REVIEW

Androgen excess in breast cancer development: implications for prevention and treatment

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Abstract

The aim of this review is to highlight the pivotal role of androgen excess in the development of breast cancer. Available evidence suggests that testosterone controls breast epithelial growth through a balanced interaction between its two active metabolites: cell proliferation is promoted by estradiol while it is inhibited by dihydrotestosterone. A chronic overproduction of testosterone (e.g. ovarian stromal hyperplasia) results in an increased estrogen production and cell proliferation that are no longer counterbalanced by dihydrotestosterone. This shift in the androgen/estrogen balance partakes in the genesis of ER-positive tumors. The mammary gland is a modified apocrine gland, a fact rarely considered in breast carcinogenesis. When stimulated by androgens, apocrine cells synthesize epidermal growth factor (EGF) that triggers the ErbB family receptors. These include the EGF receptor and the human epithelial growth factor 2, both well known for stimulating cellular proliferation. As a result, an excessive production of androgens is capable of directly stimulating growth in apocrine and apocrine-like tumors, a subset of ER-negative/AR-positive tumors. The key role of androgen excess in the genesis of different subtypes of breast cancer has significant clinical implications for both treatment and prevention. Our belief stems from a thorough analysis of the literature, where an abundance of evidence is present to justify a clinical trial that would investigate the effectiveness of treating the underlying excessive androgen production.

Key Words

- ▶ breast cancer
- ▶ ER-positive
- ▶ ER-negative
- ▶ androgen/estrogen balance
- ▶ androgen excess
- ▶ testosterone
- ▶ estradiol
- ▶ dihydrotestosterone

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Introduction

It is a well-known fact that androgens act mostly as growth inhibitors on breast cancer.

In apparent contrast with this common belief, the androgen-excess theory of breast cancer (Secreto 2012a,b) postulates that androgen excess is the pivotal endocrine derangement in women with breast cancer. In this review, we propose a novel approach to breast cancer development by emphasizing the central role of excessive androgen production in this process.

We will describe the mechanisms of tumor promotion by androgens and we will discuss the protective role of androgens in breast cancer, providing the available evidence from the literature to support our claim. Lastly, we will briefly reiterate the clinical implications of androgen excess discussed in previous papers on the prevention (Secreto *et al.* 2016) and treatment (Secreto *et al.* 2017) of estrogen receptor (ER)-positive breast cancer.

Androgen excess in the development of ER-positive breast cancer

Studies on normal breast development have shown how androgens display growth inhibitory properties in puberty and anti-proliferative and anti-estrogenic properties during adulthood (Casey & Wilson 1984, Liao & Dickson 2002, Peters *et al.* 2011). In contrast, estrogens and progesterone have been found to promote breast development in puberty and induce profound changes in the gland's structure. With each menstrual cycle the mammary gland undergoes proliferation, differentiation and regression, a process that repeats itself from puberty to menopause. After menopause, regressive phenomena prevail, resulting in progressive replacement of the glandular structure with adipose tissue. A more refined growth control mechanism is enforced by the local production of polypeptide growth factors that modulate steroid activity through autocrine and paracrine mechanisms (Borellini & Oka 1989, Cunha 1994, Reid *et al.* 1996). The interactions between mesenchymal and epithelial tissue are mediated by a paracrine signaling network comprising growth factors, cytokines and extracellular matrix molecules, all of which are part of a unique anatomo-functional entity (Imagawa *et al.* 2002, Shekhar *et al.* 2003, Fleming *et al.* 2010).

The androgen/estrogen balance in the hormonal control network of epithelial proliferation

Under physiological conditions, estrogens stimulate cellular proliferation while androgens inhibit it. These two hormones can be regarded as an endocrine control mechanism just like the insulin/glucagon pair in controlling glycemia or the parathyroid hormone/calcitonin pair in regulating calcemia. Unlike the hormonal pairs mentioned above, androgens are the obligated precursors for estrogen synthesis and thus can either directly inhibit breast epithelium growth or stimulate it via conversion into estrogens. The dynamic equilibrium between androgens and estrogens provides proper control of mammary epithelial growth in response to the different needs of puberty, menstrual cycles and menopause. In pathological or para-physiological states (i.e. polycystic ovarian disease, ovarian stromal hyperplasia, ovarian hyperthecosis, congenital adrenal hyperplasia), excessive androgen production results in increased estrogen synthesis, which, in turn, overstimulates cell proliferation. At the same time, those same elevated androgen levels attempt to counteract the stimulatory influence of increased estrogen activity. At first, the androgen/estrogen balance is maintained,

albeit at a higher than normal level. Nonetheless, the estrogens' pro-proliferative effect will ultimately prevail if the source of excessive androgen production is not removed and the pathological spiral (high androgens → high estrogens → high proliferation) is not broken (Fig. 1). This chain of events emphasizes the central role of androgen excess in the development of diseases, such as breast cancer, often regarded as estrogen dependent. We suggest that the losing struggle of androgens to maintain the androgen/estrogen balance may explain why the development of breast cancer occurs in stages: simple epithelial hyperplasia, atypical hyperplasia, carcinoma *in situ*, infiltrating carcinoma.

A recent study in women with benign breast disease who underwent serial biopsies has shown the progression of epithelial proliferation from the first to the subsequent biopsies (i.e. from non-proliferative to proliferative or from simple proliferative to atypical histological subtypes) to be associated with an increased breast cancer risk when compared to no change. However, the risk decreased when the initial histological diagnosis of a proliferative subtype regressed to non-proliferative in later biopsies (Visscher *et al.* 2017). These findings support the hypothesis that breast cancer develops in stages. We believe that the androgen/estrogen imbalance might either promote the progression through these stages or halt the process, depending on which hormone prevails.

The protective role of androgens in breast cancer

Clinical evidence shows a 20–30% remission rate from metastases in patients treated with high-dose androgens (Goldenberg 1964, Talley *et al.* 1973). A similar percentage was observed with high-dose estrogens (Carter *et al.* 1977, Ingle 2002, Iwase *et al.* 2013) and high-dose progestins (Hortobagyi *et al.* 1985). These results suggest that the use of a high-dose sex hormone (i.e. androgens, estrogens or progestins) may halt the progression of metastatic breast cancer by blocking the hypothalamic–pituitary–gonadal axis. However, these data alone do not support a specific protective role of androgens since all sex hormones, when administered at high doses, can have the same effect.

Further evidence on the beneficial role of androgens can be found in the link between androgen receptor (AR) positivity and improved outcomes in ER-positive tumors, as shown in several clinical studies (Niemeier *et al.* 2010, Hu *et al.* 2011, Vera-Badillo *et al.* 2014, Elebro *et al.* 2015). We believe that the androgens' favorable effect is consistent with our androgen/estrogen balance theory. These findings reveal but a fraction of the intricate mechanism

Figure 1

Simplified illustration of the androgen/estrogen imbalance in the development of estrogen receptor (ER)-positive breast cancer. The figure shows the cascade of events inside cancer cells that starts with the excess testosterone being converted into its two biologically active metabolites: estradiol and dihydrotestosterone (DHT). Testosterone represents androgen excess. Persistently elevated testosterone levels, in conjunction with increased aromatase activity, fuel the continuous synthesis of high estradiol levels resulting, on the one hand, in constant cell proliferation and, on the other, in the upregulation of ER synthesis. This creates a vicious circle resulting in the continuous binding of estradiol to its receptor. The estrogen-induced proliferation cannot be adequately controlled by the same high testosterone levels that first triggered the process. At the same time, high 5 α -reductase activity increases the conversion of testosterone into its stronger, non-aromatizable metabolite, DHT, exerting an anti-estrogenic and a growth inhibitory effect. DHT hinders epithelial cell proliferation for some time but is unable to revert the proliferative process back to normal unless the source of the androgen excess is removed, effectively halting the excessive estradiol production.

that is cancer growth control, where androgens exert both inhibitory and stimulatory influences.

In a number of preclinical studies on animals, androgens were shown to inhibit cancer growth and favor the regression of already established tumors (Liao & Dickson 2002, Choi *et al.* 2014). Yet, in other papers, androgens were shown to enhance tumor growth (Liao & Dickson 2002, Choi *et al.* 2014) and possibly synergize with estrogens in breast carcinogenesis (Liao & Dickson 2002). The discrepancy between these studies might be due to differences in their design, in the use of aromatizable or non-aromatizable androgens and in mechanisms of tumor induction (Choi *et al.* 2014). Similar apparently inconclusive results were obtained in cell-culture studies, where the findings might largely depend on cell type and experimental conditions. Nonetheless, we suggest administering non-aromatizable androgens (dihydrotestosterone (DHT), mibolerone) to already established ER-positive breast cancer cells may exert only

an anti-proliferative effect. However, it should be noted that 5 α -androstane-3 β ,17 β -diol, a metabolite of DHT, can stimulate growth in breast cancer cells under severely estrogen-deprived conditions (Sikora *et al.* 2009, Hanamura *et al.* 2013). On the other hand, adding aromatizable androgens (androstenedione and testosterone) may result in both anti-proliferative and pro-proliferative influences depending on several variables, i.e. the prevailing activity of estrogen-synthesizing (aromatase) or androgen-synthesizing enzymes (5 α -reductase), the relative intracellular expression of ER and AR, and the dose of androgen that was administered. The same rationale applies to carcinogenesis in the animal model. Studies in transgenic male mice overexpressing aromatase (AROM+) have found that the induction of severe gynecomastia and other abnormalities secondary to excessive estrogen production could be reverted by administering aromatase inhibitors (Li *et al.* 2004, Li 2010, Hickey *et al.* 2012). These findings provide a clear example of an unbalanced

androgen/estrogen network: testosterone is mainly converted into estrogens with only a fraction metabolized to DHT, too little to restore the balance.

Supporting evidence for the mechanism of altered androgen/estrogen balance

There are several papers supporting the central role of androgen excess in breast cancer development: (1) prospective studies in healthy women have consistently shown a strong relationship between high serum androgen levels and increased risk of developing ER-positive breast cancers (Key *et al.* 2002, Missmer *et al.* 2004, Cummings *et al.* 2005). These studies also reported an association between increased risk of breast cancer and high serum levels of estrogens, a finding consistent with increased production of estrogens fueled by high androgen levels; (2) prospective studies in women with ER-positive breast cancer show an increased risk of relapse in patients with high testosterone levels, thus highlighting the role of androgen excess in favoring disease progression (Berrino *et al.* 2005, Micheli *et al.* 2007, Emond *et al.* 2011, Secreto 2012b); (3) ER positivity in tumors was associated with high serum testosterone levels but not with high serum estradiol levels (Secreto *et al.* 2009, 2011), thus suggesting a direct role of androgens in the development of estrogen-dependent breast cancers; (4) AR is present in up to 90% of ER-positive tumors, suggesting once again that the interplay between estrogens and androgens is a crucial determinant in cancer growth (Peters *et al.* 2009, Suzuki *et al.* 2010, Fioretti *et al.* 2014); (5) the abundant expression of androgen-producing enzymes (i.e. 17 β -hydroxysteroid dehydrogenase type 5 (HSD5) and 5 α -reductase) and estrogen-producing enzymes (i.e. 17 β -HSD1, aromatase and steroid sulfatase) in breast cancer tissue suggests an increased intratumor synthesis of sex hormones leading to an androgen/estrogen balance at a higher level compared to non-cancerous tissue (Suzuki *et al.* 2003, 2006, 2008, 2010, Bulun *et al.* 2005, Santen *et al.* 2009, Nagasaki *et al.* 2009, Sasano *et al.* 2009a). Figure 2 shows the biosynthetic pathways of sex steroids in peripheral tissues and the enzymes involved in their production; (6) studies concerning androgens and estrogens concentrations in breast cancer tissue provide further support to our androgen/estrogen imbalance theory. Estradiol levels within breast cancer tissue were found to be significantly higher when compared to non-cancerous tissue and blood levels, suggesting an increase in local production of estrogens from androgen precursors (Van Landeghem *et al.* 1985, Vermeulen *et al.* 1986, Mistry *et al.* 1986,

Recchione *et al.* 1995, Shibuya *et al.* 2008, Sasano *et al.* 2009b, Yaghjian & Colditz 2011). Furthermore, Kakugawa *et al.* (2017) reported an intra-tissue concentration of estradiol about ten times higher in ER-positive compared to ER-negative tumors, while circulating estradiol was similar in the two groups. Only a few papers have dealt with intratumor concentrations of testosterone (Vermeulen *et al.* 1986, Mistry *et al.* 1986, Recchione *et al.* 1995) and DHT (Mistry *et al.* 1986, Recchione *et al.* 1995). Intratumor levels of DHT were found to be significantly higher than blood's, with a tissue-to-plasma ratio of 3:1. Suzuki *et al.* (2006) reported intratumor DHT concentrations similar to those observed in previous studies, though they did not measure DHT serum levels. Shibuya *et al.* (2008) found a tissue concentration of DHT three times higher in ductal carcinoma *in situ* than in non-cancerous tissue. The local production of DHT is suggested by the strong correlation between intratumor testosterone and DHT levels ($r=0.71$) (Mistry *et al.* 1986, Recchione *et al.* 1995). The results from studies on DHT concentration in breast cancer tissue may be interpreted as the cell's attempt to maintain the androgen/estrogen balance by increasing DHT production to counter the excessive estradiol activity. Unlike the sex hormones ratio mentioned above, the tissue/plasma ratio of testosterone was close to one in the few studies that measured it (Vermeulen *et al.* 1986, Mistry *et al.* 1986, Recchione *et al.* 1995). Tumor/plasma testosterone ratio displayed a wide dispersion, ranging from 0.05 to 5.0 in one study (Vermeulen *et al.* 1986) and from 0.3 to 4.0 in another (Recchione *et al.* 1995). This implies that tissue testosterone levels were higher than blood testosterone levels in roughly half the tumors while the other half showed higher blood testosterone levels. Whether this finding has biological relevance or not is yet to be established. Nonetheless, we tentatively suggest that elevated intratumor testosterone concentrations might relate to the aromatase pathway of estradiol synthesis, whereas low concentrations might be associated with the sulfatase pathway (Fig. 2).

In conclusion, a large body of evidence supports our hypothesis of androgen excess and androgen/estrogen imbalance as important stimulants of ER-positive tumors development.

Androgen excess in development of ER-negative breast cancer

The mammary gland is a modified apocrine gland that shares the same embryological origin with salivary and sweat glands (Oftedal 2002, Javed & Lteif 2013).

Figure 2

Simplified illustration of the sex steroids biosynthetic pathway in peripheral tissues after menopause. The two light blue boxes show the contribution of adrenal and ovarian hormones to the pool of peripherally synthesized hormones. Starting from inactive androgen precursors (androstenediol, DHEA, DHEAS), testosterone can be synthesized through several pathways while estrogen formation can only occur by aromatization of androgen precursors, namely androstenedione and testosterone. Two pathways are involved in the synthesis of estradiol: the sulfatase pathway and the aromatase pathway. The activity of sulfatase is counterbalanced by sulfotransferase through the conversion of estrone to estrone sulfate. However, estrogens cannot be reverted to their androgen precursors since there is no 'de-aromatizing enzyme'; thus, aromatase is counterbalanced by the 5 α -reductase enzyme via DHT synthesis. The interplay between aromatase and 5 α -reductase in breast cancer cells has been well documented by Suzuki *et al.* (2006). The figure illustrates the role of androgen precursors in the biosynthesis of estradiol and DHT in support of our androgen/estrogen balance model. It must be noted that DHT may be metabolized to 3 α -androstenediol and to 3 β -androstenediol by the 3 α - and 3 β -HSD enzymes, respectively. These metabolites possess mild estrogenic activity and are capable of stimulating cancer growth in an estrogen-depleted environment (e.g. women undergoing AIs therapy). An in-depth analysis of DHT metabolism is covered by the works of McNamara *et al.* (2014a,b) and Swerdloff (2017). AIs, aromatase inhibitors; DHEA, dehydro-epi-androsterone; DHT, dihydrotestosterone; HSD, hydroxysteroid dehydrogenase. Blue box, sulfatase pathway; Green box, aromatase pathway; Red box, aromatase and sulfatase pathway; Yellow box, 5 α -reductase.

The apocrine epithelium is influenced by androgens and is an active site for androgen metabolism (Wales & Ebling 1971, Bradlow *et al.* 1981, Miller *et al.* 1985, Bélanger *et al.* 1990, Lai 1995, Gatalica 1997, Selim & Wells 1999).

Androgen excess, EGF synthesis and activation of the ErbB family of tyrosine kinase receptors

Apocrine cysts arise from areas of apocrine metaplasia in the terminal-ductal-lobular-unit (TDLU) (Wellings & Alpers 1987). They are a common finding in human breasts and are associated with hyperplastic alteration of TDLU and an increased risk of breast cancer (Haagensen *et al.* 1977, Schuerch *et al.* 1982, Mazoujian *et al.* 1983, Dixon *et al.* 1985, Wellings & Alpers 1987). Per se, these

cysts cannot be regarded as precancerous lesions since cancer rarely arises from their epithelial lining. Therefore, the association of apocrine cysts with hyperplasia and breast cancer hints at a possible common endogenous milieu for cysts growth and for cancer development.

Available evidence shows that (1) apocrine cells are AR positive and ER negative; (2) apocrine cysts are characterized by high concentrations of DHEAS (Bradlow *et al.* 1981, Miller *et al.* 1983); (3) studies on apocrine breast cyst fluid composition have shown elevated levels of sex steroids (Bradlow *et al.* 1981, Boccardo *et al.* 1988, Bélanger *et al.* 1990, Angeli *et al.* 1994, Selim & Wells 1999, Wells & El-Ayat 2007) and androgen-induced proteins, namely gross cystic disease fluid proteins and prostate-specific antigen (Haagensen *et al.* 1977, Mazoujian *et al.*

1983, Diamandis *et al.* 1996, Lai *et al.* 1996, Malatesta *et al.* 1999). All together, these findings support the idea of androgens being an important component of the endogenous milieu that favors apocrine cyst growth. Furthermore, a number of studies (Collette *et al.* 1986, Boccardo *et al.* 1988, Lai *et al.* 1989, Pascall 1997, Boccardo *et al.* 2001) found high levels of epidermal growth factor (EGF), a known stimulant of breast epithelial cell proliferation (Osborne *et al.* 1980, Lippman *et al.* 1986, Borellini & Oka 1989), in apocrine cysts' fluid and assumed that androgens were responsible for EGF secretion in the apocrine cells of the human breast, in analogy with what was observed in the apocrine glands of animals (Wales & Ebling 1971, Barthe *et al.* 1974, Sheflin *et al.* 1996).

The EGF receptor (EGFR), also known as human epidermal growth factor1 (HER1), is part of the ErbB family of tyrosine kinase receptors, together with HER2, HER3 and HER4. The binding of EGF and/or EGF-related peptides (e.g. transforming growth factor- α , amphiregulin, heparin binding EGF, betacellulin, neuregulins and others –see Table 1 in Normanno *et al.* 2001) with EGFR, HER3 and HER4 triggers homo- or heterodimerization of these receptors followed by phosphorylation of receptor tyrosine kinase and activation of the intracellular signaling pathway. However, neither EGF nor EGF-related peptides directly bind with HER2, which is activated by heterodimerization with either EGFR or HER3, or HER4. ErbB receptors, together with their ligands and homo- and hetero-dimers, may interact with each other to form a complex signaling network that influences breast epithelium growth and may favor breast cancer development (Tzahar *et al.* 1996, Graus-Porta *et al.* 1997, Normanno *et al.* 2001, Prenzel *et al.* 2001, Wieduwilt & Moasser 2008).

The oncogenic role of androgens in molecular apocrine tumors is well known (Farmer *et al.* 2005, Doane *et al.* 2006). In these tumors, AR positivity was repeatedly found associated with an overexpression of HER2, suggesting a cross-talk between the AR and HER2 signaling pathways. These two, in turn, act in synergy to stimulate cell proliferation (Naderi & Hughes-Davies 2008, Vranic *et al.* 2010, Chia *et al.* 2015). Lastly, androgens are capable of inducing cancer proliferation in a certain subgroup of triple-negative tumors, the luminal androgen receptor (LAR), which share several features with molecular apocrine tumors (Lehmann *et al.* 2011).

The evidence reported above identifies the activation of AR and ErbB receptors as two pathways for AR-positive/ER-negative cancer development. We speculate that androgens can trigger both these pathways. Currently,

ER-negative tumors are roughly classified in two molecular subtypes based on the expression of ER, progesterone receptor (PR) and HER2: (1) HER2-enriched subtype comprising ER-negative, PR-negative and HER2-positive tumors; (2) triple-negative tumors (TNBC) (ER-negative, PR-negative and HER2-negative) (McNamara *et al.* 2014a, Chia *et al.* 2015, Hon *et al.* 2016). Overall, approximately 50% of ER-negative tumors express AR, with a rate of 50–81% in HER2-positive subtypes and 12–35% in triple-negative subtypes (Proverbs-Singh *et al.* 2015).

EGFR is not routinely measured and, as such, is not considered for tumor classification; yet, it is often overexpressed in basal-like tumors, a particularly aggressive subtype of TNBC (Nielsen *et al.* 2004, Rakha *et al.* 2007, Cheang *et al.* 2008, McNamara *et al.* 2013, 2014b, Changavi *et al.* 2015). It is plausible that an androgen-driven overproduction of EGF in apocrine breast epithelial cells might also aid tumor growth in EGFR overexpressing non-apocrine tumors.

However, studies concerning the role of androgens in TNBC report lower levels of AR and androgen-synthesizing enzymes (5 α -reductase and 17 β -HSD5) in the more aggressive subset of tumors compared to the less aggressive ones (i.e. lower levels in infiltrating cancers than in *in situ* ones and in basal-like cancers than in non-basal ones) (McNamara *et al.* 2013, 2014b), thus suggesting a possible protective role of androgens against developing more aggressive subtypes of TNBC. Alternatively, the reduction in AR and androgen-synthesizing enzymes might be interpreted as an attempt to hinder the androgen-driven excessive EGF production and restore the balance between androgens, EGF and ErbB receptors activation and cell proliferation, through yet unknown regulatory pathways.

Figure 3 illustrates our hypothesis of how the AR and the ErbB pathways may be triggered by androgens during the development of ER-negative/AR-positive tumors.

Anti-androgen therapy in breast cancer

Emerging evidence of the role of androgens in the development of ER-negative tumors led to consider AR as a viable therapeutic target. The success of preclinical and clinical trials testing for tolerance and antitumor activity of bicalutamide and enzalutamide provided the foundations for phase II clinical trials investigating a possible use of next generation anti-androgen drugs in breast cancer (Proverbs-Singh *et al.* 2015, Chia *et al.* 2015, Rahim & O'Regan 2017). These trials enrolled both ER-negative patients and ER-positive, resistant to anti-estrogen therapy, patients. The latter were included

Figure 3

A diagram of the two pathways that may be activated by androgens in the development of ER-negative breast cancer: AR and ErbB family receptors (EGFR and HER2) pathways. The activation of AR stimulates the development of apocrine and LAR tumors, a subset of triple-negative cancers. Activation of ErbB receptors cooperate in the development of apocrine tumors and might favor the growth of EGFR overexpressing tumors. The role of androgens in the progression of basal-like tumors is unclear, as stated above. AR, androgen receptor; ER, estrogen receptor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor2; LAR, luminal androgen receptor.

since overexpression of AR, EGFR and HER2 all are well-known mechanisms of endocrine resistance (Massarweh *et al.* 2008, De Amicis *et al.* 2010, Osborne & Schiff 2011, Rechoum *et al.* 2014, Ciupek *et al.* 2015) that suggest a role of androgens in the activation of both the AR and the ErbB receptors pathways. As a result, addressing this excessive androgen production via AR blockade, alongside standard chemotherapy, might result in better outcomes when compared to conventional treatment alone. However, it must be noted that blocking AR in an androgen-rich environment might cause androgens to shift toward an increased activation of ErbB receptors, thus hindering or even nullifying the initial therapeutic effort with anti-androgens. As previously stated, a reduced expression of AR was found to be associated with more aggressive forms of TNBC, thus suggesting that anti-androgen therapy might not be indicated for this kind of tumors (McNamara *et al.* 2013, 2014b).

The use of 17 α hydroxylase/17–20 lyase (CYP17) inhibitors that block androgen, estrogen and glucocorticoid synthesis is worthy of note. The efficacy of abiraterone acetate, a CYP17 inhibitor, has been evaluated in patients with ER-negative (Bonnetfoi *et al.* 2016) or ER-positive (O'Shaughnessy *et al.* 2016) metastatic breast cancer and was found to be less than expected. However, studies on abiraterone acetate and other CYP17 inhibitors are still ongoing (Rahim & O'Regan 2017). A more in-depth analysis of these studies is beyond the scope of this review and has been covered by the works of Proverbs-Singh *et al.* (2015), Chia *et al.* (2015), and Rahim & O'Regan (2017).

Anti-androgen therapy is still at an early stage. The results from ongoing trials might provide more

precise recommendations for a potential clinical use. For the time being, the possible role of androgen excess in breast cancer development is certainly gaining momentum and, with it, the opportunity for a novel therapeutic approach.

Clinical implication of androgen excess

Breast cancer prevention

Based on our hypothesis on the genesis of ER-positive tumors, addressing the overproduction of androgens in otherwise healthy women may be an effective preventive measure.

Anti-estrogen therapy has been widely proven to reduce the incidence of breast cancer in high-risk healthy women (Goss *et al.* 2011, Cuzick *et al.* 2013, Cuzick *et al.* 2014). However, these drugs cause a steep and symptomatic drop in estrogen levels, with a subsequent worsening in quality of life and, in turn, in poor adherence to therapy (Ropka *et al.* 2010, Waters *et al.* 2010). In a recent publication (Secreto *et al.* 2016), we proposed a treatment regimen with a GnRH analog (i.e. medical ovariectomy) for menopausal women with both high breast cancer risk and high testosterone levels, the latter being a marker of ovarian hyperandrogenemia. The goal of this intervention is to restore the normal balance between androgens and estrogens before the excessive proliferative stimulus results in infiltrating cancer. Furthermore, adherence to therapy is expected to be higher since the choice of GnRH analogs avoids all the severe side effects of hypoestrogenism caused by conventional therapy.

Adjuvant treatment of early breast cancer

The goal of hormonal therapy in ER-positive tumors is to block estrogen production. For many years, ER-negative tumors were considered non-hormone dependent thus not responsive to hormonal therapy. Lately, a possible role of androgens in the genesis of these tumors has led to clinical testing of anti-androgen treatment. In this paper we propose a possible role of androgen excess in promoting both ER-positive and ER-negative breast cancer. As a result, the excessive production of androgens might be the common predisposing factor shared by many different tumors; yet, it is not regarded as a possible therapeutic target and hormonal treatment is aimed solely at estrogens and/or the overexpression of HER2 and/or EGFR and/or AR.

The use of anti-estrogen therapy in ER-positive tumors is a clear example of this approach. These drugs are capable of markedly reducing disease progression rates by halting the excessive estrogen synthesis secondary to abnormal androgen production. Furthermore, androgens partake in the endocrine resistance mechanisms (i.e. AR and EGFR overexpression) and are the culprit behind renewed estrogen overproduction and increased risk of late relapse after anti-estrogen discontinuation. Thus, we believe that combining anti-estrogen therapy with the correction of androgen excess might yield better outcomes compared to anti-estrogens alone (Secreto *et al.* 2017). The same holds true for tumors overexpressing HER2 and EGFR and the ER-negative/AR-positive tumors as well. Once the source of androgen overproduction has been identified via a simple blood assay, it can be addressed with medical ovariectomy, thus providing a useful aid to standard treatment. As a result, we believe that testosterone blood levels should be part of the routine workup for patients with newly diagnosed breast cancer.

We suggest a therapeutic strategy aimed at diagnosing and blocking excessive androgen production, thus differing greatly from the ongoing studies on the efficacy of anti-androgen drugs in advanced stage AR-positive tumors. In particular, our approach cannot be compared to the use of abiraterone acetate in patients heavily pretreated with hormone therapy, chemotherapy and not selected for their high androgen levels.

Concluding remarks

This paper dwelled on the mechanisms employed by androgens in regulating breast epithelial growth and suggested how a chronic overproduction of these hormones

might be crucial in breast cancer development. It is well established that the cut-off value for elevated androgen blood levels varies between different populations, different laboratories and different analytical methods. As a result, we referred to 'androgen excess' and 'high levels of androgens' to indicate an increased production of these hormones, as reported in the studies setting the diagnostic criteria for hyperandrogenic syndromes (Rotterdam ESHRE/ASRM 2004, Legro *et al.* 2013, El Hayek *et al.* 2016). Nonetheless, it must be noted that androgens play a crucial role in cell proliferation regardless of the criteria defining their overproduction.

We highlighted how androgen excess and breast cancer are associated regardless of the source of the overproduction. With the exception of dire ailments such as androgen-secreting tumors, Cushing's syndrome and classical congenital adrenal hyperplasia (CAH), the main etiologies of hyperandrogenism are ovarian stromal hyperplasia, polycystic ovarian syndrome (PCOS) and non-classical CAH.

Ovarian stromal hyperplasia is consistently found within polycystic ovaries and often is present in postmenopausal ovaries, ranging from low to medium up to severe grade.

In their 1952 paper, Sommers & Teloh reported ovarian stromal hyperplasia in 83 out of 100 deceased breast cancer patients and in 50 out of 133 deceased women (37.6%) without any discernable malignancy after autopsy (Sommers & Teloh 1952). The authors concluded that ovarian stromal hyperplasia was more frequent and of a higher grade in cancer patients compared to non-cancer patients.

A number of studies showed an association between androgen levels within ovarian veins and the grade of stromal hyperplasia with a steady drop in testosterone and androstenedione levels after ovariectomy (Lucisano *et al.* 1986, Sluijmer *et al.* 1998, Jongen *et al.* 2003, Secreto *et al.* 2016). The papers cited above support the link between ovarian stromal hyperplasia, increased androgen production and breast cancer.

PCOS is a common endocrine disorder among fertile women defined as hyperandrogenism (diagnosed with clinical signs and symptoms or laboratoristically) and chronic anovulation, with or without a polycystic appearance of the ovaries (Rotterdam ESHRE/ASRM 2004, Azziz *et al.* 2006, Legro *et al.* 2013, Dumesic *et al.* 2015, El Hayek *et al.* 2016). Hyperandrogenism is responsible for anovulatory cycles, ovarian stromal hyperplasia and is associated with insulin resistance, obesity, dyslipidemia and thus is considered a key factor in the development

Table 1 Summary of the possible role of androgen excess in the development of different subsets of breast cancer.

Mechanisms of growth stimulation	Tumor type
Androgen excess	
Conversion to estrogens and activation of the ER pathway	ER+/HER2– (Luminal A) ER+/HER2+ (Luminal B)
Binding to AR and activation of the AR pathway	ER–/AR (apocrine tumors) ER–/AR+/HER2+ (apocrine tumors) ER–/AR+ (TNBC LAR subtype)
Increased production of EGF and activation of the ErbB receptors family	ER+/HER2+, ER–/AR+/HER2+, other HER2 and EGFR overexpressing tumors ^a

^aIn theory, androgens are capable of indirect stimulation of all HER2 and EGFR overexpressing tumors; yet, there is still insufficient evidence to support our claim. We discussed in the main text above how androgens are likely to protect against the development of basal-like TNBC.

AR, androgen receptor; ER, estrogen receptor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor2; LAR, luminal androgen receptor; TNBC, triple-negative breast cancer.

of PCOS (Azziz *et al.* 2006, Dumesic *et al.* 2015). High androgen levels, infertility, ovarian stromal hyperplasia, obesity and insulin resistance are all linked to an increased risk of developing breast cancer. As a result, a strong relationship between PCOS and breast cancer would be expected yet current evidence does not support this association. (Coulam *et al.* 1983, Balen 2001, Ghasemi *et al.* 2010, Barry *et al.* 2014, Shen *et al.* 2015). There is a general agreement on how the variability among the PCOS' diagnostic criteria can mislead risk evaluation.

PCOS is a complex syndrome with genetic, metabolic and environmental factors all concurring in yielding different clinical manifestations. Alongside the classical phenotype (defined as hyperandrogenism and chronic anovulation with or without the polycystic appearance of the ovaries) milder forms are present such as non-classical ovulatory PCOS (i.e. hyperandrogenism, polycystic ovaries, regular menstrual cycles) and non-classical mild or normoandrogenic PCOS (i.e. chronic anovulation, polycystic ovaries, normal androgens) (El Hayek *et al.* 2016). Such a wide range of clinical manifestations suggests that women with non-classical PCOS might be deemed normal when the hyperandrogenism and the metabolic derangements are asymptomatic. In time, these women are more likely to develop PCOS-associated morbidities (i.e. obesity, insulin resistance and dyslipidemia) and, we believe, are at a greater risk of developing breast cancer.

This hypothesis would partially explain the inconsistencies that emerged from the studies on breast cancer risk in PCOS patients, since non-classical PCOS women might have been assigned to the control group. For instance, in some studies, the diagnosis of PCOS among the subjects was solely based on self-certification provided by the patients themselves (Balen 2001, Barry *et al.* 2014).

CAH is an autosomal recessive disorder caused by a deficiency of the 21-hydroxylase enzyme, characterized

by insufficient cortisol production and excessive androgen synthesis. The non-classical variant is quite frequent, albeit a precise estimate of its incidence in the general population is unobtainable due to the often-mild symptoms and late onset (Speiser *et al.* 1985, Merke & Bornstein 2005, New 2006, Bidet *et al.* 2010). Women affected by this variant will only have a slight cortisol deficiency and some of them require no therapy to live a normal life. Signs of hyperandrogenism, such as hypertrichosis, menstrual irregularity, infertility and, in some cases, PCOS, are present and may vary in intensity (Merke & Bornstein 2005, New 2006). All the guidelines for the diagnosis of PCOS (El Hayek *et al.* 2016) require the exclusion of CAH, since the two conditions share common traits such as hyperandrogenism.

Just as mentioned before regarding the non-classical PCOS, we believe that women with undiagnosed non-classical CAH might be part of the general population and thus considered normal. This can introduce an additional confounding factor in those studies regarding breast cancer risk in PCOS patients.

A summary table (Table 1) of our hypothesis on the role of androgens in the development of different subsets of breast cancer concludes this section.

Conclusion

Breast cancer is a hormone-dependent disease that encompasses biologically and clinically different tumor types. In the past years, basic research led to a better understanding of the different subtypes of breast cancer and, consequently, to a substantial improvement in therapy.

The aim of this review is to offer clinicians and researchers a novel approach to breast cancer development by emphasizing the pivotal role of androgen excess. We believe that there is sufficient evidence in the literature to

support a randomized clinical trial. This may prove how correcting an excessive androgen production is beneficial to both prevent and treat breast cancer. We are well aware that some of our conclusions might be modified by new findings in this ever-changing field of research. Still, we are certain that our fresh approach to this somewhat unexplored matter might yield significant results and further deepen our understanding of breast cancer.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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