as the leukocyte enhancing factor (LEF) and T-cell factor (TCF) families of HMG box transcription factors.[12,13] It has been shown that co-transfection of β-catenin with TCF/LEF results in the nuclear accumulation of β-catenin.[12,14] The effect of Wnt-1 on β-catenin and the implication of β-catenin in cell-cell adhesion and signal transduction suggest that alteration of β-catenin may be involved in tumor formation.[15] The APC protein downregulates β-catenin levels by cooperating with glycogen synthase kinase 3 (GSK-3β), inducing phosphorylation of the serine-threonine residues coded in exon 3 of the β-catenin gene,[16,17] and the degradation of β-catenin through the ubiquitin-proteasome pathway. Mutation in exon 3 of the β-catenin gene results in stabilization of the protein, cytoplasmic and nuclear accumulation, and participation in signal transduction and transcriptional activation through the formation of complexes with DNA binding proteins.[18] β-Catenin mutation plays a critical role in the development of some cancers, e.g., in the prostate,[19] thyroid,[20] colon,[21,22] liver,[22] and ovary,[18] and, in rodents, molecular abnormalities of the β-catenin gene have also been reported in some endometrial cancers, in which β-catenin abnormally accumulates in the nuclei.[24,25] Recently, we showed that intensive staining of β-catenin in the nuclei occurred in 60.0% of endometrial hyperplasia samples and 30.0% of endometrial cancer samples and, furthermore, in normal endometrium, β-catenin showed nuclear localization according to cell proliferation during the menstrual cycle, suggesting that the phenomenon of nuclear localization of β-catenin observed in endometrial hyperplasia and endometrial cancer means that β-catenin/WNT-1 signal transduction is highly activated in carcinogenesis of the endometrium, as well as in the physiological condition.[25] However, at which stage of the endometrial carcinogenesis the mutation of β-catenin occurs is still unclear.

Considering this evidence, we focused on mutations in the exon 3 region of the β-catenin gene in endometrial hyperplasia and endometrial cancer associated with endometrial hyperplasia, and their roles in the carcinogenesis and tumor progression of endometrial cancer. Thus, in this study, we performed direct sequencing of exon 3 of the β-catenin gene in 25 patients with endometrial hyperplasia and 20 patients with endometrial cancer associated with endometrial hyperplasia. Furthermore, for the samples of endometrial cancer associated with endometrial hyperplasia, we analyzed both the cancer and the hyperplasia parts, dissecting their DNAs separately.

Patients, samples, and methods

Patients and samples

Samples of endometrial tissues were obtained from 80 women who had undergone hysterectomy or curettage at the Sapporo Medical University Hospital. Biopsy samples were obtained according to institutional guidelines (university hospital), and informed consent was obtained from the patients. Endometrial hyperplasia tissues (n = 25) were taken from the endometrial curettage and diagnosed according to the system of the World Health Organization. Ten cases were found to be simple hyperplasia, 7 were complex hyperplasia, and 8 were atypical hyperplasia. The endometrial hyperplasia samples were fixed in 10% buffered formalin for immunohistochemistry, and parts of them were frozen and kept at −80°C until the analysis. Patients with endometrial adenocarcinoma received modified radical hysterectomy, salpingo-oophorectomy, or selective pelvic lymphadenectomy, with or without para-aortic lymphadenectomy, and the tissues were fixed overnight in 10% buffered formalin, dehydrated, and embedded in paraffin. The endometrial carcinomas were graded according to the system of the World Health Organization. Of the endometrial cancer specimens, 20 endometrial cancer samples that were associated with endometrial hyperplasia were selected and used for the analysis. Of the 20 endometrial cancer samples, 11 were associated with simple endometrial hyperplasia and 9 with complex endometrial hyperplasia; both the cancer and hyperplasia were evaluated by immunohistochemistry and mutational analysis.

Immunohistochemistry

Five-micrometer serial sections of each sample were used in this study. Sections were cut, floated onto albumin-coated slides, dried at 56°C, deparaffinized in xylene, rehydrated, and washed with phosphate-buffered saline (PBS) for 15 min at room temperature. The specimens were treated in a microwave oven in 0.01 mol/l citrate buffer (pH 6.0) for 30 min at 100°C, slowly cooled to room temperature, and then washed with PBS for 5 min at room temperature. After the quenching of endogenous peroxidase with 3% hydrogen peroxide in PBS for 10 min at room temperature, the sections were incubated with a blocking solution (PBS containing 5% skim milk) for 60 min at room temperature. Then the slides were incubated overnight at 4°C with a 1:500 dilution of anti-β-catenin (Transduction Laboratories, Lexington, KY, USA). After several washes with PBS, they were incubated with a second antibody, a 1:200 dilution of peroxidase-conjugated anti-mouse immunoglobulin (Dakopatts, Glostrup, Denmark), for 2 h. The color reaction was developed by the silver intensification procedure described previously.[26] For the negative control, the same dilution of non-immunized mouse immunogloblin was used as for the first antibody.

Staining evaluation was performed by two independent observers (K. A. and T. S.) without knowledge of the clinical outcome. For each tissue sample, the nuclear localization of β-catenin was evaluated as: 0, negative; 1+, positive; or 2+, intensely positive. For the samples that contained both the endometrial hyperplasia and cancer, the localization was analyzed in both parts. One of the serial sections from each subject was stained with H&E for histological evaluation.

DNA extraction from paraffin-embedded and frozen tissues

For the paraffin-embedded and formalin-fixed tissue samples, in 20 endometrial cancers associated with hyperplasia and 5 endometrial hyperplasias, several serial sections (5-μm-thick) were prepared; the first slide was stained with H&E and the others were left unstained. On review of the H&E-stained section, the tumor area was encircled with a 23-gauge needle. The tissue in the selected area was dissolved by heated DEXPAT (Takara, Tokyo, Japan) solution, using a micropipette, and DNA was extracted according the manufacturer's protocol. For the samples that contained both endometrial cancer and endometrial hyperplasia, each part was selected separately and DNA was extracted from each part. For the frozen tissue samples, in the 20 endometrial hyperplasias, DNA was extracted from each tumor, using Proteinase K, sodium dodecyl-sulfate, and phenol-chloroform.

Polymerase chain reaction (PCR) and direct sequencing of the β-catenin gene

Total DNA was extracted from the 25 endometrial hyperplasias and 20 endometrial cancers (20 hyperplasia parts and 20 cancer parts). A supernatant of DEXPAT was applied to 25μl of PCR mixture, containing 2.5U of AmpliTaq DNA polymerase (Takara, Tokyo, Japan), 1.5mmol/l $MgCl_2$, 1× Taq buffer, and 0.2mmol/l of four deoxynucleotide triphosphates (dNTPs). The forward primer was 5′-ttgatggagttggacatgg-3′ and the reverse primer was 5′-caggacttgggaggtatcca-3′. Thirty-five cycles of PCR were carried out, with a program of 30s at 94°C, 1min at 55°C, and 1min at 72°C. The DNA sequence of the PCR products was determined using an ABI 100 Model 377, version 3.0 (PE-Biosystem, Foster City, CA, USA), with the same primers as those used for the PCR reaction.

Results

Subcellular localization of β-catenin and mutation of the β-catenin gene in endometrial hyperplasia

In this study, we analyzed the subcellular localization of β-catenin by immunohistochemistry and mutation in the exon 3 region of the β-catenin gene by direct sequencing of 25 endometrial hyperplasia samples (10 simple endometrial hyperplasias, 7 complex endometrial hyperplasias, and 8 atypical endometrial hyperplasias). The results are summarized in Table 1. In the 25 endometrial hyperplasia samples, β-catenin was detected only in the membrane in 11 samples (44.0%), and in the other 14 samples (56.0%) β-catenin showed nuclear localization. Although all the 25 hyperplasia samples were analyzed for genetic alteration in the exon 3 region of the β-catenin gene, we could not find any mutation in them.

Table 1. Mutation and subcellular localization of β-catenin in endometrial hyperplasia

Sample no.	Histology	Nuclear localization of β-catenin	Mutation of β-catenin
1	Simple	0	No
2	Simple	0	No
3	Simple	0	No
4	Simple	0	No
5	Simple	1+	No
6	Simple	1+	No
7	Simple	1+	No
8	Simple	2+	No
9	Simple	2+	No
10	Simple	2+	No
11	Complex	0	No
12	Complex	0	No
13	Complex	0	No
14	Complex	2+	No
15	Complex	2+	No
16	Complex	2+	No
17	Complex	2+	No
18	Atypical	0	No
19	Atypical	0	No
20	Atypical	0	No
21	Atypical	0	No
22	Atypical	1+	No
23	Atypical	2+	No
24	Atypical	2+	No
25	Atypical	2+	No

0, Negative; 1+, Positive; 2+, Intensely positive

Subcellular localization of β-catenin and mutation of the β-catenin gene in endometrial cancer associated with endometrial hyperplasia

In this study, we analyzed the subcellular localization of β-catenin and mutation in the exon 3 region of the β-catenin gene in 20 endometrial cancers associated with endometrial hyperplasia (16 were G1, 3 were G2, and 1 was G3). Furthermore, to determine genetic alterations of β-catenin in endometrial hyperplasia and in cancer, we separately extracted DNA from the cancer part (Fig. 1a) and the hyperplasia part (Fig. 1b) in the 20 endometrial cancer samples associated with endometrial hyperplasia. The results for subcellular localization of β-catenin and mutation of the β-catenin gene are shown in Table 2. In the 20 endometrial cancer samples, β-catenin was detected in the membrane (Fig. 2a) in 8 samples (40.0%), and in the other 12 samples (60.0%) it showed nuclear localization (Fig. 2b). In the associated hyperplasia, β-catenin was localized only in the membrane in 9 samples (45.0%), and nuclear localization was detected in 11 samples (55.0%). In 10 samples, the nuclear localization was detected both in the cancer and in the associated hyperplasia.

As shown in Table 2, in the 20 endometrial cancer samples, mutation in the exon 3 region of the β-catenin gene was found in two samples, no. 9 (GCT^{Ser} to GTT^{Val} in codon 43; Fig. 3a) and no. 15 (GAC^{Asp} to GAG^{Glu} in codon 32; Fig. 3c), both of which had the nuclear localization of β-catenin. However, in the associated endometrial hyperplasias, the mutation was not found in any of the 20 samples (Fig. 3b,d).

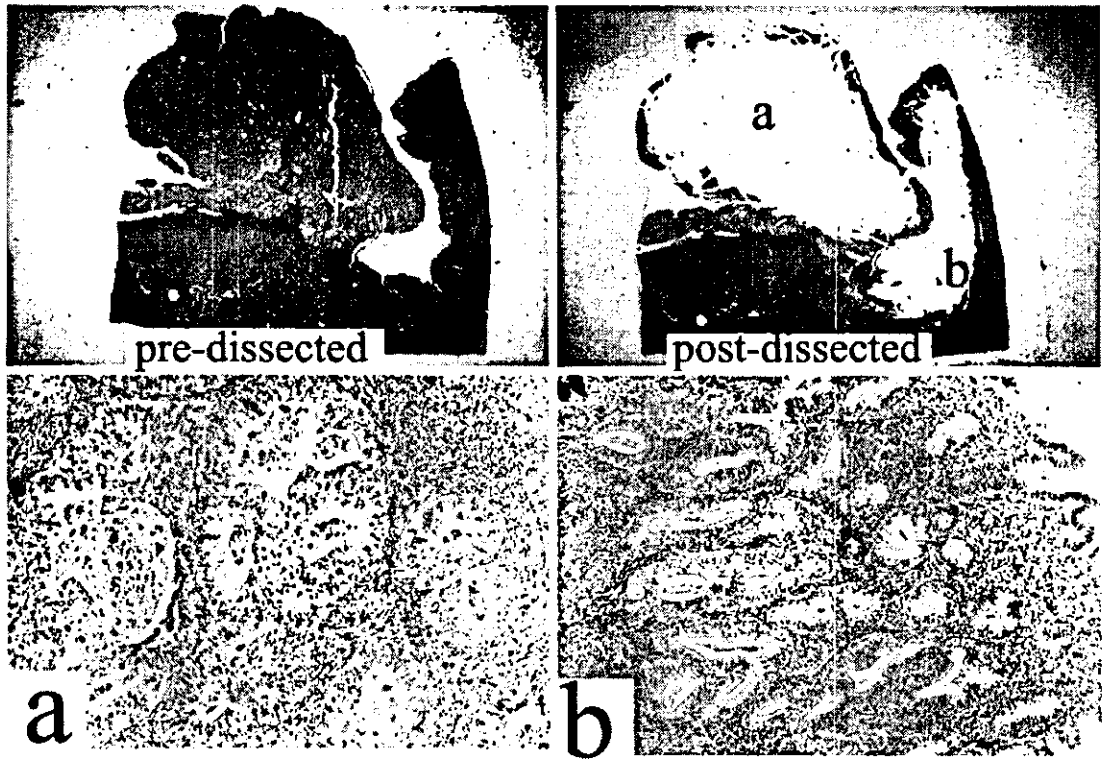

Fig. 1a,b. Dissection and extraction of DNA from endometrial cancer and associated hyperplasia. DNA was extracted from both the endometrial cancer part and the associated hyperplasia part (sample no. 9 in Table 2). **a** Endometrial cancer part and **b** associated hyperplasia part **a** H&E, ×10; **b** H&E, ×100

Table 2. Subcellular localization and mutation of β-catenin in samples of endometrial cancer associated with hyperplasia

Sample no.	Histology of		Nuclear localization of β-catenin in		Mutation of β-catenin in	
	Cancer	Hyperplasia	Cancer	Hyperplasia	Cancer	Hyperplasia
1	G1	Simple	1+	1+	No	No
2	G1	Simple	0	0	No	No
3	G1	Simple	0	0	No	No
4	G1	Simple	0	2+	No	No
5	G1	Simple	0	0	No	No
6	G1	Simple	0	0	No	No
7	G1	Simple	2+	2+	No	No
8	G1	Simple	2+	2+	No	No
9	G1	Complex	2+	2+	Codon 43 GCT^{Ser}-GTT^{Val}	No
10	G1	Simple	1+	1+	No	No
11	G1	Complex	2+	0	No	No
12	G1	Complex	0	0	No	No
13	G1	Complex	1+	0	No	No
14	G1	Complex	1+	1+	No	No
15	G1	Complex	2+	2+	No	No
16	G1	Complex	2+	2+	Codon 32 GAC^{Asp}-GAG^{Glu}	No
17	G2	Simple	0	0	No	No
18	G2	Complex	2+	1+	No	No
19	G2	Complex	2+	2+	No	No
20	G3	Complex	0	0	No	No

0, Negative; 1+, Positive; 2+, Intensely positive

Fig. 2a–d. Immunohistochemistry of β-catenin in endometrial cancer and associated hyperplasia. In the 20 endometrial cancer samples, β-catenin was detected in the membrane (**a**) in eight samples (40.0%), and in the other 12 samples (60.0%) it showed nuclear localization (**b**). In the associated hyperplasia, β-catenin was localized only in the membrane (**c**) in 9 samples (45.0%), and nuclear localization (**d**) was detected in 11 samples (55.0%). **a–d** ×400

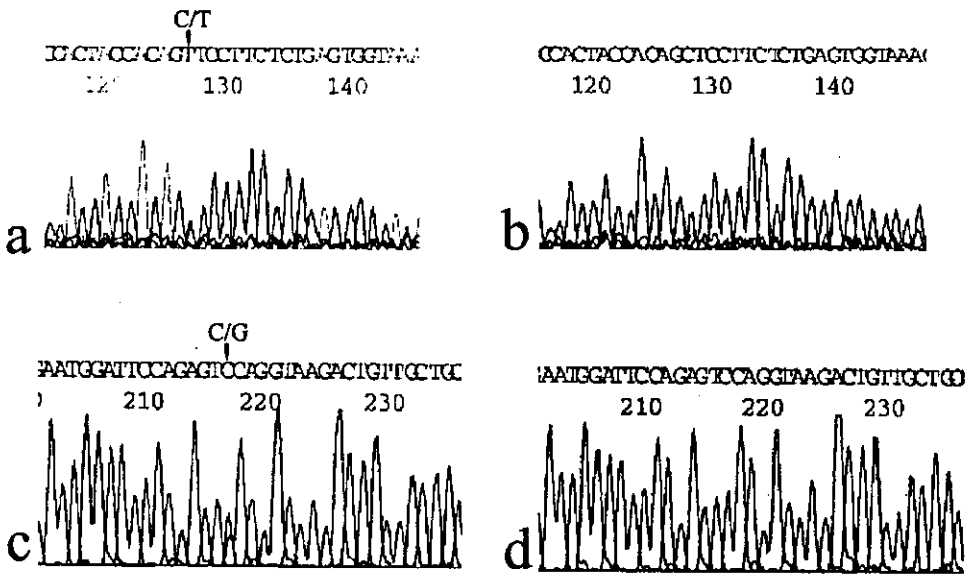

Fig. 3a–d. Mutation in exon 3 of the β-catenin gene in endometrial cancer but not in hyperplasia. **a** Cancerous part of sample no. 9; mutation, GCT^{Ser} to GTT^{Val} in codon 43, was detected. **b** Hyperplastic part of sample no. 9; mutation was not detected. **c** Cancerous part of sample no. 15; mutation, GAC^{Asp} to GAG^{Glu} in codon 32, was detected. **d** Hyperplastic part of sample no. 15; mutation was not detected

Discussion

In this study, we analyzed the β-catenin gene in 25 endometrial hyperplasia samples (10 simple hyperplasias, 7 complex hyperplasias, and 8 atypical endometrial hyperplasias) and in 20 endometrial cancer samples that were associated with endometrial hyperplasia. For the endometrial cancer samples, DNA was digested from the cancer part and the hyperplasia part separately. Thus, a total of 65 DNA samples were analyzed in this study.

In this study, 14 of the 25 endometrial hyperplasia samples (56.0%) showed nuclear localization of β-catenin by immunohistochemistry. However, we could not find any mutation in the exon 3 region of the β-catenin gene in any of the endometrial hyperplasia samples. In a previous study, we observed intense staining of β-catenin in the nuclei in 60.0% of endometrial hyperplasia samples and, furthermore, in normal endometrium, β-catenin showed nuclear localization according to cell proliferation during the menstrual cycle.[25] In our previous study, 9 of 30 (30.0%) endometrioid adenocarcinoma samples showed intense nuclear staining of β-catenin, whereas, in this study, nuclear staining was observed in 12 of 20 (60.0%) endometrioid adenocarcinomas. We think that the difference was caused by the differences in the materials, because, in the previous study, we used endometrioid adenocarcinomas, most of which were not associated with hyperplasia, whereas, in the present study, we used endometrioid adenocarcinomas associated with hyperplasia for all samples. The difference in the nuclear staining of β-catenin may indicate the importance of the activation of wnt-1 signaling and the nuclear localization of β-catenin, for endometrial carcinogenesis occurring via endometrial hyperplasia.

As described above, in endometrial cancer, mutation of the β-catenin gene has been reported in our recent study, and by others, suggesting that genetic alteration occurs during endometrial carcinogenesis.[24,25] However, at which stage of endometrial carcinogenesis the mutation occurs is still unclear. To clarify this, we analyzed the genetic alteration in endometrial cancer associated with hyperplasia, collecting DNA from each part by dissection, because it is well known that the majority of endometrial adenocarcinomas occur in a uterus with benign hyperplastic endometrium.[1] In the study, 40 DNA samples, 20 from endometrial cancer and 20 from endometrial hyperplasia associated with the cancer, were analyzed. Although mutation of the β-catenin gene was not detected in the 20 associated endometrial hyperplasia samples, it was detected in 2 of the 20 endometrial cancers (CGT^{Ser} to GTT^{Val} in codon 43, and GAC^{Asp} to GAG^{Glu} in codon 32). This result suggests that genetic alteration of β-catenin had occurred between the complex hyperplasia and the cancer. This inference is supported by the result that the 25 endometrial hyperplasias did not have any mutation of the β-catenin gene, as shown in Table 1. Unfortunately, we could not show any data about the atypical hyperplasia with the cancer in Table 2. Although we have some data about it, and it did not have mutation of β-catenin either, it was difficult to delineate the boundary between the atypical hyperplasia part and the well-differentiated adenocarcinoma part histologically, so we decided that it was impossible to digest DNA from the cancer and hyperplasia part separately and excluded them from this study. Recently, Saegusa et al.[27] demonstrated that mutations of the β-catenin gene were observed in 16 (22.9%) of 70 endometrial carcinomas, as well as in 3 (12.5%) of 24 atypical hyperplasias; however, the mutation was not found in 37 simple or complex hyperplasias. In this study, although we found only two mutations in endometrial cancer and no mutation in endometrial hyperplasias, 10 of 17 (58.8%) simple and complex hyperplasias, 4 of 8 (50.0%) atypical hyperplasias, and 10 of 16 (62.5%) well-differentiated adenocarcinomas showed nuclear localization of β-catenin. This evidence suggests that the activation

of the β-catenin signal pathway, which is, possibly, caused by the activation of β-catenin-associated proteins, e.g., APC and GSK-3β, plays an important role in the early stage of carcinogenesis of the endometrium, rather than the mutation of β-catenin. Furthermore, we must also consider lack of APC transcription caused by promoter hypermethylation, which was found in colorectal carcinomas.[28]

Recently, Samowitz et al.[29] analyzed the sequence of exon 3 of the β-catenin gene from 202 sporadic colorectal tumors; they found that the percentage of β-catenin mutations in small colorectal adenomas was significantly greater than that in large adenomas and invasive cancers. They concluded that mutation of the β-catenin gene could be an early, perhaps initiating, event in colorectal tumorigenesis. Ogawa et al.[30] analyzed exon 3 of the β-catenin gene in the hepatic tumors of B6C3F1 mice, and detected seven mutations in 13 samples dissected from hepatocellular carcinomas, induced by diethylnitrosamine, but none in the 14 hepatic adenomas. These results suggest that, although molecular alteration of the β-catenin gene contributes to carcinogenesis in several organs,[18-25,31] the period at which the molecular alteration of β-catenin occurs during the carcinogenesis may be different among the organs.

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References

1. Westhoff C, Heller D, Drosinos S, Tancer L (2000) Risk factors for hyperplasia-associated versus atrophy-associated endometrial carcinoma. Am J Obstet Gynecol 182:506–508
2. Sherman ME, Sturgeon S, Brinton L, Kurman RJ (1995) Endometrial cancer chemoprevention: implications of diverse pathways of carcinogenesis. J Cell Biochem Suppl 23:160–164
3. Kohler MF, Nishii H, Humphrey PA, Saski H, Marks J, Bast RC, Clarke-Pearson DL, Boyd J, Berchuck A (1993) Mutation of the *p53* tumor-suppressor gene is not a feature of endometrial hyperplasias. Am J Obstet Gynecol 169:690–694
4. Kurman RJ, Kaminski PF, Norris HJ (1985) The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. Cancer 56:403–412
5. Wentz WB (1974) Progestin therapy in endometrial hyperplasia. Gynecol Oncol 2:362–367
6. Kojima T, Sawada N, Kokai Y, Yamamoto M, Mori M, Mochizuki Y (1998) Occludin expression and tight junction strand formation during replicative DNA synthesis in primary cultures of rat hepatocytes. Med Electron Microsc 31:169–176
7. Mori M, Sawada N, Kikai Y, Satoh M (1999) Role of tight junctions in the occurrence of cancer invasion and metastasis. Med Electron Microsc 32:193–198
8. Oyamada M, Oyamada Y, Takamatsu T (1998) Gap junctions in health and disease. Med Electron Microsc 31:115–120
9. Shibata Y, Kumai M, Nishi K, Nakamura K (2001) Diversity and molecular anatomy of gap junctions. Med Electron Microsc 34:153–159
10. Birchmeier W, Behrens J (1994) Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim Biophys Acta 1198:11–26
11. Miller JR, Moon RT (1996) Signal transduction through beta-catenin and specification of cell fate during embryogenesis. Genes Dev 10:2527–2539
12. Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W (1996) Functional interaction of beta-catenin with the transcription factor LEF-1. Nature 382:638–642
13. Korinek V, Barker N, Morin PJ, van Wichen D, de Weger R, Kinzler KW, Vogelstein B, Clevers H (1997) Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. Science 275:1784–1787
14. Huber O, Korn R, McLaughlin J, Ohsugi M, Herrmann BG, Kemler R (1996) Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. Mech Dev 59:3–10
15. Munemitsu S, Albert I, Souza B, Rubinfeld B, Polakis P (1995) Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. Proc Natl Acad Sci U S A 92:3046–3050
16. Rubinfeld B, Souza B, Albert I, Muller O, Chamberlain SH, Masiarz FR, Munemitsu S, Polakis P (1993) Association of the APC gene product with beta-catenin. Science 262:1731–1734
17. Su LK, Vogelstein B, Kinzler KW (1993) Association of the APC tumor suppressor protein with catenins. Science 262:1734–1737
18. Palacios J, Gamallo C (1998) Mutations in the beta-catenin gene (*CTNNB1*) in endometrioid ovarian carcinomas. Cancer Res 58:1344–1347
19. Voeller HJ, Truica CI, Gelmann EP (1998) Beta-catenin mutations in human prostate cancer. Cancer Res 58:2520–2523
20. Garcia-Rostan G, Tallini G, Herrero A, D'Aquila TG, Carcangiu ML, Rimm DL (1999) Frequent mutation and nuclear localization of beta-catenin in anaplastic thyroid carcinoma. Cancer Res 59:1811–1815
21. Iwao K, Nakamori S, Kameyama M, Imaoka S, Kinoshita M, Fukui T, Ishiguro S, Nakamura Y, Miyoshi Y (1998) Activation of the beta-catenin gene by interstitial deletions involving exon 3 in primary colorectal carcinomas without adenomatous polyposis coli mutations. Cancer Res 58:1021–1026
22. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW (1997) Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC [see comments]. Science 275:1787–1790
23. Koch A, Denkhaus D, Albrecht S, Leuschner I, von Schweinitz D, Pietsch T (1999) Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene. Cancer Res 59:269–273
24. Fukuchi T, Sakamoto M, Tsuda H, Maruyama K, Nozawa S, Hirohashi S (1998) Beta-catenin mutation in carcinoma of the uterine endometrium. Cancer Res 58:3526–3528
25. Nei H, Saito T, Yamasaki H, Mizumoto H, Ito E, Kudo R (1999) Nuclear localization of beta-catenin in normal and carcinogenic endometrium. Mol Carcinog 25:207–218
26. Saito T, Schlegel R, Andresson T, Yuge L, Yamamoto M, Yamasaki H (1998) Induction of cell transformation by mutated 16K vacuolar H+-atpase (ductin) is accompanied by down-regulation of gap junctional intercellular communication and translocation of connexin 43 in NIH3T3 cells. Oncogene 17:1673–1680
27. Saegusa M, Hashimura M, Yoshida T, Okayasu I (2001) beta-Catenin mutations and aberrant nuclear expression during endometrial tumorigenesis. Br J Cancer 84:209–217
28. Hiltunen MO, Alhonen L, Koistinaho J, Myohanen S, Paakkonen M, Marin S, Kosma VM, Janne J (1997) Hypermethylation of the APC (adenomatous polyposis coli) gene promoter region in human colorectal carcinoma. Int J Cancer 70:644–648
29. Samowitz WS, Powers MD, Spirio LN, Nollet F, van Roy F, Slattery ML (1999) Beta-catenin mutations are more frequent in small colorectal adenomas than in larger adenomas and invasive carcinomas. Cancer Res 59:1442–1444
30. Ogawa K, Yamada Y, Kishibe K, Ishizaki K, Tokusashi Y (1999) Beta-catenin mutations are frequent in hepatocellular carcinomas but absent in adenomas induced by diethylnitrosamine in B6C3F1 mice. Cancer Res 59:1830–1833
31. Rubinfeld B, Souza B, Albert I, Munemitsu S, Polakis P (1995) The APC protein and E-cadherin form similar but independent complexes with alpha-catenin, beta-catenin, and plakoglobin. J Biol Chem 270:5549–5555

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Loss of γ-Catenin Expression in Squamous Differentiation in Endometrial Carcinomas

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Summary: Squamous differentiation occurs in 25 to 50% of endometrial endometrioid adenocarcinomas. In this study, we analyzed the immunohistochemical expression and localization of β- and γ-catenin, molecular changes in the β-catenin gene, and the subcellular localization of the desmosomal component protein, desmoplakin, by laser scanning microscopy in 35 endometrial carcinomas with squamous differentiation. In the glandular component, β-catenin showed nuclear localization in 10 of 35 (28.5%) samples, whereas in the squamous component, nuclear localization was found in 15 of 35 (42.9%). Of the 15 samples that showed nuclear localization of β-catenin in the squamous component, seven samples (46.7%) did not express γ-catenin. The phenomenon was not correlated with mutation in exon 3 region of β-catenin gene. Furthermore, in these samples, there was diffuse cytoplasmic staining for desmoplakin. These observations have not been reported in other tumors. Our results suggest that unique molecular events, i.e., stimulation of β-catenin and suppression of γ-catenin expression, occur within endometrial carcinomas with squamous differentiation. **Key words:** Endometrium—Endometrioid carcinoma—Squamous differentiation—Desmosome—Catenin—Cell adhesion.

Focal squamous differentiation occurs in 25 to 50% of endometrioid adenocarcinomas of the endometrium (1,2). In some of these tumors, so-called adenoacanthomas, the squamous component consists of benign appearing intraglandular squamous morules, whereas in adenosquamous carcinomas, the squamous component is cytologically malignant and usually invades the stroma. Adenosquamous carcinomas have a poorer prognosis than pure adenocarcinoma and adenoacanthomas (3), but in most studies this difference is thought to be due to the more poorly differentiated glandular component of adenosquamous carcinomas (4).

Catenins constitute the major component of adherens junctions in vertebrates in association with E-cadherin and consist of α-, β-, and γ-catenins (Fig. 1A) (5). Both β- and γ-catenin bind directly to the cytoplasmic domain of E-cadherin whereas α-catenin links the bound β- and γ-catenin to the microfilament network of the cytoskeleton (5). This binding is essential for the establishment of tight physical cell–cell adhesion. In addition to simply supporting cell–cell adhesion, a role for β-catenin in signal transduction has been proposed. The effect of Wnt-1 on β-catenin and implication of β-catenin in cell–cell adhesion and signal transduction suggest that alteration of β-catenin may be involved in tumor formation (6). The adenomatous polyposis coli protein down-regulates β-catenin levels by cooperating with glycogen synthase kinase 3, inducing phosphorylation of the serine-theronine residues coded in exon 3 of the β-catenin gene (7,8) and its degradation through the ubiquitin-proteasome pathway. Mutation in exon 3 of β-catenin results in stabilization of the protein, cytoplasmic and nuclear accumulation, and participation in signal transduction and transcriptional activation through the formation of complexes with DNA binding proteins (9). β-catenin mutation plays a critical role in the development of some cancers, including those of the prostate (10), thyroid (11), colon (12,13), liver (14), and ovary (9), and molecular abnormalities of the β-catenin gene

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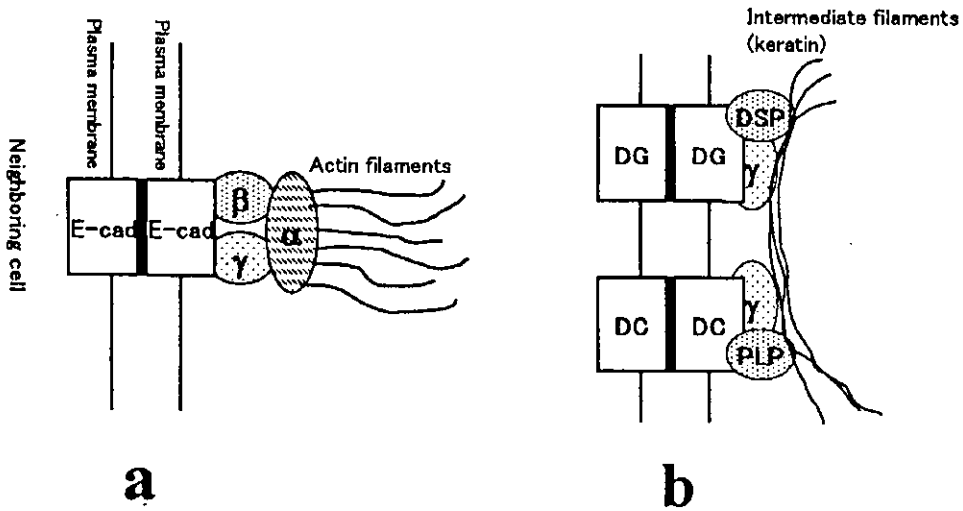

FIG. 1. Component proteins in an adherens junction and a desmosome. (a) Adherens junction: β-catenin (β) and γ-catenin (γ = plakoglobin) can bind, independently, to the cytoplasmic tail of E-cadherin (E-cad) in an adherens junction. Via α-catenin (α), they mediate cadherin association with the actin cytoskeleton. (b) Desmosome: γ-catenin (γ = plakoglobin) binds desmosomal cadherins, desmocollin, and desmoglein in desmosomes, mediating their interaction, via desmoplakin (DSP) and plakophilin (PLP), with intermediate filaments.

have also been reported in some endometrial cancers in which β-catenin abnormally accumulates in the nuclei (15,16). α-catenin, also known as plakoglobin, another vertebrate catenin, is highly homologous to β-catenin (17). Functions of γ-catenin in cell adhesion that are similar to (in adherens junctions) and different (in desmosomes) from those of β-catenin are well established (18). Recent studies indicate the γ-catenin plays a unique role in the Wnt signaling pathway, one that is different from that of β-catenin (19).

Desmosomes are major components of the junctional complex of epithelial cells in which bundles of intermediate filaments connect the two contacting plasma membranes (Fig. 1B). They also appear to be involved in maintaining the structure and function of adjacent cells (20,21). They are composed of several proteins: desmoplakin (DSP) I, DSP II, desmoglein (DG), desmocollin (DC) I, DC II, plakoglobin (γ-catenin), and other minor proteins (17,20). γ-catenin binds to α-catenin and anchors adherens junctions to actin, whereas in desmosomes, it binds to the desmosomal cadherins, DC and DG, and to DSP and keratins (17). In adherens junctions γ-catenin (plakoglobin) binds desmosomal cadherins, DC, and DG in desmosomes, mediating their interaction via DSP and plakophilin with intermediate filaments (17).

We have analyzed the expression and localization of various component proteins of cell-to-cell junctions in endometrial carcinogenesis (16,22–25), and accidentally found that some endometrioid adenocarcinomas with squamous differentiation (EASDs) had lost the expression of γ-catenin. From this evidence, we hypothesized that molecular changes of β- and γ-catenin occur during squamous differentiation of endometrioid adenocarcinoma. To clarify this, we analyzed EASDs for the immunohistochemical expression and localization of γ- and β-catenin and the molecular changes of exon 3 in the γ-catenin gene.

METHODS

Patients and Samples

Samples of endometrial tissues were obtained from 35 EASD derived from 125 (28.0%) endometrial adenocarcinomas removed by hysterectomy at the Sapporo Medical University Hospital. Patients with EASD received modified radical hysterectomy, salpingo-oophorectomy, selective pelvic lymphadenectomy, para-aortic lymphadenectomy, and peritoneal washings. None of the patients had received any preoperative therapy.

The tumors were staged using the International Federation of Gynecology and Obstetrics system and classified and graded according to World Health Organization criteria. Seven of the tumors were grade 1 (G1) (well-differentiated), 23 grade 2 (G2) (moderately differentiated), and 5 grade 3 (G3) (poorly differentiated). In the EASDs that had a benign squamous component, 7 (29.1%) were G1 and 1 (4.2%) was G3, whereas in the tumors that had a malignant squamous component, 4 of 11 (36.4%) were G3 and none were G1. Five tumors (20.8%) with a benign squamous component were stage

III or IV compared with eight cases (72.7%) of those that had malignant squamous component.

Immunohistochemistry

Tissues were fixed overnight in 10% buffered formalin, dehydrated, and embedded in paraffin. Five-micrometer sections were cut, floated onto albumin-coated slides, dried at 56°C, deparaffinized in xylene, rehydrated, and washed with phosphate-buffered saline (PBS) for 15 minutes at room temperature. Specimens were treated in a microwave oven in 0.01 mol/L citrate buffer (pH 6.0) for 30 minutes at 100°C, slowly cooled to room temperature, and then washed with PBS for 5 minutes at room temperature. After quenching endogenous peroxidase with 3% hydrogen peroxide in PBS for 10 minutes at room temperature, the sections were incubated with a blocking solution (PBS containing 5% skim milk) for 60 minutes at room temperature. Then the slides were incubated overnight at 4°C with a 1:500 dilution of anti-β-catenin (Transduction Laboratories, Lexington, KY) and anti-γ-catenin (anti-plakoglobin, Transduction Laboratories). After several washes with PBS, they were incubated with a second antibody, a 1:200 dilution of anti-mouse immunoglobulin (Dakopatts, Glostrup, Denmark), for 2 hours. The color reaction was developed by the silver intensification procedure described previously (25). For the negative control, the same dilution of non-immunized mouse immunoglobulin was used as the first antibody.

Confocal laser scanning microscopy (LSM) observation of DSP was performed for frozen sections of four samples in which the squamous component was histologically malignant and had shown negative expression of γ-catenin. The slides were incubated overnight at 4°C with a 1:5 dilution of anti-DSP I and II (Boehringer Mannheim Biochemica, Indianapolis, IN). A 1:100 dilution of FITC-conjugated anti-mouse immunoglobulin (Dakopatts) was employed as a second antibody, and then the slides were mounted using fluorescent mounting medium (Dakopatts). As the control, G1 and G3 endometrial adenocarcinomas, which were known to have normal expression and localization of β- and γ-catenin, were used. The observation by LSM was performed using an MRC-1024ES (Bio-Rad, Hercules, CA) at 0.2 μm thickness.

Staining Evaluation

Staining evaluation was performed by two independent observers (K.A. and T.S.) without knowledge of clinical outcome. For each tissue sample, the subcellular localizations of β- and γ-catenin were observed in the squamous component and glandular component. The subcellular localization was classified M, N, diffuse and negative: M, the cell-membrane was mostly stained; N, nucleus was mostly stained; diffuse, cytoplasm was diffusely stained; negative, immunostaining was not detected. One of the serial sections was stained with hematoxylin eosin for histological evaluation. Statistical analyses were performed using the Mann-Whitney test.

Polymerase Chain Reaction and Direct Sequencing of the β-Catenin Gene

Total DNA was extracted from the paraffin sections of the 35 tumors. A supernatant of DEXPAT (Takara, Tokyo, Japan) was applied to 25 μl of polymerase chain reaction (PCR) mixture containing 2.5U of AmpliTaq DNA polymerase (Takara), 1.5 mmol/L $MgCl_2$, 1x Taq buffer, and 0.2 mmol/L of four deoxynucleotide triphosphates. The forward primer was 5′-ttgatggagttggacatgg-3′ and the reverse primer 5′-caggacttgggaggtatcca-3′. Thirty-five cycles of PCR were carried out with a program of 30 seconds at 94°C, 1 minute at 55°C, and 1 minute at 72°C. The DNA sequence of the PCR products was determined using an ABI100 Model 377 (PE-Biosystem, Foster City, CA), version 3.0 with the same primers used for the PCR reaction.

RESULTS

Immunohistochemistry

The results of immunohistochemistry are shown in Table 1. As a positive control, a G1 endometrioid adenocarcinoma (Fig. 2A) was used and both β- (Fig. 2B) and γ-catenin (Fig. 2C) was detected at cell–cell contacts. As shown in Table 2, β-catenin showed nuclear localization in the glandular component in 10 of 35 (28.5%) samples: 2 of 7 (28.6%) G1 tumors; 5 of 23 (21.7%) G2 tumors; and 3 of 5 (60.0%) G3 tumors. We found no significant difference between the tumor grade and the nuclear localization of β-catenin because of the small number of samples. γ-catenin showed normal localization in 32 samples (91.4%); in the remainder, it was diffuse in 1, nuclear in 1, and negative in the glandular component in 1. In the 10 samples that showed nuclear localization of β-catenin, 8 cases had cell membrane localization of γ-catenin, 1 had nuclear localization, and 1 case had negative staining (Table 2).

In the squamous component, 15 of 35 samples showed nuclear localization. 15 of the 24 (62.5%) samples that had a benign squamous component showed normal localization of β-catenin in the squamous component (Table 3, Fig. 3A). In all 15 of these cases, γ-catenin was detected in the cell membrane. In the other 9 of 24 (37.5%) samples, β-catenin had nuclear localization in

TABLE 1. *Subcellular localization of β- and γ-catenin and mutation of β-catenin gene in endometrial adenocarcinoma with squamous differentiation*

No.	Histology of squamous component	Grade of glandular component	Surgical stage	Subcellular localization				Mutation of β-catenin
				Squamous component		Glandular component		
				β-catenin	γ-catenin	β-catenin	γ-catenin	
1	benign	G1	Ib	M	M	M	M	negative
2	benign	G1	IIIc	N	negative	M	M	negative
3	benign	G1	IIb	N	negative	N	M	GAC^{Asp} to CAC^{His} in codon 34
4	benign	G1	Ib	N	M	N	M	negative
5	benign	G1	Ib	N	negative	M	M	negative
6	benign	G1	Ib	M	M	M	M	negative
7	benign	G1	Ia	M	M	M	M	negative
8	benign	G2	Ib	N	negative	M	M	negative
9	benign	G2	Ib	M	M	M	M	negative
10	benign	G2	IIIa	M	M	N	M	negative
11	benign	G2	Ib	M	M	M	M	negative
12	benign	G2	Ib	M	M	N	M	negative
13	benign	G2	Ib	M	M	M	M	negative
14	benign	G2	Ic	M	M	M	M	negative
15	benign	G2	Ic	N	negative	M	M	negative
16	benign	G2	Ic	M	M	M	M	negative
17	benign	G2	Ib	N	negative	M	M	negative
18	benign	G2	IVa	M	M	M	M	negative
19	benign	G2	Ic	M	diffuse	M	diffuse	negative
20	benign	G2	Ia	M	M	M	M	negative
21	benign	G2	IIIc	N	N	M	M	negative
22	benign	G2	Ib	N	M	N	M	TCT^{Ser} to TGT^{Cys} in codon 37
23	benign	G2	IIIa	M	M	M	M	negative
24	benign	G3	Ib	M	M	M	M	negative
25	malignant	G2	Ia	N	M	N	M	negative
26	malignant	G2	Ib	M	M	N	N	negative
27	malignant	G2	IIIc	N	negative	M	M	negative
28	malignant	G2	IIIc	N	negative	M	M	negative
29	malignant	G2	IIIa	N	negative	M	M	negative
30	malignant	G2	Ic	M	M	M	M	negative
31	malignant	G2	IIIc	M	M	M	M	negative
32	malignant	G3	IIIa	M	M	N	M	negative
33	malignant	G3	IIIa	N	M	N	M	negative
34	malignant	G3	IVa	N	negative	N	negative	negative
35	malignant	G3	IIIa	M	M	M	M	negative

M, membrane; N, nuclei.

the squamous component (Table 3, Fig. 2B, arrow). In 7 of the 9 (77.8%, $p < 0.05$ compared with samples that had normal localization of γ-catenin in the squamous component), the expression of β-catenin was absent in the squamous component or diffuse in the cytoplasm (Fig. 3C, arrow), although it was normally detected in the glandular component (Fig. 3C arrowhead). Only one case showed cell membrane localization in the squamous component. 6 of 11 (54.5%) samples that had a malignant squamous component (Fig. 4A) showed nuclear localization of β-catenin in the squamous component (Fig. 4B, arrow). In 2 of the 6 (33.3%) samples, γ-catenin showed cell membrane localization in the squamous component, whereas in the other 4 cases, γ-catenin was absent (66.7%, $p = 0.058$ compared with samples that had normal localization of β-catenin in the squamous component), although it was expressed in the glandular component (Fig. 4C). In 4 of 11 samples that had a malignant squamous component, β-catenin showed normal localization, and in these cases γ-catenin was also normal.

As shown in Table 4, 5 of 21 (23.8%) stage I or II tumors showed the loss of γ-catenin expression in the squamous component compared with 5 of 14 (35.7%) stage III or IV tumors. However, there was no significant difference between the groups.

Direct Sequencing of Exon 3 of β-Catenin Gene

In this study, we analyzed the exon 3 region of the β-catenin gene in the 35 samples that were analyzed by immunohistochemistry to determine if they had a mutation in the exon 3 region. As shown in Table 1, in 2 of the 35 samples, mutation was found. Number 4 was GAC^{Asp} to CAC^{His} in codon 34, and number 22 was TCT^{Ser} to TGT^{Cys} in codon 37. In the other 33 samples, no muta-

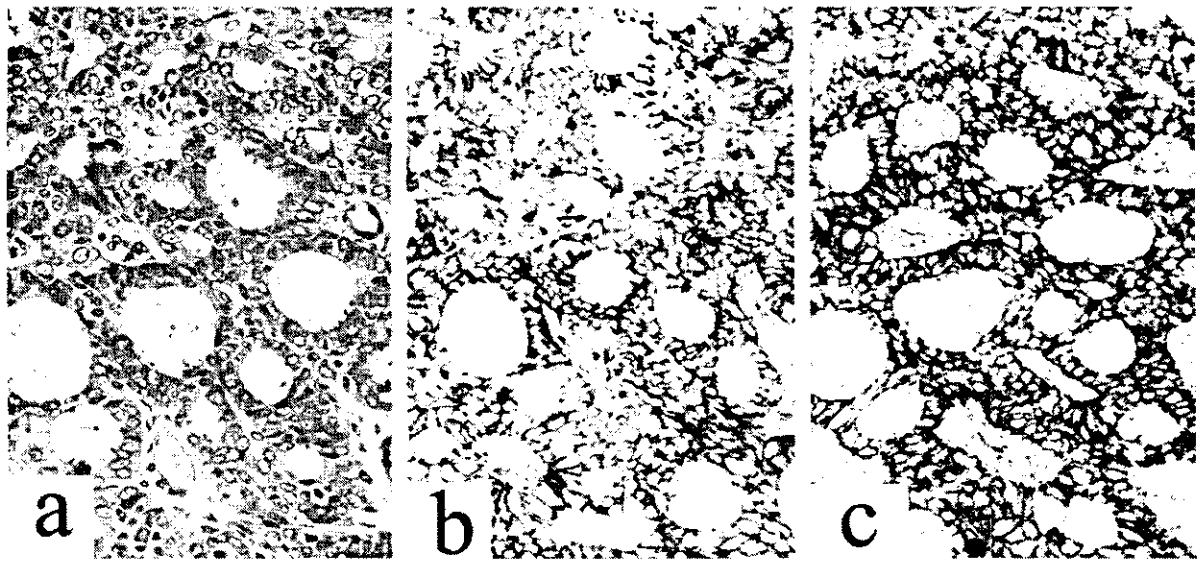

FIG. 2. Immunoreactivity of β- and γ-catenin in grade 1 endometrial adenocarcinoma. As a positive control, a grade 1 endometrial adenocarcinoma was used (a). β- (b) and γ-catenin (c) are localized at cell-to-cell contacts. Bar, 100μm.

tion was found in the exon 3 region. As shown in Table 1, both number 4 and number 22 were among the 10 cases that showed nuclear localization of β-catenin in the glandular component by immunohistochemistry. However, in the 15 cases that showed nuclear localization of β-catenin in the squamous component, mutation was not found.

LSM Observation of Desmosomal Component Proteins

In this study, the subcellular localization of DSP I and II was analyzed by LSM for four frozen sections from the endometrial carcinomas with negative expression of γ-catenin. As a positive control, G1 and G3 endometrial adenocarcinomas, which had normal localization of β- and γ-catenin, were used. As shown in Fig. 5A and 5B, the immunofluorescence was confined to the lateral aspect of individual epithelial cells and appeared as an assembly of spots. On the other hand, all the samples that had shown negative expression of γ-catenin showed diffuse localization in the cytoplasm (Fig. 5C).

DISCUSSION

In this study, in an attempt to confirm that molecular changes of β- and γ-catenin occurred during squamous differentiation in endometrioid adenocarcinoma, we analyzed the immunohistochemical expression and localization of β- and γ-catenin and the mutation of exon 3 in the β-catenin gene in 35 endometrial carcinomas with squamous differentiation.

Findings in this study that were in accord with previous observations included 1.) the proportion of EASDs was 28%; 2.) there was a higher frequency of G3 tumors in those with a malignant squamous component compared with those with a benign squamous component; and 3.) there was a higher frequency of advanced stage (stage III or IV) tumors in those that had a malignant squamous component compared with those with a benign squamous component.

In the glandular component, 10 of 35 (28.5%) tumors showed nuclear localization of β-catenin, and 2 of them had mutations of exon 3 of the β-catenin gene. The other

TABLE 2. *Nuclear localization of β-catenin and expression of γ-catenin in glandular component*

	Grade 1 (n = 7)*		Grade 2 (n = 23)		Grade 3 (n = 5)	
	Membrane	Nucleus	Membrane	Nucleus	Membrane	Nucleus
Subcellular localization of β-catenin	5	2	18	5	2	3
Loss of γ-catenin expression	0	0	0	0	0	1

* n is number of samples.

TABLE 3. *Nuclear localization of β-catenin and expression of γ-catenin in squamous component*

	Benign squamous component (n = 24)*		Malignant squamous component (n = 11)	
	Membrane	Nucleus	Membrane	Nucleus
Subcellular localization of β-catenin	15	9	5	6
Loss of γ-catenin expression	0†	7†	0‡	4‡

* n is number of tumors.
† $p < 0.05$.
‡ $p = 0.058$.

25 of 35 (71.4%) tumors showed normal cell-to-cell localization in the glandular component. β-catenin, via Wnt signaling, is accumulated in the nucleus, complexing with LEF/TCF transcription factors and transactivation of LEF/TCF target genes (26). In our previous study, moderate or strong staining of β-catenin in the nuclei was observed in 60.0% of endometrial hyperplasias, 30.0% of endometrial adenocarcinomas, and in proliferating endometrium, although the β-catenin gene had only two mutations in the nine samples that showed intensive nuclear staining, implying that β-catenin/Wnt-1 signal transduction is highly activated in carcinogenesis of the endometrium without molecular changes of the β-catenin gene (16). The findings in the present study analyzing EASD was in accord with our previous study.

In the squamous component, β-catenin showed nuclear localization in 15 of 35 (42.9%) tumors; only 1 of them had a mutation of the β-catenin gene. Additionally, in 10 samples that showed nuclear localization of β-catenin in the squamous component, it was not observed in the glandular component. Wnt proteins are recognized as one of the major families of developmentally important signaling molecules whose functions may include embryonic induction, the generation of cell polarity, and the specification of cell fate (27). In the endometrium, the activation of Wnt signaling may play an important role not only in carcinogenesis but also squamous differentiation, regardless of the mutation of the β-catenin gene.

In this study, nuclear localization of β-catenin was observed in the squamous component in 15 of the 35 tumors. In 11 of the 15, the expression of γ-catenin was absent in the squamous component, although it was detected in the glandular component. γ-catenin, also known as plakoglobin, another vertebrate catenin, is highly homologous to β-catenin (28). Functions of γ-catenin in cell adhesion that are similar to (in adherens junctions) and different from (in desmosomes) those of β-catenin

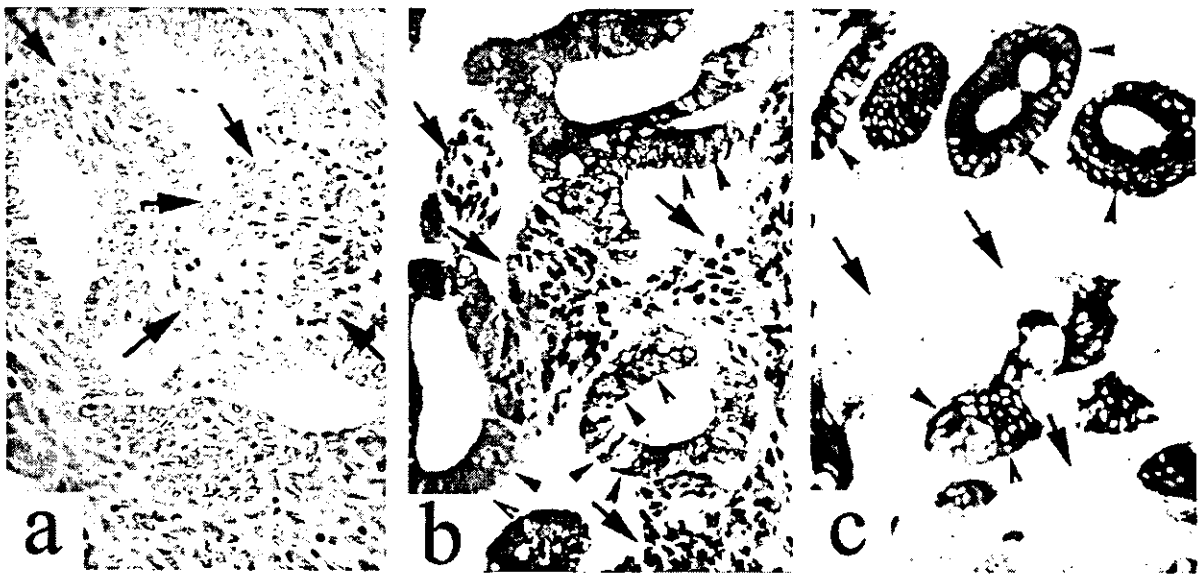

FIG. 3. Immunoreactivity of β- and γ-catenin in an endometrioid adenocarcinoma with a benign squamous component. In the glandular component, β-catenin and γ-catenin are localized at cell-to-cell contacts (b and c, arrowhead), whereas β-catenin shows nuclear localization (b, arrow). Moreover, γ-catenin is absent in the squamous component (c, arrow). (a) hematoxylin eosin; (b) β-catenin; (c) γ-catenin. Bar, 100 μm.

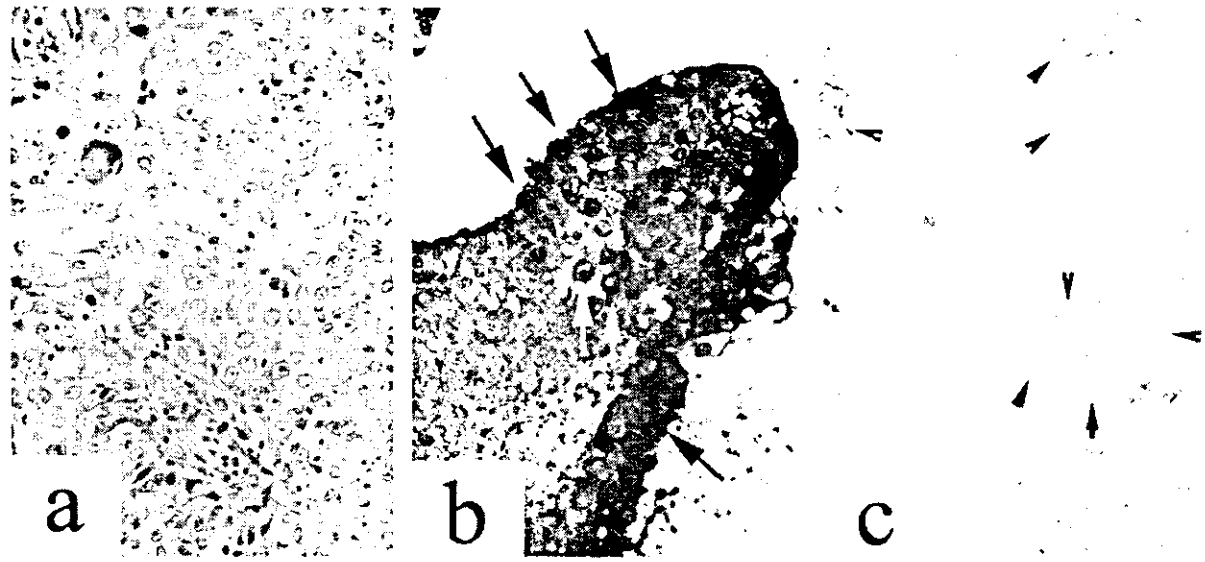

FIG. 4. Immunoreactivity of β- and γ-catenin in an endometrioid adenocarcinoma with a malignant squamous component. β-catenin shows nuclear localization (b, arrow), and γ-catenin is absent or weakly detected at cell-cell contacts (c, arrowhead) in the squamous component. (a) hematoxylin eosin; (b) β-catenin; (c) γ-catenin. Bar, 100 μm.

are well established (18). In contrast to frequent mutations in β-catenin in tumors of different origin (9,13), only one case of γ-catenin mutation has been reported in gastric cancer (29). Moreover, γ-catenin expression is often lost during cancer progression (28) and restoration of γ-catenin expression in several highly tumorigenic cells lacking γ-catenin can suppress their tumorigenicity (30,31). Our results suggest that unique molecular events involving the stimulation of β-catenin and suppression of γ-catenin expression occur during morphological changes in endometrial adenocarcinoma.

Mutation in exon 3 of β-catenin results in stabilization of the protein, cytoplasmic and nuclear accumulation, and participation in signal transduction and transcriptional activation through the formation of complexes with DNA binding proteins. However, as reported in previous studies (15,16), the nuclear localization of β-catenin has been found in 30% of endometrial adenocarcinomas, although most such tumors did not have a β-catenin mutation. In the 15 cases in the present study that showed nuclear localization of β-catenin in the squamous component, mutation was not found. This result suggests that γ-catenin expression is negatively regulated at the transcriptional level when β-catenin is accumulated and Wnt-1 is activated during squamous differentiation in endometrial carcinomas.

In this study, we analyzed the subcellular localization of the desmosomal component proteins DSP I and II by LSM in the samples that had shown the negative expression of γ-catenin. In the control samples, the immunofluorescence was confined to the lateral aspect of individual epithelial cells and appeared as an assembly of spots. However, in the samples that did not have the γ-catenin expression, the fluorescence was observed diffusely in the cytoplasm. Desmosomes are major components of the junctional complex of epithelial cells, by which bundles of intermediate filaments connect the two contacting plasma membranes. They also appear to be involved in maintaining the structures and functions of adjacent cells (20,21). They are composed of several proteins: DSP I, DSP II, DG, DC I, DC II, plakoglobin (γ-catenin), and other minor proteins (17,20). In desmo-

TABLE 4. *Surgical stage and loss of γ-catenin expression*

	Surgical stage I and II (n = 21)*		Surgical stage III and IV (n = 14)	
	Membrane	Nucleus	Membrane	Nucleus
Subcellular localization of β-catenin	14	7	6	8
Loss of γ-catenin expression	0	5	0	5

* n is number of tumors.

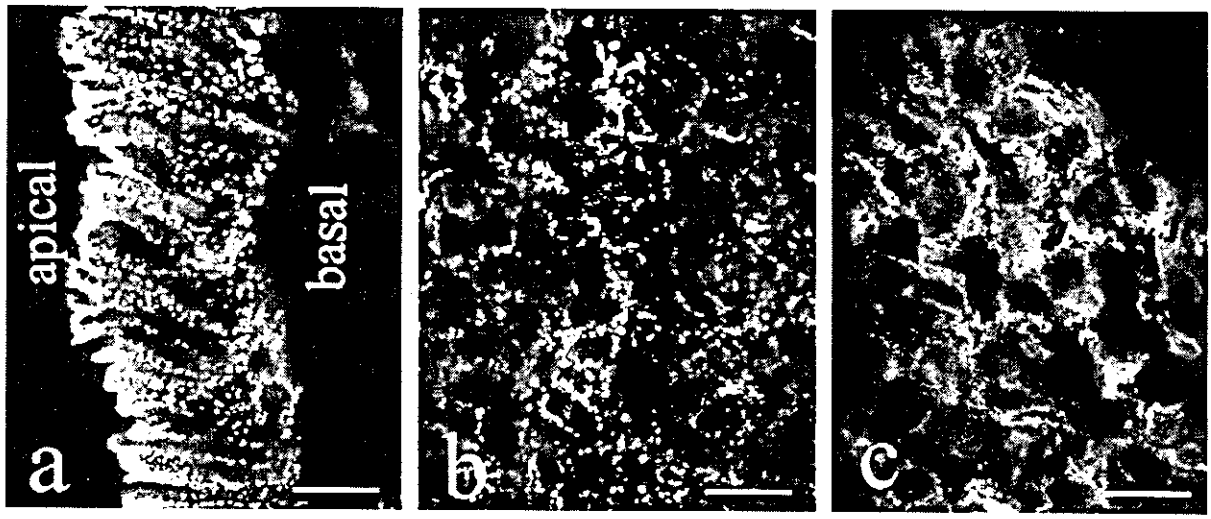

FIG. 5. Laser scanning microscopy observation of desmoplakin I and II in endometrioid adenocarcinomas with squamous differentiation that did not express γ-catenin. In the control samples, a grade 1 (a) and a grade 3 (b) endometrial adenocarcinoma, the immunofluorescence is confined to the lateral aspect of individual epithelial cells and appears as an assembly of spots. In the squamous component (c), where γ-catenin was not detected, desmoplakins are detected diffusely in the cytoplasm. Bar, 5 μm.

somes, γ-catenin binds to the desmosomal cadherins, DC, and DG, and DSP and keratins (17). Thus, the diffuse localization in the cytoplasm of DSP is caused by loss of γ-catenin from the desmosome structure.

Previously, we semi-quantified the desmosomal components, DSP and DG, in tissue sections using LSM and examined their relationship to the pathological type, the occurrence of lymph node metastasis, and the extent of myometrial invasion. Their expression decreased with loss of differentiation and was associated with tumor progression (20). In 4 of 11 tumors in the present study that had a malignant squamous component, the expression of γ-catenin was not detected in the squamous component, and the subcellular localization of DSP was diffuse in the cytoplasm. These tumors were all stage III or IV. Although disturbance of the desmosome structure could be an effect of tumor progression and not a cause, the disturbance may promote tumor progression in some adenosquamous carcinomas.

Recently, Saegusa et al. (32) reported that β-catenin mutations were seen in early tumors and were less likely to be seen in advanced endometrial carcinomas with lymph node metastasis. In this study, we found two mutations in the exon 3 region of the β-catenin gene, and both tumors were early stage (stage Ib), and thus, on this point, our study was in accord with the recent study. In our study, 5 of 21 (23.8%) stage I or II tumors showed the loss of γ-catenin expression in the squamous component compared with 5 of 14 (35.7%) stage III or IV tumors. These results seem to contradict those of Saegusa et al. because the loss of γ-catenin expression was in accord with the nuclear localization of β-catenin. However, the study of Saegusa et al. analyzed endometrial adenocarcinomas in general, whereas in the present study we focused on the squamous component of these tumors, making it difficult to compare the results of the two studies.

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REFERENCES

1. Zaino RJ, Kurman RJ. Squamous differentiation in carcinoma of the endometrium: a critical appraisal of adenoacanthoma and adenosquamous carcinoma. *Semin Diagn Pathol* 1988;5:154–71.
2. Silverberg SG. Problems in the differential diagnosis of endometrial hyperplasia and carcinoma. *Mod Pathol* 2000;13:309–27.
3. Demopoulos RI, Dubin N, Noumoff J, Blaustein A, Sommers GM. Prognostic significance of squamous differentiation in stage I endometrial adenocarcinoma. *Obstet Gynecol* 1986;68:245–50.
4. Zaino RJ, Kurman R, Herbold D, Gliedman J, Bundy BN, Voet R, Advani H. The significance of squamous differentiation in endometrial carcinoma: data from a Gynecologic Oncology Group study. *Cancer* 1991;68:2293–302.
5. Nagafuchi A, Ishihara S, Tsukita S. The roles of catenins in the cadherin-mediated cell adhesion: functional analysis of E-cadherin-alpha catenin fusion molecules. *J Cell Biol* 1994;127:235–45.
6. Munemitsu S, Albert I, Souza B, Rubinfeld B, Polakis P. Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. *Proc Natl Acad Sci U S A* 1995;92:3046–50.
7. Rubinfeld B, Souza B, Albert I, Munemitsu S, Polakis P. The APC

protein and E-cadherin form similar but independent complexes with alpha-catenin, beta-catenin, and plakoglobin. *J Biol Chem* 1995;270:5549–55.
8. Su LK, Steinbach G, Sawyer JC, Hindi M, Ward PA, Lynch PM. Genomic rearrangements of the APC tumor-suppressor gene in familial adenomatous polyposis. *Hum Genet* 2000;106:101–7.
9. Palacios J, Gamallo C. Mutations in the beta-catenin gene (CTNNB1) in endometrioid ovarian carcinomas. *Cancer Res* 1998; 58:1344–7.
10. Voeller HJ, Truica CI, Gelmann EP. Beta-catenin mutations in human prostate cancer. *Cancer Res* 1998;58:2520–3.
11. Garcia-Rostan G, Tallini G, Herrero A, D'Aquila TG, Carcangiu ML, Rimm DL. Frequent mutation and nuclear localization of beta-catenin in anaplastic thyroid carcinoma. *Cancer Res* 1999;59: 1811–5.
12. Iwao K, Nakamori S, Kameyama M, et al. Activation of the beta-catenin gene by interstitial deletions involving exon 3 in primary colorectal carcinomas without adenomatous polyposis coli mutations. *Cancer Res* 1998;58:1021–6.
13. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC [Comments]. *Science* 1997;275:1787–90.
14. Koch A, Denkhaus D, Albrecht S, Leuschner I, von Schweinitz D, Pietsch T. Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene. *Cancer Res* 1999;59:269–73.
15. Fukuchi T, Sakamoto M, Tsuda H, Maruyama K, Nozawa S, Hirohashi S. Beta-catenin mutation in carcinoma of the uterine endometrium. *Cancer Res* 1998;58:3526–8.
16. Nei H, Saito T, Yamasaki H, Mizumoto H, Ito E, Kudo R. Nuclear localization of beta-catenin in normal and carcinogenic endometrium. *Mol Carcinog* 1999;25:207–18.
17. Zhurinsky J, Shtutman M, Ben-Ze'ev A. Plakoglobin and beta-catenin: protein interactions, regulation and biological roles. *J Cell Sci* 2000;113:3127–39.
18. Cowin P, Burke B. Cytoskeleton-membrane interactions. *Curr Opin Cell Biol* 1996;8:56–65.
19. Charpentier E, Lavker RM, Acquista E, Cowin P. Plakoglobin suppresses epithelial proliferation and hair growth in vivo. *J Cell Biol* 2000;149:503–20.
20. Nei H, Saito T, Tobioka H, Itoh E, Mori M, Kudo R. Expression of component desmosomal proteins in uterine endometrial carcinoma and their relation to cellular differentiation. *Cancer* 1996; 78:461–70.
21. Pasdar M, Nelson WJ. Kinetics of desmosome assembly in Madin-Darby canine kidney epithelial cells: temporal and spatial regulation of desmoplakin organization and stabilization upon cell-cell contact. II. Morphological analysis. *J Cell Biol* 1988;106:687–95.
22. Ashihara K, Saito T, Mizumoto H, Nishimura M, Tanaka R, Kudo R. Mutation of β-catenin gene in endometrial cancer but not in associated hyperplasia. *Med Electron Microsc* 2002;35:9–15.
23. Saito T, Schneider A, Martel N, Mizumoto H, Bulgay-Moerschel M, Kudo R, Nakazawa H. Proliferation-associated regulation of telomerase activity in human endometrium and its potential implication in early cancer diagnosis. *Biochem Biophys Res Commun* 1997;231:610–4.
24. Saito T, Oyamada M, Yamasaki H, Mori M, Kudo R. Coordinated expression of connexins 26 and 32 in human endometrial glandular epithelium during the reproductive cycle and the influence of hormone replacement therapy. *Int J Cancer* 1997;73:479–85.
25. Saito T, Schlegel R, Andresson T, Yuge L, Yamamoto M, Yamasaki H. Induction of cell transformation by mutated 16K vacuolar H+-atpase (ductin) is accompanied by down-regulation of gap junctional intercellular communication and translocation of connexin 43 in NIH3T3 cells. *Oncogene* 1998;17:1673–80.
26. Eastman Q, Grosschedl R. Regulation of LEF-1/TCF transcription factors by Wnt and other signals. *Curr Opin Cell Biol* 1999;11: 233–40.
27. Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. *Genes Dev* 1997;11:3286–305.
28. Aberle H, Bierkamp C, Torchard D, et al. The human plakoglobin gene localizes on chromosome 17q21 and is subjected to loss of heterozygosity in breast and ovarian cancers. *Proc Natl Acad Sci U S A* 1995;92:6384–8.
29. Caca K, Kolligs FT, Ji X, et al. Beta- and gamma-catenin mutations, but not E-cadherin inactivation, underlie T-cell factor/lymphoid enhancer factor transcriptional deregulation in gastric and pancreatic cancer. *Cell Growth Differ* 1999;10:369–76.
30. Ben-Ze'ev A, Geiger B. Differential molecular interactions of beta-catenin and plakoglobin in adhesion, signaling and cancer. *Curr Opin Cell Biol* 1998;10:629–39.
31. Simcha I, Geiger B, Yehuda-Levenberg S, Salomon D, Ben-Ze'ev A. Suppression of tumorigenicity by plakoglobin: an augmenting effect of N-cadherin. *J Cell Biol* 1996;133:199–209.
32. Saegusa M, Hashimura M, Yoshida T, Okayasu I. beta-Catenin mutations and aberrant nuclear expression during endometrial tumorigenesis. *Br J Cancer* 2001;84:209–17.

特　集　増えてきた子宮体癌　その診断から最新の治療まで

子宮体癌の手術療法

Operative management of uterine endometrial cancer

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子宮体癌の治療の中心は手術療法である．実際FIGOのAnnual Report（2001）によると97.7％が手術療法を受けている．わが国でも同様に94.6％となっている．しかし，その術式に関しては，様々な意見がある．わが国では子宮摘出については広汎性あるいは準広汎性子宮全摘が多く選択される傾向にあるが，欧米では単純子宮全摘出術が行われることが多い．またリンパ節郭清については傍大動脈リンパ節まですべての症例に実施するか，リンパ節郭清の個別化をはかるか，また傍大動脈リンパ節を徹底的に実施するか，生検にとどめるかといった種々の考えで手術術式が選択されている．現在は傍大動脈リンパ節も子宮体部癌の所属リンパ節に考えられているため，画一的傍大動脈リンパ節郭清と骨盤内リンパ節郭清を行うという意見と，リンパ節転移がきわめてまれな症例の場合，骨盤内リンパ節郭清は手術侵襲がそれほど高くないので実施するが，傍大動脈リンパ節は生検程度にとどめるという考えの基に手術を実施している施設が多い．
著者らは子宮摘出における子宮周囲組織の切除の程度，すなわち広汎度を個別化し，傍大動脈リンパ節郭清も画一的に行うのではなく，個別化して術式を選択すべきであるとの考えに立って述べた．

Key Words　子宮体癌，手術療法，腹腔鏡下手術

はじめに

子宮体癌の治療は手術療法が中心となり，ハイリスクの症例に対して放射線治療あるいは化学療法が併用されている．そして併用療法は欧米では放射線治療，わが国では化学療法が多く選択されている．手術術式の選択にあたっては子宮摘出における広汎度について，また後腹膜リンパ節郭清にあたっては，傍大動脈リンパ節郭清を画一的に行うか，生検あるいは省略可能かといった術式の個別化について述べたい．

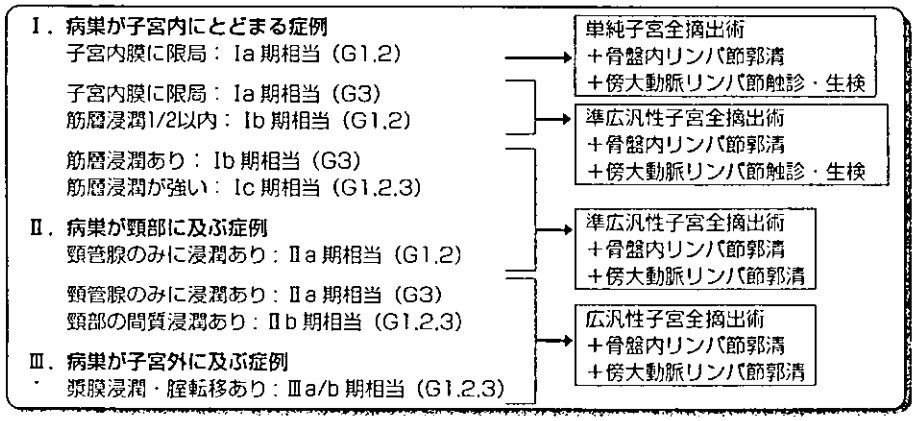
当科での手術適応の決定と標準術式（表1）

われわれの手術治療に対する考え方は，必ずしもデータにもとづくものではないが，臨床進行期，筋層浸潤の程度，頸部浸潤・脈管浸潤，組織学的分化度などの要因により可及的に個別化されるべきと考えている．つまり，画一的にすべての症例に対し，傍大動脈リンパ節の郭清を行ったり，準広汎性子宮全摘出術あるいは広汎性子宮全摘出術を実施すべきでないと考えている．

現在当科の手術計画は，術前の病巣の進展度診断にもとづいて実施されている．すなわち，筋層浸潤の程度や病巣の広がり，リンパ節の腫大などをCTやMRIなどの画像診断と，組織分化度によるリンパ節転移の可能性にもとづいて手術術式を計画している．頸管への進展については，必ずfractional curettageを行い，その組織学的な浸潤の程度で子宮頸部周囲の結合織の切除範囲を決定している．

具体的には，画像診断で子宮体部の筋層浸潤を否定はできないが内膜に限局していると考えられるgrade 1，2の症例には単純子宮全摘出術を行う．この場合，骨盤リンパ節郭清はルーチンに行われるが傍大動脈節については触診と生検で十分であると考えている．しかし，筋層浸潤が1/2以内と考えられる症例には，準広汎性子宮全摘出術を行っている．われわれの行っている準広汎性子宮

表1　当科における子宮体癌の手術療法



全摘出術は，膀胱子宮靱帯前層を切断し，尿管を側方に寄せた後に全子宮支帯と腟壁を子宮頸部からやや離れて切断する術式である．腟壁の切除は約1 cm 程度行っている．

以上のような症例よりもさらに深い筋層浸潤がある場合や，grade 3の症例には，準広汎性子宮全摘出術とリンパ節の郭清は骨盤内リンパ節に加え傍大動脈リンパ節の郭清を行っている．

広汎性子宮全摘出術が行われるのは，

1）明らかな頸部間質浸潤が認められる症例，

2）腟壁浸潤がある症例，

3）体部の筋層浸潤が深く，Grade 3の症例

である．広汎性子宮全摘出術を実施する症例には当然傍大動脈リンパ節郭清を含む，定型的な後腹膜リンパ節郭清術を行っている．

進行癌に対しては，遠隔転移のある症例も含め，原発巣周囲が肉眼的に摘出可能な場合，子宮摘出は準広汎程度を行い，リンパ節郭清を行う．しかしこの場合，侵襲はできるだけ軽度とすることを心がけていて，その後の化学療法を速やかに行うことが大切と考えている．腫瘍が肉眼的に容易に摘出することができない場合は，無理に摘出術は行わず化学療法や放射線療法を行っている．このような考えで手術の適応を計画しているのは，これまで卵巣癌のように reduction surgery を行った後に化学療法を行っても効果が認められた症例をほとんど経験していないためである．

これまで述べてきた手術術式選択の基準は，類内膜癌の場合であるが，組織型による個別化も必要と考えている．とくに漿液性腺癌の症例は卵巣癌と同様に扱われるべきであり，術式は子宮の摘出に関して準広汎以上は行わず単純子宮全摘出術でもよいが，大網切除や上腹部の生検を行ない，さらに傍大動脈リンパ節の郭清も行うべきである．何故ならば漿液性腺癌の場合，子宮体部の原発巣がきわめて小さくかつ筋層浸潤が浅くとも遠隔転移が多いためである．

わが国での子宮体癌手術の現状と欧米との比較

1．子宮頸部周囲組織の切除について

子宮体癌の手術治療を考えるうえでの重要な点の一つは子宮頸部周囲組織をどの程度切除すべきかであり，この点では欧米とわが国では考え方を異にする．

欧米では，進行期に関係なく基靱帯の処理に関しては問題にせずに基本術式は単純子宮全摘出術である[1]．拡大手術を行ったとしても頸部に比較的大きな浸潤が考えられるとき，すなわちⅡB期が fractional curettage による組織診断，または画像で診断される場合のみに modified radical hysterectomy が選択されるにすぎない．すなわち準広汎性子宮全摘出術に相当する手術が実施されている．単純子宮全摘出術を行って子宮外に病

巣が認められた場合には，術後に放射線治療がなされている．

このような事実からもFIGO stagingには子宮傍結合組織浸潤の症例をいかなる進行期に分類するか決められていない．しかしわが国の子宮体癌取扱いでは子宮傍結合組織浸潤が認められる症例をⅢc期とすることにしている．これはわが国では広汎または準広汎性子宮全摘出術を行う症例が多いため，子宮傍結合組織浸潤が認められた場合どのような進行期にすべきかといった疑問が多く出され，癌の進行度からⅢc期が妥当として，わが国で独自に決定した進行期分類への追記である．

実際，わが国では欧米に比較して子宮の拡大手術が実施される傾向にあり，Ⅰ期の症例にもすべて広汎性子宮全摘出術を行う施設もある．つまり頸部浸潤さらには子宮傍結合組織への浸潤・転移の可能性を否定できないとする立場で広汎性子宮全摘出術が行われていると解釈される．今野ら[2]の報告によればⅡ期症例に広汎子宮全摘術を行っているのは84%であり，準広汎子宮全摘術を行っている施設は13%である．

子宮傍結合組織浸潤・転移の頻度に関しては，Yuraら[3]は旧進行期分類のⅠ期で0%，Ⅱ期で11.5%，Ⅲ期で52.8%に認められ，この子宮傍結合組織に癌が認められる症例は予後不良と報告している．また，蔵本ら[4]は実際に頸部浸潤を認める場合の傍結合組織転移を14.5%，頸部浸潤を認めない場合でも3.6%あるとしている．両者の報告では共通して子宮体癌での傍結合組織の検索の必要性が強調されている．

しかし予後に関しては，単純子宮全摘術と広汎性子宮全摘出術を行った症例の検討から，両者に差はないとする報告も多い．さらに，単純子宮全摘出術と準広汎性子宮全摘出術との比較では，全国子宮体癌調査成績（1989～1990）[5]で，両術式はほとんど同じ頻度で行われているが，その予後に関しては大きな差を認めていない．

このことからも，広汎性子宮全摘出術は，明確な頸部浸潤を認める場合のみに適応となると考えられる．さらに個々の症例の年齢や全身状態などを十分考慮し決定されるべきであると考える．

2．リンパ節郭清の問題点

子宮体癌の手術療法で議論となるのは，骨盤内リンパ節と傍大動脈節リンパ節の郭清あるいは生検を行う対象症例の選択と摘出の程度についてである．リンパ節転移の危険因子としては，筋層浸潤1/2以上，Grade 2，3，類内膜癌以外の特殊な組織型，摘出物の脈管浸潤の有無である．このなかで術前または術中に診断し，手術計画を立てることができるのは，組織的分化度，組織型，筋層浸潤の程度であり，これらの因子とリンパ節転移の関係がより重要になってくる．筋層浸潤の程度はMRIなどの画像診断の進歩により比較的容易に行われるが，術中の迅速凍結病理検査も有効であるとする報告がある．また術前の生検による分化度の判定は不可欠であり重要であるが，30～50%で術後診断と違いが認められるという報告[6]もあり注意が必要である．

筋層浸潤の程度ならびに分化度との関係では，Lasonら[7]は筋層浸潤1/2以下でgrade 1の群でリンパ節を摘出しなかったにもかかわらず，5年後の再発は3.5%であることを報告していて，ハイリスク症例のみの傍大動脈リンパ節の郭清で十分であるとしている．またGeislerら[8]はⅠ期21症例，Ⅱ期4症例と症例数は少ないものの，grade 1では0%，grade 2では19.4%，grade 3では22.2%に転移を認めており，分化度と転移の関係を強調している．

リンパ節の転移率に関してはGOGの報告[9]ではⅠ期の体癌の9%に骨盤内リンパ節に転移が，傍大動脈リンパ節には5.5%に認められ，骨盤内リンパ節に転移が陰性であれば傍大動脈リンパ節転移のリスクは2%であり，陽性の場合1/3に傍大動脈リンパ節転移が認めらている．

傍大動脈リンパ節の郭清については，臨床的研究の意味も含めてすべての症例に対し行っている施設もある．Hirahatakeら[10]は，1982年以降すべての体癌症例に傍大動脈リンパ節郭清を行った200例の成績を報告している．そのなかで，傍大

動脈リンパ節転移は臨床進行期Ⅰa期で2.5%，Ⅰb期で8.5%，Ⅱ期で15.7%，Ⅲ期で33.3%，全体では9.0%に認められ，Ⅰ期でも無視できない頻度であると強調している．しかし，この頻度は現在の術後進行期と異なることを考慮しておく必要があろう．また，すべてのgradeで筋層浸潤1/2以内の症例の傍大動脈リンパ節の転移は3.6%であったと報告している．

しかし，傍大動脈リンパ節を含む後腹膜リンパ節摘除を完全に行った彼らのデータからの5年生存率は約59%である．一方，全国子宮体癌調査成績[7]では26.4%の傍大動脈リンパ節摘出率であるがリンパ節転移陽性群の5年生存率は53.4%となっており，徹底した傍大動脈リンパ節のリンパ節郭清を行っても有意に予後の改善には至らないことを示している．

リンパ節摘出の程度について，FIGO stagingでは記載はないものの，Chungら[11]は，ハイリスク症例295例に対し選択的に骨盤内リンパ節と傍大動脈リンパ節の生検を行った研究で，触診などで転移陰性と診断した部位の後腹膜に再発が認められなかったことから，リンパ節の生検の診断価値を評価している．

以上より，現在の傍大動脈リンパ節郭清の適応を考えてみると，筋層浸潤1/2以内でgrade1などのリスクの低いと考えられる症例では，傍大動脈リンパ節の生検でも十分であり，全症例に画一的に郭清を行うことの意義は現在のところ認められず，患者のQOLを意識した症例の選択が望ましいと思われる．

腹腔鏡下手術

近年，多くの施設で腹腔鏡下手術が行われるようになり，子宮体癌においても，積極的に腹腔鏡下に治療を行う施設も見られるようになってきた．これまでは，傍大動脈リンパ節の摘出の技術的な問題点が解決されていないことが最大の問題であった．しかし，この点においても種々の工夫[12]と術者の熟練により解決されつつあり，今後さらに広く行われると考えられる．

具体的には，まず腹腔内の十分な観察を行い，付属器への転移がないか，子宮の漿膜面への浸潤はないか，腸管などの腹腔内臓器への浸潤がないかなどを確認したうえで，腹腔鏡下に後腹膜リンパ節の廓清を行い，LAVHにて単純子宮全摘出を行うのが一般的である．つまりその適応は現時点では，病巣が子宮内に限局し筋層浸潤は浅い，grade 1の症例が主な対象となると思われる．

これまでのいくつかの報告では，開腹術に遜色なく十分な手術が可能であるとされる．Framら[13]は，Ⅰ期の子宮体癌で29症例の腹腔鏡下手術を行った群と32例の開腹手術を行った群を比較し，出血量と病院滞在日数では有意に腹腔鏡下手術がすぐれており，摘出したリンパ節の数や副損傷の頻度も同程度であることを報告し，腹腔鏡下手術は安全かつ有効な手段であると結論づけている．またヨーロッパの多施設共同研究[14]では，臨床進行期Ⅰa期でgrade 1の221症例に対し，177例に腹腔鏡下手術を44例に開腹術を行った群とを比較して，中間観察期間でそれぞれ腹腔鏡下手術群45.2ヵ月，開腹術群33.6ヵ月で予後に相違がないことを報告している．

当科では，以前より初期の子宮頸癌において腟式広汎性子宮全摘術を多数行っている経験から，腟式に準広汎性子宮全摘出術を行い，腹腔鏡下にリンパ節郭清を行うことは十分可能であり，適応を広げることも可能と考えている．この腹腔鏡下リンパ節郭清術と腟式子宮全摘出術あるいは準広汎性腟式子宮全摘出術は，肥満度の高い患者で，筋層浸潤が浅く，CTなどでリンパ節転移が考えられない症例に対する手術術式として検討する必要があると考えている．

おわりに

子宮体癌の手術法を計画するうえで，子宮頸部周囲組織の切除範囲や傍大動脈リンパ節の郭清は，画一的に行われるべきでない．個々の症例の正確な術前あるいは術中の診断にもとづいて，患者の

予後を損なうことがない条件で，患者のQOLを高めるための手術法の選択がなされる必要がある．

そのためにはさらなるエビデンスの蓄積と，多施設共同研究のようなより大規模で客観性のある臨床研究が行われることが望まれている．また欧米で行われている術前の放射線療法や術前化学療法は，手術症例の適応を拡大したり，より完遂度の高い手術ができる可能性を秘めており，術後の放射線療法を含め，わが国の実情にあわせたさらなる検討が必要である．

文　献

1) Berek JS, Hacker NF：Practical Gynecologic Oncology, 3rd ed Lipincott Williams &Wilkins, 2001.
2) 今野良，佐藤信二，矢嶋聡：子宮体癌大動脈リンパ節郭清の意義，適応，術式，予後，多施設アンケート調査の解析．産科と婦人科 63：1197−202, 1996.
3) Yura Y, Tauchi K, et al：Parametrial involvement in endometrial carcinoma；its incidence and correlation with other histological parameters. Gynecol Oncol 63：114−119, 1996.
4) 蔵本 博行，上坊敏子ほか：子宮体癌にたいするStaging Laparatomyの意義．産科と婦人科 62：1273−1281, 1995.
5) 日本産婦人科学会子宮癌委員会：全国子宮体癌調査成績．第4報．1989〜1990年度症例．1999.
6) Chuang L, Burke TW, Tornos C, et al：Staging laparotomy for endometrial carcinoma：Assessment of retroperitoneal lymph node. Gynecol Oncol 58：189−193, 1995.
7) Lason D, Broste SK, Krawisz B：Surgery without radiotherapy for primary treatment of endometrial cancer. Obstet Gynecol 91：355−359, 1998.
8) Geisler JP, Wieman MC, Zhou Z, et al：Using FIGO histological grade to determine when to perform lymphadenectomies endometrioid adenocarcinoma of endometrium. Eur Jgynaecol Oncol 17：204−207, 1996.
9) Creasman WT, Morrow CP, et al：Surgical pathologic spread pattern of endometrial cancer. Cancer 60：2035−2041, 1987.
10) Hirahatake K, Hareyama H, Sakuragi N, et al：A clinical and pathologic study on para-aortic lympho node metastasis in endometrial carcinoma. J Surg Oncol 65：82−87, 1997.
11) 安藤正明，伊熊健一郎，吉岡保：婦人科悪性腫瘍における後腹膜リンパ節郭清．臨婦産 55：266−269, 2001.
12) Fram KM: Laparoscopically assisted vaginal hysterectomy versus abdominal hysterectomy in stage I endometrial cancer. Int J Gynecol Cancer 12：57−61, 2002.
13) Holub Z, Jabor A, et al：Laparoscopic surgery for endometrial cancer. Long-term results of a multicentric study. Eur J Gyneaecol Oncol 23：305−310, 2002.