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REVIEW ARTICLE

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Mesenchymal Subtype of Triple-Negative Breast Cancer: A Review of Specific Features

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Abstract

Triple-negative breast cancer (TNBC) is characterized by high invasiveness, high metastatic potential, proneness to relapse, and poor prognosis. Currently, four subtypes in the classification of TNBC are distinguished, which differ from each other in morphological manifestations, molecular genetic features, survival rates, prognosis parameters, and tumor resistance to therapy. A special place in this breast tumors group is occupied by the mesenchymal subtype, the frequency percentage of which varies from 7% to 28%, according to different data. The mesenchymal subtype of TNBC (M-TNBC) is characterized by the expression of molecular markers related to the epithelial-mesenchymal transition (EMT) program and cancer stem cells. M-TNBC has a highly aggressive behavior and worse prognosis due to its invasive and stem-like features, which correlate with metastatic dissemination and resistance to therapies. This review discusses the current knowledge regarding the mesenchymal TNBC subtype and its response to conventional therapeutic strategies. The complex approach to finding effective treatment options to restore immunocompetence in mesenchymal breast cancer patients is the final goal for further extended studies.(**International Journal of Biomedicine. 2023;13(1):14-19.**)

Keywords: triple-negative breast cancer • epithelial-mesenchymal transition • molecular markers

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Abbreviations

TNBC, triple negative breast cancer; **EMT**, epithelial-mesenchymal transition; **LAR**, luminal androgen receptor; **M-TNBC**, the mesenchymal subtype of TNBC; **pCR**, pathologic complete response.

Introduction

Triple-negative breast cancer (TNBC), a specific subtype of breast cancer that does not express estrogen receptor (ER) or progesterone receptor (PR) and lacks human epidermal growth factor receptor 2 (HER2) overexpression or amplification, is characterized by high invasiveness, high metastatic potential, proneness to relapse, and poor prognosis. Recently, it has been demonstrated that TNBCs are transcriptionally heterogeneous and can be grouped into subtypes with vastly differing biologies and responses to chemotherapy and targeted therapies. The mesenchymal subtype of TNBC (M-TNBC) is characterized by the expression of molecular markers related to the epithelial-mesenchymal transition (EMT) program and cancer stem cells. M-TNBC has a highly aggressive behavior and worse prognosis due to its invasive and stem-like features, which correlate with metastatic dissemination and resistance to therapies. This review discusses the current knowledge regarding the mesenchymal TNBC subtype and its response to conventional therapeutic strategies.

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TNBC classifications

In 2011, The Journal of Clinical Investigation published an article entitled "Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies" by Lehmann et al.[1] In the presented work, the authors analyzed gene expression (GE) profiles from 21 breast cancer data sets and identified 587 TNBC cases. Cluster analysis identified 6 TNBC subtypes displaying unique GE and ontologies, including 2 basal-like (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem-like (MSL), and a luminal androgen receptor (LAR) subtype. Furthermore, using a GE signature derived from TNBC patient tumors, authors identified cell-line models for each TNBC subtypes and differential responses to standard-of-care chemotherapy.

A review of the literature within the framework of the designated topic showed that the smallest number of studies, and, accordingly, scientific publications among the described TNBC subtypes over the past decade, is devoted to breast tumors with a distinct "mesenchymal" molecular genetic pattern (M and MSL types).

Lehmann et al.[1] showed that the M subtype displays a variety of unique gene ontologies that were heavily enriched in components and pathways involved in cell motility, ECM receptor interaction, and cell differentiation pathways (Wnt pathway, anaplastic lymphoma kinase pathway, and TGF-β signaling).

The MSL subtype is enriched with genes for similar biological processes with the M subtype, including cell motility and differentiation pathways. According to Lehmann et al.,[1] genes representing components and processes linked to growth factor signaling pathways are unique to the MSL. So, the prevalence of cell differentiation and growth factor signaling pathways was illustrated by the expression of TGF-β signaling pathway components (*TGFB1L1, BGN SMAD6, SMAD7, NOTCH1, TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, and TGFBR3*), EMT-associated genes (*MMP2, ACTA2, SNAI2, SPARC, TAGLN, TCF4, TWIST1, ZEB1, COL3A1, COL5A2, GNG11, ZEB2*, and decreased E-cadherin [*CDH1*] expression), growth factors (*FGF, IGF*, and PDGF pathways), and Wnt/β-catenin signaling (*CTNNB1, DKK2, DKK3, SFRP4, TCF4, TCF7L2, FZD4, CAV1, CAV2*, and *CCND2*). In addition, it was shown that the MSL subtype was also uniquely enriched in genes involved in angiogenesis (*VEGFR2 (KDR), TEK, TIE1,* and *EPAS1*). Thus, both M and MSL subtypes share elevated expression of genes involved in epithelial-mesenchymal-transition and growth factor pathways. Unlike the M subtype, the MSL subtype has decreased expression of genes involved in proliferation that were accompanied by enrichment in the expression of genes associated with stem cells (*ABCA8, PROCR, ENG, ALDHA1, PER1, ABCB1, TERF2IP BCL2, BMP2*, and *THY1*), numerous HOX genes (*HOXA5, HOXA10, MEIS1, MEIS2, MEOX1, MEOX2*, and *MSX1*), and mesenchymal stem cell-specific markers (*BMP2, ENG, ITGAV, KDR, NGFR, NT5E, PDGFRB, THY1*, and *VCAM1*).

In 2016, considering the complexity of the varying histological landscape of tumor specimens, Lehmann et al.[2] performed histological evaluation, laser-capture microdissection, RNA isolation, and gene expression analysis on a panel of TNBC tumors and provided significant evidence that the previously described IM and MSL TNBC subtypes represent tumors with substantial infiltrating lymphocytes and tumor-associated mesenchymal cells, respectively. Therefore, the authors refined TNBC molecular subtypes from six into four tumor-specific subtypes (TNBCtype-4: BL1, BL2, M, and LAR).[2]

It is important to point out that a year earlier, Burstein et al.[3], based on RNA and DNA profiling analyses conducted on 198 TNBC tumors, also identified and confirmed four distinct TNBC subtypes: luminal androgen receptor, mesenchymal, basal-like immunosuppressed, and basal-like immune-activated. The description by Burstein et al.[3] of the molecular genetic features of the isolated groups of TNBC allows us to assume that the basal-like immune-activated type correlates in its characteristics with the BL1 subtype proposed by Lehmann et al., in turn, the basal-like immunosuppressed type is similar to the BL2 according to the classification of Lehmann et al.[2] The LAR subtype and M subtype in Burstein et al.[3] and Lehmann et al.[2] have similar molecular features, respectively. These data allow us to conclude that in the TNBC classification, the 4 variants described above are firmly established, among which the mesenchymal subtype, despite a significantly lower percentage of its occurrence among TNBC, occupies a special place. The presented review is devoted to the morphology, genetic and molecular features of TNBC tumors, which have a distinct "mesenchymal" profile. In addition, we attempted to reflect on the information available at this stage regarding the disease's course, its progression parameters, resistance to therapy, and survival rates for this subtype of breast carcinoma.

Mesenchymal subtype of TNBC

Morphology and molecular genetic features

A review of literature devoted to aspects of the molecular nature and features of the clinical course of the mesenchymal subtype of TNBC (M-TNBC) suggests that the incidence of such neoplasms varies significantly.

Classification by TNBCtype-4[2] resulted in a distribution of 35% BL1, 22% BL2, 25% M, 16% LAR, and 2% unclassified.

In a study by Harano et al.,[4] among the 88 patients with TNBC, 21 (23.86%) had M-TNBC. Kim et al. [5] and Hartung et al. [6] defined M-TNBC in 11.5% and 16% of cases, respectively. In a study by Zhao et al.,[7] the identification of the TNBC subtypes by the protein expression of AR, CD8, FOXC1, and DCLK1 detected by immunohistochemistry, found M-TNBC (AR−, CD8−, FOXC1− and DCLK1+) in 13.3% of cases (28/210). In several types of cancer, DCLK1 (Doublecortin Like Kinase 1), a microtubule-associated gene, has been recognized as a marker of cancer stem cells that may serve as a potential therapeutic target. [7-10]

Kumar et al.,[11] for the classification of TNBCs (n=245), performed immunohistochemistry on tissue microarrays for cytokeratin 5/6, 4/14 (CK5/6, CK4/14), epidermal growth factor receptor (EGFR), vimentin, E-cadherin, claudin 3 and 7, androgen receptor (AR) and aldehyde dehydrogenase. The authors identified mesenchymal type (Vimentin+, E-cadherin-, claudin 3- and 7-) in 28.6% of cases.

The morphology feature of such tumors is the presence of metaplastic signs and foci of sarcomatoid and squamous cell differentiation, specifically for dedifferentiated and aggressive metaplastic breast carcinoma.[1,5,12-14] Although metaplastic carcinoma is a rare histological variant of breast cancer, it is known that the vast majority of such tumors have a TNBC phenotype and aggressive properties.[15] Clinically, most TNBCs are claudin-low tumors with a high frequency of metaplastic and medullary differentiation.[14] In other studies, it was noted that among the cases of TNBC having a molecular genetic pattern of the mesenchymal subtype, medullary carcinomas are absent, but metaplastic carcinomas occurring among them have a clearly defined fusiform, chondroid, and osteoid morphology.[2] A distinctive morphological feature of such breast neoplasms is also the poorly cohesive pattern of tumor growth, characterized by the fact that more than 50% of carcinoma cells are scattered or discretely located in the stroma.[5]

Mesenchymal TNBCs are enriched in EMT-associated genes and contain a high rate of aberrations in the PI3K/AKT/mTOR pathway.[16] However, Kumar et al.[17] showed PIK3CA (Phosphatidylinositol-4-5-bisphosphate-3-kinase catalytic subunit-α) mutations in 16.25% (13/80) of TNBC cases. PIK3CA mutations were frequent in the LAR subtype (33.3%), followed by the unclassified type (31.5%), mesenchymal (10.5%), and BL1 (5%) subtypes. PIK3CA is an integral component of the PIK3CA/AKT signaling pathway, and the evaluation of such aberrations aims to consider them as potential therapeutic targets in the treatment of TNBC.

Hill et al.[18] evaluated the expression of αvβ3 integrin in two M-TNBC cell lines, MDA-MB-231, and BT-549, by flow cytometry. The authors observed that both cell lines express very high levels of αvβ3. As known, the altered expression of $\alpha v\beta_3$ integrin has been well established as a driver of cancer progression, stemness, and metastasis. M-TNBC cells were treated with a novel peptide, ψRGDechi, developed by authors and characterized with the ability to selectively bind and inhibit $\alpha v\beta_3$ integrin. ψRGDechi was able to hamper adhesion, migration, and invasion of M-TNBC cells, as well as the capability of these cells to form vascular-like structures and mammospheres. In addition, the ψRGDechi-reversed EMT program inhibited mesenchymal markers.

The literature provides evidence that the mesenchymal subtype of TNBC is also characterized by high IL6 and JAK1 mRNA expression. IL6 and JAK1 are crucial in the activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway.[7,19] The JAK/STAT signaling pathway is regarded as one of the central communication nodes in the cell function. Many papers have reported the importance of this pathway in malignancies.[20-22] Zhao et al.[7] found that the M subtype of TNBC showed higher mRNA expression of both IL6 and JAK1, which are crucial upstream activators of the JAK/STAT3 pathway.[19] In addition, the phosphorylated STAT3 signature score was higher in the mesenchymal subtype than in the other subtypes of TNBC.[7]

The M-TNBC samples have high expression of PDGFR (platelet-derived growth factor receptor], but this subtype is not sensitive to the corresponding targeted therapy.[23] Platelet-derived growth factor (PDGF) promotes cell proliferation, survival, and migration, primarily of mesenchymal origin cells.[24] Reported abnormalities of the PDGF pathway include overexpression or amplification of PDGF receptors (PDGFRs), a gain of function point mutations, or activating chromosomal translocations.

Zheng et al.[25] found the overexpression of CD155, known as poliovirus receptor (PVR) or nectin-like 5, in both TNBC cell lines and tumor tissues. CD155 was associated with a mesenchymal phenotype and a poor prognosis in breast cancer patients. CD155 knockdown induced a mesenchymal-epithelial transition in TNBC cells and suppressed TNBC cell migration, invasion, and metastasis in vitro and in vivo. Moreover, CD155 knockdown inhibited TNBC cell growth and survival, reduced IL-6, TGF-β, and Smad3 expression, and inhibited Stat3 phosphorylation. The authors concluded that CD155 contributes to the aggressive behavior of TNBC, and targeting CD155 may be beneficial to M-TNBC patients.

The study of the molecular features of TNBC cell lines with a mesenchymal phenotype made it possible to show the presence of a high expression of the AXL receptor tyrosine kinase in them. Zajac et al.[26] found that AXL controls directed cell migration, most likely by regulating cell polarity. Given the role of AXL in cancer development, metastasis, and drug resistance, AXL holds great promise as a prognostic biomarker and therapeutic target.

Analysis of the literature data showed that only a few publications are devoted to studying the relationship between different subtypes of TNBC and specific clinical parameters of patients. Kumar et al.[11] showed that M-TNBC was associated with a younger age group. In the literature, we did not find other research results regarding the characteristics of clinical parameters (primary tumor size, localization, menstrual function, the presence of a multifocal growth form, etc.) in different types of TNBC.

Lymph node metastasis and distant metastasis

M-TNBC is characterized by the expression of molecular markers related to the EMT program and cancer stem cells. It has a highly aggressive behavior and a worse prognosis due to its invasive and stem-like features that correlate with metastatic dissemination and resistance to therapies.[18] The literature presents data indicating TNBC carcinomas' ability to form a "vascular-like" network (the phenomenon of vascular mimicry), significantly contributing to metastasis.[27-29]

In a study by Lehmann et al.,[2] regional spread to lymph nodes occurred in 34% of TNBC. There was a significant enrichment of lymph node metastasis in LAR-TNBC, with nearly half (47%) of these patients displaying regional spread (P=0.0278). Lymph node involvement was lower for M-TNBC (21%). Using published datasets with metastasis-site annotations (GSE12276, GSE2034, and GSE2603), Lehmann et al.[2] identified 124 patients with site-specific metastasis data and examined the metastatic pattern in TNBC subtypes. In TNBC, the brain, bone, and lung metastasis incidence was found in 11%, 19%, and 31%, respectively. Stratification by the TNBCs subtype did not show any statistical differences in the brain (P=0.1238) and lung (P=0.0776) metastasis.

However, the mesenchymal subtype displayed a significantly higher frequency of lung metastasis (46%) compared to all other subtypes (25%) (P=0.0388).

Response to therapy and prognosis

In the treatment of TNBC, given the lack of the possibility of using targeted hormonal methods, chemotherapy remains the standard of care for TNBC treatment, but unfortunately, patients frequently develop resistance. [30-33] It has become evident that the development of TNBC chemoresistance is multifaceted and based on the elaborate interplay of the tumor microenvironment, drug efflux, cancer stem cells, and bulk tumor cells.[34]

The mesenchymal subtype, characterized by more aggressive behavior due to increased invasive properties and more pronounced stem-like features, has a worse prognosis in terms of drug sensitivity and resistance.[18,35] However, the data on this issue is ambiguous.

Masuda et al. [36] investigated the clinical relevancy of TNBC heterogeneity by determining pathologic complete response (pCR) rates after neoadjuvant chemotherapy based on TNBC subtypes. The pCR rate for all patients was 28% (37/130). BL1 had the highest pCR rate (52%); BL2 and LAR had the lowest pCR rates (0% and 10%, respectively), and a mesenchymal subtype had a pCR rate of 31%. However, despite its lower pCR rate, LAR had the best overall survival rate; the mesenchymal subtype had the worst.

Similar results were presented in work by Zhao S. et al.[7] The authors developed an immunohistochemistry (IHC)-based approach by the protein expression of AR, CD8, FOXC1, and DCLK1 to classify TNBCs into molecular subtypes. After adjustment for other prognostic factors in multivariate analysis, the IHC-IM, IHC-LAR, and IHC-BLIS subtypes were associated with better relapse-free survival than the IHC-MES subtype (AR−, CD8−, FOXC1− and DCLK1+).

As known, carcinoma cells can undergo EMT that confers mesenchymal traits on carcinoma cells and drives their metastatic dissemination. These mesenchymal-like cells display the functional behavior of mesenchymal stromal cells. Notably, mesenchymal stromal cells can inhibit the anti-tumor immune response through either carcinoma-associated fibroblasts or bone marrow stromal cells. Experimental data have indicated their relevance in regulating cytolytic effector lymphocytes of the innate and adaptive arms of the immune system. The phenotypic and functional features of mesenchymal-like cells can support tumor growth and proliferation.[37]

Dongre et al.[38] demonstrated that tumors arising from more mesenchymal carcinoma cell lines exhibiting EMT markers expressed low levels of MHC-I, high levels of PD-L1, and contained within their stroma regulatory T cells, M2 (protumor) macrophages, and exhausted CD8+ T cells. Accordingly, such tumors were less susceptible to therapeutic regimens.

In a study by Harano et al.,[4] analysis of 88 cases of TNBC, taking into account the obtained data on the expression of immunomodulatory genes and the assessment of tumor-infiltrating lymphocytes (TILs), revealed 39 tumors that were identified as tumors with high expression of immunomodulatory genes (IM+) involved in immune cellular processes. One of the important results was the complete absence of mesenchymal carcinomas among IM+ TNBC tumors.

However, some studies show no significant differences in response to neoadjuvant chemotherapy between different subtypes of TNBC, which requires further extended searches in this direction.

Conclusion

TNBC is a group of malignant tumors with pronounced intratumoral heterogeneity, characterized by an aggressive course, poor prognosis, high incidence of recurrence and metastasis, and resistance to ongoing chemotherapy. To date, there is compelling evidence that these carcinomas also differ from each other in terms of biological behavior and manifestations, as well as in terms of progression and sensitivity to therapy. TNBC accounts for 15–25% of all breast cancers.[23] Tumors with morphological and molecular manifestations of the mesenchymal phenotype (M-TNBC) are clearly defined in the TNBC group.[1,39-47] The M-TNBC has a highly aggressive behavior and worse prognosis due to its invasive and stem-like features that correlate with metastatic dissemination, resistance to therapies, and poor prognosis. Finding effective treatment options for TNBC subtypes, especially M-TNBC, remains a critical clinical need.

Competing Interests

The authors declare that they have no competing interests.

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