Steroid Sulfatase and Estrogen Sulfotransferase in Human Endometrial Carcinoma

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ABSTRACT

Purpose: Intratumoral metabolism and synthesis of estrogens are considered to play important roles in the pathogenesis and/or development of human endometrial carcinoma. Steroid sulfatase hydrolyzes biologically inactive estrogen sulfates to active estrogens, whereas estrogen sulfotransferase sulfonates estrogens to estrogen sulfates. However, the status of steroid sulfatase and/or estrogen sulfotransferase in human endometrial carcinoma has not been examined.

Experimental Design: We first examined the expression of steroid sulfatase and estrogen sulfotransferase in 6 normal endometrium and 76 endometrial carcinomas using immunohistochemistry to elucidate the possible involvement of steroid sulfatase and estrogen sulfotransferase. We then evaluated the enzymatic activity and the semiquantitative analysis of mRNA using reverse transcription-PCR in 21 carcinomas. We correlated these findings with various clinicopathological parameters including the expression of aromatase, 17β-hydroxysteroid dehydrogenase type 1 and type 2.

Results: Steroid sulfatase and estrogen sulfotransferase immunoreactivity was detected in 65 of 76 (86%) and 22 of 76 (29%) cases, respectively. Results of immunoreactivity for steroid sulfatase and estrogen sulfotransferase were significantly correlated with those of enzymatic activity and semiquantitative analysis of mRNA. No significant correlations were detected among the expression of the enzymes involved in intratumoral estrogen metabolism. There was a significant correlation between steroid sulfatase/estrogen sulfotransferase ratio and clinical outcomes of the patients. However, there were no significant differences between steroid sulfatase or estrogen sulfotransferase and estrogen receptors, progesterone receptor, Ki67, histologic grade, or clinical outcomes of the patients.

Conclusions: Results of our study demonstrated that increased steroid sulfatase and decreased estrogen sulfotransferase expression in human endometrial carcinomas may result in increased availability of biologically active estrogens and may be related to estrogen-dependent biological features of carcinoma.

INTRODUCTION

Endometrial carcinoma is one of the most common malignancies in developed countries, and its incidence has increased recently (1). In situ estrogen metabolism, including its synthesis and degradation, has been considered to play a very important role in the development and/or progression of various human estrogen-dependent neoplasms including endometrial carcinoma (2). In endometrial carcinoma, in situ 17β-estradiol availability has been demonstrated to be closely related to the pathogenesis and development of endometrial proliferative disorders including endometrial hyperplasia and carcinoma, especially of the endometrioid type (3). Aromatase catalyzes circulating androgens, which are androstenedione and testosterone, into estrone (E1) and 17β-estradiol, respectively (4, 5). The enzyme 17β-hydroxysteroid dehydrogenase catalyzes the reversible interconversion of E1 and 17β-estradiol. 17β-Hydroxysteroid dehydrogenase type 1 catalyzes the 17β-reduction of biologically weak E1 to strong 17β-estradiol (6–8), whereas 17β-hydroxysteroid dehydrogenase type 2 preferentially catalyzes the oxidation of 17β-estradiol to E1 (9).

A major circulating form of plasma estrogen is estrogen sulfates (E1S and E2S), a biologically inactive form of estrogen. E1S and E2S have a relatively long half-life in the peripheral blood (10), where serum levels of E1S and E2S are known to be 10-fold higher than those of unconjugated E1 or 17β-estradiol (11). It was reported recently that in situ estrogen activity in breast cancer, which is estrogen dependent as well as endometrial cancer, may be mainly regulated by the status of intratumoral steroid sulfatase (12, 13). Steroid sulfatase hydrolyzes biologically inactive estrogen sulfates to active estrogens. Estrogen sulfotransferase (SULT1E1 or STE gene) is a member of the superfamily of steroid sulfotransferases and sulfonates estrogens to estrogen sulfates (14–16). Therefore, it is suggested that estrogen sulfotransferase, especially the balance between the levels of intratumoral steroid sulfatase and estrogen sulfotransferase.
transf erase, may also play an important role in the regulation of in situ estrogen levels in human endometrial carcinoma. Steroid sulfatase and estrogen sulfotransferase activities have been examined in estrogen-dependent neoplasms such as endometrial and breast cancers (17). However, to date, steroid sulfatase and estrogen sulfotransferase mRNA and protein expressions have not been examined in human endometrial carcinoma. In addition, the comparison among the enzymes involved in intratumoral estrogen production and metabolism has not been reported in human endometrial carcinoma. Therefore, in this study we first examined the expression of steroid sulfatase and estrogen sulfotransferase in 6 normal cycling endometrium and 76 endometrial carcinomas using immunohistochemistry to elucidate the possible involvement of steroid sulfatase and estrogen sulfotransferase. We then studied the enzymatic activity and semiquantitative analysis of mRNA by reverse transcription-PCR (RT-PCR) for steroid sulfatase and estrogen sulfotransferase in endometrial carcinomas. We subsequently correlated these findings with the results of Aromatase, 17β-hydroxysteroid dehydrogenase type 1, type 2, and clinicopathological parameters to study the biological and/or clinical significance of steroid sulfatase and estrogen sulfotransferase.

MATERIALS AND METHODS

Tissue Preparation. Six normal cycling human endometria (3 proliferative phase and 3 secretory phase) and 76 endometrial endometrioid adenocarcinomas (33 well differentiated, 26 moderately differentiated, and 17 poorly differentiated; 44 stage I, 14 stage II, 17 stage III, and 1 stage IV) were obtained from hysterectomy specimens performed due to endometrial carcinoma from hysterectomy. All of the patients were examined for progesterone receptor, progesterone receptor, Ki67, 1:5 17β-hydroxysteroid dehydrogenase type 1, type 2, and 1:50 Ki67.

The specimens were all routinely processed (i.e., 10% formalin fixed for 24 to 48 h), paraffin embedded, and thin sectioned (3 μm).

Antibodies. Rabbit polyclonal antibody for estrogen sulfotransferase (PV-P2237) was purchased from Medical Biological Laboratory (Nagoya, Japan). This antibody was raised against the synthetic NH2-terminal peptide of human estrogen sulfotransferase corresponding to amino acids 1–13. The affinity-purified monoclonal antibody for steroid sulfatase (KM1049) was raised against the enzyme purified from human placenta, which recognized the steroid sulfatase peptide corresponding to amino acids 420–428. 17β-Hydroxysteroid dehydrogenase type 1 antibody (polyclonal) and 17β-hydroxysteroid dehydrogenase type 2 antibody (monoclonal) were kindly provided by Dr. Matti Poutanen at the University of Oulu (Oulu, Finland) and Dr. Stefan Andersson at the University of Texas Southwestern Medical Center (Dallas, TX), respectively. Aromatase antibody (polyclonal) was provided by Dr. Nobuhiro Harada at the Fujita Health University School of Medicine (Aichi, Japan). Monoclonal antibodies for estrogen receptor (ER1D5), progesterone receptor (MAB429), and Ki67 (MIB1) were purchased from Immunootech (Marseille, France), Chemicon (Temecula, CA), and DAKO (Carpinteria, CA), respectively. Utilization of these antibodies for immunohistochemistry has been reported previously (19).

Immunohistochemistry. Immunohistochemical analyses were performed using the streptavidin-biotin amplification method using EnVision (DAKO Co. Ltd., Carpinteria, CA) for steroid sulfatase and using a Histofine kit (Nichirei, Tokyo, Japan) for estrogen sulfotransferase, Aromatase, estrogen receptor, progesterone receptor, Ki67, 17β-hydroxysteroid dehydrogenase type 1, and type 2. The dilutions of the primary antibodies used in this study were as follows: 1:1,500 estrogen sulfotransferase, 1:9,000 steroid sulfatase, 1:800 17β-hydroxysteroid dehydrogenase type 1, 1:5 17β-hydroxysteroid dehydrogenase type 2, 1:750 Aromatase, 1:2 estrogen receptor, 1:30 progesterone receptor, and 1:50 Ki67.

The antigen-antibody complex was visualized with 3,3′-diaminobenzidine solution [1 mmol/L 3,3′-diaminobenzidine, 50 mmol/L Tris-HCl buffer (pH 7,6), and 0.006% H2O2], and counterstained with hematoxylin. Tissue sections of full-term placenta were used as positive control for steroid sulfatase, and normal liver was also used as positive control for estrogen sulfotransferase. For negative controls, normal rabbit or mouse IgG was used instead of the primary antibodies. No specific immunoreactivity was detected in these tissue sections.

Scoring of Immunoreactivity. For evaluation of steroid sulfatase and estrogen sulfotransferase immunoreactivity, we determined the labeling index (LI; i.e., the percentage of positive cells) according to the report by Sasano et al. (20). As in previous studies, two of the authors (H. U. and T. S.) independently divided the cases into the following two groups: +, > 5% positive cells and -: <5% positive-cell immunoreactivity (20, 21). Scoring of 17β-hydroxysteroid dehydrogenase type 1, type 2, estrogen receptor, progesterone receptor, and Ki67 in gland or carcinoma cells was reported previously (21).

Enzyme Assay. Twenty one carcinoma cases of fresh-frozen tissues (i.e., the cases immediately frozen in liquid nitrogen and stored at −80°C) were available for examination of enzymatic assay. Estrogen sulfotransferase was assayed as described previously (15). The samples were homogenized at 4°C in phosphate buffer [100 mmol/L KCl, 10 mmol/L KH2PO4, 10 mmol/L Na2HPO4, and 1 mmol/L EDTA (pH 7,5)], and centrifuged for 15 minutes at 1000 × g. The upper layer was used as the enzyme source. Approximately 0.2 mg of protein were added in each assay, and the reaction contained 50 mmol/L Tris-HCl (pH 7.4) and 7 mmol/L MgCl2, and E1 contained [3H]E1 at 20 nm. Reactions were started with the addition of 3′-phosphoadenosine 5′-phosphosulfate to final concentration of 20 μmol/L in a final volume of 0.125 ml. The reaction mixtures were incubated at 37°C for 30 minutes, and the reactions were terminated with the addition of 4.0 mL of chloroform followed by the addition of 0.375 mL 0.25 mol/L Tris-HCl (pH 8.7) to alkalize the solution. The reaction mixtures were centrifuged at 600 × g for 5 minutes to separate E1S (aqueous phase) from E1 (organic phase). Synthesis of the tritiated E1S...
was determined with a liquid scintillation counter (Beckman, LC-6500). The steroid sulfatase activity was assayed according to Utaker and Stoa (22) with slight modifications.

Briefly, enzyme solution (~0.2 mg protein) was mixed with E1S containing [6,7-3H]E1S (1.6 × 10^4 dpm, 0.5 pmol/L) at 20 μmol/L and added to a reaction volume up to 15 mL with PBS (-) containing 25 mmol/L sucrose and 4 mmol/L Nicotinamide. The reaction mixture was incubated at 37°C for 60 minutes in a shaking water bath. The enzyme reaction was terminated with the addition of toluene and mixed by vortex mixer for 1 minute. The reaction mixtures were centrifuged at 600 × g for 5 minutes to separate E1S (aqueous phase) from E1 (organic phase). The toluene layer was collected, and [3H] radioactivity was measured by liquid scintillation counting (LC-1100, Beckman), which is equivalent to E1 formed. Incubation condition of these assays was designed so that the formation of product was linear.

Semiquantitative Analysis of mRNA. Twenty one specimen of fresh-frozen tissues (i.e., the cases immediately frozen in liquid nitrogen and stored at ~80°C) were available for examination. Total RNA was extracted by homogenizing frozen tissue samples in 1 mL of TRizol reagent (Life Technologies, Inc., Grand Island, NY) followed by a phenol-chloroform phase extraction and isopropanol precipitation. All of the RNA samples were quantified by spectrophotometry and stored at ~80°C until they were processed for reverse transcription. The SUPERSCHRipt Preamplification system reverse transcription kit (Life Technologies, Inc.) was used in the synthesis and amplification of cDNA. cDNA was synthesized from total RNA (2 μg) using 25 ng/μL oligo(dT)12-18 primer (Life Technologies, Inc., Gaithersburg, MD) on a PTC-200 Peltier Thermal Cycler DNA Engine (MJ Research, Inc., Watertown, MA). To test for the presence of genomic DNA contamination, we performed the reverse transcription step in the absence of SUPERSCRIPT II RNase H1 Reverse Transcriptase (Life Technologies, Inc.) followed by PCR. RT-PCR products lacking reverse transcriptase in the initial reverse transcription step were run on an ethidium bromide-stained 2% agarose gel. No band was observed in these samples (data not shown). The resulting cDNA was used as a template for real-time PCR. Real-time PCR was carried out with the Light Cycler System (Roche Diagnostics GmbH, Mannheim, Germany) using the DNA binding dye SYBER Green I (Roche Diagnostics GmbH, Mannheim, Germany) for the detection of PCR products. The primer sequences used in this study are as follows: estrogen sulfotransferase [NM005420; FWD 5'-AGAGGAGCTTGAGCAAGGA-3' and REV 5'-GGCGACCATTTCCTGTTGAT-3'] (14), steroid sulfatase [M16505; FWD 5'-AGGGTCTGGTGTCTGTC-3' and REV 5'-AC-TGCAACGCTACTAAATG-3'] (23), and glyceraldehyde-3-phosphate dehydrogenase [M33197; FWD 5'-TGAACCGGAACGCTACTAAATG-3' and REV 5'-TCCACACCCGTGCTGTA-3'] (24). PCR was set up using 2 mmol/L MgCl2, 10 pmol/L of each primer, and 2.5 units of TaqDNA polymerase (Life Technologies, Inc.). An initial denaturing step of 95°C for 1 minute was followed by 40 cycles, respectively, of 95°C for 0 seconds; 15 seconds annealing at 60°C (steroid sulfatase and glyceraldehyde-3-phosphate dehydrogenase) and 58°C (estrogen sulfotransferase); and extension for 15 seconds at 72°C. The fluorescence intensity of the double strand-specific SYBER Green I, which reflects the amount of formed specific PCR products, was read by the LightCycler at 85°C after the end of each extension step. After PCR, these products were resolved on a 2% agarose ethidium bromide gel. Images were captured with Polaroid film under UV transillumination. In initial experiments, PCR products were purified and subjected to direct sequencing (ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit and ABI PRISM 310 Genetic Analyzer, Perkin-Elmer Corp., PE Applied Biosystems, Foster City, CA) to verify amplification of the correct sequences. As a positive control, frozen tissues of placenta were used for steroid sulfatase (25), and liver (HuH7 human hepatocellular carcinoma cells) was used for estrogen sulfotransferase (16). Negative control experiments lacked cDNA substrate to check for the presence of exogenous contamination DNA. No amplified products were observed under these conditions. The mRNA levels of steroid sulfatase and estrogen sulfotransferase in each case are summarized as a ratio of glyceraldehyde-3-phosphate dehydrogenase and evaluated as a ratio (%) compared with that of each positive control. Although conventional quantitative PCR requires the utilization of a purified plasma cDNA in the construction of a standard curve, we found that we were able to semiquantify our PCR products with the Light Cycler using purified cDNA of known concentrations. Other studies to date have used a similar protocol to semiquantify PCR products with the LightCycler (26, 27).

Statistical Analysis. Values for patient age and LI of estrogen receptor, progesterone receptor, and Ki67 were presented as mean ± 95% confidence interval. Association between steroid sulfatase/estrogen sulfotransferase and these parameters was evaluated using Welch’s t test. We also studied the statistical differences between patients for steroid sulfatase/estrogen sulfotransferase and histologic grade in a cross-table using the χ² test. Overall and disease-free survival curves were generated according to the Kaplan-Meier method. P values < 0.05 were considered as significant.

RESULTS

Normal Cycling Endometrium. Estrogen sulfotransferase immunoreactive protein was detected only in the cytoplasm of glandular cells in the secretory phase (Fig. 1A). Estrogen sulfotransferase immunoreactivity was not detected in either epithelium or stromal cells of proliferative phase endometrium (Fig. 1B). Steroid sulfatase immunoreactivity was not detected in any of the cases examined.

Endometrial Carcinoma. Steroid sulfatase and estrogen sulfotransferase immunoreactivity was detected in the cytoplasm of carcinoma cells (Fig. 2, A and B) but not in stromal cells. Steroid sulfatase and estrogen sulfotransferase immunoreactivity was detected in 65 of 76 (86%) and 22 of 76 (29%) cases, respectively. There were no significant correlations between steroid sulfatase or estrogen sulfotransferase and estrogen receptor LI, progesterone receptor LI, Ki67 LI, histologic grade, or clinical outcomes of the patients (Tables 1 and 2). However, there was a significant positive correlation between steroid sulfatase/estrogen sulfotransferase ratio and clinical outcomes of the patients [P < 0.05; alive (3.93 ± 2.95) versus dead (8.35 ± 4.53)]. There were
no associations among the expression of steroid sulfatase, estrogen sulfotransferase, Aromatase, 17β-hydroxysteroid dehydrogenase type 1, and type 2 (Table 3).

For enzyme activity, the results of all of the cases are summarized in Table 4. There was a statistically significant positive correlation between immunoreactivity and enzyme activity for steroid sulfatase \( P < 0.01; + (0.48 \pm 0.22 \text{ nmol/mg/hour}) \) versus \(- (0.18 \pm 0.10 \text{ nmol/mg/hour}) \) and estrogen sulfotransferase \( P < 0.05; + (0.41 \pm 0.46 \text{ nmol/mg/hour}) \) versus \(- (0.09 \pm 0.03 \text{ nmol/mg/hour}) \).

For RT-PCR, the results of all cases are summarized in Table 4. There was a statistically significant positive correlation between steroid sulfatase immunoreactivity and results of steroid sulfatase semiquantitative analysis of mRNA (RT-PCR) \( P < 0.05; + (42.2 \pm 22.3) \) versus \(- (20.2 \pm 9.3) \). There was also significant positive correlation between estrogen sulfotransferase immunoreactivity and results of estrogen sulfotransferase RT-PCR analysis \( P < 0.05; + (35.0 \pm 28.1) \) versus \(- (15.9 \pm 7.4) \).

DISCUSSION

Estrogens, especially 17β-estradiol, have been demonstrated to contribute greatly to the development and progression of the great majority of endometrial carcinoma (28, 29). In addition, in situ estrogen metabolism, including its synthesis and degradation, has been considered recently to play a very important role in the development and progression of various human estrogen-dependent neoplasms including endometrial carcinoma especially in the postmenopausal subjects (2).

Two principal pathways are implicated in the last steps of 17β-estradiol formation in humans. One is through the aromatization of androstenedione or testosterone to E1 or 17β-estradiol, which was considered a major pathway for peripheral estrogen production (4, 5). However, conversion of E1S and E2S into E1 and 17β-estradiol has been also postulated as a source of peripheral estrogen production (30, 31). For instance, the concentrations of E1S and E2S in breast cancer tissues have been reported to be significantly higher than the circulating plasma levels (32). In addition, quantitative determinations of estrogens in breast cancer tissues demonstrated that the “estrogen sulfatase pathway” is 40–500 times higher than that of the “aromatase pathway” (32, 33). Therefore, peripheral steroid sulfatase/estrogen sulfotransferase is considered to play very...
important roles in estrogen metabolism and actions in human breast cancer.

Steroid sulfatase is known as a single enzyme that hydrolyzes several sulfated steroids, including not only E1S and E2S but also dehydroepiandrosterone sulfate and cholesterol sulfate (34, 35). Recent studies demonstrated that the bioactive androgen 5α-dihydrotestosterone is locally produced from dehydroepiandrosterone (36, 37) in human breast cancer tissues. A great majority of estrogen receptor-positive breast carcinomas are also positive for androgen receptor (38). In vitro studies also demonstrated that 5α-dihydrotestosterone decreases estrogen-induced cell proliferation in human breast cancer cells (39). Therefore, steroid sulfatase may possibly be involved in the local production of both androgens and estrogens in endometrial carcinoma as well as breast carcinomas. Additional investigations are required to clarify the biological significance of these interesting findings.

Estrogen sulfotransferase expression has been reported to be induced by progesterone in the uterus (40-42). In addition, estrogen sulfotransferase activities were reported to be highest during the secretory phase of the menstrual cycle in the porcine uterus, which suggests a possible correlation between uterine estrogen sulfotransferase activity and plasma progesterone levels (43). However, we reported previously that 17β-hydroxysteroid dehydrogenase type 2 was also present in the endometrial glandular epithelium of secretory phase, but 17β-hydroxysteroid dehydrogenase type 1 was not detected in any of the phases (21). Estrogen sulfotransferase in endometrial carcinoma was markedly diminished compared with normal endometrium of secretory phase, which is in the same manner as pattern of 17β-hydroxysteroid dehydrogenase type 2 expression.

### Table 1 Correlation between STS immunoreactivity and other parameters in human endometrial carcinoma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EST LI</th>
<th>STS LI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER (%)</td>
<td>50.7 ± 13.7</td>
<td>52.1 ± 9.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>PR (%)</td>
<td>41.9 ± 9.8</td>
<td>37.4 ± 13.9</td>
<td>N.S.</td>
</tr>
<tr>
<td>Ki67 (%)</td>
<td>31.0 ± 16.8</td>
<td>24.0 ± 12.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.4 ± 7.6</td>
<td>44.1 ± 8.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>Grade 1</td>
<td>32</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>20</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>13</td>
<td>4</td>
<td>N.S.</td>
</tr>
<tr>
<td>Overall survival (5 years)</td>
<td>87.5%</td>
<td>87.5%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Disease-free survival (5 years)</td>
<td>80.0%</td>
<td>80.0%</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

NOTE. All data except for grade and survival are presented as mean ± 95% confidence interval.

Abbreviations: STS, steroid sulfatase; N.S., not significant; PR, progesterone receptor; ER, estrogen receptor.

### Table 2 Correlation between EST immunoreactivity and other parameters in human endometrial carcinoma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EST LI</th>
<th>STS LI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER (%)</td>
<td>48.0 ± 12.2</td>
<td>54.6 ± 15.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>PR (%)</td>
<td>44.2 ± 12.0</td>
<td>36.2 ± 9.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>Ki67 (%)</td>
<td>27.6 ± 12.9</td>
<td>30.3 ± 11.6</td>
<td>N.S.</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.3 ± 8.4</td>
<td>46.8 ± 7.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>Grade 1</td>
<td>10</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>6</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>6</td>
<td>11</td>
<td>N.S.</td>
</tr>
<tr>
<td>Overall survival (5 years)</td>
<td>91.2%</td>
<td>85.3%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Disease-free survival (5 years)</td>
<td>83.3%</td>
<td>79.4%</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

NOTE. All data except for grade and survival are presented as mean ± 95% confidence interval.

Abbreviations: EST, estrogen sulfotransferase; N.S., not significant; ER, estrogen receptor; PR, progesterone receptor.

### Table 3 Correlation between immunoreactivity of the enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EST LI</th>
<th>STS LI</th>
<th>17βHSD Type2 LI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cases</td>
<td>22.6 ± 38.4</td>
<td>6.8 ± 4.1</td>
<td>7.9 ± 4.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>Negative cases</td>
<td>11.5 ± 11.7</td>
<td>11.1 ± 13.1</td>
<td>8.6 ± 3.9</td>
<td>N.S.</td>
</tr>
<tr>
<td>EST Positive cases</td>
<td>36.2 ± 16.6</td>
<td>12.0 ± 7.7</td>
<td>7.3 ± 9.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>Negative cases</td>
<td>45.1 ± 20.9</td>
<td>8.2 ± 9.6</td>
<td>8.1 ± 4.0</td>
<td>N.S.</td>
</tr>
<tr>
<td>17βHSD Type2 Positive cases</td>
<td>50.2 ± 26.2</td>
<td>13.5 ± 12.0</td>
<td>9.1 ± 5.0</td>
<td>N.S.</td>
</tr>
<tr>
<td>Negative cases</td>
<td>35.7 ± 21.6</td>
<td>21.1 ± 35.4</td>
<td>7.3 ± 2.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>Arom. Positive cases</td>
<td>44.5 ± 30.0</td>
<td>14.6 ± 9.4</td>
<td>9.0 ± 4.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>Negative cases</td>
<td>38.5 ± 19.3</td>
<td>24.6 ± 37.0</td>
<td>9.5 ± 7.8</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

NOTE. All data are presented as mean ± 95% confidence interval.

Abbreviations: EST, estrogen sulfotransferase; STS, steroid sulfatase; Arom., aromatase; 17βHSD, 17β-hydroxysteroid dehydrogenase.

### Table 4 Twenty one cases for enzyme assay and RT-PCR

<table>
<thead>
<tr>
<th>Case</th>
<th>Enzyme Assay (nmol/mg protein/hr)</th>
<th>mRNA level (%)</th>
<th>Immunoreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.84</td>
<td>58.3</td>
<td>28.4</td>
</tr>
<tr>
<td>2</td>
<td>0.29</td>
<td>11.4</td>
<td>6.3</td>
</tr>
<tr>
<td>3</td>
<td>0.57</td>
<td>25.0</td>
<td>12.9</td>
</tr>
<tr>
<td>4</td>
<td>0.39</td>
<td>43.3</td>
<td>24.0</td>
</tr>
<tr>
<td>5</td>
<td>0.16</td>
<td>27.7</td>
<td>33.3</td>
</tr>
<tr>
<td>6</td>
<td>0.10</td>
<td>11.3</td>
<td>89.5</td>
</tr>
<tr>
<td>7</td>
<td>0.10</td>
<td>13.5</td>
<td>11.1</td>
</tr>
<tr>
<td>8</td>
<td>0.30</td>
<td>7.7</td>
<td>13.1</td>
</tr>
<tr>
<td>9</td>
<td>0.75</td>
<td>81.1</td>
<td>21.6</td>
</tr>
<tr>
<td>10</td>
<td>0.60</td>
<td>44.0</td>
<td>18.8</td>
</tr>
<tr>
<td>11</td>
<td>0.43</td>
<td>16.7</td>
<td>9.9</td>
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<tr>
<td>12</td>
<td>0.38</td>
<td>40.5</td>
<td>3.8</td>
</tr>
<tr>
<td>13</td>
<td>0.29</td>
<td>22.3</td>
<td>16.4</td>
</tr>
<tr>
<td>14</td>
<td>0.14</td>
<td>31.7</td>
<td>9.7</td>
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<tr>
<td>15</td>
<td>0.39</td>
<td>52.8</td>
<td>25.0</td>
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<tr>
<td>16</td>
<td>0.17</td>
<td>47.1</td>
<td>13.7</td>
</tr>
<tr>
<td>17</td>
<td>0.69</td>
<td>30.6</td>
<td>32.2</td>
</tr>
<tr>
<td>18</td>
<td>0.63</td>
<td>87.9</td>
<td>11.1</td>
</tr>
<tr>
<td>19</td>
<td>0.08</td>
<td>19.3</td>
<td>15.9</td>
</tr>
<tr>
<td>20</td>
<td>0.29</td>
<td>34.0</td>
<td>26.4</td>
</tr>
<tr>
<td>21</td>
<td>0.66</td>
<td>50.4</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Abbreviations: EST, estrogen sulfotransferase; STS, steroid sulfatase.
in human endometrium (21). These results suggest that the expression of estrogen sulfotransferase and 17β-hydroxysteroid dehydrogenase type 2, both of which decrease in situ estrogen activity, may be regulated by progesterone.

It was reported that steroid sulfatase activities in endometrial cancer tissues were significantly higher than those in normal endometrial tissues (17). In addition, estrogen sulfotransferase activities were reported to be highest during the secretory phase of the menstrual cycle (40, 41). In our present study, steroid sulfatase was absent through the menstrual cycle, and estrogen sulfotransferase was highly expressed in normal endometrium of secretory phase, which were consistent with the results of previous studies. On the other hand, steroid sulfatase was strongly expressed in 86% of the endometrial carcinoma cases, but estrogen sulfotransferase was expressed only in 29% of the cases. Decreased expression of estrogen sulfotransferase and 17β-hydroxydehydrogenase type 2 in endometrial carcinoma may be related to abundant in situ availability of 17β-estradiol. Therefore, in situ estrogen activity in endometrial carcinoma is much higher than that in normal endometrium. However, there are no correlations between steroid sulfatase or estrogen sulfotransferase and clinicopathological parameters and clinical outcomes of the patients. Therefore, these data indicated that not only steroid sulfatase and estrogen sulfotransferase but also Aromatase and 17β-hydroxydehydrogenase type 2 may independently contribute to the regulation of in situ estrogen availability and/or activity in human endometrial carcinoma. In addition, there was a significant positive correlation between steroid sulfatase/estrogen sulfotransferase ratio and clinical outcomes of the patients. Therefore, the results of the present study demonstrated that increased steroid sulfatase and decreased estrogen sulfotransferase expression in human endometrial carcinomas result in increased in situ availability of biologically active estrogens and may be related to various estrogen-dependent features of carcinoma.

In endometrial carcinoma, 17β-hydroxydehydrogenase type 1 expression was not detected (21). This is in contrast to the study of 17β-hydroxydehydrogenase type 1 in breast cancer in which nearly half of the cases demonstrated 17β-hydroxydehydrogenase type 1 immunoreactivity in carcinoma cells, and 17β-hydroxydehydrogenase type 2 was not expressed (20, 44). We have reported recently that estrogen sulfotransferase is an independent prognostic factor in human breast carcinoma (45). However, there is no correlation between estrogen sulfotransferase and clinical outcomes of the patients in this study. These results indicate that intratumoral estrogen metabolism is different between human breast and endometrial carcinoma, although both of them are sex steroid-dependent malignancies.

In conclusion, the results from our study also suggest that induction of estrogen sulfotransferase and 17β-hydroxysteroid dehydrogenase type 2 and/or inhibition of steroid sulfatase and aromatase may also have possible important therapeutic potential as an endocrine therapy for endometrial carcinoma. Utilization of selective agents designed with the intent to block the expression of enzyme related to intratumor estrogen production can result in a more specific inhibition of estrogen actions in endometrial carcinoma, which may, in turn, eventually lead to an improvement in the prognosis in some of the patients, but additional investigations are required for clarification.

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