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TRIPLE NEGATIVE BREAST CANCER AND DRUG-RESISTANT CELLS

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Abstract:

The triple negative breast cancer (TNBC) subtype is known to have a more aggressive clinical course compared to other breast cancer subtypes. Targeted therapy for this type of breast cancer is limited, and patients are primarily treated with conventional chemotherapy and radiotherapies, which are not specific and do not target resistant cells. Thus, one of the major clinical challenges is to find compounds that target drug-resistant cell populations responsible for the transformation of secondary tumors. Molecular profiling of different TNBC subtypes offers hope for better identifying these tumor-specific resistant cells. To this end, a better understanding of TNBC heterogeneity and cancer stemness is required . and extensive genomic analysis can help understand the complexity of the disease and highlight new molecular drivers that can be targeted in the clinic. The use of therapies targeting persistent cancer cells in combination with other treatments may provide major advances in improving survival for patients with TNBC.

Keywords: Triple Negative Breast Cancer , differentiation , persistent cells , tumor heterogeneity

RELEVANCE. Breast cancer (BC) comprises a diverse group of breast diseases in terms of molecular characteristics, clinical manifestations, and therapeutic response. Various biological and clinical subtypes of breast cancer have been identified using gene expression profiles, molecular pathology and histopathology [1]. Molecular subtyping facilitates the stratification of patients with breast cancer and helps in tailoring treatment to improve patient response to therapy [2]. The triple negative breast cancer (TNBC) subtype includes breast cancer tumors with negative estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2) expression status (ER-, PR-, HER2-) [2 , 3]. According to epidemiological studies, TNBC accounts for 10–20% of all breast cancer cases and is usually diagnosed in younger patients under the age of 35 years [4] . Patients typically present at later stages of the disease [5], and TNBC tumors are known to have a more aggressive clinical course compared to other subtypes [6].

1. Heterogeneity of TNBC

TNBC tumors are usually of the basal histopathological subtype [1 , 7] and are diagnosed at high pathological grade and tumor stage (stage III/IV) [5 , 7]. At the time of diagnosis, these tumors are more likely to show lymph node involvement [7 , 8] , and lymph node metastasis has been identified as an independent prognostic factor for TNBC [7]. Despite a high initial clinical response to neoadjuvant therapy, patients with TNBC continue to demonstrate a worse prognosis with a higher risk of distant metastases compared with other BC patients [5 , 9 , 10] and a short disease-free survival (DFS) at presentation. at a younger age [7]. A study conducted at the University of Toronto found that distant recurrence of TNBC after treatment peaked at approximately three years and showed that the mortality rate for TNBC was about 40% within 5 years of diagnosis [11] . Conventional treatments are ineffective in many cases, leading to tumor relapse due to long-term residual TNBC cancer cells in primary tumors and/or metastatic lesions [12]. Therefore, there is an urgent need to develop effective treatments for this aggressive subtype of breast cancer [14].

Currently, most studies of molecular subtyping of TNBC are based on tumor gene expression (GE) clustering [2]. TNBC tumors are classified into four to six different molecular subtypes based on the expression of characteristic GE profiles [10]. In 2011, Lehmann et al . classified TNBC tumors into six molecular subtypes using GE cluster analysis in a large number of TNBC samples (publicly available RNA profiling datasets of 14 patients and confirmed their results using seven other available datasets, n = 386 *)* . Based on gene ontologies and differential GE, analysis identified seven tumor clusters, of which six stable clusters exhibit a distinctive gene signature, namely: mesenchymal (M), mesenchymal stem-like (MSL), basal-like-1 (BL-1).) and -2, (BL-2), luminal androgen

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receptor (LAR), immunomodulatory (IM) subtype, and one additional unstable (UNS) tumor cluster that expresses genes found in the other six clusters [14 **]** . The study found that with adequate patient sample size, TNBC GE analysis can identify different subtypes and identify molecular targets, providing predictive biomarkers that help stratify patients for individualized treatment and therefore help improve patient response to therapy through the design of appropriate clinical trials. [8]. Lehmann et al also used the GE signature derived from tumors of TNBC patients to identify TNBC cell lines for each of these subtypes that represent clinically relevant models for functional testing of new targeted agents. Molecular stratification within six molecular subtypes described by Lehmann et al . also identified a subgroup with aberrant PTEN expression, five specific miRNA aberrations , high MYC expression, TP53 mutation, loss of RB1, and WNT signaling. These tumors were associated with poor clinical outcomes. Indeed, poor prognosis and high hazard ratios were associated with RhoA PTEN-low/ microRNA -low-high signaling in BL-1 tumors, increased AKT1 copy number/high mRNA expression in BL-2, and high programmed cell death 1 (PD1). expression in MI [**7**].

Further studies have classified TNBC into different subtypes. In 2015, Burstein et al . analyzed RNA and DNA profiles on 198 TNBC tumors from Baylor College of Medicine (Houston, TX, USA) and validated their results using seven publicly available TNBC datasets [9]. Their analysis identified four distinct subtypes of TNBC, including mesenchymal (MES or cluster 1), luminal androgen receptor (LAR or cluster 2), basal-like immunosuppressive (BLIS or cluster 3) and basal-like immune activated (BLIA or cluster 4) . . Each of these stable TNBC subtypes was characterized by the expression of different molecular profiles with different prognoses: BLIA tumors with better outcome and BLIS tumors with worse prognosis. These authors compared their work with Lehmann 's and found comparable results. They showed that cluster 1 included LAR tumors, clusters 2 and 3 included MSL tumors and some low claudin M tumors , cluster 3 and cluster 4 included BL-1 and BL-2 tumors without division into different subtypes, and finally IM tumors could be found in clusters 2 and 4 [15]. Interestingly, both laboratories found clusters of stromal , immune, and basal genes.

In a subsequent study, a hierarchical cluster analysis was performed including 2188 genes from the Lehmann study and TNBC's own data set, allowing the identification of four major GE clusters: luminal , immune, basal epithelial and stromal signature clusters [16 **]** . A similar hierarchical clustering was performed with 2188 genes and a dataset containing breast cancer samples, xenografts , normal breast samples, and breast cancer cell lines. This test showed that any of the cell lines or xenografts expresses genes with a high expression signature of the stromal /MSL or IM gene. This was the first experimental evidence that tumor samples belonging to the IM and MSL signature could be contaminated by cells of the tumor microenvironment, such as fibroblasts or immune cells [10] . Indeed, tumor infiltrating lymphocytes (TILs) are a major contributor to gene expression profiles, and correlations with this signature have been proposed as a descriptor of the immune status of TNBC tumors rather than as an independent subtype [1, 2] . Immune infiltration also has a positive effect on tumor prognosis [15], supporting the prognostic value of TIL-related gene expression for better relapse-free survival (RFS) regardless of TNBC subtype [13]. Lehmann et al later confirmed these results and refined the molecular subtypes into four different ones: BL-1, BL-2, M and LAR, thus eliminating IM and MSL as intrinsic subtypes [8, 14] .

2. TNBC and drug-resistant cells

2.1. Stem cells and cellular origin of TNBC

The cellular origin of TNBC is still debated [**1-3**] **HYPERLINK "https://www.mdpi.com/2072-6694/14/17/4280" \l "B35-cancers-14-04280"** . It is unclear whether the different subtypes of TNBC originate from mammary stem cells or progenitor cells. Stem cells (SCs) are unspecialized cells that have the ability to self-renew and can differentiate into the various cell types that make up the body's tissues. In normal human tissues, SCs give rise to new cells to maintain healthy organs. Likewise for cancer SCs support the persistence of malignant tumors by producing more cancer cells. The process of tumor initiation can be caused by the transformation of resident SCs. This cellular transformation can occur during tissue regeneration. Alternatively, they may be initiated and/or accelerated in response to metabolic dysregulation, toxins, infections, or radiation leading to genomic mutations . In 2003, Al-Haj et al identified a cell subpopulation in breast tissue capable of causing tumors. These cells were called " tumorigenic cancer cells".

Shackleton et al discovered rare mammary stem cells (MaSCs) in mouse mammary tissue that express neither the endothelial marker CD31 nor the hematopoietic markers CD45 and TER1. Cells expressing high levels of CD29 (β1 integrin) and the epithelial marker CD24 can restore milk protein-producing alveolar structures. These cells are also capable of generating new breast tissue and maintaining a stable pool of tissue-resident stem progenitor cells. This capacity for self-renewal and multipotency is also a property of human BC SCs (CSCs), which can be demonstrated in BC subtypes with shared gene ontologies similar to MaSCs [15 **]** .

Regardless of breast tumor molecular subtypes, breast CSCs exist in distinct mesenchymal -like and

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epithelial-like states based on their expression of CD44, CD24, and aldehyde dehydrogenase 1 (ALDH) markers. RNA sequencing analysis and immunofluorescence staining of breast cancer samples showed that mesenchymal-like CSCs mainly express CD44 $^{+}$/CD24 - ^{and} are predominantly inactive/inactive and localized at the tumor margins. In contrast, epithelial-like CSCs are proliferative, centrally located, and highly express ALDH genes . Breast CSCs exhibit high plasticity, allowing them to transition between epithelial-like and mesenchymal-like states. Interestingly, an abundance of CD44 $^{+}$/CD24 $^{-}$ cells was found in TNBC tumors compared to the luminal and HER2 subtypes. It has been proposed that the growth of the TNBC subtype originates from a population of CSCs or tumor-initiating cells bearing oncogenic gene mutations that are critical for tumor growth and response to therapy. CSCs play an important role in the aggressive behavior of TNBC. Studies in mice have shown that only 2% of these tumor-initiating cells can form secondary tumors . It should be noted that this aggressive cell population is mainly observed in the BL2 and MSL subtypes of TNBC. GE and gene ontology analysis showed enrichment of EMT and stemness -related pathways in these subtypes, including FGFR, mTOR , TGF-β, Rac1/ Rho , Wnt /β-catenin , PDGFR, and VEGF signaling [14 **]** . These pathways are commonly observed in normal breast tissue in CD44 $^{+}$/CD24 cells . Various studies have revealed the importance of SC markers and their prognostic role in TNBC. Indeed, CSC markers were closely associated with advanced tumor stage, tumor size, higher tumor grade, metastasis, and lymphatic involvement in TNBC patients [11].

2.2. Persister cells in TNBC

Recently, new research has focused on a discrete population of cells in tumors known as " persistent cancer cells." These are usually undetected cells that survive anticancer therapy and are considered a major cause of treatment failure [14]. Acquired resistance is observed in many pathologies, including infectious diseases and malignancies, often due to long-term treatment to select for these persistent/resistant cells [15] . This was recently demonstrated by Ramirez et al . that drug tolerance represents a phase between therapeutic sensitivity and resistance from which resistant/persistent clones can arise. These cells can survive and develop progressive drug tolerance, thus acquiring the ability to proliferate during treatment and correlate with high rates of cancer recurrence . Interestingly, it has been suggested that the terms "quiescent", "dormant", "tolerant" and " persistent " cells in cancer all describe one discrete population of tumor cells. In some cases, these cells show similarities to the molecular profile of SCs and are therefore described as " cancer stem cells." Connections between the properties of dormancy and stemness have already been established in many types of cancer, including breast cancer.

Persistent cells are very flexible in energy consumption and adaptation to the microenvironment. They have a slow proliferation rate due to their quiescent properties, but have the ability to re-enter the cell cycle, allowing them to proliferate to cause tumor recurrence. Reduced proliferation rates provide a selective advantage to resist treatment, thereby causing dormant cells to be enriched in a stem-like phenotype. Additional properties by which persister cells resist include their ability to take over their environment, creating an immunotolerant niche and . The mechanisms involved in the selection process are still unclear, but it is becoming increasingly clear that resistance is associated with the heterogeneity of cancer cells and that multiple mechanisms underlie the emergence of drug-resistant subpopulations [1 4 **]** . The mechanisms that drive their persistence offer highly sought after therapeutic targets, including epigenetic, transcriptional and translational regulatory processes, as well as complex cell-cell interactions [10 **]** .

Resistance to conventional treatments is commonly observed in TNBC tumors [12 , 13]. It is assumed that the residual resistant genotype, adaptively selected by chemotherapy, is responsible for treatment failure. This was confirmed by single cell sequencing analysis obtained from TNBC patients showing adaptive selection during neoadjuvant chemotherapy and resulting in high treatment failure in TNBC patients. In addition, many pathways are known to regulate the survival of TNBC CSCs, including Hedgehog .

An interesting mechanism by which TNBC tumors resist therapy is therapy-induced senescence (TIS) [8 , 13]. Senescence, or cell growth arrest, is a cell fate originally discovered in relation to growth arrest in cultured cells and is now recognized as an important mediator of numerous physiological and pathological processes. Oncogene-induced senescence (OIS) is thought to be a contributing factor to TIS in many cancer types, including breast tumors. Indeed, aging may contribute to cancer stemness formation and tumor aggressiveness, with the stemness state being identified as a mediator of the development of drug-resistant aggressive tumors. clones in these tumors. A study using paired gene expression analysis of 17 primary breast cancer biopsies before and after neoadjuvant chemotherapy found an increase in TGF-β signatures, a cytokine that was associated with breast CSC in treated samples. It was also noted that this unique gene signature was similarly altered in the human TNBC cell line SUM159 following treatment with paclitaxel , suggesting an enrichment of the population of CSCs with upregulation of genes involved in the TGF-β pathway following chemotherapy treatment. TGF-β is

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thought to be key for TIS due to its autocrine / paracrine pro-aging role in aging/aging-associated pathologies. Numerous potential TGF-β inhibitors are currently being tested in clinical trials as possible new treatments to improve the prognosis of patients with TNBC. These data suggest that targeting senescence may further be used in TNBC cancer therapy [10 , 1 , 7].

3. NEW THERAPEUTIC APPROACHES FOR TNBC

Despite advances in the discovery of new treatments, treating patients with TNBC remains extremely challenging [13]. This is due to the high heterogeneity of the disease and the lack of expression of receptors that are targeted by available treatment methods [15]. TNBC tumors do not respond to endocrine (hormonal) therapy or HER-2-targeting agents, so chemotherapy remains the primary systemic course of treatment [12]. Chemotherapy can be given in the neoadjuvant and/or adjuvant setting, and there is no significant difference in the likelihood of patient survival between these two types of treatment. However, neoadjuvant chemotherapy is now considered the standard therapeutic approach for high-risk TNBC because it helps reduce primary and metastatic disease. tumor prior to surgical resection and to evaluate tumor response and the potential need for adjuvant therapy. Treatment. The key role of immune checkpoint inhibitors in cancer treatment has recently been identified [9, 13]. BC immunogram is suggested to be a potential application that evaluates the tumor microenvironment and helps guide precision immunotherapy. Trials are currently ongoing on various combinations that will improve the effectiveness of immunotherapy.

Combination therapy has now also proven effective in enhancing the effect of innate immune responses against TNBC. Indeed, a viral mimicry response can be induced by using epigenetic inhibitors (histone methyltransferase inhibitors EZH2 and PRMT1), which induce the expression of double-stranded RNA derived from transposable elements [16, 17]. This response is capable of activating the interferon response, leading to a powerful antitumor effect. Additionally, a recent study linked H3K4me3 (histone H3 trimethylation at lysine 4) and H3K27me3 (histone H3 trimethylation at lysine 27) to the expression program of the persister cell population in TNBC tumors. H3K27me3 has been identified as a key activator of the persister transcriptional program , and its suppression reduces chemotherapy tolerance in TNBC models in vivo [16]. Current treatments available for TNBC subtypes

4. CONCLUSIONS

Over the past decade, great progress has been made in the treatment of TNBC, particularly through the use of molecular characterization of tumors to guide strategies for the development of specific treatments. Growing understanding of the importance of tumor heterogeneity, degree of tumor stemness and differentiation, as well as the development of resistant therapies for persistent cancer, opens new opportunities for targeted therapy for the treatment of TNBC. Molecular profiling of TNBC subtypes has identified therapeutic vulnerabilities that can be exploited to precisely treat patients that can be stratified according to the expression of subtype-specific gene signatures and mutations. However, the nature of CSCs in TNBC still remains unclear, since stemness and self-renewal are complex processes established and maintained by dynamic molecular networks and the tumor microenvironment. Thus, identifying new factors influencing TNBC stemness and drug resistance may help resolve this complexity and help identify new effective therapeutic strategies for TNBC.

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