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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Analysis of surrogate markers for target-specific therapy in breast carcinomas using archival materials

Hironobu Sasano*, Takashi Suzuki, Takuya Moriya

Department of Pathology, Tohoku University School of Medicine and Tohoku University Hospital, 2-1 Seiryou-machi, Sendai 980-8575, Japan

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Abstract

Recent development of target-specific therapy may have the potential to revolutionize cancer therapy. Target-specific therapy such as trastuzumab or imatinib requires the presence of its specific target in cancer cells. Therefore, it has become very important to identify these targets as a surrogate marker such as HER2/neu for trastuzumab in breast cancer or c-kit for imatinib in gastrointesitinal stromal tumor for these treatments to exert maximum clinical benefits on the patients with these malignancies. Archival or 10% formalin-fixed and paraffin-embedded materials could be the most accessible materials available for examining these surrogate markers for therapy, especially in patients with breast carcinoma. In addition, correlation of the findings with histological features that are pivotal in an evaluation of the findings obtained and retrospective analysis are both possible in these analyses. Immunohistochemistry or FISH for HER2/neu have been widely performed in numerous institutions and provide the gold standard for the treatment of trastuzumab in patients with breast carcinoma. In addition, an analysis of potential surrogate markers at DNA, mRNA and protein levels has become possible using archival materials of human breast carcinoma. However, it is very important to make these analyses widely available or possible to perform in any of the regular diagnostic laboratories without technical difficulties and financial burden on the patients. In addition, it is necessary to standardize the following when using surgical pathology materials for the analysis of these markers, especially in terms of quality control or reproducibility of the results obtained by the analysis: (1) fixation or specimens preparation, (2) methodology to be used and (3) interpretation and/or assessment of the findings.

Keywords: Surrogate markers; Pathology; Immunohistochemistry; Molecular therapy; Archival materials

1. Introduction

Recent development of target-specific therapy such as the humanized anti-HER2 monoclonal antibody trastuzumab has the potential to revolutionize breast cancer therapy by individualizing the treatment among patients. However, it is also true that these therapies may be associated with marked side effects or clinical complications in some patients and no therapeutic effects are expected unless the specific targets for these therapies are demonstrated in these tumor tissues prior to the therapy. Therefore, it has become very important to study the surrogate markers for the target-specific therapy in order to confer the maximum benefits upon patients with breast carcinoma through selecting the appropriate targets in carcinoma tissues.

For the analysis of surrogate markers in the breast cancer patients, the analysis of tumor cells in circulation or peripheral blood is scientifically of great interests but far from clinical relevance [1], at least at this juncture. In addition, the analysis of genomic DNA including SNP in these patients does and will provide little information as to the efficacy of target-specific therapy, at least in those patients with breast malignancies. Therefore, an examination of these surrogate markers should be directed toward the analysis of resected tissue specimens of cancer in cases of breast cancer patients. It is also very important to note that the analysis of surrogate markers should be scientifically accurate; however, more important aspects in terms of clinical relevance and management of the patients are how widely the methods can be applied in

* Corresponding author. Tel.: +81 22 717 7450; fax: +81 22 273 5976.
E-mail address: hsasano@patholo2.med.tohoku.ac.jp (H. Sasano).

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most regular diagnostic laboratories, including conditions of cost-effectiveness without financial burden and technical difficulties, and how reproducible the results are. The former is especially important because the breast cancer patients toward whom target-specific therapy may be initiated by oncologists and who could benefit from these therapies should receive the benefits wherever he or she lives. The great majority of the resected tissue specimens of cancers has been and is being stored as 10% formalin-fixed and paraffin-embedded materials or archival materials throughout the world. Therefore, the analysis of surrogate markers of target-specific therapy must be able to be performed on archival materials, otherwise the techniques will be of only academic interest without any clinical relevance. This is especially important in administering the therapy to cancer patients with advanced stages of cancer who usually lose the windows of opportunity to procure snap-frozen tumor tissues, and archival materials of the primary tumors are the only available specimens containing the cancer.

Therefore, in this short review we will briefly summarize what methods of detecting surrogate markers of target-specific therapies could be applied in archival materials of human breast cancer and summarize the advantages and pitfalls or disadvantages of using stored 10% formalin-fixed and paraffin-embedded tissue materials. We will also emphasize the factors which influence the results of examination of surrogate markers and eventually of the therapeutic outcome of the patients, such as the modes or methods of preparation of 10% formalin-fixed and paraffin-embedded specimens and interpretation or assessment of the findings.

2. Methods that can be applied to 10% formalin-fixed and paraffin-embedded tissue materials of breast cancer

The surrogate markers are usually examined at the levels of DNA, mRNA and protein. Therefore, it is very important for those involved in the target-specific therapy of breast cancer patients to understand what can be performed, how reliable the results are, and how difficult or expensive the methods are when submitting the archival tissues of primary tumors of breast cancer patients retrieved from surgical pathology files of diagnostic laboratories. Herein we will briefly review the methodology that can be applied to archival materials, including its advantages and disadvantages compared to the analysis of fresh or snap-frozen tissue materials.

3. General methodological advantages and disadvantages or pitfalls using archival materials

The three most important advantages of using archival tissues are summarized as follows: (1) Availability of specimens in the great majority of communities worldwide. This means that the archival materials-based analysis can be theoretically applied to any breast cancer patients in any country. (2). Relatively stable preservation of the target molecules in paraffin-embedded tissue blocks at room temperature. This indicates that one does not need an expensive deep freezer or liquid nitrogen to preserve the carcinoma tissues of the patients in the laboratory. (3) Correlation of the findings with morphological features of the carcinoma. This is especially important in cases of breast carcinoma which contain parenchymal, inflammatory and stromal cells, and the methods which treat the tissue as a mass do not provide any clinically relevant findings.

On the other hand, the major potential disadvantages or pitfalls in using archival tissues are summarized as follows: (1) Denaturation of the targets through the process of fixation employing 10% formalin. This is especially marked in the study of mRNA or DNA of relatively large sizes, which makes the precise quantitation of the results extremely difficult. (2) Marked variations in the methods of the preparation of the specimens among the institutions, especially the duration of fixation, which influence the final results. (3) Relative difficulty of obtaining the quantitative data.

4. Analysis of DNA in archival materials

As shown in the demonstration of DNA sequence extracted from relatively old archival materials [2], DNA can be extracted from archival materials and with the combination of laser capture microscopy, DNA from specific types of carcinoma cells can be extracted using archival tissues in a reasonably accurate manner [3]. Dot blot analysis was first performed in the analysis of DNA from archival tissues [4] because Southern blot analysis was and is still nearly impossible in DNA extracted from specimens due to its degradation. However, with the advent of PCR, the great majority of examinations using DNA extracted from paraffin blocks involves the application of PCR and provides important information such as point mutations of various genes of interest which may be related to the efficacy of target-specific therapy [5]. The ability to utilize archival specimens reliably for high-resolution molecular genetic analysis would be of immense practical application in the study of human cancer [6,7]. The combination of whole genome amplification and laser capture microscopy may make it possible to perform analysis such as comparative genomic hybridization in archival tissue specimens, which has been considered to be able to be performed only in high molecular weight DNA extracted from snap-frozen tissue specimens [6,7], but it still requires further investigations to determine the clinical application and validity of whole genome amplification in the study of archival tissues specimens.

Results of the analysis of DNA extracted from archival materials usually cannot localize the findings unless labor-intensive laser capture microscopy is employed. Fluorescent in situ hybridization (FISH) could provide in situ abnormalities of DNA such as deletion or amplification of certain sizes of DNA in tissue sections. FISH analysis is currently indispensable to the determination of HER2/neu status in patients with breast cancer for trastuzumab therapy [8,9]. However, the standardization of FISH is also considered very important to provide appropriate results, including which areas of breast carcinoma tissues are to be examined, how many cells are necessary to be counted, the thickness of the tissue sections, the duration of fixation or others [8,9], although Pathvysion

appears to be established as the gold standard methodology in HER2/neu FISH analysis in the great majority of diagnostic laboratories [10]. One of the disadvantages of FISH is that hybridization signals are visualized by fluorescence, which easily decays through time after staining and requires fluorescent microscopy for evaluation, which is not always available in the laboratory. Therefore, various chromogenic in situ hybridization methods have been proposed but the clinical significance is yet to be determined by further analysis [11,12].

DNA in archival materials is relatively stable and could be preserved for a long time in paraffin wax. However, it is very important to note that formalin fixation usually cannot maintain the integrity of relatively large sized DNA, especially in the specimens treated with long duration of fixation, and that the study of surrogate markers requiring a large sized DNA is nearly impossible unless a wide genome amplification, a research tool at the developing stage at this juncture, is employed.

5. Analysis of RNA in archival materials

Analysis of RNA in archival specimens has usually been performed by mRNA in situ hybridization. This technique was considered as state of the art because of the labor intensiveness and time-consuming nature of the method, and of the requirement of specialized preparation of the specimens [13]. However, the technical advancement such as development of automatic or semiautomatic staining instruments has made it possible to perform the technique in 10% formalin-fixed and paraffin-embedded tissue specimens in a reproducible manner [14–16], and has become widely applicable in the great majority of diagnostic laboratories.

However, it is very important to recognize that results obtained by mRNA in situ hybridization could provide additional information to more widely available immunohistochemistry mainly in the following circumstances in clinical setting: the absence of reliable antibodies and rapid cellular turnover of the protein. Therefore, mRNA in situ hybridization does not necessarily play an important role in determination of surrogate markers of target-specific therapy in breast caner patients at this juncture where reliable antibodies against the targets employed for immunohistochemistry are increasingly available.

An extraction of RNA from resected human cancer specimens has traditionally employed fresh or snap-frozen tissue specimens due to the presumed fragility of the molecules. However, several studies have reported extraction of reasonable size and quality of RNA from archival materials including those with more than 40-year-old autopsy specimens or others using RT–PCR analysis [17–19]. If RNA of reasonable quality could be extracted from archival materials after amplification, it then becomes important to study to what extent such RNA can be used for analysis. Recently, high-throughput technologies such as gene-expression profiling using microarray have provided important information. However, gene-expression profiling could not be reliably applied to relatively old archival specimens and RT–PCR methods are the only reliable laboratory methods to evaluate RNA extracted from 10%formalin-fixed and paraffin-embedded tissue specimens [20]. Paik et al. reported the results of a RT–PCR assay of 21 prospectively selected genes examined in RNA extracted from paraffin-embedded tumor tissue, correlated with the likelihood of distant recurrence and magnitude of chemotherapy benefits in patients with node-negative, tamoxifen-treated breast cancer who were enrolled in the National Surgical Adjuvant Breast and Bowel Project clinical trial B-14 [21,22]. Based on the results of these studies, Oncotype Dx was devised and is commercially available at least in the United States [23], but its clinical value especially in the populations of breast cancer patients outside the United States should be determined by further analyses.

The analysis of RNA in archival tissues usually depends highly on the degree of preservation of the molecule. This preservation of integrity of RNA in the archival specimens is not only influenced by duration of fixation, as in DNA, but also the duration until the time of fixation, because of the well-known potential deleterious activation of intracellular RNase. Prompt and appropriate modes of fixation are therefore more important in evaluation of RNA in 10% formalin-fixed and paraffin-embedded specimens than in the analysis of protein or DNA.

6. Analysis of protein in archival materials

The gold standard of analysis of target protein in human breast cancer is immunohistochemistry. Immunoblotting is usually not feasible in protein extracted from archival materials due to irreversible denaturation caused by fixatives employed. Immunohistochemistry is no longer a technique used solely for research but is employed increasingly for surgical pathology diagnosis and for the assessment of therapeutic biomarkers [24]. Immunohistochemistry has provided very important information as to the targets of specific therapy, as in estrogen receptor, HER2/neu and others. The method is available in the great majority of diagnostic laboratories throughout the world and will also continue to be the gold standard of detection of targets for future molecular therapy. However, the following pitfalls should be noted when applying the results of immunohistochemistry of targets to the determination of feasibility of target-specific therapy in patients with breast carcinoma.

6.1. Preparation of the specimens

Immunohistochemistry is a reliable technique which has continuously advanced over the years for wide applicability, but the modes of fixation are still one of the most important factors influencing the outcome of the results. Ideally, the breast cancer specimens should be sliced into 4–5 mm to allow rapid and even fixation as early as possible. This is important not only in immunohistochemistry but also in the analysis of DNA and RNA above. In addition, it is usually better to perform immunohistochemistry in freshly cut slides due to gradual deterioration of immunoreactivity in unstained tissue sections [25].

6.2. Methodology of immunohistochemistry

The methodology of immunohistochemistry has been recently standardized due to the introduction of all-inclusive staining kits such as Hercept Test or of automatic stainers such as Benchmark. However, it is still important to note that the optimal modes of antigen retrieval may be different among the antibodies employed and different detection systems could influence the results, which can yield false information as to the suitability of target-specific therapy in patients with breast cancer. Therefore, it is advisable that the laboratories should use an all-inclusive staining kit or automatic stainer instruments in immunohistochemical demonstration of surrogate markers in order to minimize inter- and intralaboratory differences as much as possible, since the results directly influence the treatment outcome and eventually the prognosis of breast cancer patients.

6.3. Interpretation and assessment of the findings

Immunohistochemistry provides valuable information as to the localization and quantitation of target molecules. However, assessment or interpretation of the findings are very controversial areas and it is absolutely necessary to establish reproducible scoring systems in reporting the final results to clinicians. Results of immunohistochemistry are usually based on three factors, viz. the number of positive cells, immunointensity and intracellular location of the antigens, i.e., membrane, cytoplasmic and/or nucleus. Therefore, if the antigen is located in one compartment of the carcinoma cells, it then becomes very important to evaluate both the number of the positive cells and relative immunointensity when scoring the immunoreactivity of the specimens, as in the Allred score for determining the status of estrogen receptor in breast carcinoma tissues [26]. We have also recently introduced the criteria of scoring aromatase immunoreactivity in specimens using a newly developed monoclonal antibody, #677, in order to determine the possible candidates for aromatase inhibitor therapy [27]. Aromatase is located in both stromal and parenchymal cell components. Therefore, the scoring systems established evaluates the proportion of each compartment, the proportion of positive cells in each compartment, and relative immunointensity in each compartment of the cells [27]. Summation of these scores was demonstrated to be correlated with biochemical activities.

However, it is very important to note the following points when applying these scoring systems in an evaluation of surrogate markers in breast cancer specimens. The first is the definition of immunopositivity. When positive control is present in the same tissue section, this does not pose difficulties but the employment of positive control sections at the time of immunostaining is usually not sufficient because every specimen is handled in a different manner. The second is the sites of evaluation. Breast carcinoma is associated with marked intratumoral heterogeneity and the location where the cells are examined largely influences the eventual results. For instance, the potential surrogate markers may be determined at sites of the invasive front. The third is the fact that immunointensity is markedly influenced by the duration of colorimetric reaction, which should be noted when interpreting or assessing immunointensity at the time of scoring.

For the analysis of these findings, computerized image analysis systems of immunohistochemistry have been applied [28,29]. The potential advantages of these image analyses include more accurate determination of the amounts of antigens in the cells by quantitative analysis based on absorption. However, there is only a linear correlation between the amount of antigen and immunointensity at low levels, with the exception of aromatase [24,28,30] and a non-linear relationship is detected at higher amounts of antigens in the cells, which results in inaccurate determination of the amount of antigens. Therefore, assessment and reporting of immunoreactivity of surrogate markers for target-specific therapy in breast cancer tissues should be done manually by experienced and alerted pathologists at this juncture in order to avoid false-positive or -negative immunohistochemical findings.

7. Summary

The analysis of surrogate markers for target-specific therapy in breast carcinoma tissues is very important in determining the appropriate candidates for treatment. These surrogate markers can now be examined in archival or 10% formalin-fixed and paraffin-embedded materials, but great care should be taken to standardize the modes of preparation of the specimens, methodology, and assessment of interpretation of the findings.

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Aromatase inhibitor and bone

Yasuhiro Miki, Takashi Suzuki, Hironobu Sasano*

Department of Pathology, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980 8575, Japan

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Abstract

Aromatase is a key enzyme of intratumoral production of estrogen in breast cancers. Aromatase inhibitors are commonly used as hormone therapy in postmenopausal estrogen sensitive breast cancer patients. Type I aromatase inhibitors such as exemestane are steroidal inhibitors, which have androstenedione like structure and bind to androgen receptor with low affinity. Type II aromatase inhibitors such as anastrozole and letrozole are known as non-steroidal inhibitors, which are non-competitive inhibitors of aromatase. Sex steroid hormones such as estrogen and androgen play important roles in the maintenances of female and male bone tissues. It is well known that adult women have less bone mass than men. Especially after menopause, adult women lose their bone mass more rapidly than men of comparable age do. Therefore, many clinical reports of breast cancer patients treated with aromatase inhibitors have emphasized potential bone loss caused by aromatase inhibition. Several basic researches using animal model or *in vitro* model demonstrated the different effects of steroid and non-steroid aromatase inhibitors on bone tissues and cells. In this review, we summarize the effects of AIs on bone tissues reported in clinical studies and animal/*in vitro* studies.

Keywords: Aromatase inhibitor; Bone; Osteoblast

1. Introduction

Estrogens play important roles in the development of hormone-dependent breast carcinomas. The postmenopausal women have low levels of circulating estrogens, however, local synthesis of estrogens takes place in breast tissue [1,2]. Local production of estrogens in human breast carcinoma tissues through aromatization caused by cytochrome P450 19 (aromatase) of androgens into estrogens has been demonstrated [3]. Intratumoral aromatase has been established as the important target of the breast cancer endocrine therapy in hormone-dependent postmenopausal patients. Two types of aromatase inhibitor (AI) currently available (Table 1) have different mechanisms of actions. Agents that interfere with the substrate-binding sites of the enzyme are androgen analogues known as steroidal AI (type 1 AI; e.g., exemestane, formestane) [4]. Agents that block the electron transfer chain by the cytochrome P450 prosthetic group of aromatase are known as non-steroidal AI (type II AI; e.g., letrozole, anastrozole, aminoglutethimide) [4].

It is well known that sex steroid hormones such as estrogen and androgen play an important role in the maintenance of bone tissues [5]. The reductions in circulating estrogen levels that occur at the menopause are related with a rapid deterioration in bone density by as much as 3% per year for the first 5 years following the menopause [6]. Therefore, several bone damage such as osteoporosis and fracture have been reported to arise along with further estrogen depletion caused by AI treatment in postmenopausal breast cancer patients.

2. AIs and bone: clinical studies

Several clinical trials in postmenopausal breast cancer patients treated with aromatase inhibitors evaluated the risks of bone fractures. In ATAC [Arimidex (anastrozole) and Tamoxifen Alone or in Combination] trial at a median follow-up of 68 months, an increase in clinical bone fracture occurred in the patients treated with anastrozole despite greater clinical efficacy of anastrozole over tamoxifen [7]. Similar results were also reported in BIG (Breast International Group) 1-98 trial

* Corresponding author. Tel.: +81 22 717 7450; fax: +81 22 273 5976.
E-mail address: hsasano@patholo2.med.tohoku.ac.jp (H. Sasano).

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Table 1
Aromatase inhibitors by generation and type

Generation	Type 1 (steroidal inhibitor)	Type 2 (non-steroidal inhibitor)
First	Testolactone	Aminoglutethimide
Second	Formestane	Fadrozole
Third	Exemestane	Vorozole
	Atamestane	Anastrozole
		Letrozole

Refs. [4,20].

comparing adjuvant letrozole, tamoxifen, and sequential letrozole–tamoxifen therapy for more than 5 years [8]. In the IES, exemestane had a higher incidence of bone fracture and osteoporosis compared with tamoxifen [9]. The LEAP (letrozole, exemestane, and anastrozole pharmacodynamics) trial is Phase I pharmacodynamic study comparing the effects of the AIs, letrozole, exemestane, and anastrozole on the safety parameters such as serum markers of bone formation and resorption in total of 102 healthy postmenopausal women with normal bone mineral density [10]. Results of this study demonstrated that all 3 inhibitors treated for 24 weeks caused an increment of bone resorption marker such as C-telopeptide crosslinks, while only exemestane increased (no significant) the bone formation marker such as bone alkaline phosphatase and propeptides of type 1 collagen [10]. There is a significant decrease in parathyroid hormone with exemestane than with anastrozole reported in this study [10].

Zolendronic acid, which is a potent bisphosphonate, prevents the bone loss in premenopausal women who received adjuvant estrogen suppression therapy. A twin study to Z-FAST [ZOMETA (zolendronic acid)/Femara (letrozole) Adjuvant Synergy Trial; USA and Canada] trial and ZO-FAST (approximately 30 countries outside USA and Canada) trial has been started [11]. The goal of these trials is to investigate how to best combine zolendronic acid with letrozole in postmenopausal women. Patients will be randomized to zolendronic acid either at the initiation of letrozole therapy or after a decrease in T-score below normal, or in the case of a nontraumatic fracture, with a primary end point of change in lumber bone mineral density [11]. Results of this study demonstrated that bisphosphonate therapy in combination with an AIs offers the potential to prevent AI induced bone loss, but its additional costs may provide financial burdens in the great majority of the patients.

3. AIs and bone: experimental studies

3.1. Animal model

Goss et al. reported that steroidal inhibitor, exemestane and its principal metabolite form, 17-hydroexemestane but not non-steroidal inhibitor, letrozole significantly prevented bone loss in ovariectomized (OVX) rats [12,13]. There were several reports regarding the effects of non-steroidal inhibitors on rat bone tissues [14–17]. Both vorozole [14,15] and aminoglutethimide [16] were reported to impair skeletal development and maintenance in growing and/or aged male rats. However, arimidex had no effects on bone tissues in OVX rats [17]. Exemestane and its principal metabolite, 17-hydroexemestane are structurally related to androstenedione and bind to androgen receptor with relatively low affinity compared to natural ligand of 5α-dihydrotestosterone [18]. These finding suggest that exemestane may demonstrate protective effects toward bone tissues through its androgenic actions. However, clinical studies described above could not confirm these findings and suggest that switching from tamoxifen to exemestane results in significant bone loss [19]. Furthermore, very recently, Goss et al. also reported the effects of atamestane, which is a third generation steroidal aromatase inhibitor, on bone tissues of OVX rats [20]. In this report, atamestane significantly prevent bone loss but androgen blocker, flutamide, does not block this prevention [20]. The mechanisms of atamestane's bone protective effects observed in Goss's report have remained largely unclear. Gasser et al. investigated that the effects of the bisphosphonate, zolendronic acid on bone tissues in 8-month-old female rats treated with letrozole [21]. Zolendronic acid protected against bone loss induced by letrozole treatment in a dose dependent manner. This finding is considered a useful model case reflecting clinical trial such as Z-FAST/ZO-FAST trials described above.

3.2. In vitro model

Various studies using human or animal bone tissues [22,23] and osteoblast cell culture using osteosarcoma cells [24,25] demonstrated that aromatase mRNA or protein was detected in osteoblast cells, which play an important role in bone remodeling. Therefore, AIs are considered to effect directly on osteoblast cells. Recently, we reported the direct effects of aromatase inhibitors on osteoblast using osteoblast cell line, hFOB, and osteoblast-like cell lines, Saos-2 and MG-63 [26]. We recently demonstrated that there was a significant increment in the number of the cells treated with steroidal aromatase inhibitor such as exemestane in hFOB and Saos-2 but not in MG-63. Androgen is well known to stimulate osteoblast proliferation [27] and differentiation [28]. Pretreatment with the androgen receptor blocker, flutamide, partially inhibited the effects of exemestane [26]. Non-steroidal aromatase inhibitors such as experimental reagent, aromatase inhibitor I [4-(Imidazolylmethyl)-1-nitro-9H-9-xanthenone; EMD Biosciences, Inc.] [26], and letrozole (personal finding) exerted no effects on osteoblast cell proliferation. Furthermore, first generation of non-steroidal inhibitor, aminoglutethimide significantly diminished the number of cells of hFOB, MG-63, and Saos-2, respectively [26]. Fadrozole was also reported to demonstrate no effects on cell proliferation of human osteoblast-like cell line HOS [29], but relatively high dose (1 μM) of steroidal inhibitor, formestane (4-hydroxyandrostenedione) significantly reduced proliferation of male rat long bone-derived osteoblast-like cells [30].

Bone mass is maintained when the removal of old bone, resorption and the synthesis of new bone, formation performed by osteoclast and osteoblast are balanced (coupled). Osteoclasts,

which are responsible for bone resorption, are target cells of many anti-osteoporosis therapeutic agents such as bisphosphonate of postmenopausal women. Human mononuclear leukemia derived THP-1 cells have been shown to be capable of high rates of aromatase activity, especially following differentiation into osteoclast-like cells with vitamin D treatment [31]. However, it is unclear whether both steroidal and non-steroidal AIs act on osteoclast and osteoclast-like cells directly.

4. Conclusion

Results of many clinical trials compared the effects of AIs on bone tissues with that of tamoxifen. Tamoxifen is a selective estrogen receptor modulator and has a potent anti-estrogenic effect. Tamoxifen has also partial estrogen agonistic effects on uterus as well as bone tissues. Therefore, the treatment with tamoxifen therapy may be related to direct effects on gynecological tissues. AIs are all associated with lower rates of gynecological symptoms and endometrial cancer compared with tamoxifen [7,8]. AIs will replace tamoxifen as the treatment of choice in several type of breast. More than 50 of aromatase inhibition materials such as steroidal, non-steroidal inhibitor, and flavonoids have been discovered [32]. Damages of the bone tissues by the estrogen depletion for aromatase inhibitor administration are unavoidable. However, selection of the hormone therapy that minimizes the damage of bone tissues is important.

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Review

Intracrinology of estrogens and androgens in breast carcinoma

Hironobu Sasano *, Takashi Suzuki, Yasuhiro Miki, Takuya Moriya

Department of Pathology, Tohoku University School of Medicine, 2-1 Seiryou-machi, Aoba-ku, Sendai 980-8575, Japan

Abstract

Intratumoral metabolism and synthesis of biologically active steroids such as estradiol and 5α-dihydrotestosterone as a result of interactions of various enzymes are considered to play very important roles in the pathogenesis and development of hormone-dependent breast carcinoma. Among these enzymes involved in estrogen metabolism, intratumoral aromatase play an important role in converting androgens to estrogens *in situ* from serum and serving as the source of estrogens, especially in postmenopausal patients with breast carcinoma. However, other enzymes such as 17β-hydroxysteroid dehydrogenase (17β-HSD) isozymes, estrogen sulfatase (STS), and estrogen sulfotransferase, which contribute to *in situ* availability of biologically active estrogens, also play pivotal roles in this intratumoral estrogen production above. Androgen action on human breast carcinoma has not been well-studied but are considered important not only in hormonal regulation but also other biological features of carcinoma cells. Intracrine mechanisms also play important roles in androgen actions on human breast carcinoma cells. Among the enzymes involved in biologically active androgen metabolism and/or synthesis, both 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5; conversion from circulating androstenedione to testosterone) and 5α-reductase (5αRed; reduction of testosterone to DHT (5α-dihydrotestosterone) were expressed in breast carcinoma tissues, and *in situ* production of DHT has been proposed in human breast cancer tissues. However, intracrine mechanisms of androgens as well as their biological or clinical significance in the patients with breast cancer have not been fully elucidated in contrast to those in estrogens.

Keywords: Estrogen; Androgen; Breast; Cancer; Intracrinology

Contents

1. Introduction

1.1. Development of intracrinology

Biologically active hormones, whether peptides or steroids, are synthesized and secreted from the endocrine organs such as adrenal cortex, or ovary, or pituitary glands. These hormones are transported through the circulation, and act on their target tissues where their specific receptors are expressed (Fig. 1). This system of hormone actions has been called "endocrine", and various biological/clinical features of endocrine target tissues are well-known to be influenced by plasma concentration of the biologically active hormones. Therefore, in the fields of endocrinology, it is very important to evaluate serum or urinary concentrations of hormones in order to obtain a better understanding of physiology and pathology of hormones actions. These locally produced hormones can also act in the same cell (autocrine) or neighboring cells (paracrine) without their release into the circulation.

However, it is also true that a large proportion of androgens in men (approximately 50%) and estrogens in women

* Corresponding author. Fax: +81 22 273 5976.
E-mail address: hsasano@patholo2.med.tohoku.ac.jp (H. Sasano).

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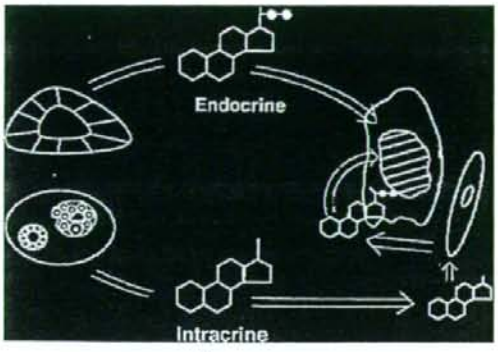

Fig. 1. Illustrations of intracrine and endocrine mechanisms. In endocrine mechanism, biologically active hormones are produced in the endocrine organs, transported through the circulation to the target tissues where they exert their effects. In contrast, in an intracrine mechanism, biologically active hormones are locally converted from biologically inactive precursor hormones produced in the endocrine organs in the tissues where they exert their effects.

(approximately 75% before menopause, and close to 100% after menopause) were produced in peripheral hormone-target tissues from abundantly present circulating precursor steroids [1], where the enzymes involved in the formation of androgens and estrogens are expressed (Fig. 1). These locally produced bioactive androgens and/or estrogens exert their action in the cells where synthesis occurs without release in the extracellular space including circulation. This phenomenon is different from the classical concept of endocrinology such as autocrine, paracrine, and endocrine. This mechanism has been termed "intracrine". It is Labrie and colleagues who elegantly described the formation of active androgens such as dehydrotestosterone (DHT) from the inactive adrenal precursors, dehydroepiandrosterone (DHEA), (DHEA-S (sulfate)) and/or androstenedione locally in the some tissues or cells in adenocarcinoma of the prostate where biosynthesis takes place without release into the extracellular space as "intracrine activity" [1,2].

It then becomes very important to evaluate physiological and/or pathological significance of this intracrine activity compared to endocrine activity. In classical endocrine systems, among those produced and secreted from the endocrine organs, only a small amount of hormones secreted is in general utilized in the target tissues or exerts their effects. The great majority of these hormones is actually metabolized or converted to inactive forms. In contrast, an intracrine system requires minimal amounts of biologically active hormones to exert their maximum hormonal effects. Therefore, the intracrine system is considered a markedly efficient mode of hormone action and plays an important role, especially in the development of hormone-dependent neoplasms including human prostate, breast, endometrial, and ovarian malignancies. It is also important to note that, in an intracrine system, serum concentrations of hormones do not necessarily reflect the local hormonal activities in the target tissues. Therefore, it becomes very important to study how the hormones are metabolized and/or synthesized in the tissue where they exert their actions.

In this review, we summarize intratumoral production of sex steroids including estrogens and androgens in human breast carcinoma tissues, and discuss the potential biological and/or clinical significance of intratumoral production of sex steroids in these carcinomas.

1.2. Intracrinology of estrogens in breast cancer

The great majority of human breast carcinomas express estrogen receptor (ER) in carcinoma or parenchymal cells. These cases are termed hormone- or estrogen-dependent breast carcinoma, and estrogens, especially 17β-estradiol (E2), a biologically potent estrogen, contribute greatly to the growth and development of carcinoma cells and some of these carcinoma cases actually require estrogens for their continued growth and other biological behaviors [3].

It then becomes very important to determine the possible sources of these estrogens that influence various biological behaviors of breast cancers. It is well-known that estradiol originated from different sources before and after the menopause in women. In premenopausal women, the ovary or membrana granulosa of dominant follicles is the main source of abundant circulating estrogens [4,5]. However, as mentioned above after menopause, estrogens are produced primarily through conversion of androgens of both adrenal and ovarian origins, especially of zona reticularis origin of adrenal cortex [6]. The conversion of androgens to estrone occurs principally in peripheral tissues, including skin [7], muscle [8], fat [8], and bone [9]. This conversion is catalyzed by the aromatase enzyme complex [3,5]. However, the great majority of estrone in circulation, including postmenopausal women, is present as sulfated form or estrone sulfate (E1-S) and steroid sulfatase (STS) hydrolyzes circulating E1-S to E1 in various human tissues [10,11]. Estrogen sulfotransferase (EST) (*SULT 1E1* or *STE* gene) is a member of the superfamilly of steroid-sulfotransferases, and sulfonates estrogens to biologically inactive estrogen sulfates [12–14]. Therefore, EST and STS play very important roles in maintaining an availability of biologically active estrogens in the tissues. Estrone is subsequently reduced to 17β-estradiol by 17β-hydroxysteroid dehydrogenase (HSD) type 1, which is also widely distributed in various peripheral tissues [15–17].

Increased peripheral conversion of androgens to estrogens may result in elevated serum levels of estrogens. Therefore, numerous studies have been performed to examine the subtle differences of serum estrogen concentrations between breast cancer patients and their age matched control population. Several epidemiological studies did indicate that plasma estradiol, adrenal androgens, and testosterone levels are higher in women who will develop neoplasms over a period of several years than in those who do not [18]. However, results of other studies [19,20] were not necessarily consistent with those above.

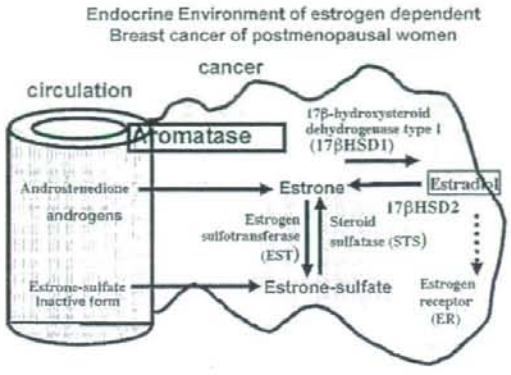

Fig. 2. Illustration of intratumoral estrogen metabolism and production in human breast carcinoma tissues.

Fig. 3. Immunohistochemistry of androgen receptor in human breast carcinoma. Immunoreactivity was detected in the nuclei of carcinoma cells.

Miller et al. [21] and Perel et al. [22] both independently demonstrated that human breast and its neoplasms can produce 17β-estradiol *in vitro*. Yue et al. subsequently reported that *in situ* synthesis of estrogen predominates over uptake from plasma as a means of maintaining breast tissue estradiol concentrations after menopause [23]. These findings clearly indicate the biological importance of elevated *in situ* estrogen concentrations as a result of intratumoral estrogen production in postmenopausal human breast cancer patients. The pathways or cascades of intratumoral estrogen production in human breast cancer tissue is summarized in Fig. 2. It is true that the biological significance of *in situ* estrogen production still remains controversial with regard to development and biological behavior of breast cancer but an increasing number of studies have indicated that in patients with estrogen-dependent breast carcinoma, especially in postmenopausal women, intratumoral estrogens derived from *in situ* aromatization could function as an autocrine growth and a mitogenic factor and could impart a growth advantage to these cancer cells, regardless of serum concentration of estrogens. Therefore, estrogen-dependent breast carcinoma in which *in situ* conversions from serum androgen to biologically active estrogens occur should also be considered as "intracrine" tissues [24].

1.3. Intracrinology of androgens in human breast cancer

AR or androgen receptor is expressed in a majority of human breast carcinoma tissues, especially in carcinoma cells (Fig. 3), more widely than estrogen receptor [25–28]. In addition, Farmer et al. recently identified the group of breast carcinoma cases with increased androgen signaling and some apocrine features in a microarray study [29]. These tumors are ER negative and retention of androgenic signals may further subclassify ER negative breast carcinoma into subtypes which may respond to various target specific therapy. These findings all suggest potential important roles of androgens in human breast carcinomas. However, the possible effects or actions of androgens on human breast carcinoma cells have not necessarily been well-examined.

The effects of androgens are considered to predominantly exert anti-proliferative effects *via* androgen receptor or AR in human breast carcinoma cells [25–28], although some divergent or conflicting findings have been reported. AR is expressed in a majority of human breast carcinoma tissues [30–33], suggesting important roles of androgens in breast carcinomas. 5α-Dihydrotestosterone (DHT) binds with the highest affinity to AR, and together with testosterone promotes AR transcriptional activity [34]. Androgen concentrations have been previously examined in breast cancers by two groups [35,36], and the potent androgen DHT was demonstrated to be significantly higher in breast carcinoma tissues than in plasma in these studies. Androgen-producing enzymes, such as 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5; conversion from circulating androstenedione to testosterone) and 5α-reductase (5αRed; reduction of testosterone to DHT) were expressed in breast carcinoma tissues [33], and *in situ* production of DHT has been proposed in breast cancer tissues [34]. The enzymes involved in *in situ* androgen production in human breast carcinoma is summarized in Fig. 4.

Androgen actions are mediated through an interaction with androgens and AR. Therefore, it becomes very important to evaluate both AR expression and intratumoral DHT concentration in the breast carcinoma tissues, in order to obtain a better understanding of the androgenic actions.

Correlation between AR status and clinical outcome of breast carcinoma patients has been examined by several groups, but the results were not necessarily consistent. Bryan et al. [35] found a significant association between AR status evaluated by AR assays and overall survival of the patients. Soreide et al. [30], however, did not detect any significant correlation between AR status and relapse-free survival. In more recent study, Kuenen-Boumeester et al. [32] performed immunohistochemistry for AR in 153 breast carcinomas, and reported that AR status was a significant

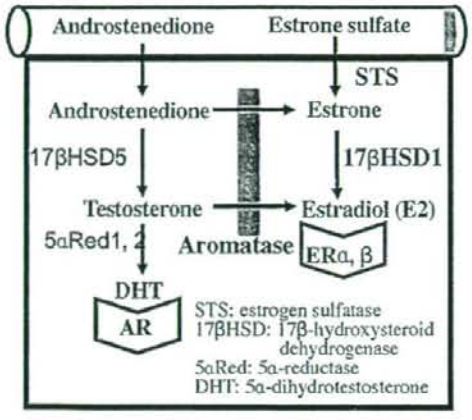

Fig. 4. Illustration of intratumoral androgen metabolism and production in human breast carcinoma tissues.

prognostic factor for disease-free survival, but was not an independent factor following a multiple analysis. If DHT is involved in growth inhibition through AR, residual cancer cells following surgical treatment in AR and 5αRed1 double-positive breast carcinomas possibly grow slowly in the presence of locally produced DHT, which may subsequently result in a better clinical outcome of these patients. Inconsistent results regarding the correlation between AR status and prognosis in previous studies may partly be due to different ratios of breast carcinomas positive for both AR and 5αRed1 examined. Previous studies demonstrated a significant association of intratumoral concentrations between DHT and testosterone in breast carcinomas, suggesting that the intratumoral DHT concentrations were influenced by amounts of the precursor [35,36]. Aromatase catalyzes the conversion of androstenedione and testosterone, which are precursors of DHT, to estrone and estradiol, respectively. However, DHT itself is nonaromatizable. Previously, Spinola et al. reported that treatment with an aromatase inhibitor (4-hydroxyandrostenedione) markedly elevated intratumoral testosterone concentrations in dimethylbenz(*a*)anthracene (DMBA)-induced rat mammary tumors [36], and Sonne-Hansen and Lykkesfeldt [37] recently showed that aromatase preferred testosterone as a substrate in MCF-7 cells. In addition, the aromatase inhibitor letrozole was demonstrated to block conversion of androgens into estrogens with a subsequent increment of intraovarian androgens [38,39]. Therefore, aromatase is suggested a negative regulator for *in situ* production of DHT in breast carcinoma tissues by possibly reducing concentrations or availability of the precursor testosterone. Administration of androgens combined with anti-estrogen has been more effective than that of anti-estrogen alone in breast cancer patients, and the additive inhibitory effects were exerted in part by different mechanisms [40]. In addition, results of recent treatments, such as anastrolozole letrozole and exemestane, compared to anti-estrogen tamoxifen [40–42], although it might be due to agonistic effects of tamoxifen in estrogen-deprived environment [43]. It awaits further investigations to clarify the correlation between *in situ* androgen production or intracrinology of androgens and androgen actions in human breast carcinoma cells.

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Review

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Hironobu Sasano*, Takashi Suzuki, Yasuhiro Miki, Takuya Moriya

Department of Pathology, Tohoku University School of Medicine, 2-1 Seiryou-machi, Aoba-ku, Sendai 980-8575, Japan

Abstract

Intratumoral metabolism and synthesis of biologically active steroids such as estradiol and 5α-dihydrotestosterone as a result of interactions of various enzymes are considered to play very important roles in the pathogenesis and development of hormone-dependent breast carcinoma. Among these enzymes involved in estrogen metabolism, intratumoral aromatase play an important role in converting androgens to estrogens *in situ* from serum and serving as the source of estrogens, especially in postmenopausal patients with breast carcinoma. However, other enzymes such as 17β-hydroxysteroid dehydrogenase (17β-HSD) isozymes, estrogen sulfatase (STS), and estrogen sulfotransferase, which contribute to *in situ* availability of biologically active estrogens, also play pivotal roles in this intratumoral estrogen production above. Androgen action on human breast carcinoma has not been well-studied but are considered important not only in hormonal regulation but also other biological features of carcinoma cells. Intracrine mechanisms also play important roles in androgen actions on human breast carcinoma cells. Among the enzymes involved in biologically active androgen metabolism and/or synthesis, both 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5; conversion from circulating androstenedione to testosterone) and 5α-reductase (5αRed; reduction of testosterone to DHT (5α-dihydrotestosterone) were expressed in breast carcinoma tissues, and *in situ* production of DHT has been proposed in human breast cancer tissues. However, intracrine mechanisms of androgens as well as their biological or clinical significance in the patients with breast cancer have not been fully elucidated in contrast to those in estrogens.

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1. Introduction

1.1. Development of intracrinology

Biologically active hormones, whether peptides or steroids, are synthesized and secreted from the endocrine organs such as adrenal cortex, or ovary, or pituitary glands. These hormones are transported through the circulation, and act on their target tissues where their specific receptors are expressed (Fig. 1). This system of hormone actions has been called "endocrine", and various biological/clinical features of endocrine target tissues are well-known to be influenced by plasma concentration of the biologically active hormones. Therefore, in the fields of endocrinology, it is very important to evaluate serum or urinary concentrations of hormones in order to obtain a better understanding of physiology and pathology of hormones actions. These locally produced hormones can also act in the same cell (autocrine) or neighboring cells (paracrine) without their release into the circulation.

However, it is also true that a large proportion of androgens in men (approximately 50%) and estrogens in women

* Corresponding author. Fax: +81 22 273 5976.
E-mail address: hsasano@patholo2.med.tohoku.ac.jp (H. Sasano).

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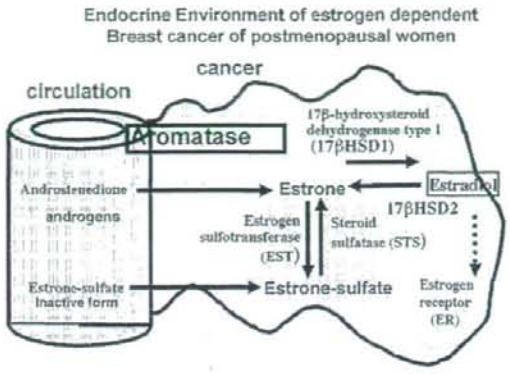

Fig. 2. Illustration of intratumoral estrogen metabolism and production in human breast carcinoma tissues.

Fig. 3. Immunohistochemistry of androgen receptor in human breast carcinoma. Immunoreactivity was detected in the nuclei of carcinoma cells.

Miller et al. [21] and Perel et al. [22] both independently demonstrated that human breast and its neoplasms can produce 17β-estradiol *in vitro*. Yue et al. subsequently reported that *in situ* synthesis of estrogen predominates over uptake from plasma as a means of maintaining breast tissue estradiol concentrations after menopause [23]. These findings clearly indicate the biological importance of elevated *in situ* estrogen concentrations as a result of intratumoral estrogen production in postmenopausal human breast cancer patients. The pathways or cascades of intratumoral estrogen production in human breast cancer tissue is summarized in Fig. 2. It is true that the biological significance of *in situ* estrogen production still remains controversial with regard to development and biological behavior of breast cancer but an increasing number of studies have indicated that in patients with estrogen-dependent breast carcinoma, especially in postmenopausal women, intratumoral estrogens derived from *in situ* aromatization could function as an autocrine growth and a mitogenic factor and could impart a growth advantage to these cancer cells, regardless of serum concentration of estrogens. Therefore, estrogen-dependent breast carcinoma in which *in situ* conversions from serum androgen to biologically active estrogens occur should also be considered as "intracrine" tissues [24].

1.3. Intracrinology of androgens in human breast cancer

AR or androgen receptor is expressed in a majority of human breast carcinoma tissues, especially in carcinoma cells (Fig. 3), more widely than estrogen receptor [25–28]. In addition, Farmer et al. recently identified the group of breast carcinoma cases with increased androgen signaling and some apocrine features in a microarray study [29]. These tumors are ER negative and retention of androgenic signals may further subclassify ER negative breast carcinoma into subtypes which may respond to various target specific therapy. These findings all suggest potential important roles of androgens in human breast carcinomas. However, the possible effects or actions of androgens on human breast carcinoma cells have not necessarily been well-examined.

The effects of androgens are considered to predominantly exert anti-proliferative effects *via* androgen receptor or AR in human breast carcinoma cells [25–28], although some divergent or conflicting findings have been reported. AR is expressed in a majority of human breast carcinoma tissues [30–33], suggesting important roles of androgens in breast carcinomas. 5α-Dihydrotestosterone (DHT) binds with the highest affinity to AR, and together with testosterone promotes AR transcriptional activity [34]. Androgen concentrations have been previously examined in breast cancers by two groups [35,36], and the potent androgen DHT was demonstrated to be significantly higher in breast carcinoma tissues than in plasma in these studies. Androgen-producing enzymes, such as 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5; conversion from circulating androstenedione to testosterone) and 5α-reductase (5αRed; reduction of testosterone to DHT) were expressed in breast carcinoma tissues [33], and *in situ* production of DHT has been proposed in breast cancer tissues [34]. The enzymes involved in *in situ* androgen production in human breast carcinoma is summarized in Fig. 4.

Androgen actions are mediated through an interaction with androgens and AR. Therefore, it becomes very important to evaluate both AR expression and intratumoral DHT concentration in the breast carcinoma tissues, in order to obtain a better understanding of the androgenic actions.

Correlation between AR status and clinical outcome of breast carcinoma patients has been examined by several groups, but the results were not necessarily consistent. Bryan et al. [35] found a significant association between AR status evaluated by AR assays and overall survival of the patients. Soreide et al. [30], however, did not detect any significant correlation between AR status and relapse-free survival. In more recent study, Kuenen-Boumeester et al. [32] performed immunohistochemistry for AR in 153 breast carcinomas, and reported that AR status was a significant

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