PTEN/MMAC1 Mutations in Endometrial Cancers

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Abstract

Endometrial carcinomas represent the most common gynecological cancer in the United States, yet the molecular genetic events that underlie the development of these tumors remain obscure. Chromosome 10 is implicated in the pathogenesis of endometrial carcinoma based on loss of heterozygosity (LOH), comparative genomic hybridization, and cytogentic analysis. Recently, a potential tumor suppressor gene, PTEN/MMAC1, with homology to dual-specificity phosphatases and to the cytoskeletal proteins tensin and auxillin was identified on chromosome 10. This gene is mutated in several types of advanced tumors that display frequent LOH on chromosome 10, most notably glioblastomas. Additionally, germ-line mutations of PTEN/MMAC1 are responsible for several familial neoplastic disorders, including Cowden disease and Bannayan-Zonana syndrome. Because this locus is included in the region of LOH in many endometrial carcinomas, we examined 70 endometrial carcinomas for alterations in PTEN/MMAC1. Somatic mutations were detected in 24 cases (34%) including 21 cases that resulted in premature truncation of the protein, 2 tumors with missense alterations in the conserved phosphatase domain, and 1 tumor with a large insertion. These data indicate that PTEN/MMAC1 is more commonly mutated than any other known gene in endometrial cancers.

Introduction

The molecular pathogenesis of endometrial carcinoma is largely unknown (1). The most commonly observed defect is a profound instability of microsatellite sequences found in about 20% of tumors (2, 3). This type of microsatellite instability is seen in sporadic as well as tumors from hereditary nonpolyposis colorectal cancer, of which endometrial carcinoma is the second most common noncolonic tumor (4, 5). Microsatellite instability is likely a result of defective DNA mismatch repair. Additionally, a smaller percentage of tumors have activating alterations of the K-ras oncogene or alterations in the p53 tumor suppressor gene (1). Recently, several studies have examined LOH in endometrial carcinoma to elucidate regions of the genome that could harbor tumor suppressor genes. Chromosome 10 has been shown to contain significant LOH in endometrial carcinomas (6, 7). These studies have implicated two commonly deleted areas, one in the 10q22—24 region and a more distal region at l0q25—26 (7, 8). Additionally, conventional cytogenetic banding techniques and comparative genomic hybridization methods have implicated chromosome 10 in endometrial carcinomas (9, 10).

A candidate tumor suppressor gene, PTEN/MMAC1, was recently isolated from the 10q23—24 region and found to be mutated in several cancer types that display LOH in this region (11, 12). Of these mutations in PTEN/MMAC1 are most frequently found in advanced glioblastomas. The fact that mutations were seen in advanced tumors suggested that the gene was involved in disease progression and thus led to its nomenclature (mutated in multiple advanced cancer 1). PTEN/MMAC1 is also mutated in several other tumor types including those of the prostate and the breast. Germ-line mutations of PTEN/MMAC1 are also responsible for Cowden disease and Bannayan-Zonana syndrome (12, 13). These syndromes are characterized by several neoplastic conditions, some of which are benign in nature. These data strongly suggest that the PTEN/MMAC1 gene is involved in the pathogenesis of several varied neoplasms. Based on these findings and the clear involvement of this chromosomal region in endometrial cancers, we examined the PTEN/MMAC1 gene for mutational inactivation in a large panel of endometrial carcinomas.

Materials and Methods

Clinical Specimens. Snap-frozen tissue samples were obtained from 70 women who underwent hysterectomy for endometrial adenocarcinoma at Duke University Medical Center between 1990 and 1996. There were 16 well-differentiated cancers, 29 moderately differentiated cancers, and 25 poorly differentiated cancers. Staging was performed in accordance with the Fédération Internationale des Gynaecologistes et Obstetricistes staging system. Histological evidence of metastatic disease was found outside the uterus in 17 of 70 cases (24%). Complete clinicopathological information was abstracted from the hospital records. Genomic DNA and total RNA were isolated according to established protocols. Cases with identical mutations were genotyped with the D1S80 marker using the AmpliFlp kit (Applied Biosystems, Foster City, CA).

PTT. Five µg of total RNA were reverse-transcribed into cDNA using Superscript II reverse transcriptase and random hexamers according to the manufacturer’s recommendations (Life Technologies Inc., Gaithersburg, MD). The entire PTEN/MMAC1 gene was amplified using the Expand high-fidelity PCR system (Boehringer Mannheim, Indianapolis, IN) with a PCR cycling profile of 20 s at 95°C, 20 s at 55°C, and 2 min at 70°C, repeated 35 times. Primers for PCR were GATCTCTAAATGCTACTATAGGGAGACCCACATGACCCAGCATCAACAGAG and GTTCACATTCCCTCATTCAAC. PCR product (3 µl) was used in a coupled transcription and translation reaction (Promega, Madison, WI) that incorporated [35S]methionine to label the resultant proteins. Labeled proteins were analyzed on 10 and 4–15% SDS polyacrylamide gels.

SSCA. This assay was performed as described previously (14). The intron sequences of PTEN/MMAC1 were determined by sequencing the product of FA/RP primers described (11). The 9 exons of PTEN/MMAC1 were amplified using 11 pairs of primers based on the FA/RP sequences. Exons 5 and 8 were divided and amplified with two overlapping primer pairs. Normal and tumor DNAs were amplified using a step-down cycling profile of 7 cycles of 20 s at 95°C, 20 s at 55°C, and 30 s at 72°C, followed by an additional 30 cycles with an annealing temperature of 48°C, or alternatively, 37 cycles with an annealing temperature of 48°C. PCR was performed using AmpliTaq or AmpliTaq Gold (for exon 1) with appropriate buffers from the manufacturer (Applied Biosystems). The products were labeled with [35P]dATP, which was incorporated during the PCR. Primers for the exons were: exon 1, CAGAGAGAGC-CGGCCACAG and AGAGGAGAGCCGAGAGAAG; exon 2, TTACATATTCTCCTACA and AACAGAAATAATTCACATA; exon 3, TATTTCACATTTAAGCACAAGTACA and AAGATATGGCAGGGAGTG; exon 4, GTTGGTATTTAGCTTCTTCA and AACAACGATAGTACGATCATT; exon 5, TATTTCGATTTGATTTCTTCA and CTTTACCCTGTTTGA-CAGTGA (first pair) and GCCAAGGGTAAATGATACATCA and AGAGAAACACACAAAAAATA (second pair); exon 6, TTOCCCTTCTC-
Table 1 PTEN/MMAC1 mutations in primary endometrial carcinomas

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*Grade: 1, well differentiated; 2, moderately differentiated; 3, poorly differentiated.

*Histology: E, endometrioid; AS, adenosquamous.

Fig. 1. PTT of PTEN/MMAC1 in primary endometrial cancers. Lane 1 represents the protein generated from normal uterus and its position is denoted by wt (wild-type). Lanes 2–5 contain shorter peptides indicative of mutant PTEN/MMAC1 and are marked with an arrowhead, residual wild-type product is present in all cases, most likely due to the presence of nonneoplastic cells in the tissue biopsies.

Fig. 2. SSCA analysis of PTEN/MMAC1 exon 3. Altered mobility bands present in PCR products amplified from the patients' tumor DNA are seen in Lane 2 (case 6) and Lane 4 (case 5). PCR products amplified from the patients' normal DNA do not contain alterations indicating the somatic nature of these changes.

Results and Discussion

The entire coding region of PTEN/MMAC1 was examined using both an RNA-based (PTT) and a DNA-based (SSCA) mutation screening test. Both tests were used to maximize mutation detection. Mutations were identified in 24 of 70 (34.3%) unselected endometrial cancers (Table 1). Most mutations resulted in premature termination of the PTEN/MMAC1 message, either by small deletions or insertions and in several cases by missense alteration. Examples of cases detected by the protein truncation test are depicted in Fig. 1, Lane 2 (case 20), Lane 3 (case 23), Lane 4 (case 22), and Lane 5 (case 17), which clearly show peptides smaller than the wild-type peptide (Lane 1). Representative mutations detected by SSCA are shown in Fig. 2 for two cases with different alterations in exon 3. PCR products amplified from the patient's tumor DNA contain aberrantly migrating bands, whereas those amplified from normal lymphocyte DNA migrated with the wild-type pattern (Fig. 2). The exact nucleotide changes and subsequent effect on possible translated proteins is summarized for these and all mutations in Table 1. Three representative examples of the direct sequence analysis are depicted in Fig. 3. Several other missense alterations were detected in tumors. These changes were present in the corresponding normal DNA and were not additionally analyzed.
Tumors from two patients (cases 9 and 10, Table 1) have missense alteration in the PCR product generated from normal DNA, sequence 6.963—969; the change is not present in the patient's normal tissue (sequence 4). Single alteration is absent in the patient's germ-line DNA, sequence 2. Sequence 3 (case 18) was not assessed; it is possible that these lesions may harbor a higher frequency of alteration.

The frequency of PTEN/MMAC1 mutations described in this report is severalfold higher than that described for any other gene mutated in endometrial cancers, including K-ras and p53 (1). Based on these data, PTEN/MMAC1 mutations seem to represent the most common defined genetic alteration identified to date in endometrial cancers.

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**References**


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