Molecular Characteristics of Triple-Negative Breast Cancer

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Researchers have investigated the molecular mechanisms of breast cancer initiation and progression, especially triple-negative breast cancer (TNBC), in order to identify specific biomarkers that could serve as feasible targets for innovative therapeutic strategies development. TNBC is characterized by a dynamic and aggressive nature, due to the absence of estrogen, progesterone and human epidermal growth factor 2 receptors.

triple-negative breast cancer | inflammasome | pyroptosis

1. Introduction

The breast is a complex structure, which is organized in 15 to 25 lobes of different sizes that are connected with lactiferous ducts that terminate in the nipple, ductal formations and glandular tissue, all of these being surrounded by fibro-adipose tissue [1]. Specifically, the adult breast is a tissue highly variable in conformation, adiposity and volume, due to the different proportions between adipose, fibrous and glandular tissue, among which are also found blood and lymphatic vessels. The corresponding distribution of fat and collagenous components differs among women and is influenced by hormonal, physiologic and environmental factors [2].

Breast cancer (BC) is a dynamic, aggressive and heterogeneous disease that is the principle cause of death among women worldwide, but that also affects men [3]. This neoplasm has the potential to be determined by exposure to both genetic and non-genetic risk factors such as gender, age, menopause, nulliparity, obesity, alcohol abuse and exposure to hormones, radiation or therapy [1]. The early detection and diagnosis of BC is based on screening techniques (ultrasound, mammography, contrast-enhanced digital mammography, magnetic resonance imaging and positron emission tomography), microwave imaging techniques (microwave tomographic, radar-based microwave imaging and radiometry), biomarker-based techniques (radioimmunoassay, immunohistochemistry, enzyme-linked immunosorbent assay and fluoroimmunoassay) and breast tissue biopsies, which are used to differentiate between malign and benign tumors [4].

Following a diagnosis of BC, it is necessary to stage it according to the American Joint Committee on Cancer (AJCC) tumor, nodes, and metastasis (TNM) system, as TNM staging is used to define and stratify the size of the tumors (T), the status of regional lymph nodes (N), and distant metastasis (M) [5]. TNM staging is divided into four classes: (I) clinical staging, which includes information from clinical examination; (II) pathological staging, which includes the affected anatomical formations; (III) post-therapy staging, which includes clinical and pathologic information; and (IV) restaging, if necessary [6].

In order to establish the most efficient treatment for patients, the histological classification is completed via the molecular classification of BC that is based on the expression profiles of the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki67 antigen. The main molecular subtypes of breast cancer are luminal A (ER positive, PR positive and HER2 negative), luminal B (ER positive, PR negative and HER2 negative), HER2-enriched (ER negative, PR negative and HER2 positive), triple-negative breast cancer (TNBC), basal-like (ER negative, PR negative and HER2 negative), claudin low (ER negative, claudin negative, vimentin positive, E-cadherin low) and normal breast-like (adipose tissue gene signature) [7]. Luminal and HER2-enriched subtypes are associated with good prognoses and are highly responsive to therapy, resulting in a greatly improved outcome, while TNBC is the most aggressive subtype of BC, which is characterized by a high cell proliferation rate and a tendency to relapse [8]. Experimental studies indicate the presence of two main pathways involved in low-grade and high-grade breast tumorigenesis; low-grade BCs are regularly ER positive, PR positive and HER2 negative, while high-grade BCs are ER negative, PR negative and HER2 positive [9].

The mechanism of BC is dependent on the genetic modification and molecular processes that determine initiation, transformation and progression from normal tissue to tumor tissue [10]. More than 90% of diagnosed BCs are associated with the mutation of specific genes, such as breast cancer genes 1 and *2* (*BRCA1* and *BRCA2*), *TP53*, phosphatase and tensin homolog (*PTEN*), serine/threonine kinase 11 (*STK11*), ataxia-telangiectasia mutated (*ATM*), BRCA1 interacting protein 1 (*BRIP1*) or the partner and localizer of BRCA2 (*PALB2*), etc. [11].

In addition, BC aggressiveness is supported by chronic inflammation considering the up-regulation of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, IL-18 or tumor necrosis factor-α (TNF-α), growth factors or free radicals [12]. The secretion and release of pro-inflammatory cytokines are associated with inflammasome complex activation. Inflammasome is a cytoplasmic multiprotein complex, composed of a sensor (NOD-like receptor protein (NLRP)), an adaptor (an apoptosis-associated speck-like protein containing a caspase-activation and recruitment domain (CARD) (ASC)) and effector molecules (pro-caspase). The inflammasome complex is involved in numerous physiological and pathological mechanisms, including different types of cancer, although the activation of this complex can have both a positive or negative impact on carcinogenesis [13]. First of all, inflammasome activity stimulates epithelial mesenchymal transition (EMT), metastasis and angiogenesis, while inhibiting apoptosis and enhancing tumor development. On the other hand, the inflammasome pathway is also associated with immune reactions and the programmed death of tumor cells through pyroptosis [14].

The dynamic interaction between BC and inflammasome is regulated by a complex molecular network, including non-coding RNAs (ncRNAs). NcRNAs can be classified into short non-coding RNAs (sncRNAs) or long non-coding RNAs (lncRNAs), based on their number of nucleotides [15]. Among the most studied sncRNAs are microRNAs (miRNAs), which are single-stranded ribonucleic acid molecules involved in diverse cellular processes (cell survival, proliferation and adhesion, motility, cell death, inflammation, carcinogenesis, etc.). According to their implication in tumorigenic mechanisms and metastasis, miRNA molecules can be classified into oncogenic miRNAs and suppressor miRNAs [16]. Increasing evidence has indicated that many miRNAs are involved in the regulation of the inflammasome complex (miR-7, miR-9, miR-20a, miR-21, miR-23a, miR-30e, miR-33, miR-132, miR-133, miR-146, miR-155, miR-223, miR-296, miR-377, miR-711, etc.) and that these molecules represent a link between

inflammasome activity and BC, especially TNBC [17]. Recently, researchers' attention has been directed towards targeting these molecules as possible therapeutic strategies for the treatment of BC, due to their significance in post-transcriptional regulation [18].

2. Molecular Characteristics of TNBC

TNBC is a highly invasive and aggressive type of BC, which is characterized by the absence of ER, PR and HER2 expression. TNBC represents almost 20% of all diagnosed BC, being associated with resistance to chemotherapy, a predisposition to metastasis, poor prognoses and reduced survival rates [19]. Over the years, researchers have studied the molecular signatures of TNBC using advanced techniques and, according to gene expression profiles, Lehmann et al. [20], Burstein et al. [21] and Jezequel et al. [22] have all proposed different classification of TNBC in order to investigate possible therapeutic targets to improve the outcomes of TNBC (**Table 1**). Moreover, according to Lehmann et al., TNBC can be subdivided into six groups: basal-like 1, basal-like 2, mesenchymal, mesenchymal stem-like, immunomodulatory, and luminal androgen receptor TNBC [20]. Burstein et al. proposed a TNBC classification divided into four subtypes: basal-like immune-activated, basal-like immune-suppressed, mesenchymal, and luminal androgen receptor TNBC [21], while Jezequel et al. divided triple-negative tumors into three clusters: C1 (22.4%), C2 (44.9%) and C3 (32.7%) [22]. Classifying and understanding the particularities of TNBC allow for the development of personalized medicine, because each subtype has different characteristics and responses to anti-tumor therapy [23].

Table 1. Molecular comparison between three proposed classifications of TNBC.

TNBC Classification	Method of Analyses	Number of Patients	Subtypes	Abnormal Mechanisms	Relevant Markers	Therapeutic Strategies	Refs.
The Vanderbilt Subtype	K-means clustering	586	Basal-like 1	Cell cycle Cell proliferation DNA damage response	MYC, PIK3CA, CDK6, AKT2, KRAS, FGFR1, IGF1R, CCNE1, CDKN2A/B, BRCA2, PTEN, MDM2, RB1, TP53, KI67	PARP inhibitors HDAC/DNMT inhibitors Natural-killer therapy Cisplatin,	[20] [24] [25] [26]
			Basal-like 2	EGFR, MET, NGF, Wnt/β-catenin, TP63, IGF1R signaling pathway	TP53, TP63, EGFR, MET, BRCA1, RB1, PTEN, CDKN2A, UTX	mTOR inhibitors Growth factor inhibitors (lapatinib, gefitinib, cetuximab, etc.)	[20] [24] [27]

TNBC Classification	Method of Analyses	Number of Patients	Subtypes	Abnormal Mechanisms	Relevant Markers	Therapeutic Strategies	Refs.
				Glycolysis Gluconeogenesis			
			Immunomodulatory	Th1/2, IL-7, IL-12 signaling pathway	TP53, CTNNA1, DDX18, HUWE1, NFKBIA, APC, BRAF, MAP K4, RB1, CTLA4, PDL1	PD1/PDL1/CTLA4 inhibitors Cisplatin PARP inhibitors	[20] [24]
			Mesenchymal-like	Cell motility Cell proliferation Cell differentiation Wnt, TGFβ, Notch signaling pathway Epithelial-mesenchymal transition	PTEN, RB1, TP53, PIK3CA, VEGFR2, PI3KCA	mTOR inhibitors Drugs targeting epithelial–mesenchymal transition Abl/Src inhibitor Dasatinib	[20] [24] [28]
			Mesenchymal stem-like	Cell motility Cell differentiation Growth factor signaling Epithelial–mesenchymal transition Low proliferation	ABCA8, PROCR, ENG, ALDHA1, PER1, ABCB1, TERT2IP, BCL2, BMP2, THY, HOXA5, HOXA10, MEIS1, MEIS2, MEOX1, MEOX2, MSX1, BMP2, ENG, ITGAV, KDR, NGFR, NT5E, PDGFR, THY1, VCAM1, VEGFR2	mTOR/MEK/PI3K inhibitors, Src antagonists Antiangiogenic drugs Abl/Src inhibitor Dasatinib	[20] [24]
			Luminal androgen receptor	Steroid synthesis, porphyrin metabolism,	DHCR24, CD166, FASN, FKBP5,	Anti-AR therapy PI3K/CDK4/6 inhibitors	[20] [24] [26] [29]

TNBC Classification	Method of Analyses	Number of Patients	Subtypes	Abnormal Mechanisms	Relevant Markers	Therapeutic Strategies	Refs.
				Androgen/estrogen metabolism	APOD, PIP, SPDEF, CLDN8		
The Baylor Subtype	Non-negative matrix factorization	198	Luminal androgen receptor	Steroid hormone biosynthesis Porphyrin and chlorophyll metabolism PPAR signaling pathway Androgen and estrogen metabolism Hormonale-mediated signaling	TP53, PI3KCA, AKT1, ERBB2, ERBB4, CDK4/6, AR, MUC1, ER, CDH1, KRT7, KRT8, KRT18, KRT19, XBP1, FOXA1	Anti-AR/MUC1 therapy	[21] [30] [31] [32] [33] [34]
			Mesenchymal	Cell motility Epithelial–mesenchymal transition Focal adhesion TGF-β signaling pathway Adipocytokine signaling pathway	PIK3CA, PTEN, STAT3, IGF1, prostaglandin, TGF-β, Wnt, β-catenin, PDGFRα, c-Kit, ABC transporter	TKI/RAS/mTOR inhibitor Growth factor inhibitors	[21] [30] [31] [32] [33]
			Basal-like immunosuppressed	Mitotic cell cycle Mitotic prometaphase M phase of mitotic cell cycle DNA replication DNA repair Immune response Innate immune response	VTCN1, TP53, CENPF, BUB1, PRC1, VTCN1, MS4A6A, MTBP, FGFR2, BARD1, RNASE6	VTCN1 inhibition	[21] [31]
			Basal-like immune-activated	Cytokine–cytokine receptor interaction T cell receptor signaling pathway B cell receptor signaling pathway Chemokine signaling pathway	CCR2, CXCL13, CXCL11, CD1C, CXCL10, CCL5, STAT	Drugs targeting stat signal transduction molecules and cytokines	[21] [31] [35]

TNBC Classification	Method of Analyses	Number of Patients	Subtypes	Abnormal Mechanisms	Relevant Markers	Therapeutic Strategies	Refs.
The French Subtype	Fuzzy clustering	194 [47]		NF-kB signaling pathway [46]			
			Cluster 1	Luminal androgen receptor enriched	AR, Hsp90, PI3K, FGFR4, TTN, TNR, PKHD1L1, SPTA1, NCKAP5, COL15A1, ANKRD11, MYLK	Anti-AR therapy	[36] [37] [38]
			Cluster 2	Basal-like with low immune response High M2-like macrophages High pro-tumorigenic Low anti-tumor immune response [49]	CCL2, CCL5, CCL18, CCL10, CXCL22, IL4, IL8, IL10, IL13, TGFβ1, CD206, CD204, VEGF, Aginase1, PIK3CA, NF1, AKT1, FBN3, ABCC1, DNHD1	M2 inhibition Repolarization of M2 into M1 macrophages	[20] [38] [39] [40] [41] [42] [43] [44] [45]
			Cluster 3	Basal-enriched High immune response Low M2-like macrophages Low pro-tumorigenic High anti-tumor immune response	IL-1β, IL-6, IL-12, IL-23,CXCL9, TNF-α, CCL2, IFNγ, GSF10, DNAH1, CDH23, AHNAK2, GTF3C1	Repolarization of M2 into M1 macrophages	[38] [41] [45]

involved in the development of T nesis, to s are the nzymes, sis. Pro- na; IL-1, , TNBC, BC is the being the n [48]. up new genesis, dard for bine and braxane, d drugs), requires several tion, but alopecia, uropathy, neutropenia, pyrexia, anemia, thrombocytopenia, electrolyte abnormalities, infection, etc.) and do not lower the relapse rate [50].

Table 2. SOC for TNBC.

Approach	Class of Agents	Examples of Therapy	Mechanism of Action	Refs.
Neoadjuvant	Anthracycline + Taxane	Doxorubicin + Cyclophosphamide + Paclitaxel Epirubicin + Cyclophosphamide + Nab-paclitaxel	Inhibition of DNA and RNA synthesis Inhibition of topoisomerase II enzyme Generation of reactive oxygen species (ROS) Stabilization of microtubules	[49] [51]
	Fluoropyrimidine + Taxane	Capecitabine + Docetaxel		
	Fluoropyrimidine + Epothilone	Capecitabine + Ixabepilone		

P-binding ne-protein 1 family, protein D 2 (BCL2), ncer type tif) ligand (CCL), cyclin E1 (CCNE1), C-C chemokine receptor type 2 (CCR2), cl cadherin (CDH),

Approach	Class of Agents	Examples of Therapy	Mechanism of Action	Refs.
Adjuvant	Anthracycline + Taxane	Doxorubicin + Cyclophosphamide + Docetaxel		[49] [52]

potential of other agents and interfere with various molecular mechanisms (such as angiogenesis, the immune response, the cell cycle, etc.) have been tested or are currently under testing and approval (Table 3). The optimal treatment scheme for TNBC is a great challenge due to the disease's heterogeneity and increased risk of relapse, so the researchers have explored several promising anti-tumor agents (pembrolizumab, bevacizumab, olaparib, sacituzumab govitecan, etc.) [53]. Other therapeutic approaches are represented by platinum salts (carboplatin, cisplatin, etc.) Immunotherapies that target the programmed cell death-1 (PD-1) receptor/PD-L1 pathway, immune checkpoint inhibitors, poly-adenosine diphosphate ribose polymerase (PARP) inhibitors or AKT inhibitors [54].

Table 3. Novel strategies for TNBC treatment.

Class of Agents	Examples of Therapy	Mechanism of Action	Refs.
PD-1 and PD-L1 inhibitors	Pembrolizumab + Paclitaxel Doxorubicin + Cyclophosphamide Pembrolizumab + Paclitaxel + Carboplatin Durvalumab + Nab-paclitaxel Atezolizumab + Nab-paclitaxel Atezolizumab + Nab-paclitaxel + Carboplatin	Reactivation of the anti-tumor immune response PD-1/PD-L1 complex formation inhibition	[55]
Platinum-based therapy	Carboplatin + Eribulin Gemcitabine + Carboplatin + Iniparib Carboplatin + Bevacizumab Cisplatin + Paclitaxel + Everolimus Paclitaxel + Carboplatin	Double-strand DNA break Apoptosis initiation	[56] [57] [58]
Cell cycle inhibitors	Trilaciclib, etoposide, abemaciclib, prexasertib	Activate the spindle assembly/mitotic checkpoint Prolonged mitotic arrest Cell death initiation	[59]
Angiogenesis inhibitors	Cisplatin + Bevacizumab Anlotinib, apatinib, afatinib, lenvatinib, erlotinib, famitinib, pyrotinib	Blocking new blood vessel formation Tumor growth inhibition VEGF signaling pathway disruption	[60]
PI3K/AKT/mTOR inhibitors	Rapamycin, ipatasertip, buparlisib, pictilisib, alpelisib	Cancer cells migration and invasion inhibition Apoptosis initiation	[61] [62]
PARP inhibitors	Olaparib + Carboplatin + Paclitaxel Veliparib + Carboplatin Cisplatin + Rucaparib Veliparib, niraparib, talazoparib	Double-strand DNA break Cell death initiation Base excision repair	[63]

Class of Agents	Examples of Therapy	Mechanism of Action	Refs.
		Relax/condense chromatin bind nucleosom PARylate H1/H2B	
EGFR inhibitors	Bintrafusp Alfa, dasatinib, geftinib, sorafenib, nimotuzumab, panitumumab, erlotinib, osimertinib	Cell death initiation Inhibition of cancer cell proliferation Blocking dimerization of receptors, auto-phosphorylation and downstream signaling Inducing receptor internalization, degradation and stable downregulation	[64]
Androgen receptor (AR) antagonists	Bicalutamide, enzalutamide, abiraterone, palbociclib	Decrease in cancer cell viability G1 phase arrest Apoptosis induction	[30] [65]
Antibody drug conjugates	Sacituzumab govitecan, Ladiratuzumab vedotin, Trastuzumab deruxtecan	Cell growth and migration inhibition Binding to the topoisomerase in DNA replication inhibition S-phase-specific cell death initiation DNA damage	[66]

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strategies
nsport (or
enhanced permeability and retention (nanoparticles)) and active transport (miRNA and aptamers) [49].

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