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Prognostic significance of CUB domain containing protein expression in endometrioid adenocarcinoma

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Abstract. CDCP1, a transmembrane protein with intracellular tyrosine residues which are phosphorylated upon activation, is supposed to be engaged in proliferative activities and resistance to apoptosis of cancer cells. High level of CDCP1 expression proved to be a poor prognosticator for lung adenocarcinoma. Here, expression level of CDCP1 was immunohistochemically examined in 110 cases (median age of 54.7 years) of endometrioid adenocarcinoma, and its clinical implications were evaluated. Tumor stage was stage I in 71 cases (64.5%), II in 5 (4.5%), III in 28 (25.5%), and IV in 6 (5.5%). Staining intensity of tumor cells was divided into two categories; tumor cells with no to weak and moderate to strong membrane staining. The intensity of CDCP1 expression in each case was defined by the staining of major population of cells as follows; cases with tumor cells showing no to weak and moderate to high membrane staining were categorized as CDCP1-low and CDCP1-high, respectively. Eighty-seven of 110 cases were categorized as CDCP1-high, and the remaining as CDCP1-low. Significant positive correlation was observed between low CDCP1 expression and stage (p=0.0091), relapse rate (p=0.0017), and poor prognosis (p=0.0009). Multivariate analysis revealed that low CDCP1 and advanced stage were independent poor prognostic factors for both OS and DFS. As compared to cancer cells, normal endometrium continuously expressed CDCP1. These suggested that the attitude of CDCP1 in cancers of lung and endometrium was different. Different role of CDCP1 by tissues and cancers is discussed.

Introduction

CDCP1 is originally identified as an epithelial tumor antigen expressed in the lung cancer cell lines but not in normal lung tissues (1). CDCP1 is a transmembrane protein with three extracellular CUB domains, which are important for cell-cell interactions, and intracellular tyrosine residues, which are phosphorylated upon activation (1-4). Physiologically, CDCP1 expression is limited to stem or progenitor cells in the hematopoietic and mesenchymal tissues, and no significant expression has been reported in developed tissues (5,6). CDCP1 protects cells from anoikis, a form of apoptosis triggered by loss of cell survival signals generated from interaction of cells with extracellular matrix (7).

We previously reported the epigenetic regulation of CDCP1 expression in the cell lines derived from various malignancies, such as leukemia and colon adenocarcinoma, and in the clinical samples of breast cancer (8,9). CDCP1 expression level is correlated with proliferative activities of tumor cells in breast cancer (8). Uekita *et al* reported that the knocked-down expression of CDCP1 in lung adenocarcinoma cell line by RNA interference abolishes *in vitro* colony formation and *in vivo* metastatic abilities (7). Among cases with lung adenocarcinoma, high CDCP1 expression proved to be an independent factor for poor prognosis of patients (10). Recent study revealed CDCP1 expression to be an independent negative prognostic factor for renal cell carcinoma (11). These findings indicate that CDCP1 plays an important role in tumorigenesis and metastasis, and that the high level of CDCP1 expression suggests aggressiveness of malignancies.

Endometrioid adenocarcinoma is the most common invasive malignancy of the female genital system (12,13). Despite the advances in the methods for detection and treatment, prognosis of patients with endometrioid adenocarcinoma still remains unfavorable. In the present study, CDCP1 expression was immunohistochemically examined in clinical samples with endometrioid adenocarcinoma, and its clinical implications were evaluated.

Materials and methods

Patients and methods. One hundred and ten patients who underwent surgery for endometrioid adenocarcinoma at Osaka University Hospital during the period from January 1998 to January 2007 were examined. Clinicopathological findings in

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Table I. Summary of characteristics in 110 endometrioid adenocarcinoma patients.

	No. of patients
Tumor	
T1	79
T2	10
T3	20
Stage	
I	71
II	5
III	28
IV	6
Tumor histological grade	
1	44
2	43
3	23
Menopausal status	
Premenopausal	
Postmenopausal	
Estrogen receptor status	
Positive	75
Negative	35
Progesterone receptor status	
Positive	66
Negative	44
Recurrence	
Positive	22
Negative	87
Prognosis	
Deceased	15
Alive (with recurrence)	8
Alive (with no recurrence)	87

these 110 patients are summarized in Table I. There were 110 women with ages ranging from 22 to 75 (median, 54.7 years). Resected specimens were macroscopically examined to determine the location and size of the tumors. Normal (3 cases of proliferative and 4 of secretory phases), atrophic (3 cases), and hyperplastic endometrium (6 cases), together with endometrial polyp (2 cases), obtained from patients with functional bleeding, were included as control tissues. Histologic specimens were fixed in 10% formalin and routinely processed for paraffin-embedding. Paraffin-embedded specimens were stored in the dark room in the Department of Pathology of Osaka University Hospital at room temperature, sectioned at 4 μm thickness at the time of staining, and stained with hematoxylin and eosin and immunoperoxidase procedure. The histological stage was determined according to the International Federation of Obstetricians and Gynecologists (FIGO) staging system (14). All patients were followed up with laboratory examinations including routine peripheral blood cell counts at 1-6-month intervals, roentgenogram, computed tomographic scan and pelvic examination at 6-12-month intervals. The follow-up period for survivors ranged from 7 to 122 (median, 82) months. The study was approved by the ethics review board of Graduate School of Medicine, Osaka University.

Immunohistochemistry for CDCP1, estrogen receptor (ER), progesterone receptor (PgR) and MIB-1. CDCP1 expression was immunohistochemically examined with use of anti-CDCP1 antibody (Abcam Ltd., Cambridge, UK). The proliferative activity of cancer cells was examined with monoclonal antibody MIB-1 (Immunotech, Marseilles, France), recognizing the proliferation-associated antigen Ki-67. Expression status of ER and PgR was estimated with anti-ER (Dako) and anti-PgR (Dako) antibodies, respectively. After antigen retrieval with Pascal pressurised heating chamber (Dako A/S, Glostrup, Denmark), the sections were incubated with anti-CDCP1, anti-ER, anti-PgR antibody and MIB-1, diluted at x200, x2, x6 and x100, respectively. Then, the sections were treated with biotin-conjugated anti-goat IgG (Zymed, South San Francisco, CA, USA) for CDCP1 staining, or with biotin-conjugated anti-mouse IgG (Dako A/S) for ER, PgR and MIB-1 staining. After washing, the sections were incubated with the peroxidase-conjugated biotin-avidin complex (Vectastain ABC kit, Vector, Burlingame, CA). DAB (Vector) was used as a chromogen. As the negative control, staining was carried out in the absence of primary antibody. Stained sections were evaluated independently by two pathologists (J.I. and E.M.) based on the manner described previously (10). Briefly, staining intensity of tumor cells was divided into two categories; tumor cells with no to faint and moderate to strong membrane staining. The intensity of CDCP1 expression in each case was defined by the staining of major population of cells as follows; cases with tumor cells showing no to faint staining were categorized as CDCP1-low, and those showing moderate to strong membrane staining as CDCP1-high. Proportion and intensity of ER and PgR expression were evaluated as described by Allred *et al* (15). The MIB-1 labeling index was defined as the percentage of stained nuclei per 1000 cells. The cases were divided into MIB-1-high and MIB-1-low groups using the median as cut-off value.

RNA extraction and quantification of CDCP1 mRNA level by real-time reverse transcription (RT)-PCR. RNA was extracted from paraffin sections using an RNeasy FFPE kit (Qiagen, Valencia, CA). Total RNA was subjected to reverse transcription by Superscript III (Invitrogen, Carlsbad, CA), and the single strand cDNA was obtained. The mRNA levels for CDCP1 and GAPDH genes were verified using TaqMan gene expression assays (Applied Biosystems, Foster City, CA) as recommended by the manufacturer. The amount of CDCP1 mRNA was normalized to that of GAPDH mRNA, and the normalized value was shown as relative mRNA amount.

Statistical analysis. Statistical analyses were performed using StatView software (SAS Institute Inc., Cary, NC). The χ^2 and Fisher's exact probability test were used to analyze the

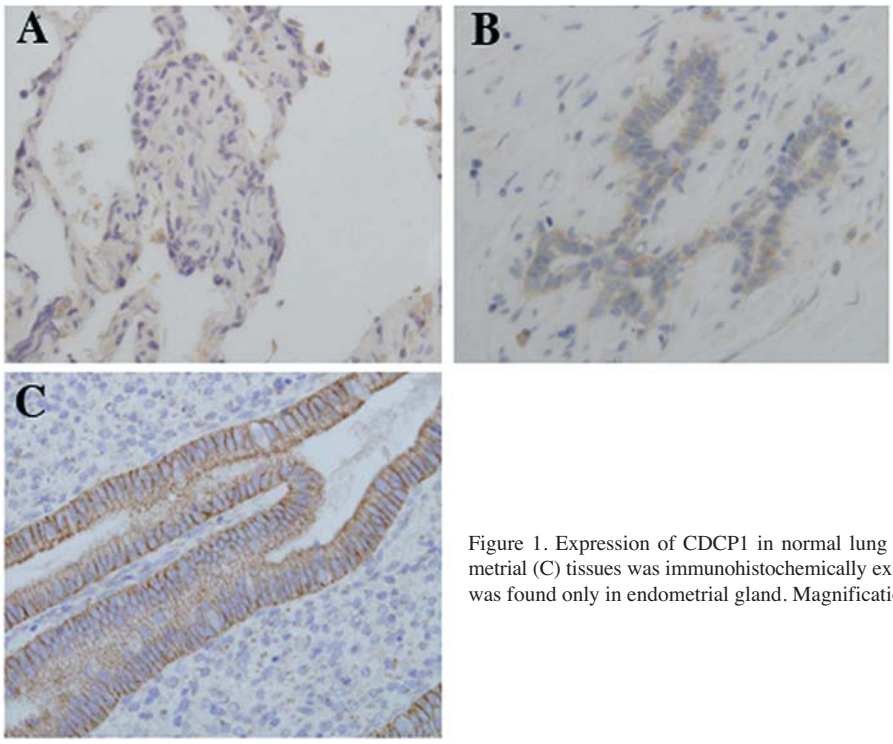

Figure 1. Expression of CDCP1 in normal lung (A), breast (B), and endometrial (C) tissues was immunohistochemically examined. CDCP1 expression was found only in endometrial gland. Magnification, x400.

Table II. Correlation between CDCP1 expression and MIB-1 index in normal and non-cancerous endometrium.

	CDCP1 expression		
	Low	High	P-value
MIB-1 labeling index			
≥20	4	12	
<20%	2	0	0.0339

correlation between CDCP1 expression and clinicopathological factors in endometrioid adenocarcinoma. Kaplan-Meier methods were used to calculate overall survival (OS) and disease-free survival (DFS) rate, and differences in survival curves were evaluated with the log-rank test. Cox's proportional hazards regression model with a stepwise manner was used to analyze the independent prognostic factors. The p-values <0.05 were considered to be statistically significant.

Results

Immunohistochemical findings. Our previous study on lung and breast carcinomas revealed that CDCP1 expression was hardly detected in adjacent normal tissues (Fig. 1A and B) (9). Whereas high level of CDCP1 expression was found not only in endometrioid adenocarcinoma cells but also in adjacent normal endometrium (Fig. 1C). Then, CDCP1 expression was examined in normal (proliferative and secretory phases, Fig. 2A and B), atrophic (Fig. 2C), and hyperplastic endometrium (Fig. 2D), together with endometrial polyp (Fig. 2E). Strong CDCP1 expression was detected in all examined endometrial tissues, although the expression level was slightly lower in atrophic and secretory phase endometrium. The MIB-1 labeling index correlated with the CDCP1 expression level in the non-neoplastic endometrial glands (Table II).

Next, the expression of CDCP1 was examined in 110 endometrioid adenocarcinoma tissues. Staining intensity of tumor cells was divided into two categories; tumor cells with no to faint, and moderate to strong, membrane staining (Fig. 3). Eighty-seven (79.1%) of 110 cases were categorized as CDCP1-high, and the remaining as CDCP1-low.

Correlation of intensity in immunohistochemical staining with level of CDCP1 mRNA expression. RNAs were extracted from the endometrioid adenocarcinomas immunohistochemically showing CDCP1-high and CDCP1-low expression, and normal endometium (each three cases). Level of CDCP1 mRNA expression was higher in CDCP1-high adenocarcinoma and normal endometrium than in CDCP1-low cases (Fig. 4). Level of CDCP1 expression in CDCP1-high adenocarcinoma was comparable to that of normal endometrium.

Correlation of CDCP1 expression with clinical variables. Correlation of CDCP1 expression with clinicopathological features was evaluated. Significant positive correlation was observed between CDCP1-low expression and relapse rate (p=0.0017), stage (p=0.0091), poor prognosis (p=0.0009), and tumor grade (p=0.0108). Other parameters including tumor size, Ki-67 index, ER and PgR did not correlate with CDCP1 expression (Table III). Tumor stage in the present patients was stage I in 71 cases (64.5%), II in 5 (4.5%), III in 28 (25.5%), and IV in 6 cases (5.5%). The 5-year DFS and OS were 86.7%

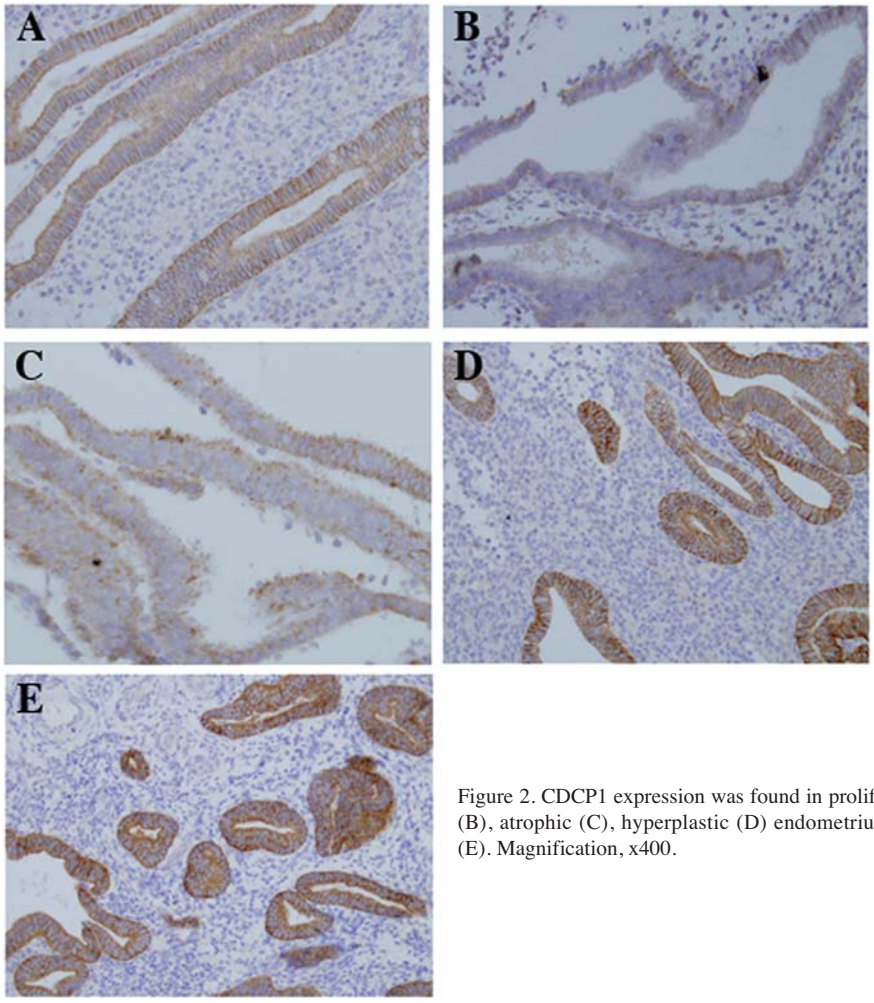

Figure 2. CDCP1 expression was found in proliferative (A), secretory phase (B), atrophic (C), hyperplastic (D) endometrium, and endometrial polyps (E). Magnification, x400.

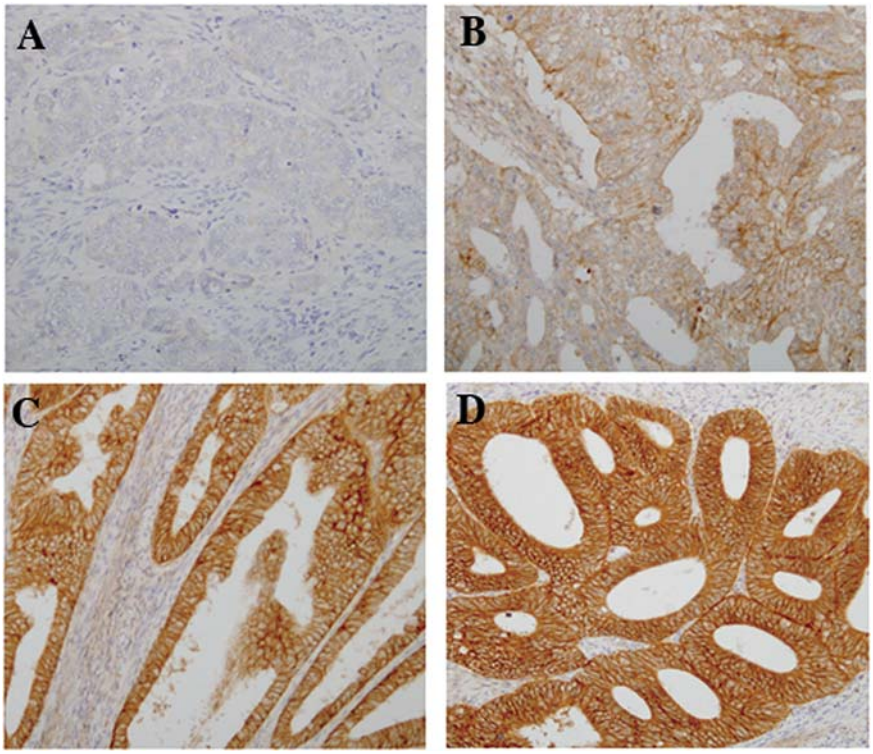

Figure 3. Staining of CDCP1 in endometrioid adenocarcinoma. Representative fields of CDCP1-low (A and B) and -high (C and D) cases are shown. Magnification, x400.

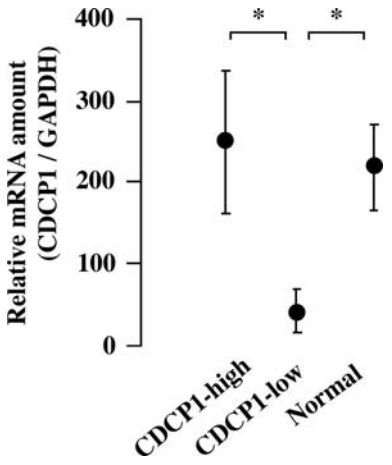

Figure 4. Correlation of CDCP1 expression at mRNA and protein level. RNAs were obtained from CDCP1-high and CDCP1-low endometrioid adenocarcinoma, and normal endometrium. The values represent the mean ± SE of three cases. *p<0.05 by the Student's t-test.

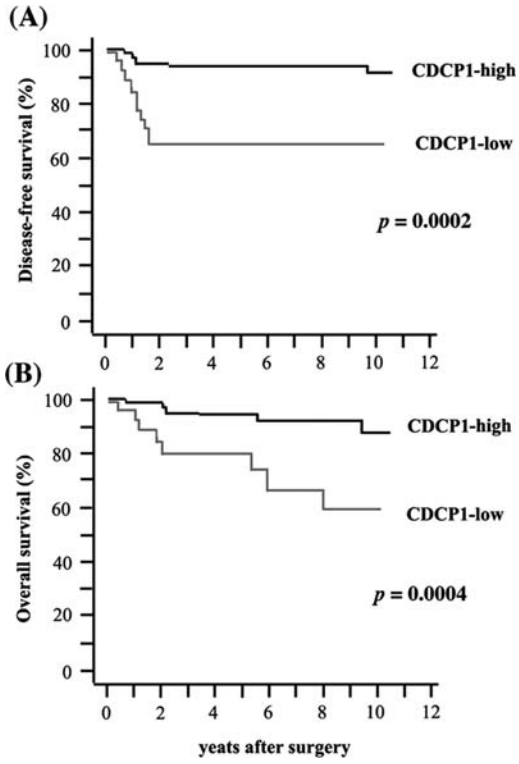

Figure 5. Kaplan-Meier plots. Survival curves of disease-free (A) and overall (B) are shown. CDCP1-low cases showed less favorable disease-free and overall survival.

and 90.6%, respectively. Tumors recurred in 22 patients. Of these, 15 patients died of their tumors. There was a statistically significant difference in DFS rates (p=0.0002) and OS rates (p=0.0004) between patients with CDCP1-high and CDCP1-low tumors (Fig. 5).

Univariate analysis showed that stage, tumor grade, and CDCP1 expression were significant factors for both OS and DFS (Table IV). The multivariate analysis revealed that CDCP1 expression and stage were independent prognostic factors for both OS and DFS (Table IV); the patients with CDCP1-low expression showed a less favorable prognosis than those with the high expression.

Table III. Correlation between CDCP1 expression and clinicopathological parameters.

	CDCP1 expression		
	Low	High	P-value
Tumor			
T1	13	66	
T2	4	6	
T3	6	14	0.0718
Stage			
I	10	61	
II	0	5	
III	12	16	
IV	1	5	0.0091
Tumor histological grade			
1	6	38	
2	7	36	
3	10	13	0.0108
Estrogen receptor status			
Positive	16	64	
Negative	8	23	0.5375
Progesterone receptor status			
Positive	15	64	
Negative	8	23	0.4288
MIB-1 labeling index			
≥20	9	37	
<20%	13	50	0.8907
Recurrence			
Positive	10	12	
Negative	13	74	0.0017
Prognosis			
Deceased	8	7	
Alive (with recurrence)	2	6	
Alive (with no recurrence)	13	74	0.0032

Discussion

The characteristics of patients, such as age and stage, in the current study were similar to those in a previous report, indicating that the results obtained from the current study are commonly applicable to endometrioid adenocarcinoma worldwide (16). Our previous data on lung adenocarcinoma showed that the high level of CDCP1 expression in the tumor cells was a factor of poor prognosis (10). As in the cases of lung adenocarcinoma, CDCP1-high expression is a negative prognosticator in breast and renal cancers (8,11). In the

Table IV. Univariate and multivariate analyses of prognostic factors for overall and disease-free survivals.

	Overall survival				Disease-free survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Tumor grade	3.32 (1.58-7.00)	0.002	1.59 (0.66-3.80)	0.302	3.29 (1.58-6.83)	0.002	1.49 (0.61-3.61)	0.383
Stage	2.64 (1.66-4.21)	<0.001	2.19 (1.21-3.97)	0.010	2.67 (1.66-4.28)	<0.001	2.15 (1.17-3.98)	0.014
MIB-1 labeling index	2.56 (0.34-19.5)	0.364			2.85 (0.38-21.7)	0.311		
CDCP1 expression	0.44 (0.26-0.73)	0.002	0.57 (0.33-0.98)	0.040	0.43 (0.26-0.71)	0.001	0.54 (0.32-0.94)	0.028

HR, hazard ratio; CI, confidence interval.

current study on endometrioid adenocarcinoma, the opposite results were obtained; the high level of CDCP1 expression in the tumor cells was a favorable sign for prognosis. This might suggest a different role of CDCP1 molecules for cancers of endometrium from those of other organs, such as lung, breast, and kidney.

In a physiologic or non-neoplastic condition, lung, breast, and kidney tissues hardly express CDCP1. Whereas, CDCP1 was overexpressed in the cancers of lung, breast, and kidney, suggesting that CDCP1 might render the resistance of cancer cells to anoikis as observed in the cell lines from lung adenocarcinoma (7). In the endometrium, CDCP1 expression was detected in the endometrial glands of both proliferative and secretory phases. The present RT-PCR study showed that level of mRNA expression was well-correlated with that of protein expression as revealed by immunohistochemistry in both the normal and diseases endometrial glands, indicating that the immunohistochemistry is a reliable method for detection of CDCP1. Non-cancerous endometrial tissues, such as endometrial polyp, also expressed CDCP1 at a high level. These findings suggest that CDCP1 plays some physiological roles in the endometrial tissues. In endometrioid adenocarcinoma, the CDCP1 expression level was variable; cancer cells in some cases showed comparable level of expression to that in the normal endometrial glands, whereas others showed a lower level. These findings suggested that a disruption of CDCP1 expression might render a malignant potential to endometrioid adenocarcinoma.

Level of CDCP1 expression correlated with proliferative potential of the lung and breast adenocarcinomas (8,10) but not with that in endometrioid ones. In contrast to cancer cells, proliferative potential of normal endometrial glands correlated with CDCP1 expression level. CDCP1 seems to behave as a cancer suppressive factor in the endometrium, while it promotes cancer development in the lung, breast, and kidney. Because CDCP1 expression in various organs of normal condition has not been studied in detail, further studies are necessary to elucidate whether the disruption of CDCP1 expression is related to malignant progression in tissues that physiologically express CDCP1. First of all, more precise information for action mechanism of CDCP1 in cell survival and death and its regulatory mechanism will shed light on this point.

ER and PgR are abundantly expressed in normal endometrium. Expression levels of ER and PgR are reported to be correlated with a favorable prognosis of endometrioid adenocarcinoma (13,17), although the level of CDCP1 expression was not correlated with those of ER and PgR expressions. ER and PgR might not regulate CDCP1 expression. These findings are consistent with our previous report that CDCP1 expression was transcriptionally regulated by Zfp67, a member of zinc-finger proteins, but not by ER and PgR (9).

In conclusion, low CDCP1 expression is a negative prognostic factor in endometrioid adenocarcinoma.

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