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REVIEW

Aromatase in Human Breast Carcinoma as a Key Regulator of Intratumoral Sex Steroid Concentrations

TAKASHI SUZUKI, YASUHIRO MIKI*, JUN-ICHI AKAHIRA*, TAKUYA MORIYA**, NORIAKI OHUCHI*** AND HIRONOBU SASANO*

Department of Pathology, Tohoku University School of Health Sciences, Sendai, 980-8575, Japan
**Department of Pathology, Tohoku University School of Medicine, Sendai, 980-8575, Japan*
***Department of Pathology 2, Kawasaki Medical School, Kurashiki, 701-0192, Japan*
****Department of Surgical Oncology, Tohoku University School of Medicine, Sendai, 980-8575, Japan*

Abstract. It is well-known that estrogens are closely involved in the growth of human breast carcinomas, and that the great majority of breast carcinoma express estrogen receptors. Recent studies have demonstrated that estrogens are locally produced and act on the breast carcinoma tissue. Among these pathways, aromatase is a key enzyme for intratumoral production of estrogens in breast carcinomas, and aromatase inhibitors are currently used in the breast carcinoma in postmenopausal women as an estrogen deprivation therapy. This review summarizes the results of recent studies on the expression and regulation of aromatase in breast carcinoma tissues, and discusses the potential biological and/or clinical significance of aromatase. Aromatase is abundantly expressed in various cell types, such as carcinoma cells, intratumoral stromal cells, and adipocytes adjacent to the carcinoma, in breast carcinoma tissues. Further, a key regulator for aromatase expression differed according to cell type. In addition, aromatase suppressed *in situ* production of bioactive androgen, 5α-dihydrotestosterone (DHT), in breast carcinoma. Aromatase inhibitors may thus have additional antiproliferative effects through increasing local DHT concentration with estrogen deprivation.

Key words: Androgen, Aromatase, Breast carcinoma, Estrogen

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Intratumoral production of estrogens in the breast carcinoma

IT is well-known that estrogens contribute immensely to the development of hormone-dependent human breast carcinoma, and that estrogen deprivation is an effective treatment for breast carcinoma as an endocrine therapy. Estrogens are mainly secreted from the ovary into plasma in premenopausal women. Ovarian suppression therapies, such as ovariectomy and treatment with gonadotropin releasing hormone (GnRH) agonists, thus are frequently considered in breast carcinoma patients in premenopausal women [1] (Table 1). On the other hand, since the biological effects of estrogens are mediated through the estrogen receptor (ER), antiestrogens such as tamoxifen have been used in breast carcinoma regardless of menopausal status [2] (Table 1).

ER is expressed in a great majority of breast carcinoma tissues, but the great majority of these carcinomas arise after menopause when ovaries are no longer functional. In postmenopausal women and men, estrogens are mainly biosynthesized in various peripheral tissues such as adipose tissue, skin, and muscle, through conversion of circulating inactive androgens from the adrenal cortex or gonads [3]. Increased peripheral conversion of androgen to estrogen might result in elevated serum levels of estrogen, and numerous studies have been performed to study the subtle differences of serum estrogen concentration. However, there is no consistent evidence of increased serum estrogens concentration or other systemic estrogen abnormalities in women with breast carcinoma.

Miller *et al*. and Miller [4, 5] have shown that tissue

Correspondence to: Takashi Suzuki, M.D., Department of Pathology, Tohoku University School of Health Sciences, 2-1 Seiryo-machi, Aoba-ku, Sendai, 980-8575, Japan

Table 1. Representative estrogen deprivation therapies currently used in hormone-receptor positive breast carcinoma

Treatment	Effect	Patients
Ovariectomy	Decrease of estrogen concentration in plasma	Premenopausal women
GnRH agonists* (Goserelin and leuprolide)	Suppression of ovarian function (decrease of estrogen concentration in plasma)	Premenopausal women
Anti-estrogens (Tamoxifen, fulvestrant and toremifene)	Inhibition of estrogenic actions	Premenopausal and postmenopausal women
Aromatase inhibitors (Anastrozole, letrozole and exemestane)	Inhibition of estrogen production	Postmenopausal women

GnRH; gonadotropin releasing hormone

concentrations of bioactive estrogen estradiol were more than ten times higher in breast carcinoma than in plasma, and demonstrated that human breast neoplasms can produce estradiol *in vitro*. Intratumoral estradiol levels were not significantly different between premenopausal and postmenopausal breast carcinoma patients, but the intratumoral estradiol/estrone ratio was significantly higher in postmenopausal than premenopausal breast carcinoma [6]. In addition, the concentration of estradiol was 2.3-times higher in breast carcinoma tissues than in the areas considered as morphologically normal [7]. To date, bioactive estrogens are thought to be locally produced from circulating inactive steroids and to act on breast carcinoma tissue. Since this phenomenon is different from the classical endocrine system, it is called an "intracrine" system (Fig. 1).

In the classical endocrine system, only a small amount of hormone is generally utilized in the target tissues, and thereafter the great majority of it is either metabolized or converted into inactive forms. On the other hand, an intracrine system requires a minimal amount of biologically active hormone to exert its maximum effect. Therefore, the intracrine system is an efficient mode of hormone action and plays important roles especially in the development of hormone dependent neoplasms. A large proportion of estrogens in women (approximately 75% before menopause, and close to 100% after menopause) were synthesized in peripheral hormone-target tissues from abundantly present circulating precursor steroids [8]. Intratumoral production of estrogens plays an important role in the proliferation of breast carcinoma cells, especially in postmenopausal women, and the blockade of this pathway is suggested to lead to the inhibition of growth of breast carcinoma.

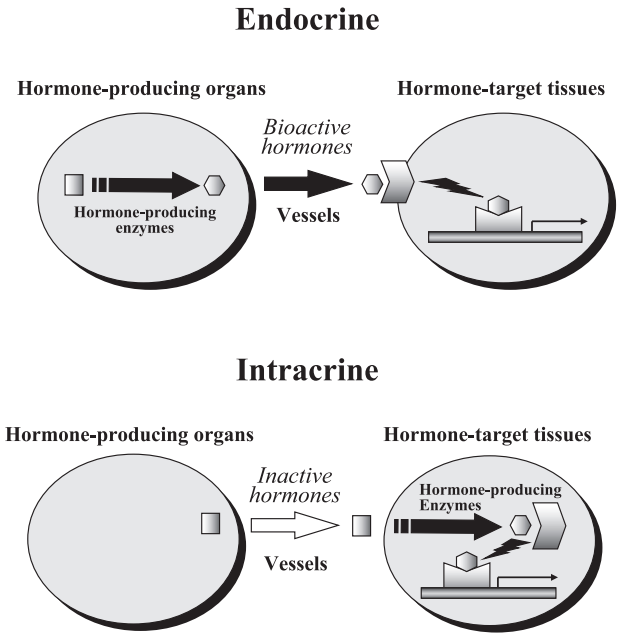

Fig. 1. Summary of endocrine and intracrine actions. In a classical endocrine action (upper panel), biologically active hormones are produced and secreted from endocrine organs, transported through the circulation, and act on their target tissues where their specific receptors are expressed. On the other hand, in an intracrine action (lower panel), bioactive hormones are synthesized in peripheral hormone-target tissues from abundantly present circulating precursor steroids, where hormone-producing enzymes are expressed, and act on the tissue. ▯; inactive hormone, ⬡; bioactive hormone, ⟩; receptor, and ▭; promoter region of the target gene.

Aromatase as a potent estrogen-producing enzyme in breast carcinoma

Fig. 2 summarizes the representative pathways of *in situ* production of sex steroids in breast carcinoma tis-

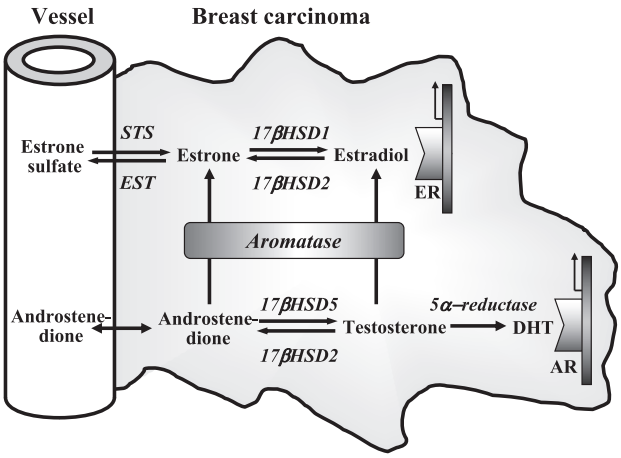

Fig. 2. Scheme representing *in situ* production of sex steroids in human breast carcinoma tissues. High concentrations of circulating inactive steroids, such as estrone sulfate and androstenedione are precursor substrates of local production of estrogens and/or androgens in breast carcinomas. Bioactive sex steroids such as estradiol and 5a-dihydrotestosterone (DHT), are locally produced, and act on the carcinoma cells through estrogen (ER) and androgen (AR) receptor, respectively. STS; steroid sulfatase, EST; estrogen sulfotransferase, and 17bHSD; 17b-hydroxysteroid dehydrogenase.

sues, which are currently postulated. High concentrations of circulating inactive steroids, such as androstenedione and estrone sulfate, are considered major precursor substrates of local estrogen production. Briefly, aromatase catalyzes the conversion of androgens (androstenedione and testosterone) to estrogens (estrone and estradiol, respectively), while 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, estrogens were metabolized by 17βHSD2 (oxidation of estradiol to estrone) and estrogen sulfotransferase (EST; designated SULT 1E1) (sulfonation of estrone to estrone sulfate).

Among the pathways of intratumoral production of estrogens, only aromatase route is irreversible, suggesting that aromatase is a key enzyme for the incremental increase of intratumoral estrogen level in breast carcinoma. Inhibition of aromatase is clinically useful for reducing the progression of breast tumors especially in postmenopausal women, and third-generation aromatase inhibitors, such as anastrozole, letrozole and exemestane, are currently available [9] (Table 1). Aromatase inhibitors have been shown to efficiently suppress estrogen levels in plasma [10] and breast carcinoma tissue [11]. Results of large multicenter trials, such as ATAC trial, NCIC MA-17 trial, and Intergroup exemestane study, all demonstrated that aromatase inhibitors are significantly associated with the improved disease-free survival and good tolerability in breast cancer patients [12–16], with anastrolozole demonstrating superior efficacy to tamoxifen in the ATAC trial.

Expression and localization of aromatase in breast carcinoma tissue

Previous studies reported an association between aromatase activity in breast carcinoma tissues and the response to treatment with aromatase inhibitors [17, 18]. Therefore, it is very important to obtain a better understanding of aromatase expression in breast carcinoma to improve clinical effects of aromatase inhibitor in the breast carcinoma patients. Approximately 70% of breast carcinoma specimens had aromatase activity comparable with or greater than that found in other tissues [19]. Aromatase mRNA levels were significantly increased in invasive [20] and noninvasive [21] breast carcinoma compared to nonmalignant breast tissue. Therefore, aromatase is abundantly expressed in breast carcinoma tissues.

Immunolocalization of aromatase in breast carcinomas was examined by several groups, but the results reported are inconsistent. Previously, Sasano *et al*. [22] showed aromatase immunoreactivity in stromal cells such as intratumoral fibroblasts and adipocytes in breast carcinoma tissues (Fig. 3A). Santen *et al*. [23] also demonstrated aromatase immunoreactivity predominantly in the stromal cells, On the other hand, Esteban *et al*. [24] and Brodie *et al*. [25] reported aromatase immunoreactivity in breast carcinoma cells. Recently, Sasano *et al*. [26] validated several new aromatase antibodies for immunohistochemistry, and demonstrated that aromatase immunoreactivity was detected in various types of cells such as stromal cells, carcinoma cells and normal duct epithelial cells (Fig. 3B). When we examined localization of aromatase mRNA in breast carcinoma tissues by laser capture microdissection (LCM)/real-time polymerase chain reaction (real-time PCR), aromatase mRNA was detected in both carcinoma and intratumoral stromal

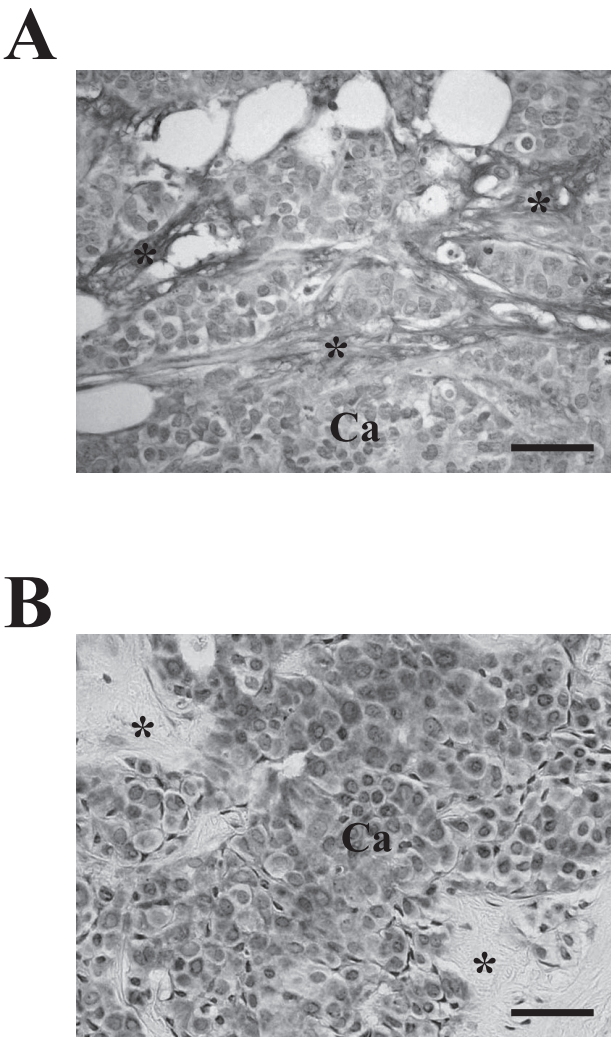

Fig. 3. Immunohistochemistry for aromatase in the breast carcinoma. A: Aromatase immunoreactivity was detected in the intratumoral stromal cells (*), but not in the carcinoma cells (Ca), when we used the rabbit polyclonal antibody same as Ref. 22. B: On the other hand, aromatase immunoreactivity was detected in the carcinoma cells, when we used the mouse monoclonal antibody same as Ref. 26. Bar = 50 mm, respectively.

cells [19]. This suggested that aromatase is expressed in various types of cells, such as carcinoma cells, intratumoral stromal cells, and adipose tissues adjacent to the carcinoma.

Discrepant results of aromatase immunolocalization in previous studies may be due to the different nature of the aromatase antibodies employed. Since immunohistochemistry for aromatase is expected to be the most attractive method to evaluate aromatase expression, considering its great success in detecting ER, progesterone receptor (PR) and HER2 in breast carcinoma tissues, further examinations are required to establish a standardized approach, including the determination of aromatase antibody, immunohistochemical procedure and the evaluation system.

Regulation of aromatase expression in breast carcinoma

It remains largely unclear which mechanism increases aromatase expression in various types of cells in breast carcinoma tissues. In a preliminary study we examined expression of aromatase mRNA in breast carcinoma tissues by LCM/real-time PCR analysis, the aromatase mRNA levels both in carcinoma cells and intratumoral stromal cells were significantly higher in invasive breast carcinoma than noninvasive breast carcinoma tissues ($P = 0.03$ and $P = 0.01$, respectively) (Fig. 4). Previous *in vitro* studies demonstrated that breast carcinoma cells secrete various factors that induce aromatase expression in adipose fibroblasts [27], including prostaglandin E2 [28], interleukin (IL)-1, IL-6, IL-11 and tumor necrosis factor [29]. On the other hand, it has also been reported that exogenous growth factors, such as epidermal growth factor [30], transforming growth factor [30], and keratinocyte growth factor [31], stimulated aromatase activity in MCF-7 breast carcinoma cells. Very recently, Miki *et al*. [32] reported that mRNA level and enzymatic activity of aromatase in MCF-7 breast carcinoma cells were significantly increased on coculture with primary stromal cells isolated from breast carcinoma tissue. Therefore, aromatase expression is suggested to be, at least in part, regulated by tumor-stromal interactions in breast carcinoma tissues, which may be promoted by invasion of the carcinoma cells into the stroma.

Previous studies also demonstrated the regulation of aromatase expression by various transcriptional factors. Transcription of aromatase is activated by steroidogenic factor 1/adrenal 4 binding protein (SF1; designated NR5A1) in the ovary, which binds to a nuclear receptor half site within their promoter regions to mediate basal transcription and in part cAMP-induced transcription. However, SF1 is not expressed in breast carcinoma tissues [33]. Clyne *et al*. and Zhou *et al.* examined various orphan nuclear receptors known to bind to such a nuclear receptor half site in 3T3-L1 preadipocytes, and reported the induction of aromatase

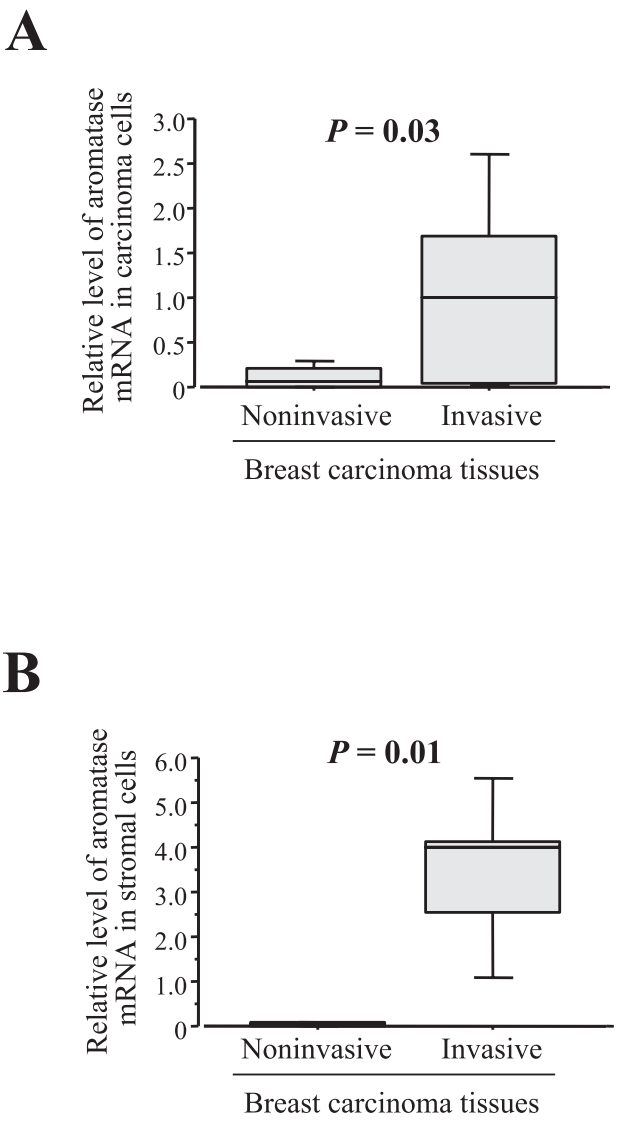

Fig. 4. Expression of aromatase mRNA in carcinoma cells (**A**) and intratumoral stromal cells (**B**) in noninvasive (n = 8) and invasive (n = 9) breast carcinoma tissues. The carcinoma cells and intratumoral stromal cells in breast carcinoma were separately collected by laser capture microdissection (LCM), and subsequently, mRNA level of aromatase was examined by real-time polymerase chain reaction (real-time PCR). Data were summarized as a relative ratio ($X10^{-2}$%) of aromatase mRNA to an internal standard (mRNA of ribosomal protein L 13a). The data were represented as box and whisker plots, and statistical analyses were performed using a Mann-Whitney's U test.

expression by liver receptor homologue-1 (LRH-1; NR5A2) in adipose stromal cells in the breast carcinoma [33, 34]. LRH-1 immunoreactivity was detected in adipocytes adjacent to the carcinoma invasion and carcinoma cells, and significant association was detected between LRH-1 and aromatase mRNA levels in the adipose tissues adjacent to the carcinoma [34]. On the other hand, LRH-1 in breast carcinoma cells was not associated with the aromatase expression [35]. Therefore, LRH-1 may mainly regulate aromatase expression in adipose tissue adjacent to the breast carcinoma.

On the other hand, estrogen-related receptor a (ERRa; NR3B1) has a positive regulatory function of aromatase in SK-BR-3 breast carcinoma cells [32, 36], but not in 3T3-L1 preadipocytes [33]. ERRa was mainly immunolocalized in breast carcinoma cells, but not in the intratumoral stromal cells or adipocytes [37], and expression of ERRa mRNA in carcinoma cells was positively associated with that of aromatase mRNA in breast carcinoma tissues [32]. Therefore, aromatase expression is regulated by various transcriptional factors in breast carcinoma tissues, and the key regulator may be different according to the types of cells expressing aromatase.

Regulation of intratumoral androgen production by aromatase in breast carcinoma tissues

In contrast to estrogens, androgens are considered to exert antiproliferative effects predominantly *via* androgen receptor (AR) in breast carcinoma cells [38, 39], although some divergent findings have been reported. Tissue concentration of androgens was investigated in breast carcinomas by three groups [40–42]. Biologically active and potent androgen, 5α-dihydrotestosterone (DHT), was significantly higher in breast carcinoma tissues than in plasma [41], and *in situ* production of DHT has been proposed in breast carcinoma tissues (Fig. 2). AR is expressed in a majority of human breast carcinoma tissues [43–45], suggesting important roles of androgens in breast carcinoma as well as estrogenic actions.

The substrates of aromatase, *i.e.* androstenedione and testosterone, are not only precursors of estradiol synthesis but also precursors of DHT production (Fig. 2). DHT itself is nonaromatizable. Intratumoral concentration of DHT was significantly associated with that of testosterone in breast carcinoma tissues [40, 41], suggesting that DHT concentration in breast carcinoma is influenced by the amount of precursor. Spinola *et al*. [46] showed that treatment with an aromatase inhibitor markedly elevated intratumoral test-

osterone concentrations in dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumors, and Sonne-Hansen and Lykkesfeldt [47] reported that aromatase preferred testosterone as a substrate in MCF-7 breast carcinoma cells. In addition, very recently, Suzuki *et al*. [42] demonstrated that aromatase expression was inversely associated with intratumoral DHT concentration in breast carcinoma tissues, and aromatase suppressed DHT synthesis from androstenedione in coculture experiments of MCF-7 cells and primary intratumoral stromal cells isolated from breast carcinoma. Aromatase is thus suggested as a negative regulator for *in situ* production of DHT in breast carcinoma tissues possibly by reducing concentrations or availability of the precursor testosterone.

Administration of androgens combined with antiestrogen has been more effective than that of antiestrogen alone in breast cancer patients, and the additive inhibitory effects were exerted in part by different mechanisms [8]. Results of large multicenter trials demonstrated superior efficacy of aromatase inhibitors compared to antiestrogen tamoxifen [12–16]. Although it might be due to the agonistic effects of tamoxifen in estrogen-deprived environment [14], it is speculated that aromatase inhibitors have additional antiproliferative effects through increasing local DHT concentration with estrogen deprivation. Further examinations are required to clarify the clinical importance of androgenic actions in association with a response to aromatase inhibitors in breast cancer patients.

Estrogen deprivation therapies in breast carcinoma patients

Aromatase is a key enzyme of intratumoral production of estrogen in breast carcinomas. However, it is also true that other enzymes such as STS and 17βHSD1 are involved in the intratumoral production of estrogen in breast carcinoma (Fig. 2). STS immunoreactivity was detected in carcinoma cells in 60% to 90% of breast carcinoma tissues [48, 49]. STS immunoreactivity was correlated with the tumor size, and it was significantly associated with an increased risk of recurrence in breast carcinoma patients [49]. On the other hand, 17βHSD1 immunoreactivity was detected in carcinoma cells in approximately 60% of the cases [50, 51], and 17βHSD1 mRNA levels and intratumoral estradiol/estrone ratios were significantly higher in postmenopausal than premenopausal breast carcinoma [6]. Therefore, other estrogen-producing enzymes with the exception of aromatase, including STS and17βHSD1, may also have important therapeutic potential as an endocrine therapy for total blockade of local estrogen in breast cancer tissues.

Reed *et al.* [52] 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 the results of a phase I study suggested that STS inhibitor may be effective in hormone-dependent breast carcinoma including those that progressed on aromatase inhibitors [53]. The design of 17βHSD1 inhibitors has also been attempted [54–56]. In addition, Pasqualini [57] reported that progestin medrogestone stimulates EST in breast carcinoma cells through decreasing the estrogen-dependent cell proliferation. Therefore, induction of estrogen-metabolizing enzymes is also considered to result in a decrement of estrogenic actions in breast carcinoma tissues, which may eventually contribute to an improved prognosis in breast cancer patients.

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