

Short Review

## Role of Androgen Excess in the Development of Estrogen Receptor-positive and Estrogen Receptor-negative Breast Cancer

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**Abstract.** *The androgen-excess theory posits a central role of androgens in promoting breast cancer development. At first glance, this appears to contradict the currently accepted central role of estrogens in this process, but as we will show, the apparent contradiction is not a real one. In the present article, we review the mechanisms by which androgen excess may stimulate cancer growth in different subsets of estrogen receptor-positive and estrogen receptor-negative tumors. We also propose an endocrine classification of postmenopausal breast cancer based on the simultaneous evaluation of a patient's serum testosterone levels and the estrogen receptor status of the tumor. This classification identifies several different subsets of tumors and may have important clinical implications.*

Estrogen has always been recognized as playing a primary role in breast carcinogenesis, and the discovery of intratumoral estrogen receptor (ER) provided further evidence of estrogen involvement in this process. ER-positive status is considered unequivocal proof of hormone-dependent cancer, and the evaluation of ER status is used in clinical practice to select patients for endocrine therapy.

In a series of studies during the last 40 years, we have repeatedly found that increased androgenic activity is the principal endocrine abnormality of women with hormone-dependent breast cancer, a finding that prompted us to propose the androgen-excess theory. The theory points to

androgen excess as being a stimulatory hormonal alteration common to several breast cancer types, both ER-positive and ER-negative, and proposes a novel viewpoint about breast cancer without refuting, indeed rather reinforcing, the important role commonly attributed to estrogen.

### Origin of the Androgen-Excess Theory

The theory takes its origin from articles that Grattarola published in the 1970s which showed that increased urinary androgen excretion and endometrial hyperplasia are typical features of patients with hormone-dependent breast cancer (1-5). These original findings have been subsequently confirmed and extended in several studies reviewed in (6), and have found substantial support in a large series of prospective studies (7-13). More recently, further support to the theory has been provided by the identification of a subset of androgen receptor (AR)-positive tumors with increased androgen signaling (14), and of a subset of ER-negative tumors whose growth is directly stimulated by androgens in an AR-dependent way (15).

The theory can be summarized very briefly as follows: Elevated androgen levels are frequently present in women with breast cancer (6) and are a marker of hormone-dependent disease, as shown by the more frequent remission of metastases after ovariectomy in patients with high pre-surgical urinary androgen levels than in those with normal pre-surgical androgen excretion (2, 4, 16, 17). Urinary androgen excretion and ER status have approximately the same ability to predict the clinical outcome from ovariectomy in patients with metastatic breast cancer (18-20). One study showed that remission of metastases occurred in almost 90% of patients with high androgen excretion and positive ER status but in none of the patients with normal androgen excretion and negative ER status (20);

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Table I. Endocrine classification of postmenopausal breast cancer obtained by combining estrogen receptor (ER) status (positive/negative) and serum testosterone levels (high/low) in a cohort of 534 patients. The cut-off value between high and low testosterone levels is 0.39 ng/ml (median of all values).

	No. of patients	%
ER-positive group	443/534	82.9
High testosterone level	229/443	51.7
Low testosterone level	214/443	48.3
ER-negative group	91/534	17.1
High testosterone level	30/91	33.0
Low testosterone level	61/91	67.0

these findings suggest that androgen excretion and ER status are independent prognostic factors for response to ovariectomy, able to identify different populations of hormone-dependent breast cancer. The androgen excess originates in the ovary: after ovariectomy androgen levels revert to normal in patients with elevated pre-surgical levels (4, 16, 17), and on histological examination of the removed ovaries, ovarian interstitial-cell hyperplasia is found in all the high androgen excretors (2, 4). The interstitial cells are the physiological source of ovarian androgens (21), and a hyperplastic pattern points to increased androgen production by the gland, thus providing anatomical support to the biochemical evidence for androgen excess in patients with breast cancer.

### Endocrine Classification of Breast Cancer

In two recently published articles (22, 23) we found a significant association between elevated serum testosterone levels and ER-positive status of the tumors in a cohort of 534 postmenopausal patients with breast cancer. This finding confirms the results of previous studies on the role of androgen excess as a marker of hormone-dependent disease (4, 16, 17) and suggests that the hormonal status of the patient should be evaluated together with the characteristics of the tumor. We believe that a comprehensive characterization of the patient and tumor would be helpful in selecting different subsets of breast cancer to submit to different treatments, and would constitute a further step toward personalized therapy, which is the objective of modern oncology. Accordingly, we propose an endocrine classification of breast cancer that takes into account both the serum testosterone level (high/low) of the patient and the ER status (positive/negative) of the tumor.

We evaluated both parameters in our cohort of 534 postmenopausal patients with breast cancer and identified

Table II. Outcomes of 361 postmenopausal patients with estrogen receptor (ER)-positive breast cancer divided into two subgroups by serum testosterone level (high/low). The cut-off value between high and low testosterone levels is 0.39 ng/ml (median of all values).

	N	%	Disease progression		p-Value <sup>†</sup>
			N	%	
ER-positive group	361				
High testosterone subset	184	51	27	14.7	0.037
Low testosterone subset	177	49	14	7.9	

N, Number of patients; <sup>†</sup>Pearson's chi-square test.

four different groups of tumors, two ER-positive groups and two ER-negative groups, each divided into subgroups with high or low testosterone levels (Table I).

Within these four major groups, other subgroups can be further defined according to other tumor characteristics, such as progesterone receptor (PR), AR, epidermal growth factor receptor (EGFR), human epidermal growth factor type-2 receptor (HER2), and others.

Preliminary data on the outcomes in the patients of our cohort validate the clinical implications of our classification. Studies on 434 patients (81%), 361 ER-positive and 73 ER-negative, with a median follow-up of 67.7 months, showed that high serum testosterone levels were present in 184 (51%) patients with ER-positive tumor and in 16 (22%) of those with ER-negative tumors. Disease progression was observed in 27 patients with ER-positive tumor and high serum testosterone levels (14.7%) and in 14 with ER-positive tumor and low serum testosterone levels (7.9%) ( $p=0.037$ ) (data presented at the BIT's 4th Annual World Congress and Expo of Molecular Diagnostics in Beijing, China, September 22-24, 2011) (Table II).

This finding suggests that the serum testosterone level identifies two different subsets of ER-positive tumors with different outcomes. Previous studies (2, 4, 16, 17, 19) have shown that the ovary is the main source of the elevated testosterone, and suppression of ovarian androgen production with gonadotropin-releasing hormone (Gn-RH) analogs (medical ovariectomy) might be considered as a possible additional therapeutic option for these patients.

Interesting data on the outcomes of patients with ER-negative tumor were found when the AR status (positive/negative) was taken into account (Table III).

AR status identified two different subgroups of patients with ER-negative tumor and high testosterone level: disease progression occurred in one out of the seven patients with AR-positive disease (14%) but in 8 out of the 18 with AR-

Table III. Outcomes of 73 postmenopausal patients with estrogen receptor (ER)-negative breast cancer classified by testosterone level (high/low) and androgen receptor (AR) status (positive/negative).

	N	Disease progression		<i>p</i> -Value <sup>†</sup>
		N	%	
ER-negative group	73			
High testosterone and AR-positive	7	1/7	14.3	n.e.
High testosterone and AR-negative	18	8/18	44.4	
Low testosterone and AR-positive	22	9/22	40.9	0.464
Low testosterone and AR-negative	26	8/26	30.8	

N, Number of patients; <sup>†</sup>Pearson's chi-square test; n.e., not evaluable because of the small number of cases.

Table IV. Enzymes involved in intratumoral production of active estrogens and androgens.

Estrogen-producing enzymes	
Aromatase	→ Aromatization of androstenedione to estrone and testosterone to estradiol
17β-HSD1	→ Reduction of estrone to estradiol
Sulfatase	→ Conversion of estrone sulfate to estrone
Androgen-producing enzymes	
17β-HSD5	→ Reduction of androstenedione to testosterone
5α-reductase	→ Reduction of testosterone to dihydro-testosterone

17β-HSD, 17 β-hydroxysteroid dehydrogenase.

negative disease (44%). Thus AR-positive status appeared to be a factor for better outcome in the ER-negative disease with high testosterone level, although the number of cases was too small to achieve statistical significance. The value of categorizing patients by testosterone level is clear: if we had considered only the AR status in these patients, we would have observed disease progression in 10/29 patients with AR-positive disease (34.5%) and in 16/44 of those with AR-negative disease (36.4%), and we would have concluded, incorrectly, that AR status is not prognostic in patients with ER-negative disease.

### Implication of Androgen Excess in the Development of ER-positive Breast Cancer

Most cases of breast cancer are ER-positive, and such tumors are generally regarded as being hormone-dependent. We

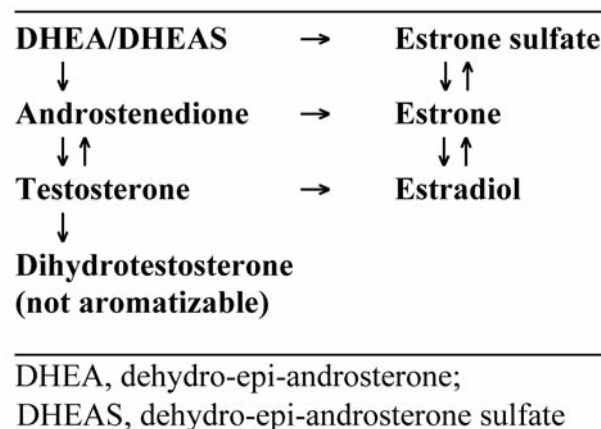


Figure 1. Simplified scheme of the sex-steroid biosynthetic pathways.

Table V. Classification of estrogen receptor (ER)-negative tumors by simultaneous evaluation of testosterone levels and androgen receptor (AR) status in a cohort of 91 patients.

ER-negative subsets	N	%
High testosterone and AR-positive	9	9.9
High testosterone and AR-negative	21	23.1
Low testosterone and AR-positive	28	30.8
Low testosterone and AR-negative	33	36.2

N, Number of patients.

suggest that androgen excess stimulates the growth of ER-positive tumors by increasing conversion into estrogens in the tumoral tissue; this is consistent with the widely recognized role of estrogens in breast cancer.

Intratumoral estradiol concentrations are about ten times as high as those in blood (24-29), suggesting increased local production of this hormone. Two principal pathways are involved in the synthesis of estradiol: the aromatase pathway and the sulfatase pathway (Figure 1).

As shown in the Figure, estrogens can be synthesized only from androgen precursors. Elevated concentrations of such androgen precursors are present in breast-cancer tissue (24, 27, 30, 31), and there is clear evidence that estrogen-producing enzymes [aromatase, 17β-hydroxysteroid dehydrogenase-1 (17β-HSD1), and sulfatase], as well as enzymes that produce the active androgens testosterone and dihydrotestosterone (17β-HSD5 and 5α-reductase), are abundantly expressed in breast tumors (32-36) (Table IV).

An elevated intratumoral level of androgen precursors in the presence of increased activity of estrogen-producing

Table VI. Proposed targeted therapies for the four groups of estrogen receptor (ER)-negative tumors obtained by simultaneous evaluation of testosterone levels and androgen receptor (AR) status.

ER-negative subsets	Proposed targeted therapies
High testosterone and AR-positive	Antiandrogens (and medical ovariectomy)*
High testosterone and AR-negative	EGFR inhibitors <sup>†</sup> (and medical ovariectomy)*
Low testosterone and AR-positive	Antiandrogens
Low testosterone and AR-negative	No hormonal therapy

\*Hypertestosteronemia largely originates from ovarian interstitial-cell hyperplasia. <sup>†</sup>Synthesis and function of EGF is under the control of androgens; EGFR is frequently expressed in ER-negative tumors.

enzymes results in increased local synthesis of estradiol, which is the final stimulator of cancer growth through binding to the ER. At the same time, increased 5 $\alpha$ -reductase activity favors increased conversion of testosterone into the stronger (and non-aromatizable) androgen dihydrotestosterone (DHT), thus explaining the finding that DHT concentrations are three times as high in tumor tissue as in blood (28, 29).

As shown in Table I, about half of patients with ER-positive tumor have high circulating levels of testosterone. In these patients, androgen excess originates mainly from hyperplastic ovarian stromal tissue (2, 4, 16, 17). In patients with low circulating testosterone levels, androgen excess probably derives from increased production of adrenal androgens, which provide cancer cells with substrates for intratumoral estrogen formation. Increased adrenal androgen production may also result in increased synthesis of estrone sulfate in peripheral tissues, as suggested by the significant relationship between elevated serum levels of estrone sulfate and an increased risk of breast cancer reported in the four prospective studies that have examined this association in postmenopausal women (7, 37-39).

Standard therapy of ER-positive tumor with antiestrogens and antiaromatase drugs is effective against the result of the androgen excess, *i.e.* increased estrogen production and activity, but is ineffective against the androgen excess itself, which persists in these patients. Additional therapy to eliminate the androgen excess might therefore be useful. Identification of the origin of the androgen excess in a particular patient, *i.e.* whether it is predominantly ovarian or predominantly adrenal, would be important in the choice of such additional therapy: medical ovariectomy would be indicated for patients with high serum testosterone levels (presumably of ovarian origin), and sulfatase inhibitors (which inhibit the conversion of estrone sulfate to estrone and the conversion of DHEAS to DHEA, thus substantially reducing the intratumoral synthesis of estradiol from adrenal precursors) would be indicated for patients with low serum testosterone levels.

### Implications of Androgen Excess in the Development of ER-negative Breast Cancer

ER-negative breast carcinomas are a very heterogeneous class of tumors that express AR in about half of all cases (40-43). The measurement of circulating testosterone levels identifies two groups of ER-negative tumors that can be further subdivided by AR status, thus identifying four different subsets of ER-negative cancer (Table V).

Direct stimulation of cancer growth *via* the androgen/AR pathway is likely in the high testosterone/AR-positive subset, and therapy with antiandrogens would be appropriate for them. Medical ovariectomy might be considered for patients with an ovarian origin of their hyperandrogenemia.

In the high testosterone/AR-negative subset, androgen excess cannot stimulate tumor growth directly nor *via* conversion to estrogens, but it can have a stimulatory effect by increasing the production of EGF; the synthesis of EGF is known to be under the control of androgens (44), and EGFR has been found in 6 to 30% of ER-negative tumors (45). Treatment with EGFR inhibitors might be appropriate in these patients, and medical ovariectomy might also be considered.

It is accepted that hormones can up-regulate the synthesis of their receptors (46-48), and therefore AR-positivity may develop due to intratumoral androgen excess (of adrenal-precursor origin), even in the low serum testosterone level subset; therapy with antiandrogens may also be appropriate for these patients. Finally, we regard the low testosterone/AR-negative subset as a non-hormone-dependent group of tumors that cannot benefit from hormonal therapy.

The clinical implications of our endocrine classification of ER-negative tumors are summarized in Table VI.

### Conclusion

In the present article, we propose a novel view of breast cancer development, suggesting that androgen excess is the principal growth stimulator of ER-positive, as well as ER-



negative, tumors. We propose routine measurement of circulating testosterone levels as a component of an endocrine classification of postmenopausal breast cancer that takes into account both the characteristics of the patient and those of the tumor. Such a classification identifies subsets of tumors not otherwise identifiable by tumor characteristics alone, and may have important clinical implications regarding therapy and outcome. The proposed endocrine classification can be further enriched by including other tumor characteristics, among which HER2 overexpression is of major interest.

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