PTEN Mutation in Endometrial Cancers Is Associated with Favorable Clinical and Pathologic Characteristics[1]

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ABSTRACT

Mutation of the *PTEN* tumor suppressor gene is a frequent event in endometrial cancers. In other types of cancers, *PTEN* mutation has been associated with metastatic behavior and advanced stage. To examine the relationship between *PTEN* mutation and clinical features of endometrial cancers, we screened 136 cases for mutations in the nine exons and intronic splice sites of the *PTEN* gene, using single-strand conformation analysis, and aberrant bands were sequenced. Mutations were noted in 44 of 136 (32%) endometrial cancers, and two mutations were present in 8 cases. There were 36 cases with mutations resulting in truncated protein products, 6 cases with missense mutations in the phosphatase domain, 1 case with an in-frame deletion, and 1 case with a large insertion. Mutation of the *PTEN* gene correlated most closely with endometrioid histology; mutations were seen in only 5% (1 of 21) of serous/clear cell cancers compared with 37% (43 of 115) of endometrioid cancers ($P = 0.004$). *PTEN* mutation was associated with early stage, nonmetastatic disease and more favorable survival in both the entire group of 136 cases and in the 115 endometrioid cases. In addition, *PTEN* mutation correlated with other molecular features associated with favorable clinical behavior, including microsatellite instability and absence of p53 overexpression. Microsatellite instability was found in 60% of cases with *PTEN* mutations compared with only 25% of cases without mutations ($P = 0.004$). Overexpression of p53 was seen in only 14% of cases with *PTEN* mutations compared to 39% of cases without mutations ($P = 0.006$). In conclusion, *PTEN* mutation is associated with endometrioid histology and other favorable pathological, clinical, and molecular features rather than with increased metastatic potential as has been noted in some other types of cancers.

INTRODUCTION

Analysis of homozygous deletions on chromosome 10q23 in human cancers led to the discovery of the *PTEN* gene, which was named on the basis of its phosphatase domain and homology to tensin (1, 2). Several lines of evidence suggest that *PTEN* acts as a tumor suppressor gene. First, germ-line mutations of the *PTEN* gene are found in individuals with increased cancer susceptibility, including those with Cowden's syndrome, Bannayan-Zonana syndrome, and juvenile polyposis (3–7). The high frequency of deletion and mutation of the *PTEN* gene in some types of sporadic cancers also is consistent with the classic tumor suppressor model (1, 2). In addition, the presence of a phosphatase domain in *PTEN* suggests that it normally acts to oppose the activity of tyrosine kinase oncogene products (1, 2, 8). Finally, because of its homology to the cytoskeleton proteins tensin and auxilin, it has been postulated that *PTEN* might act to inhibit invasion and metastasis through modulation of the cytoskeleton (1).

Chromosome 10q23 is one of the most frequent sites of loss of heterozygosity in endometrial cancers, and this occurs in ~40% of cases (9). Following the discovery of the *PTEN* gene in this region, our group and others (10–12) demonstrated that *PTEN* mutations occur in ~30–50% of endometrial cancers. This represents the most frequent genetic alteration described thus far in this relatively common malignancy. The *PTEN* gene also is a frequent target of mutational inactivation in other human cancers, including glioblastomas (1, 13, 14), prostate cancers (15), and melanomas (16). In these other cancers, there is evidence to suggest that *PTEN* mutation is associated with a metastatic phenotype; one of the groups that identified this gene named it *MMAC1*, for "Mutated in Multiple Advanced Cancers" (2).

In contrast, initial small studies in endometrial cancer suggested that *PTEN* mutation is associated with less virulent clinical behavior and a lower likelihood of metastasis (10, 11). Previously, we found that 24 of 70 endometrial cancers had *PTEN* mutations (11). More recently we analyzed an additional 66 of these cancers and found mutations in 20 cases. To elucidate the clinical behavior of endometrial cancers with *PTEN* mutations, we examined the relationship between mutation of this gene and clinico-pathological features in the entire group of 136 endometrial cancers. We also explored the relationship between *PTEN* mutation and other molecular alterations known to correlate with clinical behavior, such as p53 overexpression and microsatellite instability.

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Table 1 Mutations in the *PTEN* gene in endometrial cancers

Case	Nucleotide(s)	Codon	Alteration	Change	Stage/grade
1	50–57	17–19	Del GGAT	Stop 23	IIB/3
	799–800	267	Del AA	Stop 296	
2	89	30	Del C	Stop 53	IC/3
3	96	32	Del T	Stop 53	IB/2
4	97–100	34–35	Del ATTG	Stop 53	IB/2
5	296	99	Ins TA	Stop 112	IVB/1
6	319	107	G→T	Asp to Tyr	IB/2
7	356	119	T→A	Phe to Tyr	IIIC/1
	952–955	318–319	Del CTTA	Stop 320	
8	382–390	128–130	Del 9 bp	Deletion Lys, Gly, Arg	IC/2
9	388	130	G→T	Arg to Leu	IIIA/2
10	387	130	C→T	Arg to Stop	IB/2
11	519	173	C→T	Arg to Cys	IIIC/2
12	530–534	177–178	Del ATTAT	Stop 178	IB/3
	969	323	Ins A	Stop 324	
13	762–775	254–259	Del 14 bp	Stop 298	IIIC/3
14	800	267	Del A	Stop 275	IB/1
15	800	267	Del A	Stop 275	IB/1
	968	323	Del A	Stop 343	
16	956–959	319–320	Del CTTT	Stop 343	IVB/3
17	968	323	Del A	Stop 343	IB/2
18	968	323	Del A	Stop 343	IVB/2
19	967–968	323	Del AA	Stop 324	IC/3
20	989–990	330	Del AA	Stop 341	IIIA/2

PATIENTS AND METHODS

Patients. Snap-frozen tissue samples from the Duke Gynecological Oncology tumor bank were analyzed; these samples came from 136 women (99 Caucasian, 34 African-American, and 3 Native American) who underwent treatment for endometrial adenocarcinoma at Duke University Medical Center between 1985 and 1997. All but five patients have been followed for more than 2 years after initial surgery. Staging was performed in accordance with the Fédération Internationale des Gynaecologistes et Obstetristes staging system. Advanced-stage cancers were intentionally overrepresented in the study group to increase our power to examine the relationship between alteration of *PTEN* and the presence of metastatic disease. In 82 cases (60%), the cancer appeared to be confined to the uterus, whereas in 54 cases (40%), there was histological evidence of metastatic disease. Among the 82 patients with nonmetastatic disease, full surgical staging including pelvic and aortic lymph node sampling was performed in 54 cases (66%). Lymph node sampling was not performed in some well or moderately differentiated, minimally invasive cases in which the risk of metastasis was low and in other cases because the patient was a poor candidate for an extended operation because of advanced age or comorbid conditions. All of the histological material was reviewed at diagnosis by expert gynecological pathologists at our institution.

PCR. Eleven primer pairs were used to individually amplify the nine exons and intronic splice sites of the *PTEN* gene from genomic endometrial cancer DNA as described previously (11). PCR mixtures consisted of 1–10 μg of genomic DNA; 200 μM each of dGTP, dCTP, and dTTP; 20 μM of dATP; 10× Taq buffer; 1 unit of Taq DNA polymerase, 0.1 μl [α ^{33}P]dATP; and 1.0 μM of each primer in a reaction volume of 10 μl. Amplification was then performed using a Perkin-Elmer Cetus 9700 thermocycler (Perkin-Elmer; Sunnyville, CA) using the following protocol: 7 cycles of 95°C for 20 s, 55°C for 20 s, and 72°C for 30 s; and 30 cycles of 95°C for 20 s, 48°C for 20 s, and 72°C for 30 s. Exon 1 was amplified using Amplitaq Gold with the corresponding buffer.

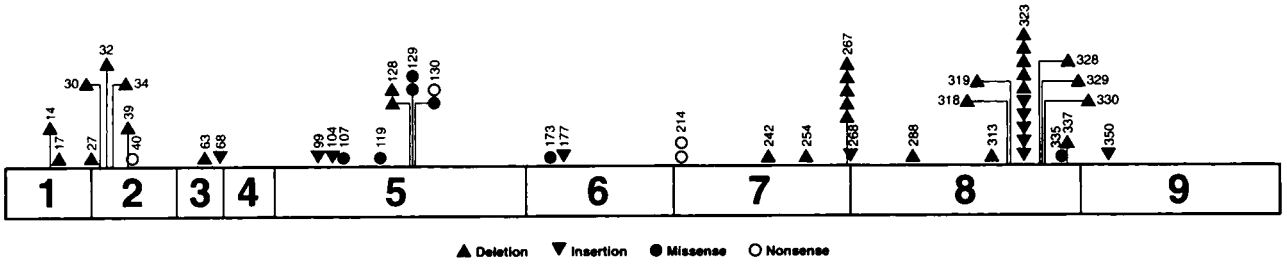

Fig. 1 Spectrum of mutations in the nine exons of the *PTEN* gene in endometrial cancers. There were 52 mutations identified in 44 endometrial adenocarcinomas.

Table 2 Relationship between *PTEN* mutation and clinico-pathologic features of endometrial cancers

	PTEN mutation					
	All cases (n = 136)			Endometrioid cases (n = 115)		
	No./total	(%)	*P*	No./total	(%)	*P*
Race						
Caucasian	37/99	(37%)	0.06	36/90	(40%)	0.48
African American	7/34	(21%)		7/24	(29%)	
Native American	0/3			0/1		
Age						
<65	17/53	(32%)	1.00	17/47	(36%)	0.85
≥65	27/83	(33%)		26/68	(38%)	
Histologic grade						
Well differentiated	14/29	(48%)	0.1	14/28	(50%)	0.49
Moderately differentiated	16/57	(28%)		15/52	(29%)	
Poorly differentiated	14/50	(28%)		14/35	(40%)	
Histologic type						
Endometrioid	43/115	(37%)	0.004	43/115	(37%)	
Serous/clear cell	1/21	(5%)				
Myometrial invasion[a]						
None	7/14	(50%)	0.06	7/13	(54%)	0.22
Inner third	14/44	(32%)		14/41	(34%)	
Middle third	13/34	(38%)		13/32	(41%)	
Outer third	9/44	(20%)		8/29	(28%)	
Stage						
IA	6/11	(55%)	0.01	6/11	(55%)	0.08
IB	16/37	(43%)		16/37	(43%)	
IC	7/17	(41%)		7/16	(44%)	
II	1/5	(20%)		1/3	(33%)	
IIIA[b]	3/18	(17%)		3/18	(17%)	
IIIB						
IIIC	5/24	(21%)		4/15	(27%)	
IV	6/24	(25%)		6/15	(40%)	
Recurrence						
No	38/93	(41%)	0.003	38/90	(42%)	0.06
Yes	6/43	(14%)		5/25	(20%)	

[a] One patient with advanced disease did not undergo hysterectomy, and the depth of invasion was not determined.
[b] Includes 12 cases with malignant cells in pelvic peritoneal cytology, but no histologic evidence of metastatic disease.

Single-Strand Conformation Polymorphism Analysis. PCR products were diluted 1:5 with denaturing loading buffer, heated to 95°C for 5 min, and then cooled on ice. Three μl of each sample were then loaded on nondenaturing 0.5% mutation detection enhancement gels and underwent electrophoresis at 8 W for ~16 h. Gels were dried and exposed to Kodak Biomax film. Exposure time ranged from 3 to 24 h.

Sequencing. Abnormally migrating bands were excised from gels after autoradiography and suspended in 100 μl of deionized water; 1 μl of this solution was used as a template for a subsequent PCR reaction. The PCR product was purified on a Wizard column (Promega, Madison, WI) and sequenced directly using the Thermosequenase kit according to the manufacturer's recommendations (Amersham, Arlington Heights, IN).

Analysis of Microsatellite Instability, p53 Expression, and Ploidy. We also examined the relationship between *PTEN* mutation and other molecular alterations that we had studied previously in these cancers (17–20). Normal and tumor DNA from 101 cases were amplified using a panel of three simple sequence repeat microsatellite loci: *BAT 26* $(A)_n$, *D14S65* $(CA)_n$, and *D14S297* $(GATA)_n$. Samples in which the tumor displayed additional alleles compared to normal DNA were scored as unstable; cases in which two of the three markers were unstable were classified as having microsatellite instability. In addition, p53 immunostaining had been performed previously on frozen sections of 125 of the endometrial cancers (18–20). Finally, ploidy had been assessed using a computerized image analysis system to quantitate feulgen staining in touch preparations of 76 endometrial cancers (19).

Statistics. Proportions among unordered categories were compared using Fisher's exact test, while those for ordered categories were compared using the χ^2 test for trend. Survival curves were constructed using the Kaplan-Meier method and differences were tested using the log-rank statistic.

RESULTS

Previously, we found mutations in the *PTEN* gene in 24 of 70 (34%) endometrial adenocarcinomas (11). More recently, we screened the *PTEN* gene in an additional 66 endometrial cancers and found 20 additional mutations (Table 1). In the two studies combined, mutations in the *PTEN* gene were found in 44 of 136 (32%) endometrial adenocarcinomas, and in 8 cases two mutations were present. There were 36 cases in which one or more mutations resulted in truncated protein products. In six cases, there were missense mutations in the phosphatase domain, in one case there was an in-frame deletion of three amino acids, and in another case, there was one large insertion. All 52

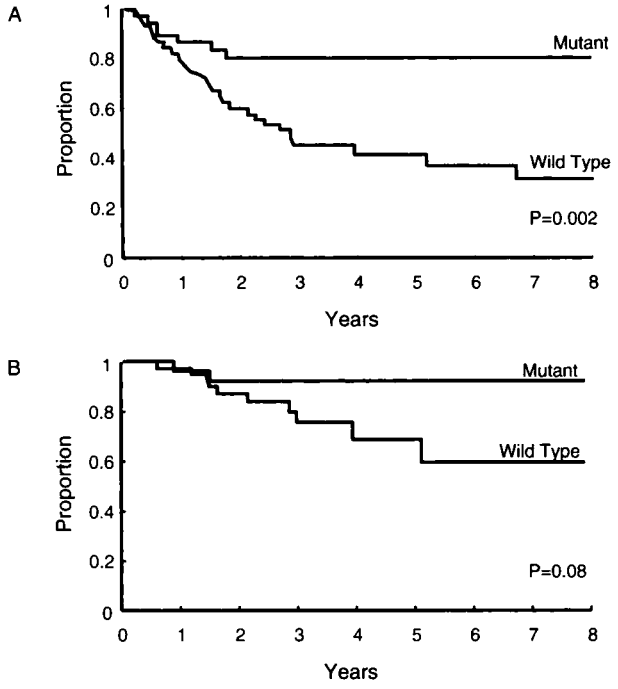

Fig. 2 Relationship between *PTEN* mutation and survival in endometrial cancer. *A*, all stages (n = 136); *B*, stage I/II (n = 82).

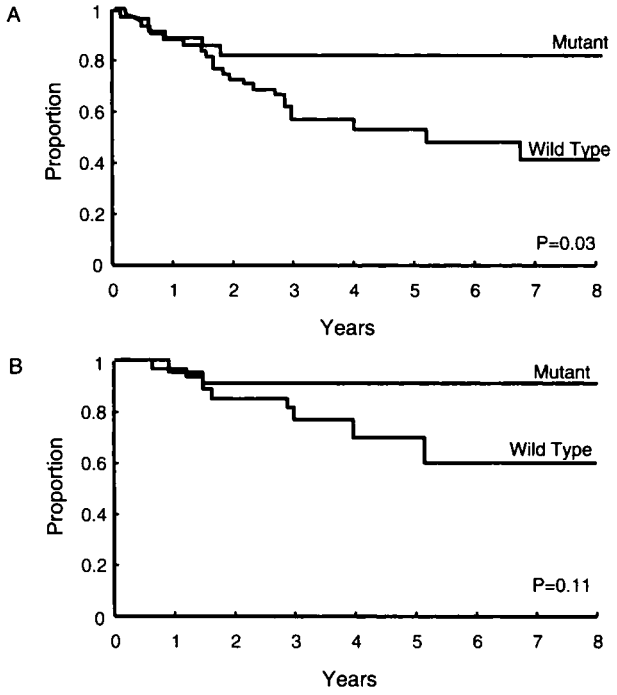

Fig. 3 Relationship between *PTEN* mutation and survival in endometrioid endometrial cancer. *A*, all stages (n = 115); *B*, stage I/II (n = 79).

mutations identified are illustrated in Fig. 1. In 20 advanced-stage cancers, both the primary tumor and one metastatic site were screened for *PTEN* mutations. In one of these cases an identical mutation was found in both sites, whereas in the other 19 a mutation was not found in either site.

The relationship between *PTEN* mutation and clinical and pathological features of all 136 endometrial cancers is presented in Table 2. *PTEN* mutation was associated with favorable pathological features such as endometrioid histology, low grade, and lack of myometrial invasion. The frequency of *PTEN* mutations in papillary serous (n = 18) and clear cell (n = 3) cancers (1 of 21, 5%) was significantly lower than that seen in endometrioid cancers (43 of 115, 37%), including both pure adenocarcinomas (37 of 103, 36%) and adenosquamous cancers (6 of 12, 50%). The highest frequency of *PTEN* mutations was seen in stage IA endometrioid cases that were confined to the endometrium without evidence of myometrial invasion or other spread of disease (6 of 11, 55%). In the entire group of 136 cancers, *PTEN* mutations were more common in cases in which the cancer was confined to the uterus (33 of 82, 40%) relative to those in which histological evidence of metastatic disease was present (11 of 54, 20%; P = 0.02). In addition, cancers with mutations had a lower recurrence rate (Table 2) and better survival (Fig. 2*A*) than those lacking mutations. This survival advantage also was seen in the subset of 82 early-stage cases (Fig. 2*B*). There was no relationship between *PTEN* mutation and estrogen/progesterone receptor expression, obesity (Quetelet index > 30), or history of hormone replacement (data not shown).

The frequency of *PTEN* alterations was somewhat lower in African Americans than in Caucasians (Table 2); however, as we have noted previously, African Americans have a higher frequency of advanced-stage disease (20). In this study, metastatic disease was present in 21 of 34 (62%) African Americans compared with only 32 of 99 (32%) Caucasians (P = 0.004). Because the frequency of *PTEN* mutations is lower in advanced-stage cases, it is not surprising that African Americans have a lower overall incidence of *PTEN* mutations. Among cases with advanced disease, however, there was an even more striking racial difference in the frequency of *PTEN* mutations. None of 21 African Americans with advanced (stage III/IV) disease had *PTEN* mutations, compared with 11 of 32 (34%) Caucasians (P = 0.004).

Because *PTEN* mutation is primarily a feature of endometrioid cancers, we examined the relationship between *PTEN* mutation and clinical and pathological features in the subset of 115 endometrioid cancers (Table 2). Similar to what was observed in the entire group, *PTEN* mutation in endometrioid cases was associated with favorable features. Mutations occurred in 33 of 79 (42%) cancers that were confined to the uterus and in 10 of 36 (28%) cases with metastatic disease (P = 0.21). In addition, cases with mutations had a lower recurrence rate and more favorable survival (Fig. 3) than those lacking mutations.

We examined the relationship between *PTEN* mutation and several other molecular features, including microsatellite instability, p53 overexpression, and DNA ploidy (Table 3). Microsatellite instability, which is associated with favorable clinical behavior (17), was noted in 20 of 101 (20%) cases and correlated strongly with mutation of the *PTEN* gene (P = 0.004). Immunohistostaining for p53 is indicative of the presence of mutant p53 protein and has been associated with poor prognosis (19, 20). Overexpression of p53 was seen in 36 of 125 (29%)

Table 3 Relationship between *PTEN* mutation and other molecular features of endometrial cancers

	PTEN mutation					
	All cases (n = 136)			Endometrioid cases (n = 115)		
	No./total	(%)	*P*	No./total	(%)	*P*
DNA ploidy						
Diploid (DNA index <1.2)	21/54	(39%)	0.20	21/52	(40%)	0.57
Aneuploid (DNA index ≥1.2)	5/22	(23%)		5/16	(31%)	
p53 overexpression						
No	35/89	(39%)	0.006	35/80	(44%)	0.03
Yes	5/36	(14%)		4/24	(17%)	
Microsatellite instability						
No	20/81	(25%)	0.004	17/59	(29%)	0.04
Yes	12/20	(60%)		11/19	(58%)	

cases and was less frequent in those with *PTEN* mutations. Aneuploidy (DNA index ≥1.2), which is associated with poor survival (19), was seen in 22 of 76 (29%) cases. Although cancers with *PTEN* mutations were less likely to be aneuploid than cases without mutations, the difference was not significant.

DISCUSSION

Mutation of the *PTEN* gene is the most frequent molecular event described thus far in endometrial cancers. The spectrum of mutations in the *PTEN* gene that we have observed in endometrial cancers was similar to that seen in this gene in other types of sporadic cancers (21) and in cancer syndromes due to germline *PTEN* mutations (3–7). Deletions, insertions, and nonsense mutations that lead to truncated protein products account for ~80% of mutations, whereas 15% have missense mutations in the critical phosphatase domain and 5% involve in-frame deletions or insertions.

Studies in prostate cancers, melanomas, and glioblastomas have suggested that *PTEN* mutation may be a characteristic of tumor progression and acquisition of the metastatic phenotype (1, 2, 15). It has been postulated that the tensin homology domain of *PTEN* might be involved in cell adhesion and that loss of this function due to inactivating mutations could increase the ability of a cancer cell to invade and metastasize (1, 2). In contrast there is now strong evidence to suggest that *PTEN* mutation in the endometrium is both an early event in carcinogenesis and associated with the development of cancers that are less likely to metastasize. In this regard, studies by our group (22) and Levine *et al.* (23) have demonstrated *PTEN* mutations in endometrial hyperplasias, which are premalignant precursors of invasive endometrial cancers. In addition, in the present study *PTEN* mutations were seen in both early- and advanced-stage endometrial cancers, but the highest frequency of mutations was observed in early-stage cases that were confined to the uterus. In addition, we did not observe *PTEN* mutations in metastases that were not also present in the primary tumor.

In the present study, we found mutations in the *PTEN* gene in 32% of cases. The study population was intentionally weighted toward advanced-stage cases, however, to facilitate examination of the relationship between metastatic behavior and *PTEN* mutation. Because advanced-stage cases have a lower incidence of mutations, this study undoubtedly underestimates the frequency of *PTEN* mutations in endometrial cancers. On the basis of the frequency of mutations observed in various sub-sets in this study, ~40% of endometrial cancers in a population-based sample would be predicted to have *PTEN* mutations.

Tashiro *et al.* (10) found a somewhat higher (50%) incidence of *PTEN* mutations in 32 endometrial cancers; however, in this study there was an overrepresentation of cancers with microsatellite instability, which have a higher incidence of *PTEN* mutations. Similarly, Kong *et al.* (12) found a 55% incidence of *PTEN* mutations in 38 endometrial cancers; again, however, cases with microsatellite instability were overrepresented. Overall, in the studies by these two other groups and our group, *PTEN* mutations have been noted in 38 of 52 cases (73%) with microsatellite instability. These combined data are strongly suggestive that the *PTEN* gene is a target for mutations in endometrial cancers that have deficiencies in DNA repair. It is not clear whether the *PTEN* gene is mutated at a higher frequency in cells with mismatch repair deficiency or whether there is a selection for cells that are *PTEN*-mutant and mismatch repair-deficient. Although microsatellite instability has been noted in ~20–30% of endometrial cancers, mutations in *hMSH2*, *hMLH1*, and other genes known to be involved in DNA repair have not been found in the majority of endometrial cancers with microsatellite instability (24). The origin of microsatellite instability in these cancers remains unclear, but may be due to down-regulation of DNA repair activity by mechanisms other than genomic deletion and/or mutation.

The frequency of *PTEN* mutations is clearly lower in endometrial cancers that do not exhibit microsatellite instability. When the data from this study and two other series of studies are combined, *PTEN* mutations have been noted in 31 of 113 cases (27%; Refs. 10, 12). Although *PTEN* mutations in cancers with microsatellite instability may arise due to failure of DNA repair mechanisms, it appears that *PTEN* mutations also can arise in some tumors that do not manifest DNA repair deficiencies. Because 70–80% of endometrial cancers lack microsatellite instability, about one-half of the *PTEN* mutations in a population-based sample of endometrial cancers would be predicted to occur in such cases despite the lower frequency of mutations.

Our group and others have shown that mutation and overexpression of the p53 tumor suppressor gene occurs in ~20–30% of endometrial cancers and is associated with advanced stage and poor survival (18–20, 25). Conversely, in this study *PTEN* mutation was associated with early stage and more fa-

vorable survival. Thus, it is not surprising that there was an inverse relationship between p53 overexpression and *PTEN* mutation. In five cases, alterations were seen in both p53 and *PTEN*, however, indicating that these are not mutually exclusive events. Among the five cancers with alterations in both genes, three had metastasized.

We have proposed previously that the increased frequency of p53 alterations observed in endometrial cancers of African Americans contributes to their relatively poor survival (20)–which is ~30% worse than that of Caucasians (26). In advanced-stage endometrial cancers, we have reported that p53 overexpression correlates strongly with decreased survival, and the frequency of p53 overexpression in African Americans (57%) was twice that seen in Caucasians (26%; Ref. 20). However, even among advanced-stage cases with p53 overexpression, survival of African Americans was worse than that of Caucasians. This suggests that factors other than p53 also contribute to the racial disparity in outcome. In the present study, *PTEN* mutations, which are associated with more favorable prognosis, were found in about one-third of Caucasians with advanced-stage disease but in none of the 21 African Americans with advanced-stage disease. Thus, the relatively poor prognosis of African Americans with advanced-stage disease may be due not only to the high frequency of p53 overexpression, but also partly to the absence of *PTEN* mutations.

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