Mutation of the PTEN Tumor Suppressor Gene in Endometrial Hyperplasias

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Abstract

Mutation and deletion of the PTEN tumor suppressor gene occurs in about 40% of endometrial carcinomas. The purpose of this study was to determine whether PTEN mutations also are present in endometrial hyperplasias, which are premalignant precursors of invasive endometrial adenocarcinomas. Genomic DNA from 51 endometrial hyperplasias was extracted from paraffin blocks, and PCR was used to amplify the nine exons of the PTEN gene. These products were screened using single-strand conformation analysis, and variant bands were sequenced. Somatic mutations in the PTEN gene were seen in 10 of 51 cases (20%), and two mutations were found in one case. An identical 4-bp deletion in exon 8 was seen in three cases, and 8 of 11 PTEN mutations predicted truncated protein products. There was no higher frequency of PTEN mutations in endometrial hyperplasias with atypia (6 of 32; 19%) relative to those without atypia (4 of 19; 21%). These data suggest that inactivation of the PTEN tumor suppressor gene is an early event in the development of some endometrial cancers.

Introduction

Endometrial cancer is the most common gynecological malignancy, and about 33,000 new cases arise annually in American women (1). Several molecular alterations have been identified that accompany malignant transformation in the endometrium. Overexpression of the HER-2/neu oncogene (2) and mutation of K-ras oncogene (3) occur in 10–20% of endometrial cancers. Mutation and overexpression of the p53 tumor suppressor gene (2) and microsatellite instability (4, 5) have been detected in 20–30% of endometrial adenocarcinomas. The molecular pathogenesis of these cancers remains poorly understood, however.

Endometrial hyperplasia is a premalignant lesion, and it is estimated that progression to invasive cancer occurs in about 5–10% of adenomatous hyperplasias and in 20–30% of cases with accompanying atypia (6). Previously, our group and others have shown that the frequency of K-ras mutations in endometrial hyperplasias (15%) is similar to that seen in endometrial adenocarcinomas, suggesting that this may represent an early event in endometrial carcinogenesis (3). In contrast, we found that overexpression of mutant p53 is not a feature of endometrial hyperplasias (7). This may signify that alterations in p53 may occur late in neoplastic progression. Alternatively, p53 mutation may be primarily a feature of endometrial cancers that do not arise from hyperplasia.

Recently, our group and others have found that deletion and mutation of the PTEN tumor suppressor gene on chromosome 10q23 occurs in about 40% of endometrial cancers (8–11). Mutations in PTEN also have been observed in glioblastomas, malignant melanomas, and prostate and renal carcinomas and have been associated with high grade and/or metastasis (12–17). Conversely, initial studies have suggested that endometrial cancers with PTEN mutations tend to be endometrioid, well differentiated, and minimally invasive (8, 9). Because these pathological characteristics are typical of cancers that arise in a background of hyperplastic endometrium, we sought to determine whether PTEN mutations are present in endometrial hyperplasias.

Materials and Methods

Tissue Specimens. Fifty-one patients were identified in whom endometrial hyperplasia had been diagnosed in either an endometrial biopsy or hysterectomy specimen at Duke University Medical Center between 1971 and 1991. Hematoxylin and eosin-stained slides were prepared from the paraffin-embedded tissue samples and examined by a pathologist to confirm the original diagnosis of hyperplasia. Histological examination revealed hyperplasia with atypia in 32 patients and hyperplasia without atypia in 19 patients. Ten sections (10 μm thick) were cut from the selected paraffin blocks. In hysterectomy specimens, microdissection was performed to assure that greater than 75% of the tissue sample analyzed contained endometrial hyperplasia. Genomic DNA was extracted from the tissue sections as previously described (4).

Polymerase Chain Amplification. The nine exons of PTEN were amplified using 11 intron-based primer pairs as described previously (10). PCRs consisted of 50–250 ng of genomic DNA; 10× Taq buffer; 1 unit of Taq DNA polymerase; 1.0 μM of each oligonucleotide primer (forward and reverse); 200 μM each of dGTP, dCTP, and dTTP; and 20 μM dATP in a reaction volume of 10 μL. Amplification was then performed using a Perkin Elmer Corp. 9600 thermocycler (Perkin Elmer; Sunnyville, CA) using the following protocol: 95°C for 3 min; seven cycles of 95°C for 20 s, 55°C for 20 s, and 72°C for 30 s; and then 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 corresponding buffers using the following protocol: 95°C for 9 min, 43 cycles of 94°C for 60 s and 60°C for 60 s, and 60°C for 10 min.

Single-Strand Conformational Polymorphism Analysis. PCR products were diluted 1:1 with denaturing loading buffer, heated to 95°C for 5 minutes, and then cooled on ice. Three ml of each sample were then loaded on nondenaturing 0.75% mutation detection enhancement gels, which were run at 5 W for approximately 16 h. Gels were dried and exposed to Kodak XAR film at −70 degrees with intensifying screens. Exposure time ranged from 0.5 to 3 h.

DNA Sequencing. Variant bands were excised from gels following autoradiography and suspended in deionized water for 3 h. Samples were reamplified, as described above, and purified using 4% low-melt agarose. DNA samples were then extracted from gel cuts and further purified using the QiAquick gel extraction kit (Qiagen, Santa Clarita, CA). Amplification of genomic DNA was performed in cases in which the original excised bands failed to yield adequate material for sequencing. PCR products were sequenced using either the Thermosequenase kit (Amersham Corp., Arlington Heights, IL) or the ABI dye terminator sequencing kit (Perkin Elmer). Samples were analyzed using the ABI Prism 377 fluorescent DNA sequencer (Perkin Elmer). In cases in which direct sequence analysis proved inadequate to determine clearly the sequence of the aberrant bands, the involved exon was reamplified and cloned into a T-tailed pBluescript plasmid. Several independent clones

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were sequenced using T3 and T7 plasmid primers to determine the exact sequence changes.

**Results**

The nine exons of the PTEN gene were amplified using PCR and screened for mutations using single-stranded conformation analysis in 51 endometrial hyperplasias. Examples of cases in which PCR products with altered mobility were observed are demonstrated in Fig. 1. DNA sequencing revealed 13 sequence variants, including one case in which two alterations were noted (Table 1). DNA sequence analysis of a mutation in codons 318-319 of exon 8 is demonstrated in Fig. 2, and the histological appearance of this atypical adenomatous hyperplasia is demonstrated in Fig. 3.

We found eight mutations that predict truncated PTEN protein products (Table 1). In seven cases, deletions were seen that lead to frameshifts with subsequent chain termination. This included three cases with an identical 4-bp deletion in codons 318–319 of exon 8. In two of these cases, we were able to obtain germ-line DNA from a paraffin block of normal tissue and the codon 318–319 deletion was not observed, indicating that this does not represent a polymorphism. One endometrial hyperplasia with this codon 318–319 deletion also had a more proximal nonsense mutation in codon 130. In addition, a nonsense mutation in codon 139 was seen in another case.

Five missense changes were observed that alter a single amino acid residue. A previously unreported missense mutation (Arg to Cys) was seen in codon 173, which resides in a highly conserved region of exon 6. This variant was not noted when exon 6 was sequenced in germ-line DNA obtained from a corresponding normal tissue block, and it presumably represents a somatic mutation. A missense variant also was found in codon 130, which is in the catalytic domain of the phosphatase. Although corresponding normal DNA was not available for analysis, this also likely represents a deleterious mutation. In one case, a missense alteration was seen in codon 36. This alteration also was observed in the corresponding normal tissue block and represents a polymorphism. In two cases, previously unreported missense alterations were seen in codons 191 and 348. Because corresponding normal tissue blocks were not available, it is unclear whether these represent polymorphisms or somatic mutations.

There was no relationship between the presence of PTEN mutations and clinical or pathological features. Mutations were seen in 4 of 19 (21%) adenomatous hyperplasias and 6 of 32 (19%) adenomatous hyperplasias with atypia.

**Discussion**

Endometrial hyperplasia is a premalignant lesion that usually develops in pre- or perimenopausal women due to stimulation of the endometrium by unopposed endogenous or exogenous estrogen. Although fewer than 10% of hyperplasias progress to invasive cancer in the absence of atypia, the risk of malignant transformation rises to about 20–30% when atypia is present (6). Endometrial cancers that develop in a background of hyperplasia usually are well-differentiated, endometrioid lesions that are minimally invasive and have a favorable prognosis. In contrast, endometrial carcinomas in older, postmenopausal patients usually arise within an atrophic endometrium, and these tumors more often exhibit poor prognostic features, such as nonendometrioid histology and poor grade.

Little is known regarding the molecular pathogenesis of endometrial hyperplasias and cancers. Mutations of the K-ras oncogene occur

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**Table 1 PTEN sequence alterations in endometrial hyperplasias**

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<tr>
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<th>Nucleotide</th>
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<th>Amino acid change</th>
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<td>128-132</td>
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<td>130</td>
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<tr>
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**Polymorphisms**

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**Variants of uncertain significance**

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<td>T&gt;C</td>
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<td>1043</td>
<td>348</td>
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PTEN MUTATIONS IN ENDOMETRIAL HYPERPLASIAS

in both endometrial hyperplasias and cancers (2), whereas mutations in the p53 tumor suppressor gene are present only in invasive cancers (2, 7). This has been interpreted as evidence that mutation of K-ras occurs early in the process of endometrial carcinogenesis, whereas mutation of p53 is a late event. Alternatively, alterations in various cancer-causing genes may lead to the development of endometrial cancers that differ with respect to their biological and clinical behavior. Identification of other molecular events that accompany the development of endometrial hyperplasia and cancer is needed to elucidate the pathogenesis of this disease process.

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 (12, 13). Several lines of evidence suggest that PTEN acts as a tumor suppressor. First, germ-line mutations of the PTEN gene are found in several hereditary cancer susceptibility syndromes, including Cowden disease (18). 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 (12–17). In addition, the presence of a phosphatase domain in PTEN suggests that it normally acts to oppose the activity of tyrosine kinase oncogene products (12, 13, 19, 20).

Mutation and deletion of the PTEN gene recently was shown to occur in about 40% of endometrioid endometrial cancers (8–11). Because these lesions often arise in association with hyperplasia, we screened the PTEN gene for mutations in these premalignant lesions and found alterations in 20% of cases. The 20% frequency of PTEN mutations in endometrial hyperplasias is somewhat less than has been reported in endometrial cancers (30–50%), but most hyperplasias do not evolve into fully invasive cancers. Perhaps PTEN mutation represents one of the critical genetic alterations involved in malignant progression of endometrial hyperplasias. Although hyperplasias with accompanying atypia have a somewhat greater propensity to progress to invasive cancer (6), a higher frequency of mutations was not observed in such cases.

The presence of PTEN mutations in endometrial hyperplasias suggests that this is an early event in the development of some endometrioid endometrial cancers. In contrast, PTEN mutations and allelic loss at 10q23 have been associated with tumor progression in glioblastoma melanomas and prostate cancers (12–17). It is possible that inactivation of the PTEN tumor suppressor gene may be an early event in the pathogenesis of some cancers and a later event in others. The spectrum of PTEN mutations in endometrial hyperplasias is similar to that seen in invasive endometrial cancers (9–11) and other types of cancers (12–17). Most of the mutations resulted in truncated protein products, but we also observed some missense mutations. Although many of the mutations directly involve the highly conserved central phosphatase domain, more distal mutations also are observed. It has been postulated that the distal portion of the PTEN molecule may play an important functional role by creating an acceptor cleft for the substrate that is to be dephosphorylated (19).

The highest frequency of PTEN mutations in invasive endometrial cancers occurs in cases with microsatellite instability (9, 11). In an analysis of 32 primary endometrial carcinomas, Tashiro et al. noted PTEN mutations in 86% of tumors with instability compared to only 33% of tumors without instability (9). This suggests that some PTEN mutations may arise secondary to loss of DNA repair mechanisms. In the present study, an identical deletion in codons 318–319 was seen

Fig. 2. Demonstration of PTEN mutation in exon 8 (Table 1, case 1) using DNA sequencing. This figure demonstrates sequencing of the reverse strand: A, wild-type sequence; B, deletion of CTTA in codon 318 of exon 8.

Fig. 3. Representative adenomatous hyperplasia with atypia in which a PTEN mutation was found in codon 318 of exon 8 (Table 1, case 1).
in three independent hyperplasia samples. A fourth case was noted to have a 1-bp deletion in codon 323. This area has been found to be a target for mutations in various cancers, including endometrial cancers (10, 14). Inspection of this region reveals a repeated sequence element (CTTACTTTA) that might be more susceptible to mutation in cells that lack intact DNA repair pathways.

Although microsatellite instability has been observed in about 20% of endometrial cancers (4, 5, 9, 11), germ-line or somatic mutations in DNA repair genes have been identified in only a minority of these cases (21). It is possible that known DNA repair genes may be inactivated in a higher frequency of cases than appreciated thus far. Alternatively, microsatellite instability in endometrial cancers may be due to loss of other DNA repair systems or as-yet-unknown mechanisms. Microsatellite instability in endometrial cancer is associated with well-differentiated grade and other favorable features that also are characteristic of cases that arise in hyperplasia. Although extensive analyses of microsatellite instability have not been performed in endometrial hyperplasias, Mutter and coworkers observed instability in both well-differentiated endometrial cancer and adjacent atypical hyperplasia in three cases (22). Additional studies are needed to determine whether PTEN mutations in endometrial hyperplasias arise due to loss of DNA repair mechanisms or by way of other mechanisms.

References

Mutation of the *PTEN* Tumor Suppressor Gene in Endometrial Hyperplasias


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