Body Mass Index is Associated with Immunohistochemical Nuclear Phosphatase and Tensin Homolog Deleted on Chromosome 10 (PTEN) Expression in Stage IB-IC Endometrioid Endometrial Carcinoma*

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Abstract

Objective: To examine the relationship between clinicopathologic features and nuclear phosphatase and tensin homolog deleted on chromosome 10 (PTEN) expression in a homogeneous group of Stage IB-IC sporadic endometrioid endometrial cancer (EEC) patients.

Materials and Methods: This study was conducted on 65 consecutive EEC patients with FIGO Stage IB-IC disease who underwent initial complete surgical staging. Age, body mass index (BMI), tumor grade, immunohistochemical nuclear PTEN expression and clinical outcome were examined. The median follow-up period was 49 months (range, 8 to 78 months).

Results: Immunohistochemical staining revealed positive nuclear PTEN expression in 20 cases (30.8%). Nuclear PTEN was found out to be reduced in 36 cases (55.4%), whereas it was completely lost in nine cases (13.8%). After evaluating the staining status, 45 cases (69.2%) were judged as "negative" for nuclear PTEN expression. During the follow-up period, clinical recurrence of disease was documented in five of 65 women (7.7%). The 5-year disease free survival rate for patients with positive nuclear PTEN expression was similar to that for patients with negative nuclear PTEN expression (75.2% vs. 91.3%, respectively; p=0.728 [log-rank test]). The mean body mass index (BMI) of positive nuclear PTEN expressing cases was significantly greater than that of patients with negative nuclear PTEN expression (32.5±6.5 vs. 28.5±5.0, respectively; p=0.009).

Discussion: Lost/reduced nuclear PTEN expression was frequent in FIGO Stage IB/IC EEC. BMI is the only clinicopathologic variable affecting immunohistochemical nuclear PTEN expression.

Keywords: nuclear PTEN expression, immunohistochemistry, body mass index, endometrial cancer

Özet

Evre IB-IC Endometrioid Endometriyum Kanserinde Vücut Kitle İndeksi İmmünohistokimyasal Nükleer PTEN Ekspresyonuyla İlişkilidir

Amaç: Sporadik Evre IB-IC endometrioid endometriyum kanser olgularında nükleer kromozom 10 üstünde fosfataz ve tensin homologu silinmesi (PTEN: phosphatase and tensin homolog deleted on chromosome 10) ekspresyonu ile klinikopatolojik özellikler arasındaki ilişkiyi araştırmak.

Materyal ve Metot: Komplet cerrahi evreleme işlemine tabi tutulan 65 Evre IB-IC endometrioid endometriyum kanser olgusu üzerinde retrospektif olarak çalışıldı. Hasta yaşı, vücut kitle indeksi, tümor gradı, immünohistokimyasal nükleer PTEN ekspresyonu ve klinik sonuç incelendi. Medyan izlem süresi 49 aydı (8-78 ay).

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Sonuçlar: İmmünohistokimyasal çalışma sonucunda olguların 20'sinde (%30.8) nükleer PTEN ekspresyonu pozitif olarak bulundu. Olguların 36'sında (%55.4) nükleer PTEN ekspresyonu azalmışken 9 olguda (%13.8) tamamen kayıptı. Boyanma durumu değerlendirildiğinde 45 olguda (%69.2) nükleer PTEN ekspresyonunun negatif olduğu bulundu. İzlem sürecinde 65 olgudan 5'inde (%7.7) klinik nüks saptandı. Nükleer PTEN ekspresyonunun pozitif bulunduğu olgulardaki 5 yıllık hastalıksız sağkalım, nükleer PTEN ekspresyonunun negatif bulunduğu hastalardaki 5 yıllık hastalıksız sağkalımdan farklılık göstermiyordu (%75.2'ye karşın %91.3, p=0.728 [log-rank test]). Nükleer PTEN ekspresyonu pozitif bulunan olguların ortalama vücut kitle indeksinin nükleer PTEN ekspresyonu negatif bulunan olguların ortalama vücut kitle indeksinden belirgin şekilde daha büyük olduğu saptandı (32.5±6.5'e karşın 28.5±5.0, p=0.009).
Tartışma: Kayıp/azalmış nükleer PTEN ekspresyonu, Evre IB-IC endometrioid endometriyum kanserinde sık rastlanılan bir bulgudur. İmmünohistokimyasal nükleer PTEN ekspresyonunu etkileyen tek klinikopatolojik değişken, vücut kitle indeksidir.

Anahtar sözcükler: nükleer PTEN ekspresyonu, immünohistokimya, vücut kitle indeksi, endometriyum kanseri

Introduction

In 1997, PTEN (phosphatase and tensin homolog deleted on chromosome 10) was cloned and mapped to cytoband 10q23, a region undergoing frequent somatic deletion in several tumors (1-3). PTEN is a tumor suppressor and lipid phosphatase that antagonizes phosphoinositide 3-kinase-dependent signaling through metabolism of the membrane-localized lipid second messenger phosphatidylinositol [3,4,5] P_3 (PIP_3) (4-6). The relationship of PTEN with cancer appears to be centered mainly on its capability to antagonize the PIP_3 kinase activity, modulating negatively the PIP_3-Akt signaling pathway (7). Although, other 3-phosphorylated inositol lipids and phosphates have been proposed as substrates for PTEN, current evidence indicates that PIP_3 may well be its only physiological substrate of this type (8). The balance between PTEN and phosphoinositide 3-kinase [PI3K] determines PIP_3 levels at the plasma membrane (9), which in turn, regulates numerous cellular processes.

Six characteristics have been defined that are usually necessary for tumor formation (10): evasion of apoptosis, self sufficiency in growth signals, insensitivity to anti-growth signals, sustained angiogenesis, tissue invasion and metastasis, and limitless replicative potential. It is notable that PIP_3 signaling appears to be involved in the regulation of at least first five of these six (11). Briefly, induction of apoptosis, G1 cell cycle arrest, cell-cell and cell-matrix adhesion, migration and angiogenesis are the main biological processes in which PTEN is involved (12,13).

The mean frequencies of PTEN mutation, loss of heterozygosity, and loss of cytoplasmic PTEN expression in endometrial cancer have been reported to be 42%, 50%, and 35%, respectively (11); making PTEN the most commonly mutated gene in endometrial cancer. Loss of PTEN expression in the vast majority of endometrioid endometrial cancers (EEC) and precancerous lesions, probably in the earliest stages of tumor development as shown by Mutter (14), suggests a "gatekeeper role" (15) for the PTEN gene in the endometrium. This gate keeping function of the PTEN gene appears to be selective for the endometrioid cell lineage that proliferates in a hormone-rich environment (16). PTEN mutations occur as an early event in endometrial carcinogenesis (17,18), even before the neoplastic phenotype is fully developed (19).

PTEN is expressed in the cytoplasm of many tumor cells. However, PTEN is expressed both in the cytoplasm and in the nucleus of normal cells, with a preferential nuclear localization in differentiated or resting cells (20-22). This inverse correlation between nuclear PTEN and the transformed phenotype suggests a tumor suppressor function for PTEN in the nucleus. An increased level of nuclear PTEN is associated with G0-G1 in MCF-7 cells, and nuclear PTEN has been postulated to be directly involved in regulating cell cycle progression (23). Chung and Eng (24) have reported that nuclear PTEN is required for the inhibition of cell growth, whereas cytoplasmic PTEN is required for apoptosis. Nuclear PTEN has been very recently reported to play a fundamental role in the maintenance of chromosomal stability through physical interaction with centromers and control of DNA repair (25), and to be essential for tumor suppression (26). PTEN functions at distinct cellular compartments are complex and the tumor suppressor and/or proapoptotic functions of PTEN, at least in part, are executed in the nucleus (27).

Previous studies have examined clinicopathologic features associated with immunohistochemical cytoplasmic PTEN expression, but to our knowledge, there is only one study (28) in the literature associated with nuclear PTEN expression in endometrial carcinoma. Since PTEN alternations have been found to be an early event in endometrial carcinogenesis (14), we sought to examine the relationship between clinicopathologic features and nuclear PTEN expression in a homogeneous group of Stage IB-IC EEC patients.

Materials and Methods

Patient sample

This study was conducted on 65 consecutive endometrial cancer patients with Stage IB-IC disease who were admitted and treated between 2000 and 2003 at two centers; Gynecologic Oncology Department of Etlik Maternity and Women's Health Training and Research Hospital of Ankara, and Department of Obstetrics and Gynecology of Inönü University of Malatya. Patients who had a history of concomitant or previous malignant disease, who did not undergo initial surgical intervention including pelvic-paraaortic lymphadenectomy, and who had pathologic diagnosis other than endometrioid type endometrial

carcinoma were not included in this study. No patients received any therapy before surgery. Surgical staging was applied according to "International Federation of Gynecology and Obstetrics" (FIGO) guidelines (29).

All patients underwent initial surgical operation consisting of peritoneal cytology, omental biopsy, total extrafascial abdominal hysterectomy, bilateral salpingo-oopherectomy with complete pelvic and paraaortic lymph node dissection. All cases were considered sporadic on the basis of a questionnaire targeted to establish the presence of tumors in first and second-degree relatives revealing the absence of familial history of cancer (30). All patients gave informed consent before collection of specimens. The histologic type was endometrioid adenocarcinoma in all subjects. There were 37 well differentiated (G1) cancers (56.9%), 22 moderately differentiated (G2) cancers (33.8%), and 6 poorly differentiated (G3) cancers (9.2%). The study population consisted of 44 women with FIGO Stage IB disease (67.7%) and 21 women with FIGO Stage IC disease (32.3%). The median age of the patients was 57 years (range, 31 to 82 years).

Complete clinicopathologic information and follow-up data were obtained from the hospital records. Adjuvant therapy was not administered to patients with Stage IB disease, whereas all patients with Stage IC disease received high-dose-rate brachytherapy in a dose of 27.5 cGy at a depth of 0.5 cm in five fractions using remote controlled afterloading system.

After completing treatment, patients were seen every three months for the first two years, every six months from the third up to fifth year, and yearly thereafter. Recurrence was considered as only documented relapse of the tumor, either locally in the pelvis or distant failure. Disease free survival (DFS) was calculated from the day of surgery until the day of clinical recurrence (uncensored) or the date last seen (censored). The median follow-up period was 49 months (range, 8 to 78 months).

Immunohistochemical staining

A section from each case was stained with hemotoxylin and eosin (H&E), and examined microscopically by one of the authors (N.K.) in order to confirm tissue adequacy. Thin sections (5 μm) of the selected formalin fixed and paraffin embedded specimens were then used for immunohisto-chemistry. The sections were mounted on Poly-L-lysine coated slides. Sections were then deparaffinized in xylene and rehydrated through a series of graded alcohol. Antigen retrival was performed for 10 min at 95°C in 0.01 M sodium citrate buffer (pH 6.0) in a microwave oven. Hydrogen peroxide (0.3%) in methanol was used to block endogenous peroxidase activity. According to the manufacturer's protocol, Protein Block Serum-Free Solution (Dako, Carpenteria, CA, USA) was used to incubate the sections in order to prevent non-specific staining.

Anti-PTEN antibody (28H6; Novocastra, Balliol Business Park West, UK) was used at 1:100 dilutions for 60 min at room temperature. Pre-immune serum was used as a negative control. The sections were washed with phosphate-buffered saline (PBS) three times each, for five minutes. After washing the sections with PBS, the DAKO LSAB2 kit was used based on the manufacturer's protocol. Visualization was performed by immersing 3-3'-diaminobenzidine in chromogene substrate for one minute. The stained slides were counterstained with hematoxylin and cover-slipped with EUKITT (O. Kindler, Freiburg, Germany).

Evaluation of immunohistochemical staining

Blinded for patient characteristics and outcome, the slides were examined with a standard light microscope for immunohistochemical staining by one of the authors (N.K.). Staining intensity and distribution were used to evaluate the status of nuclear PTEN staining. Staining intensity was judged as strong, moderate, or weak. Staining distribution was scored as diffuse (≥50% tumor staining), regional (15-50% tumor staining), and focal (<15% tumor staining).

Cases were considered "positive for nuclear PTEN expression" if staining intensity was strong with diffuse or regional staining distribution. Moderate intensity with regional or focal distribution was evaluated as "reduced nuclear PTEN expression", whereas weak staining intensity with any distribution pattern was judged as "lost nuclear PTEN expression".

It is important to note that regions of atypical endometrial hyperplasia adjacent to the tumors were not involved when staining status were recorded. Reading of nuclear PTEN immunohistochemistry blinded to patient and treatment status ensured that immunohistochemical staining evaluation was not affected by interpretive bias.

Statistical analysis

Statistical analysis was performed using SPSS Base 10.0. χ^2 and Fisher's exact tests were used to compare categorical data and distribution of patients between the two groups of nuclear PTEN expression. Student *t*-test was used where appropriate. Survival estimates were obtained via the Kaplan-Meier method. The log-rank test was used to assess the prognostic significance of nuclear PTEN expression on DFS. Multivariate analysis was performed by Cox's regression analysis (forward wald method). *P* values <0.05 were considered significant.

Results

Immunohistochemical staining revealed positive nuclear PTEN expression in 20 cases (30.8%) (Figure 1A). Nuclear PTEN was found out to be reduced in 36 cases (55.4%) (Figure 1B), whereas it was completely lost in nine cases (13.8%) (Figure 1C). After evaluating the staining status, 45 cases (69.2%) were judged as "negative" for nuclear PTEN expression, which suggests lost or reduced nuclear PTEN (dysfunctional PTEN) in those cases. According to the analysis, we compared nuclear PTEN lost/reduced cases (negative nuclear PTEN expression) versus cases with positive nuclear PTEN expression.

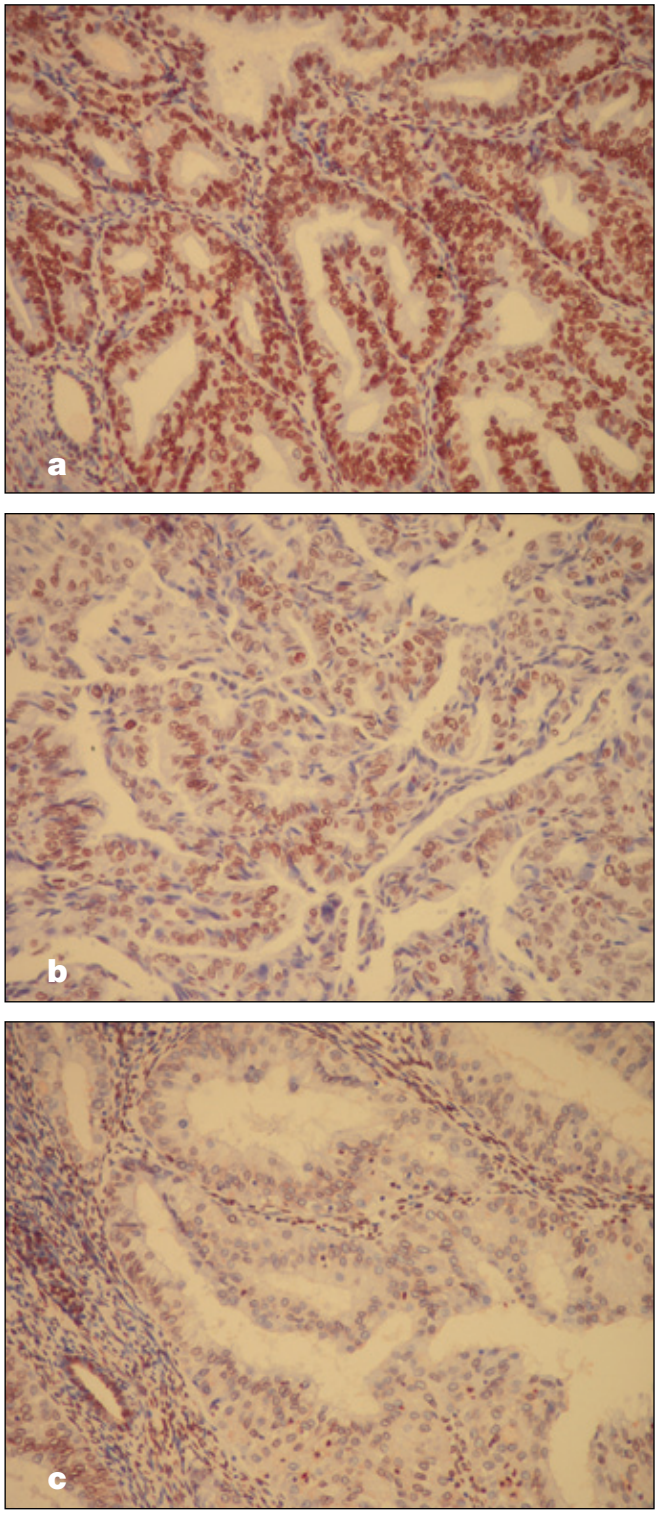

Figure 1. (**a**) There were cancer cells possessing abundant PTEN expression in the nuclei (positive nuclear PTEN status), x200 PTEN. In contrast, (**b**) there were cells in which staining for PTEN was dramatically reduced (reduced nuclear PTEN expression), x200 PTEN, or (**c**) completely lost (lost nuclear PTEN expression), x200 PTEN.

During the follow-up period, clinical recurrence of disease was documented in five of 65 women (7.7%). Characteristics of patients with recurrence were shown in Table 1. The 5-year DFS rate for patients with positive nuclear PTEN expression was similar to that for patients with negative nuclear PTEN expression (75.2% vs. 91.3%, respectively; p=0.728 [log-rank test]). Figure 2 shows DFS curves in relation to immunohistochemical nuclear PTEN expression.

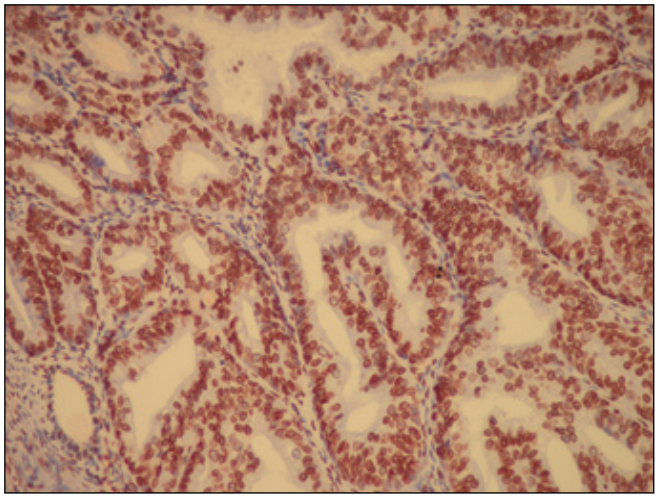

Figure 2. Disease free survival curves in relation to immunohistochemical nuclear PTEN expression.

Nuclear PTEN expression was not associated with age, FIGO Stage (IB vs. IC), histologic grade, menopausal status, initial serum CA 125 levels, or lymphovascular space invasion (LVSI) (Table 2). However, the mean body mass index (BMI) of positive nuclear PTEN expressing cases was significantly greater than that of patients with negative nuclear PTEN expression (32.5±6.5 vs. 28.5±5.0, respectively; p=0.009). BMI was the only clinicopathologic variable associated with immunohistochemical PTEN expression.

However, we were not able to define any independent prognostic factors associated with DFS according to subsequent multivariate analysis (Cox regression, forward Wald method).

Discussion

This study investigated the relationship between immunohistochemical nuclear PTEN expression with clinicopathologic features and clinical outcome in a homogenous group of FIGO Stage IB-IC primary sporadic EEC patients.

In the original study of Risinger (31), the frequency of PTEN mutations has been reported to be 43.4% (23/53) in FIGO Stage IB-IC endometrial cancer. Reduced nuclear PTEN expression has been reported to occur at 34% (19/56) of FIGO Stage I endometrial cancers (28). However, it should be emphasized that histological subtypes such as papillary serous, clear cell and adenosquamous carcinomas were also included in both of the above mentioned studies. We have found out that 69% of FIGO Stage IB-IC EEC patients have tumors retaining lost/reduced nuclear PTEN expression. Loss of PTEN expression may occur in the absence of PTEN gene mutations (11) although, it is currently unclear how PTEN expression is lost in the absence of molecular alternations.

Table 1. Characteristics of patients with recurrence

Stage	Grade	Nuclear PTEN	LVSI	Site of recurrence	Time to recurrence (months)	Treatment after recurrence	Final status
IB	1	Lost	Negative	Vaginal vault	38	Brachytherapy	NED[1]
IC	1	Positive	Negative	Abdomen + lung	30	Chemotherapy	DOD[2]
IC	1	Reduced	Positive	Vaginal vault	15	Chemotherapy	DOD[2]
IC	2	Reduced	Positive	Bone	4	Chemotherapy	AWD[3]
IB	1	Positive	Negative	Vaginal vault	27	Brachytherapy	NED[1]

LVSI: lymphovascular space invasion.
NED[1]: no evidence of disease.
DOD[2]: dead of disease.
AWD[3]: alive with disease.

Table 2. Clinicopathological variables and immunohistochemical nuclear PTEN expression

	Nuclear PTEN staining lost/reduced (n=45)	Nuclear PTEN staining positive (n=20)	*p* value
Age			
<60 y	27	11	0.7
≥60 y	18	9	
BMI[a]			
<27 kg/m²	20	2	0.007
≥27 kg/m²	25	18	
Parity			
0	9	7	0.1
1+	36	13	
Serum CA 125 level			
Normal	42	18	0.4
Elevated	3	2	
Menopausal status			
Premenopause	14	3	0.1
Postmenopause	31	17	
Histologic grade			
Grade 1	25	12	0.7
Grade 2 and 3	20	8	
LVSI[b]			
None	34	17	0.3
Positive	11	3	
Concomittant hyperplasia			
None	43	18	0.39
Positive	2	2	
FIGO stage			
IB	33	11	0.1
IC	12	9	
Recurrence			
No	42	18	0.6
Yes	3	2	

[a]BMI: body mass index.
[b]LVSI: lymphovascular space invasion.

An important question is whether external stimuli modulate the levels and/or activity of nuclear PTEN, and thereby regulate nuclear PTEN function. BMI has been identified as the only clinicopathologic feature associated with nuclear PTEN expression in our study. Women with nuclear PTEN-positive tumors had significantly greater body mass indices compared to women with tumors retaining negative nuclear PTEN expression. The association between increased weight and endometrial cancer is hypothesized to be the result of a mitogenic milieu of relative unopposed or elevated peripheral estrogen. Obesity (as quantified by BMI) is associated with increased levels of estrone via peripheral conversion of adrenal androstenedione within adipocytes by aromatization, as well as decreased sex hormone binding globulin (32-35). Obese women with endometrial cancer tend to have less aggressive disease by both stage and histology (36-39). Temkin (39) has recently reported that as BMI increases, endometrial cancer patients are more likely to have been diagnosed with histologically less aggressive disease, and increased BMI may confer a survival advantage.

PTEN is preferentially localized in the nuclei of differentiated or resting cells (20-22). As a mitogen, estrogen's indirect effect via high BMI on endometrial cellular proliferation seems to result in prominent nuclear PTEN expression. This might be the reason for type I endometrial cancer (40) arising in women with hyperestrogenic states, to be generally well differentiated.

Güzeloğlu-Kayışlı (41) has reported that short-term estradiol treatment (15 minutes) induces a marked increase in nuclear PTEN levels, whereas long-term estradiol treatment (3-24 hours) does not cause a further increase in nuclear PTEN. These *in vitro* findings indirectly support our findings associated with nuclear PTEN and BMI. On the other hand, immunohistochemical detection of cytoplasmic PTEN in cycling endometrium showed high levels of protein in all cell types during the proliferative phase with diminution or absence of epithelial PTEN protein in mid to late secretory glands (42). This implies that cytoplasmic PTEN is expressed specifically during the mitotically active, high-estrogen phase of the cycle, when regulation of replication is

most required. It is unknown whether an estrogen-associated increase in endometrial PTEN expression is a part of mitogenic response or whether there is a direct upstream link between estrogen response pathway and the PTEN gene (43). Whatever the case, it seems that a rapidly dividing estrogen-stimulated endometrial gland has a greater PTEN requirement than a quiescent progesterone-exposed nonmitotic gland, and it is reasonable to conclude that these settings would respond differently to loss of PTEN function (43).

Cytoplasmic PTEN protein has been suggested to be expressed as a negative feedback response to estrogen in order to control cellular overgrowth (44,45). In addition, cytoplasmic PTEN expression has been reported to be positively correlated with the expression of cell cycle regulators indicating higher proliferative activity (46). If the physiologic expression of nuclear PTEN also corresponds to a functional requirement, it is not surprising to detect more prominent nuclear PTEN staining as BMI increases.

Several studies have shown an association between microsatellite instability (MSI) and mutations in the PTEN gene, with PTEN mutations identified in 60-86% of EECs with MSI and in only 24-35% of EECs that were microsatellite stable (19,47). Since EECs with MSI were found more often to harbor PTEN mutations (48), our finding associated with BMI indirectly supports the findings of McCourt (49), who reported that BMI was significantly greater in microsatellite stable tumors compared to microsatellite instable tumors in endometrial carcinoma.

FIGO Stage IB and IC disease represent 45.3% (595/1312) of all endometrial cancers (50). Although early-stage endometrial cancer often follows a favorable course, 10% of Stage I patients experience recurrence and eventually die of the disease (51). In this context, the biologic factors correlating with aggressive tumor behavior would be very important in the selection of primary and adjuvant treatment options. Nuclear PTEN staining has been reported to be a good indicator of stage and probability of tumor recurrence in endometrial cancer (28). However, Salvesen (52) has reported that loss of cytoplasmic PTEN expression has no prognostic impact on recurrence free survival in FIGO Stage I EEC. Similar to the findings of the mentioned study (52), nuclear PTEN expression has been found out to have no prognostic impact on DFS in FIGO Stage IB/IC disease in the present study. However, we believe that no definitive conclusions associated with prognostic significance of nuclear PTEN expression can be drawn from the limited number of patients and events in our study.

In conclusion, we found that lost/reduced nuclear PTEN expression was frequent in FIGO Stage IB/IC EEC. BMI has been found to be the only clinicopathologic variable affecting immunohistochemical nuclear PTEN expression.

Nuclear PTEN may have growth-regulatory roles that are distinctive from those of cytoplasmic PTEN. The biologic role and clinical significance of nuclear PTEN in EECs need to be investigated further.

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