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SPECIAL REVIEW SERIES: Carcinogenesis of breast carcinoma and its development

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In situ production of sex steroids in human breast carcinoma

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Abstract It is well known that sex steroids are closely involved in the growth of human breast carcinomas, and the great majority of breast carcinomas express sex steroid receptors. In particular, recent studies have demonstrated that estrogens and androgens are locally produced and act in breast carcinoma tissues without release into plasma. Blockade of intratumoral estrogen production potentially leads to an improvement in the prognosis of invasive breast carcinoma patients, and, therefore, it is important to obtain a better understanding of sex steroid-producing enzymes in breast carcinoma. In this review, we summarize recent studies on tissue concentration of sex steroids and expression of enzymes related to intratumoral production of estrogens [aromatase, steroid sulfatase (STS), and 17β-hydroxysteroid dehydrogenase type 1 (17βHSD1)], and androgens (17βHSD5 and 5α-reductase) in invasive and in situ (noninvasive) breast carcinomas, and discuss the significance of intratumoral production of sex steroids in breast carcinoma.

Key words Androgen · Aromatase · Breast cancer · DCIS · Estrogen · Sex steroid

Introduction

Sex steroids, such as estrogens and androgens, play important roles in various target tissues including the reproductive organs. A majority of breast carcinoma tissues express sex steroid receptors, such as estrogen (ER), progesterone (PR), and androgen (AR) receptors,[1,2] and recent studies have demonstrated that biologically active sex steroids are locally produced and act in breast carcinoma tissues. This mechanism is considered to play a pivotal role in the proliferation of breast carcinoma cells. In particular, blockade of intratumoral estrogen production potentially reduces cell proliferation of breast carcinoma, and it is very important to obtain a better understanding of sex steroid-producing enzymes in breast carcinoma as potential therapeutic targets of endocrine therapy. Therefore, in this review, we summarize results of recent studies on tissue concentration of sex steroids and expression of sex steroid-producing enzymes in invasive and in situ (noninvasive) breast carcinomas, and we discuss the potential biological and clinical significance of intratumoral production of sex steroids in human breast carcinomas.

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In situ production of sex steroids in invasive breast carcinoma

Breast carcinoma is the most common malignant neoplasm in women worldwide, and the great majority of breast carcinoma is invasive. Among sex steroids, estrogens immensely contribute to growth of invasive breast carcinoma through binding with ER.[3] Circulating estrogens are mainly secreted from the ovary in premenopausal women, but it is also true that the majority of invasive breast carcinomas arise after menopause when the ovaries cease to be functional. Miller et al.[4] have shown that the concentration of biologically active estrogen, estradiol, was more than 10 times higher in breast carcinoma tissue than in plasma, and the intratumoral estradiol level was not significantly different between premenopausal and postmenopausal breast carcinoma patients.[5] In addition, tissue concentration of estradiol was 2.3 times higher in breast carcinoma than in the areas considered as morphologically normal.[6] Considering that invasive breast carcinomas occurring after menopause frequently express ER, local production of estrogens plays an impor-

tant role in the proliferation of invasive breast carcinoma cells in postmenopausal women.

In contrast to estrogens, androgens are considered to predominantly exert antiproliferative effects via AR in breast carcinoma cells,[7,8] although some divergent findings have been reported. Tissue concentration of androgens was investigated in invasive breast carcinomas by three groups.[9–11] Biologically active and potent androgen, 5α-dihydrotestosterone (DHT), was significantly higher in breast carcinoma tissues than in plasma,[10] and in situ production of DHT has been proposed in breast carcinoma tissues. Intratumoral DHT concentration was not significantly altered according to menopausal status in invasive breast carcinoma tissues.[11]

Figure 1 summarizes representative pathways of the local production of sex steroids in human breast carcinoma tissues, which is currently postulated. Circulating inactive steroids, such as androsteoneione and estrone sulfate, are major precursor substrates of local estrogen production. Aromatase catalyzes androstenedione into estrone, and steroid sulfatase (STS) hydrolyzes estrone sulfate to estrone. Estrone is subsequently converted to estradiol by 17β-hydroxysteroid dehydrogenase type 1 (17βHSD1) and acts locally on breast carcinoma cells through ER. On the other hand, circulating androstenedione is also converted to DHT by androgen-producing enzymes, such as 17βHSD5 (conversion from androstenedione to testosterone) and 5α-reductase (reduction of testosterone to DHT). Therefore, it is very important to examine these sex steroid-producing enzymes in breast carcinoma tissues to obtain a better understanding of the biological and clinical significance of sex steroids in breast carcinoma.

Expression of estrogen-producing enzymes in invasive breast carcinoma

Aromatase

Aromatase is an enzyme located in the endoplasmic reticulum of cells, and a single gene (CYP19) encodes aromatase in humans. Aromatase catalyzes the aromatization of androgens (androstenedione or testosterone) to estrogens (estrone or estradiol) (see Fig. 1). Aromatase is a key enzyme in estrogen biosynthesis, and aromatase inhibitors are currently used in postmenopausal patients with invasive breast carcinoma as an estrogen deprivation therapy. Approximately 70% of breast carcinoma specimens had aromatase activity comparable with or greater than that found in other tissues,[12–14] and aromatase mRNA levels

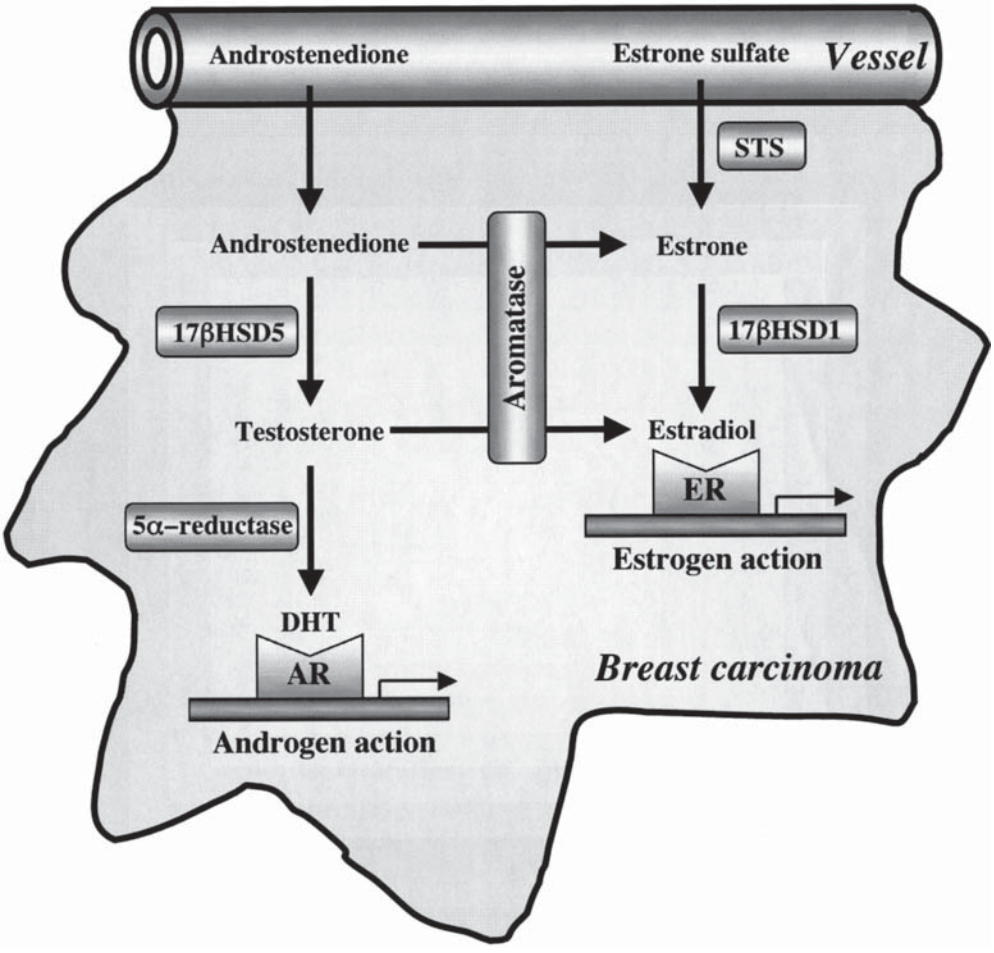

Fig. 1. Scheme representing local production of sex steroids in human breast carcinoma tissues. *STS*, steroid sulfatase; *17βHSD1*, 17β-hydroxysteroid dehydrogenase type 1; *17βHSD5*, 17β-hydroxysteroid dehydrogenase type 5; *ER*, estrogen receptor; *AR*, androgen receptor; *DHT*, 5α-dihydrotestosterone

were significantly increased in the breast carcinomas compared to those in nonmalignant tissues.[15] Aromatase was expressed in invasive breast carcinoma cells and stromal cells such as intratumoral fibroblasts and adipocytes at both mRNA and protein levels.[16] No consistent correlations between aromatase immunoreactivity and known clinicopathological factors are reported in invasive breast carcinomas.

The substrates of aromatase, i.e., androstenedione and testosterone, are not only precursors of estradiol synthesis but also precursors of DHT production (see Fig. 1). DHT itself is nonaromatizable. Intratumoral concentration of DHT was significantly associated with that of testosterone in invasive breast carcinoma tissues,[9,10] suggesting that DHT concentration in invasive breast carcinoma is possibly influenced by amount of precursor. Spinola et al.[17] showed that treatment with an aromatase inhibitor markedly elevated intratumoral testosterone concentrations in dimethylbenz (a)anthracene (DMBA)-induced rat mammary tumors, and Sonne-Hansen and Lykkesfeldt[18] reported that aromatase preferred testosterone as a substrate in MCF-7 breast carcinoma cells. In addition, very recently, Suzuki et al.[11] demonstrated that aromatase expression was inversely associated with intratumoral DHT concentration in invasive breast carcinoma tissues, and that aromatase suppressed DHT synthesis from androstenedione in coculture experiments. Therefore, aromatase is suggested to be a negative regulator of local DHT production, as well as a key enzyme of intratumoral estrogen production, in invasive breast carcinomas.

Previous in vitro studies demonstrated that breast carcinoma cells secrete various factors that induce aromatase expression in adipose fibroblasts,[19] including prostaglandin E_2,[20] interleukin (IL)-1, IL-6, IL-11, and tumor necrosis factor-α.[21,22] On the other hand, it has been also reported that exogenous growth factors such as epidermal growth factor,[23] transforming growth factor,[23] and keratinocyte growth factor[24] stimulated aromatase activity in MCF-7 cells. Very recently, Miki et al.[25] reported that mRNA level and enzymatic activity of aromatase in MCF-7 cells were significantly increased by coculture with primary stromal cells isolated from breast carcinoma tissue. Therefore, aromatase expression is suggested to be, at least in a part, regulated by tumor–stromal interactions in breast carcinoma tissues, which may be promoted by invasion of breast carcinoma into the stroma.

Other studies have demonstrated the regulation of aromatase transcription by nuclear receptors such as liver receptor homologue-1 (LRH-1)[26] and estrogen-related receptor-α (ERRα).[27] The mRNA level of aromatase was significantly associated with that of LRH-1 in adipose tissue adjacent to the invasive breast carcinoma,[26] whereas it was significantly correlated with that of ERRα in the carcinoma cells.[27] Therefore, aromatase expression in invasive breast carcinoma may be differently regulated according to the cell types.

STS

A major circulating form of plasma estrogens is estrone sulfate, a biologically inactive form of estrogen, in postmenopausal women. Estrone sulfate has a long half-life in the peripheral blood, and the level of estrone sulfate is five to ten times higher than that of unconjugated estrogens, such as estrone, estradiol, and estriol, during the menstrual cycle and in postmenopausal women.[28] STS is a single enzyme that hydrolyzes estrone sulfate to estrone (see Fig. 1).

The enzymatic activity of STS is detected in a great majority of invasive breast carcinomas, which is considerably higher than aromatase activity in breast tumors.[29] STS immunoreactivity was detected in carcinoma cells in 60%–90% of breast carcinoma cases.[30,31] STS immunoreactivity was correlated with tumor size and was significantly associated with an increased risk of recurrence in invasive breast carcinomas.[31] STS mRNA expression was also reported to be higher in breast carcinoma tissues than that in normal tissues and was significantly associated with poor clinical outcome of the patients.[32,33]

Reed et al.[34] proposed that the sulfatase pathway might be more important than the aromatase route for intratumoral estrogen synthesis in breast carcinomas, because aromatase mRNA expression was reported to have no significant prognostic value. STS inhibitors are currently being developed by several groups, and results of the phase I study suggested that STS inhibitor may be effective in hormone-dependent invasive breast carcinomas including those that progressed on aromatase inhibitors.[35]

17βHSD1

17βHSD catalyzes an interconversion of estrogens or androgens. Twelve isozymes of 17βHSD have been cloned, and 17β-reduction (17βHSD1, -3, -5, -7, etc.) or oxidation (17βHSD2, -4, -6, etc.) of estrogens and/or androgens is catalyzed by different 17βHSD isozymes. Among these isozymes, the 17βHSD1 enzyme uses NADPH as a cofactor, and mainly catalyzes the reduction of estrone to estradiol (see Fig. 1). Oxidative 17βHSD activity is the preferential direction in normal breast tissues, but the reductive 17βHSD pathway is dominant in invasive breast carcinomas.[36,37] Miyoshi et al.[5] reported that 17βHSD1 mRNA levels and intratumoral estradiol/estrone ratios were significantly higher in postmenopausal than premenopausal breast carcinomas. 17βHSD1 immunoreactivity was detected in carcinoma cells in approximately 60% of invasive breast carcinoma tissues, and it was correlated with ER and PR.[38] Therefore, it is suggested that the majority of estradiol, which is synthesized by 17βHSD1 in carcinoma cells, directly acts on these cells in breast carcinoma tissues without release into the extracellular space or plasma. Gunnarsson et al.[39] showed that breast carcinoma patients with a high level of 17βHSD1 mRNA were associated with increased risk to develop late relapse of breast carcinoma. Therefore, 17βHSD1 is considered responsible for regulating the

process leading to the accumulation of estradiol in human breast carcinoma tissues.

Retinoic acid induces the expression of 17βHSD1 mRNA in breast carcinoma cells,[40] and a significant correlation was detected between retinoic acid receptor (RAR)-α and 17βHSD1 immunoreactivity.[41] Progestines also induced 17βHSD1 expression in breast carcinoma cells.[42]

Expression of androgen-producing enzymes in invasive breast carcinoma

17βHSD5

Testosterone is secreted from Leydig cells of the testis in men, and it is biosynthesized from androstenedione by 17βHSD3.[43] However, the testis provides approximately 50% of the total amount in men, and the remaining amount is converted from circulating androstenedione in peripheral tissues.[44] 17βHSD3 is mainly expressed in the testis, whereas the same enzymatic reaction in peripheral tissues is catalyzed by different enzymes, namely 17βHSD5[45] (see Fig. 1). 17βHSD5 is a member of the aldo-keto reductase (AKR) superfamily and is formally termed AKR1C3.[46]

Expression of 17βHSD5 mRNA was detected in carcinoma cells in 70%–80% of breast carcinomas,[47,48] and it was significantly higher in breast tumor specimens than in normal tissues.[48] 17βHSD5 immunoreactivity was detected in carcinoma cells in approximately 50% of invasive breast carcinomas, and it was significantly associated with that of 5α-reductase,[49] which catalyzes the reduction of testosterone to DHT. Therefore, 17βHSD5 is involved in in situ DHT production in invasive breast carcinomas.

5α-Reductases

5α-Reductase catalyzes the conversion of testosterone to a more potent androgen DHT (see Fig. 1) and is considered as an important regulator of local actions of androgens. Two isoforms of 5α-reductase have been cloned and characterized in mammals. 5α-reductase type 1 is located on the distal short arm of chromosome 5 and is mainly expressed in the liver and skin.[50,51] Type 2 5α-reductase is located in band p23 of chromosome 2 and is expressed in the liver, prostate, seminal vesicle, and epididymis.[50,51]

Activity of 5α-reductase was detected in human breast carcinoma cell lines, and 5α-reductase activity was elevated four- to eightfold in breast carcinoma tissues compared to nontumorous breast tissues.[52] mRNA expression of 5α-reductase type 1 was detected in all the breast carcinoma tissues examined, whereas that of 5α-reductase type 2 was detected in 40%–100% of the tumors.[49,53] Lewis et al.[53] also demonstrated that mRNA expression levels of 5α-reductase type 1 and type 2 were significantly higher in the tumors than that in corresponding normal tissues. Immunoreactivity of 5α-reductase type 1 was detected in carcinoma cells in 60% of invasive breast carcinomas, while that of 5α-reductase type 2 was positive only in 15% of the cases.[49] In addition, intratumoral DHT concentration was significantly associated with the expression of 5α-reductase type 1 but not type 2.[11] Therefore, 5α-reductase type 1 is suggested to mainly determine DHT concentration in invasive breast carcinoma tissues. Invasive breast carcinomas positive for both AR and 5α-reductase type 1 were inversely associated with tumor size and Ki-67, and these patients showed significant association with a decreased risk of recurrence and improved prognosis for overall survival.[11] Therefore, antiproliferative effects of DHT may primarily occur in these invasive breast carcinomas.

Local production of sex steroids in in situ breast carcinoma

In situ breast carcinoma is regarded as a precursor lesion of invasive breast carcinoma. A great majority of in situ breast carcinoma is histologically diagnosed as a ductal carcinoma in situ (DCIS),[54] and a risk of invasive ductal carcinoma developing after the diagnosis of DCIS was reported as four to ten times higher than in normal women.[55,56] Incidence of DCIS has been markedly increased during the past two decades with advancement of mammographic screening,[57,58] and DCIS now comprises approximately 20% of all human breast carcinomas diagnosed.[59,60]

Because estrogens play a pivotal role on the growth of invasive breast carcinoma, antiestrogens such as tamoxifen, aromatase inhibitors, and luteinizing hormone-releasing hormone (LH-RH) agonists are currently used in patients with invasive breast carcinoma positive for ER and/or PR to block the intratumoral estrogen actions. Sex steroid receptors such as ER, PR, and AR were also positive in a great majority of DCIS,[59,61–63] which suggests important roles of sex steroids in DCIS as in invasive breast carcinoma. Tamoxifen was reported to inhibit the growth of premalignant mammary lesions and the progression to invasive carcinoma in a transplantable mouse model of DCIS.[64] The National Surgical Adjuvant Breast Project (NSABP) P-1 trial demonstrated that tamoxifen significantly reduced the risk of in situ breast carcinoma by 50%,[65] and results of NSABP B-24 trial indicated that adjuvant tamoxifen therapy was clinically effective in ER-positive DCIS and reduced the recurrence of noninvasive breast carcinoma by 30%.[66]

Immunolocalization of aromatase[67,68] and 17βHSD1[69] has been previously reported in DCIS, suggesting a possible importance of in situ production of sex steroids in DCIS. However, no information is available regarding the expression of other sex steroid-producing enzymes in in situ breast carcinomas. Moreover, intratumoral concentration of sex steroids has not been reported in in situ breast carcinoma tissues. Information on sex steroids is very limited in in situ breast carcinoma compared to that in invasive breast carcinoma as described in the foregoing sections, and the clinical and/or biological significance of sex steroids in in situ breast carcinomas remains largely unclear.

When we examined intratumoral concentrations of sex steroids in DCIS as a preliminary study, both estradiol and

Table 1. Tissue concentration of sex steroids and expression of sex-steroid-producing enzymes in nonneoplastic breast and DCIS tissues

	Nonneoplastic breast ($n = 7$)	DCIS ($n = 7$)	P value
Tissue concentration of estradiol	23 ± 9 pg/g	209 ± 82 pg/g	**0.04**
Tissue concentration of DHT	97 ± 9 pg/g	319 ± 30 pg/g	**0.02**
Estrogen-producing enzymes			
Aromatase mRNA	0.4% ± 0.1%	1.4% ± 0.5%	**0.04**
STS mRNA	0.1% ± 0.1%	1.4% ± 1.1%	**0.03**
17βHSD1 mRNA	0.1% ± 0.1%	0.6% ± 0.2%	**0.04**
Androgen-producing enzymes			
17βHSD5 mRNA	0.1% ± 0.1%	1.1% ± 0.3%	**0.01**
5α-Reductase type 1 mRNA	1.1% ± 0.2%	5.7% ± 2.6%	**0.04**
5α-Reductase type 2 mRNA	0.4% ± 0.2%	0.4% ± 0.3%	0.89

Data are presented as mean ± 95% confidence interval (95% CI)
Tissue concentration of sex steroids was examined by liquid chromatography/electrospray tandem mass spectrometry
mRNA of sex-steroid-producing enzymes was examined by real-time polymerase chain reaction; the mRNA level was summarized as a ratio (%) of that of ribosomal protein L 13a
DCIS, ductal carcinoma in situ; DHT, 5α-dihydrotestosterone; STS, steroid sulfatase; 17βHSD, 17β-hydroxysteroid dehydrogenase
Statistical analysis was performed using unpaired two-group t test; P values less than 0.05 were considered significant (shown in boldface)

DHT concentrations were significantly (9.1 fold and 3.3 fold, respectively) higher in DCIS than nonneoplastic breast tissues (Table 1). Results of the study also demonstrated that mRNA expression of estrogen- (aromatase, STS, and 17βHSD1) and androgen- (17βHSD5 and 5α-reductase type 1) producing enzymes were significantly higher in DCIS than their corresponding nonneoplastic breast tissues (Table 1). Therefore, it is suggested that both estradiol and DHT are locally produced in DCIS tissues as invasive breast carcinomas, and that endocrine therapies may be clinically effective in a selective group of DCIS patients. Further examinations are required to clarify the significance of sex steroids in in situ breast carcinomas.

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