overall survival (OS), according to J.M. Lebert study [12]. The best neo-adjuvant therapeutic regimen for TNBC patients has not been identified yet. However, it has been found that in addition of Carboplatin results in higher rates of (PCR). But, unfortunately, it leads to greater toxicity effects.

Adjuvant Therapy

This regimen is recommended to be used for TNBC tumor with size larger than 0.5 cm because of their aggressiveness. The guidelines of European Society for Medical Oncology (ESMO) recommended not using adjuvant treatment if there is a residual disease after the completion of neoadjuvant treatment. However, this indication is completely reversed after the "create-x" trial results [13]. The optimal adjuvant option for TNBC is still not identified despite the use of chemotherapy containing anthracyclines and taxanes that are supported by European society guidelines [14]. Using Platinum based chemotherapy in metastatic TNBC usually recommended rather than non-platinum based chemotherapy whose use results in longer PFS in patients with no differences in their OS rates.

Molecular Signaling Pathways Underlying the Molecular Heterogeneity of TNBC and Their Potential Molecular Targeting Weapons

Different signaling pathways contribute to TNBC development, metastasis, and resistance to current therapeutic regimens [15]. NF-κB is greatly expressed in TNBC cells [16], it is the key regulator of TNBC cells apoptosis, angiogenesis, and resistance. It is one of soy isoflavones agents "Genistein" down regulate the expression of "BCL-2, BCL-xL and Cyclin B1" proteins and lead to TNBC cells apoptosis [17]. "Plumbagin" also induces apoptosis in TNBC cells through inactivation of DNA binding activity of NF-κB and BCL-2 and has no effect on normal breast cancer cells [18]. "Fenofibrate" cause apoptosis to TNBC cells through up regulation of "Bad", activation of "Caspase3" and down regulation of "BCL-xL" [19].

JAK/STAT signaling pathway

STAT3 overexpressed in TNBC tissues giving higher aggressiveness and poor prognosis [20]. JAK2 gene greater expressed in TNBC cells following chemotherapy indicating that it has a role in developing chemo-resistance in TNBC cells. Therefore, using JAK2 specific inhibitor "BSK805" with chemotherapy in mice cause tumor regression [21]. Drugs inhibit STAT3 (as Metformin) control tumor growth [22].

PI3K/AKT/mTOR Signaling Pathway

PI3K is over expressed in 60% of TNBC which leads to deletion or mutation in PTEN gene. AKT regulates "Bad" and thus can controls TNBC cells apoptosis [23]. PI3K/AKT pathway activation in TNBC cells which has been knocked down from ELK3 protein leads

to increase the chemosesitivity of cells to doxorubicin [24]. Using PI3K/AKT inhibitor as "Buparlisib" activates ERK and MEK1, which decrease expression of BRCA1. "Everolimus" (mTOR inhibitor) has antitumor activity against TNBC cancer cells [25].

Wnt/β-Catenin Signaling Pathway

Wnt/β-catenin is associated with poor prognosis in TNBC cells [26]. Using "ICRT-3" (Wnt/β-catenin pathway inhibitor) has been reported to inhibit TNBC cells proliferation [27]"Salinomycin"(anticancer drug) induces degradation of RLP6 (Wnt/β-catenin co-receptor) inhibiting TNBC cells' proliferation [28].

Hedgehog signaling pathway

The dysregulation of Hedgehog signaling pathway (Hh) enhances invasion and migration as well as metastasis in TNBC cells [29]. Furthermore, its activation in BC induces angiogenesis in a way that is independent on VEGF activation [29]. SMO receptors and GLI1 signaling components of this pathway are highly expressed in TNBC. This overexpression is responsible of poor prognosis and recurrence of the tumor [30]. GLI1 inhibitors reduce GLI1 expression and inhibit its binding to target DNA sequences [31]. Decreased GLI1 expression has been found to reduce tumor growth and viability of breast CSCs in a xenograft model [32].

Notch Signaling Pathway

Abnormal NOTCH signaling activation initiates tumor formation and enhances angiogenesis [33]. NOTCH signaling pathway has been inhibited by GSI (γ-secretase inhibitors) [34] NOTCH-1 has been found to be overexpressed in TNBC NOTCH-4 has a great role in initiation of TNBC [35].

Targeting Receptor Tyrosine Kinases in TNBC

RTKs are a group of cell surface receptors. They have specific structural features make them with high affinity to different polypeptides signaling molecules as: hormones, cytokines, and growth factor. It is responsible for regulation of many downstream signaling pathways as MAPK, JAK/STAT and PI3K/AKT. This regulation makes it a key factor in controlling cell growth, metabolism cell differentiation and proliferation [36]. The deregulation of these molecules has a key role in regulating tumorigenesis, angiogenesis, and metastasis. RTKs' overexpression in BC increases tumor aggressiveness and metastasis. Their multi-faceted roles make them promise molecular targets for targeted therapy in many cancers. However, gene amplification and alternate pathway activation make anti-RTK therapy challenging. The RTK family members, which are overexpressed, in TNBC are VEGFR [37], TGFβR [38], EGFR (HER1) [39], FGFR [40], PDGF [41] and IGF-IR [42]. TKI (tyrosine kinase inhibitor), known as "Erlotinib" and acts on EGFR, increases the expression of E-Cadherin and down regulates Vimentin expression in TNBC cells. This leads to change of mesenchymal phenotype to epithelial phenotype (EMT) [43]. Other TKI agents act on EGFR as

"Cetuximab", "geftinib" and "Panitumumab" does not produce any significant results when tested clinically [44]. "Sunitinib" inhibits PDGF& VEGF which induce reduction in tumor volume in TNBC cells when tested in xenograft models [45]. "Bevacizumab" inhibits VEGF, thus, decreases the progression of tumor in 35% of TNBC patients [46]. "Nintedanib" is a multi-targeted angiokinase inhibitor that acts against many growth factors, as PDGFR, VEGFR and FGFR [47]. In BC, the combination of chemotherapy paclitaxel enhances tumor regression in 50% of patients with HER2 negative BC [48]. It inhibits the effect of STAT3 activated molecules. This is the main mechanism by which Nintedanib induces apoptosis in TNBC cells. That is why it decreases the average tumor size in mice bearing MDA-MB-231 tumor.

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