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Correlative Expression of Cyclooxygenase-1 (Cox-1) and Human Epidermal Growth Factor Receptor Type-2 (Her-2) in Endometrial Cancer

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Key words: Cyclooxygenase-1 (Cox-1); Human epidermal growth factor receptor type-2 (Her-2); Cyclooxygenase-2 (Cox-2); Vascular endothelial growth factor (VEGF); Endometrial cancer

Objectives: Cyclooxygenase-2 (Cox-2) is known to be associated with tumorigenesis in many cancers including endometrial cancer, while there is substantial evidence for the tumorigenicity of cyclooxygenase-1 (Cox-1). However, little is known about the involvement of Cox-1 in the development of endometrial cancer. The aim of this study was to determine whether cyclooxygenase-1 or -2 (Cox-1, Cox-2) is tumorigenic, as well as whether these two cyclooxygenase isoforms correlate with the clinicopathological characteristics or with another two biomarkers, human epidermal growth factor receptor type-2 (Her-2) and vascular endothelial growth factor (VEGF), of endometrial cancer.

Methods: At first, Cox-1 and Cox-2 levels in eight endometrial cancer cell lines were determined by means of real-time PCR. At second, the levels of four biomarkers (Cox-1, Cox-2, Her-2, and VEGF) in 70 endometrial cancer samples were determined by means of real-time PCR. Pairs of these biomarkers were subjected to correlation as each biomarker and clinical status or survival.

Results: In the eight cell lines, the expression of Cox-1 and Cox-2 showed major variations in their mRNA levels. Analysis of the patient samples showed that the mRNA expression of Cox-1 was elevated significantly in the G1 (P=0.021) and G2 (P=0.036) groups, as was the mRNA expression of Her-2 in the two groups (P=0.036 and P=0.0029, respectively). The mRNA expression of Cox-1 and Her-2 were correlated (CI=0.671). None of the three biomarkers, Cox-1, Cox-2, and Her-2, was correlated with clinical status such as FIGO classification, myometrial invasion, or clinical outcome.

Conclusion: Cox-1, together with Her-2, may be involved in the early stage of endometrial cancer development.

Endometrial cancer (endometrial adenocarcinoma) is the most common type of female genital cancer worldwide. Most endometrial neoplasias are diagnosed while they are still restricted to the uterus, although endometrial cancer may spread along the uterine cavity to the cervix, penetrate the uterine wall, or spread through the fallopian tubes. Once

disseminated, it is as lethal as ovarian cancer (9). Despite an increasing mortality rate, the mechanism underlying the pathogenesis of endometrial cancer remains to be clarified. Recent investigations have found that endometrial cancer is characterized by a multi-step progression from adenoma to carcinoma, and a putative pathogenetic model has been constructed which resembles the Vogelstein progression model for colorectal cancer (1,21,33). This model suggests that alterations of several genes may be related to the formation of this adenoma-carcinoma sequence (21).

Human epidermal growth factor receptor type-2 (Her-2) is overexpressed in many types of tumor such as breast, lung, and ovarian cancers, and evidence of overexpression of Her-2 in endometrial cancer is accumulating. In fact, Her-2 overexpression has been reported in 10-30 % of all endometrial cancers (21, 25), and is suspected to be strongly related to the tumorigenesis of type II endometrial cancer (9). As for the clinical aspect of endometrial cancer, several reports indicate that Her-2 overexpression correlates with clinical characteristics and survival (9,25,33). Although the exact role of Her-2 is not yet fully understood, and it remains a matter of debate whether Her-2 exerts its effect on tumorigenesis in the initial stage (21,25), or during tumor progression (9).

The role of cyclooxygenase (Cox) (also known as prostaglandin H synthetase) in cancer development has been the subject of close scrutiny recently. Cox is the rate-limiting enzyme that is responsible for the conversion of arachidonic acid into prostaglandins. Two Cox isoforms, cyclooxygenase-1 (Cox-1) and cyclooxygenase-2 (Cox-2), have been identified in human. Cox-1 has been identified as the constitutive form, and is expressed in many tissues for the regulation and maintenance of normal cellular function, while Cox-2 has been classified as the stimulating form, which is expressed via growth factors, cytokines, and tumor promoters (5). Especially, the tumorigenic effect of Cox-2 has been discussed in relation to many types of tumors including colorectal, gastric, lung, and ovarian cancers. Cox-2 is linked with tumorigenesis through, among others, promotion of angiogenesis, inhibition of apoptosis, promotion of cell proliferation (8,10). The involvement of Cox-1 in tumor growth has also been discussed in relation to several cancers, and is thought to be a cyclooxygenase expressed at constant levels, but several studies have shown that Cox-1 is expressed with major variations in some cancers (7,32). Within the limits of endometrial cancer, while Tong et al. (36) reported low expression levels of Cox-1, and few studies have dealt with Cox-1 and Cox-2 expression, the expression patterns of Cox-1 or Cox-2 are not yet fully understood. In addition, one study found that both Cox-1 and Cox-2 are expressed and regulated in the uterine endometrium, where estrogen and progesterone stimulate Cox-1 but not Cox-2 (4). Because the development of endometrial cancer is thought to affect the status of estrogen and/or progesterone, further evidence of the involvement of Cox-1 and Cox-2 in cancer development is required.

Vascular endothelial growth factor (VEGF) is a well-characterized tumorigenic molecule and is known to have the potentiality of tumor invasion. In vivo and in vitro studies have shown that Cox-2 expression is implicated in VEGF expression in many cancers in terms of angiogenesis (5,12). In a limited number of studies about the relationship between VEGF and Cox in gynecological malignancy, VEGF has been found to correlate with Cox-2 in endometrial cancer (12), and with Cox-1 in ovarian cancer (18). However, VEGF expression in endometrial cancer along with that of Cox-1 and Cox-2 has not been adequately analyzed.

Against this background, this study was performed to determine whether Cox-1 and/or Cox-2 is involved in endometrial cancer development, and whether either of these two Cox

COX-1 AND HER-2 IN ENDOMETRIAL CANCER

isoforms correlates with the clinicopathological characteristics or with another two biomarkers, i.e. Her-2 or VEGF, of endometrial cancer.

MATERIALS AND METHODS

Patients

This study used 70 samples of primary untreated endometrial cancer (endometrial adenocarcinoma) patients admitted and treated at the Gynecology and Oncology Division, Hyogo Medical Center for Adults, between January 1998 and October 2003. All research was conducted with informed consent. The mean age of the patients was 57 years (range, 38-79). Staging of endometrial cancer was done according to the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system (1988) (15), which showed that 51 patients (72.9 %) had stage I - II, and 19 (27.1 %) stage III-IV disease. For histological classification, the World Health Organization (WHO) criteria were used, resulting in 26 patients (37.1 %) classified as well-differentiated, 34 (48.6 %) as moderately differentiated, and 10 (14.3 %) as poorly undifferentiated. Other clinicopathologic characteristics are shown in Table 1.

Table.1 Clinicopathological characteristics and biomarker status in endometrial cancer patients

	Cox-1			Cox-2			Her-2			VEGF		
	low	high	p	low	high	p	low	high	p	low	high	p
	population	(<med.)	(≥ med.)	(<med.)	(≥ med.)	p	(<med.)	(≥ med.)	p	(<med.)	(≥ med.)	p
Age												
< 60	18	10	8	8	10	0.58	11	7	0.27	8	10	0.58
≥ 60	52	25	27	27	25		24	28		27	25	
WHO grade												
G1	26	13	13	9	17	0.36	13	13	0.77	14	12	0.76
G2	34	15	19	19	15		16	18		17	17	
G3	10	7	3	7	3		6	4		4	6	
FIGO												
I - II	51	27	24	26	25	0.42	26	25	0.79	27	24	0.42
III-IV	19	8	11	9	10		9	10		8	11	
Myometrial invasion												
No	20	9	11	5	15	0.60	7	13	0.11	8	10	0.58
Yes	50	26	24	30	20		28	22		27	25	
Menopausal status												
Premenopausal	19	8	11	8	11	0.42	7	12	0.18	12	7	0.18
Postmenopausal	51	27	24	27	24		28	23		23	28	
Total	70	35	35	35	35		35	35		35	35	

χ^2 test, med.: median

Patients were treated with total abdominal or modified radical hysterectomy plus bilateral salpingo-oophorectomy. Fluid samples for cytological examination were obtained during laparoscopy. Postoperative adjuvant therapy including irradiation or systemic chemotherapy was administered to patients carrying at least one of the following high risk factors: Grade 3 disease, deep myometrial invasion, cervical involvement, para-aortic lymph node metastasis, and a positive result for cytological fluid examination. The median follow-up of surviving patients was 40.5 months. Seven patients died, and ten suffered relapse. For control, 15 samples were obtained at the same institute from normal subjects, consisting of five in the proliferative phase, nine in the secretory phase, and one postmenopausal subject.

Cell Lines and Culture

Endometrial cancer cell lines, SNG-II (JCRB 0175) and SNG-M (JCRB 0179) were obtained from the JCRB Cell Bank (Japan), HHUA (RCB 0658), HOUA-1 (RCB 0659), JHUEM-1 (RCB 1548), and JHUEM-2 (RCB 1551) from the Riken Cell Bank (Japan), and RL95-2 (ATCC CRL 1671) was obtained from the American Type Culture Collection

(Rockville, MD, USA). Ishikawa cells (Ishikawa 3-H-12 No.86) were a gift from Dr. Nishida (Kasumigaura Medical Center, Ibaragi, Japan), and were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco-BRL, Grand island, NY, USA) supplemented with 10 % heat complement-inactivated fetal bovine serum (FBS). SNG-II and SNG-M cells were grown in Ham's F12 medium (Gibco-BRL) with 20 % FBS. HHUA and HOUA-1 cells were grown in Ham's F12 medium with 15 % FBS, and JHUEM-1, JHUEM-2, and RL95-2 cells in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 (DMEM/F12) with 10 % FBS. All media contained 100 IU/ml penicillin (Sigma, St. Louis, MO, USA) and 100 µg/ml streptomycin (Sigma). All cell lines were kept as monolayers in an atmosphere of 95 % O₂, 5 % CO₂, at 37 °C, and collected at approximately 80 % confluence by two washes with phosphate-buffered saline (PBS) before being used for the experiments.

Quantitative mRNA analysis

Total RNA was extracted with the aid of TRIzol (Life Technologies, Gaithersburg, MD, USA) from the frozen tissues of the patient samples (n=70), normal endometrium specimens (n=15), and endometrial cancer cell lines (n=9). Each 1 µg of total RNA was then reverse transcribed using MgCl₂ (5 mM), dNTPs (1 mM each), oligo dT adaptor primer (0.125 M), RNAase inhibitor (1 U/µl) and reverse transcriptase (0.25 U/µl) (all from the RNA PCR Kit; Takara, Shiga, Japan) according to the manufacturer's protocol. An aliquot of 1/20th of the resulting cDNA was then used for quantitative real-time PCR amplification.

The Cox-1, Cox-2, Her-2, and VEGF sequences of primers and probes for real-time PCR were designed with Primer Express software (Perkin-Elmer, Foster City, CA, USA). The following sequences of primers and probes were used. For Cox-1: forward, 5'- AGC AGC TTT TCC AGA CGA CC -3'; reverse, 5'- CGG TTG CGG TAT TGG AAC TG -3'; probe (FAM labeled), 5'- CGT GCA GCA GCT GAG TGG CTA TTT CC -3'. For Cox-2: forward, 5'- CCA GCA CTT CAC GCA TCA GT -3'; reverse, 5'- ACG CTG TCT AGC CAG AGT TTC AC -3'; Probe (FAM labeled), 5'- GGC TGG GCC ATG GGG TGG ACT TAA AT -3'. For Her-2, the forward primers and probe were: forward, 5'- GTA TAC ATT CGG CGC CAG CT -3'; reverse, 5'- GCA GAC GAG GGT GCA GGA T -3'; probe (FAM labeled), 5'- CTG CCT GTC CCT ACA ACT ACC TTT CTA CGG A -3'. For VEGF, the forward primers and probe were: forward, 5'- GCA GAC CAA AGA AAG ATA GAG CAA G -3'; Reverse, 5'- CGC CTC GGC TTG TCA CAT -3'; Probe (FAM labeled), 5'- AGA AAA TCC CTG TGG GCC TTG CTC -3'.

Standard curves of Cox-1 and Cox-2 mRNA expression were constructed from original or 2-fold diluted c-DNA samples of Ishikawa cells. Standard expressions of Her-2 and VEGF were constructed from original or 2-fold diluted c-DNA samples of JHUEM-1 cells. The expression level of each original sample (copy numbers per 1 µg of total RNA) was defined as 1. The standard curve of Glyceraldehyde Phosphate Dehydrogenase (GAPDH) consisted of 10-fold diluted c-DNA of the GAPDH cloning vector.

Reactions were performed with a qPCR Master Mix (Eurogentec, Seraing, Belgium) and with the aid of the ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA) using the following thermal cycling conditions: 50 °C for 2 min and 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 sec and 60 °C for 1 min. All expression data were normalized using GAPDH expression as the internal standard, and presented in relation to that standard.

Statistical analysis

SPSS Inc. software (Version 11.0) was used for all of the statistical analyses. Comparisons between the normalized mRNA levels were made by means of Student's t-test (parametric data) or Welch's t-test (nonparametric data). The χ^2 test was used for comparison

COX-1 AND HER-2 IN ENDOMETRIAL CANCER

of categorical data. The correlation analysis of mRNA quantitative expression by each of the biomarkers was performed with Spearman's correlation coefficient test (R_s). Overall survival (OS) and relapse free survival (RFS) were analyzed with the aid of the Kaplan-Meier method, and comparison between curves was performed with the log rank test.

RESULTS

mRNA expression levels of Cox-1 and Cox-2 in endometrial carcinoma cell lines

mRNA expression analysis of two Cox isoforms in eight endometrial cancer cell lines was performed by using real-time quantitative PCR analysis (Fig. 1).

Cox-1 mRNA expression was detected within 40 cycles in all eight cell lines, indicating that Cox-1 expression is recognized in all cell lines. However, Cox-1 expression showed major variations among these cell lines. The descending order of magnitude of mRNA expression levels for Cox-1 was SNG-M > JHUEM-1 > HOUA-1 > Ishikawa > HHUA > SNG-II > JHUEM-2 > RL95-2.

On the other hand, Cox-2 mRNA expression was detected within 40 cycles in only five cell lines (Ishikawa, RL95, SNG-M, SNG-II, JHUEM-1), and between 40 and 45 cycles in the remaining three cell lines (HHUA, HOUA-1, JHUEM-2). The descending order of magnitude of mRNA expression levels for Cox-2 was Ishikawa > RL95-2 > SNG-M > SNG-II > JHUEM-2 > JHUEM-1 > HOUA-1 > HHUA.

These results suggest that Cox-1 and Cox-2 mRNA expression both vary in different endometrial carcinoma cell lines.

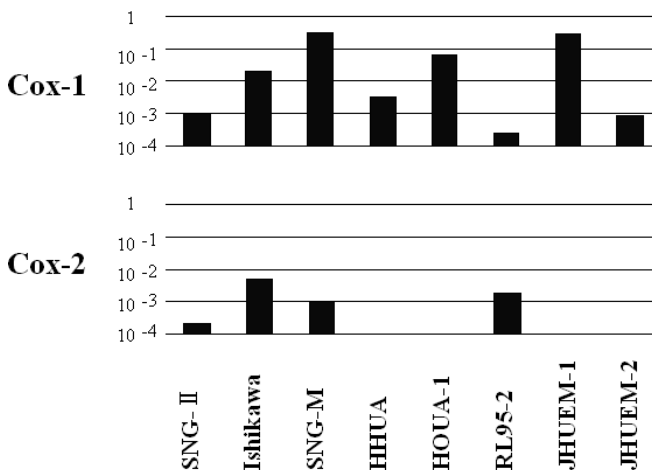


Fig. 1: Quantitative mRNA expression levels of Cox-1 and Cox-2 in eight endometrial cancer cell lines. The Cox-1 or Cox-2 data obtained from real-time PCR analysis was normalized by GAPDH. (Note: Cox-2 expression levels below 10⁻⁴ are not shown in this bar graph.)

Analysis of mRNA expression levels of four biomarkers in endometrial cancer patients

Seventy endometrial carcinoma patient samples were subjected to mRNA quantitative expression analysis. The distributions of the mRNA expression levels of four biomarkers (Cox-1, Cox-2, Her-2, and VEGF) normalized by GAPDH are shown in Fig.2. The median values of Cox-1 expression levels for each of the G1-G3 groups and the control group were 0.017 (range; 0.0015-0.79), 0.018 (0.0018-1.0), 0.0068 (0.0016-0.032), and 0.0062 (0.00010-0.079), respectively. Significant elevation of Cox-1 in cancer samples compared to that in normal samples was found in the G1 ($P=0.021$) and G2 ($P=0.036$) groups.

The median values of Cox-2 expression level for each of the G1-G3 groups and the control group were 0.0032 (0.0033-0.021), 0.0017 (0.00028-0.085), 0.0014 (0.00012-0.0069),

0.0030 (0.00026-0.015), respectively. No significant differences from normal samples were observed in any group.

The respective median values of the Her-2 expression levels for each of the G1-G3 groups and the control group were 0.033 (0.0046-6.4), 0.036 (0.00083-5.5), 0.0026 (0.00060-0.44), 0.0075 (0.00012-0.22). Significant elevation of Her-2 in cancer samples comparing to that in normal samples was detected in the G1 ($P=0.036$) and G2 ($P=0.0029$) groups.

VEGF expression was below detectable levels in four samples (all four samples were in group G2). The data of these four samples was excluded from the analysis due to the difficulty of quantitative assessment. The respective median values of VEGF expression levels for each of the G1-G3 group and the control group were 0.15 (0.029-4.4), 0.17 (0.024-5.6), 0.22 (0.084-1.4), 0.084 (0.00026-0.015). No significant differences from normal samples were observed in any group.

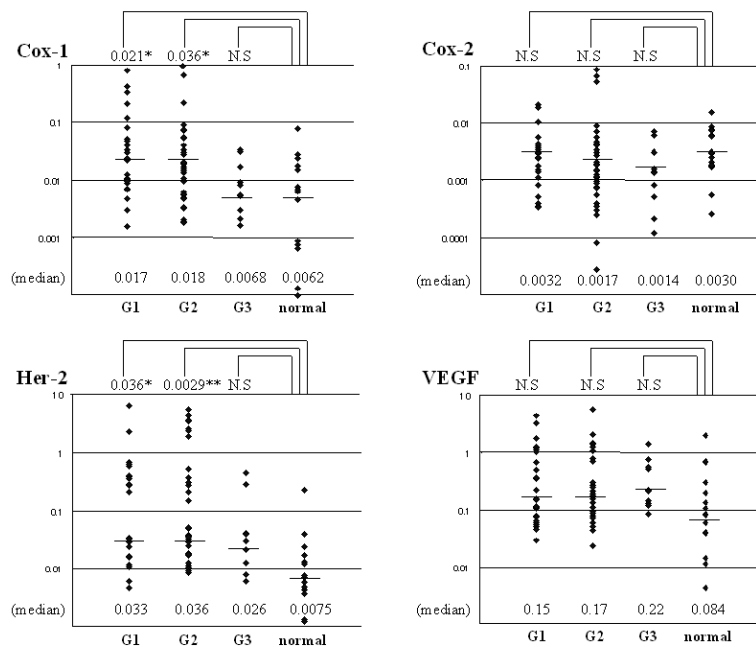


Fig. 2: Distribution of quantitative mRNA levels in Cox-1, Cox-2, Her-2, and VEGF. Horizontal axis: G1-G3 (WHO classification) and normal samples. Vertical axis: relative expression level normalized by GAPDH expression on a log10 scale. The median values of each population are shown in the graphs. Relationships between mRNA expression levels of each of the WHO groups and the normal group were assessed Student's t-test or Welch's t-test (*: 5% significance; **: 1% significance; N.S.: not significant).

Correlation between two Cox isoforms and other biomarkers in endometrial carcinoma patients

We studied the relationship between mRNA expression levels of each of the two Cox isoforms and that of Her-2 or VEGF in endometrial carcinoma patients (Fig. 3). Spearman's correlation coefficients (R_s) were 0.671 (Cox-1 and Her-2), 0.361 (Cox-1 and VEGF), 0.364

COX-1 AND HER-2 IN ENDOMETRIAL CANCER

(Cox-2 and Her-2), and 0.385 (Cox-2 and VEGF). These findings demonstrate that Cox-1 and Her-2 correlated significantly in terms of mRNA expression.

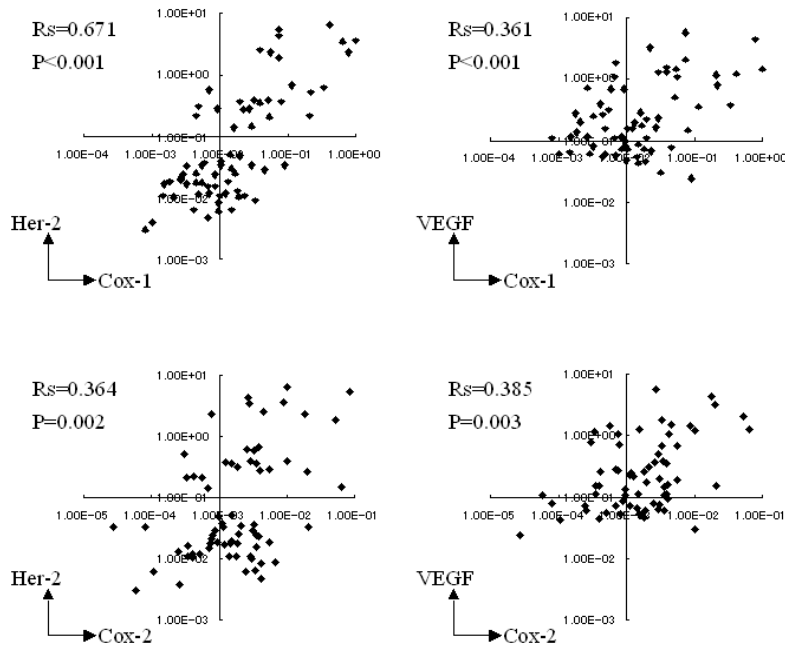


Fig. 3: Correlation of mRNA expression of each of the Cox isoforms (Cox-1, Cox-2) and Her-2 or VEGF was determined by Spearman's correlation coefficient test (R_s). Strong correlation was demonstrated between Cox-1 and Her-2 ($R_s=0.671$, $P<0.001$).

Relationship between each of the four biomarkers and clinicopathological characteristics

The study population was divided into two groups in accordance with the expression levels (lower or higher) of the four biomarkers. The statistical χ^2 test demonstrated that none of the biomarkers showed a significant relationship with age, WHO grading, FIGO staging, menopausal status, or myometrial invasion (Table 1).

Furthermore, to investigate the relationship between the biomarkers and prognosis, the Kaplan-Meier analysis was used to investigate the two groups described above, and the log rank method was used to calculate significance. No significant correlation for disease-free survival (DFS) was found among Cox-1 (log rank test; 0.63), Cox-2 (0.18), Her-2 (0.57), and VEGF (0.72), nor (any correlation) for overall survival (OS): Cox-1 (0.37), Cox-2 (0.67), Her-2 (0.32), and VEGF (0.43) (data not shown).

DISCUSSION

In terms of the role of cyclooxygenase (Cox) in cancer development, cyclooxygenase-2 (Cox-2) is known to be tumorigenetically involved in a wide range of tumors, including colorectal, non-small cell lung, gastric, breast, pancreas, cervical, prostate, and bladder cancers (27,34). Prostaglandin, which is the down-stream product of the cyclooxygenase pathway, is thought to contribute to the inhibition of apoptosis and immune function, as well

as the promotion of angiogenesis, invasion and metastasis (20,23). As in other cancers, Cox-2 expression is upregulated in endometrial cancer *in vitro* (17), and localized in both neoplastic epithelial and endothelial cells (30). In clinical terms, there are substantial evidences that Cox-2 is upregulated in endometrial cancer samples (10,12,36). Furthermore, a clinical study found that up-regulated Cox-2 correlates with FIGO classification, or is associated with extra-uterine spread, high-grade, deep invasion and shorter disease-free survival (10).

Cox-1, on the other hand, is often referred to as a housekeeping enzyme because it is constitutively expressed throughout the body in almost all tissues and performs an important function in mediating various normal physiological processes (27). The induction of Cox-1, however, has also been reported, although it is not as extensive as Cox-2 and not as widely observed (8). As for the tumorigenesis of Cox-1, several reports mention that its effects are beyond those of a mere housekeeping gene (2,13,22,24,26). In the case of gynecological cancer, some studies have provided evidence that Cox-1 is up-regulated and plays an important role in cervical (18,31) or in ovarian cancer (22,26). Roland *et al.* (29) reported that Cox-1 was expressed more frequently than Cox-2 in ovarian cell lines. More recently, Kino *et al.* (19) and Daikoku *et al.* (6) showed that up-regulation of Cox-1 frequently occurs in ovarian cells, and suggested that Cox-1 is the major contributor to the production of prostaglandins in ovarian cancer cells. As for endometrial carcinoma, however, only a few studies have dealt with Cox-1 expression, and one of these reported that the Cox-1 level was unaltered (36). The lack of data about the significance of Cox-1 for the tumorigenesis of endometrial cancer has been the main impetus for this study.

Our investigation showed that Cox-1 is expressed with a wide variety of levels and up-regulated significantly in endometrial cancer at the mRNA and protein levels. The variety of Cox-1 expression among cell lines indicates that regulation of Cox-1 may cover a wide range, resulting in far from uniform patterns. In addition, Cox-1 expression levels in patient samples were found to be significantly up-regulated compared to mRNA levels. On the other hand, the level of Cox-2 expression also differs among cell lines, although no Cox-2 mRNA elevation was found in our patient samples. These results indicate that the up-regulation of Cox-1 rather than of Cox-2 may have an important role in tumor development in endometrial cancer. Our study is thus the first study to provide evidence of the up-regulation of Cox-1 in endometrial cancer. Although the regulation of Cox-1 may seem somewhat surprising, our results are compatible with those of recent studies that indicate the regulation of Cox-1 in ovarian cancer (13,29). Sales & Jabbour (30) demonstrated the autocrine-paracrine-intracrine regulation of Cox enzyme expression in epithelial and endothelial cells. Positive feedback circulation, constructed from prostaglandin (PG), c-AMP, inositol triphosphate (IP₃), mitogen-associated protein kinase (MAPK), and phosphatidyl inositol 3 kinase-protein kinase B (PI3K/Akt), may promote Cox-1 or Cox-2 expression, and may thus result in tumor promotion. In our study, no correlation between Cox-1 and Cox-2 expression was observed. In view of these results, we speculate that both Cox-1 and Cox-2 may each be capable of constructing this positive feedback circulation for tumorigenesis in endometrial cancer. In addition, Dore *et al.* (8) demonstrated that these two Cox isoforms could play distinct roles in an organism because the development of Cox-1- or Cox-2-deficient mice by means of target gene disruption resulted in different phenotype changes. The significance of the role of Cox-1 in endometrial cancer therefore warrants further study.

Human epidermal growth factor-2 (Her-2) was up-regulated significantly in this study, which is compatible with previously reported results for endometrial cancer (21,25). The role of the Her-2 elevation is still controversial, that is, it is not clear whether the up-regulation of

Her-2 is involved in the early stage of tumorigenesis (21,25) or in tumor progression (9). The correlation coefficient analysis of our study disclosed that Cox-1 correlates more closely with Her-2 than does Cox-2. Several reported findings suggest that Cox-2 correlates with Her-2 in cancers such as breast cancer (3,6,19,29). As for endometrial cancer, however, there are no reports of a positive relationship between one of the Cox isoforms and Her-2. Interestingly, though, Ferrandina et al. (11) reported that Cox-2 did not correlate with Her-2 in 90 primary untreated endometrial cancer patients, which is consistent with our finding of a stronger relationship between Cox-1 and Her-2 than between Cox-2 and Her-2.

Neither Cox-1 nor Her-2 showed any correlation with clinicopathological status or survival. These results lead us to speculate that the elevation of both Cox-1 and Her-2 may occur in the early stage. Several studies support the hypothesis that Cox-1 contributes to the early stage of cancer development. Kim et al. (18) demonstrated that the up-regulation of Cox-1 is not associated with the clinicopathologic features of cervical cancer. Takada et al. (35) reported a significant contribution of Cox-1 to the early stage of tumor development because the prostaglandin of early stage polyps is supplied only by way of the Cox-1 pathway, while Cox-2 is essential for the later development of intestinal polyposis. In addition, Li et al. (22) suggested that Cox-1 may be involved in the initiation of ovarian cancer because inclusion cysts, which are thought to be the precursor lesions of ovarian cancer, show a high expression of Cox-1. On the other hand, Roland et al. (29) suggest that Cox-2 contributes to tumor initiation, and that either Cox-1 or Cox-2 contributes to tumor progression. Further study is required whether Cox-1 has the early tumorigenesis or not.

No significant up-regulation of VEGF was found in this study. It should be taken into consideration that the precise VEGF status in endometrial cancer was difficult to assess in our study because the patients tended to be in the early FIGO stage. However, VEGF showed a tendency to correlate with Cox-1 ($R_s=0.361$) as well as with Cox-2 ($R_s=0.385$), indicating that both Cox-1 and Cox-2 may have an effect on VEGF in endometrial cancer. Previous studies of gynecological malignancy showed that VEGF tended to correlate with Cox-2 in endometrial cancer (12), or to correlate with Cox-1 in ovarian cancer (18). Since our findings show a tendency of Cox-1 and VEGF to correlate, the relationship between Cox-1 and angiogenesis should be investigated further.

In conclusion, the present results reported here suggest that Cox-1, together with Her-2, may be involved in the early stage of endometrial cancer development.

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