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Title:	Future oncology (London, England)
Title Abbrev:	Future Oncol
Citation:	2008 Feb;4(1):15-21
Article:	Androgen receptor as a target for the treatment of
Author:	Nahleh Z
NLM Unique ID:	101256629 Verify: PubMed
PubMed UI:	18240997
ISSN:	1479-6694 (Print) 1744-8301 (Electronic)
Fill from:	**Any format**
Publisher:	Future Medicine Ltd., London :
Copyright:	Copyright Compliance Guidelines
Authorization:	barb
Need By:	N/A
Maximum Cost:	**$20.00**
Patron Name:	Glaser, Rebecca - TN: 213109
Referral Reason:	Other
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Androgen receptor as a target for the treatment of hormone receptor-negative breast cancer: an unchartered territory

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Estrogen receptor-negative (ER^-) and progesterone receptor-negative (PR^-) breast cancers represent approximately 30% of all breast cancers and, in general, have a more aggressive clinical course. They are unresponsive to antiestrogens, more likely to be poorly differentiated, of higher histological grade and are associated with a higher recurrence rate and decreased overall survival. Androgen receptor (AR) expression has been reported in over 70% of breast cancer and in 45–50% of patients with ER-negative breast cancer. There is emerging evidence that the androgen signaling pathway plays a critical role in breast carcinogenesis, independent of ER. Preclinical data have suggested the inhibitory role of adrenal steroids, such as dehydroepiandosterone (DHEA) and its sulfate on the growth of human ER-negative breast cancer cell lines, when these demonstrate a strong expression of AR. This potentially results in decreased AR gene expression. However, DHEA has been shown to stimulate growth in breast cancer cells when an ER is expressed in ER-positive breast cancer cells. Therefore, the effect of adrenal steroids may differ based on the tumor hormone receptor status and ER^-/PR^- breast tumors may not be truly hormone 'insensitive'. Exploration of new androgen-based hormonal therapy is warranted in this patient population. This article reviews the role of the AR in breast cancer and discusses potential avenues for the treatment of $ER^-/PR^-/AR^+$ tumors with 'hormonal therapy'.

The androgen receptor in breast cancer

The androgen receptor (AR) is a member of the steroid receptor subfamily, which also contains the glucocorticoid receptor, progesterone receptor (PR) and mineralocorticoid receptor, and it binds to the same response elements as these receptors. There is emerging evidence that the androgen signaling pathway plays a critical role in breast carcinogenesis [1–4]. The AR is expressed in over 70% of breast cancers [1–3,5–16] and has been implicated in the pathogenesis of breast cancer [16,17]. This could be through the activation of a number of estrogen-responsive genes, as observed in other tumors [18]. However, many pathology studies have demonstrated that the direct AR-mediated action of androgens is the major mechanism used by androgens to influence the growth of breast carcinomas, independent of the estrogen and progesterone receptors [3,19,20]. Androgens have a predominantly inhibitory effect on the growth of breast cancer cells, both *in vitro* and *in vivo* [21–26], potentially through the induction of apoptosis [27–29]. However, preclinical studies have shown that androgen action in breast cancer cell lines is cell type-specific and has been reported to result in either stimulation or inhibition of proliferation [21]. Birrell *et al.* have experimented using the androgenic agents, dihydrotestosterone (DHT) and mibolerone, on six human breast cancer cell lines [21]. The resultant data suggests that androgens regulate the proliferation of T47-D and ZR-75–1 cells via an interaction with the AR. However, in the case of MDA-MB-453 and MCF-7 breast cancer cells, androgen-induced stimulation of proliferation was observed, and both AR-mediated and AR-independent mechanisms appear to be involved. Antisense oligonucleotides to the AR were used to determine whether both the inhibitory and stimulatory effects of androgens on the proliferation of the breast cancer cell lines were mediated by the AR, using computer-assisted video image analysis to measure cellular levels of AR. This demonstrated that co-incubation with AR antisense oligonucleotides, but not sense oligonucleotides, resulted in complete reversal of the growth inhibitory effect of androgens on ZR-75–1 cells, but did not alter the growth stimulatory effect of androgens on MDA-MB-453 cells, suggesting that the stimulatory effects of DHT and mibolerone on MDA-MB-453 cell growth are not mediated solely by the AR. The other cell lines examined, MDA-MB-231 and BT-20, which expressed very low or undetectable levels of AR, were not affected by androgens. All stimulatory and inhibitory proliferative responses were reversed by androgen antagonists (hydroxyflutamide or anandron); however, the androgen antagonists alone had no significant effect on cell proliferation. This study suggests: first, that

Keywords: androgen receptor, breast cancer, dehydroepiandosterone (DHEA), triple negative

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androgens regulate the proliferation of T47-D and ZR-75–1 via a direct interaction with the AR, causing the inhibitory effect; second, in the case of MDA-MB-453 and MCF-7, AR-independent growth regulatory pathways are involved in androgen-induced stimulation. Other studies have suggested that the activation of AR-independent pathways could result from the action of active metabolites of DHT with estrogenic-like actions [30]. In addition, one of the metabolites of DHT, 5 α androstane-3B, 17 β-diol, was shown to increase proliferation of MCF-7 cell lines via interaction with estrogen receptor (ER) [30]. Therefore, it is possible that, in the absence of ER, androgenic action may be mediated via direct interaction of DHT metabolites with AR, leading to an inhibitory growth effect, as observed in ZR-75–1 and T47-D; In the case of MCF-7 cell lines, there is interaction of DHT metabolites with ER, which is expressed in these cells, leading to proliferation. Therefore, while it is clear that the expression of the AR is necessary for androgens to modulate the growth of breast cancer cells *in vitro*, additional cell factors, such as androgen binding to receptors other than the AR, for example ER, may explain divergent responses to androgens observed in different breast cell lines. A recent genome-wide expression analysis of 99 primary breast cancer samples and eight breast cancer cell lines identified a subset of ER^-/PR^- tumors, ER (-) class A with paradoxical expression of genes known to be either direct targets of ER, responsive to estrogen, or typically expressed in ER^+ breast cancer [19]. The ER^- class A samples described were distinct from the luminal A and basal subtypes identified by Perou *et al* [31]. This ER^- class A cell line, MDA-MB-453, demonstrated a proliferative response to androgen.

Macedo *et al.* have presented data that sheds more light on the role of androgens in malignant human breast tissue [32]. MCF-7 and AC1 cells (MCF-7 cells transfected with the human aromatase gene), were used as models for studying the balance between the androgenic and estrogenic effect in breast cancer, since they express significant levels of both AR and ER. These cells were treated with both the aromatizable androgen, androstenedione, and the nonaromatizable androgen, 5α-DHT. DHT inhibited the growth of both cell lines, while androstenedione had an inhibitory effect on MCF-7 cell growth, but a cell-proliferative effect on AC1 cells, similar to estradiol. The proliferative effect of androstenedione was associated with its metabolic transformation into estrogens by the AC1 cells with high aromatase activity, and not from its direct interaction with the AR. This was confirmed by the inhibition of androstenedione-induced proliferation of AC1 cells by the aromatase inhibitor, letrozole. On the other hand, the antiproliferative effect of androgens on breast cells is mediated by the AR; the addition of the antiandrogen casodex suppressed the effect of DHT on MCF-7 and AC1 cells and the effect of androstenedione on MCF-7 cells, while casodex showed no effect on androstenedione-induced AC1 cell growth or on estradiol-induced MCF-7 and AC1 cell growth as casodex does not block the ER. These results suggest that there are two hormonal forces regulating proliferation in AR^- and ER-positive breast cancer cells. The androgenic signaling, through the AR, induces cell-growth inhibition, whereas the estrogen-mitogenic signaling is mediated by the ER. During aromatase inhibition, precursor androgens, such as androstenedione, can act directly, or be converted to DHT and exert their antiproliferative effect by interacting with the AR [32]. This would have significant clinical implications when we consider the development of AR-targeted therapy, as the addition of an aromatase inhibitor to an androgenic agent may be necessary to achieve the maximum inhibitory effect. Furthermore, targeting ER^-/PR^- tumors would eliminate potential interactions of androgens with ER-related pathways.

Role of androgens in breast cancer treatment

In vivo studies have shown that androgens may affect the growth of breast carcinoma [34]; pharmacologic administration of androgens to rats bearing dimethylbenzanthracene-induced breast carcinoma led to tumor regression [34]. Tumor proliferation in human mammary carcinoma is also significantly altered by androgens [35]. Historically, androgens have been used successfully as hormonal therapy for advanced breast cancer. Approximately 20% of patients with metastatic breast carcinoma may experience tumor regression after treatment with androgens [36,37]. However, androgen therapy (e.g., fluoxymesterone and testosterone) has not gained popularity owing to a high incidence of undesirable, virilizing side effects. In addition, the advent of ER-targeted therapy and aromatase inhibitors for the treatment of ER-positive breast cancer has focused hormonal therapy on those agents. Of interest is the role of aromatase

inhibitors, which block the conversion of adrenal steroids (mainly androgens) into estrogens in the treatment of breast cancer [38–42]. This would also underscore the important role of androgens (albeit in an indirect way, through estrogens) in the stimulation of human mammary carcinoma growth. Thus, androgens can have either stimulatory or inhibitory effects on tumor growth. These seemingly paradoxical effects may depend on carcinoma cell type and/or may be related to the presence or absence of other steroid receptors, such as ER and PR. In addition, the heterogeneity of carcinoma cells in terms of steroid receptor positivity and the proportional distribution of each steroid receptor among carcinoma cells may influence the activity of androgens in either a proliferative or inhibitory manner.

AR as a hormonal therapy target for ER$^-$ & PR$^-$ breast cancer

ER-negative and PR-negative breast cancers represent approximately 30% of all breast cancers and are known to have a more aggressive clinical course. Hormone receptor-negative tumors are more likely to be poorly differentiated, of higher histological grade and are associated with a higher recurrence rate and decreased overall survival [43–45]. ER-/PR-tumors lack the benefit of specific targeted therapy and have more limited treatment options, especially for those with no evidence of HER 2 neu overexpression, known as triple negative ER$^-$/PR$^-$/HER 2 neu$^-$. AR expression has been reported in 45–50% of patients with ER-breast cancer [16,45]. Moinfar *et al.* have studied the frequency of AR expression in 200 cases of breast carcinoma [16]. A total of 60% of invasive carcinomas and 82% of ductal carcinomas *in situ* were AR-positive. In addition, 46% of all ER-negative invasive carcinomas were AR-positive; and among the poorly differentiated invasive carcinomas, 39% were both ER$^-$ and PR$^-$, but AR$^+$. Among noninvasive carcinomas, 68% were ER$^-$ but AR$^+$. In view of the prevalence of AR expression in ER$^-$ and/or PR$^-$ tumors and preclinical work suggesting that ER$^-$/PR$^-$ breast cancer cells will respond to hormone therapy in the form of the androgen [29,33], it is likely that ER- and/or PR-negative breast tumors may not be truly hormone 'insensitive' and exploration of androgen-based hormone therapy may be warranted in this population. Hardin *et al.* [29] have recently published preclinical data using the androgen dehydroepiandosterone sulfate (DHEAS) on three human ER-negative, PR-negative breast cancer cell lines (HCC1937, 1954, and 38). DHEAS is an adrenal steroid that has reportedly minimal androgenic side effects and is available as an over-the-counter dietary supplement. Hepatocellular carcinoma (HCC) 1937 and 1954 cells that demonstrate high AR expression showed a decrease in cell proliferation after treatment with DHEAS for 7 days by 47% (HCC 1937 cells), and 29% (HCC 1954 cells), compared with untreated cells; while HCC 38 cells, which have barely detectable levels of AR expression, were unaffected by DHEAS treatment. Cells that receive continuous DHEAS treatment show a 1.76-fold decrease in cell proliferation by day 14 and a 2.8-fold decrease by day 18. After day 8 of DHEAS treatment, there was a 2.8-fold increase in cells positive for apoptosis in HCC 1937 cells, a 1.9-fold increase in HCC 1954 cells, and no significant change in HCC 38 cells, relative to the baseline number of cells positive for apoptosis, using the TdT-mediated dUTP nick end labeling (TUNNEL) assay. It is worth noting that these cell lines were pretreated with anastrozole to prevent any possible conversion of DHEAS to estrogens. Quantitative reverse transcriptase PCR demonstrated that AR gene expression decreases after treatment with DHEAS in AR-positive cell lines. Upon co-treatment with the AR antagonist bicalutamide, the downregulatory effect on the AR by DHEAS was not observed, localizing the action of DHEAS to the AR. This study suggests that a subset of ER- and PR-negative breast cancers may respond to hormonal manipulation with an endogenous precursor of androgens and estrogens, such as dehydroepiandosterone (DHEA) or DHEAS, combined with an aromatase inhibitor. DHEA treatment appears to act as an AR antagonist, resulting in the downregulation of AR as demonstrated by decreased AR gene expression after treatment with the androgenic agent. The ultimate effect is increased cell death, as demonstrated by increased apoptosis, using the TUNNEL assay.

Garreau *et al.* have demonstrated that ER$^-$/PR$^-$ breast cancer cells will respond to hormone therapy in the form of the androgen DHEAS, provided there is AR expression [33]. In a series of experiments, ER$^-$/PR$^-$/AR$^-$ HCC 1806 cells were shown to be unaffected by DHEA and anastrozole. However, when these cells were transfected with AR, these ER$^-$/PR$^-$/AR$^+$ cells showed significant cell death after DHEAS/anastrozole. The results of this and above reported studies support proceeding with the development of an effective AR-targeted hormonal therapy option for patients with ER-/PR-tumors that express AR [33].

AR interaction with other 'targets'

Targeting AR with androgen-based hormonal therapy in ER^-/PR^- breast cancer should take into consideration potential interactions between AR and other tumor growth-related factors. To our knowledge, the interaction of AR with HER 2 neu-related pathways has not been explored in breast cancer. In human prostate cancer cell lines, research has provided strong evidence to suggest that HER2/Neu induces AR transactivation through the MAP kinase pathway [46]. This has potential clinical relevance since antiandrogens did not inhibit AR in the presence of HER 2 [46]. This potential interaction should be further elucidated. The prevalence and role of AR expression in triple negative $ER^-/PR^-/HER2neu^-$ breast cancer has not been well-described and would also need to be explored further. A recent study evaluated 1944 consecutive patients with breast cancer in the UK and found 282 cases with the triple negative phenotype; 87% of whom had absence of AR expression [47]. This study was not designed to study AR in triple negative breast cancer, and, therefore, may not accurately estimate the prevalence of AR expression in this population. Futhermore, studying other cancer risk modifiers should provide important clues as to the impact of therapeutic strategies on breast cancer in different groups. Of interest, BRCA1 mutant breast cancers exhibit a basal-like phenotype [48]. It has been suggested that the androgen signaling pathways in breast cells may be modulated by the tumor suppressor, BRCA1 [49]. Many studies have shown that BRCA1 enhances the activity of the AR in prostate and breast cancer cell lines [49,50]. The potentiation of AR signaling by BRCA1 occurs predominantly via direct interaction of BRCA1 with AR and stimulation of its activity [48,49]. BRCA1 upregulates the AR-mediated expression of the G1 cell-cycle inhibitor, $p21^{WAF}$, and enhances DHT-induced cell death in human prostate cancer cells [48]. BRCA1 was also found to interact directly with both AR and the coactivator, glucocorticoid receptor interacting protein 1 (GRIP1), and to stimulate AR activity via the activation factor (AF-1) domain of AR [48]. The ability of BRCA1 to stimulate AR activity is enhanced by several coactivators, including CBP, ARA55, ARA70 and GRIP1 [49]. The AR exhibits genetic polymorphisms in the number of polyglutamine (CAG) repeats in its AF-1 domain, with the repeat length inversely correlated with p160 coactivator binding and AR activity. Some studies suggest an association between a long CAG repeat length and an early age of breast cancer onset in *BRCA1* mutation carriers, while others do not show such a correlation [51]. The correlation of BRCA1 mutant tumors in breast cancer with AR and androgens should be pursued in further research.

Future perspective

Preclinical and clinical evidence suggest that androgens and the androgen signaling pathway play a critical role in breast carcinogenesis, and may provide a novel treatment approach in ER-negative/AR-positive tumors. This territory should be further explored. The adrenal steroid, DHEA, an endogenous precursor of androgens and its sulfate, DHEAS, have minimal androgenic side effects and have been reported to have downregulatory effects on the growth of human ER-negative breast cancer cell lines, through direct interaction with AR, in cells that strongly express AR. This effect causes decreased AR gene expression in AR-positive cell lines. It is therefore possible that a subset of ER^-/PR^- breast tumors that express AR may not be truly hormone 'insensitive' and respond to hormonal manipulation with an androgen-like DHEA. Exploration of new androgen-based hormonal therapy is warranted in this patient population. This is especially important in view of the more aggressive clinical course, higher recurrence rate and decreased overall survival associated with ER^-/PR^- breast tumors with overall limited treatment options. Methods of therapeutic targeting and downregulation of AR should be studied in patients with ER-negative, PR-negative, and AR-positive breast cancer. The characteristics of AR-positive breast tumors should also be elucidated. Specific gene expression profiles associated with *AR*, *HER 2* neu and *BRCA* genes should also be investigated. The development of AR-targeted hormone therapy will open an avenue for effective, tolerable therapy for nearly half of those patients with ER/PR^- tumors.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Androgens and androgen signaling pathway play a critical role in breast carcinogenesis

- Androgen receptor (AR) expression has been reported in 45–50% of patients with estrogen receptor (ER)-negative breast cancer.
- AR may represent a novel target for ER-negative/AR-positive tumors.

Dehydroepiandosterone and the growth of breast cancer

- Dehydroepiandosterone (DHEA) and DHEA sulfate inhibit the growth of human ER-negative breast cancer cell lines, when these demonstrate a strong expression of AR.
- The treatment of AR-positive cell lines is associated with decreased AR gene expression.
- Upon co-treatment with an AR antagonist, the downregulatory effect on the AR by DHEA is not observed, localizing the action of DHEA to the AR.

Conclusions

- ER^-/progesterone receptor (PR)-negative breast tumors may not be truly hormone 'insensitive' and exploration of new androgen-based hormonal therapy is warranted in patients with breast cancer expressing AR.
- DHEA, combined with an aromatase inhibitor to prevent any possible conversion of DHEA to estrogens, maintains its specificity as an androgen, and preserves its availability as an AR agonist, which may result in loss of AR and decreased tumor proliferation.
- This approach, if clinically proven, would open new avenues for the treatment of ER^-/PR^- breast tumors, a population lacking the benefit of specific targeted therapy overall and with currently limited treatment options.

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