Aromatase in Colon Carcinoma

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Abstract. *Aromatase is one of the key estrogen-producing enzymes and is regarded as one of the therapeutic targets in estrogen receptor-positive breast cancer patients. Human colon carcinoma has also been recently proposed as being an estrogen-responsive malignancy, but the detailed status of aromatase has not yet been reported. Therefore, in this study, we evaluated the aromatase expression in colon carcinoma using immunohistochemistry and real-time polymerase chain reaction. Aromatase mRNA was significantly higher (p=0.03) in colon carcinoma than in the corresponding non-neoplastic mucosa (n=31). Aromatase immunoreactivity tended to be positively associated with the intratumoral concentration of estrogens (n=53), and in particular, the concentration of estradiol was significantly higher (p=0.02) in aromatase-positive cases in men. Aromatase immunoreactivity was detected in the cytoplasm of the carcinoma cells in 217/328 (65%) examined colon carcinoma cases. Aromatase immunoreactivity was significantly positively correlated with tubular differentiation, and inversely correlated with Ki-67 labeling index, although not necessarily correlated with the clinical outcome of the patients. All these results demonstrate that colon carcinoma expresses functional aromatase, and that estrogens are locally synthesized in the tumor tissues. The findings reported here could contribute to a better understanding of the actions of estrogen in colon carcinoma.*

Aromatase is one of the key estrogen-producing enzymes. In breast cancer, estrogens are locally produced from circulating inactive steroids through two major pathways, the aromatase and the sulfatase pathways (1). In the aromatase pathway, aromatase converts androstenedione and testosterone into estrone and estradiol, the most potent estrogen, respectively. In the sulfatase pathway, steroid sulfatase (STS) synthesizes estrone from circulating estrone sulfate, or estrogen sulfotransferase (EST) inversely inactivates estrone into estrone sulfate. Estrone is subsequently converted to estradiol by reductive 17β-hydroxysteroid dehydrogenases (17βHSDs) (1). Results of previous studies in post-menopausal breast cancer patients (2) demonstrated that the intratumoral concentration of estradiol in breast carcinoma tissue was 2.3-fold higher than that in the corresponding non-neoplastic breast tissue. Therefore, *in situ* production of estrogens is considered to play pivotal roles in providing estrogens for carcinoma tissue, and these enzymes are generally regarded as important therapeutic targets in estrogen-dependent neoplasms (3). The STS activity was reported to be 50 to 200 times greater than the aromatase activity (4), but the aromatase inhibitor has been widely used for patients with breast cancer, and has significantly improved their survival (3).

An increasing body of evidence suggests the importance of estrogen actions in human colon carcinoma. Results of a number of observational studies (5-7) and a randomized trial (8-9) demonstrated that hormone replacement therapy (HRT) affected the overall incidence of colon carcinoma and the recurrence of colorectal adenoma (10) in post-menopausal women. In addition, a great majority of colon carcinoma specimens and cell lines expressed estrogen receptor β (ERβ) (11), and some of these cell lines were actually responsive to estrogens (12-14). Moreover, colon carcinoma has been shown to express aromatase (15, 16), STS (16-18), EST (16), and 17βHSDs (15, 19). We previously demonstrated (16) that concentrations of total estrogen (estrone and estradiol), and estrone in colon carcinoma tissue were significantly (2.0- and 2.4-fold, respectively) higher than those in normal colonic mucosa, and higher intratumoral concentrations of total estrogen and estrone were significantly associated with poorer survival. We also reported that the STS/EST status determined the intratumoral concentration of total estrogen in colon carcinoma, and was significantly associated with

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clinical outcome of the patients. All these results indicated that estrogens are locally produced in colon carcinoma, mainly through the sulfatase pathway and play important roles in the progression of the disease. Therefore in this study, we examined the clinical significance of another estrogen-producing pathway, the aromatase pathway, in order to obtain a better understanding of intratumoral estrogens in colon carcinoma.

Materials and Methods

Patients and tissues. Detailed characteristics of the patients and the tissues used in this study have been published previously (16). Briefly, colon carcinoma specimens were obtained from 328 consecutive patients (184 men and 144 women including 138 post-menopausal women) who underwent surgery between 1994 and 2000. Two hundred and six patients were operated on at Miyagi Cancer Center (Natori, Japan) and 122 patients at Tohoku University Hospital (Sendai, Japan). The mean age of the patients was 66.0 years (range, 22-91 years). The mean follow-up time was 78.8 months (range, 0-161 months), and survival data of all the patients were available. The surgical specimens were fixed in 10% formalin and embedded in paraffin-wax. Snap-frozen tissues and serum were not obtainable in these cases.

Another set of colon carcinoma tissues was obtained from 53 patients (28 men and 25 post-menopausal women) who underwent surgery from 2000 to 2008 at Tohoku University Hospital (mean age=69.1 years; range, 45-83 years). Among these 53 cases, specimens of non-neoplastic colonic mucosa were available in 31 (16 men and 15 women). The specimens used for measurement of estrogen concentrations and real-time polymerase chain reaction (PCR) were snap-frozen and stored at –80˚C until use. For immunohistochemistry, 10% formalin fixed and paraffin-wax embedded tissues were used. Serum samples of these patients were not available in this study.

Patients clinically suspected of having hereditary non-polyposis 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, received HRT, nor underwent irradiation or chemotherapy prior to 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 (2007-369) and the Miyagi Cancer Center (2007-6).

Immunohistochemistry. The characteristics of the antibodies to aromatase, STS and EST used for immunohistochemistry, have been described previously (16, 20, 21). Briefly, the aromatase monoclonal antibody (#677) was raised against recombinant baculovirus-expressed human aromatase protein (20). 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 414–434. A rabbit polyclonal antibody for EST (PV-P2237) was purchased from the Medical Biological Laboratory (Nagoya, Japan) (21). Mouse monoclonal antibodies for ERβ (MS-ERB13-PX1) and Ki-67 (MIB1) were purchased from GeneTex (San Antonio, TX, USA) and DAKO (Carpinteria, CA, USA), respectively.

A Histofine Kit (Nichirei, Tokyo, Japan), which employs the streptavidin-biotin amplification method was used for immunohistochemistry in this study. Antigen retrieval for ERβ and Ki-67 immunostaining was performed by heating the slides in citric acid buffer (2 mM citric acid and 9 mM trisodium citrate dehydrate, pH 6.0), in an autoclave at 120˚C for 5 min. Antigen retrieval for EST was carried out 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: aromatase: 1/6,000; STS: 1/9,000; EST: 1/1,500; ERβ: 1/1,000; and Ki-67: 1/50. The antigen-antibody complex was visualized with 3,3'-diaminobenzidine solution [1 mM 3,3'-diaminobenzidine, 50 mM Tris-HCl buffer (pH 7.6), and 0.006% H_2O_2] and counterstained with hematoxylin. As a negative control for immunostaining, normal rabbit or mouse IgG was used instead of the primary antibodies.

Immunoreactivities for aromatase, STS and EST were detected in the cytoplasm, and the cases that had more than 10% of positive carcinoma cells were considered as being positive (16, 22). Immunoreactivities for ERβ and Ki-67 were detected in the nucleus, which were evaluated in more than 1,000 carcinoma cells for each case, and subsequently the percentage of the positive cells, i.e. labeling index (LI), was determined (22). Cases with ERβ LI of more than 10% were considered ERβ-positive colon carcinoma in this study (11, 16).

Total RNA extraction and real-time PCR. RNA was extracted from the 31 paired colon carcinoma specimens and from corresponding non-pathological colonic mucosa using the RNeasy Mini Kit (Quiagen, Duesseldorf, Germany). A reverse transcription kit (Superscript II Preamplification system; Invitrogen, San Diego, CA, USA) was used in the synthesis of cDNA. Real-time PCR was carried out using the LightCycler System (Roche Diagnostics GmbH, Mannheim, Germany). The primer sequences of aromatase and ribosomal protein L13a (RPL13A) were reported previously (23). The cDNAs of known concentrations for target genes were used to generate standard curves for real-time quantitative PCR, and the quantity of the target cDNA transcripts were determined. The mRNA level in each case was determined as previously described (23). Negative controls, in which the reaction mixture lacked cDNA template, were included to rule out the possibility of exogenous DNA contamination.

Liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS) analysis. Concentrations of estrone and estradiol were measured using LC-ESI-MS at Teikoku Hormone Medical (Kawasaki, Japan) as described previously (16, 22, 24, 25). Results were also reported in the previous studies (16).

Statistical analysis. Values for patients' age, estrogen concentrations, mRNA level, and Ki-67 LI are presented as the mean±SEM. Associations between these parameters and aromatase immunoreactivitiy were evaluated using Welch's *t*-test. Paired *t*-test was used for analysis of the paired samples. Associations between aromatase immunoreactivity and other clinicopathological parameters were evaluated in a cross-table using the χ^2 test. Overall survival curves were generated according to the Kaplan-Meier method and the log-rank test was used for analysis. The StatView 5.0 software (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses and differences with p-values<0.05 were considered significant.

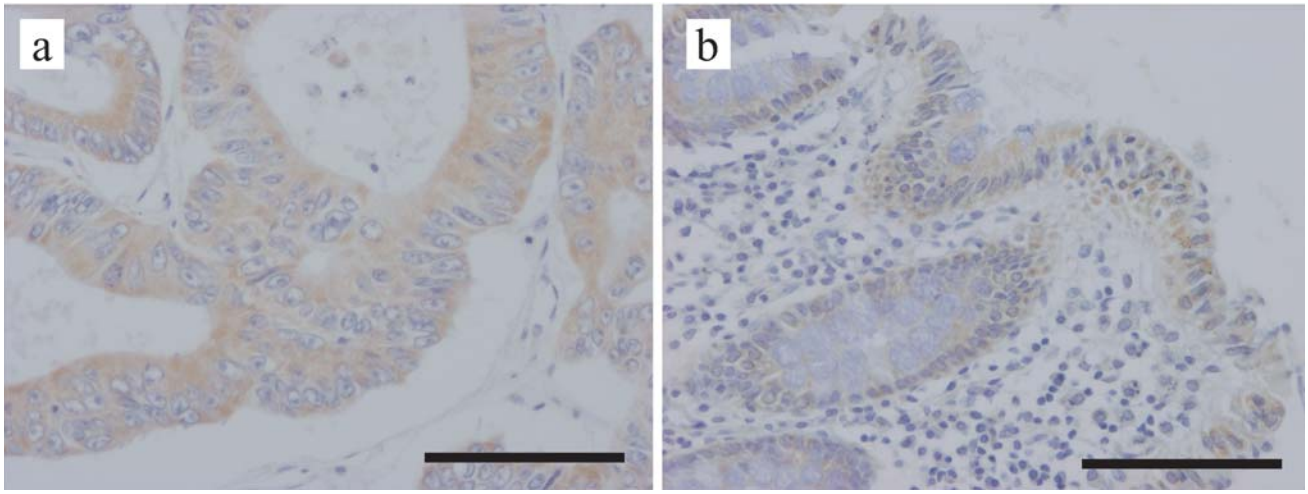

Figure 1. *Immunohistochemistry for aromatase in colon carcinoma. Aromatase immunoreactivity was detected in the cytoplasm of carcinoma cells (a), but not in stromal cells. Non-neoplastic colonic epithelial cells were weakly positive for aromatase (b). Bar=100 μm.*

Results

Immunoreactivity for aromatase and correlation with clinicopathological factors and clinical outcome in 328 colon carcinomas. We performed immunohistochemistry for aromatase in 328 colon carcinomas in order to examine the clinical and prognostic significance of the aromatase status in colon carcinoma. Aromatase immunoreactivity was detected in the cytoplasm of carcinoma cells in 217 (66%) out of 328 cases (Figure 1a). Non-neoplastic colonic epithelium was weakly positive for aromatase in approximately one third of the cases examined, as described previously (Figure 1b) (16). Aromatase immunoreactivity was negative in stromal cells both in carcinoma and non-neoplastic tissues. As summarized in Table I, aromatase immunoreactivity in the carcinoma cells was inversely correlated with Ki-67 LI (p=0.02). Aromatase-positive cases were frequently detected in tubular adenocarcinoma (p=0.01) and in cases other than STS^{-}/EST^{+} carcinomas (p=0.001). Other clinicopathological factors, including gender, Dukes' stage, and ERβ immunoreactivity, were not associated with the aromatase status in this group of patients.

The aromatase status was not significantly associated with the clinical outcome of the 328 examined colon carcinoma patients (Figure 2).

mRNA expression of aromatase in 31 pairs of colon carcinomas. We evaluated the aromatase expression using real-time PCR in the 31 paired samples. As demonstrated in Table II, aromatase mRNA level was significantly (p=0.03 and 5.2-fold) higher in carcinoma tissue than in non-neoplastic mucosa. When the cases were divided according to gender, aromatase mRNA level was significantly higher in male (p=0.03) but not in female patients.

Correlation between aromatase status and intratumoral estrogen concentrations in the 53 colon carcinoma cases. Since aromatase converts androgens into estrogens, we examined the correlation between aromatase immunoreactivity of the carcinoma tissue and intratumoral concentrations of estrogens in the 53 cases. Aromatase immunoreactivity was detected in 36 (68%) examined cases. Intratumoral concentration of estrogens tended to be higher in aromatase-positive cases, as described previously (16). When the cases were stratified by gender, the intratumoral estradiol concentration was significantly (p=0.02) higher in aromatase-positive cases in men (Table III).

We previously reported that the intratumoral concentrations of estrogens were strongly regulated by the sulfatase pathway, represented by STS/EST status (16). Therefore, we divided the cases according to the STS/EST status, and further examined associations between intratumoral estrogen levels and aromatase status. As demonstrated in Table IV, the intratumoral estradiol level was 1.8 to 2.0 times higher in aromatase-positive cases, and the difference was significant (p=0.02) in the cases other than the STS^{-}/EST^{+} group.

Discussion

This is the first study to evaluate the clinical and prognostic significance of aromatase in human colon carcinoma. In the present study, aromatase immunoreactivity was detected in 217 out of 328 (66%) colon carcinoma cases, while it was weakly and focally detected in normal colonic epithelium,

Table I. *Association between the aromatase status and clinicopathological parameters in 328 colon carcinomas.*

Value	Aromatase status		*p*-Value
	– (n=111)	+ (n=217)	
Age (years)	65.6±1.1	66.2±0.7	0.61
(Min-max)	(35-89)	(22-91)	
Gender			
Male	61	123	0.77
Female	50	94	
Tumor site†			
Proximal	62	116	0.68
Distal	49	101	
Dukes' stage			
A + B	53	119	0.22
C + D	58	98	
Depth of invasion (T stage)			
Submucosa - muscularis propria (T1+2)	28	38	0.10
Beyond muscularis propria (T3+4)	83	179	
Lymph node metastasis			
–	58	131	0.16
+	53	86	
Distant metastasis			
–	94	173	0.61
+	17	38	
Histological type			
Tubular adenocarcinoma	96	205	0.01
Mucinous adenocarcinoma	15	12	
Histological differentiation‡			
Well	25	67	0.24
Moderate + poor	71	138	
ERβ			
–	27	72	0.10
+	84	145	
STS/EST status§			
–/+	27	22	0.001
Other	84	195	
Ki67-LI (%)	54.0±1.9	48.5±1.5	0.02
(Min-max)	(5.9-96.5)	(2.3-95.3)	

ER: Estrogen receptor; STS/EST: steroid sulfatase/estrogen sulfotransferase; LI: labeling index. Data are presented as the mean±SEM, and were evaluated by Welch's *t*-test test. All other values represent the number of cases, and were evaluated using a cross-table using the chi-square test. *p*-Values less than 0.05 were considered significant. †Proximal colon includes ascending and transverse colon. ‡Cases of mucinous adenocarcinoma were excluded. §The STS/EST status was evaluated by immunohistochemistry, and "–/+" represents colon carcinomas negative for STS and positive for EST, respectively.

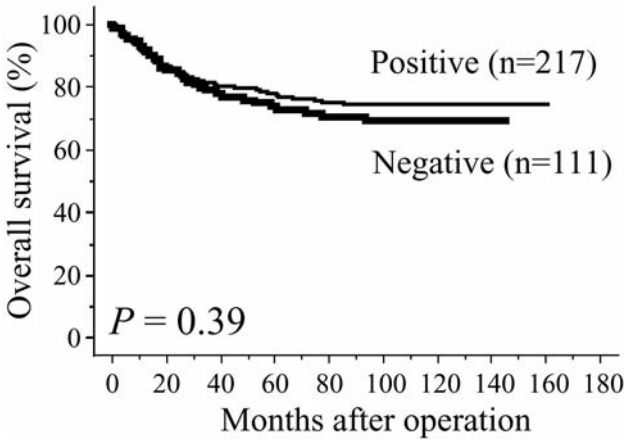

Figure 2. *Overall survival curves of 328 colon carcinoma patients according to the aromatase status. The log-rank test was used for statistical analysis.*

Table II. *Aromatase mRNA level in 31 colon carcinoma tissue and corresponding non-neoplastic epithelium.*

	n	Aromatase mRNA†		*p*-Value
		Normal tissue	Tumor tissue	
Total	31	0.26±0.12	1.36±0.49	0.03
Men	16	0.41±0.23	2.59±0.85	0.03
Post-menopausal women	15	0.10±0.03	0.06±0.04	0.29

Data are presented as the mean±SEM, and were evaluated by the paired *t*-test. *p*-Values less than 0.05 were considered significant. †Relative to expression of *RPL13A*.

and the aromatase mRNA level was significantly (5.2-fold) higher in colon carcinoma than in the corresponding non-neoplastic mucosa. Intratumoral aromatase has been reported in several malignancies, including breast (2, 4, 22), lung (26, 27), gastric (28), ovarian carcinomas (29), and others (30). As for colon carcinoma, English and colleagues (15) reported that human colon epithelial and carcinoma tissue had aromatase activity and converted androstenedione into estrone. They also demonstrated aromatase activity in some colon cancer cell lines, such as Caco-2, HT-29 and SW620. Fiorelli and colleagues (31) showed that human colon carcinoma cell lines HCT8 and HCT116 had aromatase mRNA and enzymatic activity, although the maximum capacity was 10- and 4-fold lower than that of adipose tissue. Our present results were consistent with these previous findings, and also suggested that aromatase was frequently overexpressed in human colon adenocarcinoma.

We revealed that the intratumoral concentration of estorogens tended to be higher in aromatase-positive colon carcinomas, and the estradiol level was significantly associated with the aromatase status in men. In human lung carcinoma, Niikawa (27) and colleagues demonstrated that intratumoral estradiol concentration was closely associated with the aromatase mRNA level, and was significantly higher in men than in post-menopausal women. The serum concentrations of androstenedione and testosterone, the substrates for aromatase,

Table III. *Intratumoral concentration of estrogens in 53 colon carcinomas according to the aromatase status stratified by gender.*

Parameter	Aromatase-positive cases		Aromatase-negative cases		*p*-Value
	n	Concentration (pmol/g)	n	Concentration (pmol/g)	
Estrone					
Total	36	0.30±0.07	17	0.22±0.04	0.36
Men	20	0.32±0.07	8	0.28±0.05	0.56
Post-menopausal women	16	0.27±0.14	9	0.18±0.06	0.56
Estradiol					
Total	36	0.06±0.01	17	0.04±0.01	0.08
Men	20	0.08±0.02	8	0.04±0.01	0.02
Post-menopausal women	16	0.04±0.01	9	0.04±0.01	0.08
Total estrogen					
Total	36	0.36±0.08	17	0.26±0.04	0.07
Men	20	0.41±0.08	8	0.31±0.05	0.34
Post-menopausal women	16	0.30±0.14	9	0.22±0.06	0.60

Data are presented as the mean±SEM, and were evaluated by Welch's *t*-test. *p*-Values less than 0.05 were considered significant.

Table IV. *Intratumoral concentration of estrogens in 53 colon carcinomas according to the aromatase status, stratified by the steroid sulfatase(STS)/estrogen sulfotransferase(EST) status.*

Parameter	Aromatase-positive cases		Aromatase-negative cases		*p*-Value
	n	Concentration (pmol/g)	n	Concentration (pmol/g)	
Estorone					
STS^-/EST^+	6	0.07±0.02	3	0.11±0.03	0.48
Other	30	0.34±0.08	14	0.25±0.04	0.32
Estradiol					
STS^-/EST^+	6	0.04±0.02	3	0.02±0.01	0.25
Other	30	0.07±0.01	14	0.04±0.01	0.02
Total estrogen					
STS^-/EST^+	6	0.09±0.03	3	0.15±0.03	0.20
Other	30	0.41±0.09	14	0.29±0.05	0.22

Data are presented as the mean±SEM, and were evaluated by Welch's *t*-test. *p*-Values less than 0.05 were considered significant.

are 1.7 and 20 times higher in men than in postmenopausal women, respectively (32, 33). Therefore, circulating testosterone is reasonably postulated to be a major precursor substrate of local estradiol production by aromatase in colon carcinoma. Our results also demonstrated that aromatase immunoreactivity was frequently detected in the colon carcinomas other than the STS^-/EST^+ group, and that the aromatase status was positively associated with the intratumoral estradiol level in these cases. EST is the only sulfotransferase that displays affinity for estradiol in a physiological concentration range, and the sulfating activity for estradiol was stronger than that for estrone (34-36). Thus, EST might offset the estradiol production through the aromatase pathway, but it awaits further studies for clarification. The results of the present study suggested that aromatase was also involved in the local estradiol production in cooperation with the sulfatase pathway in colon carcinoma.

It is well known that aromatase is expressed not only in the carcinoma cells but also in the intratumoral stromal cells in breast carinoma, and their interaction regulates the local production of estrogens (37). However, aromatase immunoreactivity was detected only in the colon carcinoma cells in our study, and similar localization has been also reported in lung (38), gastric (28), and oral squamous cell carcinomas (30). Therefore, the characteristics of stromal cells in breast carcinoma may be different from those in non-classical estrogen-dependent malignancies including colon carcinoma.

In our current study, aromatase immunoreactivity was not correlated with survival of the patients. Aromatase inhibitors are frequently used in patients with breast carcinoma, and improves their clinical outcome (3), but the actual correlation between intratumoral aromatase expression and prognosis of the patients remains unclear. Salhab and colleagues (39) demonstrated that higher aromatase mRNA expression in breast carcinoma tissues was correlated with poorer survival. However, Miyoshi and colleagues (40) failed to show that intratumoral aromatase mRNA expression had prognostic value, and Marigilka and colleagues (41) reported the absence of correlation between aromatase enzymatic activity and survival of the patients. Hence, our present result does not necessarily deny the significance of aromatase in colon carcinoma. Considering that aromatase expression was associated with the intratumoral estrogen levels, aromatase is considered to play an important role in the pathogenesis of colon carcinoma. Further investigations are required to clarify the clinical importance of estrogen actions and aromatase in colon carcinoma.

Disclosure Statement

The Authors have no conflicts of interest.

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References

1 Sasano H, Suzuki T, Nakata T and Moriya T: New development in intracrinology of breast carcinoma. Breast Cancer *13*: 129-136, 2006.

2 Chetrite GS, Cortes-Prieto J, Philippe JC, Wright F and Pasqualini JR: Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. J Steroid Biochem Mol Biol *72*: 23-27, 2000.

3 Mauri D, Pavlidis N, Polyzos NP and Ioannidis JP: Survival with aromatase inhibitors and inactivators versus standard hormonal therapy in advanced breast cancer: meta-analysis. J Natl Cancer Inst *98*: 1285-1291, 2006.

4 Pasqualini JR, Chetrite G, Blacker C, Feinstein MC, Delalonde L, Talbi M and Maloche C: Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients. J Clin Endocrinol Metab *81*: 1460-1464, 1996.

5 Newcomb PA, Zheng Y, Chia VM, Morimoto LM, Doria-Rose VP, Templeton A, Thibodeau SN and Potter JD: Estrogen plus progestin use, microsatellite instability, and the risk of colorectal cancer in women. Cancer Res *67*: 7534-7539, 2007.

6 Grodstein F, Newcomb PA and Stampfer MJ: Postmenopausal hormone therapy and the risk of colorectal cancer: a review and meta-analysis. Am J Med *106*: 574-582, 1999.

7 Prihartono N, Palmer JR, Louik C, Shapiro S and Rosenberg L: A case-control study of use of postmenopausal female hormone supplements in relation to the risk of large bowel cancer. Cancer Epidemiol Biomarkers Prev *9*: 443-447, 2000.

8 Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, Bonds D, Brunner R, Brzyski R, Caan B, Chlebowski R, Curb D, Gass M, Hays J, Heiss G, Hendrix S, Howard BV, Hsia J, Hubbell A, Jackson R, Johnson KC, Judd H, Kotchen JM, Kuller L, LaCroix AZ, Lane D, Langer RD, Lasser N, Lewis CE, Manson J, Margolis K, Ockene J, O'Sullivan MJ, Phillips L, Prentice RL, Ritenbaugh C, Robbins J, Rossouw JE, Sarto G, Stefanick ML, Van Horn L, Wactawski-Wende J, Wallace R and Wassertheil-Smoller S: Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA *291*: 1701-1712, 2004.

9 Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM and Ockene J: Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA *288*: 321-333, 2002.

10 Woodson K, Lanza E, Tangrea JA, Albert PS, Slattery M, Pinsky J, Caan B, Paskett E, Iber F, Kikendall JW, Lance P, Shike M, Weissfeld J and Schatzkin A: Hormone replacement therapy and colorectal adenoma recurrence among women in the Polyp Prevention Trial. J Natl Cancer Inst *93*: 1799-1805, 2001.

11 Wong NA, Malcomson RD, Jodrell DI, Groome NP, Harrison DJ and Saunders PT: ERβ isoform expression in colorectal carcinoma: an *in vivo* and *in vitro* study of clinicopathological and molecular correlates. J Pathol *207*: 53-60, 2005.

12 Fiorelli G, Picariello L, Martineti V, Tonelli F and Brandi ML: Functional estrogen receptor beta in colon cancer cells. Biochem Biophys Res Commun *261*: 521-527, 1999.

13 Qiu Y, Waters CE, Lewis AE, Langman MJ and Eggo MC: Oestrogen-induced apoptosis in colonocytes expressing oestrogen receptor beta. J Endocrinol *174*: 369-377, 2002.

14 Arai N, Strom A, Rafter JJ and Gustafsson JA: Estrogen receptor beta mRNA in colon cancer cells: growth effects of estrogen and genistein. Biochem Biophys Res Commun *270*: 425-431, 2000.

15 English MA, Kane KF, Cruickshank N, Langman MJ, Stewart PM and Hewison M: Loss of estrogen inactivation in colonic cancer. J Clin Endocrinol Metab *84*: 2080-2085, 1999.

16 Sato R, Suzuki T, Katayose Y, Miura K, Shiiba K, Tateno H, Miki Y, Akahira J, Kamogawa Y, Nagasaki S, Yamamoto K, Ii T, Egawa S, Evans DB, Unno M and Sasano H: Steroid sulfatase and estrogen sulfotransferase in colon carcinoma: regulators of intratumoral estrogen concentrations and potent prognostic factors. Cancer Res *69*: 914-922, 2009.

17 Koudstaal J: The histochemical demonstration of arylsulphatase and acid hydrolases in carcinoma of the colon and the breast. Eur J Cancer *11*: 479-482, 1975.

18 Double JA, Cooper EH and Goligher JC: Hydrolytic enzymes in colorectal cancer. Biomedicine *27*: 11-13, 1977.

19 English MA, Stewart PM and Hewison M: Estrogen metabolism and malignancy: analysis of the expression and function of 17beta-hydroxysteroid dehydrogenases in colonic cancer. Mol Cell Endocrinol *171*: 53-60, 2001.

20 Sasano H, Anderson TJ, Silverberg SG, Santen RJ, Conway M, Edwards DP, Krause A, Bhatnagar AS, Evans DB and Miller WR: The validation of new aromatase monoclonal antibodies for immunohistochemistry-a correlation with biochemical activities in 46 cases of breast cancer. J Steroid Biochem Mol Biol *95*: 35-39, 2005.

21 Suzuki T, Nakata T, Miki Y, Kaneko C, Moriya T, Ishida T, Akinaga S, Hirakawa H, Kimura M and Sasano H: Estrogen sulfotransferase and steroid sulfatase in human breast carcinoma. Cancer Res *63*: 2762-2770, 2003.

22 Suzuki T, Miki Y, Moriya T, Akahira J, Ishida T, Hirakawa H, Yamaguchi Y, Hayashi S and Sasano H: 5Alpha-reductase type 1 and aromatase in breast carcinoma as regulators of *in situ* androgen production. Int J Cancer *120*: 285-291, 2007.

23 Suzuki T, Miki Y, Moriya T, Shimada N, Ishida T, Hirakawa H, Ohuchi N and Sasano H: Estrogen-related receptor alpha in human breast carcinoma as a potent prognostic factor. Cancer Res *64*: 4670-4676, 2004.

24 Yamashita K, Okuyama M, Watanabe Y, Honma S, Kobayashi S and Numazawa M: Highly sensitive determination of estrone and estradiol in human serum by liquid chromatography-electrospray ionization tandem mass spectrometry. Steroids *72*: 819-827, 2007.

25 Mitamura K, Setaka M, Shimada K, Honma S, Namiki M, Koh E and Mizokami A: Determination of sulfates of androsterone and epiandrosterone in human serum using isotope diluted liquid chromatography-electrospray ionization-mass spectrometry. Biomed Chromatogr *19*: 796-801, 2005.

26 Mah V, Seligson DB, Li A, Marquez DC, Wistuba II, Elshimali Y, Fishbein MC, Chia D, Pietras RJ and Goodglick L: Aromatase expression predicts survival in women with early-stage non small cell lung cancer. Cancer Res *67*: 10484-10490, 2007.

27 Niikawa H, Suzuki T, Miki Y, Suzuki S, Nagasaki S, Akahira J, Honma S, Evans DB, Hayashi S, Kondo T and Sasano H: Intratumoral estrogens and estrogen receptors in human non-small cell lung carcinoma. Clin Cancer Res *14*: 4417-4426, 2008.
28 Izawa M, Inoue M, Osaki M, Ito H, Harada T, Terakawa N and Ikeguchi M: Cytochrome P450 aromatase gene (*CYP19*) expression in gastric cancer. Gastric Cancer *11*: 103-110, 2008.
29 Cunat S, Rabenoelina F, Daures JP, Katsaros D, Sasano H, Miller WR, Maudelonde T and Pujol P: Aromatase expression in ovarian epithelial cancers. J Steroid Biochem Mol Biol *93*: 15-24, 2005.
30 Cheng YS, Mues G, Wood D and Ding J: Aromatase expression in normal human oral keratinocytes and oral squamous cell carcinoma. Arch Oral Biol *51*: 612-620, 2006.
31 Fiorelli G, Picariello L, Martineti V, Tonelli F and Brandi ML: Estrogen synthesis in human colon cancer epithelial cells. J Steroid Biochem Mol Biol *71*: 223-230, 1999.
32 Leder BZ, Longcope C, Catlin DH, Ahrens B, Schoenfeld DA and Finkelstein JS: Oral androstenedione administration and serum testosterone concentrations in young men. JAMA *283*: 779-782, 2000.
33 Leder BZ, Leblanc KM, Longcope C, Lee H, Catlin DH and Finkelstein JS: Effects of oral androstenedione administration on serum testosterone and estradiol levels in postmenopausal women. J Clin Endocrinol Metab *87*: 5449-5454, 2002.
34 Pasqualini JR: Estrogen sulfotransferases in breast and endometrial cancers. Ann NY Acad Sci *1155*: 88-98, 2009.
35 Zhang H, Varlamova O, Vargas FM, Falany CN, Leyh TS and Varmalova O: Sulfuryl transfer: the catalytic mechanism of human estrogen sulfotransferase. J Biol Chem *273*: 10888-10892, 1998.
36 Hui Y, Yasuda S, Liu MY, Wu YY and Liu MC: On the sulfation and methylation of catecholestrogens in human mammary epithelial cells and breast cancer cells. Biol Pharm Bull *31*: 769-773, 2008.
37 Miki Y, Suzuki T, Tazawa C, Yamaguchi Y, Kitada K, Honma S, Moriya T, Hirakawa H, Evans DB, Hayashi S, Ohuchi N and Sasano H: Aromatase localization in human breast cancer tissues: possible interactions between intratumoral stromal and parenchymal cells. Cancer Res *67*: 3945-3954, 2007.
38 Miki Y, Suzuki T, Abe K, Suzuki S, Niikawa H, Iida S, Hata S, Akahira J, Mori K, Evans DB, Kondo T, Yamada-Okabe H and Sasano H: Intratumoral localization of aromatase and interaction between stromal and parenchymal cells in the non-small cell lung carcinoma microenvironment. Cancer Res *70*: 6659-6669, 2010.
39 Salhab M, Reed MJ, Al Sarakbi W, Jiang WG and Mokbel K: The role of aromatase and 17-beta-hydroxysteroid dehydrogenase type 1 mRNA expression in predicting the clinical outcome of human breast cancer. Breast Cancer Res Treat *99*: 155-162, 2006.
40 Miyoshi Y, Ando A, Hasegawa S, Ishitobi M, Taguchi T, Tamaki Y and Noguchi S: High expression of steroid sulfatase mRNA predicts poor prognosis in patients with estrogen receptor-positive breast cancer. Clin Cancer Res *9*: 2288-2293, 2003.
41 Silva MC, Rowlands MG, Dowsett M, Gusterson B, McKinna JA, Fryatt I and Coombes RC: Intratumoral aromatase as a prognostic factor in human breast carcinoma. Cancer Res *49*: 2588-2591, 1989.

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