

B. Vogelstein for Ad-LacZ and Ad-p53. This work was supported by the University of Pennsylvania Comprehensive Cancer Center. W.S.E.-D. is an Assistant Investigator at the Howard Hughes Medical Institute.

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## PTEN1 is frequently mutated in primary endometrial carcinomas

Endometrial cancer is the most common cancer of the female genital tract in America<sup>1</sup> and Japan<sup>2</sup>. However, our knowledge regarding the molecular mechanisms underlying endometrial carcinogenesis is limited. Although mutations of *p53* (also known as *TP53*; ref. 3) and *ras*<sup>4</sup> have been reported in endometrial cancers, the prevalence of alterations in these two genes is low.

The *PTEN* (phosphatase and tensin homologue deleted on chromosome 10) gene, a candidate tumour-suppressor gene, was recently identified at chromosome 10q23.3 (refs 5,6). Studies thus far have demonstrated alterations of *PTEN* in Cowden disease<sup>7</sup>, as well as in human brain, breast, prostate and kidney cancer cells<sup>5,6</sup>. In addition, a high rate of loss of heterozygosity (LOH) has been reported at chromosome 10q23–q26 in endometrial cancers<sup>8</sup>. To determine the possible involvement of *PTEN* in endometrial and other malignancies, we investigated a series of endometrial, colorectal, gastric and pancreatic carcinomas for intragenic sequence alterations affecting the *PTEN* gene.

Paired normal and tumour genomic DNAs were purified from 41 colorectal, 29 gastric, 9 pancreatic and 38 endometrial carcinomas. We performed a pilot study of 10q-LOH, employing a PCR-based approach. LOH was identified in 6 (17%) of 35 informative colorectal cancers and 3 (15%) of 20 informative gastric tumours with three microsatellite markers surrounding *PTEN* (*D10S579*, *D10S215* and *D10S541*). Thirty-eight endometrial-cancer DNAs were assessed for LOH at three or more of the following seven loci: *D2S123*, *D9S162*, *D9S165*, *D10S215*, *D10S197*, *D10S541* and *D10S579*. Of 23 total infor-

mative endometrial-cancer samples, LOH was found in eleven cases (48%).

To determine whether intragenic *PTEN* DNA sequence alterations occurred in endometrial, colorectal, gastric and pancreatic cancers, PCR-SSCP analysis or direct DNA sequencing was performed for

all exons and intron–exon boundaries. SSCP was performed as an initial screen on eighteen colorectal, eleven gastric and nine pancreatic cancers. Direct sequencing was performed on DNA samples from all thirty-eight endometrial cancers, an additional six 10q-LOH+ colorectal cancers and

**Fig. 1** *PTEN* mutations in endometrial cancers.

**a, b, d**, Nucleotide substitutions: somatic DNA sequence alterations are shown on the coding DNA strand in cases JE94N/JE94T, U16N/U16T and U8N/U8T. One C-to-T mutation at codon 233 and a T-to-A mutation at codon 250 are indicated in sample JE94T, one G-to-A substitution at the first base of intron 4 is shown in sample U16T and a C-to-A substitution is shown at codon 336 in sample U8T. Mutant bases are indicated by arrows. **c, e**, Nucleotide deletions or insertions: a 4-bp deletion at codons 317–320 and a one-A insertion at codons 321–323 are indicated in samples U6T and U14T, respectively. A one-G deletion at codon 246 is shown in sample U10T. The arrow in **c** denotes the position of a one-G deletion in sample U10T (sense strand). The antisense strand sequence is shown in **e**. The arrow on the left in **e** indicates the position of a 4-bp deletion in sample U6T, while the arrow on the right indicates the position of a one-A insertion in sample U14T. Starting at each of these arrows, an upward displacement of the DNA sequence is visible in lanes G, A, T and C for tumours U6 and U14. As a first screening step, PCR-SSCP was performed according to methods described previously<sup>9</sup>, with some modifications<sup>10</sup>. Primer sequences were modified from Steck *et al.*<sup>6</sup> and are available on request; PCR annealing temperature was 54 °C. For confirmation of insertion and deletion mutations, a simple PCR-based assay was also performed<sup>11</sup>.

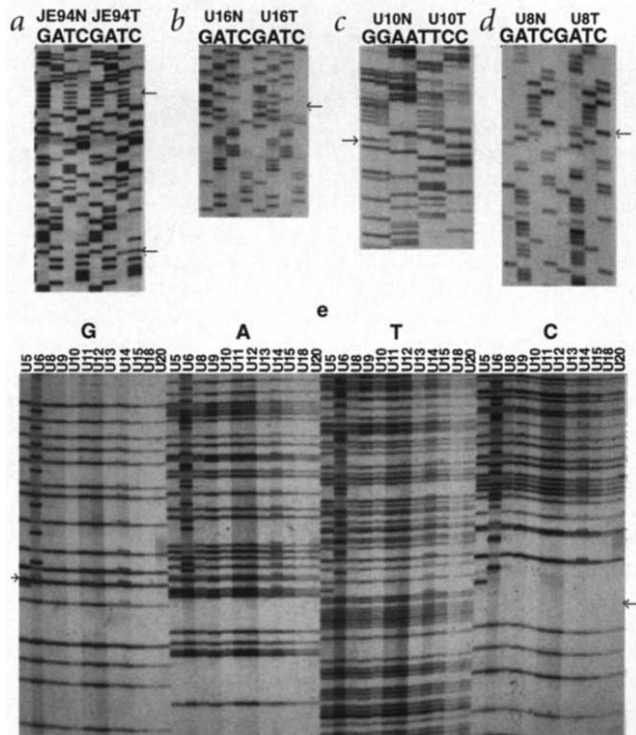


Table 1 • Summary of *PTEN* mutations in endometrial and other cancers

Samples	MI status	10q-LOH	Exon/intron	Codon	Nt position	Mutation	Predicted effect
U13T	-	-	exon 2	33	98	ATT to AGT	Ile to Ser
U17T	+	-	exon 2	32	95/	T del	stop at codon 53
U16T	+	+	intron 4	first base		G to A	splice donor
U18T	+	-	exon 5	93	277	CAT to TAT	His to Tyr
U10T	+	+	exon 7	246	738	G del	stop at codon 255
U6T	+	+	exon 8	317-320	950-953/	4 bp del	stop at codon 319
U14T	-	-	exon 8	321-323	963/	A ins	stop at codon 324
U8T	-	+	exon 8	336	1008	TAC to TAA	Tyr to stop
JE113T	-	NI	exon 2	32-33	94-96/	3 bp del	Ile del
			exon 8	from 326	976-intron 8	55 bp del	17 amino acids del, splice donor
JE103T	+	+	intron 4	first base		G to A	splice donor
JE89T	-	NI	exon 5	135-136	405	A ins	stop at codon 179
			exon 7	218	654	TGC to TGA	Cys to stop
JE92T	+	NI	exon 5	130	388	CGA to GGA	Arg to Gly
JE96T	+	+	exon 5	130	388	CGA to TGA	Arg to stop
JE94T	+	-	exon 7	233	697	CGA to TGA	Arg to stop
				250	750	TGT to TGA	Cys to stop
JE102T	-	+	exon 7	214	640	CAG to TAG	Gln to stop
JE86T	+	-	exon 7	265-267	795/	A del	stop at codon 275
			exon 8	317-320	950-953/	4 bp del	stop at codon 319
JE83T	+	NI	exon 8	321-323	963/	A del	stop at codon 343
JE90T	+	NI	exon 8	321-323	963/	A del	stop at codon 343
JE93T	-	NI	exon 8	321-323	963/	A ins	stop at codon 324
JE115T	+	-	exon 8	321-323	963/	A ins	stop at codon 324
			intron 3	1st-7th base		4bp del	splice donor
JE201T	+	+	exon 8	293	877	GGA to TGA	Gly to stop
G23T	+	NI	exon 8	321-323	963/	A ins	stop at codon 324

U and JE, endometrial cancers; G, gastric cancers; MI, showing microsatellite instability at more than one locus; 10q-LOH, loss of heterozygosity on chromosome 10q; NI, not informative; ins, insertion; del, deletion; Nt position, nucleotide number mutated (GenBank accession no. U93051). Front slash, frameshift mutations within repeat regions; first base of repeat indicated. No nucleotide number is given for intron mutations. For microsatellite instability (MI) and loss of heterozygosity (LOH) analysis, PCR, electrophoresis and gel analysis were performed as described<sup>12</sup>.

three 10q-LOH+ gastric cancers. No mutations were found in *PTEN* in the colorectal and pancreatic tumours, and only one mutation was identified in a gastric cancer with microsatellite instability (MI+). However, frequent mutations (21/38, 55%) were identified in endometrial carcinomas: 14/18 (78%) of MI+ and 7/20 (35%) of MI- cases (Fig. 1, Table 1). Eighteen (86%) of twenty-one mutations are predicted to produce truncated protein products. Two mutations were seen in each of five endometrial tumours without LOH; a variety of mutations were identified, including frameshifts, splice-site mutations and point mutations. Six (27%) of twenty-two tumours, four of which were MI+, had either an insertion or a deletion within a poly(A)<sub>n</sub> stretch in codons 321-323. Tumours U6 and JE86 had a 4-bp deletion in the region of nucleotides 950-958 in the sequence 5'-AGTACTTACTTT-3', which contains two direct repeats (TACT-TACT and ACTT-ACTT), as well as a palindromic structure (AGTA-NN-TACT). As these eight tumours had frameshift mutations between codons 317 and 323, the mutations were confirmed by simple PCR amplification. Two samples, U16 and JE103, had an identical base substitution (G-to-A transition) at the first base of intron 4. In sample JE115, a four-base deletion was present in the first seven bases of

intron 3. Missense mutations were identified in tumours U13, U18 and JE92, two in the putative phosphatase domain.

In addition, an insertion/deletion polymorphism containing the nucleotides ATCTT, spanning the 108th to the 112th base from the splice donor site, was observed in intron 4 with a frequency of 54% (with ATCTT) and 46% (lacking ATCTT; data not shown). Because heterozygosity is high in this polymorphic system, it may prove useful for analysing LOH at the *PTEN* locus.

Frequent LOH at 10q23-q26 in several recent reports strongly suggests the presence of a tumour-suppressor gene in this region. The high frequency of *PTEN* mutations in endometrial cancers in the present study suggests that this putative tumour-suppressor gene may be the target of allelic losses in endometrial carcinogenesis. The particularly high frequency of mutation in MI+ tumours further implies that *PTEN* may constitute a target of microsatellite instability; furthermore, the MI+ phenotype may predispose these tumours to simple base substitution mutations, as well as to the frameshift mutations that are typical of microsatellite instability. *PTEN* may be the most frequently mutated gene studied thus far in endometrial cancers, particularly those of the MI+ type. *PTEN* mutation appears less important in colorectal, gastric or pan-

creatic cancers. To completely elucidate the role of *PTEN* in human carcinogenesis, larger sample sizes, the function of the *PTEN* protein, levels of *PTEN* mRNA and protein expression in these and other tumour types should be investigated.

#### Acknowledgements

This work was supported by grants CA67497, DK47717, the Robert & Sally D. Funderburg Award for Gastric Cancer Biology and the Medical Research Service, the Department of Veterans Affairs (S.J.M.), the Ministry of Education, Science, Sports and Culture of Japan, the Vehicle Racing Commemorative Foundation and Japanese Foundation for Multi-disciplinary Treatment of Cancer and the Terumo Life Science Foundation (A.H.).

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