E-Cadherin and Alpha-Catenin Expression in Normal, Hyperplastic and Neoplastic Endometrium

ELISABETTA CARICO[1], MARCO ATLANTE[2], ENRICO GIARNIERI[1], SALVATORE RAFFA[3], BARBARA BUCCI[4], MARIA ROSARIA GIOVAGNOLI[1] and ALDO VECCHIONE[5]

[1]U.O.D Citopatologia II and [3]U.O.C. Diagnostica Cellulare II, Faculty of Medicine, University La Sapienza, A.O. Sant'Andrea, 00189 Rome, Italy;
[2]Divisione di Ginecologia, Fabia Mater, 00171 Rome, Italy;
[4]AFAR Centro Ricerca, S. Pietro Fatebenefratelli Hospital, 00189, Rome, Italy;
[5]Direzione Scientifica, Istituto Tumori G. Pascale, 80131 Naples, Italy

Abstract. *The aim of this study was to determine whether modulation of expression of cell adhesion molecules may occur in neoplastic transformation of endometrial epithelium. Materials and Methods: E-Cadherin and α-catenin protein expression were evaluated by immunohistochemistry in 124 biopsies representative of normal, hyperplastic and neoplastic endometrium. Results: In normal endometrium (proliferative, secretive and atrophic endometrium) strong homogeneous, E-cadherin and α-catenin reactivity was found; 58.3% and 66.6% of biopsies representative of simple hyperplastic endometrium were homogeneously positive for E-cadherin and α-catenin, respectively, whereas no samples representative of atypical hyperplasia showed evidence of homogeneous E-cadherin or α-catenin expression. No expression of homogeneous E-cadherin was seen in endometrial adenocarcinomas; α-catenin homogeneous immunostaining was observed in 2 G1 and 2 G2 out of 22 adenocarcinoma samples (18.2%). A homogeneous co-expression of both molecules was seen only in normal (70%) and simple hyperplastic (46%) endometrium. Conclusion: These results suggest that E-cadherin and α-catenin down-regulation might be associated with neoplastic transformation of endometrial tissues.*

Cell adhesion molecules such as cadherins have been recognized to be important biomarkers of tumor differentiation (1, 2). They regulate epithelial, endothelial and neural cell adhesion with different cadherins expressed on different cell types (3). E-Cadherin, which is an epithelial cell adhesion molecule, is a transmembrane protein with a cytoplasmic domain that connects the actin cytoskeleton through a complex with its associated cytoplasmic proteins, α-, β- and γ-catenins (4, 5). Cell adhesive properties mediated by E-cadherin are regulated by the association with catenins: adhesiveness at cell adhesion sites is enhanced when E-cadherin links to α-catenin (6, 7). Protein p120cas has also been defined as a member of the cadherin–cell–cell adhesion complex and binds directly to the same region of E-cadherin as the catenins (8, 9); p120cas can be phosphorylated after activation by several growth factors and oncogenes, suggesting a possible role for this protein in the regulation of E-cadherin function by these factors (10).

Loss of E-cadherin expression has been correlated with the *in vitro* invasive phenotype of cancer cell lines (11, 12). Furthermore, previous *in vivo* studies reported the reduced or aberrant expression of E-cadherin and/or catenins in different human cancers (13): in neoplastic thyroid tissue (14), in esophageal cancer (15), breast cancer (16, 17), gastric (18) and pancreatic carcinoma (19) bladder (20) and prostatic cancer (21), melanoma (22) and meningioma (23). Defects in the E-cadherin/catenin adhesion complex have been described in several gynecologic carcinomas, including cervical (24-26), endometrial (27-30) and ovarian carcinomas (31, 32).

This study evaluated the tissue distribution of E-cadherin and α-catenin in normal, hyperplastic and neoplastic endometrium using immunohistochemical analysis, with the aim to investigate whether differences of expression of these molecules exist. The possibility that modulation of expression of E-cadherin and α-catenin may have a prognostic role is discussed.

Correspondence to: Elisabetta Carico, MD, Ph.D., U.O.D. Cytopathology II, Faculty of Medicine, University La Sapienza, Rome, A.O. Sant'Andrea, Via di Grottarossa 1035, 00198 Rome, Italy. Tel/Fax: +39 0633775345, e-mail: elisabetta.carico@uniroma1.it

Key Words: α-Catenin, E-cadherin, down-regulation, immunohistochemistry, endometrium.

Table I. *E-Cadherin and α-catenin immunohistochemical expression.*

	E-Cadherin			α-Catenin		
Histology	Negative	Heterogeneous	Homogeneous	Negative	Heterogeneous	Homogeneous
Proliferative endometrium	4/23 (17.4%)	4/23 (17.4%)	15/23 (65.2%)	1/23 (4.4%)	4/23 (17.4%)	18/23 (78.2%)
Secretory endometrium	4/23 (17.4%)	3/23 (13%)	16/23 (69.6%)	2/23 (8.7%)	2/23 (8.7%)	19/23 (82.6%)
Atrophic endometrium	3/21 (14.3%)	0/21 (0)	18/21 (85.7%)	3/21 (14.3%)	0/21 (0)	18/21 (85.7%)
Simple hyperplasia	6/24 (25%)	4/24 (16.7%)	14/24 (58.3%)	6/24 (25%)	2/24 (8.4%)	16/24 (66.6%)
Atypical hyperplasia	4/11 (36.4%)	7/11 (63.6%)	0/11 (0)	6/11 (54.5%)	5/11 (45.5%)	0/11 (0)
Adenocarcinoma	7/22 (31.8%)	15/22 (68.2%)	0/22 (0)	6/22 (27.3%)	12/22 (54.5%)	4/22 (18.2%)

Materials and Methods

Immunohistochemical analysis. A total of 124 endometrial tissue specimens (biopsy or surgical samples) were fixed in 10% buffered formalin. Hematoxylin end eosin-stained sections from paraffin-embedded blocks were classified as: normal proliferative endometrium (23 cases), normal secreting endometrium (23 cases), atrophic endometrium in postmenopausal women (21 cases), simple endometrial hyperplasia (24 cases), atypical endometrial hyperplasia (11 cases) and 22 endometrial adenocarcinomas. The hormonal phases of all normal endometrium specimens were established according to the criteria of Noyes *et al*. (33). The endometrial adenocarcinomas were graded according the World Health Organization grading system: seven G1 cases (well-differentiated adenocarcinomas), nine G2 cases (moderately differentiated adenocarcinomas) and six G3 cases (poorly differentiated adenocarcinomas). The patients had not received any preoperative therapy.

Immunohistochemistry was performed according to a standard streptavidin-biotin peroxidase complex method (Zymed, S. Francisco, CA, USA). E-Cadherin and α-catenin expression were assessed using commercially available specific mouse monoclonal antibodies (Zymed, San Francisco, CA, USA): HECD-1, specific for human E-cadherin and α-CAT-7A4 raised against a synthetic peptide corresponding to the *C*-terminus of mouse α-catenin (dilution 1:50). Sections (4 μm) were deparaffinized in xylene, rehydrated in graded ethanol and washed with phosphate-buffered saline (PBS), then treated in a microwave oven for 15 min in 0.01 M citrate buffer (pH 6.0) and allowed to cool for 20 min to room temperature. Endogenous peroxidases were quenched by incubation in 0.3% hydrogen peroxide and sections were then incubated in serum blocking solution to reduce non-specific labeling. Anti E-cadherin primary antibody and anti α-catenin primary antisera were added and sections were then incubated for 1 h at room temperature. Positive controls were known E-cadherin- and α-catenin-expressing epithelial tissues; negative controls were carried out by using unrelated isotype matching antibody. The secondary biotinylated antibody was incubated as previously described (26). The reaction was revealed by adding diaminobenzidine-tetrahydrochloride (DAB) chromogen mixture (Zymed). After hematoxylin counterstaining, slides were permanently mounted and analyzed for the presence and distribution of the immunostaining. Staining was scored based on semiquantitative assessment of the distribution pattern of staining (plasma membrane and cytoplasm) and number of immunoreactive epithelial cells. Following a previous study (17), homogeneous expression was present when immunostaining of E-cadherin and α-catenin was observed more than 70% of epithelial cells for each tissue section in (with strong membrane immunostaining) as a normal control; heterogeneous expression was presentation immunostaining of less than 70% of epithelial cells was observed. Samples were considered negative when they showed no immunoreactivity.

Statistical analysis. Pearson χ^2 test was used to assess the statistical difference of E-cadherin and α-catenin expression in relation to the histological classification ($p<0.05$ was accepted as statistically significant).

Results

The results of the investigations on E-cadherin and α-catenin expression and their different patterns of distribution in normal, hyperplastic and neoplastic endometrium are summarized in Table I.

In normal endometrium (proliferative, secretory phases and atrophic endometrium), E-cadherin and α-catenin were expressed in 65.2%, 69.6% and 85.7% of samples, respectively: a homogeneous pattern of immunostaining was observed with intense reactivity at the cell-to-cell borders, whereas a weak immunostaining was present in the cytoplasm (Figure 1A). No substantial difference in protein expression was evidenced among the different phases of menstrual cycle for normal endometrium. No nuclear staining of E-cadherin and α-catenin was observed in glandular cells; no stromal cells showed any immunoreactivity. Atrophic endometrium

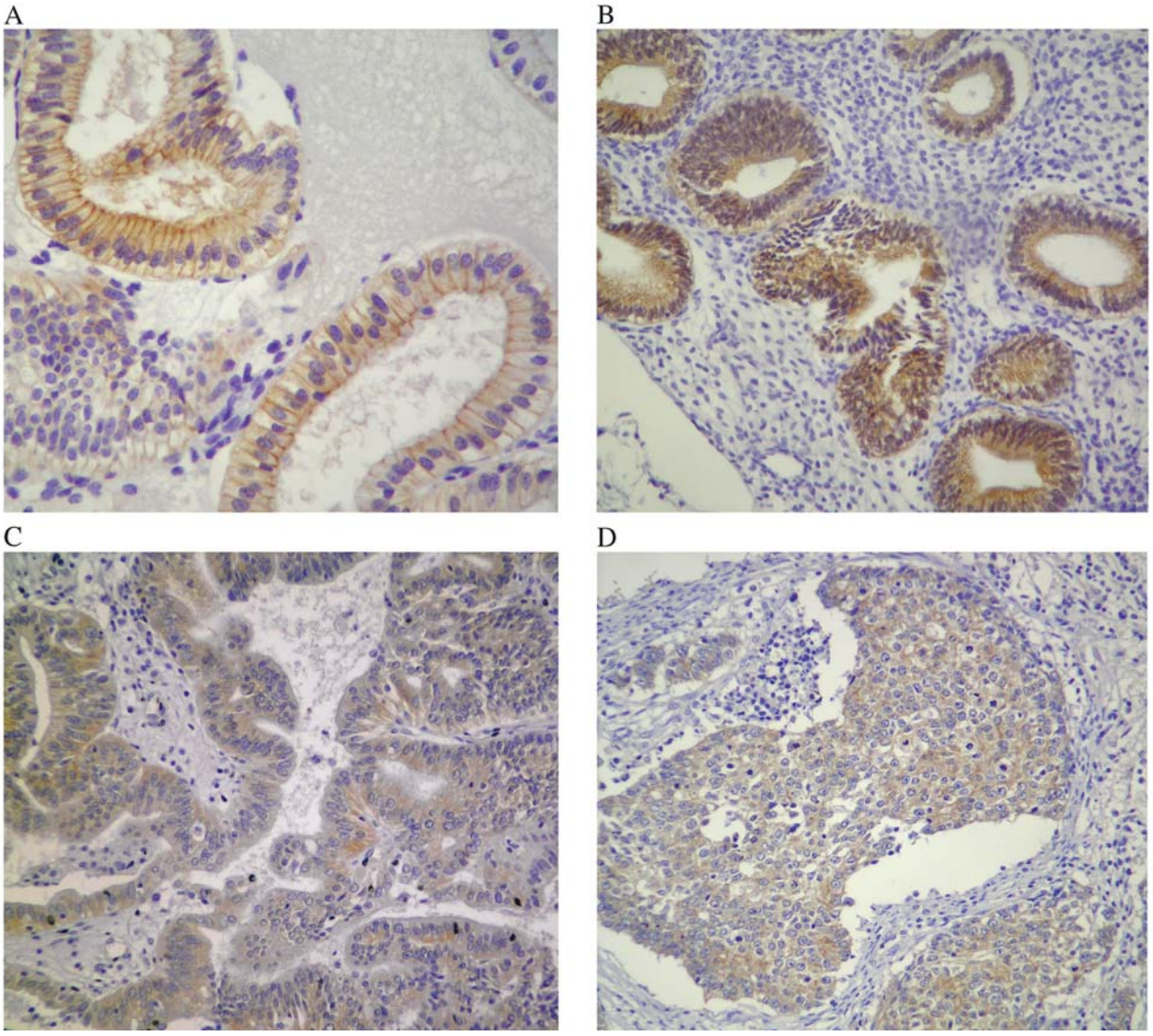

Figure 1. *E-Cadherin and a-catenin immunohistochemical staining. A: E-Cadherin immunoreactivity at the cell borders in normal glandular epithelium (original magnification, ×40). B: Homogenous a-catenin immunoreactivity in simple hyperplasia (original magnification, ×20). C: Hetereogenous a-catenin expression in atypical hyperplasia (original magnification, x40). D: Homogeneous expression restricted to focal areas of well-differentiated carcinoma (original magnification, ×20).*

cases in postmenopausal women interestingly showed the same pattern for E-cadherin and α-catenin expression. A homogeneous distribution was seen in 18 out of 21 specimens (85.7%), whereas only 14.3% cases were negative for both molecules.

A total of 24 cases of simple hyperplasia and 11 cases of atypical hyperplasia were also analyzed. Homogeneous E-cadherin and α-catenin expression was seen in 58.3% cases and 66.6% cases respectively (Figure 1B). Homogeneous expression of E-cadherin and its associated cytoplasmatic molecule was not seen in atypical hyperplasia. E-Cadherin heterogeneous expression was seen in 63.6% cases of atypical hyperplasia; moreover 45.5% of cases of atypical hyperplasia showed heterogeneous α-catenin expression (Figure 1C) whereas 54.5% of cases were unreactive for α-catenin

Twenty-two cases of endometrial adenocarcinoma were analyzed: seven cases were classified as G1, 9 cases were classified as G2 and six cases were classified as G3. E-Cadherin homogeneous expression was never seen (68.2% of adenocarcinoma cases showed an heterogeneous expression and 31.8% were unreactive); α-catenin homogeneous expression was seen in four cases (2 G1 and 2 G2 tumor). Immunostaining was restricted to focal areas of well-differentiated carcinoma (Figure 1D).

Table II. *Statistical significance of differences in E-Cadherin and α-Catenin expression.*

	E-Cadherin*			α-Catenin**			E-Cad/α-Cat***
Histology	Negative	Heterogeneous	Homogeneous	Negative	Heterogeneous	Homogenous	Homogeneous
Normal endometrium	11/67 (16.4%)	7/67 (10.5%)	49/67 (73.1%)	6/67 (9%)	6/67 (9%)	55/67 (82%)	47/67 (70%)
Simple hyperplasia	6/24 (25%)	4/24 (16.7%)	14/24 (58.3%)	6/24 (25%)	2/24 (8.4%)	16/24 (66.6%)	11/24 (46%)
Atypical hyperplasia/ adenocarcinoma	11/33 (33.3%)	22/33 (66.7%)	0/33 (0)	12/33 (36.4%)	17/33 (51.5%)	4/33 (12.1%)	0/33 (0)

Pearson χ^2: *p=<0.0001, **p=<0.0001, ***p=<0.01.

A homogeneous co-expression of both adhesion molecules was seen in 70% and in 46.0% of cases of normal and simple hyperplastic endometrium, respectively; no atypical hyperplasia or adenocarcinoma samples showed such a co-expression.

The histological diagnoses were correlated with the presence of immunostaining and the pattern of distribution (normal endometrium, simple hyperplasia, *vs*. atypical hyperplasia and adenocarcinoma). The correlation between E-cadherin expression (p<0.0001), α-catenin expression (p<0.0001) and the histological diagnosis was statistically significant; furthermore, no co-expression of both adhesion molecules was observed in atypical hyperplasia and adenocarcinoma samples (p<0.01) (Table II).

Discussion

Alterations in cell adhesion are among the hallmark characteristics of a malignant tumor, including irregularities in expression and distribution of adhesion molecules (1, 2). Decreased expression of E-cadherin, a protein essential for the establishment of cell–cell contacts, has been detected in a significant number of tumors of different epithelial origin (12). Since the association of E-cadherin with catenins is essential for a proper anchorage to the cytoskeleton and is necessary for E-cadherin binding function (13), this study investigated the co-expression of E-cadherin and α-catenin in normal, hyperplastic and neoplastic endometrium.

According to previous studies (31, 32), E-cadherin immunostaining showed features similar to that of α-catenin in endometrial samples. In normal endometrium, a strong and homogeneous expression was seen in contrast to that observed in atypical hyperplastic or neoplastic samples, where the intensity of staining was clearly decreased. The expression of E-cadherin and α-catenin did not change during the menstrual cycle, which is consistent with the results of a previous study (32). Morphologically normal endometrial cells showed a polarized E-cadherin and α-catenin expression which was uniformly localized at cell-to-cell borders. It is worth emphasizing that such a localized expression was never seen here in atypical or neoplastic endometrial cells, where the immunostaining was weak and found mainly in the cytoplasm. This may be linked to the fact that such an altered localization can be due to abnormal accumulation of E-cadherin and α-catenin in the cytoplasm so that no adhesion function can be expressed. Moreover different areas of adenocarcinoma samples showed different pattern of E-cadherin and α-catenin expression thus reflecting the intratumoral heterogeneity of neoplastic epithelium.

In simple hyperplastic endometrium, homogeneous E-cadherin (58.3%) and α-catenin (66.6%) expression was decreased; although no direct evidence for a correlation between the E-cadherin/α-catenin status has been reported, this evidence suggests that the lost of E-cadherin interactions with the cadherin–catenin complex might be impaired early in the hyperplastic process.

No atypical hyperplasia or adenocarcinoma samples showed a homogeneous co-expression for both adhesion molecules, suggesting that their expression might provide an additional criterion to define endometrial malignancies.

These findings indicate that alterations of these adhesion proteins are involved in endometrial cancers. Further studies are necessary in order to evaluate the biologic significance of adhesive functions, during endometrial carcinoma progression.

Acknowledgements

We thank Dr. Carlo Falasca for technical assistance and Dr. Armando Bartolazzi for expert advice.

References

1 Takeichi M: Cadherins in cancer: implication for invasion and metastasis. Curr Opin Cell Biol *5*: 806-811, 1993.

2 Shiozaki H, Oka H, Inoue M, Tamura A and Monden M: E-Cadherin mediated adhesion system in cancer cells. Cancer *77*: 1605-1613, 1996.

3 Aplin AE, Howe A, Alahari SK and Juliano RL: Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobulin–cell–adhesion molecules, and selectins. Pharmacol Rev *50*: 197-263, 1998.

4 Piepenhagen PA and Nelson WJ: Defining E-cadherin associated protein complexes in epithelial cells: plakoglobin, β-catenin and γ-catenin are distinct components. J Cell Sci *104*: 751-762, 1993.
5 Hinck L, Nathke IS, Papkoff J and Nelson WJ: Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. J Cell Biol *125*: 1327-1340, 1994.
6 Shimoyama Y, Nagafuchi A, Fujita S, Gotoh M, Takeichi M, Tsukita S and Hirohashi S: Cadherin dysfunction in a human cancer cell line: possible involvement of loss of α-catenin expression in reduced cell–cell adhesiveness. Cancer Res *52*: 5770-5774, 1992.
7 Aberle H, Schwartz H and Kemler R: Cadherin–catenin complex: protein interactions and their implications for cadherin function. J Cell Biochem *61*: 514-523, 1996.
8 Shibamoto S, Hayakawa M, Takeuchi K, Hori T, Miyazawa K, Kitamura N, Johnson KR, Wheelock MJ, Matsuyoshi N, Takeichi M and Ito F: Association of p120, a tyrosine-kinase substrate, with E-cadherin/catenin complexes. J Cell Biol *128*: 949-957, 1995.
9 Daniel JM and Reynolds AB: The tyrosine kinase substrate p120cas binds directly to E-cadherin but not to the adenomatous polyposis coli protein β, or, α-catenin. Mol Cell Biol *15*: 4819-4824, 1995.
10 Downing JR and Reynolds AB: PDGF, CSF-1 and EGF induce tyrosine phosphorylation of p120, a pp60src transformation substrate. Oncogene *6*: 607-613, 1991.
11 Hashimoto M, Niwa O, Nitta Y, Takeichi M and Yokoro K: Unstable expression of E-cadherin adhesion molecules in metastatic ovarian tumor cells. Jpn J Cancer Res *80*: 459-463, 1989.
12 Frixen UH, Beherens J, Sachs M, Eberle G, Voss B, Warda A, lochner D and Birchmeier W: E-Cadherin mediated cell–cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol *113*: 173-185, 1991.
13 Van Aken E, De Wever O, Correia da Rocha AS and Mareel M: Defective E-cadherin/catenin complexes in human cancer. Virchows Arch *439*: 725-751, 2001.
14 Serini G, Trusolino L, Maggiorato E, Cremona O, de Rossi M, Angeli A, Orlandi F and Marchisio PC: Changes in integrin and E-cadherin expression in neoplastic *versus* normal thyroid tissue. J Natl Cancer Inst *88*: 442-449, 1996.
15 Buongiorno PF, al-Kasspooles M, Lee SW, Rachwal WJ, Moore JH, Whyte RI, Orringer MB and Beer DG: E-Cadherin expression in primary and metastatic thoracic neoplasms and in Barrett's oesophagus. Br J Cancer *71*: 166-172,1995.
16 Siitonen SM, Kononen JTT, Helin HJ, Rantala IS, Holli KA and Isola JJ: Reduced E-cadherin expression is associated with invasiveness and unfavorable prognosis in breast cancer. Am J Clin Pathol *105*: 394-402, 1996.
17 Sung-Chul L and Mi-Sook L: Significance of E-cadherin/β-catenin complex and cyclin D1 in breast cancer. Oncol Rep *9*: 915-928, 2002.
18 Oka H, H Shiozaki H, Kobayashi K, Tahara H, Tamura S, Miyata M, Doki Y, Iihara K, Mattsuyoshi N and Hirano S: Immunohistochemical evaluation of E-cadherin adhesion molecule expression in human gastric cancer. Virchows Arch A Pathol Anat Histopathol *421*: 149-156, 1992.
19 Weinel RJ, Neumann K, Kisker O and Rosendahl A: Expression and potential role of E-cadherin in pancreatic carcinoma. Int J Pancreatol *19*: 25-30, 1996.
20 Wakatsuki S, Watanabe R, Saito K, Katagiri A, Sato S and Tomita Y: Loss of human E-cadherin (ECD) correlated with invasiveness of transitional cell cancer in the renal pelvis, ureter and urinary bladder. Cancer Lett *103*: 11-17, 1996.
21 Cheng L, Nagabhushan M, Pretlow TP, Amini SB and Pretlow TG: Expression of E-cadherin in primary and metastatic prostate cancer. Am J Pathol *148*: 1375-1380, 1996.
22 Danen EH, de Vries TJ, Morandini R, Ghanem GG, Ruiter DJ and van Muijen GN: E-Cadherin expression in human melanoma. Melanoma Res *6*: 127-131, 1996.
23 Tohma Y, Yamashima T and Yamashita J: Immunohistochemical localization of cell adhesion molecule epithelial cadherin in human arachnoid villi and meningiomas. Cancer Res *52*: 1981-1987, 1992.
24 Vessey CJ, Wilding J, Folarin N, Hirano S, Takeichi M, Soutter P, Stamp GW and Pignatelli M: Altered expression and function of E-cadherin in cervical intraepithelial neoplasia and invasive squamous cell carcinoma. J Pathol *176*: 151-159, 1995.
25 Carico E, Atlante M, Bucci B, Nofroni I and Vecchione A: E-Cadherin and α-catenin expression during tumor progression of cervical epithelium. Gynecol Oncol *80*: 156-161, 2001.
26 Carico E, Fulciniti F. Giovagnoli MR, Losito NS, Botti G, Benincasa G, Farnetano MG and Vecchione A: Adhesion molecules and p16 expression in endocervical adenocarcinoma. Virchows Arch *455*: 245-251, 2009.
27 Hideyuki N, Tsuyoshi S, Hiroshi Y, Hisanobu M, Eiki I and Ryuichi K: Nuclear localization of β-catenin in normal and carcinogenic endometrium. Mol Carcinog *25*: 207-218, 1999.
28 Moreno-Bueno G, Hardisson D, Sarrio D, Sanchez C, Cassia R, Prat J, Herman JG, Esteller M, Matias-Guiu X and Palacios J: Abnormalities of E- and P-cadherin and catenin (beta-, gamma catenin, and p120ctn) expression in endometrial cancer and endometrial atypical hyperplasia. J Pathol *199*: 471-478, 2003.
29 Shih HC, Shiozawa T, Miyamoto T, Kashima H, Feng YZ, Kurai M and Konishi I: Immunohistochemical expression of E-cadherin and beta-catenin in the normal and malignant human endometrium: an inverse correlation between E-cadherin and nuclear beta-catenin expression. Anticancer Res *24*: 3843-3850, 2004.
30 Tsuchiya B, Sato Y, Kameya T, Okayasu I and Mukai K: Differential expression of N-cadherin and E-cadherin in normal human tissues. Arch Histol Cytol *69*: 135-145, 2006.
31 Veatch AL, Carson LF and Ramakrishnan S: Differential expression of the cell–cell adhesion molecule E-cadherin in ascites and solid human ovarian tumor cells. Int J Cancer *58*: 393-399, 1994.
32 Risinger JI, Berchuck A, Kohler MF and Boyd J: Mutations of the E-cadherin gene in human gynecologic cancers. Nat Genet *7*: 98-102, 1994.
33 Noyes R, Hertig A and Rock J: Dating the endometrial biopsy. Am J Obstet Gynecol *122*: 262-263, 1975.

Received September 30, 2010
Revised November 9, 2010
Accepted November 11, 2010