Steroid Sulfatase and Estrogen Sulfotransferase in Colon Carcinoma: Regulators of Intratumoral Estrogen Concentrations and Potent Prognostic Factors

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Abstract
Previous epidemiologic and in vitro studies have indicated a potential involvement of estrogens in the pathogenesis of human colon carcinoma, but the precise roles of estrogens have remained largely unknown. Therefore, in this study, we first measured intratumoral concentrations of estrogens in 53 colon carcinomas using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS). Tissue concentrations of total estrogen (estrone (E1) + estradiol) and E1 were significantly (2.0- and 2.4-fold, respectively) higher in colon carcinoma tissues than in nonneoplastic colonic mucosa (n = 31), and higher intratumoral concentrations of total estrogen and E1 were significantly associated with adverse clinical outcome. Intratumoral concentration of total estrogen was significantly associated with the combined status of steroid sulfatase (STS) and estrogen sulfotransferase (EST), but not with that of aromatase. Thus, we subsequently examined the STS/EST status in 328 colon carcinomas using immunohistochemistry. Immunoreactivities for STS and EST were detected in 61% and 44% of the cases, respectively. The −/+ group of the STS/EST status was inversely associated with Dukes’ stage, depth of invasion, lymph node metastasis, and distant metastasis and positively correlated with Ki-67 labeling index of the carcinomas. In addition, this −/+ group had significantly longer survival, and a multivariate analysis revealed the STS/EST status as an independent prognostic factor. Results from our present study showed that the STS/EST status of carcinoma tissue determined intratumoral estrogen levels and could be a significant prognostic factor in colon carcinoma, suggesting that estrogens are locally produced mainly through the sulfatase pathway and play important roles in the progression of the disease. [Cancer Res 2009;69(3):914–22]

Introduction
Colon cancer is the third leading cause of cancer-related deaths in both men and women in the United States (1). Although the recent advances in chemotherapy have prolonged the survival of patients with advanced disease (2), the results are still unsatisfactory and further researches are required to understand the disease and to improve the outcome. A number of observational studies (3–5) and a randomized trial (6, 7) have shown that hormone replacement therapy affects the incidence of colon carcinoma and the recurrence of colorectal adenoma (8) in postmenopausal women. In addition, the great majority of colon carcinomas express estrogen receptor β (ERβ; ref. 9), and some colon carcinoma cells are responsive to estrogens (10–12). These findings all suggest a possible involvement of estrogens in the pathogenesis of human colon carcinoma.

It is well known that estrogens are locally produced from circulating inactive steroids in estrogen-dependent tumors, such as breast carcinoma, through sulfatase and aromatase pathways (ref. 13; Supplementary Fig. S1). The sulfatase route synthesizes estrone (E1) from circulating estrone sulfate (E1-S) by steroid sulfatase (STS) or inversely inactivates E1 into E1-S by estrogen sulfotransferase (EST). E1 is subsequently converted to a potent estrogen, estradiol (E2), by reductive 17β-hydroxysteroid dehydrogenases (17βHSD). In the aromatase pathway, aromatase produces E1 and E2 from circulating androstenedione and testosterone, respectively (13, 14). In breast carcinoma, STS activity is 50 to 200 times greater than aromatase activity (15), and expressions of STS and EST are both reported as significant prognostic factors (16) whereas aromatase is not (17).

In colon carcinoma, expression of 17βHSDs has been previously examined, and colon carcinoma mainly expresses 17βHSD type 2 and type 4, which metabolize E2 to E1 (18, 19). It has been also shown that colon carcinoma tissue has sulfatase (20, 21) and aromatase activities (18). However, to the best of our knowledge, intratumoral concentrations of estrogens and their clinical significance have not been reported. Therefore in this study, we first measured tissue concentrations of estrogens in 53 colon carcinoma tissues and correlated these findings with immunoreactivities for STS, EST, and aromatase. The result showed a strong association between the intratumoral estrogen concentrations and STS/EST status, so we subsequently examined the STS/EST status in 328 colon carcinomas to obtain better understanding of the role and significance of estrogenic actions in colon carcinoma.

Materials and Methods
Patients and tissues. Fifty-three specimens (28 men and 25 postmenopausal women) of colon carcinoma tissues were obtained from patients who underwent surgery from 2000 to 2008 in the Department of Surgery at Tohoku University Hospital, Sendai, Japan (mean age, 70.3 y; range, 45–93 y).
concentrations of total estrogen (E1 + E2), E1 and E2 in colon carcinoma (T; n = 53) and nonneoplastic colonic mucosa (N; n = 31). B, tissue concentration of total estrogen in males (M; n = 28) and females (F; n = 25). C, E1/E2 ratio in colon carcinoma and nonneoplastic colonic mucosa. Columns, mean; bars, SE. Statistical analyses were done by Welch’s t test. D, correlation of intratumoral E1 and E2 concentrations in the 53 colon carcinomas. Statistical analysis was done by a correlation coefficient (r) and regression equation.

Among these 53 cases, specimens of corresponding nonneoplastic colonic mucosa were also available in 31 cases (16 men and 15 women). The mean follow-up time was 22 mo (range, 0–81 mo) and clinical outcome of these patients was available. Specimens for measurement of estrogen concentrations were snap-frozen and stored at −80°C. Tissue concentration of dehydroepiandrosterone-sulfate (DHEA-S) was also measured in both colon carcinoma and corresponding nonneoplastic mucosa in 22 cases (12 men and 10 postmenopausal women). For immunohistochemistry, 10% formalin-fixed and paraffin-embedded tissues were used. Serum samples of the patients were not available in this study.

Another set of colon carcinoma specimens was obtained from 328 consecutive patients (184 men and 144 women including 138 postmenopausal women) from 1994 to 2000. Two hundred six patients were operated on at Miyagi Cancer Center (Natori, Japan) and 122 patients at Tohoku University Hospital. The mean age of the patients was 66.0 y (range, 22–91 y). The mean follow-up time was 78.8 mo (range, 0–161 mo), and survival data of all the patients were available. The specimens were fixed in 10% formalin and embedded in paraffin wax. Snap-frozen tissues and serum were not available in these cases.

Patients clinically suspected to have hereditary nonpolyposis colorectal cancer, carcinoma associated with inflammatory bowel disease, or rectal carcinoma were excluded from this study. Review of the patients’ charts revealed that no patients used oral contraceptives or received hormone replacement therapy, irradiation, or chemotherapy before the surgery. Informed consent was obtained from all the patients examined in this study, and the research protocol for this study was approved by the Ethics Committees at both Tohoku University School of Medicine and Miyagi Cancer Center.

LC-ESI-MS analysis. Concentrations of E1, E2 and DHEA-S were measured using LC-ESI-MS at Teikoku Hormone Medical (Kawasaki, Japan; refs. 22–24).

Briefly, colon carcinoma specimens (~150 mg for each sample) were homogenized in 1 mL of distilled water. For measurement of E1 and E2, 13C-E1 (100 pg) and 13C-E2 (100 pg) were added to the homogenate as internal standards. The steroid fraction was extracted with diethyl ether, and the separated organic layer was evaporated. The extracts were subsequently derivatized with picolinyl anhydride in tetrahydrofuran. After application to a Bond Elut C18 column (Varian, Inc.), the steroid derivatives were eluted with 80% acetonitrile solution.

An API-5000 triple stage quadrupole mass spectrometer equipped with an ESI ion source (Applied Biosystems) and a Shimadzu high-performance liquid chromatography system (Shimadzu Co. Ltd.) were used in our study. The chromatographic separation was done on a Cadenza CD-C18 column (150 mm × 3 mm id., 3 μm Intakt) at 40°C. The mobile phase consisting of CH3CN-Ch3OH (50:50, v/v; solvent A) and 0.1% HCOOH (solvent B) was used with a gradient elution of A/B = 60:40 to 90:10 (0–5.5 min), 90:10 to 1000 (5.5–7.5 min), 1000 (7.5–8.5 min), and 4060 (8.5–10 min) at a flow rate of 0.4 mL/min. The ESI/MS conditions were as follows: spray voltage, 3,300 V; collision gas, nitrogen, 1.5 psi; curtain gas, nitrogen, 11 psi; ion source temperature, 600°C; and positive ion polarity. The derived E1 and E2 were determined using product ions (m/z 157 and 264, respectively) produced from their individual protonated molecular ions. The limit of quantification was 4 fmol/g for E1 and 2 fmol/g for E2. Absolute recovery was high (97.5–103.1%), and intra-day accuracy and precision were 96% and 9.9% for E1 and 84% and 12.8% for E2, respectively, in our present study.

For measurement of DHEA-S, the homogenate was treated with acetonitrile for deproteinization, and 1H-DHEA-S was added as an internal standard. The steroid fraction was extracted using an Oasis HLB cartridge (Waters) and washed with hexane. After the solvent was evaporated under nitrogen gas stream, the residue was dissolved in the LC mobile phase, methanol/5 mmol/L AcONH4 (1:1, v/v; 50 μL). A LC-10AT chromatograph (Shimadzu) coupled with an API 2000 triple-stage quadrupole mass spectrometer (Applied Biosystems) was operated with ESI in negative ion mode. A semi-micro column, Develosil ODS-HG-5 (5 μm, 150 × 2.0 mm id.; Nomura Chemical), was used at a flow rate of 0.2 mL/min at 40°C. The ionization conditions were as follows: ion spray voltage, −4 kV; turbo gas temperature, 500°C; ion source gas 1 (nebulizer gas), 40 psi; ion source gas 2 (turbo gas), 80 psi; declustering potential, −71 V; focusing potential, −310 V; entrance potential, −10 V; curtain gas, 55 psi; detection, selected.
ion monitoring mode, m/z 367. The analytic recovery was satisfactory (89.4–109.2%) and the limit of quantification was 50 pmol/g.

**Immunohistochemistry.** The characteristics of STS, EST, and aromatase antibodies used for immunohistochemistry were described previously (16, 25). Briefly, the affinity-purified monoclonal STS (KM1049) antibody was raised against the STS enzyme purified from human placenta and recognizes the peptides corresponding to amino acids 414 to 434. Rabbit polyclonal antibody for EST (PV-P2237) was purchased from the Medical Biological Laboratory. This antibody was raised against the synthetic NH2-terminal peptides of human EST, corresponding to amino acids 1 to 13. Aromatase monoclonal antibody (#677) was raised against recombinant baculovirus-expressed human aromatase protein. Mouse monoclonal antibodies for ERβ (MS-ERB13-PX1) and Ki-67 (MIB1) were purchased from GeneTex and DAKO, respectively.

A Histofine Kit (Nichirei), which uses the streptavidin-biotin amplification method, was used for immunohistochemistry in this study. Antibody retrieval for ERβ and Ki-67 immunostaining was done by heating the slides in an autoclave at 120°C for 5 min in citric acid buffer (2 mmol/L citric acid and 9 mmol/L trisodium citrate dehydrate, pH 6.0), and antibody retrieval for EST was done by heating the slides in a microwave oven for 15 min in citric acid buffer. The dilutions of the primary antibodies used in this study were as follows: STS, 1:9,000; EST, 1:1,500; aromatase, 1:6,000; ERβ, 1:1,000; and Ki-67, 1:50. 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. As a negative control, normal rabbit or mouse IgG was used instead of the primary antibodies.

Immunoreactivities for STS, EST, and aromatase were detected in the cytoplasm, and the cases that had >10% of positive carcinoma cells were considered positive (23). Immunoreactivities for ERβ and Ki-67 were detected in the nucleus. These immunoreactivities were evaluated in more than 1,000 carcinoma cells for each case, and subsequently the percentage of positive cells [i.e., labeling index (LI)] was determined (23). Cases with ERβ LI of >10% were considered ERβ-positive colon carcinoma in this study (9).

**Statistical analysis.** Values for patients’ age, estrogen and DHEA-S concentrations, and Ki-67 LI were presented as mean ± SE. Associations between these parameters and STS, EST, and aromatase immunoreactivities were evaluated using Wilcoxon’s rank sum test. Paired t test was used for analyses of the paired samples. Statistical analysis between E1 and E2 concentrations was done using a correlation coefficient (r) and regression equation. Associations between STS and EST immunoreactivities and other clinicopathologic parameters were evaluated in a cross-table using the χ² test. Overall survival curves were generated according to the Kaplan-Meier method, and Cox’s proportional hazards model was used for univariate and multivariate analyses. StatView 5.0 software (SAS Institute, Inc.) was used for statistical analyses, and differences with P < 0.05 were considered significant.

**Results**

Intratumoral concentrations of estrogens in 53 colon carcinomas. We first examined tissue concentrations of estrogens in 53 colon carcinomas and 31 nonneoplastic colonic mucosal tissues using LC-ESI-MS. As shown in Fig. 1A, mean values ± SE of tissue concentrations of total estrogen (E1 + E2), E1, and E2 were 0.33 ± 0.05, 0.27 ± 0.05, and 0.06 ± 0.01 pmol/g in colon carcinoma and 0.16 ± 0.02, 0.12 ± 0.01, and 0.05 ± 0.01 pmol/g in nonneoplastic colonic mucosa, respectively. Intratumoral concentrations of total estrogen and E2 were significantly higher (P = 0.004 and 2.0-fold, and P = 0.003 and 2.4-fold, respectively) than those in nonneoplastic colonic mucosa, whereas E2 concentration was not significantly different between these two tissues (P = 0.53 and 1.2-fold). Similar tendencies were detected regardless of the gender of the patients (Fig. 1B), although the P values did not reach statistical significance in women [men: P = 0.002 (total estrogen), P = 0.004 (E1), and P = 0.005 (E2), and women: P = 0.23 (total estrogen), P = 0.12 (E1), and P = 0.24 (E2); Supplementary Table S1]. An analysis of the 31 paired samples showed that tissue concentrations of total estrogen, E1, and E2 were higher in carcinoma tissue than in the corresponding nonneoplastic colonic mucosa in 19 (61%), 19 (61%), and 18 (58%) cases, respectively, and the intratumoral concentrations of total estrogen and E1 were significantly higher (P = 0.048 and 1.3-fold, and P = 0.006 and 1.5-fold, respectively) than those in their corresponding nonneoplastic colonic mucosa. On the other hand, DHEA-S concentration in tumor tissue was not significantly different from that in corresponding nonneoplastic colonic mucosa in 22 cases examined (P = 0.22). The E2/E1 ratio was comparable (P = 0.30) between colon carcinoma and nonneoplastic mucosa (6.2 ± 0.9 and 11.8 ± 5.3, respectively).
respectively; Fig. 1C). The intratumoral concentrations of E₁ and E₂ were significantly correlated (P < 0.001 and r = 0.47) in the 53 colon carcinomas (Fig. 1D).

Correlations between the intratumoral estrogen levels and clinical outcome of the 53 colon carcinoma patients were shown in Fig. 2. Higher intratumoral concentrations of total estrogen (Fig. 2A) and E₁ (Fig. 2B) were significantly (P = 0.003 and P = 0.002, respectively) associated with adverse clinical outcome of the patients. A similar tendency was also detected in intratumoral concentration of E₂, but the association did not reach statistical significance (P = 0.10; Fig. 2C). This trend was also observed regardless of the gender of the patients [men: P = 0.75 (E), P = 0.82 (E₁), and P = 0.56 (E₂); women: the P values could not be calculated; Supplementary Fig. S2].

Intratumoral concentration of DHEA-S was not associated with clinical outcome of the colon carcinoma patients (P = 0.61). No significant correlations were observed between the intratumoral concentrations of estrogens and other clinicopathologic factors examined in this study, such as patients' age, gender, tumor site, and Dukes' stage (data not shown).

**Immunohistochemistry for STS, EST, and aromatase in the 53 colon carcinomas and their association with intratumoral estrogen concentrations.** We next evaluated associations between the intratumoral estrogen levels and enzymes related to the local estrogen production pathways in the 53 colon carcinomas. Immunoreactivities for STS, EST, and aromatase were detected in the cytoplasm of carcinoma cells in 32 (60%), 24 (45%), and 36 (68%) of the 53 colon carcinomas, respectively (Fig. 3A). Nonneoplastic colonic epithelium was positive for EST but negative for STS, as reported previously (26). Aromatase immunoreactivity was weakly detected in nonneoplastic colonic epithelium in approximately one third of the cases examined.

The sulfatase pathway is mediated by both STS and EST, so we subsequently classified the carcinomas into the following four groups according to the STS and EST status of carcinoma tissues: −/+ , cases negative for STS and positive for EST (n = 9); +/−, cases positive for both STS and EST (n = 12); and +/+ , cases positive for STS but negative for EST (n = 17). C, association between intratumoral concentrations of total estrogen (E; E₁ + E₂), E₁, and E₂ and the STS/EST status in 53 colon carcinomas. The cases were divided into −/+ (n = 9) and others (n = 44). D, association between intratumoral concentrations of estrogens and aromatase immunostaining. Columns, mean; bars, SE. Statistical analyses were done by Welch's t test.

![Figure 3](image-url)

**Figure 3.** Immunoreactivities for STS, EST, and aromatase in colon carcinoma and their associations with intratumoral estrogen concentrations. A, STS, EST, or aromatase immunoreactivity was detected in the cytoplasm of carcinoma cells. Bar, 100 mm. B, intratumoral concentration of total estrogen in 53 colon carcinomas according to the STS/EST status evaluated by immunohistochemistry. −/+ , cases negative for STS and positive for EST (n = 9); +/−, cases positive for both STS and EST (n = 12); and +/+ , cases positive for STS but negative for EST (n = 17). C, association between intratumoral concentrations of total estrogen (E; E₁ + E₂), E₁, and E₂ and the STS/EST status in 53 colon carcinomas. The cases were divided into −/+ (n = 9) and others (n = 44). D, association between intratumoral concentrations of estrogens and aromatase immunostaining. Columns, mean; bars, SE. Statistical analyses were done by Welch's t test.
(0.11 ± 0.02 pmol/g) and highest in the +/+ group (0.45 ± 0.12 pmol/g). STS or EST immunoreactivity alone was not associated with the intratumoral concentration of total estrogen when evaluated as continuous values (i.e., percent of positive carcinoma cells; STS: P = 0.74, r = −0.05, and EST: P = 0.11, r = −0.22). When the cases were divided into −/+ and others, the STS/EST status was significantly associated with the intratumoral concentrations of total estrogen (P < 0.001), E1 (P < 0.001), and E2 (P = 0.002) in the 53 colon carcinoma tissues examined (Fig. 3C). The intratumoral concentrations of estrogens were higher in aromatase-positive cases, but the differences were not statistically significant (P = 0.07 in total estrogen, P = 0.36 in E1, and P = 0.08 in E2; Fig. 3D).

**Correlation between the STS/EST status and clinicopathologic factors in 328 colon carcinomas.** Because the sulfatase pathway represented by the STS/EST status determined the intratumoral estrogen concentrations rather than the aromatase pathway, we then performed immunohistochemistry for STS and EST in 328 colon carcinoma cases to further examine the clinical significance of the STS/EST status in colon carcinoma. STS and EST immunoreactivities were detected in 200 (61%) and 144 (44%) of 328 cases, respectively, and 49 (15%) cases were in the +/+ group.

As shown in Table 1, the −/+ group was inversely associated with Dukes’ stage (P = 0.01), depth of invasion (P = 0.002), lymph node metastasis (P = 0.03), and distant metastasis (P = 0.01) and positively correlated with Ki-67 LI (P = 0.006). The −/+ group was also frequently (P < 0.001) detected in mucinous adenocarcinoma. Other clinicopathologic parameters were not associated with the STS/EST status in this group of patients.

**Correlation between the STS/EST status and clinical outcome in the 328 patients.** The −/+ group of the STS/EST status was significantly (P = 0.003) associated with better clinical outcome (Fig. 4B).

### Table 1. Association between STS/EST status and clinicopathologic parameters in 328 colon carcinomas

<table>
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<th>Value</th>
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<th>P</th>
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<tr>
<td></td>
<td>−/+ (n = 49)</td>
<td>Others (n = 279)</td>
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<tr>
<td>Age (y)*</td>
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<td>[22–91]</td>
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<td>C + D</td>
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<td>[min–max]</td>
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<td>[2.3–95.3]</td>
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NOTE: The STS/EST status was evaluated by immunohistochemistry, and “−/+” represents colon carcinomas negative for STS and positive for EST.

*Data are presented as mean ± SE and were evaluated by Welch’s t test. All other values represent the number of cases and were evaluated using a cross-table using the χ² test. P < 0.05 was considered significant, and shown in boldface.

†Proximal colon includes ascending and transverse colon.

‡Cases of mucinous adenocarcinoma were excluded.
outcome of the 328 colon carcinoma patients examined (Fig. 4A). Similar tendencies were detected regardless of the Dukes' stage (Fig. 4B and C), although the P value could not be calculated in the group of Dukes' stages A and B because no patients died in the −/+ group (Fig. 4B), and the P value did not reach a significant level (P = 0.08) in the group of Dukes' stages C and D (Fig. 4C). The −/+ cases had significantly longer survival in ERβ-positive cases (P = 0.009; Fig. 4D) but not in the ERβ-negative group (P = 0.18).

Using a univariate analysis (Table 2), distant metastasis (P < 0.0001), lymph node metastasis (P < 0.0001), depth of invasion (P = 0.001), and the STS/EST status (P = 0.03; Table 2) were identified as significant prognostic factors for overall survival in these 328 patients. However, STS alone (P = 0.10) or EST alone (P = 0.09) did not possess statistically significant prognostic value. Multivariate analysis showed that the STS/EST status (P = 0.03) was an independent prognostic factor for overall survival, as well as distant metastasis (P < 0.0001), lymph node metastasis (P = 0.02), and depth of invasion (P = 0.03; Table 2).

Discussion

To the best of our knowledge, this is the first reported study to show intratumoral concentrations of estrogens in colon carcinoma. In the present study, tissue concentration of total estrogen was 0.33 ± 0.05 pmol/g in colon carcinoma, and it was significantly and 2.0-fold higher than that in nonneoplastic colonic mucosa. Previous study in breast carcinoma showed that tissue concentration of total estrogen was 1.9-fold higher in breast carcinoma than in corresponding nonneoplastic breast tissue in postmenopausal women (27), whereas the plasma levels of E1 and E2 were similar in normal women and breast carcinoma patients both before and after menopause (28). Therefore, estrogens are considered to be locally produced in breast carcinoma tissue from circulating inactive steroids and to act on the breast carcinoma cells without release into the circulation (13). The intratumoral concentration of total estrogen in colon carcinoma in this study was approximately four times lower than that in breast carcinoma reported previously (15, 27), but the relative ratio of total estrogen between carcinoma and nonneoplastic tissue was similar between these two malignancies. Although we could not examine serum concentrations of estrogens in colon carcinoma patients, our results indicated that estrogens are locally synthesized in colon carcinoma as in breast carcinoma.

Results of our present study also showed that the STS/EST status was significantly associated with the intratumoral concentrations of total estrogen and E2, whereas aromatase was not. Previously, Suzuki and colleagues (16) reported that STS and EST immunoreactivities were significantly correlated with their mRNA expression levels and enzymatic activities in breast carcinomas using the same antibodies used in the current study. Interconversion of E1-S and E2 is regulated by STS and EST, and biologically inactive estrogen E1-S is a major circulating form of plasma estrogen in both men and women (15, 29). Thus, the sulfatase pathway, rather than the aromatase pathway, may be a potent regulatory pathway of intratumoral estrogens in colon carcinoma.

In our present study, E1 was higher than E2 in tumor tissue whereas E2 was reported to be higher in breast carcinoma (15, 27). Interconversion of E1 and E2 is mediated by 17βHSDs. To date, 14 isoforms of 17βHSD have been identified (14, 30), and reduction (E1 to E2) or oxidation (E2 to E1) of estrogen is catalyzed by different 17βHSD isozymes (reduction: 17βHSD types 1, 7, and 12, and oxidation: 17βHSD types 2, 4, and 14). Previous studies have shown that reductive 17βHSD pathway is dominant in breast carcinoma (13) and oxidative 17βHSD activity is a preferential direction in colon carcinoma, which is in good agreement with the result of our present study. Our data also revealed that the E1/E2

Figure 4. Overall survival curves of 328 colon carcinoma patients according to the STS/EST status. A, overall survival curves of all the patients (n = 328). B and C, overall survival curves of patients in the group of Dukes’ stages A and B (B; n = 172) and in the group of Dukes’ stages C and D (C; n = 156). D, overall survival curves of ERβ-positive cases (n = 229). Cox’s proportional hazards model was used for statistical analyses. The P value was not available in B because no patients died in the −/+ group.
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Table 2. Univariate and multivariate analyses of overall survival of 328 colon carcinoma patients

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<td>$P$</td>
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<td>$P$</td>
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<td>Distant metastasis (+ vs –)</td>
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<td>Lymph node metastasis (+ vs –)</td>
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<td>STS/EST status (Others vs +/–)</td>
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</tr>
<tr>
<td>Ki67 LI (others vs highest quartile)</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EST (– vs +)</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STS (+ vs –)</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histologic grade (moderate + poor vs well)</td>
<td>0.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age [a]</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki67 LI [b]</td>
<td>0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERβ (– vs +)</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site (distal vs proximal)</td>
<td>0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (women vs men)</td>
<td>0.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histologic type (mucinous vs tubular)</td>
<td>0.98</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Statistically significant values ($P < 0.05$) are in boldface. Abbreviation: 95% CI, 95% confidence interval.
[a] Data were considered significant in univariate analysis and examined in multivariate analysis. [b] Cases of mucinous adenocarcinoma were excluded. [c] Data were evaluated as continuous values.

ratio was similar between colon carcinoma and nonneoplastic colonic mucosa, and the intratumoral concentrations of E1 and E2 were closely correlated. Therefore, it is suggested that 17βHSD activity is relatively constant in nonneoplastic and neoplastic colon tissues.

In our study, lower concentrations of intratumoral total estrogen and E1, and the –/+ group of the STS/EST status were significantly associated with better clinical outcome of the colon carcinoma patients, with the STS/EST status being an independent prognostic factor. Previous reports showed that hormone replacement therapy reduced the risk of colon cancer (4, 5) and improved the survival of the patients with colon cancer (31), which seems to be inconsistent with our present findings. However, it is also true that hormone replacement therapy often contains progestin with estrogens. The biological activity of E1 is weaker than E2, and E1 is generally considered as a precursor of E2. However, it was reported that E1 could also bind to ERs, and its binding affinity relative to E2 was 60% for ERα and 37% for ERβ (39). In addition, E1 induced the transcriptional activity of ERs, and its relative potency compared with E2 was 65% to 110% in ERα and 55% to 70% in ERβ (40). Because the intratumoral concentration of E1 was ~6-fold higher than that of E2 in colon carcinoma in this study, E1 might also act on colon carcinoma cells in addition to serving as a precursor of E2. Oduwole and colleagues (41) reported that higher mRNA expression of 17βHSD type 2 was significantly associated with shorter survival in female patients with distal colorectal carcinoma.
and suggested a possible protective effect of E₂ against colon carcinoma. However, considering our present results, it is also possible to interpret this finding as evidence that increment of intratumoral E₁ by 17βHSD type 2 is, at least in part, involved in the adverse clinical outcome of colon carcinoma patients. Further examinations are required to clarify the biological significance of E₁ in colon carcinoma.

Results of our present study showed that STS⁻ and EST⁺ colon carcinoma was frequently detected in mucinous adenocarcinoma, which was reported to have worse prognosis (41, 42). However, the American Committee on Cancer Prognostic Factors Consensus Conference concluded that histologic type is of no prognostic significance in colon carcinoma (43), and such an association between histologic type and clinical outcome of the patients was not detected in our present study, as summarized in Table 2.

The −/+ group of the STS/EST status was inversely associated with Dukes’ stage, depth of invasion, lymph node metastasis, and distant metastasis and positively correlated with Ki-67 LI. Ki-67 LI has been frequently used as a marker for the proliferative activity in various human malignancies, and higher Ki-67 LI in tumor tissues is associated with shorter survival of the patients with breast and lung carcinomas, astrocytoma, and meningioma (43). However, overexpression of Ki-67 induced growth arrest (44), and Hilska and colleagues (45) suggested that strongly stained tumor cells had a slow cell cycle and a low proliferation rate. Furthermore, several investigators reported that higher Ki-67 LI was associated with longer survival in colon carcinoma patients (45–47). Therefore, taken together with these previous reports and our present results, it is indicated that the decrement of intratumoral total estrogen concentration in the STS⁻ and EST⁺ colon carcinoma may contribute to lower proliferative activity of carcinoma cells, diminished invasive and metastatic potentials, and better clinical outcome in colon carcinoma despite elevated Ki-67-LI, but it awaits further investigations for clarification.

In summary, we showed that the intratumoral concentrations of total estrogen and E₁ were significantly higher than those in nonneoplastic colonic mucosa, and higher intratumoral concentrations of total estrogen and E₁ were significantly associated with adverse clinical outcome of the patients. Immunoreactivities for STS, EST, and aromatase were detected in 61%, 44%, and 68% of colon carcinomas, respectively, and the STS/EST status determined the intratumoral concentration of estrogens in colon carcinoma. The −/+ group of the STS/EST status was inversely associated with Dukes’ stage, depth of invasion, lymph node metastasis, and distant metastasis. Moreover, this −/+ group was significantly associated with better clinical outcome of the patients, and a multivariate analysis revealed the STS/EST status as an independent prognostic factor. Results from our present study suggest that estrogens are locally produced in colon carcinoma mainly through the sulfatase pathway and play important roles in the progression of the disease.

Disclosure of Potential Conflicts of Interest

D.B. Evans: ownership interest, Novartis Pharma, AG. The other authors disclosed no potential conflicts of interest.

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References

25. Sasano H, Anderson TJ, Silverberg SG, et al. The validation of new aromatase monoclonal antibodies for...


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