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Is androgen receptor targeting an emerging treatment strategy for triple negative breast cancer?

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ABSTRACT

Triple negative breast cancer (TNBC) is an aggressive breast cancer subtype. The absence of expression and/or amplification of estrogen and progesterone receptor as well as ERBB-2 prevent the use of currently available endocrine options and/or ERBB-2-directed drugs and indicates chemotherapy as the main current therapy. TNBC represents approximately 15% of breast cancer cases with high index of heterogeneity. Here, we review the role of androgen receptor in breast carcinogenesis and its association with alterations in the expression pattern and functional roles of regulatory molecules and signal transduction pathways in TNBC. Additionally, based on the so far preclinical and clinical published data, we evaluate the perspectives for using and/or developing androgen receptor targeting strategies for specific TNBC subtypes.

Introduction

Breast cancer is one of the most common and clinical challenging type of cancer in women and among the leading causes of death in the female population worldwide, highlighting the need for developing effective strategies for breast cancer treatment [1]. Breast cancer represents a heterogeneous complex of distinct subtypes that are characterized by variable clinical outcome and different expression patterns of steroid hormone receptors and transcription factors that are involved in carcinogenesis [2,3]. Based on whole genome microarray analysis, breast tumors were classified into major subtypes distinguished by differences in their molecular portraits [4].

In recent years, a new breast cancer type has been identified and considered as a difficult to treat and aggressive malignancy. Triple negative breast cancer (TNBC) is a group of tumors, characterized by the absence of estrogen receptor (ER) and progesterone receptor (PR) expression as well as ERBB-2 over-expression and/or amplification [5]. Furthermore, it is associated with several chromosomal abnormalities and genomic instability, resulting in poor prognosis and increased rate of mortality [6,7]. In addition, TNBC shares common immunohistochemical features with basal-like cancer (BLC) type, expressing specific proteins such as epidermal growth factor receptor (EGFR) and cytokeratins 5/6 that are usually correlated with high histological tumor grade and enhanced invasive capacity [8]. Therefore, the in-depth elucidation of the molecular events modulating triple negative breast carcinogenesis will provide further prognostic and therapeutic avenues [9].

Such field of intense investigation is the role of androgen receptor (AR) and the associated signaling cascades. AR is a steroid hormone and is expressed in about 90% of breast carcinomas [10]. In TNBC, AR is detected in about 30% of them and its significance in the initiation and progression of tumor remains obscure [11]. Emerging studies have revealed conflicting data on the biological role of androgens in TNBC and the association of AR expression with prognosis and clinical outcome [12]. However, agents targeting AR are being evaluated in TNBC patients in early stage clinical trials with promising results [13]. The aim of this review is to summarize the current data in preclinical and clinical level regarding AR targeting in TNBC, as well as to highlight the future perspectives of the current and future AR-directed strategies in breast cancer therapeutics.

Classification of TNBC and associated signaling cascades

For many decades, breast cancer was categorized according to histological features with insufficient impact on the clinic. Thus, an accurate classification of clinically significant breast cancer subgroups based on clinic-pathological parameters and the expression of predictive biomarkers such as ER, PR and ERBB-2 was needed [14]. Studies based on microarray gene expression analyses have

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classified breast cancers according to an 'intrinsic' gene set into five molecular subtypes of breast cancer: luminal A, luminal B, BLC, HER2-enriched and normal breast-like [4,15].

Although the majority of TNBC have been identified as BLC phenotype, according to molecular analysis, these two categories are not considered as synonymous in clinical and research setting [16]. Further genomic and molecular analysis led the current classification of TNBC which is essential in the development of novel effective therapies based on crucial molecular drivers. Genomic studies using 587 TNBC cases led to the establishment of six TNBC subgroups with unique gene expression profiles and ontologies: basal-like 1 and basal-like 2 (BL1, BL2), mesenchymal (M), mesenchymal stem-like (MSL), immunomodulatory (IM) and luminal androgen receptor (LAR) [17]. More extensive studies have elucidated the molecular signaling pathways implicated in each subtype separately, suggesting molecular events that can be pharmacologically targeted (Table 1). Gene amplifications, deletions or mutations in well-recognized signaling pathways have been observed in 90% of TNBC patients [18]. Mutations in the *p53* tumor-suppressor gene and increased expression of genes associated with proliferation, such as *myc* and *ki67* and lack of the retinoblastoma (Rb) protein are mainly observed in BL TNBC [19]. Along with this, BL TNBC show elevated levels of proteins involved in DNA damage response pathways [17]. In addition, BL TNBC have common clinic-pathological features with mutant-*BRCA1* breast cancer and constitute the main malignant subtype identified in carriers of mutant *BRCA1* allowing the use of novel therapeutic approaches, such as poly (ADP-ribose) polymerase (PARP) inhibitors [17]. Research in this subtype of TNBC has also focused on epidermal growth factor receptor (EGFR), demonstrating that mutations in *EGFR* are rare but high/increased *EGFR* gene copies are related to worse outcome [20], while EGFR inhibitors are already being tested in the clinical setting [21].

Mesenchymal and mesenchymal stem-like types share similar expression profiles of upregulated growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR) and overexpressed signal transduction pathways, such as phosphatidylinositol-3-kinase (PI3K)/mammalian target of rapamycin (mTOR) and Wnt/β-catenin [22]. M and MSL subtypes have been also found to be enriched in gene ontologies for epithelial–mesenchymal transition (EMT) and cell motility pathways, which is indicated by the overexpression of Src kinase [23]. These subtypes can be distinguished through the different expression patterns of proteins involved in breast carcinogenesis [22]. In detail, the M TNBC exhibits high expression levels of cell proliferation proteins (Ki-67), while the MSL subtype overexpresses EGFR, the phosphorylated protein kinase B (AKT) and the mutant form of PI3K [17].

Immunomodulatory TNBC subtype has similar phenotype with the gene expression profile of immune system. In particular, clinical studies have highlighted the upregulated pattern of various immuno-regulated molecules that determine the profile of immune-modulatory cells [17]. Immunohistochemical analysis have indicated elevated levels of transcriptional factors, including interferons and tumor necrosis factor (TNF) as well as hyperactive Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and Nuclear factor-kB (NF-kB) signaling cascades [2,17]. Although JAK inhibitors are currently being tested in clinical trials, preclinical data support that a crosstalk between signaling pathways participate in TNBC progression [24].

LAR subtype is characterized by elevated levels of AR, upregulated endogenous AR-ligands and *PIC3CA* mutations [17]. LAR tumors represent 11% of TNBC and almost 2% of all breast carcinomas, while the majority is classified as luminal A or luminal B [17]. However, clinical studies have shown that AR inhibitors might be effective also in BL TNBC patients, suggesting the existence of at least two subgroups of AR-expressing TNBCs [25]. Additionally, recently published data support the induction of protein kinase C (PKC)-associated pathway as a crucial modulator of LAR TNBC growth and metastasis, creating opportunities for novel therapeutic targets [26].

Table 1
Molecular subtypes of TNBC.

Subtype	Main mutations	Cellular pathways
BL-1	*BRCA1/2*	Cell cycle division, DNA damage response
BL-2	*BRCA1/2*, *TP53*, *ATM*	Cell cycle division, DNA damage response, Growth factor signaling pathways
IM	*TP53*	Cytokine pathways, Immune cell signaling pathways
M	*PI3KCA*, *PTEN*	Cell motility, Cell differentiation pathways
MSL	*TP53*	Cell motility, Cell differentiation pathways, Growth factor signaling pathways
LAR	*PI3KCA*, *PTEN*	Hormone regulated pathways, including AR signaling

The role of AR in TNBC

As nuclear receptor, AR is composed of four distinct domains that determine its' activity. The main active region is the amino-terminal domain (NTD), including multiple binding sides for TFs and/or transcription co-factors as well as the activator function (AF)-1 side, which regulates the transcriptional function of receptor. The carboxyl-terminal domain (CTD) is, also, necessary for the association of AR with endogenous ligands, such as testosterone and 5α-dihydrosterone (DHT), while the DNA binding domain (DBD) interacts with specific sequences of DNA, the androgen response elements (AREs) to modulate AR-related genes transcription. Finally, the hinge region completes the structure of AR [27] (Fig. 1).

AR is prevalent in early-stage and metastatic breast carcinomas [28] but its' level and effect varies among patients depending on ER status [29]. Although AR is expressed in a considerably low rate of TNBC, recent sub-classification of TNBC has emerged AR as a new biomarker beyond the traditional ones [17,30]. Interestingly, invasive ductal carcinomas appear to show higher AR positivity than lobular ones, which might be explained by the less frequent TNBC phenotype in the latter [10]. The prognostic value of AR has been evaluated in several studies with controversial results [20,31–36] (Table 2). Strong supportive data for the prognostic role of AR is given by a more analytical study of AR positivity in combination with androgen metabolizing enzymes, thus assessing not only the receptor's expression but also its' ligands [37]. In this study, the discordance regarding the prognostic role of AR expression and the role of its actions on the biological behavior of TNBC was further evaluated. Thus, AR expression in tandem with the presence of androgen synthesizing enzymes 5α-reductase type 1 (5αR1) and 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5) was analyzed. Although AR expression pattern in tumor cells did not show any significant effect on prognosis of TNBC patients, the presence of androgen synthesizing pathway in addition to AR expression conferred a contribution to decreased cell proliferation, indicating that not only the receptor's expression but also its' ligands are factors governing tumor growth [37].

Recent clinical data indicate worse clinical outcome in the AR expressing TNBCs [38]. A few meta-analyses have also been conducted evaluating the correlation of AR expression with clinical outcome. The more recent provides evidence supporting the positive prognostic role of AR in ER-negative breast carcinomas [39], whereas previous reports showed better disease-free survival but