

Frequent PTEN/MMAC Mutations in Endometrioid but not Serous or Mucinous Epithelial Ovarian Tumors¹

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Abstract

Epithelial ovarian cancer comprises three major histological subtypes (serous, mucinous, and endometrioid), and it is becoming clear that the developmental pathways for these subtypes are fundamentally different. In particular, endometrioid ovarian cancers probably arise by the malignant transformation of ectopic endometrial implants called endometriosis and not the ovarian surface epithelium. The *PTEN/MMAC* gene on chromosome 10q23 is a tumor suppressor implicated in the pathogenesis of a wide variety of malignancies, but to date, somatic mutations in *PTEN* have not been identified in studies of predominantly serous ovarian cancers. In endometrial cancers, *PTEN* mutations are very common in tumors of the endometrioid type but have rarely been found in serous types, and we hypothesized that a similar histological subtype bias might be occurring in ovarian cancer. We have analyzed 81 ovarian tumors, including 34 endometrioid, 29 serous, 10 mucinous, and 8 clear cell tumors, for loss of heterozygosity (LOH) on 10q23 and for mutations in all 9 coding exons of *PTEN*. LOH was common among the endometrioid (43%) and serous (28%) tumors but was infrequent among the other histological subtypes. Somatic *PTEN* mutations were detected in seven (21%) of the endometrioid tumors, and in all informative cases, the mutation was accompanied by loss of the wild-type allele. One mucinous tumor without 10q23 LOH was shown to harbor two somatic *PTEN* mutations. In this tumor, the histological appearance of the mucinous areas was atypical, and the mucinous areas contained foci of endometrioid differentiation. The majority of tumors with *PTEN* mutations were grade 1 and/or stage 1, suggesting that inactivation of *PTEN* is an early event in ovarian tumorigenesis. No *PTEN* mutations were found among the serous or clear cell tumors. The identification of frequent somatic *PTEN* mutations in endometrioid ovarian tumors indicates that it plays a significant role in the etiology of this subtype. The absence of mutations in other histological subtypes is consistent with the hypothesis that epithelial ovarian cancers arise through distinct developmental pathways.

Introduction

The molecular genetic events that underlie the development of ovarian cancer are largely unknown, and it is unclear whether the major histological subtypes (serous, endometrioid, and mucinous) are derived from a common precursor, or whether they arise through different developmental pathways. In particular, ECs³ are frequently found in association with endometriosis (1), and there is substantial evidence to suggest that they arise through the malignant transformation of these ectopic endometrial implants (2) and not the ovarian

surface epithelium, as is thought to be the case for serous and mucinous ovarian cancers.

Recently, the tumor suppressor gene *PTEN/MMAC/TEP1* on chromosome 10q23 has been shown to be somatically mutated in a wide range of tumors including those of the brain, prostate, breast, and thyroid (3–5). *PTEN* mutations are particularly common in endometrial tumors but are found predominantly in those of endometrioid histology and not in the more aggressive serous histological subtype (6–8). Mutations have not been found in ovarian cancer, but in the studies reported so far, the tumors have been predominantly of serous histology (8–10). Considering the histological subtype distribution of *PTEN* mutations in endometrial cancers, we reasoned that a similar bias may occur for ECs. Furthermore, if endometrioid ovarian tumors are derived from endometriosis, then in a sense they are of endometrial rather than ovarian origin, which provides a plausible developmental link between the two tumor types. Consequently, we analyzed 81 ovarian tumors, which included 34 endometrioid tumors, for LOH at 10q23 and for mutation in all 9 coding exons of *PTEN*.

Materials and Methods

Tumor Specimens and DNA Extraction. Eighty-one epithelial ovarian tumors were obtained from patients undergoing surgery for primary epithelial ovarian cancer. Matching tumor DNA and normal DNA were extracted from fresh tumor biopsies and from blood lymphocytes or by microdissection of normal and tumor areas of paraffin-embedded archival tissue as described previously (11). The collection included 34 endometrioid cancers, 29 serous tumors (27 malignant and 2 borderline), 10 mucinous tumors (6 malignant and 4 borderline), and 8 clear cell cancers.

PCR and LOH Analysis. PCR was carried out using 10–100 ng of genomic DNA in a reaction volume of 10 μ l with the inclusion of 1 μ Ci of [α -³²P]dCTP. Three microsatellite markers flanking the *PTEN* locus were used to assess LOH: D10S1696; D10S215, and D10S1765. The alleles were separated on 8% nondenaturing polyacrylamide gels and examined after autoradiographic exposure. Assessment of LOH was based on visual comparison of the intensities of the normal DNA alleles and those of the tumor.

SSCP and HD Mutation Analysis. All nine exons of *PTEN* were amplified separately using flanking intronic primers. Exon 1 was amplified using the primer set described by Risinger *et al.* (6). Exons 2–4 were amplified using the primer sequences described by Steck *et al.* (3) modified by removal of the M13 sequence from each primer. Exons 5–9 were amplified using the primers and conditions described previously (12). PCR products were denatured by the addition of an equal volume of 95% formamide and heating to 95°C for 5 min, followed by rapid cooling on ice. Denatured products were subjected to SSCP/HD analysis by electrophoresis through 0.5 \times mutation detection enhancement gel matrix (Flowgen, Lichfield, United Kingdom) as well as 6% polyacrylamide gels containing 5% glycerol (13). Cases showing aberrant band shifts were reamplified, and the PCR product was purified on a Wizard PCR prep column (Promega, Southampton, United Kingdom) and directly sequenced using the ThermoSequenase kit (Amersham, Little Chalfont, United Kingdom). Matching normal DNA was also sequenced to assess whether the alteration was germ line or somatic.

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³ The abbreviations used are: EC, endometrioid ovarian cancer; HD, heteroduplex; LOH, loss of heterozygosity; SSCP, single-strand conformational polymorphism.

Table 1 Summary of 10q23 LOH and PTEN mutations in ovarian tumors

Histological subtype	No. analyzed	10q LOH ^a	PTEN mutation
Endometrioid	34	13/30 (43%)	7/34
Serous ^b	29	7/25 (28%)	0/29
Clear cell	8	1/7 (14%)	0/8
Mucinous ^c	10	1/10 (10%)	1/10

^a 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. The percentage of cases with LOH is shown in parentheses.

^b Two of these tumors were of borderline malignancy. Neither showed 10q23 LOH.

^c Four tumors were of borderline malignancy; none showed 10q23 LOH. One tumor, case 144, was classified as mucinous but was atypical and contained foci of endometrioid differentiation.

Results

LOH analysis was performed on all tumors using three markers flanking the *PTEN* locus at 10q23. LOH was detected with at least 1 marker in 22 of 72 (31%) tumors, and with the exception of 2 cases, the LOH involved all informative markers. The highest frequency of LOH was detected in the endometrioid cancers (43%), but LOH was also relatively frequent among the serous tumors (28%; Table 1). Clear cell tumors exhibited a low frequency of LOH, although the number of cases examined was small. Only one mucinous tumor (a grade 1, stage 1 carcinoma) demonstrated LOH at 10q23 consistent with the lower overall frequency of LOH often reported at all chromosomal loci in mucinous ovarian tumors. However, four of the mucinous tumors were of borderline malignancy that may in part have contributed to the low 10q23 LOH frequency.

PTEN mutation analysis of all nine exons identified eight tumors with *PTEN* mutations (Table 2). Sequencing of matching normal DNA demonstrated that in all eight cases, these represented somatic alterations, examples of which are shown in Fig. 1. In six of the seven cases informative for 10q23 microsatellite markers, the *PTEN* mutation was accompanied by LOH of the wild-type allele consistent with a tumor suppressor function for *PTEN*. In the informative tumor without LOH (case 144), two somatic *PTEN* mutations were identified that are likely to be on different alleles, although this has not been confirmed. Seven of the tumors with *PTEN* mutations were of endometrioid histology, although in one of these (case 10.2) there were occasional small foci of mucinous differentiation. Tumor 144, which harbored two somatic *PTEN* mutations, had the overall appearance of a mucinous tumor, but the histological structure was atypical, and in many areas there were features suggestive of endometrioid differentiation.

The majority of the tumors with *PTEN* mutations were well or moderately differentiated, and six were stage 1, suggesting that in at least a subset of ovarian tumors, *PTEN* inactivation is an early event.

Discussion

Ovarian cancer has been the focus of extensive molecular genetic studies aimed at identifying the genes that underlie the development

of the disease. However, to date, only *TP53* and, to a lesser extent, *RASK* have been shown to be somatically altered in a significant proportion of ovarian tumors. Identification of all the genes involved in ovarian tumorigenesis will be essential for an understanding of the relationship between the different histological subtypes of ovarian cancer and between the benign, borderline, and malignant forms of the disease.

The *PTEN* gene has been shown to be mutated in a diverse range of cancers, and because 10q23 LOH has been observed in ovarian cancers (14, 15), it was an attractive candidate as an ovarian tumor suppressor gene as well. However, in 3 separate studies (8–10), no mutations were identified in a total of 41 primary ovarian cancers and 3 ovarian cancer cell lines, and it was concluded that *PTEN* was of no relevance in ovarian tumorigenesis.

Our interest in *PTEN* stemmed from the observation in endometrial cancer that *PTEN* mutations were common in tumors of endometrioid but not serous differentiation (6–8). Recently, we have provided genetic evidence that endometriosis, which arises by implantation and proliferation of refluxed endometrial cells, may be the precursor of EC (2, 11). If this were true, then we speculated that *PTEN* mutations were likely to be as common in endometrioid ovarian tumors as they were in endometrial carcinomas of endometrioid differentiation. The failure of previous studies to identify *PTEN* mutations may simply have been due to a bias toward the analysis of the more common serous tumors.

In this study, we have shown that *PTEN* is mutated in 21% of all endometrioid ovarian tumors and in 46% of those with 10q23 LOH, making *PTEN* the most common gene mutation reported so far in this histological subtype. This is likely to be an underestimate, because

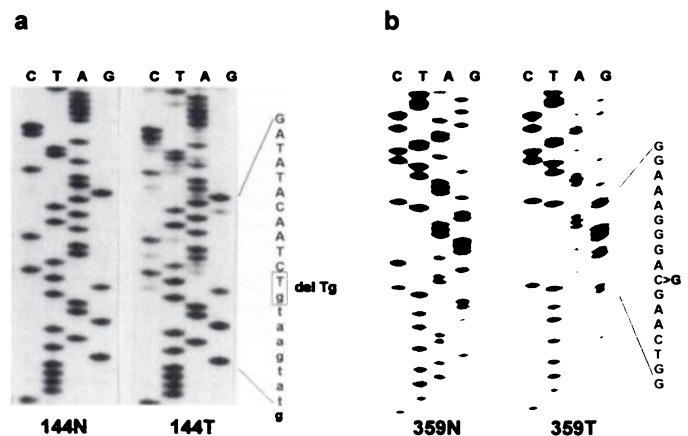


Fig. 1. Representative examples of *PTEN* sequence alterations. *a*, portion of a sequencing gel showing a frameshift/splice mutation caused by the deletion of TG in codon 70 in the tumor (T) DNA but not in the matching normal (N) DNA of case 144. *b*, portion of a sequencing gel showing a C → G transversion in codon 130 in the tumor DNA but not in the matching normal DNA of case 359.

Table 2 Summary of PTEN mutations detected

Case	Histology	Stage	Grade	10q LOH ^a	Exon	Codon	Base change	Amino acid change
2.2	EC	1b	1	+	5	126	GCT → GTT	Arg → Leu
4.2	EC	2	1	+	5	130	CGA → TGA	Arg → stop
10.2	EC	1c	2	+	7	233	CGA → TGA	Arg → stop
10.5	EC	1	1	+	5	130	CGA → GGA	Arg → Gly
144	MC ^b	1c	1	-	5	130	CGA → TGA	Arg → stop
					3	70	Del TG	Frameshift
253	EC	3a	2	+	8	317–318	Del 4bp	Frameshift
359	EC	1a	2	+	5	130	CGA → GGA	Arg → Gly
1148	EC	1	1	NI	8	308	Del G	Frameshift

^a +, detection of 10q23 LOH with at least one microsatellite marker; -, tumor informative for at least one 10q23 microsatellite marker but no LOH was detected; NI, tumor not informative for any microsatellite marker.

^b MC, mucinous ovarian cancer.

SSCP/HD mutation analysis is not 100% sensitive, and we did not analyze promoter regions of the gene. The only other gene mutations described for ECs are in *TP53* that are typically found in less than 15% (2, 16) of cases and in *RASK* that occur in less than 3% (2) of cases. The absence of *PTEN* mutation in any of the 29 serous tumors examined is consistent with previous studies of ovarian tumors and suggests that at best *PTEN* is rarely involved in this histological subtype. It is likely that *PTEN* is not involved in mucinous ovarian tumors, because 10q23 LOH was uncommon, and only one tumor harbored *PTEN* mutations. Additionally, the classification of this tumor as mucinous was uncertain, because the overall histological appearance was atypical, and there were foci of endometrioid-type differentiation. Nevertheless, the number of mucinous tumors analyzed was small and included four tumors of borderline malignancy; clearly, there is a need to examine a larger series before a role for *PTEN* in this subtype can be excluded.

It was surprising that no *PTEN* mutations were detected among the clear cell ovarian cancers, because these are frequently found in association with endometriosis and are believed to represent a histological variant of the endometrioid type (17). This may be due to the small number of cases examined, but it may indicate that the role of *PTEN* is related more to the histological differentiation rather than the tissue of origin.

In most tumor types such as glioblastoma (18) and prostate cancer (19), somatic *PTEN* mutations occur only in advanced tumors, suggesting that *PTEN* regulates cellular functions relevant in disease progression rather than initiation (6). However, germ-line mutations of *PTEN* are responsible for Cowden disease, which predisposes individuals to a variety of benign and malignant neoplasms. The contradictory nature of *PTEN* function suggests that it may regulate a range of cellular processes. The hallmark of Cowden disease is the development of multiple hamartomas that are composed of disorganized cell masses and has led to suggestions that one of the functions of *PTEN* is to control cellular proliferation and organization (12). Our data show that inactivation is an early event in endometrioid ovarian tumorigenesis, suggesting that it is the cellular proliferation function of *PTEN* that is most relevant in the development of this tumor type. This is consistent with the idea that ECs arise from endometriosis that essentially results from the inappropriate proliferation of ectopic endometrial implants. We have previously shown that ECs that are adjacent to or contiguous with endometriotic cysts harbor similar genetic alterations, suggesting a common lineage (2). It will be interesting to determine whether *PTEN* mutations are common in endometriosis, because this would provide an important genetic marker for verifying whether endometriosis is the precursor of ECs.

In summary, this study has demonstrated frequent *PTEN* alterations in ECs and supports the view that they develop along a fundamentally different pathway from those of serous and mucinous ovarian tumors.

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