2. Role of the androgen receptor in triple-negative breast cancer

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Abstract. Triple-negative breast cancer (TNBC) accounts for 15% to 25% of breast-cancer diagnoses. TNBC expresses neither estrogen nor progesterone receptors, nor does it overexpress human epidermal growth factor type-2 receptor. It is an aggressive form of cancer and has not been a specific target of therapies for breast cancer. While it is initially sensitive to chemotherapy, TNBC has a high rate of relapse and poor prognosis after such chemotherapy. More effective therapeutic options for this subset of breast cancer are needed. Androgen receptor (AR) is expressed in one-third of TNBCs, and recent studies have clarified its action in breast cancer. AR-targeted therapy is one of the envisioned new therapies for TNBC; the therapeutic approach can involve the inhibition of androgen synthesis, use of non-androgenic AR-binding hormones, inhibition of co-regulators, activation of membrane AR, and reassessment of medroxyprogesterone acetate. These approaches are being evaluated in studies at the preclinical and phase II levels. Targeted therapies that improve outcome in patients with TNBC are expected to be developed in the near future.

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Introduction

Breast cancer comprises a heterogeneous group of tumors that are diverse in behavior, outcome, and response to therapy. Recently, the clinical management of breast cancer has come to rely on biomarkers [1-3]. DNA microarray profiling of breast cancer has identified 5 distinct subtypes—luminal A, luminal B, HER2-positive, basal-like, and normal-like breast cancer [4,5]. Considering these distinct subtypes is necessary for the management of breast cancer, in order to identify a proper therapeutic approach for the improvement of patient outcomes [1]. These genetic subtypes have overlapping immunohistochemical features [6-8]. Basal-like breast cancer (BLBC) likely represents a subset of triple-negative breast cancer (TNBC) that is estrogen-receptor (ER)-negative, progesterone-receptor (PR)-negative, and human epidermal-growth-factor-type-2 (HER2)-negative. ER, PR, and HER2 are the most valuable biomarkers in predicting the response to targeted therapy [2,3]. TNBC, which represents 15% to 25% of all breast cancers [9-12], has a more aggressive clinical course and does not benefit from conventional endocrine- and HER2-targeted therapy. Although patients with TNBC show a better initial response to chemotherapy, they do not have better overall survival; the short duration of the effects of cytotoxic agents in TNBC is associated with a higher likelihood of relapse and poorer prognosis in these patients [13-15].

While HER2-positive patients have a poor prognosis, similar to that of TNBC [16], the introduction of trastuzumab has significantly improved their prognosis [17]. Standardized targeting therapy and optimal standardized care have been lacking in the management of TNBC, but several pathways of interest are being studied. One such area of investigation is the role of the androgen receptor (AR) [11,18], which is expressed in one-third of TNBC patients. If AR is confirmed as biologically relevant in TNBC, it is possible that hormonal manipulations targeting it could be useful in the treatment of these tumors. This section reviews the expression of AR in TNBC and future plans for the development of AR-targeted therapies.

AR expression in TNBC

AR is expressed in 70% to 90% of primary breast cancers, an incidence comparable to or higher than that of either ER or PR [19,20]. Compared with other subtypes, TNBC has 25% to 45% lower AR levels [12,21-24]. AR expression is associated with good prognosis in ER-negative cancer [20,25] as well as in TNBC [21,23]. The relationship between AR expression and prognosis in ER-positive breast cancer is controversial: some studies have
shown that AR expression is related to good prognosis [26], others have reported no relationship between AR expression and prognosis [27]. In addition to prognostic prediction, it has been hoped that AR could predict the response to hormonal therapy in patients with AR- and ER/PR-positive breast cancer, or in patients with AR-positive and PIK3C-mutant TNBC [21].

Despite the prevalence of AR expression in both normal breast tissue and breast cancer, the clinical significance and functional role of AR and androgens in breast cancer are not well characterized [28,29]. Some epidemiologic studies report that the therapeutic use of testosterone in females is not associated with an increased risk of breast cancer [30]. Others show increased risk of breast cancer in postmenopausal women treated with estrogen and testosterone during 24 years follow-up [31]. Still others have recently reported that high plasma androgen levels are associated with an increased risk of breast cancer [32,33] and poor prognosis [34]. Early preclinical studies demonstrated that androgens exerted predominantly anti-proliferative effects on breast-cancer cells [35,36], but contradictory findings have been reported more recently [37-39]: androgens were found to induce proliferative changes in breast-cancer cell lines and to promote tumorigenesis in animal models by AR-mediated pathways [40,41]. These differences in the reports about AR and androgens in breast cancer may represent differences in experimental conditions.

AR and ER are co-expressed in normal breast epithelial cells and in 80–90% of breast cancers. When AR is activated by 5α-dihydrotestosterone (5α-DHT), ER activity is inhibited in a dose-dependent fashion [26]. Co-expression of ER with AR has been shown to decrease AR transactivation [42]. High concentrations of 5α-DHT stimulate the proliferation of cells of the estrogen-responsive human breast-cancer cell lines MCF-7 and EFM-19 [38]. Androgen-induced proliferation also has been demonstrated in the AR-positive, ER/PR-negative MDA-MB-453 breast-cancer cells [39]. Studies using MDA-MB-453 cells have demonstrated that both estrogens and anti-estrogens have no effect on cell proliferation or viability. AR signaling is intact in MDA-MB-453, and cell growth and survival are responsive to androgen in an AR-dependent and ER-independent manner [26].

It is well known that ER is related to the development of normal breast tissue and the progression of breast cancer, ER expression is associated with increased proliferation of breast-cancer cells, but the cancers are of relatively low malignancy and good prognosis. The expression of AR is likewise associated with increased cell proliferation, with cancers of relatively low malignancy and good prognosis.
AR-targeted therapy in TNBC

Adjuvant therapy for breast cancer can be generally cytotoxic or specifically biomolecule-targeted. General cytotoxic agents have a DNA-damaging effect on most dividing cells and often induce adverse effects [10]. Targeted therapies interfere with the action of specific biomolecules that are typically overexpressed in the cancer cells [43]. There are only a few targeted-treatment options for TNBC; those of recent clinical interest include poly-(ADP-ribose) polymerase (PARP) inhibitors and epidermal growth factor receptor (EGFR) inhibitors.

TNBCs do not constitute a homogeneous group (Table 1). AR is expressed in 25–45% of them [12,22-24], and the use of AR-targeted therapies for such cancers is being investigated (Table 2). Most efforts to modulate AR activity have focused on inhibiting the synthesis of androgens or on the identification of androgens that bind to the receptor. A more recent approach is screening for small molecules that inhibit other sites in the steroid-receptor pathways [49]. Furthermore, membrane androgen receptors (mARs), which have biological actions different from intracellular AR, have been identified in prostate and breast-cancer cells [50].

**Table 1.** Rates of AR expression, BRCA1 mutation and EGFR expression in triple-negative breast cancer.

<table>
<thead>
<tr>
<th>Association</th>
<th>Prevalence rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR positive in TNBC</td>
<td>25% to 45%*</td>
</tr>
<tr>
<td>BRCA1 mutation in TNBC</td>
<td>10%*</td>
</tr>
<tr>
<td>EGFR expression in TNBC</td>
<td>42% to 71%*</td>
</tr>
</tbody>
</table>

TNBC: triple-negative breast cancer. *1, Ref [12,22-24]; *2, Ref [44,45]; *3, Ref [46-48].

**Table 2.** AR-related targeting therapies in triple-negative breast cancer.

<table>
<thead>
<tr>
<th>Category</th>
<th>Target</th>
<th>Agent</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen synthesis</td>
<td>5α-red 17β-HSD5</td>
<td>(Inhibitor)</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Agonist hormone</td>
<td>AR</td>
<td>Bicalutamid</td>
<td>Phase II</td>
</tr>
<tr>
<td>Co-regulation factor</td>
<td>ARA70β</td>
<td>(Inhibitor)</td>
<td>Preclinical</td>
</tr>
<tr>
<td>New receptor</td>
<td>membrane AR</td>
<td>Albumin-conjugated testosterone</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Others</td>
<td>-----</td>
<td>Medroxyprogesterone acetate</td>
<td>Phase II</td>
</tr>
</tbody>
</table>
AR has two endogenous ligands, testosterone and DHT. 17β-hydroxysteroid dehydrogenase type 5 (17β-HSD5) converts circulating androstenedione to testosterone. In breast tissue, testosterone is converted by 5α-reductase (5α-red) to DHT, and by aromatase to estradiol [51]. 5α-red activity is 4 to 8 times as high in breast-cancer cells as in normal cells [52]. The concentration of circulating DHT is very low both in healthy women and in breast-cancer patients [53], but DHT concentration in breast-cancer tissue is three times as high as that of plasma [54]. Treatment with an aromatase inhibitor increases intratumoral testosterone concentration [55], and aromatase expression is inversely associated with intratumoral DHT levels in breast cancer [56]. These results suggest that high intratumoral concentrations of androgen-converting enzymes play a key role in the effects of androgens on cancer progression. Tissue-specific distribution of these enzymes in women may be suitable for targeted therapy. The usefulness of treatment with aromatase inhibitors in postmenopausal ER-positive breast cancer patients has been established [1]. Inhibition of locally over-expressed 17β-HSD5 and 5α-red may provide treatment options for patients with AR-positive and ER-negative breast cancers. While inhibitors are still at an early stage of development, preclinical studies suggest their potential in the future [57].

Since oral non-steroidal anti-androgens developed in the 1970s, including bicalutamide, flutamide, and nilutamide, are effective in the treatment of prostate cancer, a phase II study is currently being performed to evaluate the efficacy of AR blockade with bicalutamide in AR-positive and ER/PR-negative metastatic breast cancer patients (NCT 00468715). Bicalutamide (Casodex, AstraZeneca) is a widely available, orally active, non-steroidal anti-androgen that competitively inhibits the action of androgens by binding to cytosolic ARs in the target tissue. These anti-androgens have high specificity for the AR, but lack tissue selectivity [51]. An ideal selective androgen receptor modulator (SARM) should have high AR specificity and tissue-selective pharmacological actions [58]. In recent years, SARMs with greater tissue selectivity have been developed to minimize adverse effects [51]; they are currently being investigated mostly for use in prostatic cancer at different stages, but might well contribute to new treatments for AR-positive TNBC.

High-dose medroxyprogesterone acetate (MPA) has an anti-proliferative effect in ER-positive and/or PR-positive breast cancer. Before the advent of luteinizing-hormone agonists and third-generation aromatase inhibitors, MPA was a major therapeutic agent in breast cancer, with efficacy comparable to that of tamoxifen [59] and effective for the treatment of endocrine-resistant breast cancer [60]. It is still used occasionally for the treatment of metastatic...
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breast cancer, as a third- or fourth-line endocrine therapy. Clinical studies have revealed that expression of AR, but not ER or PR, predicts response to MPA in breast cancer [61, 62]. It will be necessary to reevaluate the use of MPA for AR-positive TNBC treatment. 5α-red metabolizes progesterone to 5α-dihydroprogesterone, suggesting that this enzyme is also involved in the local regulation of progesterone action [63].

Steroid receptor co-regulators are important in regulating the function of receptors in endocrine organ development and in cancer progression. Unbound AR is bound to chaperone proteins in the cytosol. Once bound by an androgen ligand, AR dissociates from the chaperone proteins and forms a homodimer with the ligand that is translocated to the nucleus and induces a cascade of molecular events, resulting in the activation of target gene transcription [64]. More than 350 nuclear receptor co-regulators have been described [49]. Of these, the AR co-activators ARA70α and ARA70β have distinct effects in mammary gland development and in the progression of breast cancer: ARA70α inhibits and ARA70β promotes proliferation in MCF7 breast-cancer cells; decreased ARA70α expression is associated with an increased tendency towards breast-cancer metastasis; ARA70β has been shown to enhance the invasive ability of MCF7 breast cancer cells [65]. These preclinical studies suggest the potential of AR co-activators for targeted therapy.

In recent years, mARs, specific G-protein-coupled receptors that mediate a rapid response in the form of non-genomic steroid action [66], have been identified in prostate- and breast-cancer cells [66-68]. mAR-dependent biological actions are different from the actions of intracellular ARs: activation of mARs with testosterone, in the presence of the antiandrogen flutamide, induces inhibition of cell motility and controlled apoptosis in prostate- and breast- cancer cells [66,68], and potentiates paclitaxel action in hormone-sensitive cells [69]; the mAR-induced apoptosis is independent of intracellular AR status. Ligands to mAR include natural androgens, albumin-conjugated androgens, and flavonol compounds [70-72]. Although free testosterone favors the growth of AR-positive cancer, albumin-conjugated testosterone probably initiates only mAR-dependent effects in cells [50]. Development of a specific human-serum-albumin-conjugated androgen for possible treatment of prostate cancer is under investigation [50], and mAR-specific compounds may have therapeutic potential in breast cancer.

Conclusions

Targeted endocrine therapy is an important treatment strategy for ER-positive and/or PR-positive breast cancer, but it rarely affects TNBC. AR has
recently received attention in breast-cancer treatment; the data reviewed suggest that it has relevance to breast-cancer biology and may be an important therapeutic target in AR-positive TNBC, especially TNBC that exhibits less AR-ER interaction. We believe that the development of AR-targeted therapy may improve the outcome in patients with TNBC.

References


52. Wiebe, J.P. et al. The 4-pregnen and 5alpha-pregnan progesterone metabolites formed in nontumorous and tumorous breast tissue have opposite effects on breast cell proliferation and adhesion. 2000, Cancer Res., 60, 936.