# PTEN Expression in Endometrial Biopsies as a Marker of Progression to Endometrial Carcinoma

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## Abstract

**Inactivation of *PTEN* tumor suppressor gene is common in endometrial carcinoma and its precursor, atypical endometrial hyperplasia (EH). We compared PTEN expression via immunohistochemistry in endometrial biopsies diagnosed as EH in 138 cases, who were diagnosed with EH and then endometrial carcinoma at least 1 year later (median, 6 years), and 241 individually matched controls, who were diagnosed with EH but did not progress to carcinoma during equivalent follow-up. We assessed PTEN status (normal versus null) in index biopsies containing EH to estimate the relative risk (RR) of developing endometrial carcinoma up to 25 years later. Analysis of 115 cases and 193 controls with satisfactory assays revealed PTEN-null glands in index biopsies of 44% of cases and 49% of controls [*P* = 0.85; RR, 1.51; 95% confidence interval (CI), 0.73–3.13]. For predicting progression to carcinoma, PTEN-null status had low sensitivity (44%; 95% CI, 45–54%) and specificity (51%; 95% CI, 44–58%). Among 105 cases with PTEN results for both index biopsy and carcinoma, 16% had a PTEN-null index biopsy, 23% had PTEN-null carcinoma, and 26% had both a PTEN-null index biopsy and carcinoma. Loss of PTEN expression in endometrial biopsies was neither associated with nor a sensitive and specific marker of subsequent progression to endometrial carcinoma.** [Cancer Res 2008;68(14):6014–20]

## Introduction

The term endometrial hyperplasia (EH) refers to endometrial abnormalities ranging from mild proliferation to incipient carcinoma (1, 2). The WHO classification scheme combines architectural (simple versus complex crowding) and cytologic (no atypia versus atypia in nuclei) features to classify EH severity. Lesions with modest glandular crowding are called simple hyperplasia (SH), whereas those with more pronounced crowding or highly branching glands are called complex hyperplasia (CH). When cytologic atypia is present, the lesions are classified as simple atypical hyperplasia (SAH) or complex atypical hyperplasia (CAH). Atypical hyperplasia (AH) is often used to describe any EH with atypia because SAH is so rare (3). Detection of AH in an endometrial biopsy specimen carries a high risk of occult (4) or subsequent (5) carcinoma. In contrast, both SH and CH have low progression risks (5) but are more common than AH and have potential for overdiagnosis and overtreatment. This has stimulated a search for biomarkers that can be used for risk prediction.

Multiple lines of evidence support a role for the phosphatase and tensin homologue (*PTEN*) tumor suppressor gene as one such marker for endometrial carcinoma. *PTEN* regulates proliferation, growth, and apoptosis in a phosphatidylinositol-3-OH kinase (PI3K)-dependent pathway (6, 7). It produces a second messenger for the AKT pathway, which inhibits other tumor suppressor genes (e.g., p53, p21, and p27; ref. 8). Inactivation of *PTEN* and activation of PI3K together produce AKT phosphorylation, β-catenin accumulation in nuclei, and activation of gene transcription (9). *PTEN*-knockout mice develop EH and endometrial carcinoma (10, 11). Germline mutations in *PTEN* occur in 85% (12, 13) of patients with Cowden syndrome, an inherited condition associated with increased endometrial carcinoma risk (14). Somatic mutations have been reported in approximately one-half of patients with type I endometrial carcinoma (15) and AH (16). Loss of PTEN expression (i.e., PTEN-null glands) tends to be diffuse in endometrial carcinoma but also occurs in morphologically normal endometrial tissue (16), which suggests that PTEN abnormalities occur early in sporadic endometrial carcinomas. However, PTEN expression in endometrial biopsies containing EH has not been evaluated in population-based studies as a potential marker for predicting the subsequent risk of carcinoma. Using data from our well-controlled study (5) of progression risk among patients with EH, we compared PTEN expression by immunohistochemistry (IHC) in endometrial biopsy specimens from patients with EH who progressed to carcinoma versus patients with EH who did not clinically progress.

## Materials and Methods

We previously described our study design[8] and methods (5) in detail but summarized them here.

**Study participants.** Participants were members of the Kaiser Permanente Northwest (KPNW) prepaid health plan (17) who were originally diagnosed with incident EH at KPNW between 1970 and 2002.

**Cases.** Potential cases were diagnosed with endometrial carcinoma at least 1 y after their initial diagnosis of EH (i.e., index biopsy). Women who were diagnosed with endometrial carcinoma <1 y after their index biopsy

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were considered to have had prevalent carcinoma at presentation and were excluded.

We retrieved original pathology reports and diagnostic slides for all endometrial procedures, including hysterectomy. One gynecologic pathologist (MES) reviewed all slides, assigned a WHO diagnosis, and chose a single representative slide from each accession. Two experienced gynecologic pathologists (BMR and OBI) independently reviewed the selected slides and assigned a WHO diagnosis to each specimen. We then assigned a single, pathology panel WHO diagnosis based on a standard algorithm from the three independent reviews (5). For analysis, diagnostic categories were negative, disordered proliferative endometrium (DPEM), which represents equivocal EH (1, 3, 18), SH, CH, AH (either SAH or CAH), and carcinoma.

We identified 229 potential cases based on community WHO diagnoses: 188 women who had EH and then carcinoma, plus 41 women who had EH and then received a community diagnosis of CAH at hysterectomy. We excluded 15 cases whose original KPNW pathology reports were miscoded as EH or carcinoma and 76 cases whose panel WHO diagnosis was negative ($n$ = 55) or carcinoma ($n$ = 13) or whose slides were unavailable for review ($n$ = 8). This left 138 eligible cases who had a panel WHO diagnosis of DPEM ($n$ = 33), SH ($n$= 42), CH ($n$ = 21), or AH ($n$ = 42). We also reviewed slides from all follow-up surgical pathology specimens to confirm the diagnosis of carcinoma and time to progression.

**Controls.** To identify individually matched controls, we identified all women at KPNW who had incident EH at the same age ($\pm$1 y) and the same date ($\pm$1 y) as the cases but did not progress to carcinoma and remained at risk (i.e., no hysterectomy, no diagnosis of uterine cancer, alive, and still a member of the KPNW health plan) for an interval at least as long as the

**Table 1.** Clinical characteristics of 127 cases and 214 controls

| | Cases ($n$ = 127) | Controls ($n$ = 214)* | $P$ |
|---|---|---|---|
| Age at EH diagnosis (y) | $n$ (%) | $n$ (%) | 0.93 |
| Less than 44 | 25 (20%) | 49 (23%) | |
| 45–48 | 26 (21%) | 46 (22%) | |
| 49–52 | 25 (20%) | 37 (17%) | |
| 53–58 | 27 (21%) | 44 (21%) | |
| 59 or older | 24 (19%) | 38 (18%) | |
| Mean (y) | 52.1 | 51.4 | |
| Median year at EH diagnosis (range) | 1989 (1973–2001) | 1989 (1972–2002) | |
| Age at carcinoma or censoring (y) | $n$ (%) | $n$ (%) | 0.82 |
| Less than 50 | 17 (13%) | 42 (20%) | |
| 50–54 | 30 (24%) | 47 (22%) | |
| 55–59 | 39 (31%) | 39 (18%) | |
| 60–67 | 44 (35%) | 44 (21%) | |
| 68 or older | 42 (33%) | 42 (20%) | |
| Median follow-up interval (y) | 7.0 | 6.4 | 0.41 |
| By final diagnosis of EH | | | |
| DPEM | 10.5 | 5.9 | |
| SH | 9.4 | 6.7 | |
| CH | 5.7 | 6.4 | |
| AH | 4.4 | 6.4 | |
| Follow-up biopsies | | | |
| At least one | 104 (75%) | 207 (86%) | 0.08 |
| At least one within 1st 6 mo | 31 (22%) | 128 (53%) | <0.0001 |
| Median number (range) | 2 (0–12) | 2 (0–13) | |
| Mean among women with at least 1 | 2.9 | 2.5 | 0.41 |
| Treatment during progression interval | | | |
| Any progestogen | 118 (86%) | 222 (92%) | 0.47 |
| Oral† | 99 (72%) | 208 (86%) | 0.15 |
| I.m.† | 29 (21%) | 42 (17%) | 0.57 |

NOTE: $\chi^2$ likelihood ratio $P$ from conditional logistic regression analysis, adjusted for age at index biopsy, date of index biopsy, and duration of follow-up and weighted based on the batch-quota and countermatched sampling. For progression interval and follow-up biopsies, $P$ value is from Student's $t$ tests.

*Four hundred thirteen controls were originally matched to cases on age at EH diagnosis, date of EH diagnosis, and duration of follow-up, based on the original community diagnoses of EH. Because the 172 controls who were ineligible based on the pathology panel diagnoses are not included above, the distribution of age, date, and follow-up interval among the 241 eligible controls above differs slightly from the distribution among all 413 potential controls. The conditional logistic regression models retained individual matching on age at index biopsy, date of index biopsy, and duration of follow-up interval.

† Not mutually exclusive.

**Table 2.** PTEN expression at index biopsy for 115 cases and 193 controls

| | Cases | Controls | Total | *P* |
|---|---|---|---|---|
| | *n* (%) | *n* (%) | | |
| PTEN | | | | |
| Normal | 64 (55.7) | 98 (50.8) | 162 | |
| Null | 51 (44.3) | 95 (49.2) | 146 | 0.85 |
| Total | 115 (100.0) | 193 (100.0) | 308 | |

NOTE: PTEN expression status at index biopsy was unavailable for 11 cases and 27 controls. IHC was unsuccessful for 12 cases (9.4%) and 21 controls (9.8%). *P* value for case control difference is 0.85.

progression interval of the case to whom they were matched. We identified, on average, 43 potential controls for each case.

For each case, we chose three controls who were countermatched (19) on the severity of the community WHO diagnosis. This approach boosts statistical power and ensures the full spectrum of EH among controls.[9] We selected two controls whose community WHO diagnoses (i.e., SH, CH, or AH) differed from the case (e.g., for a case with SH, we chose one control with CH and one control with AH). We then oversampled AH by always selecting a third control who had AH. Countermatching yielded 413 controls: 129 with SH (31%), 153 with CH (37%), and 131 with AH (32%) as their community WHO diagnoses.

**Slide review of controls.** We reviewed all of the slides of the controls, using the same procedures that were used for the cases. We excluded 172 controls who had a panel WHO diagnosis of negative ($n$ = 160) or carcinoma ($n$ = 3), or whose slides were unavailable ($n$ = 9). This left 241 eligible controls who had a panel WHO diagnosis of DPEM ($n$ = 97), SH ($n$ = 67), CH ($n$ = 43), or AH ($n$ = 34). Procedures for determining final eligibility of controls were identical to those for cases: selection was based on the original community diagnosis of EH, but eligibility was based on the panel diagnosis. We also reviewed slides from all follow-up biopsies from all controls to confirm the lack of clinical progression to carcinoma.

**Medical record review.** We used a standardized abstract form to extract demographic characteristics, height and weight, reproductive and pregnancy history, other health factors, use of exogenous hormones, and treatment for EH. Risk factors were generally assessed at the time of index biopsy. We supplemented medical record data with linked outpatient pharmacy data (available from 1986 onward).

**PTEN IHC.** IHC stains for PTEN were performed on sections of the index biopsies (containing EH) from cases and controls and on hysterectomy tissues (containing carcinoma) from cases. After microwave antigen retrieval, sections were stained with murine monoclonal anti-PTEN antibody 6H2.1 (Cascade Biosciences) at 1:300 dilution and incubated overnight at 4°C, then washed and incubated with secondary biotinylated immunoglobulin (Vectastain ABC kit; Vector Laboratories, Inc.), followed by avidin peroxidase and 3,3′-diaminobenzidine. Epithelial staining was scored by GLM using intense PTEN staining in endometrial stroma and normal glands as an internal positive control. All of the tissue fragments were examined for PTEN-null glands, which were often few in number. Individual glands were scored as PTEN-null when signal was absent in the nuclear and cytoplasmic compartments of most cells in that gland. Specimens with any PTEN-null glands were scored as PTEN-null.

We randomly selected 30 PTEN-immunostained slides to be independently interpreted by two pathologists (MES and MAD) who were masked to the original scoring (by GLM). We calculated $\kappa$ values for agreement between these two reviewers and between each reviewer and the primary reviewer (GLM). The two pathologists subsequently reassessed every slide that was originally scored as PTEN-null to evaluate whether the PTEN-null glands were within the normal or hyperplastic tissue.

**Statistical analysis.** We assessed the frequency of PTEN-null expression according to the panel WHO diagnoses and then compared frequencies in cases versus controls using $\chi^2$ tests. To test PTEN expression in index biopsy versus hysterectomy specimens among controls, we used McNemar's test for paired data. Using conditional logistic regression, we generated rate ratios (RR) and 95% confidence intervals (CI) for the association between PTEN status at index biopsy and subsequent endometrial carcinoma. Normal expression of PTEN was the reference group. The final regression models included sampling weights for both the batch-quota and countermatched sampling, which were included as an offset in standard conditional logistic regression models (20), and were adjusted for age at index biopsy (1-y intervals), date of index biopsy (1-y intervals), and duration of follow-up (in days). Among all potential confounders, parity, diabetes, oral contraceptive use, a history of irregular menses, body mass index (BMI), number of follow-up biopsies, and hormonal treatment were associated with both PTEN (normal versus null) and case status. Only BMI, follow-up biopsies, and hormonal treatment altered the PTEN-null variable estimates by at least 10% and were included in regression models. We calculated sensitivity and specificity for subsequent carcinoma based on PTEN status at index biopsies.

**PTEN mutation analysis.** We evaluated potential lineage continuity for *PTEN* mutations in index biopsies and subsequent carcinomas from a subset of 20 cases that were selected to represent the range of diagnosis dates. We used immunodirected laser-capture microdissection (LCM) to isolate PTEN-null glands and adjacent PTEN-normal glands from cases with PTEN-null cancer specimens. Using a standard proteinase-extraction protocol, we isolated DNA from both the PTEN-expressing and the PTEN-null tissues. We amplified the DNA using established GC-clamped primers (16) that define the coding region and flanking introns of all 9 *PTEN* exons (21) without accidentally amplifying the PTEN pseudogene (22). We then sequenced the PCR products using denaturing gradient gel electrophoresis. When a case had a sequence-confirmed *PTEN* mutation, we performed immunodirected LCM, DNA extraction, and denaturing gradient screening on that matched index biopsy specimen of the case to assess whether the *PTEN* mutation that was observed in the cancer specimen was also present in the index biopsy specimen that was obtained years earlier. These analyses always included matched PTEN-expressing glands from the same specimen as negative controls to confirm the absence of the mutation that was observed in the PTEN-null glands.

**Human subjects.** The KPCHR's Research Subjects Protection Office and the National Cancer Institute's (NCI) Special Studies Institutional Review Board approved this study.

## Results

Index biopsies were available for IHC from 127 (92.0%) of the 138 eligible cases and 214 (88.8%) of the 241 eligible controls. Eleven cases and 27 controls either had unavailable tissue blocks or had previously refused access to their archived tissues for research.

Cases and controls had similar ages at index biopsy and diagnosis (for cases) or censoring (for controls). All 413 selected controls were matched to eligible cases on progression interval (5), but the 214 eligible controls had progression intervals that slightly differed from cases when evaluated by EH type (Table 1).

One-half of the index biopsies of the cases and controls displayed normal PTEN expression (Table 2). PTEN expression was not associated with case-control status ($P$ = 0.85) nor was expression statistically significantly associated with EH type (DPEM, SH, CH, or AH) among cases ($P$ = 0.25) or controls ($P$ = 0.74; Table 3). Among both cases and controls, slightly higher percentages of

[9] J.V. Lacey, Jr., et al., submitted.

**Table 3.** PTEN expression by panel WHO EH classification at index biopsy for 115 cases and 193 controls

| | PTEN-normal | | | PTEN-null | | Total |
|---|---|---|---|---|---|---|
| | *n* | Row % | | *n* | Row % | |
| Cases | | | | | | |
| DPEM | 16 | 59.3 | | 11 | 40.7 | 27 |
| SH | 23 | 67.6 | | 11 | 32.4 | 34 |
| CH | 7 | 50.0 | | 7 | 50.0 | 14 |
| AH | 18 | 45.0 | | 22 | 55.0 | 40 |
| *P** | | | 0.25 | | | |
| Controls | | | | | | |
| DPEM | 35 | 50.7 | | 34 | 49.3 | 69 |
| SH | 30 | 53.6 | | 26 | 46.4 | 56 |
| CH | 19 | 54.3 | | 16 | 45.7 | 35 |
| AH | 14 | 42.4 | | 19 | 57.6 | 33 |
| *P** | | | 0.74 | | | |
| Total | 162 | 52.6 | | | 47.4 | 308 |

NOTE: PTEN expression status at index biopsy was unavailable for 11 cases and 27 controls. IHC was unsuccessful for 12 cases (9.4%) and 21 controls (9.8%).
*$P$ based on $\chi^2$ test (PTEN status vs panel WHO classification) among cases and controls separately.

women with AH than DPEM, SH, or CH had PTEN-null index biopsies.

A PTEN-null index biopsy was not statistically significantly associated with progression to carcinoma (Table 4). The RR for PTEN-null was 1.51 (95% CI, 0.73–3.13). Additional adjustment for the panel WHO diagnoses as an ordinal variable (RR, 1.45; 95% CI, 0.65–3.22) or other factors (e.g., oral contraceptive use or diabetes) did not change results (data not shown). As a predictor of progression to carcinoma, PTEN-null status had low sensitivity (44%; 95% CI, 35–54%) and specificity (51%; 95% CI, 44–58%).

PTEN status was not associated with progression after stratification on panel WHO classification. Based on a reference group of DPEM and PTEN-normal, RRs for PTEN-null and PTEN-normal were essentially equivalent among women with nonatypical EH (SH or CH) and AH (Table 4). The only statistically significant RRs were for AH among PTEN-normal (RR, 7.34; 95% CI, 1.73–31.19) and PTEN-null (RR, 10.68; 95% CI, 2.60–43.78) index biopsies, and their CIs overlapped. The difference in RRs for PTEN-null (2.40) versus PTEN-normal (0.93) among women with nonatypical EH was not statistically significant ($P$ = 0.51).

**Table 4.** RRs of developing endometrial carcinoma after a diagnosis of EH according to PTEN expression (normal vs null) at index biopsy, combined with panel WHO classification

| | Cases | Controls | RR* (95% CI) |
|---|---|---|---|
| PTEN expression at index biopsy | | | |
| Normal | 64 | 98 | 1.00 (Reference) |
| Null | 51 | 95 | 1.51 (0.73–3.13) |
| WHO and PTEN | | | |
| DPEM & PTEN-normal | 16 | 35 | 1.00 (Ref.) |
| DPEM & PTEN-null | 11 | 34 | 0.58 (0.15–2.21) |
| (SH or CH) & PTEN-normal | 30 | 49 | 0.93 (0.31–2.77) |
| (SH or CH) & PTEN-null | 18 | 42 | 2.40 (0.70–8.30) |
| AH & PTEN-normal | 18 | 14 | 7.34 (1.73–31.19) |
| AH & PTEN-null | 22 | 19 | 10.68 (2.60–43.78) |

RRs are based on conditional logistic regression analysis adjusted for age at index biopsy, date of index biopsy, interval between EH and carcinoma (or censoring, for controls), and weighted based on the batch-quota and countermatched sampling. All controls were diagnosed with EH at the same age and date as the cases and remained at risk (i.e., no hysterectomy or uterine cancer) for at least as long as the progression interval of the cases. PTEN expression status at index biopsy was unavailable for 11 cases and 27 controls (RR, 0.81; 95% CI, 0.23–2.86). IHC was unsuccessful for 12 cases and 21 controls (RR, 0.67, 95% CI, 0.21–2.14). IHC was unsuccessful for 3 cases and 13 controls with DPEM (RR, 0.27, 95% CI, 0.05–1.48), 7 cases and 8 controls with SH or CH (RR, 0.79, 95% CI, 0.15–4.12), and 2 cases with AH (RR N/A).
*Adjusted for age at index biopsy, date of index biopsy, progression interval, BMI at index biopsy, follow-up biopsies, and MPA-based treatment for EH.

**Table 5.** PTEN expression at index biopsy vs hysterectomy for 104 cases with successful IHC in both specimens

| PTEN expression at index biopsy | PTEN expression at hysterectomy | | Total | *P* |
|---|---|---|---|---|
| | Normal | Null | | |
| Normal | 37 (35.6%) | 23 (22.1%) | 60 | |
| Null | 17 (16.3%) | 27 (26.0%) | 44 | 0.34 |
| | 54 | 50 | 104 | |

NOTE: PTEN expression status was unavailable for hysterectomy specimens from three cases, index biopsy specimens from four cases, and both index biopsy and hysterectomy specimens from seven cases. IHC was unsuccessful for index biopsy specimens from 11 cases, for hysterectomy specimens from 8 cases, and both index biopsy and hysterectomy specimen for 1 case. *P* value is based on $\chi^2$ McNemar's test.

We previously reported that classification of index biopsies according to a dichotomous WHO diagnosis (DPEM or nonatypical EH versus AH) or a dichotomous endometrial intraepithelial neoplasia (EIN) diagnosis (benign versus EIN) each produced similar associations between high-risk precursors (AH or EIN) and subsequent risk of carcinoma.[10] The association between EIN and subsequent carcinoma was also similar when stratified by PTEN status (normal versus null) at index biopsy (data not shown).

There was substantial agreement on PTEN status (K = 0.71; 95% CI, 35–100%) between the two NCI reviewers. Many of the PTEN-null index biopsies contained PTEN-null glands both in areas of normal endometrium and areas containing EH. Among cases and controls with PTEN-null index biopsies, cases were more likely (46 of 51; 90.2%) than controls (72 of 95; 75.8%) to have those PTEN-null glands within the EH foci. We repeated the analyses after redefining PTEN-null biopsies as those with loss of expression within EH foci, but results were essentially unchanged (data not shown).

The association between PTEN status at index biopsy and hysterectomy was not statistically significant among cases ($P$ = 0.34; Table 5). Twenty-seven cases (26.0%) were PTEN-null at both index biopsy and hysterectomy. Both index biopsy and hysterectomy were PTEN-normal for over one-third (35.6%) of the cases. Of 60 cases with PTEN-normal index biopsies, 23 (38%) had a PTEN-null hysterectomy specimen. Of 44 cases with PTEN-null index biopsies, 17 (38%) had a PTEN-normal hysterectomy specimen.

Neither the progression risk associated with a PTEN-null index biopsy nor the distribution of PTEN status at index biopsy versus hysterectomy differed after stratification on key endometrial carcinoma risk factors, such as obesity. Although IHC was successful on >90% of all index biopsies of cases and controls, most of the unsuccessful stains were among women whose index biopsies were before 1980. Repeating the analyses after excluding all patients diagnosed with EH before 1980 did not substantially change the results (RR for PTEN-null, 1.31; 95% CI, 0.61–2.81).

Of the 20 cases whose hysterectomy cancer specimens were included in the lineage continuity analysis, 18 had successful IHC and 9 (50%) were PTEN-null. After isolating DNA from the null and normal glands from those 9 cases, 8 (89%) had sequence-confirmed *PTEN* mutations in the carcinoma that were not present in adjacent PTEN-normal tissue, and 5 of those 8 had 2 mutations. Four of those 8 cases had PTEN-null index biopsies, and 3 of those 4 cases (75%) with PTEN-null index biopsies had a sequence-confirmed *PTEN* mutation in their index biopsy (Table 6). For all three of those four cases, the *PTEN* mutation found in the index biopsy was identical to the *PTEN* mutation that was found in their subsequent cancer specimen, including one case who had two identical mutations in the paired specimens.

## Discussion

Based on our IHC analysis, loss of PTEN expression in endometrial biopsies classified as DPEM or EH was not associated with increased risk of subsequent progression to endometrial carcinoma. The prevalence of PTEN-null glands in archived endometrial biopsy specimens was relatively high—almost 50%—yet almost identical among cases (i.e., women who progressed to carcinoma) and finely matched controls (i.e., women who did not progress to carcinoma during equivalent follow-up intervals). A similar percentage of the cases had PTEN-null glands in their hysterectomy specimens, although only one-half of these cases had persistent PTEN-null glands in both index biopsy and hysterectomy specimens. Taken together, our data from this population-based, nested case-control study indicate that PTEN expression in endometrial biopsies is unlikely to usefully predict progression to endometrial carcinoma.

The hypothesis that PTEN loss is a biomarker for premalignant clones capable of progressing from EH to carcinoma arose primarily from case series and convenience samples that showed PTEN-loss or *PTEN* mutations in patient groups defined by increasing histologic severity (16). In a series of 103 patients with EH who were followed for an average of 4 years, Baak and colleagues (23) reported that all 7 patients who subsequently developed endometrial carcinoma had PTEN-null hyperplasia. None of the patients with PTEN-normal EH progressed to carcinoma, but only 16% of the 43 PTEN-null patients progressed.

None of the previously published studies on PTEN expression and progression to carcinoma included a representative control group of women who had a similar risk of progression at baseline but did not progress to carcinoma during an equivalent follow-up period. The strengths of our study therefore lend validity to our conclusions. Both cases and controls were population-based and had community diagnoses of DPEM or EH. Careful matching on age, date, and follow-up time, plus countermatching on the

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community WHO diagnosis, ensured that the control group was unbiased, had well-characterized follow-up, and received similar treatment as the cases. Original archived slides and biopsy specimens were available from cases and controls, as was extensive clinical data.

Previous studies reported that PTEN-null glands can persist across menstrual cycles in women with otherwise normal endometrial histology (21). This implies that PTEN inactivation might represent an early biomarker of endometrial carcinogenesis (i.e., latent precancer) and could initiate progression before histologic changes appear. Our study was limited to women whose index biopsies were classified as DPEM or EH by our pathology panel. However, the prevalence of PTEN-null glands (44–49%) in our study was similar to the prevalence of PTEN-null glands among premenopausal women with histologically normal endometrial tissue (21) that was diagnosed as proliferative endometrium (43%; 24 of 56) by Mutter and colleagues (18). In addition, 39% of our cases with PTEN-null index biopsies had PTEN-normal glands at hysterectomy. A similar percentage of cases lost PTEN expression during their progression to carcinoma: 22% of cases with PTEN-normal index biopsies had PTEN-null hysterectomy specimens. PTEN inactivation occurred in slightly under one-half of all women, regardless of endometrial status, and gain or loss of PTEN expression occurred at roughly equal levels among women with proliferative or hyperplastic endometrial tissue. These factors contributed to the low sensitivity of PTEN-null status as a marker of progression.

Despite similar PTEN expression among index biopsies from cases and controls, we observed conserved mutations from PTEN-null glands that persisted for years. PTEN inactivation is primarily due to irreversible structural changes in *PTEN* (21). The conserved *PTEN* mutations sequenced from matched hyperplasia and carcinoma tissue in three cases therefore suggest that these carcinomas evolved directly from glands that were present many years earlier, when EH was diagnosed. Not all such clones, however, progressed to carcinoma. These mutations might be informative markers for unique clones in individual carcinomas, or they could reflect widespread *PTEN* mutations within the endometrium when EH was diagnosed. At present, the low sensitivity of PTEN expression as a marker of progression risk argues against analysis of *PTEN* mutations as candidate progression markers.

Sampling issues inherent in endometrial biopsies might have influenced these results. PTEN status in unbiopsied areas of endometrium might differ from PTEN status in the biopsy tissue that was available for study. Those errors are likely to be completely random among cases and controls, and therefore, differential PTEN misclassification is unlikely. Endometrial biopsy itself might alter natural history by entirely removing small foci of tissue that might otherwise be at risk of progressing; that, too, should be independent of PTEN status. We used a proven PTEN antibody test that is correlated with molecular alterations (24) and performed similarly well among cases and controls. One highly experienced pathologist interpreted all of the stains, and all laboratory personnel were blinded to progression status. It is unlikely that any controls had occult carcinoma during their follow-up; of 18 controls who had a hysterectomy after their censoring date (7.5%), only 3 (1.2%) were diagnosed with endometrial carcinoma. These 3 carcinoma diagnoses came 10 years after their index biopsies, and 3, 4.5, and 9 years after their censoring dates. Although our sample size was relatively small, we captured all potential cases at KPNW from 1970 to 2003. We had 89% statistical power to detect an RR of 2.0 for the association between a PTEN-null index biopsy and subsequent progression to carcinoma.

Loss of PTEN expression in these endometrial biopsy specimens was not associated with subsequent risk of carcinoma. Although PTEN expression had low sensitivity and specificity as a marker for progression from EH to carcinoma, conserved *PTEN* mutations in matched EH and carcinoma specimens in some women indicates that PTEN alterations can occur early in and persist during endometrial carcinogenesis. That prospect, plus the associations of PTEN with other hormone-dependent sporadic cancers, such as breast and ovarian cancers (25), suggests that PTEN may have other roles in endometrial

**Table 6.** *PTEN* mutations in paired index biopsy—cancer specimens from four cases with PTEN-null glands in both their index biopsies and hysterectomy specimens

| Index biopsy | | | Time to cancer (y) | Hysterectomy | | |
|---|---|---|---|---|---|---|
| Year | Panel Dx. | *PTEN* mutation in index biopsy | | Year | Panel Dx. | *PTEN* mutation in hysterectomy |
| 1983 | CH | Exon 3: IVS3+1 ~ 2 del GT | 8.8 | 1991 | Poorly differentiated AC | Exon 3: IVS3+1 ~ 2 del GT |
| 1991 | AH | Exon 5: L140F (TTA140TTT)<br>Exon 8: K342T (AAG342ACG)<br>IVS8+1T>T | 2.1 | 1994 | Moderately differentiated AC | Exon 5: L140F (TTA140TTT)<br>Exon 8: K342T (AAG342ACG)<br>IVS8+1T>T |
| 1987 | DPEM | Exon 4: V85F (GTT85TTT) | 13.5 | 2001 | Well differentiated AC with squamous differentiation | Exon 4: V85F (GTT85TTT) |
| 1986 | CH | None | 14.7 | 2001 | Well differentiated AC with squamous differentiation | Exon 3: 166 ~ 170 ins T |

Abbreviations: Dx, diagnosis; AC, endometrioid adenocarcinoma.

carcinoma besides influencing which EH lesions progress to carcinoma. Whether PTEN-related alterations in other pathways, especially mammalian target of rapamycin and PI3K/AKT, affect development of EH, progression from EH to carcinoma, or the clinical behavior of endometrial carcinoma is currently uncertain but warrants further consideration.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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