The Korean Journal of Pathology
2005; 39: 1-8

PTEN and p53 Mutations in Endometrial Carcinomas

Jae-Sung Choi[1] · Kwang-Sun Suh
Heung-Tae Noh[1] · Yun-Ee Rhee[1]
Sun-Young Na · Hye-Kyung Lee[2]

[1]Department of Obstetrics and Gynecology, Chungnam National University School of Medicine, Department of Pathology, Chungnam National University School of Medicine, and [2]Department of Pathology, Catholic University St. Mary's Hospital, Daejeon, Korea

Received : August 9, 2004
Accepted : October 27, 2004

Corresponding Author
Kwang-Sun Suh, M.D.
Department of Pathology, Chungnam National University School of Medicine, 6 Munwha-1-dong, Jung-gu, Daejeon 301-747, Korea
Tel: 042-220-7199
Fax: 042-220-7189
E-mail: kssuh@cnu.ac.kr

Background : Endometrial carcinomas are pathogenetically classified into two major types; endometrioid carcinoma (EC) and serous carcinoma (SC). The most frequently altered gene in EC is the PTEN tumor suppressor gene (TSG). SC is usually associated with mutations in the p53 TSG. **Methods :** To further determine the role of PTEN and p53 mutation in endometrial carcinogenesis, the analysis of 33 endometrial carcinomas, including 28 ECs and 5 SCs, for loss of heterozygosity (LOH) on 10q23 and for mutation in all 9 coding exons of PTEN and the 5-8 exons of p53, using SSCP-PCR methods was carried out. **Results :** LOH was detected in at least one marker in 12 (54.5%) of 22 ECs, but in only one (20.0%) of 5 SCs. Somatic PTEN mutations were detected in 10 (35.7%) of 28 ECs. PTEN was altered in 67.9% of ECs and in 20.0% of SCs, including those with 10q23 LOH. No PTEN mutations were found among the SCs. Somatic p53 mutations were detected in 2 (7.1%) of 28 ECs and 3 (60.0%) of 5 SCs. **Conclusions :** PTEN gene alterations contribute to the pathogenesis of an endometrioid subtype of endometrial carcinoma, but not to the serous type. In contrast, p53 plays an important role in the pathogenesis of SCs.

Key Words : Carcinoma, endometrioid; PTEN gene product; Genes, p53; Mutation; Loss of heterozygosity

Bockman[1] described two pathogenetic types of endometrial carcinomas. Since then it has been accepted that type I tumors are of low-grade endometrioid carcinomas (EC) that usually develop in pre- or perimenopausal women and frequently coexist with or are preceded by complex or atypical endometrial hyperplasia (EH). In contrast, type II tumors are non-EC, less common, more aggressive, largely serous carcinomas (SC), and unrelated to estrogen stimulation. These tumors arise from precancerous lesions developing in atrophic endometrium, mainly in postmenopausal women. Endometrioid and SCs of the endometrium are distinct biologic entities, but the molecular pathogenesis of the two major types remains relatively unknown. Recent molecular studies have supported this classification by demonstrating differences in the molecular alterations that underlie in the two types of endometrial carcinomas.[2-5]

The tumor suppressor gene (TSG) PTEN (phosphatase and tensin homologue deleted from chromosome 10) on chromosome 10q23 is known to be somatically mutated, in association with frequent loss of heterozygosity (LOH) at 10q23.3, in a variety of tumors including those of the brain, breast, prostate, and thyroid gland.[6-8] The most frequently altered gene in EC is the PTEN TSG, which is mutated in 30-50% of cases,[2-4] but is very rarely involved in SC.[3,5]

Mutation or deletion of the p53 TSG on chromosome 17p13.3 is the most frequent gene mutation in human cancers.[8] p53 mutations have been identified in approximately 15% of ECs,[10] whereas they are found in more than 90% of SCs.[11] Approximately, 75% of endometrial intraepithelial neoplasias, the putative precursor of SC, have mutations in p53.[10,12]

To further determine the role of PTEN and p53 mutation in endometrial carcinogenesis, genomic DNA from 33 endometrial carcinomas was extracted from paraffin blocks. PCR was used for LOH on 10q23 and to amplify the 9 exons of the PTEN gene and the 5-8 exons of p53. These products were screened using single-strand conformation analysis (SSCP), and subsequently the sequencing of variant bands was carried out.

MATERIALS AND METHODS

Materials

Thirty-three endometrial carcinomas were retrieved from the files of Chungnam National University Hospital and St. Mary Hospital, Daejeon, Korea. The endometrial carcinomas consisted of 28 ECs and 5 SCs. Patient age ranged from 38 to 73 years. Histopathological slides were reviewed by two pathologists. Tumors were staged in accordance with the International Federation of Gynecology Oncology (FIGO, 1993).

DNA extraction

All tumor samples were taken from formalin fixed, paraffin embedded tissue samples. Tissue blocks were required to have at least 85% neoplastic cells. In order to meet this requirement, tumor areas were dissected from the surrounding normal tissues. DNA samples from endometrial carcinomas and normal tissue pairs were prepared. Segments of normal adnexa, uninvolved uterine cervix, or myometrium were obtained from the same patients to serve as normal tissue controls. A total of 4 to 5 microdissected 5 μm sections of formalin-fixed, paraffin-embedded tumor tissue were incubated at 52℃ for one or two days in 400 μL DNA extraction buffer (0.25 μg/μL proteinase K (Roche, Germany), 20 mM Tris/HCl, pH 8.3, 5 mM $MgCl_2$, 100 mM KCl, 1% Tween-20, and 1% NP-40). The mixture was boiled for 10 min to inactivate the proteinase K, followed by phenol extraction for purification and then concentrated by ethanol precipitation. The isolated DNA solution was quantified spectrophotometrically.

Table 1. Primer sets for D10S1765, D10S1696, and D10S215

Primer	Sequence (5′→3′)
D10S1765-F	FAM-ACACTTACATAGTGCTTTCTGCG
D10S1765-R	CAGCCTCCCAAAGTTGC
D10S1696-F	FAM-TCCTGGGTGACAGAGTGA
D10S1696-R	GAGACAGCATTTCCATTATGA
D10S215-F	FAM-TGGCATCATTCTGGGGA
D10S215-R	GCTTTACGTTTCTTCACATGGT

Table 3. Primer sets for the exons of p53

Exon/ primer	Sequence (5′→3′)	Product length (bp)	tempera- ture (°C)
5-F	GTCTCCTTCCTCTTCCTACAG	243	60
5-R	CAACCAGCCCTGTCGTCTCT		
6-F	CTGATTCCTCACTGATTGCTC	159	60
6-R	GACCCCAGTTGCAAACCAGAC		
7-F	GGGCCTGTGTTATCTCCTAG	148	60
7-R	TGGCAAGTGGCTCCTGAC		
8-F	TCCTATCCTGAGTAGTGG	173	58
8-R	CTTCTTGTCCTGCTTGCTTAC		

LOH analysis (Genetic analyzer method)

Fluorescent microsatellite oligonucleotide primer

The three microsatellite markers flanking the PTEN locus, D10S1765, D10S1696, and D10S215, were used to assess LOH. All primers used in this study were obtained from GenoTech (Korea) (Table 1).

Fluorescent PCR amplification of microsatellites

Fluorescent PCR amplification of microsatellites was performed in a total volume of 20 μL containing 10 μL of AmpliTagGold PCR master mix (Applied Biosystem, USA), 7.5 pmol of both fluorescent forward and reverse primers, and distilled water. PCR cycles in an automatic thermal cycler (GeneAmp PCR system 9600, Perkin Elmer, USA) consisted of 10 min at 95℃ followed by 10 sec at 96℃, 30 cycles for 3 min at 70℃, followed by one cycle for 30 min at 70℃.

Electrophoresis by the capillary method

Electrophoresis using the capillary method was performed in

Table 2. Amplification primers for SSCP/PCR mutation analysis of the PTEN gene

Exon/ primer	Sequence (5′→3′)	Product length (bp)	tempera- ture (°C)
1-F	CAGAAGAAGCCCCGCCACCAG	177	65
1-R	AGAGGAGCAGCCGCAGAAATG		
2-F	TTTCAGATATTTCTTTCCTTA	171	58
2-R	AACATGAATATAAACATCAA		
3-F	TAATTTCAAATGTTAGCTCAT	147	60
3-R	AAGATATTTGCAAGCATACAA		
4-F	GTTTGTTAGTATTAGTACTTT	150	58
4-R	ACAACATAGTACAGTACATTC		
5-1-F	TATTCTGAGGTTATCTTTTA	158	58
5-1-R	CTTTTCCAGCTTTACAGTGAA		
5-2-F	GCTAAGTGAAGATGACAATCA	186	58
5-2-R	AGGAAAAACATCAAAAAATAA		
6-F	TTGGCTTCTCTTTTTTTTCTG	202	58
6-R	ACATGGAAGGATGAGAATTTC		
7-F	CCTGTGAAATAATACTGGTATG	229	62
7-R	CTCCCAATGAAAGTAAAGTACA		
8-1-F	GTGCAGATAATGACAAGGAATA	159	58
8-1-R	ACACATCACATACATACAAGTC		
8-2-F	TTAAATATGTCATTTCATTTCTTTTTC	244	58
8-2-R	CTTTGTCTTTATTTGCTTTGT		
9-F	TTCATTTTAAATTTTCTTTCT	242	65
9-R	TGGTGTTTTATCCCTCTTGAT		

a total volume of 12 μL containing 0.5 μL of the PCR product, 0.5 μL of a Genescan size standard (Applied Biosystem, USA), and 11 μL of formamide (Applied Biosystem, USA). The mixture was denatured for 5 min at 95℃ and stored in ice. Electrophoresis was then performed using an ABI 310 Genetic analyzer (Applied Biosystem, USA).

Microsatellites were amplified using one fluorescent labeled and one unlabeled primer for each locus. Two amplifications were run for each microsatellite, one using sample genomic DNA isolated from normal cells and one using sample DNA isolated from tumor cells from the same individual. A GeneScan Internal Lane Size Standard was added to each sample before denaturation and loading. The amplification products were separated and detected using an ABI Prism 310 Genetic Analyzer. Data was analyzed using GeneScan Analysis Software.

LOH analysis

Once GeneScan analysis software determines the peak height and area for all alleles of all relevant microsatellite loci, Genotyper software was used to pool data from all independent injections of each normal (N) or tumor (T) sample to obtain an average peak height and area for every allele in every sample.

Calculation of the LOH value

LOH can be defined mathematically as follows:

$$LOH = \frac{\text{height of normal allele two/height of normal allele one}}{\text{height of tumor allele two/height of tumor allele one}}$$

An LOH value ≤ 0.5 indicates that the tumor sample shows significant loss of the longer allele whereas an LOH value ≥ 1.5 indicates that the tumor sample shows significant loss of the shorter allele. If a particular locus in a normal sample is homozygous, that locus cannot be used to diagnose LOH for the corresponding N-T pair.

PCR amplification of the PTEN and p53 genes

All 9 exons of PTEN were amplified separately, purified, and subjected to direct sequence analysis. The sequences of primers for the amplification of exons 1-9 have been published previously.[2] Exons 5 and 8 were divided, then amplified with two overlapping primer pairs (Table 2).

The analysis of exons 5 to 8 of the p53 gene was carried out (Table 3). Oligonucleotide primers used for amplification are shown in Table 3. PCR amplification of p53 exon 5-7 was performed in a total volume of 20 μL containing 500 ng of template DNA, one unit of ExTaq polymerase (Takara, Japan), 1.25 mM of dNTP, 15 pmole primers, and 2 μL of 1 X reaction buffer. PCR for exon 8 was performed under the same conditions as for exon 5-7 except for the dNTP concentration, which was 2.5 mM dNTP. PCR cycles consisted of 5 min at 94℃ followed by 35 cycles for 30 sec at 94℃, 30 sec at 55℃, and 30 sec at 72℃, followed by one cycle for 7 min at 72℃.

SSCP analysis, Silver staining, and Direct DNA sequencing

Two μL of PCR product was mixed with 6 μL of sample loading buffer containing 95% formide (deionized), 10 mM NaOH, 0.25% Bromophenol blue, and 0.25% Xylene cyanol. The samples were denatured for 3 min at 100℃ and quickly chilled on ice. They were then loaded onto 12% polyacrylamide gel containing 1 X sample buffer (33 mM Tris-sulfate, 7% Glycerol, pH 8.3), and were electrophoresed at 250 V. After electrophoresis the gels were disassembled from the glass plate, then stained using a Silver Stain Plus kit (BIO-RAD, USA), followed by air drying. Samples with abnormal bands were automatically sequenced on a Genetic analyzer (ABI, USA).

Statistical analysis

The associations between the presence of PTEN or p53 gene alterations and clinical stage or pathologic grade were analyzed by chi-square analysis and Student's T-test.

RESULTS

Thirty-three endometrial carcinomas, including 28 ECs (Fig. 1A) and 5 SCs (Fig. 1B), were analyzed. The age of the patients ranged from 38 to 73 years. Tumors were staged and graded in accordance with the International Federation of Gynecology Oncology (FIGO, 1993). Among 28 ECs, ten cases were of grade 1, 14 cases were of grade 2, and 4 cases were of grade 3. Twenty-seven carcinomas were in stage I, one was in stage II, and five were in stage III.

Three microsatellite markers flanking the PTEN locus (D10S1765, D10S1696, and D10S215) were used to assess LOH. The entire coding region of PTEN and the 5-8 exons of p53 were examined using a DNA-based SSCP mutation screening test. LOH was detected with at least one marker in 12 (54.5%) of 22 informative ECs. However, it was detected in only one (20.0%) of 5 SCs (Table 4 and Fig. 2, LOH value=0.4). Somatic PTEN

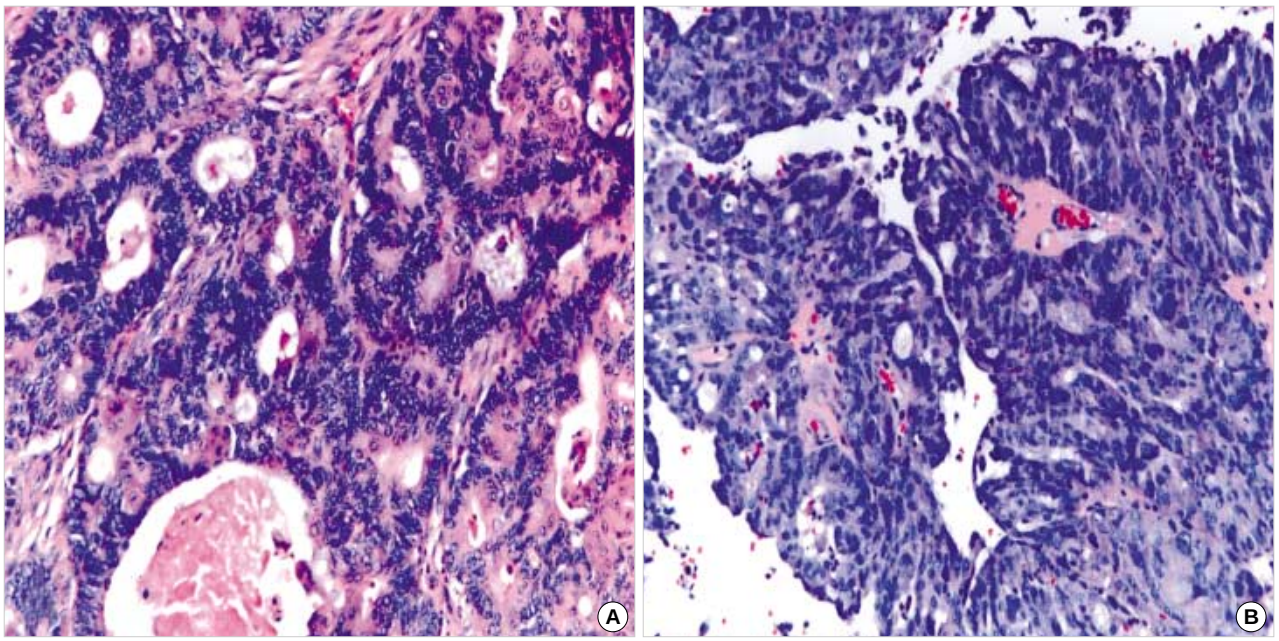

Fig. 1. (A) Endometrioid carcinoma of the endometrium, ×100. (B) Serous carcinoma of the endometrium, ×100.

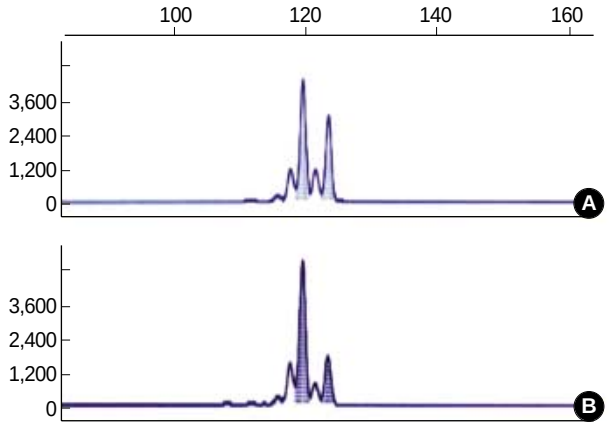

Fig. 2. Detection of LOH (D10S1765) in serous carcinoma (Case No. S2). LOH value=0.48.

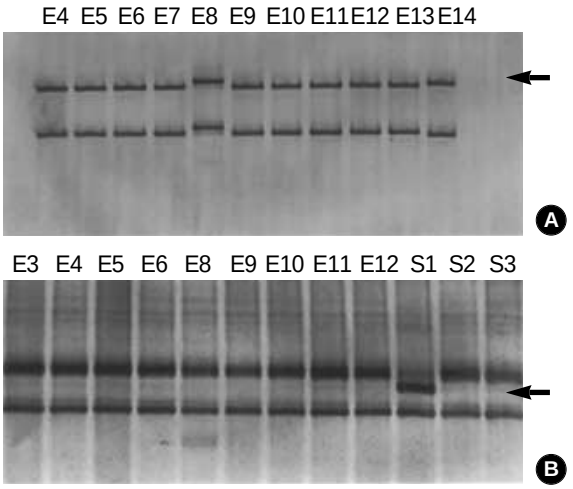

Fig. 3. (A) Detection of PTEN mutation in exon 1 in endometrioid carcinoma using SSCP analysis. The abnormal band (arrow) indicates the presence of PTEN mutations (Case No. E8). (B) Detection of p53 mutation in exon 8 in serous carcinoma using SSCP analysis. The abnormal band (arrow) indicates the presence of p53 mutations (Case No. S1).

mutations were detected in 10 (35.7%) of 28 ECs. PTEN was altered in 67.9% of ECs and 20.0% of SCs, including those with 10q23 LOH. The codons that showed the PTEN mutation were scattered between exons 1 and 8 (Fig. 3A). Two mutations were found in three cases. No PTEN mutations were found among the SCs. Somatic p53 mutations were detected in 2 (7.1%) of 28 ECs and 3 (60.0%) of 5 SCs (Table 5, Fig. 3B). Among two ECs that showed somatic p53 mutations, one case was histologic grade 1 and the other was histologic grade 3. The codons that showed the p53 mutation were at exons 5 (one case of EC) and 8 (one case of EC and three cases of SC) (Table 4).

Abnormally shifted bands that were detected from cases of both ECs and SCs were eluted from the gel and the extracted DNA was automatically sequenced. A total of 13 PTEN mutations in 10 tumors were detected, out of which, 6 were missense, 4 were frameshift, and 3 were nonsense mutations (Table 6, Fig. 4A). Three tumors showed two different mutations in the PTEN gene. A total of 5 p53 mutations in five tumors (2 ECs and 3 SCs) were detected. All 5 cases were missense mutations (Table 7, Fig. 4B). There was no correlation between the presence of PTEN or p53

Table 4. PTEN and p53 mutations in endometrioid and serous carcinomas of the endometrium

Case No.	Age (yr)	Grade	Stage	LOH D10S1765-D10S1696		D10S215	PTEN mutation	p53 (axon)
E 1	50	1	IB		NI		–	–
E 2	51	1	IA		NI		–	–
E 3	38	1	IB		NI		+ (2)	–
E 4	53	3	IB		NI		–	–
E 5	60	3	IC	–	+	–	+ (4)	–
E 6	54	2	III		NI		–	–
E 7	55	2	IIIA		NI		–	–
E 8	43	2	IA	–	–	–	+ (1,5-1)	–
E 9	42	2	IA	–	+	–	–	–
E10	43	2	IB	–	–	–	+ (8-1,8-2)	–
E11	45	2	IB	–	+	–	+ (5-2)	–
E12	54	3	IC	+	–	–	–	–
E13	43	1	IA	–	–	–	–	–
E14	67	1	IA	–	–	–	–	+ (8)
E15	38	2	IB	–	+	–	–	–
E16	53	2	IC	–	–	–	+ (1)	–
E17	51	1	IIIA	+	–	–	–	–
E18	55	2	IB	–	–	–	+ (2)	–
E19	61	2	IB	–	–	–	–	–
E20	68	3	IC	–	+	–	+ (6)	+ (5)
E21	60	2	IB	–	+	–	–	–
E22	60	2	IB	–	–	+	–	–
E23	43	1	IA	–	–	–	–	–
E24	45	1	IA	+	–	–	–	–
E25	60	2	IC	–	–	–	+ (8-1)	–
E26	58	2	IB	–	–	–	+ (6,8-2)	–
E27	43	1	IB	+	–	–	–	–
E28	60	1	IC	+	–	–	–	–
S 1	73	3	IIIA	–	–	–	–	+ (8)
S 2	62	3	IC	–	–	–	–	–
S 3	63	3	IIIA	+	+	–	–	–
S 4	60	3	IB	–	–	–	–	+ (8)
S 5	58	3	IIA	–	–	–	–	+ (8)

E, endometrioid carcinoma; S, serous carcinoma; NI, not informative.

mutations and clinical stage or pathologic grade.

DISCUSSION

Recent molecular studies have demonstrated differences in the molecular alterations that underlie the two types of endometrial carcinomas, namely, ECs and SCs. ECs show microsatellite instability (MI) in 23% to 34%,[13,14] PTEN mutations in 34% to 50%,[2,3] and beta-catenin mutations with nuclear protein accumulation in 14% to 38% of cases.[15,16] In contrast, most non-ECs show p53 mutations,[12] LOH on several chromosomes,[17] and only rarely show MI or PTEN mutations (0% to 5%),[3,18] or beta-catenin alterations.[15,16] We have shown that PTEN was mutated in 35.7% of ECs, whereas no PTEN mutations were found among SCs. 10q23

Table 5. Frequency of PTEN and p53 mutations by tumor type (%)

Tumor type	No. analyzed	LOH	PTEN mutation	p53 mutation
Endometrioid	28	12/22* (54.5)	10/28 (35.7)	2/28 (7.1)
Serous	5	1/5 (20.0)	-	3/5 (60.0)

*The number of tumors showing LOH with any 10q23 microsatellite marker divided by the total number of tumors informative for at least one microsatellite marker.

Table 6. Mutation in the PTEN gene in endometrial carcinomas

Case No.	Exon	Base change	Amino acid change	Consequence
3	2	ATT→–	ATT deletion	Frameshift
7	8-2	TTT→CTT	Phe→Leu	Missense
8	1	ATC→ACC	Ile→Thr	Missense
	5-1	GAC→GGC	Asp→Gly	Missense
10	8-1	ACTT→–	ACTT deletion	Frameshift
	8-2	TACT→–	TACT deletion	Frameshift
11	5-2	TAT→TAG	Tyr→Stop	Nonsense
16	1	GAG→TAG	Glu→Stop	Nonsense
18	2	AGG→A_G	Deletion	Frameshift
20	6	CGC→TGC	Arg→Cys	Missense
25	8-1	GCC→GCT	Ala→Ala	Missense
26	6	TAT→CAT	Tyr→His	Missense
	8-2	TGG→TAG	Trp→Stop	Nonsense

Table 7. Mutation in the p53 gene in endometrial carcinomas

Case No.	Exon	Base change	Amino acid change	Consequence
E-14	8	GAG→GTG	Glu→Val	Missense
E-20	5	ACG→ATG	Thr→Met	Missense
S-1	8	TGT→CGT	Cys→Arg	Missense
S-4	8	CGT→CAT	Arg→His	Missense
S-5	8	GCC→GCT	Ala→Ala	Missense

LOH was detected with at least one marker in 12 (54.5%) of 22 informative ECs. However, it was detected in only one (20.0%) of 5 SCs. Somatic p53 mutations were detected in 2 (7.1%) of 28 ECs and 3 (60.0%) of 5 SCs. These results fall into the range of previous studies.[2-5,18]

The PTEN, also called MMAC1 (mutated in multiple advanced cancers), is located on chromosome 10q23.3 and encodes a phosphatase.[6,7] The primary target is the lipid molecule phosphatidylinositol-3,4,5-triphosphate (PIP3), which is involved in a signal transduction pathway that regulates cell growth and apoptosis. The function of the phosphatase is to interfere the activity of PIP3.[19,20] Tensin is a protein that helps to connect the cell's internal skeleton of protein filaments to its external environment.[6,7]

The genes encoding protein phosphatases, like PTEN, act as TSGs, because their proteins may counteract the task of the pro-

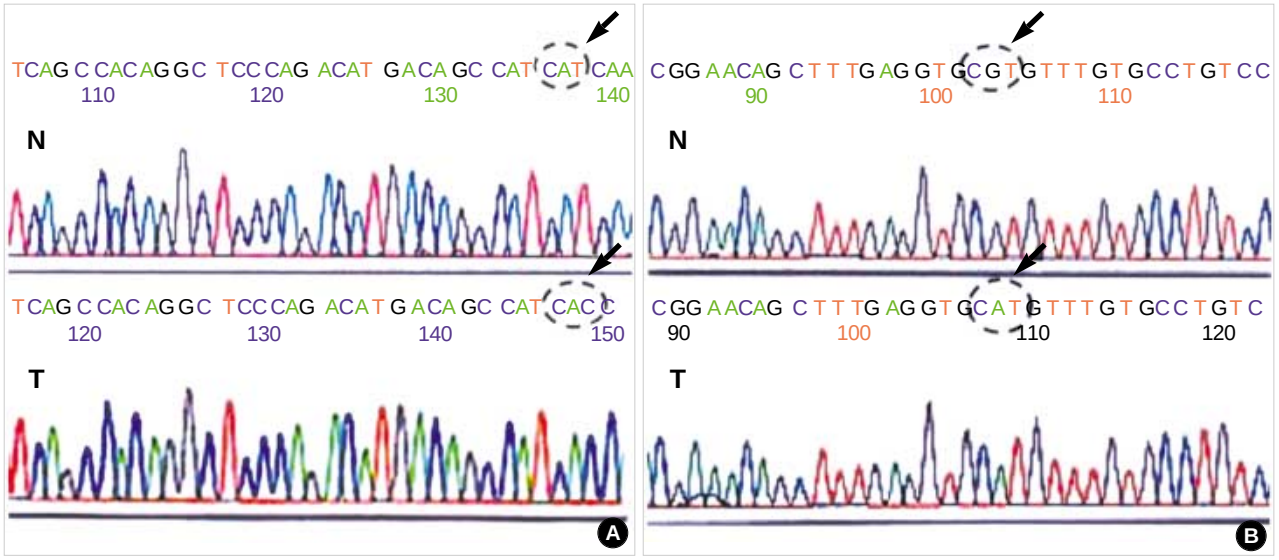

Fig. 4. (A) Demonstration of PTEN mutation (missense mutation) in exon 1, endometrioid carcinoma (Case No. E8). (B) Demonstration of p53 mutation (missense mutation) in exon 8, serous carcinoma (Case No. S4).

teins encoded for the protein kinase group of protooncogenes.[20] Activation of the signal transduction pathway can be caused by either gain-of-function mutations in protein kinases or by loss-of-function mutations in protein phosphatases.[6,7,20] Loss of function of just a single allele of PTEN is sufficient to confer a growth advantage, indicating that PTEN mutation without LOH in the PTEN region or LOH in the PTEN region without mutation can reduce the function of PTEN.[21] Herein, PTEN was altered in 67.9% of ECs, including those with 10q23 LOH.

Somatic PTEN mutations occur in advanced tumors including glioblastoma,[22] suggesting that PTEN regulates cellular functions relevant for disease progression rather than initiation.[2] Germ-line mutations of PTEN are a frequent predisposing factor for multiple cancers in women.[23] These mutations are responsible for Cowden disease, which predisposes individuals to a variety of benign and malignant neoplasms and is characterized by the development of multiple hamartomas that are composed of disorganized cell masses. Therefore, it has been suggested that one of the functions of PTEN is to control cellular proliferation and organization.[7]

PTEN mutation has been shown to be an early event in endometrial carcinomas because it is mutated in approximately 20% of atypical and nonatypical EH, which are precursor lesions of ECs.[4,12] PTEN alterations may play a central role in the initiation of proliferative lesions, which then acquire mutations in other cancer-causing genes, such as, K-ras and p53, in the progression to malignancy. Also, it has been demonstrated that PTEN inactivation is an early event in ovarian EC tumorigenesis because the majority of tumors with PTEN mutations are grade 1 and/or stage I.[24] We did not analyse endometrial hyperplasia cases. There was no correlation between PTEN mutation and stage or grade in this study.

It has been reported that SCs of the endometrium lack detectable PTEN mutations.[3,5] The frequency of PTEN gene alterations in other types of ovarian carcinoma, such as serous and mucinous carcinoma, is also small compared with ovarian EC.[24] Although the number of SC cases examined in this study was small, the absence of PTEN mutation in any of the 5 SCs is consistent with previous studies[3,24] and indicates that PTEN is rarely involved in this serous subtype.

Sato *et al.*[25] found that mutations in the endometrial cysts and clear cell carcinomas of the ovary were concentrated at exons 5-6, which encode the phosphatase domain of the PTEN gene. In contrast, we found that the codons showing the mutation were scattered between exons 1 and 8.

The biological importance of LOH and mutations of PTEN remains largely unknown. Although initial investigations reported that PTEN mutation was associated with early stage, nonmetastatic disease and prolonged survivial,[18] more recent studies have suggested that mutations present outside exons 5 to 7 of PTEN might represent a molecular predictor of favorable survival, independent of the clinical and pathological characteristics of the tumor.[26] There is only sparse information about PTEN protein expression by immunohistochemistry in hormone dependent female tumors. The loss of PTEN expression is relatively frequent in endometrial carcinoma and is associated with metastatic disease.[27]

PTEN positive staining is a significant prognostic indicator of favorable survival for patients with advanced endometrial carcinoma who underwent postoperative chemotherapy.[28]

p53 mutations, which are central to the development of SC, occur relatively at earlier stages in SC.[12] p53 mutations appear to be important in the conversion of relatively quiescent, atrophic endometrium to an intraepithelial form of SC that then sets the stage for the accumulation of alterations in yet unidentified cancer-causing genes.[29] In contrast, p53 mutations in EC are relatively uncommon. When they do occur, they are largely confined to grade 3 tumors. There is a wide range of variation of opinion in the literature regarding the proportion of endometrial carcinoma overexpressing the p53 protein. Expression of the p53 protein may occur in endometrial adenocarcinoma without mutation and may be due to stabilization of the protein during its normal function, possibly by an mdm2 mediated process.[30]

In this study, somatic p53 mutations were detected in 2 (7.1%) of 28 ECs and 3 (60.0%) of 5 SCs. Among two ECs that showed somatic p53 mutations, one case was histologic grade 1 and the other was histologic grade 3. There was no correlation between the presence of PTEN or p53 mutations and clinical or pathological features. In summary, this study has demonstrated frequent PTEN alterations in ECs, in contrast to SC cases that showed a higher p53 mutation. These findings support the hypothesis that ECs occur along a different pathway from those of SCs.

REFERENCES

1. Bockman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983; 15: 10-7.
2. Risinger JI, Hayes AK, Berchuck A, Barrett JC. PTEN/MMAC1 mutations in endometrial cancers. Cancer Res 1997; 57: 4736-8.
3. Tashiro H, Blazes MS, Wu R, *et al*. Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. Cancer Res 1997; 57: 3935-40.
4. Maxwell GL, Risinger JI, Gumbs C, Shaw H, Bentley RC, Barrett JC. Mutation of the PTEN tumor suppressor gene in endometrial hyperplasias. Cancer Res 1998; 58: 2500-3.
5. Bussaglia E, Del Rio E, Matias-Guiu X, Prat J. PTEN mutations in endometrial carcinomas: A molecular and clinicopathologic analysis of 38 cases. Hum Pathol 2000; 31: 312-7.
6. Li J, Yen C, Liaw D, *et al*. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast and pro- state cancer. Science 1997; 275: 1943-7.
7. Steck PA, Pershouse MA, Jasser SA, *et al*. Identification of a candidate tumor suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997; 15: 356-62.
8. Liaw D, Marsh DJ, Li J, *et al*. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 1997; 16: 64-7.
9. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994; 54: 4855-78.
10. Lax SF, Kendall B, Tashiro H, Slebos RJ, Hedrick L. The frequency of p53, K-ras mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathway. Cancer 2000; 88: 814-24.
11. Tashiro H, Lax SF, Gaudin PB, Isacson C, Cho KR, Hedrick L. Microsatellite instability is uncommon in uterine serous carcinoma. Am J Pathol 1997; 150: 75-9.
12. Tashiro H, Isacson C, Levine R, Kurman RJ, Cho KR, Hedrick L. p53 gene mutations are common in uterine serous carcinoma and occur early in their pathogenesis. Am J Pathol 1997; 150: 177-85.
13. Muresu R, Sini MC, Cossu A, *et al*. Chromosomal abnormalities and microsatellite instability in sporadic endometrial cancer. Eur J Cancer 2002; 38: 1802-9.
14. Baldinu P, Cossu A, Manca A, *et al*. Microsatellite instability and mutation analysis of candidate genes in unselected sardinian patients with endometrial carcinoma. Cancer 2002; 94: 3157-68.
15. Fukuchi T, Sakamoto M, Tsuda H, Maruyama K, Nozawa S, Hironashi S. Beta-catenin mutations in carcinoma of the uterine endometrium. Cancer Res 1998; 58: 3526-8.
16. Kobayashi K, Sagae S, Nishioka Y, Tokino T, Kudo R. Mutations of the beta-catenin gene in endometrial carcinomas. Jpn J Cancer Res 1999; 90: 55-9.
17. Tritz D, Pieretti M, Turner S, Powell D. Loss of heterozygosity in usual and special variant carcinomas of the endometrium. Hum Pathol 1997; 28: 607-12.
18. Risinger JI, Hayes K, Maxwell GL, *et al*. PTEN mutation in endometrial cancers is associated with favorable clinical and pathologic characteristics. Clin Cancer Res 1998; 4: 3005-10.
19. Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-triphosphate. J Biol Chem 1998; 273: 13375-8.
20. Myers MP, Stolarov JP, Eng C, *et al*. PTEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. Proc Natl Acad Sci USA 1997; 94: 9052-7.
21. Di Cristofano A, Pandolfi PP. The multiple roles of PTEN in tumor suppression. Cell 2000; 100: 387-90.
22. Liu W, James CD, Frederick L, Alderete BE, Jenkins RB. PTEN/MMAC1 mutations and EGFR amplification in glioblastoma. Cancer Res

1997; 57: 5254-7.

23. De Vivo I, Gertig DM, Nagase S, *et al*. Novel germline mutations in the PTEN tumor suppressor gene found in women with multiple cancers. J Med Genet 2000; 37: 336-41.
24. Obata K, Morland SJ, Watson RH, *et al*. Frequent PTEN/MMAC mutations in endometrioid but not serous or mucinous epithelial ovarian tumors. Cancer Res 1998; 58: 2095-7.
25. Sato N, Tsunoda H, Nishida M, *et al*. Loss of heterozygosity on 10q23.3 and mutation of the tumor suppressor gene PTEN in benign endometrial cyst of the ovary: possible sequence progression from benign endometrial cyst to endometrioid carcinoma and clear cell carcinoma of the ovary. Cancer Res 2000; 60: 7052-6.
26. Minaguchi T, Yoshikawa H, Oda K, *et al*. PTEN mutation located only outside exons 5, 6, and 7 is an independent predictor of favorable survival in endometrial carcinomas. Clin Cancer Res 2001; 7: 2636-42.
27. Salvesen HB, Stefansson I, Kalvenes MB, Das S, Akslen LA. Loss of PTEN expression is associated with metastatic disease in patients with endometrial carcinoma. Cancer 2002; 94: 2185-91.
28. Kanamori Y, Kigawa J, Itamochi H, *et al*. PTEN expression is associated with prognosis for patients with advanced endometrial carcinoma undergoing postoperative chemotherapy. Int J Cancer 2002; 100: 686-9.
29. Vogelstein B, Kinzler KW. p53 function and dysfunction. Cell 1992; 70: 523-6.
30. Stewart RL, Toyds JA, Burton JL, Heatley MK, Wells M. Direct sequencing of the p53 gene shows absence of mutations in endometrioid endometrial adenocarcinomas expressing p53 protein. Histopathology 1998; 33: 440-5.