

# Enhanced CD24 expression in endometrial carcinoma and its expression pattern in normal and hyperplastic endometrium

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**Summary.** CD24 is known to be an important diagnostic and prognostic marker of several major cancers affecting females. We aimed to determine CD24 expression in normal, hyperplastic, and carcinomatous endometrium and its correlation with estrogen and progesterone receptor expression.

A total of 271 cases including 62 normal/atrophic endometrium cases (47/15), 127 endometrial hyperplasia cases (51/52/24, simple/complex/atypical hyperplasia), and 82 endometrial carcinoma cases were immunohistochemically analyzed by using anti-CD24, ER, and PR antibodies that were embedded on paraffin blocks. Next, we assessed the CD24 mRNA expression in these tissues by using RT-PCR.

In the normal endometrium, cyclic expression of membranous CD24 was detected during the regular menstrual cycle, i.e., down-regulation in the proliferative phase and up-regulation in the secretory phase. CD24 expression was very infrequent and weak in the atrophic endometrium. In hyperplasias and carcinomas, the expression of both membranous and cytoplasmic CD24 was found to be sharply reduced in the hyperplastic lesions and significantly enhanced in the carcinomas. In the case of carcinomas, high CD24 expression showed significant correlation with high-grade (G2 and 3)

( $P < 0.05$ ). In addition, an inverse correlation was apparent between CD24 and the estrogen and progesterone receptor expressions in normal and diseased endometrium.

In conclusion, we demonstrated that CD24 was expressed in a cyclic pattern in the normal endometrium, and its expression was enhanced in case of endometrial carcinoma. These results suggest that CD24 may be involved in tumor progression and can be a useful diagnostic biomarker.

**Key words:** CD24, Endometrial carcinoma, Endometrial hyperplasia, Estrogen receptor, Progesterone receptor

## Introduction

CD24 was originally described as a B cell-specific marker that was expressed at the early stage of B cell development (Fischer et al., 1990). Subsequent studies have demonstrated a high degree of CD24 expression on neutrophils and renal tubular epithelial cells; moreover, several studies showed that CD24 can be expressed on several solid tumors, including small cell lung carcinoma (Jackson et al., 1992), neuroblastoma (Mechtersheimer et al., 1993), renal cell carcinoma (Droz et al., 1990), serous adenocarcinoma of the ovary (Choi et al., 2005), colorectal adenocarcinoma (Choi et al., 2006), and prostate adenocarcinoma (Kristiansen et al., 2004). CD24 has also been considered a therapeutic target

because it shows specific and high expression in several types of cancers (Jackson et al., 1992). In addition, CD24, along with CD29 and CD49f, was known as one of the four mammary gland stem cell markers (Wang, 2006). The cell types of CD45-Ter119-CD31-CD49<sup>hi</sup>CD24<sup>med</sup> and Lin<sup>-</sup>CD29<sup>hi</sup>CD24<sup>+</sup> are demonstrated to be mammary stem cell populations (Stingl et al., 2006; Wang, 2006). In addition, CD44<sup>+</sup>/CD24<sup>-</sup> is known to be the hallmark of cancer stem cells in breast cancer (Al-Hajj et al., 2003).

Endometrial cancer is the most frequently occurring malignancy of the female genital tract in developed countries, and it is the seventh leading cause of death among women suffering from cancer (Parkin et al., 2005). Although CD24 expression is known to be a prevalent and an important prognostic factor in female gynecologic cancers such as breast (Fogel et al., 1999; Kristiansen et al., 2003), and ovarian cancers (Choi et al., 2005; Kristiansen et al., 2002), CD24 expression during the regular menstrual cycle and endometrial tumorigenesis has never been investigated.

In this study, we immunohistochemically investigated CD24 expression at the protein level in the normal endometrium during the atrophic (AE), proliferative (PE), and secretory phases (SE); in various types of endometrial hyperplasia, including simple (SH), complex (CH), atypical (AH) hyperplasia; and in endometrial adenocarcinoma (CA). In addition, we determined CD24 mRNA expression in endometrial cancer cells by using RT-PCR. The results obtained were examined in the context of the hormone receptor status. Our results demonstrated that CD24 can be a potential therapeutic target and a diagnostic marker.

## Materials and methods

### *Patients, tissue samples, and reagents*

We investigated 271 cases of normal, atrophic, and hyperplastic endometrial lesions and endometrial carcinomas. The data was procured from the surgical pathology files kept at the pathology departments of the following hospitals: Eulji University Hospital, Chungbuk National University Hospital, Konyang University Hospital, and Kangbuk Samsung Hospital. The selected cases comprised 15 cases of atrophic endometrium; 47, normal endometrium (24 in the proliferative phase and 23 in the secretory phase); 127, endometrial hyperplasia (51, simple hyperplasia; 52, complex hyperplasia; and 24 atypical hyperplasia); and 82, endometrioid adenocarcinoma. This study was approved by the institutional review board of Eulji University Hospital, Chungbuk National University Hospital, Konyang University Hospital, and Kangbuk Samsung Hospital.

All archival materials were routinely fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections of 4  $\mu$ m were prepared on silane-coated slides

(Sigma, St Louis, MO, USA). The immunostaining kits were purchased from DAKO Inc. (Glostrup, Denmark).

### *Preparation of TMA*

Tissue microarray slides were employed to enable successful detection. To prepare these slides, we punched out tissue columns (3.0 mm in diameter) from the original blocks and inserted them into new paraffin blocks (each containing 30 holes to accommodate the abovementioned tissue columns). Consequently, the serially sectioned slides were prepared. Each microarray tissue slide (1x3 inches) held 30 specimens, thus enabling the simultaneous analysis of 30 specimens with minimum variation in the staining process. All specimens were circular with a 3.0-mm diameter, and this ensured that a sufficient amount of tissue was available for histopathological analysis.

### *The immunohistochemical staining procedure*

Immunostaining for CD24, ER, and PR proteins were performed using mouse monoclonal antibodies against CD24 (Clone SN3b; LAB VISION Inc., NeoMarkers, Fremont CA, USA; 1:50), ER (NCL-ER-6F11; Novocastra, Newcastle, United Kingdom; 1:50) and PR (NCL-PGR; Novocastra, Newcastle, United Kingdom; 1:50), respectively. Negative controls to check the specificity of the three antibodies were developed by omitting the primary antibodies; these were prepared by using mouse immunoglobulins instead of individually using each primary antibody. The tissue sections embedded in the microslides were deparaffinized with xylene, hydrated in serial dilutions of alcohol, and immersed in peroxidase-blocking solution (Dako REAL<sup>TM</sup> Peroxidase-Blocking Solution, DAKO) to quench the endogenous peroxidase activity. The sections were then microwaved in 10mM citrate buffer (pH 6.0) and 40 mM Borate buffer (pH 8.2) supplemented with 1 mM EDTA and 1 mM NaCl for 20 min to retrieve antigens (Kim et al., 2004a,b). Incubation with the primary antibody was carried out for 60 min, followed by three successive rinsings with a washing buffer. Further incubation was performed using Dako REAL<sup>TM</sup> EnVision<sup>TM</sup>/HRP, Rabbit/Mouse (ENV) detection reagent (EnVision<sup>TM</sup> Detection System Peroxidase/DAB, Rabbit/Mouse, DAKO) for an additional 20 min at room temperature. After rinsing, the chromogen was developed for 2 min. The slides were counter stained with Meyer's hematoxylin, dehydrated, and mounted with Canada balsam for examination. We used distilled water with 0.1% Tween 20 as a rinsing solution (Kim et al., 2003).

### *Evaluation of results of immunohistochemical staining*

In this study we have used the scoring method of Sinicrope et al. (1995) which was applied for evaluating

both the intensity of immunohistochemical staining and determining the proportion of stained epithelial cells. The staining intensity was further classified as follows: (i) weak; (ii) moderate; or (iii) strong. The positive cells were quantified as a percentage of the total number of epithelial cells and assigned to one of the following five categories (0, <5%; 1, 5%-25%; 2, 26%-50%; 3, 51%-75%; and 4, >75%). The percentage of positivity of the tumor cells and the staining intensities were then multiplied to generate the immunohistochemistry (IHC) score for each of the tumor specimens. Each lesion was separately examined and scored by two pathologists (K.H.K & S.H.K). The pathologists discussed the cases showing a discrepancy in scores till a consensus was reached.

#### *Reverse transcription-polymerase chain reaction (RT-PCR)*

Cancer tissues were microdissected from paraffin-embedded tissues using a laser-capture microdissection machine (PALM Microbeam, PALM Microlaser Technologies, Bernried, Germany) and then total cellular RNA was extracted from the dissected tissue slices and cDNA was synthesized using Paradise full transcript RT-reagent system (Arcturus, Mountainview, CA) in accordance with the manufacturer's instructions. The level of CD24 mRNA was determined by real time PCR using Lightcycler 2.0 (Roche, Mannheim, Germany) and the TaqMan master mix (Roche, Mannheim, Germany). PCR reactions were carried out in triplicate using fixed amounts of template DNA from each fraction at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. The threshold (CT) was set at a point where the fluorescence signal was above the baseline noise but as low as possible in the exponential amplification phase. Two micro liters of DNA were amplified in a total volume of 10  $\mu$ l with the forward (5'-TTTGACTAGATGATGAATGCCAAT-3') and reverse primers (5'-GGATGTTGCCTCTCCTTCAT-3'). For the specific detection of amplified CD24 cDNA, the probe UPL#67 (FAM-CTCCAGCA-TAMRA) (Roche Diagnostics, Mannheim, Germany) was employed. As a negative control, distilled water was supplied instead of the template DNA for each examination. As a reference gene, the GAPDH gene (sense, 5'-GGACCTGACCTGCCGTCTA-3' and anti-sense, 5'-TAGCCCAGGATGCCCTTGAG-3' was also amplified and specifically detected by TaqMan probe (DYXL-CCTCCGACGCCTGCTTCGCCACCT-BHQ2) (TIB MOLBIOL, Berlin, Germany).

#### *Statistical analysis*

Statistical analyses were conducted using Pearson's correlation coefficient, Fisher's exact tests, Pearson's  $\chi^2$  tests, ANOVA, Mann-Whitney tests, Turkey's HSD, and Duncan's test (as a *post hoc* test). *P* values less than 0.05

were regarded as statistically significant. All statistical analyses were performed using SPSS software (SPSS Inc. 233s. Wacker Drive, Chicago, Illinois).

## **Results**

### *CD24 protein expression in normal endometrium*

In the proliferative phase samples, weak immunoreactivity for CD24 protein was focally and infrequently detected on the endometrial gland cell membrane (7/24, 29.2%) and cytoplasm (10/24, 41.7%) (Figs. 1, 2, Tables 1 and 2). Stromal tissues and myometrium exhibited complete absence of immunoreactivity for CD24 protein. The reaction patterns in an atrophic (postmenopausal) endometrium for the CD24 antibody was identical to those in the proliferative phase but the detection was more infrequent (membrane: 2/15, 13.3%; cytoplasm: 1/14, 3.7%) (Table 2). In glandular cells in the secretory phase, weak to moderate immunoreactivity for CD24 protein was more frequently observed in the membrane and occasionally in the cytoplasm (membrane: 20/23, 87%; cytoplasm: 11/23, 47.8%) (Table 2). The membranous staining of CD24 showed apical localization rather than a basolateral or circumferential pattern. Immunoreactivity for non-epithelial components, including stromal cells, smooth muscle cells, and infiltrating lymphocytes was completely negative (Fig. 2). As shown in Figure 1, the average IHC scores of both cytoplasmic and membranous CD24 were significantly higher in the secretory endometrium than in the atrophic endometrium. Moreover, the IHC score of membranous CD24 was more significantly enhanced in the secretory endometrium than the proliferative endometrium (Figure 1, Table 1).

Immunostaining for both ER and PR in the nucleus of endometrial cells showed relatively homogenous distribution. A marked reduction in staining intensity was noted for both antibodies when the cells showed transition from the proliferative to the secretory phase. As shown in Table 2, the IHC scores of CD24 were inversely correlated with those of ER or PR throughout the menstrual cycle.

### *CD24 protein expression in hyperplastic lesions*

Very weak to weak membranous and cytoplasmic immunoreactivity for CD24 was detected infrequently in simple or complex hyperplastic lesions. The staining patterns in atypical hyperplasia were identical to those in simple/complex hyperplasias. Absolutely no immunoreactivity was detected in the stromal components.

The average IHC scores of membranous CD24 in either simple/complex or atypical hyperplastic lesions were significantly lower than those of the normal endometrial glands in the secretory phase and

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endometrial carcinomas (Fig. 1). The IHC scores of cytoplasmic CD24 in various types of hyperplastic lesions were also statistically lower than those of the secretory endometrium and carcinoma; this score pattern was identical to that of membranous CD24, except for the absence of a significant difference between atypical hyperplasia and carcinoma (Fig. 1). In contrast, no significant differences were noted in the CD24 scores between simple/complex and atypical lesions. As shown in Table 1, the expression rate of CD24 was in the same

pattern of the average IHC scores (Table 1). Negative correlations among the values for CD24, ER, and PR are presented in Table 2.

CD24 protein expression in endometrial carcinoma

Diffusely distributed moderate to strong membranous staining for CD24, and to a relatively limited extent, staining for cytoplasmic expression of CD24 was observed in endometrial carcinoma.

Table 1. The expressional profile of CD24 in endometrial adenocarcinoma and its precursor lesions.

	No.	CD24 membranous exp.			CD24 cytoplasmic exp.		
		absent	mild	moderate	absent	Mild	moderate
Atrophic endometrium	15	13/15 (86.7%)	2/15 (13.3%)	0/15 (0.0%)	14/15 (93.3%)	1/15 (6.7%)	0/15 (0.0%)
Proliferative Endometrium	24	17/24 (70.8%)	6/24 (25.0%)	1/24 (4.2%)	14/24 (58.3%)	9/24 (37.5%)	1/24 (4.2%)
Secretory Endometrium	23	3/23 (13.0%)	18/23 (78.3%)	2/23 (8.7%)	12/23 (52.2%)	9/23 (39.1%)	2/23 (8.7%)
Simple Hyperplasia	51	47/51 (92.2%)	4/51 (7.8%)	0/51 (0.0%)	51/51 (100.0%)	0/51 (0.0%)	0/51 (0.0%)
Complex Hyperplasia	52	42/52 (80.8%)	10/52 (19.2%)	0/52 (0.0%)	46/52 (88.5%)	6/52 (11.5%)	0/52 (0.0%)
Atypical Hyperplasia	24	16/24 (66.7%)	6/24 (25.0%)	2/24 (8.3%)	17/24 (70.8%)	7/24 (29.2%)	0/24 (0.0%)
Adeno-carcinoma	82	29/82 (35.4%)	33/82 (40.2%)	20/82 (24.4%)	50/82 (61.0%)	27/82 (32.9%)	5/82 (6.1%)

CD24 membranous expression. Atrophic endometrium vs carcinoma:  $p < 0.001$ ; Proliferative endometrium vs carcinoma:  $p < 0.01$ ; Secretory endometrium vs carcinoma:  $p < 0.01$ ; Simple hyperplasia vs carcinoma:  $p < 0.001$ ; Complex hyperplasia vs carcinoma:  $p < 0.001$ ; Atypical hyperplasia vs carcinoma:  $p < 0.025$ . CD24 cytoplasmic expression. Atrophic endometrium vs carcinoma:  $p < 0.1$ ; Proliferative endometrium vs carcinoma: N.S; Secretory endometrium vs carcinoma: N.S; Simple hyperplasia vs carcinoma:  $p < 0.001$ ; Complex hyperplasia vs carcinoma:  $p < 0.01$ ; Atypical hyperplasia vs carcinoma: N.S. Abbreviation: N.S, not significant.

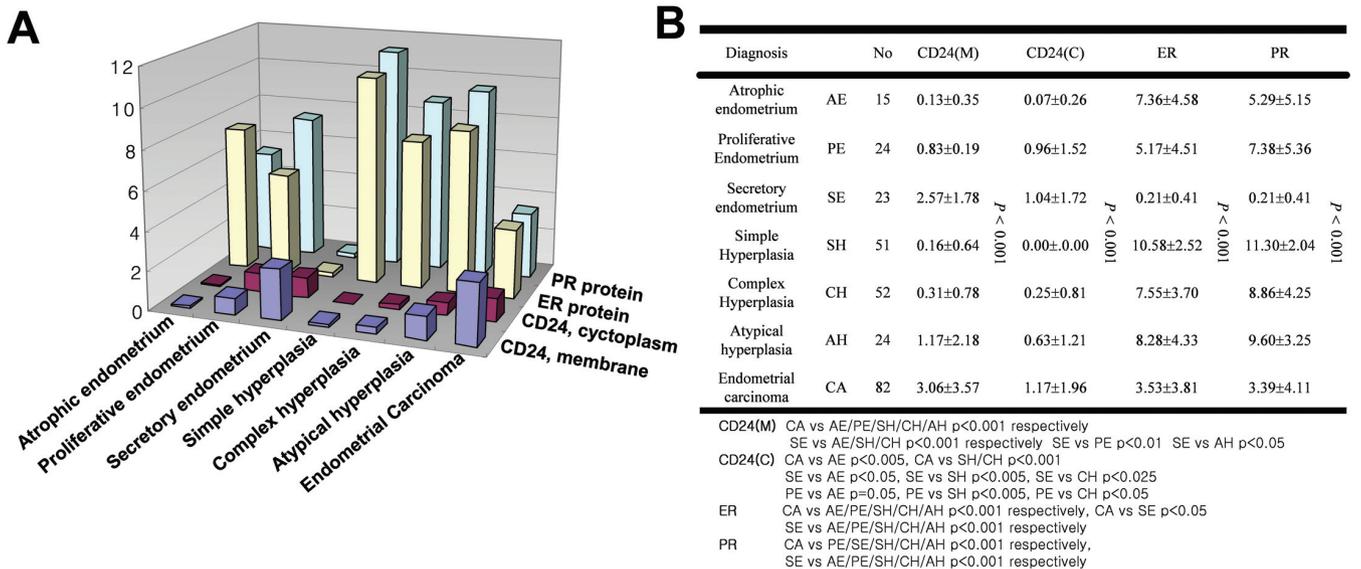


Fig. 1. The illustration of expressional profile of CD24 and ER/PR in the tissue samples of normal, hyperplastic, and carcinomatous endometrium. A. The mean of IHC scores for each protein in each lesion was illustrated graphically using a bar graph. B. The results were summarized. Abbreviation: IHC, immunohistochemistry; CA, carcinoma; AH, atypical hyperplasia; CH, complex hyperplasia; SH, simple hyperplasia; AE, atrophic endometrium; PE, proliferative endometrium; SE, secretory endometrium; CD24(M), CD24 membranous expression; CD24(C), CD24 cytoplasmic expression.

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Immunoreactivities in the stromal components were also absent, and inverse correlations were observed among CD24 and the hormone receptor immunoreactivities.

As shown in Figure 1, the CD24 IHC scores are markedly elevated in carcinomas as compared with the normal and hyperplastic lesions. In particular, the membranous expression of CD24 is significantly higher in carcinomas than that of all other normal and hyperplastic lesions, except for the secretory endometrium (Fig. 1B) ( $P < 0.001$ ). Additionally, CD24 cytoplasmic expression was significantly enhanced in carcinomas as compared with the atrophic endometrium and simple/complex hyperplastic lesions ( $P < 0.005$ ). The

expression rate of CD24 was in the same pattern of the average IHC scores (Table 1). As shown in Table 3, the CD24 expression rate correlated with the tumor grade. Both membranous and cytoplasmic expressions of CD24 were significantly greater in the high-grade tumor cells (grade 2 and 3) than in grade 1 tumor cells ( $P < 0.05$ ).

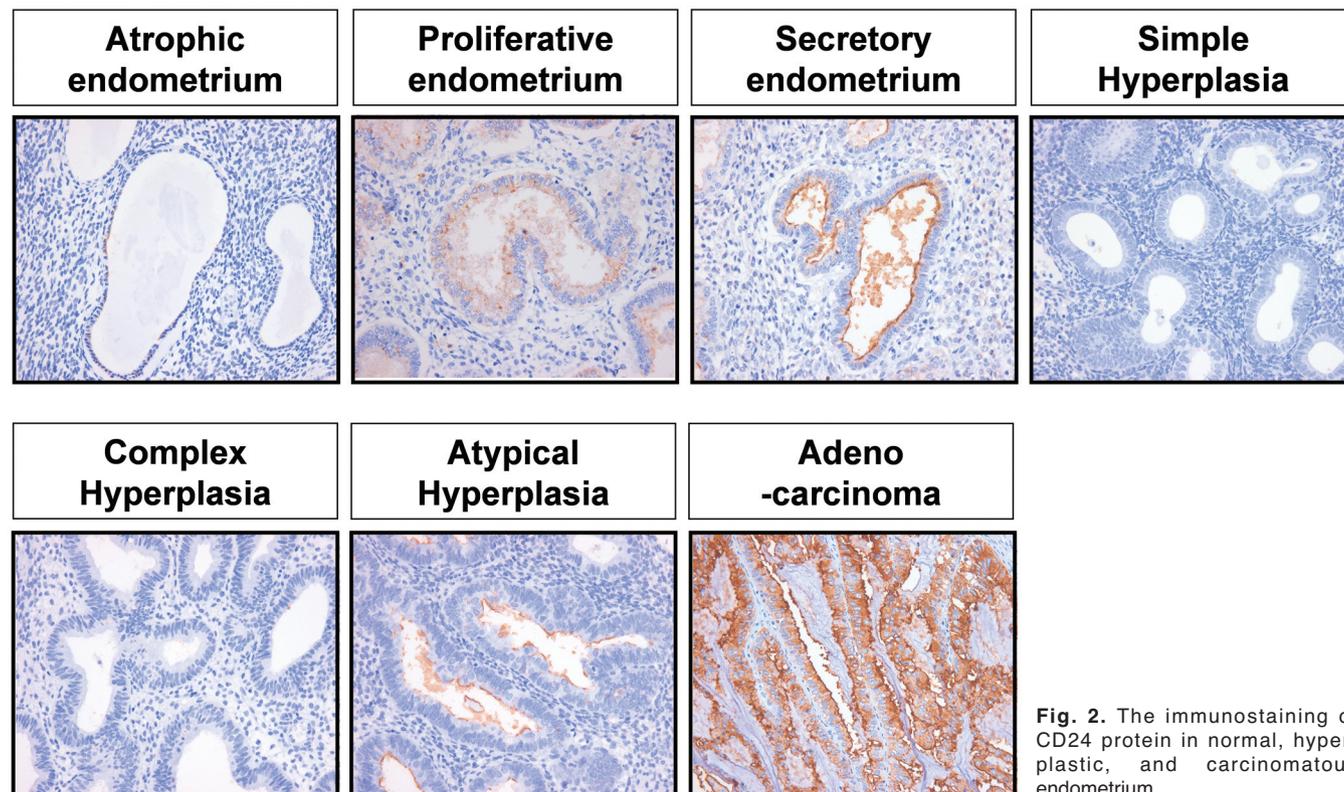
### RNA of CD24 is detected in endometrial cancer tissues.

RNA was extracted from the 15 paraffin-embedded endometrial tissue samples (endometrial cancer: 6, normal endometrium: 6, hyperplasia: 3) by using laser-captured microdissection. cDNAs were generated from

**Table 2.** Correlation of IHC scores among CD24(M), CD24(C), ER, and PR in normal, hyperplastic, and malignant endometrium.

	CD24(M) versus			CD24(C) versus		
	CD24(C) r(P)	ER r(P)	PR r(P)	CD24(M) r(P)	ER r(P)	PR r(P)
Endometrioid Carcinoma (n=82)	0.537 (p<0.001)	-0.237 (p=0.034)	-0.300 (p=0.006)	0.537 (p<0.001)	-0.193 (p=0.087)	-0.233 (p=0.035)
Atypical Hyperplasia (n=24)	0.256 (p=0.228)	-0.568 (p=0.004)	-0.407 (p=0.048)	0.256 (p=0.228)	-0.562 (p=0.004)	-0.325 (p=0.121)
Hyperplasia* (n=103)	0.487 (p<0.001)	-0.425 (p<0.001)	-0.528 (p=0.000)	0.487 (p<0.001)	-0.253 (p=0.012)	-0.321 (p=0.001)
Normal** (n=62)	0.635 (p<0.001)	-0.461 (p<0.001)	-0.375 (p=0.003)	0.635 (p<0.001)	-0.245 (p=0.057)	-0.145 (p=0.264)

r: Pearson's correlation coefficient; CD24(M): CD24 membranous expression; CD24(C): CD24 cytoplasmic expression; ER: estrogen receptor; PR: progesterone receptor; n: number of cases; \*: Hyperplasia including simple and complex types; \*\*: Normal including atrophic, proliferative, and secretory phases



**Fig. 2.** The immunostaining of CD24 protein in normal, hyperplastic, and carcinomatous endometrium.

## CD 24 in normal endometrium and its related lesions

**Table 3.** Correlation between CD24 expression and clinicopathological parameters.

	CD24 (M)				<i>p</i>	CD24(C)				<i>p</i>
	absent	mild	Mod/high			absent	mild	Mod/high		
FIGO stage										
I	17/52 (32.7%)	25/52 (48.1%)	10/52 (19.2%)	N.S	29/52 (55.8%)	19/52 (36.5%)	4/52 (7.7%)	N.S		
II/III/IV	5/13 (38.5%)	4/13 (30.8%)	4/13 (30.8%)		8/13 (61.5%)	4/13 (30.8%)	1/13 (7.7%)			
Grade										
G1	15/34 (44.1%)	16/34 (47.1%)	3/34 (8.8%)	0.041	26/34 (76.5%)	7/34 (20.6%)	1/34 (2.9%)	0.030		
G2+G3	12/39 (30.8%)	14/39 (35.9%)	13/39 (33.3%)		18/39 (46.2%)	18/39 (46.2%)	3/39 (7.7%)			
Myometrial invasion										
< 1/2	14/41 (34.1%)	18/41 (43.9%)	9/41 (22.0%)	N.S	21/41 (51.2%)	16/41 (39.0%)	4/41 (9.8%)	N.S		
≥ 1/2	8/24 (33.3%)	11/24 (45.8%)	5/24 (20.8%)		16/24 (66.7%)	7/24 (29.2%)	1/24 (4.2%)			
Lymph node metastasis										
Negative	20/60 (33.3%)	27/60 (45.0%)	13/60 (21.7%)	N.S	34/60 (56.7%)	21/60 (35.0%)	5/60 (8.3%)	N.S		
Positive	2/5 (40.0%)	2/5 (40.0%)	1/5 (20.0%)		3/5 (60.0%)	2/5 (40.0%)	0/5 (0.0%)			

CD24(M): CD24 membranous expression; CD24(C): CD24 cytoplasmic expression; N.S: not significant

the RNA and amplified by using primer sets for the CD24 and GAPDH genes. The amplified DNA products were detected using specific TaqMan probe and quantified by real-time PCR (Fig. 3A). Ct values of GAPDH (Reference gene) ranges from 20.28 to 29.32 and Ct value of CD24 ranges from 21.33 to 27.14 (Fig. 3B). CD24 mRNA level was normalized against the reference gene (GAPDH) and the concentration ratio of CD24 over reference gene (GAPDH) ranges from 0.02 to 6.55. In all (15) cases, a considerable level of CD24 mRNA expression was detected, and in most cases CD24 expressional level was higher than GAPDH except for two cases (Fig. 3B). As shown in Fig. 3, CD24 mRNA was present in all normal and disease categories, including proliferative and secretory endometrium, hyperplastic endometrium and endometrial adenocarcinoma, and there was no significant difference among these categories. Also there is no significant correlation between the level of mRNA and IHC score. These results suggest the protein level of CD24 may be regulated during post-transcriptional process such as translation or protein degradation.

## Discussion

The present study is the first to demonstrate CD24 expression in normal, hyperplastic, and carcinomatous endometrium. In the normal endometrium, the cyclic expression of membranous CD24 was exhibited during the regular menstrual cycle, i.e., down-regulation in the proliferative phase and up-regulation in the secretory phase. This finding suggests that membranous expression of CD24 may be linked to differentiation or maturation of the endometrial glandular cells during menstrual cycles. Furthermore, the observed inverse correlation between CD24 expression and hormone receptor status strongly indicates a link with ovarian hormones.

In addition, our study revealed the dynamic change of CD24 expression in hyperplastic and carcinomatous lesions, i.e., a sharp down-regulation of CD24 in the hyperplastic lesions, followed by a remarkable up-regulation in the adenocarcinomatous lesions. This finding suggests that CD24 expression may be linked with tumor progression. A similar pattern of CD24 over-expression in carcinomatous lesions with its concurrent low expression in precancerous lesions was documented in previous studies on colon (Choi et al., 2006), ovarian (Choi et al., 2005), and breast cancers (Fogel et al., 1999). Moreover, in carcinomatous lesions, the level of CD24 expression is clearly higher in high-grade tumors, implying the role of CD24 in cancer progression. Several previous studies have reported the association of CD24 over-expression with a more aggressive phenotype and poor prognosis in various types of cancer, including breast (Fogel et al., 1999; Kristiansen et al., 2003), ovarian (Kristiansen et al., 2002; Choi et al., 2005), colon (Weichert et al., 2005; Choi et al., 2006), and prostate cancers (Kristiansen et al., 2004, 2005).

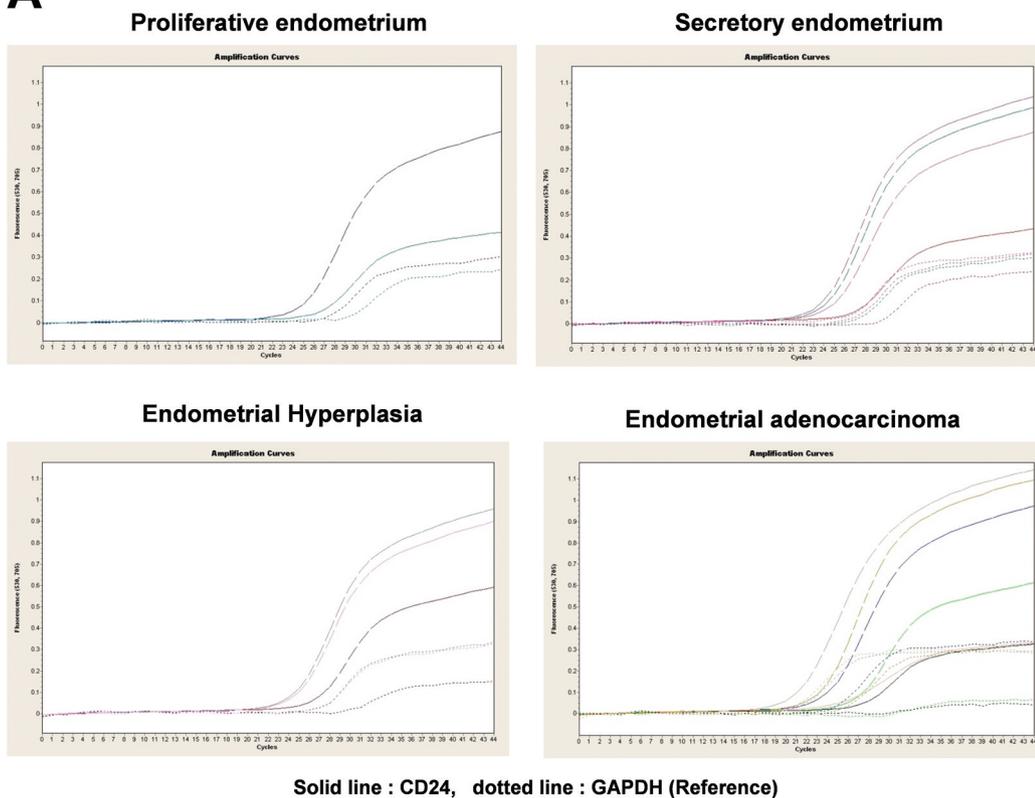
The expression pattern of ER and PR in normal, hyperplastic, and neoplastic endometrial tissues exhibited in our study are generally consistent with the results of previous studies (Bergeron et al., 1988; Papadimitriou et al., 1992). In the present study, ER/PR expressions were significantly higher than proliferative and atrophic endometrium (Fig. 1). Moreover, our study revealed that their levels dropped sharply in carcinomas. In addition, there was very clear inverse correlation between CD24 and ER/PR expression in the normal endometrium and hyperplastic lesions and this tendency was also observed in case of carcinomas. These results imply that CD24 may be a differentiation-related marker as opposed to ER/PR that are pro-proliferative proteins. To completely understand the significance of high CD24 expression in ER/PR negative cancer cases, further studies are clearly warranted.

## CD 24 in normal endometrium and its related lesions

The clinical application of CD24 in endometrial carcinoma can be speculated in terms of its therapeutic and diagnostic potential. CD24 can be considered as a therapeutic target in endometrial adenocarcinoma because of the following points. First, CD24 expression is more frequent in ER-negative endometrial cancer patients. For ER-positive cancer patients, several therapeutic modalities have already been established, therefore, CD24 can be considered as an alternative

therapeutic target for ER-negative patients. Second, most endometrial cancer patients are post-menopausal women and their endometrium is atrophic and not proliferative or secretory. CD24 expression in an atrophic endometrium is very rare and weak, therefore, the possible side effects of the CD24-targeting drug may be diminished. Third, CD24 expression is more frequent in the cell membrane of the apical border than in the cytoplasm or nucleus. If an anti-CD24 antibody is

### A



### B

Case	Diagnosis	IHC score (membrane)	IHC score (cytoplasm)	CD24 Ct	GAPDH (Ref) Ct	Concentration ratio
1	Proliferative endometrium	2	1	25.05	26.86	3.51
2	Proliferative endometrium	4	0	26.59	28.89	4.95
3	Secretory endometrium	12	4	26.81	28.66	3.6
4	Secretory endometrium	8	4	24.96	26.11	2.21
5	Secretory endometrium	8	1	24.45	26.7	4.76
6	Secretory endometrium	2	1	23.98	26.25	4.85
7	Simple hyperplasia	2	3	24.65	26.61	3.89
8	Complex hyperplasia	1	2	26.55	29.26	6.55
9	Complex hyperplasia	1	1	24.8	26.73	3.8
10	Adenocarcinoma	6	2	24.12	24.45	1.26
11	Adenocarcinoma	10	8	26.59	28.84	4.74
12	Adenocarcinoma	10	4	27.14	29.32	4.53
13	Adenocarcinoma	0	1	21.33	21.03	0.82
14	Adenocarcinoma	1	1	25.77	20.28	0.02
15	Adenocarcinoma	10	3	23.53	25.24	3.27
16	DW_CD24/GAPDH					Invalid

**Fig. 3.** The real time RT-PCR assays. **A.** The amplification curve of cDNA was depicted. **B.** The results of real time RT-PCR and its IHC score were summarized. IHC, immunohistochemistry

selected as a therapeutic modality, the presence of antigen in the membrane will greatly enhance the accessibility of the therapeutic antibody to the target.

On the other hand, the remarkable difference in CD24 expression between adenocarcinomas and hyperplastic lesions increases the diagnostic potential of CD24. A diagnostic difficulty lies in discrimination between cancer and hyperplasia, especially complex/atypical hyperplasia. CD24 membranous expression is rare and weak in these types of hyperplasias; therefore, its high expression can support the diagnosis of adenocarcinoma.

In the present study, we demonstrate the presence of CD24 mRNA in the endometrial tissue. We found that CD24 mRNA was not correlated with immunoreactivity for CD24. This result may suggest that the level of CD24 protein is regulated during the post-transcription process, such as translation or protein degradation, rather than transcription. Specifically, accelerated translation or prolonged stabilization of CD24 protein, such as an escape from ubiquitin-dependent proteolysis, may maintain its protein level high regardless of its mRNA level. However, based on the results of the present study, we can suggest that the immunohistochemical approach provides a more accurate assessment of the actual level of protein expression in the tissue rather than an RNA-based analysis.

In summary, we demonstrated CD24 expression in normal, hyperplastic, and carcinomatous lesions, and our results suggest that CD24 may be involved in tumor progression and can be a diagnostic biomarker.

## References

- Al-Hajj M., Wicha M.S., Benito-Hernandez A., Morrison S.J. and Clarke M.F. (2003). Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. USA* 100, 3983-3988.
- Bergeron C., Ferenczy A. and Shyamala G. (1988). Distribution of estrogen receptors in various cell types of normal, hyperplastic, and neoplastic human endometrial tissues. *Lab. Invest.* 58, 338-345.
- Choi Y.L., Kim S.H., Shin Y.K., Hong Y.C., Lee S.J., Kang S.Y. and Ahn G. (2005). Cytoplasmic CD24 expression in advanced ovarian serous borderline tumors. *Gynecol. Oncol.* 97, 379-386.
- Choi Y.L., Xuan Y.H., Lee S.J. and Kim S.H. (2006). Enhanced CD24 expression in colorectal cancer correlates with prognostic factors. *Kor. J. Pathol.* 40, 103-111.
- Droz D., Zachar D., Charbit L., Gogusev J., Chretien Y. and Iris L. (1990). Expression of the human nephron differentiation molecules in renal cell carcinomas. *Am. J. Pathol.* 137, 895-905.
- Fischer G.F., Majdic O., Gadd S. and Knapp W. (1990). Signal transduction in lymphocytic and myeloid cells via CD24, a new member of phosphoinositol-anchored membrane molecules. *J. Immunol.* 144, 638-641.
- Fogel M., Friederichs J., Zeller Y., Husar M., Smirnov A., Roitman L., Altevogt P. and Stthoeger Z.M. (1999). CD24 is a marker for human breast carcinoma. *Cancer Lett.* 143, 87-94.
- Jackson D., Waibel R., Weber E., Bell J. and Stahel R.A. (1992). CD24, a signal-transducing molecule expressed on human B cells, is a major surface antigen on small cell lung carcinomas. *Cancer Res.* 52, 5264-5270.
- Kim S.H., Shin Y.K., Lee K.M., Lee J.S., Yun J.H. and Lee S.M. (2003). An improved protocol of biotinylated tyramine-based immunohistochemistry minimizing nonspecific background staining. *J. Histochem. Cytochem.* 51, 129-132.
- Kim S.H., Kook M.C. and Song H.G. (2004a). Optimal conditions for the retrieval of CD4 and CD8 antigens in formalin-fixed, paraffin-embedded tissues. *J. Mol. Histol.* 35, 403-408.
- Kim S.H., Kook M.C., Shin Y.K., Park S.H. and Song H.G. (2004b). Evaluation of antigen retrieval buffer systems. *J. Mol. Histol.* 35, 409-416.
- Kristiansen G., Denkert C., Schluns K., Dahl E., Pilarsky C. and Hauptmann S. (2002). CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival. *Am. J. Pathol.* 161, 1215-1221.
- Kristiansen G., Winzer K.J., Mayordomo E., Bellach J., Schluns K., Denkert C., Dahl E., Pilarsky C., Altevogt P., Guski H. and Dietel M. (2003). CD24 expression is a new prognostic marker in breast cancer. *Clin. Cancer Res.* 9, 4906-4913.
- Kristiansen G., Pilarsky C., Pervan J., Stürzebecher B., Stephan C., Jung K., Loening S., Rosenthal A. and Dietel M. (2004). CD24 expression is a significant predictor of PSA relapse and poor prognosis in low grade or organ confined prostate cancer. *Prostate* 58, 183-192.
- Kristiansen G., Pilarsky C., Wissmann C., Kaiser S., Bruemendorf T., Roepcke S., Dahl E., Hinzmann B., Specht T., Pervan J., Stephan C., Loening S., Dietel M. and Rosenthal A. (2005). Expression profiling of microdissected matched prostate cancer samples reveals CD166/MEMD and CD24 as new prognostic markers for patient survival. *J. Pathol.* 205, 359-376.
- Mechtersheimer G., Barth T., Ludwig R., Staudter M. and Moller P. (1993). Differential expression of leukocyte differentiation antigens in small round blue cell sarcomas. *Cancer* 71, 237-248.
- Papadimitriou C.S., Athanasiadou S., Stylianidou A., Bai M.K., Demolitis J. and Stephanou D.G. (1992). Immunohistochemical detection of estrogen receptors on paraffin sections of normal, hyperplastic and carcinomatous endometrium. *Oncology* 49, 196-202.
- Parkin D.M., Bray F., Ferlay J. and Pisani P. (2005). Global cancer statistics, 2002. *CA. Cancer J. Clin.* 55, 74-108.
- Sinicrope F.A., Ruan S.B., Cleary K.R., Stephens L.C., Lee J.J. and Levin B. (1995). bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. *Cancer Res.* 55, 237-241.
- Stingl J., Eirew P., Ricketson I., Shackleton M., Vaillant F., Choi D., Li H.I. and Eaves C.J. (2006). Purification and unique properties of mammary epithelial stem cells. *Nature* 439, 993-997.
- Wang R.H. (2006). The new portrait of mammary gland stem cells. *Int. J. Biol. Sci.* 2, 186-187.
- Weichert W., Denkert C., Burkhardt M., Gansukh T., Bellach J., Altevogt P., Dietel M. and Kristiansen G. (2005). Cytoplasmic CD24 expression in colorectal cancer independently correlates with shortened patient survival. *Clin. Cancer Res.* 11, 6574-6581.