**PIK3CA and PTEN Mutations in Uterine Endometrioid Carcinoma and Complex Atypical Hyperplasia**

Monica Prasad Hayes,1,2 Hong Wang,1 Rosanny Espinal-Witter,1 Wayne Douglas,1 Garron J. Solomon,1 Suzanne J. Baker,3 and Lora Hedrick Ellenson1

Abstract  
**Purpose:** The tumor suppressor PTEN gene and the PIK3CA oncogene are frequently mutated in uterine endometrioid carcinoma (UEC). PTEN mutations are also common in complex atypical hyperplasia (CAH), the precursor lesion of UEC. The status of PIK3CA has not yet been explored in CAH. In this study, we evaluated both CAH and UEC for PTEN and PIK3CA mutations.

**Experimental Design:** Neoplastic tissue was microdissected, and DNA was extracted from 29 cases of CAH. DNA was available from 44 UEC cases previously characterized for PTEN mutations. Direct DNA sequencing of exons 9 and 20 of the PIK3CA gene was done on all 73 cases. In addition, CAH cases were analyzed for PTEN mutations. Statistical analyses were done using the Fisher's exact test.

**Results:** Two (7%) of 29 CAH and 17 (39%) of 44 UEC cases contained a PIK3CA mutation (P = 0.003). Fourteen (48%) of 29 CAH cases had a PTEN mutation, but none contained both a PTEN and PIK3CA mutation. Twenty-five (57%) of 44 UEC cases had a PTEN mutation, and 12 (48%) of these 25 cases also contained a PIK3CA mutation. Coexistent PIK3CA and PTEN mutations were significantly correlated with UEC compared with CAH (P = 0.002), but the association in UEC did not reach statistical significance (P = 0.21).

**Conclusions:** PIK3CA is the most commonly mutated oncogene in UEC; however, mutations are uncommon in CAH. Thus, mutations in PIK3CA, unlike PTEN mutations, are associated with invasion. These findings suggest that mutations in PIK3CA may serve as a marker of invasion with potential clinical use. Furthermore, PIK3CA and PTEN mutations may play distinct roles in endometrial tumorigenesis.

Within the last 10 years, our knowledge of the molecular genetics of endometrial cancer has expanded with the discovery of mutations in genes, such as PTEN, K-RAS, CTNNB1, and TP53. These discoveries have led to a better understanding of the pathogenesis of endometrial cancer and its distinct histologic subtypes, broadly categorized as type I and II cancers, which are thought to arise via different pathogenic pathways. Uterine endometrioid carcinoma (UEC) is the most common histologic subtype, representing >85% of cases. Not only is PTEN the most commonly mutated gene in UEC, the mutations also occur early in its pathogenesis as they are present at approximately the same frequency in complex atypical hyperplasias (CAH), the precursor lesion of UEC (1, 2).

Recently, mutations of a novel oncogene (PIK3CA) were discovered in multiple human epithelial cancers, including UEC (3, 4). PIK3CA encodes the catalytic p110-α subunit of phosphatidylinositol 3-kinase (PI3K), a lipid kinase that generates phosphatidylinositol 3,4,5-triphosphate by phosphorylating phosphatidylinositol 3,4-diphosphate. This in turn activates the AKT/mammalian target of rapamycin oncogenic pathway, directly opposing the actions of the lipid phosphatase PTEN (5). It is unclear what role mutations in PIK3CA play during endometrial tumorigenesis and what, if any, additional role they confer over PTEN mutations. Recent literature has suggested that PIK3CA mutations not only promote cell growth, but that they also promote invasion and are oncogenic in vitro and in vivo (6–8).

Given the importance of the PI3K pathway and the early inactivation of PTEN in the development of UEC, we investigated the status of the PIK3CA gene and its association with PTEN mutations in CAH and UEC. We found a high frequency of PIK3CA mutations in UEC similar to the only reported study thus far and, furthermore, show that mutations are infrequent in CAH. Our findings suggest that PIK3CA mutations, unlike PTEN mutations, are associated with invasion in the development of UEC.
Materials and Methods

**Specimen collection.** Twenty-nine cases of uterine CAH were retrospectively identified from an institutional database at the Weill Medical College of Cornell University. In addition, 44 cases of UEC that have been previously molecularly characterized in our lab for mutations in PTEN were evaluated for PIK3CA mutations (9). Of the UEC cases, 36 had early stage I/II disease, and eight had stage III/IV disease. Histologic grade of the UEC tumors was as follows: 23 grade 1, 14 grade 2, and 7 grade 3 tumors. A gynecologic pathologist (L.H.E.) reviewed the H&E-stained slides of all cases to confirm the diagnoses. All cases were anonymized, and Institutional Review Board approval was obtained.

**Microdissection and DNA extraction.** Five-micrometer tissue sections were prepared from formalin-fixed, paraffin-embedded endometrial curettings or hysterectomy specimens of both hyperplastic and normal tissue. Slides were stained with hematoxylin, and areas of hyperplasia were microdissected with a 26-gauge needle under direct light microscopy guidance. DNA was extracted with phenol-chloroform and precipitated with ethanol as previously described (9).

**PIK3CA and PTEN mutational analysis.** The exons that are known to be most commonly mutated in PTEN (exons 3, 4, 5, 7, and 8) were evaluated using exon-specific PCR amplification and direct DNA sequencing. In addition, exons 9 and 20 of the PIK3CA gene are the most common sites of mutations in this gene, and PCR amplification of each exon was done with subsequent DNA sequencing using the Applied Biosystems Automated 3730 DNA Analyzer (Foster City, CA).

Two to 100 ng of genomic DNA were amplified using previously described exon-specific primers for PTEN (9) and PIK3CA (3). For exon 20 of the PIK3CA gene, new primer sequences were constructed for optimal PCR conditions. A forward primer of 5′-CATTTGCTCCAAAACCT-GACCA-3′ and a reverse primer of 5′-TGTTGAATCCAGAGTGAGCTT-3′ were used with the following PCR conditions to produce a 353-bp PCR product: 5 minutes at 95°C for one cycle, 40 cycles at 95°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, followed by 5 minutes at 72°C for one cycle. Purification of PCR fragments was done with the QIAquick PCR Purification kit (Qiagen, Valencia, CA) and submitted for direct sequence analysis. All potential mutations were verified by reamplification from genomic DNA and direct sequencing of the mutated exon. In addition, insertion and deletion mutations were reamplified from genomic DNA and sequenced a third time. Novel alterations were confirmed to be somatic mutations by comparison of normal and neoplastic DNA sequences.

**Statistical analysis.** The Fisher’s exact test was used for a comparison of frequencies between two groups. All tests were two sided, and P < 0.05 was considered statistically significant.

Results

Two (7%) PIK3CA mutations were found in 29 uterine CAHs compared with 17 (39%) mutations in 44 cases of UEC (P = 0.003; Table 1). All PIK3CA mutations identified were missense mutations in a single allele. The two CAH cases with a PIK3CA mutation had identical missense mutations in exon 20, an A3140G transition mutation converting histidine to arginine (H1047R). In the UEC cases, the 17 PIK3CA mutations were evenly distributed between exons 9 and 20, with eight and nine cases mutated in each exon, respectively. The most commonly mutated codons in PIK3CA were 1047 in exon 20 and 545 in exon 9. H1047R was found in four UEC cases and H1047L (A3140T) in one case, whereas E545K (G1633A) was discovered in four cases and E545D (G1635T) in one case (Table 2). In addition, a novel mutation that has not previously been reported in the literature was confirmed in exon 20 of an UEC tumor. This mutation was an A-to-T transversion, thus replacing isoleucine with phenylalanine (I1058F; Fig. 1). DNA isolated from the patient’s normal tissue revealed a wild-type sequence, confirming the somatic nature of this alteration.

Fourteen (48%) of the 29 CAH cases harbored a PTEN mutation; however, there were no cases that contained both a PTEN and PIK3CA mutation. Of the 17 PTEN mutations found in 14 CAH cases, eight were frameshift mutations; three were nonsense mutations that coded for a truncated protein product; and six were missense mutations. The frequency of PTEN mutations was not significantly different in CAH compared with UEC. Twenty-five (57%) of the 44 UEC cases analyzed had a PTEN mutation, and 12 (48%) of these

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**Table 1. CAH and UEC cases with PIK3CA and PTEN mutations**

<table>
<thead>
<tr>
<th>PIK3CA</th>
<th>PTEN</th>
<th>PTEN + PIK3CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine CAH (n = 29)</td>
<td>2 (7%)</td>
<td>14 (48%)</td>
</tr>
<tr>
<td>UEC (n = 44)</td>
<td>17 (39%)</td>
<td>25 (57%)</td>
</tr>
</tbody>
</table>

**Table 2. PIK3CA mutations in CAH and UEC**

<table>
<thead>
<tr>
<th>PIK3CA mutation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1047R</td>
<td>2</td>
</tr>
<tr>
<td>E545K</td>
<td>4</td>
</tr>
<tr>
<td>E545D</td>
<td>1</td>
</tr>
<tr>
<td>Q546K</td>
<td>1</td>
</tr>
<tr>
<td>Q546R</td>
<td>1</td>
</tr>
<tr>
<td>Q546H</td>
<td>1</td>
</tr>
<tr>
<td>H1047R</td>
<td>4</td>
</tr>
<tr>
<td>H1047L</td>
<td>1</td>
</tr>
<tr>
<td>Y1021C</td>
<td>1</td>
</tr>
<tr>
<td>T1025A</td>
<td>1</td>
</tr>
<tr>
<td>M1043V</td>
<td>1</td>
</tr>
<tr>
<td>I1058F*</td>
<td>1</td>
</tr>
</tbody>
</table>

*Novel PIK3CA mutation not previously reported in other human cancers.*

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![Fig. 1. Novel PIK3CA mutation. A-to-T transversion, resulting in a transformation of isoleucine to phenylalanine (I1058F) in UEC.](image-url)
25 cases also contained a PIK3CA mutation (Fig. 2). PIK3CA mutations were not found to be significantly clustered with PTEN mutations and were also seen in 26% of UEC tumors without a PTEN mutation (P = 0.21). An observed difference in the coexistence of PIK3CA and PTEN mutations between CAH and UEC (0 of 29 versus 12 of 44) was highly statistically significant (P = 0.002). The frequency of two or more PTEN mutations was similar between the two groups and was found in two CAH and five UEC tumors.

The distribution of PIK3CA mutations was not correlated with grade or stage of disease in the UEC cases. Of the 23 grade 1 tumors in the UEC cohort, 11 (48%) contained a PIK3CA mutation, whereas 4 of 14 (29%) grade 2 tumors and 2 of 7 (29%) grade 3 tumors contained a PIK3CA mutation. When UEC cases with PIK3CA mutations were compared with those without a PIK3CA mutation, 11 of 17 (65%) cases with PIK3CA mutations were grade 1 tumors, whereas 12 of 27 (44%) cases without PIK3CA mutations were grade 1 tumors (P = 0.23). Five of the 44 UEC cases had associated CAH, and two of these cases contained PIK3CA mutations. Interestingly, both of these tumors were grade 1 carcinomas that also contained PTEN mutations.

### Discussion

Endometrial cancer remains the most common gynecologic malignancy in the United States with over 41,000 women diagnosed each year and 7,350 women expected to die from their disease in 2006 (10). The most common histologic subtype, UEC, has been shown to arise from the precursor lesion CAH. Observational studies have found that up to 29% of women with CAH will eventually develop invasive carcinoma if left untreated (11). Previous studies have also shown that inter-observer agreement by gynecologic pathologists is lowest when making the diagnosis of atypical hyperplasia (12). In addition, a recent study conducted by the Gynecologic Oncology Group showed that 42.6% of cases with a diagnosis of CAH on endometrial biopsy had adenocarcinoma in the uterus when hysterectomy was done shortly following the biopsy (13). Thus, treatment of uterine hyperplasia in the thousands of women diagnosed each year remains a clinical dilemma. Currently, the standard of care for women diagnosed with CAH is to recommend a hysterectomy. However, these women are often between the ages of 30 and 50 years, and many may desire future fertility. In this situation, uterine preservation becomes of critical importance, and clinicians find that hysterectomy may not be the most appropriate treatment in this patient population. These studies stress the importance of identifying those patients with CAH who will develop UEC, to potentially tailor therapeutic interventions. Currently, there are no markers that can determine which women are at risk of developing carcinoma; thus, a large number of women undergo hysterectomy unnecessarily.

Previous studies have found mutations of PTEN and K-RAS as well as the presence of microsatellite instability in CAH (1, 2, 14, 15), providing evidence that these are early genetic alterations contributing to the pathogenesis of UEC. Recently, a high percentage of mutations of the oncogene PIK3CA were identified in endometrial carcinoma (4). Given the fact that PTEN functions primarily to counteract the effect of PI3K and the recently reported role of PIK3CA mutants in invasion, we were interested in determining the status of PIK3CA in CAH and its relationship to PTEN mutations.

Our finding of a significantly lower frequency of PIK3CA mutations in CAH compared with UEC (7% versus 39%) provides evidence that PIK3CA mutations occur as a late event in endometrial cancer pathogenesis. This is comparable with colorectal tumors, in which 2 (3%) of 76 premalignant tumors were found to have a PIK3CA mutation, both of which were advanced tubulovillous adenomas, compared with a 32% prevalence of PIK3CA mutations in colon cancers (3). We found no significant difference in the frequency of PTEN mutations between CAH (48%) and UEC (57%; P = 0.64), indicating that PTEN mutations precede PIK3CA mutations in endometrial tumorigenesis. Mutations of PIK3CA and PTEN likely occur as independent events, as PIK3CA mutations were present in UEC with and without PTEN mutations. The high

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**Fig. 2.** A, H&E stain of CAH containing a PTEN mutation. B, H&E of UEC containing PIK3CA and PTEN mutations. C, DNA sequence of an A-to-T transversion in PTEN, resulting in a nonsense mutation (K144X) in CAH. D, A-to-G transition, causing a PIK3CA missense mutation (H1047R) in UEC. E, C-to-T transition, resulting in a nonsense mutation (R130X) of the PTEN gene in UEC.
frequency of PIK3CA and PTEN mutations in our UEC cohort, as well as a recent study showing that 36% of the endometrial cancers studied contained a PIK3CA mutation (4), provide evidence that the PI3K/AKT pathway can be activated by alterations in multiple genes. Furthermore, it suggests that PTEN and PIK3CA mutations may have additional nonoverlapping consequences during endometrial tumorigenesis.

CAH is often associated with progression to grade 1 UEC rather than higher-grade carcinomas (13). We examined grade as a possible factor associated with the presence of PIK3CA mutations and found that the majority of UEC cases with PIK3CA mutations were grade 1 tumors. It does not seem that PIK3CA mutations are significantly more associated with high-grade tumors. Thus, the higher rate of PIK3CA mutations in UEC versus CAH cannot be explained based on tumor differentiation.

Thirty-nine percent of the UEC cases evaluated contained a PIK3CA mutation, confirming that PIK3CA is the most commonly mutated oncogene yet identified in endometrial carcinoma. Thus far, K-RAF has been thought to be the most commonly mutated oncogene, with mutations reported in up to 26% of UEC (15–17). Twelve of the 19 PIK3CA mutations seen in the entire cohort of 73 cases were mutations in codon 545, located in the helical domain, and codon 1047, found in the kinase domain of the p110α subunit. These are the two most commonly mutated codons seen in other epithelial cancers as well (3, 18, 19), and the mutations E545K and H1047R, in particular, have been shown to be oncogenic (6, 8, 20, 21). These mutations cause increased lipid kinase activity and result in elevated PI3K expression, both in vitro in mammary epithelial cells (21, 22), colorectal cancer cell lines (8, 20), and chicken embryo fibroblasts (6) as well as in vivo in chickens (7) and nude mice (8). PI3K is a well-established enzyme that activates the AKT-mammalian target of rapamycin pathway and thus promotes tumorigenesis via increased cell proliferation, cell survival, and motility (23). Recently, functional studies of the specific mutations H1047R and E545K in the PIK3CA gene have shown that these mutations result in constitutive activation of AKT, producing a resistance to apoptosis and increased cell migration, invasion, and metastases (8). Our findings are consistent with these studies in that PIK3CA mutations are significantly associated with UEC compared with CAH and, thus, seem to serve as a marker for invasive disease. The clinical implications are apparent, and diagnostic screening tools for CAH may be developed, in which cases harboring PIK3CA mutations would be treated as invasive cancer, whereas those without this gene mutation would be candidates for a more conservative approach, such as pharmacologic treatment with progestins. In addition, agents that block the mammalian target of rapamycin, such as rapamycin and its derivative RAD001, inhibit PIK3CA-induced cellular transformation in vitro (6) and tumor growth in vivo (7). Our findings further establish the AKT pathway as a major mechanism through which uterine endometrioid tumorigenesis occurs. Thus, possible therapeutic interventions using molecular targeted agents that would regulate this pathway may play a role in the prevention and treatment of endometrial carcinoma.

References
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