The Role of Androgens in Normal and Malignant Breast Tissue

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Key Words

Androgens · Paracrine mechanisms · Breast · Breast cancer

Summary

Androgens, like estrogens, can be synthesized in the breast. As both active androgens and their corresponding receptors are present in breast tissue, we conclude that they play a role in breast physiology. This is supported by the fact that insufficient androgen production or sensitivity results in the development of gynecomastia. Complete androgen insensitivity due to receptor defects leads to normal female breast development in these XY women. While breast development is completely inhibited by male testosterone levels, partial but not total degradation of a developed breast by androgen treatment appears to be possible. Breast cancer in early stages seems to fulfill the prerequisites of androgen responsiveness. Androgen treatment of advanced breast cancer has shown similar effectiveness as anti-estrogen or estrogen-ablative therapy, but also considerable side effects. It has been speculated that the use of selective androgen modulators (SARMs), either alone or preferably in addition to anti-estrogens or aromatase inhibitors, may be a promising alternative to current therapy modalities in hormone-dependent breast cancer. In addition, future studies on the use of SARMs in prophylactic settings seem to be justified.

Schlüsselwörter

Androgene · Paracrine Mechanismen · Brust · Brustkrebs

Zusammenfassung

Androgene, wie auch Östrogene, können in der Brust synthetisiert werden. Da sowohl aktive Androgene als auch der korrespondierende Rezeptor im Brustgewebe vorhanden sind, kann angenommen werden, dass sie auch eine physiologische Rolle spielen. Es gibt mehrere Hinweise dafür: Insuffiziente Androgenproduktion oder -sensitivität resultiert häufig in einer Gynäkomastie. Komplette Androgeninsensitivität aufgrund eines Androgenrezeptordefekts führt zu normaler Brustentwicklung bei den betroffenen XY-Frauen. Während die Brustentwicklung durch männliche Testosteronspiegel vollständig verhindert werden kann, zeigt sich unter Androgenbehandlung nur ein partieller Rückgang der bereits entwickelten Brust. Brustkrebs im Frühstadium scheint alle Voraussetzungen für eine Androgensensitivität zu haben. Die Androgenbehandlung des fortgeschrittenen Mammakarzinoms war in etwa gleich effektiv wie alle anderen additiven oder ablativen Hormontherapien, aber die Nebenwirkungen waren größer. Es wird vermutet, dass die Verwendung von selektiven Androgen-Rezeptor-Modulatoren (SARMs), allein oder in Kombination mit Antiöstrogenen oder Aromatasehemmern, eine Erfolg versprechende Alternative zu derzeitigen Therapiemodalitäten beim hormonsensitiven Mammakarzinom wäre. Auch Studien zur Verwendung von SARMs in der Prophylaxe des Brustkrebses scheinen gerechtfertigt.

General Considerations

The aim of this review is to highlight the role of androgens in the female mammary gland, both in normal and cancerous tissue.

Androgens are C19-steroid hormones that are produced in the ovaries, adrenal glands and peripheral tissues. The term 'androgen' is in fact only valid for testosterone (T) and dihydrotestosterone (DHT). T can also be converted to estradiol and is thus a precursor for both the 'pure' androgen DHT and the 'pure' estrogen estradiol. Dehydroepiandrosterone (DHEA), DHEA sulfate (DHEA-S) and androstendione (A-dione) are not able to bind to and activate the androgen receptor (AR), and they serve as precursors for androgen as well as estrogen synthesis. Therefore, these substances are considered prohormones.

Only a small amount of T (1–3%) circulates free and unbound in the blood. Most of it is bound either to albumin (25–35%) or to sex hormone-binding globulin (SHBG) (65–75%) [1]. The uptake of SHBG-bound steroids into the cell has been found to be mediated by a receptor called megalin [2]. Expression of megalin mRNA has also been detected in mammary epithelial cells [3].

Most steroids circulate as precursors such as DHEA, DHEA-S or A-dione through the body. Steroid-dependent tissues are able to synthesize active steroid hormones from these precursors. Labrie calls this phenomenon 'intracrinology' [4]. However, this would mean that one and the same cell produces the hormone and the corresponding receptor. Such a construction would imply independence of a cell from others, which would be difficult to incorporate within a comprehensive model of regulation of mammary gland formation and function. The term 'paracrinology' would thus appear more appropriate as different cells communicate with each other.

Androgen-Converting Enzymes in the Breast

To form DHT from DHEA-S, steroid sulfatase (STS), 3 β -hydroxysteroid dehydrogenase (3 β -HSD), 17 β -HSD and 5 α -reductase are required. The synthesis of androgens as well as estrogens is possible from DHEA-S and DHEA; this occurs through the combination of aromatase and the enzymes named above.

STS hydrolyzes steroid sulfates to their more active, unconjugated forms. It is responsible for the conversion of DHEA-S to DHEA and estrone (E1)-S to E1. The sulfated inactive precursors possess a much longer half-life in the blood than DHEA or E1, and are therefore the ideal transportation form for steroid hormones.

As to the localization of STS in the normal human mammary gland, Tobacman et al. [5] have detected STS mRNA expression and enzymatic activity in myoepithelial cells. They therefore conclude that myoepithelial cells may play an important

role in providing unsulfated steroid hormones to the mammary secretory epithelium. In contrast to these findings, Suzuki et al. [6] and Miki et al. [7] report only very low levels of STS mRNA expression and no STS immunoreactivity in normal breast tissue.

Several groups report elevated STS expression and activity in cancerous breast tissue in comparison to non-malignant breast tissue [6, 8]. Additionally, Zaichuk et al. [9] demonstrate that STS expression is elevated only in estrogen receptor (ER) α -positive breast cancer while it is even lowered in ER α -negative cancer tissues.

Comparisons of STS versus aromatase activities have attached great importance to the sulfatase pathway [10]. Both in normal and in cancerous mammary gland tissue, the measured activity of STS has been shown to be significantly higher than the activity of aromatase [11].

3 β -HSD is necessary for the conversion of DHEA to A-dione. Two isoforms of 3 β -HSD exist. While type 2 has been found only in the adrenals, testis and ovaries, type 1 was isolated from the placenta and several peripheral tissues including normal and cancerous breast tissue [12, 13]. 3 β -HSD was present in normal mammary gland in fibroblasts and epithelial cells, but not in myoepithelial cells [14].

Currently, 15 isoforms of 17 β -HSD are known [15]. Types 2, 4, 6, 8, 9, 10, 11 and 14 catalyze the oxidation of androgens and estrogens, while types 1, 3, 5, 7 and 12 catalyze the reduction of these steroid hormones. The oxidative enzymes use NAD(P)⁺ as a cofactor and cause inactivation of their substrates, whereas the reductive isoforms lead to activation by means of NAD(P)H [16].

In normal breast tissue, oxidation of estrogens predominates, while the reductive pathway is strongly favored in hormone-dependent breast tumors [17, 18]. The conversion of 17 β -estradiol (E2) to E1 is dominant in hormone-independent breast cancer [19].

17 β -HSD type 1 mainly converts E1 to E2, and was found to be expressed in both normal and cancerous mammary epithelial cells [18]. Day et al. [20] recently demonstrated that 17 β -HSD type 1 is the main 17 β -HSD isoform in converting E1 to E2 in breast cancer tissue. In contrast, Song et al. [21] postulate that 17 β -HSD type 7 and most notably type 12 are the most widely expressed reductive isoenzymes in breast carcinoma cells and are therefore of greater interest.

Furthermore, Gangloff et al. [22] describe the inactivation of DHT by 17 β -HSD type 1 to either 3 β -androstenediol or androstandion. If this enzyme is not only capable of providing the most potent estrogen E2 but also of inactivating the most potent opponent of E2, this would imply a further support of estrogenic action.

17 β -HSD types 3 and 5 synthesize T from A-dione. Type 3 is mainly located in the testes, while type 5 is expressed in peripheral tissues such as the prostate and the breast. 17 β -HSD type 5 is the only member of the 17 β -HSDs that belongs to the aldo-keto reductase family and not to the short-chain alcohol

dehydrogenase superfamily. It has been localized immunocytochemically in the epithelium of acini and ducts of the mammary gland [14], and has also been detected in breast cancer tissue [23].

17 β -HSD type 2 converts T to A-dione and E2 to E1 [18]. It is expressed in normal mammary epithelial cells, and is believed to play a major role in inactivating E2 [18]. As to its appearance in cancerous breast cells, Suzuki et al. [24] report almost no immunoreactivity in 111 examined tissues, whereas Gunnarsson et al. [25] find 17 β -HSD type 2 expression in 69% of 230 examined tissues.

5 α -Reductase catalyzes the conversion of T to DHT. Of the two identified isoforms, type 1 is assumed to be of more importance in the mammary gland than type 2 [23]. Expression and activity of the two isoenzymes has been detected in both normal and cancerous breast tissue, but in significantly higher amounts in tumorous tissue [23, 26].

Aromatase expression and activity has been found in stromal cells in normal and cancerous breast tissue [27, 28]. It is still a matter of controversy whether it is also located in epithelial and carcinoma cells [29].

Aromatase converts A-dione to E1 and T to E2. But it has also been suggested that T may be the preferred substrate in breast cancer cells [30]. Suzuki et al. [23] propose the influence of aromatase on intratumoral DHT concentrations. Accordingly, by converting T to E2, the enzyme decreases T concentrations; the less T is available, the less DHT can be produced.

The Androgen Receptor in the Breast

Not only the uptake of androgens and/or their precursors and the conversion into biologically active forms, but also the presence of corresponding receptors is a prerequisite to enable androgen action in a tissue.

There have only been a small number of studies dealing with the localization of the AR in the normal human mammary gland. However, it has been possible to detect immunohistochemical staining of the AR in the nuclei of mammary gland tissue epithelial cells. Studies have found the AR in ductal and alveolar cells [31–35]. Additionally, Birrell et al. [35] report occasional cytoplasmic staining. Kimura et al. [33] and Birrell et al. [35] detect at least some AR immunoreactivity in myoepithelial cells, unlike Janssen et al. [34] and Ruizefeld de Winter et al. [32]. The occurrence of the AR in the stroma is still uncertain. Kimura et al. [33] and Zhuang et al. [31] observe the presence of AR in stromal cells, whereas Birrell et al. [35] and Janssen et al. [34] find only sporadic occurrence in stromal cells.

In contrast to the limited number of studies on AR expression in normal breast tissues, there have been numerous publications about the localization of AR in breast tumors. Most of them report AR expression in about 70–90% of the examined cases [23, 36–40]. Some groups report associations between

AR status and tumor type, grade and stage [36, 37], while others do not [40]. Conflicting findings are also found with regard to correlations between AR status and prognosis in terms of disease-free survival.

Androgen Concentrations in the Breast

Measurements of androgens in the mammary gland have revealed the presence of T and DHT in both normal and tumorous tissues. Suzuki et al. [41] report mean DHT concentrations in non-tumorous samples of 97 ± 9 pg/g. Measured DHT concentrations in invasive ductal carcinoma and in ductal carcinoma in situ tissue were significantly higher [23, 41–45]. Moreover, decreases in intratumoral DHT levels have been associated with increases in malignancy or dedifferentiation [42, 45].

As estradiol levels in breast cancer tissue have been found to be independent of serum levels due to local production [46, 47], the same has been assumed for DHT. Studies on tissue versus serum concentrations of DHT have supported this assumption [43, 44].

Possible Functions of Androgens in the Breast

As all the enzymes crucial for T and DHT production, measurable androgen concentrations and AR are present in the breast, one can conclude that androgens play a role in the regulation of the mammary gland, not only in cancer but also under normal conditions.

Inhibition of Proliferation in the Normal Mammary Gland

Recently published data provide evidence for the widespread theory of androgens opposing estrogens in the mammary gland. Studies on rhesus monkeys demonstrate the inhibiting influence of androgens on mammary epithelial proliferation by reducing E2-induced proliferation [48, 49]. The same results have been shown in rodent mammary gland [50].

Observations indicate similar effects of androgens in humans. The impact of T addition to combined estrogen and progesterone hormone replacement therapy on the postmenopausal breast has been studied by Hofling et al. [51]. While the estrogen and progesterone combination produced a considerable increase of breast cell proliferation, no significant changes were observed when T was added [51]. This is another indication of androgens blocking stimulatory influences in the mammary gland.

Observations regarding anabolic androgen steroids (AAS) provide further information. The use and abuse of AAS in females leads to elevated T levels within normal male ranges [52]. One of the side effects of AAS is the reduction of breast size. Both female-to-male transsexuals and athletes taking

AAS have self-reported a reduction of breast size [53]. But not all of the participants of these studies noticed this effect. The atrophy of the mammary gland has been verified histologically by several groups [54–56]. In contrast, Burgess and Shousha [57] found no atrophic changes in breast tissues among female-to-male transsexuals 1 month after stopping AAS treatment. Moreover, it should be mentioned that mastectomy is an inherent part of gender reassignment for female-to-male transsexuals. This indicates that, even with prolonged AAS treatment, it is not possible to completely reverse previous breast development.

Inhibition of Breast Development

Androgens are thought to inhibit mammary gland development. This is supported by the fact that males usually do not develop breasts. In patients with hypogonadism and androgen deficiency, however, e.g. in Klinefelter's syndrome or partial androgen insensitivity (PAIS), gynecomastia is often observed [58]. Genotypically male individuals suffering from complete androgen insensitivity (CAIS) develop morphologically normal female breasts during puberty [58].

Girls with congenital adrenal hyperplasia (CAH) and androgen excess do not undergo pubertal thelarche [59]. Overall, this may be an indicator of the complete inhibition of pubertal breast development by androgens.

Inhibition of Proliferation in Breast Cancer

Numerous studies describe the effects of androgens on breast cancer cells as inhibiting proliferation [60]. In ZR-75-1, T47-D, HCC 1500, CAMA-1 and MFM-223 cells, DHT inhibits proliferation [61, 62]. Conflicting results have been reported for MCF-7 cells. While some groups observe stimulation [62], others find inhibition of proliferation by DHT [61]. The effects of androgens on breast cancer cell lines seem to depend on the presence of the AR, the concentration and type of androgen used and the presence of co-regulatory proteins [60]. Furthermore, apoptotic effects of androgens on breast cancer cell lines have been shown [63]. Similar observations have been made for cancer cells of the prostate [64].

Androgen Receptor Mutations and Breast Cancer

The assumption that androgens protect the mammary gland from developing cancer is further supported by studies on AR polymorphisms. The AR gene contains a CAG and a GGN repeat length polymorphism. The CAG repeat length has been shown to be inversely associated with transcriptional activity of the AR gene. A long repeat length means less activity, while a shorter CAG repeat length results in higher transcriptional

activity [65]. Some groups suggest that short CAG repeats possibly reduce and long repeats possibly enhance breast cancer risk in women [66]. Others report that longer CAG repeats are associated with higher breast cancer risk in women with first-degree family history of breast cancer [67]. And still others find no association between CAG repeat length and breast cancer risk [68]. Although higher breast cancer risk via less activity of the AR through longer CAG repeats would fit perfectly with the assumption mentioned above, these inconsistent results do not permit us to draw any final conclusions.

Another approach to the role of androgens in breast cancer is through research on AR gene mutations. Thus far, there have only been three reports of such alterations [69–71]. All described mutations are located in the region encoding the DNA binding domain (DBD), and are found in male PAIS patients with breast cancer. It has been speculated that the changes in the DBD of the AR may either lead to a decrease in activity or to the binding of the AR to estrogen-responsive elements. Poujol et al. [72] observe weaker binding of the mutant AR to androgen-responsive elements, but no binding to estrogen-responsive elements. However, analysis of male non-PAIS breast cancer tissues for AR mutations revealed no AR gene alterations [73]. Furthermore, there have been neither reports of elevated breast cancer risk nor case reports of breast cancer in CAIS patients. Thus, it can be concluded that AR mutations do not necessarily lead to breast cancer development. If androgens represented the sole element inhibiting breast cell proliferation, the complete loss of AR function in CAIS would cause uncontrolled proliferation. This would result either in macromastia or in elevated breast cancer risk. As there have been no reports of breast cancer in CAIS patients, one could conclude that there is an alternative pathway that inhibits excessive breast cell proliferation.

No further AR gene mutations with impact on breast cancer development are known [74]. Currently, all identified AR mutations are provided on www.mcgill.ca/androgendb.

All attempts to detect AR gene mutations in breast cancer cells have been unsuccessful [75, 76]. However, Shan et al. [76] postulate that the loss of AR expression in AR-negative female breast cancer may be caused by inactivation of the active X-chromosome. Zhu et al. [75] discovered another irregularity regarding AR mRNA in breast cancer. An exon 3 deletion splice variant was detected in part of the cancer tissues, but not in normal breast tissue.

Androgen Serum Levels and Breast Cancer

Evaluating associations between serum steroid levels and breast cancer risk, most studies report increased risk of breast cancer in postmenopausal women with elevated serum levels [77, 78]. As the mammary gland is capable of converting adrenal precursors into estradiol and DHT via several intermediates, these results do not necessarily indicate higher pro-

liferation stimulation as a consequence of elevated serum hormone levels. It seems more probable that the breast acts as an endocrine gland that contributes significantly to postmenopausal blood levels of steroidal sex hormones. Elevated levels might then reflect higher breast turnover, including higher proliferation, and thus a higher risk of developing cancer. It has not been proved that physiological blood levels of sex steroids have a direct influence on breast homeostasis. Male T levels and pregnancy estradiol and progesterone are at least one order of magnitude higher than physiologically active concentrations, which may allow them to interfere directly with intramammary proliferation and differentiation mechanisms. Inadequately low T levels in men seem to be associated with increased risk of male breast cancer [79].

Androgen Therapy in Breast Cancer

Assuming that androgens are able to inhibit proliferation of both normal and cancerous breast tissue, androgen therapy would be a logical conclusion. Since the 1930s there have been attempts to treat breast cancer with androgens, in particular advanced breast cancer. The reported efficacy of around 20–30% is similar to that of other hormonal or anti-hormonal

approaches [80]. Karydas et al. [81] analyzed relapse-free and overall survival in patients receiving adjuvant androgen treatment (T propionate 200 mg s.c.). No survival advantage for the treated group was observed. However, no information on serum levels is provided, which could allow us to assess whether the applied doses were appropriate. Due to side effects and the advent of anti-estrogens and estrogen depletion strategies, androgen therapy in breast cancer was abandoned. Garreau et al. [82] recently demonstrated the efficacy of combined treatment with DHEA and an aromatase inhibitor in AR-transfected HC 1806 breast cancer cells. The cell death rate was notably enhanced by this treatment. The authors conclude that androgen therapy in AR-positive and ER/ progesterone receptor (PR)-negative breast cancer could increase survival rates in combination with chemotherapy.

The clinical use of androgens in breast cancer treatment has been limited due to adverse effects. To eliminate side effects such as virilization, the development of selective androgen receptor modulators (SARMs) could be a promising approach. It needs to ensure, however, that either the androgen used is non-aromatizable or that aromatase activity is blocked to prevent adverse effects on breast cancer cell proliferation. SARMs and anti-estrogenic treatment should theoretically act synergistically in inhibiting breast cancer growth.

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