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Steroid sulfatase and estrogen sulfotransferase in normal human tissue and breast carcinoma☆

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Abstract

Steroid sulfatase (STS) hydrolyzes inactive estrone sulfate (E1-S) to estrone (E1), while estrogen sulfotransferase (EST; *SULT 1E1* or *STE* gene) sulfonates estrogens to estrogen sulfates. They are considered to play important roles in the regulation of local estrogenic actions in various human tissues, however, their biological significance remains largely unknown. Therefore, we examined the expression of STS and EST in non-pathologic human tissues and breast carcinomas. STS expression was very weak except for the placenta, while EST expression was markedly detected in various tissues examined. In breast carcinoma tissues, STS and EST immunoreactivity was detected in carcinoma cells in 74 and 44% of cases, respectively, and was significantly associated with their mRNA levels and enzymatic activities. STS immunoreactivity was significantly correlated with the tumor size, and an increased risk of recurrence. EST immunoreactivity was inversely correlated with the tumor size or lymph node status. Moreover, EST immunoreactivity was significantly associated with a decreased risk of recurrence or improved prognosis. Our results suggest that EST is involved in protecting various peripheral tissues from excessive estrogenic effects. In the breast carcinoma, STS and EST are suggested to play important roles in the regulation of in situ estrogen production in the breast carcinomas.

Keywords: Breast cancer; Estrogen; Estrogen sulfotransferase (EST); Steroid sulfatase (STS)

1. Introduction

It is well known that estrogens play important roles not only in the breast carcinoma [1,2] but also in various tissues, including the female reproductive organs, bone, liver, central nervous system and vascular system. A major circulating form of plasma estrogen is estrone sulfate (E1-S), a biologically inactive form of estrogen. E1-S has a relatively long half-life in the peripheral blood, where serum levels of E1-S are known to be 10-fold higher than those of unconjugated estrone (E1) or estradiol (E2). Steroid sulfatase (STS) hydrolyzes E1-S to E1 [3,4], while estrogen sulfotransferase (EST) (*SULT 1E1* or *STE* gene), a member of the superfamily of steroid-sulfotransferases, sulfonates estrogens to biologically inactive estrogen sulfates [5–7]. Therefore, STS and EST are considered to be involved in the regulation of in situ estrogen levels in various human tissues and hormone-dependent neoplasms. However, the distribution of STS and EST in normal human tissues and breast carcinomas has not examined well, and their biological significance remains largely unknown. Therefore, in this study, we examined the expression of STS and EST in non-pathologic human tissues and breast carcinomas.

2. Materials and methods

2.1. Tissues and patients

Human tissues from seven adults (four males and three females) were obtained during autopsy at the Department of Pathology, Tohoku University Hospital, Sendai, Japan. The Ethics' Committee at Tohoku University School of Medicine approved this research protocol.

One hundred and thirteen specimens of invasive ductal carcinoma of the breast were obtained from female patients who underwent mastectomy from 1984 to 1989 in

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the Department of Surgery, Tohoku University Hospital, Sendai, Japan. The mean follow-up time was 106 months (range 5–154 months). To perform the reverse transcription (RT)/real-time polymerase chain reaction (PCR) and enzymatic assay analyses, 35 specimens of breast carcinoma were obtained from patients who underwent mastectomy in 2000 in the Departments of Surgery at Tohoku University Hospital and Tohoku Kosai Hospital, Sendai, Japan. Research protocols for this study were approved by the ethic's committee at both Tohoku University School of Medicine and Tohoku Kosai Hospital.

2.2. Immunohistochemistry

Rabbit polyclonal antibody for EST (PV-P2237) was purchased from the Medical Biological Laboratory (Nagoya, Japan). This antibody was raised against the synthetic N-terminal peptide of human EST, corresponding to amino acids 1–13. The affinity purified monoclonal STS (KM1049) antibody was raised against the STS enzyme purified from human placenta, and recognized the peptide corresponding to amino acids 420–428. STS antibody was used for immunohistochemistry analysis, as previously described [8].

Immunohistochemistry was performed by streptavidin–biotin amplification method using a Histofine Kit (Nichirei, Tokyo, Japan) in this study. The antigen–antibody complex was visualized with 3,3′-diaminobenzidine (DAB) solution (1 mM DAB, 50 mM Tris–HCl buffer (pH 7.6), and 0.006% H_2O_2), and counterstained with hematoxylin. Human tissues of placenta [9] and liver [7] were used as positive controls for STS and EST antibodies, respectively. As negative controls, normal rabbit or mouse IgG was used instead of the primary antibodies. No specific immunoreactivity was detected in these sections.

2.3. RT/real-time PCR

To semi-quantify the level of STS and EST mRNA expression by real-time PCR, the Light Cycler System (Roche Diagnositics GmbH, Mannheim, Germany) was used in this study [10]. Settings for the PCR thermal profile were: initial denaturation at 95 °C for 1 min followed by 40 amplification cycles of 95 °C for 0 s, annealing at 60 °C (STS and glyceraldehyde-3-phosphate dehydrogenase, GAPDH) or 58 °C (EST) for 15 s, and elongation at 72 °C for 15 s. The primer sequences used in this study are as follows: STS (FWD 5′-AGGGTCTGGGTGTGTCTGTC-3′ and REV 5′-ACTGCAACGCCTACTTAAATG-3′), and EST (FWD 5′-AGAGGAGCTTGTGGACAGGA-3′ (cDNA position; 751–771) and REV 5′-GGCGACAATTTCTGGTTCAT-3′ (cDNA position; 844–864)). Human placental tissues were used as positive controls of STS. As positive controls for EST, non-pathologic liver tissues and HuH7 hepatocellular carcinoma cell line were used in the study of normal tissues and breast carcinomas, respectively. The mRNA level for STS and EST in each case has been summarized as a ratio of GAPDH, and subsequently evaluated as a ratio (%) compared with that of the positive controls.

2.4. Enzyme assay

The STS activity was assayed according to Utaaker et al. [11] with slight modifications. Briefly, enzyme solution was mixed with E1-S containing [6,7-^{3}H] E1-S. The reaction mixture was incubated at 37 °C for 60 min in a shaking water bath. The enzyme reaction was terminated with the addition of toluene. The reaction mixtures were centrifuged, and [^{3}H] radioactivity in the toluene layer was measured via a liquid scintillation counter (Beckman, LC-6500).

EST was assayed as described previously [12]. Briefly, the protein was added in the reaction mixture including E1 contained [^{3}H] E1, and reactions were initiated with the addition of PAPS. The reaction mixtures were then incubated at 37 °C for 30 min. The reactions were terminated with the addition of chloroform. The reaction mixtures were subsequently centrifuged to separate the aqueous and organic phases. Synthesis of the tritiated E1-S was determined with a liquid scintillation counter (Beckman, LC-6500).

Incubation conditions for these assays were designed so that the formation of product was linear.

3. Results

3.1. STS and EST in normal human tissues

STS immunoreactivity was detected only in the cytoplasm of placental syncytiotrophoblast (Fig. 1A), but was not in other normal tissues examined. In RT/real-time PCR analyses, STS mRNA expression was marked in the placenta, but was almost negligible (less than 4% of the levels of the placenta) in other tissues. STS enzymatic activities were very low compared to that of the placenta (0.2–4.5%) in all the tissues examined.

EST immunoreactivity was detected in various tissues, including hepatocytes of the liver (Fig. 1B), ductal epithelium of the mammary gland (Fig. 1C), Leydig cells of the testis (Fig. 1D), urinary tubules of the kidney, absorptive epithelial cells of the gastrointestinal tract, the zonae fasciculata and reticularis of the adrenal cortex, aortic wall, epithelium of the urinary bladder, epithelial cells of the endometrium and placental syncytiotrophoblast. EST mRNA expression and enzymatic activity were markedly detected in various tissues (Fig. 2). Discrepant results between mRNA levels and enzymatic activities for EST were detected in the adrenal gland and pancreas. mRNA transcripts for EST were detected in the adrenal glands despite low enzymatic activity and vice versa in the pancreas.

3.2. STS and EST in breast carcinoma tissues

STS and EST immunoreactivity was detected in carcinoma cells (Fig. 3A and B), and was significantly associated

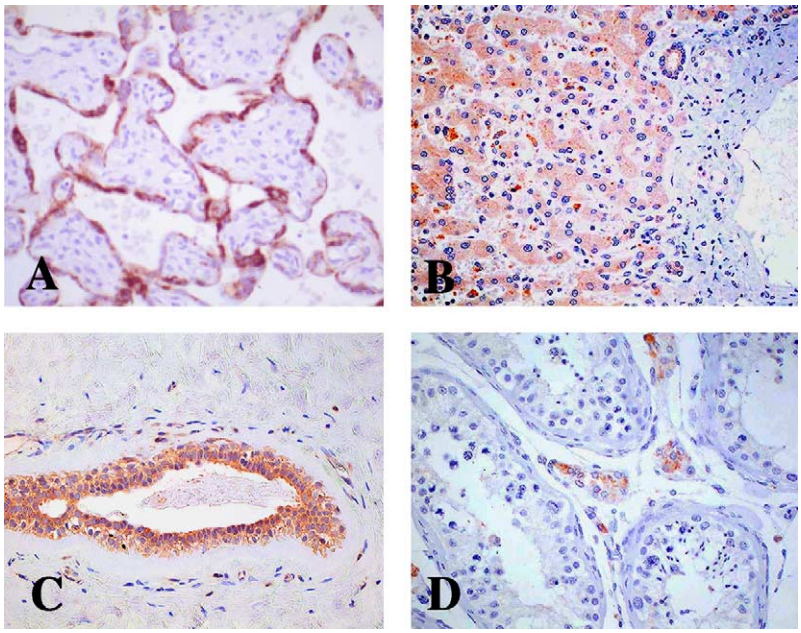

Fig. 1. Immunohistochemistry for STS (A) and EST (B–D) in normal human tissues. (A) STS immunoreactivity was detected in the cytoplasm of syncytiotrophoblasts of the placenta. (B–D) EST immunoreactivity was detected in the cytoplasm of hepatocytes of the liver (B), epithelial cells of the mammary gland (C), and Leydig cells of the testis (D). Original magnification 140×, respectively.

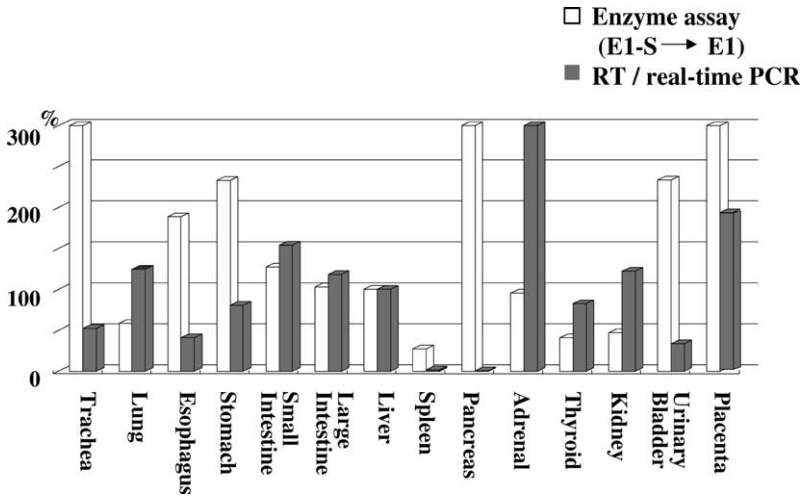

Fig. 2. EST enzymatic activities and mRNA expression in human tissues. Enzymatic activity and mRNA expression of EST were detected in the various tissues examined. mRNA level and enzymatic activity of EST was evaluated as a ratio (%) compared with that of the liver (positive control).

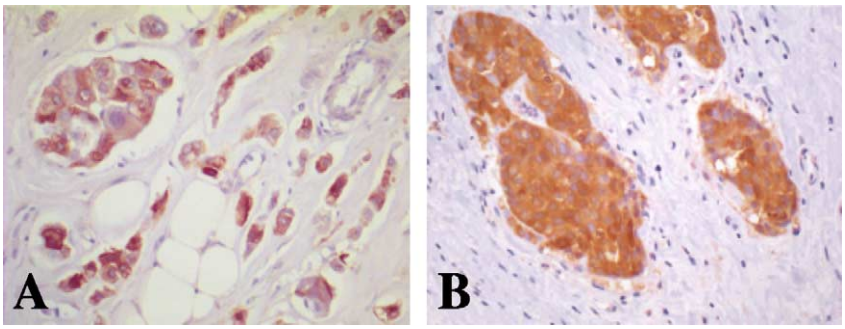

Fig. 3. Immunohistochemistry for STS (A) and EST (B) in the breast carcinoma tissues. Immunoreactivity of STS (A) and EST (B) was detected in the cytoplasm of carcinoma cells. Original magnification 140×, respectively.

Table 1
Univariate and multivariate analyses for the clinical outcome in 113 breast cancer patients examined

Variable	Disease-free survival			Overall survival		
	Univariate	Multivariate		Univariate	Multivariate	
	P	*P*	Relative risk (95% CI)	*P*	*P*	Relative risk (95% CI)
Node status (positive/negative)	<0.0001[a]	0.0011	5.455 (1.970–15.109)	0.0008[a]	0.0087	16.149 (2.023–68.883)
EST (positive/negative)	0.0044[a]	0.0429	2.425 (1.029–5.716)	0.0026[a]	0.0149	6.162 (1.426–26.621)
Tumor size (≥20 mm/<20 mm)	0.0040[a]			0.0124[a]		
STS (positive/negative)	0.0118[a]			0.0325[a]		
HER2 (positive/negative)	0.0567			0.0333[a]		
Histological grade (3/1, 2)	0.1688			0.0773		

[a] Data were considered significant in the univariate analyses, and were examined in the multivariate analyses.

with their mRNA levels ($P = 0.0158$ and 0.0027, respectively), as measured by RT/real-time PCR, and enzymatic activities ($P = 0.0089$ and 0.0005, respectively) in 35 breast carcinomas. In the 113 invasive ductal carcinomas, STS and EST immunoreactivity was detected in 84 and 50 cases (74 and 44%), respectively. In these cases, STS immunoreactivity was significantly correlated with tumor size ($P = 0.0047$). In contrast, EST immunoreactivity was inversely correlated with tumor size ($P = 0.0030$) or lymph node status ($P = 0.0027$). STS immunoreactivity, was significantly associated with an increased risk of recurrence ($P = 0.0118$) and worsened prognosis ($P = 0.0325$) by univariate analysis (Table 1). On the other hand, EST immunoreactivity was significantly associated with a decreased risk of recurrence or improved prognosis by both uni- ($P = 0.0044$ and 0.0026, respectively) and multi-variate ($P = 0.0429$ and 0.0149, respectively) analyses (Table 1).

4. Discussion

Two major pathways are considered to be involved in providing peripheral sources of estrogen in human peripheral tissues. One is the aromatization of androstenedione to E1 by aromatase, and the other is conversion of E1-S to E1 by STS. Involvement of aromatase in the local estrogen production has been reported in various tissues, including the liver, testis, thyroid, lung and aorta [13–17]. In our study, STS expression was negligible in various human tissues except for the placenta. Therefore, it is suggested that local estrogen production is mainly regulated by aromatase and involvement in STS is very limited except for the placenta.

In contrast to STS, EST expression was detected in a wide range of human tissues. 17β-Hydroxysteroid dehydrogenase type 2, which converts from E2 to E1, has been reported in human kidney, liver, and gastrointestinal tract, with a similar pattern of localization as described for EST above [18]. Therefore, EST may play an important role in metabolizing biologically active estrogens to inactive sulfonated forms with marked hydrophilic property in these organs.

EST expression was detected in the adrenal gland by RT/real-time PCR and immunohistochemical analyses, however, the EST enzymatic activity was markedly low. Previously, guinea pig adrenal cortical EST (gpEST) was purified and cloned as a pregnenolone-binding protein [19,20]. Pregnenolone and E2 effectively compete for binding to gpEST, but pregnenolone, which is not sulfonated by gpEST, dose not inhibits sulfonation of E2 [21]. Therefore, EST may participate in steroid production as a pregnenolone-binding carrier protein in the human adrenal gland.

In our study of the breast carcinoma, STS immunoreactivity was detected in 84 out of 113 human breast carcinomas (74%). STS immunoreactivity was positively correlated with tumor size, and significantly associated with an increased risk of recurrence. E1-S is the most abundant estrogen in peripheral blood [22]. Enzymatic activity for STS has been reported to be higher in breast cancer tissues than that in normal breast tissues [23,24], and Utsumi et al. [25] have reported that patients with high mRNA levels for STS were associated with an increased risk of recurrence. Results from our present study are consistent with these reports, and it is suggested that STS plays an important role in the in situ activation of E1 from E1-S, thereby contributing to the increment of estrogenic actions in human breast cancer tissues.

EST immunoreactivity was detected in carcinoma cells in 50 out of 113 human breast carcinomas (44%). EST immunoreactivity was inversely correlated with tumor size or lymph node status, and significantly associated with a decreased risk of recurrence or improved prognosis. Results of both univariate and multivariate analyses demonstrated that EST immunoreactivity is an independent prognostic factor for both recurrence and overall as well as lymph node status, a well-established diagnostic modality [26]. Previous studies have demonstrated that MCF-7 breast cancer cells transfected with EST possess EST at levels similar to normal human mammary epithelial cells, and are associated with much lower estrogen-stimulated DNA synthesis or cell proliferation than control MCF-7 cells that do not possess EST [27,28]. These findings suggest that the loss of EST may result in altered estrogen metabolism in breast cancer cells [27]. Results from our present study are also consistent with these reports, and it is possible to speculate that residual cancer cells in EST-negative breast carcinomas may be associated with an increment of in situ estrogen concentrations,

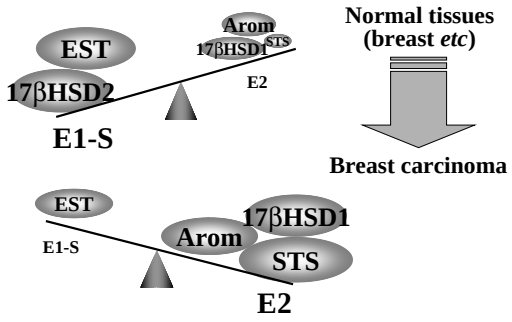

Fig. 4. Scheme representing expression of enzymes related to the local estrogen production in human normal tissue and breast carcinoma. High concentrations of circulating inactive steroids, androstenedione and E1-S, are major precursor substrates of local estrogen production in human tissue. Aromatase (Arom) catalyzes androstenedione into E1, and STS hydrolyzes E1-S to E1. E1 is subsequently converted to potent estrogen E2 by 17β-HSD type 1 (17βHSD1), while 17β-hydroxysteroid dehydrogenase type 2 (17βHSD2) converts from E2 to E1. EST and 17βHSD2 [18] are widely distributed in various normal human tissues including the breast, which suggests important roles of these enzymes to protect from excessive estrogenic effects. However, in the breast carcinoma tissues, STS, Arom [29] and 17βHSD1 [30] are tended to be overexpressed, while EST and 17βHSD2 [30] are frequently decreased, which may result in the accumulation of E2 in breast carcinoma tissues.

thereby resulting in an increased recurrence and/or poor prognosis in these patients.

In summary, we have demonstrated that EST is widely distributed in various normal human tissues, including the breast, and it may be involved in protecting peripheral tissues from excessive estrogenic effects (Fig. 4). STS expression was negligible except for the placenta. However, in the breast carcinoma tissues, STS was tended to be overexpressed and EST expression was frequently decreased, which may result in the accumulation of E2 in breast cancer tissues.

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