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PTEN Expression in Primary Prostate Carcinoma in Turkish Patients*

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Aim: Prostatic adenocarcinoma shows remarkable variability in the incidence and biological behavior between populations from different races and geographical regions of the world. PTEN has been reported as the most commonly mutated tumor suppressor gene in prostate cancer. Incidence of PTEN alterations occurring in prostatic carcinoma has not been studied previously in the Turkish population.

Materials and Methods: We examined PTEN protein expression immunohistochemically and its significance in 69 primary prostate cancer patients from Turkey. Two tissue microarrays constructed by 0.6 mm cores from radical prostatectomy specimens were used. Clinical information for all patients was obtained from hospital records.

Results: PTEN loss was found in 49% of the tumors ($P < 0.001$). Down-regulation was also prevalent in atrophic epithelium (58.7%) and in the inflamed non-neoplastic tissue (76.5%) ($P < 0.001$). Decreased PTEN was prone to segregate with unfavorable prognostic features; however, a statistically significant correlation could be obtained only with respect to the presence of positive surgical margins ($P = 0.007$).

Conclusions: Alterations in PTEN protein level occur frequently in primary prostate cancers in Turkish men, which can be a potential predictor of adverse features for outcome. Further studies with a larger cohort of patients are needed to clarify its prognostic significance. High rate of loss of tumor suppressor PTEN in atrophy and inflammation must provoke active research to clarify a possible connection of chronic inflammation-atrophy with carcinogenesis in the prostate.

Key Words: Prostate, cancer, PTEN, prognosis, carcinogenesis

Türk Hastalarda Primer Prostat Karsinomunda PTEN Ekspresyonu

Amaç: Prostatik adenokarsinom dünyada değişik coğrafi bölgeler ve ırklar arasında insidans ve biyolojik davranım açısından belirgin farklılıklar göstermektedir. Bu durumu açıklayabilecek spesifik nedenler büyük oranda gizli kalmıştır. PTEN ekspresyonu farklı organlardan köken alan birçok malignite türünde bozulmuştur. Bu genin prostat kanserinde en sık mutasyon gösteren tümör supresör gen olduğu bildirilmektedir. Şimdiye dek Türk popülasyonunda PTEN değişikliklerinin insidansı araştırılmamıştır.

Yöntem ve Gereç: Altmış dokuz Türk prostat karsinom olgusunda PTEN protein ekspresyonu ve ekspresyon değişikliklerinin önemi immünohistokimyasal yöntemle araştırıldı. Bunun için radikal prostatektomi spesmenlerinden alınan 0,6 mm.lik kor'larla oluşturulan iki doku mikrodizimi kullanıldı. Hastaların klinik bilgilerine hastane kayıtlarından ulaşıldı.

Bulgular: PTEN kaybı tümörlerin % 49'unda saptandı ($P < 0.001$). Gen ekspresyonunda baskılanma atrofik prostat epiteli (% 58.7) ve inflame non-neoplastik dokuda da mevcuttu (% 76.5) ($P < 0.001$). Azalmış PTEN, olumsuz prognostik parametrelerle birlikte olma eğilimi gösterdi, ancak bu doğrultuda istatistik olarak anlamlı korrelasyon yalnızca cerrahi sınır pozitifliği ile saptandı ($P = 0.007$).

Sonuç: PTEN protein seviye değişikliklerine Türk hastalardaki primer prostat kanserinde sık olarak rastlanmaktadır. Protein ekspresyonunda azalma kötü prognoza işaret eden bir belirleyici olma potansiyelindedir. Prognostik öneminin aydınlatılması için daha geniş serilerle yürütülecek çalışmalara ihtiyaç vardır. Tümör supresör bir gen olan PTEN'in atrofi ve inflamasyonda da yüksek oranda kaybı, prostatta kronik inflamasyon ve atrofi ile karsinogenez arasındaki ilişkinin açığa çıkarılması yönündeki aktif çalışmaları tetiklemelidir.

Anahtar Sözcükler: Prostat, kanser, PTEN, prognoz, karsinogenez.

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Introduction

There is large variability in the incidence and biological behavior of clinically manifest prostate carcinoma between different races and geographical regions. It is much rarer in East and Southeast Asia with respect to western countries (1,2), which cannot be explained solely by serum PSA screening. African-American patients present with a more advanced stage disease compared with Caucasian patients. The underlying reasons for these differences have been unclear for decades. Genetic and environmental factors including infections, different hormonal milieu and diet have been suspected. Cancer statistics are not fully satisfactory in Turkey. Nevertheless, the age-adjusted incidence of prostate carcinoma is estimated roughly as 8 per 100,000 among Turkish men (3), which is considerably less frequent than in western and northern Europe and North America.

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a tumor suppressor gene, mapped to 10q 23.31, which functions in regulating the signaling of multiple biological processes such as apoptosis, metabolism, cell proliferation and cell growth. Mutations of the PTEN have been detected in cancers of various organs including the prostate (4-9). It is thus far the most frequently mutated gene and its mutation is associated with tumorigenesis in the prostate. To the best of our knowledge, the frequency of altered PTEN in prostate cancer in Turkish men has not yet been studied. In this study, we aimed to determine the incidence of PTEN loss immunohistochemically in a group of clinically localized prostatic adenocarcinoma patients from Turkey. Presence of any correlation between PTEN expression and clinicopathological prognostic parameters was also evaluated.

Materials and Methods

Patients and Prostate Specimens

A total of 69 clinically localized prostate carcinoma patients who had undergone radical prostatectomy between 1992 and 2001 were included in this study. Sixty-nine cases had pelvic lymphadenectomy. No patient had received androgen deprivation or radiotherapy for cancer before surgery. Pre-operative data were collected on all patients from the hospital charts.

All slides for each case were retrieved from the pathology archives and were reviewed by a single pathologist (D. E. B.). Prognostically well-established pathological parameters including Gleason score, status of extraprostatic extension, seminal vesicle invasion, surgical margins, lymphovascular invasion, lymph node metastases and pathologic stage (pT) were noted. Tumor volume was estimated by simple eye-balling.

Array Construction and Tissue Sections

The index tumor was identified in all radical prostatectomies. The clinically most significant cancer nodule was chosen as the index tumor, which was the largest tumor focus with the highest Gleason score and stage in all cases. Two tissue microarrays were constructed by 0.6 mm tissue cores from 69 radical prostatectomies using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD). They contained four cores from the index tumor and an additional four cores from corresponding non-neoplastic regions for each case. Internal controls were placed in a preestablished pattern throughout each array. Five-micrometer sections from the array blocks and tissues were cut for hematoxylin and eosin (H-E) and immunostaining. H-E sections were reviewed to record the tissue type (unremarkable normal glands, non-neoplastic glands surrounded by chronic inflammation, atrophy, prostatic intraepithelial neoplasia and adenocarcinoma) represented in each core.

Immunohistochemistry

Sections were deparaffinized and rehydrated. They were then heated in citrate buffer steam (0.01 M, pH 8.0) for 20 min for antigen retrieval. Endogenous peroxidase in sections was inactivated in 2% H_2O_2 for 10 min. The sections were then blocked in 3% normal horse serum in 0.2 M phosphate buffered saline (PBS) (pH 7.4), followed by incubation with rabbit anti-PTEN antibody from Zymed (San Francisco). Anti-PTEN was used at a dilution of 1:8000 in PBS with 0.5% normal horse serum. Sections were incubated overnight in primary antibody at 4°C. They were then processed following a standard ABC immunostaining (Vector Laboratory, Burlingame, CA). Immunoreactive products were visualized using 3,3'-diaminobenzidine.

Quantitation of Immunohistochemistry

PTEN immunostaining was evaluated microscopically

for all tissue cores in the tissue microarrays. The percentage of PTEN-positive cells (labeling frequency %) as well as intensity seen at low power (graded as 0 to 3, where 0 was defined as lack of staining, 1 weak staining, 2 moderate staining, and 3 intense staining) were recorded. The extent of staining per core was stratified into three groups based on the percentage of positive cells: Group 1, < 25%; Group 2, 25 to 50%; and Group 3, >50%. Semiquatitative scores ranging from 1 to 9 for the specific staining in the represented tissue type (normal glands, normal glands with adjacent inflammation, atrophy, prostatic intraepithelial neoplasia or carcinoma) in each core were obtained by multiplying the staining intensity by the number of the group reflecting the percentage of positive cells. The labeling index (LI), as the function of labeling frequency and labeling intensity, was accepted as 1 for scores of 1 and 2, as 2 for scores of 3 and 4 and as 3 for scores of 6 and 9. Separate LIs were obtained for each tissue type available in a specimen. Results from the cores belonging to the same specimen were combined to reach one LI for each case.

The majority of normal prostatic epithelium expressed PTEN strongly and diffusely, >75% having LI of 2 and 3. Based on this observation, absence of staining (LI = 0) and LI at the level of 1 were considered as reduced PTEN expression.

Statistical Analysis

The differences in PTEN labeling were compared between normal prostate, atrophy and cancer specimens by using the Mann-Whitney test. The relationship of PTEN expression with patients' clinical and pathological variables was evaluated using Fisher's exact and chi-square tests for categorical variables and the Mann–Whitney U test for continuous variables. $P < 0.05$ was taken to indicate statistical significance.

Results

Age of the patients varied between 48 and 75 (average and median: 62). PSA at diagnosis ranged from 1.3 to 41.5 ng/ml (mean: 11.68 ng/ml). Pelvic lymph node metastasis was detected in 5 patients. Extraprostatic extension, seminal vesicle invasion, lymphovascular involvement and positive surgical margins were identified in 67.6%, 24.6%, 14.5% and 45.6% of the radical prostatectomies, respectively. Grade and stage characteristics of the cases are displayed in Table 1.

PTEN Expression

Four hundred eighty-eight tissue cores were evaluated in the two tissue microarrays. Of these, 245 represented the tumor (belonging to 69 cases). Non-neoplastic epithelium was present in 63 cases (total 239 cores, among which 46 represented atrophy). An additional 4

Table 1. Distribution of 69 cases in the arrays according to tumor stage (pT) and Gleason score.

Pathological Stage		Gleason score*			Total	
		6	7	8 or > 8		
pT2	N0	11	8	2	21	22
	NX	1	0	0	1	
pT3a	N0	5	14	9	28	30
	N1	0	2	0	2	
pT3b	N0	0	11	2	13	17
	N1	0	2	1	3	
	NX	0	0	1	1	
Total		17	37	15	69	

* Grading based on radical prostatectomy specimen.

cores represented high-grade prostatic intraepithelial neoplasia belonging to 4 different patients.

The secretory layer of normal prostatic glands showed high LI in the majority. Atrophy and inflammation led to decrease in the intensity and the extent of staining.

The intensity of staining in the normal-appearing epithelium could be classified as moderate to strong with LI of 3 or 2 in 75.5% of the tissue cores (Table 2). The PTEN immunoreactivity was predominantly localized to the secretory cells of normal prostatic glands (Figure 1).

Table 2. PTEN labeling index for different tissue types.

Tissue Type (Number of cores)	PTEN Labelling Index			
	0	1	2	3
Normal prostatic glands (176)	38 (21.5%)	5 (3%)	9 (5%)	124 (70.5%)
Atrophic prostatic glands (46)	24 (52.2%)	3 (6.5%)	2 (4.3%)	17 (37%)
Prostatic epithelium in inflammation (17)	12 (70.6%)	1 (5.9%)	3 (17.6%)	1 (5.9%)
Prostatic carcinoma glands (245)	123 (50.25%)	10 (4%)	17 (6.95%)	95 (38.8%)

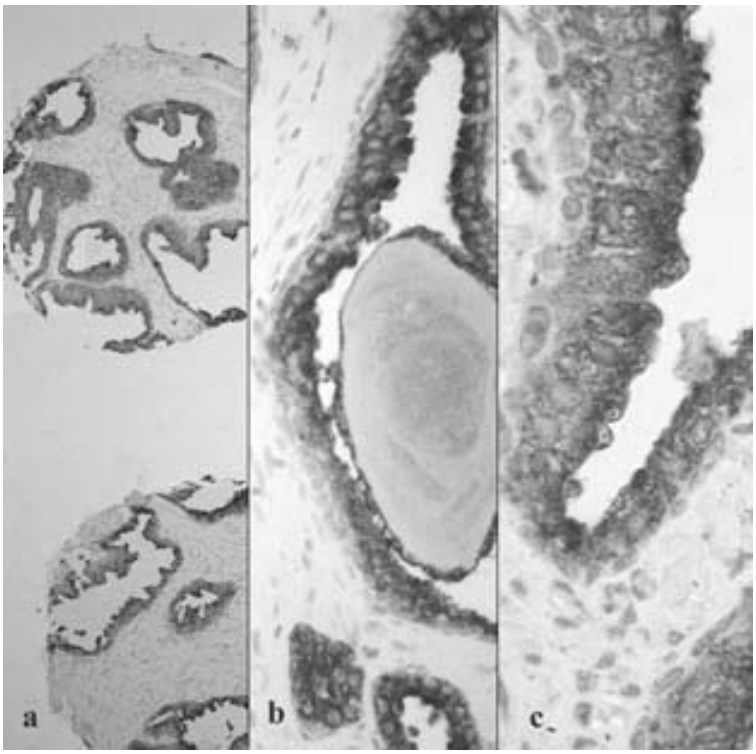

Figure 1. Normal prostatic tissue from three patients. Staining reveals high PTEN protein levels in luminal cells of prostate glands. Basal cells are generally negative. (Immunohistochemistry, anti-PTEN Ab, ABC; *a:* x 100, *b:* x 400, *c:* × 1000).

Basal cells and stromal cells were generally negative. With regard to subcellular localization, PTEN staining was in the cytoplasm. There was a noticeable reduction in cytoplasmic PTEN in tumor tissues compared with the staining in non-neoplastic normal looking glands ($P < 0.001$) (Figure 2). Reduced or loss of PTEN was observed in 54.25% of 245 tumor cores (34 cases). Among the 4 cores representing high-grade prostatic intraepithelial neoplasia, 3 had no PTEN expression. Low LI was also detected in atrophy or when non-neoplastic glands were associated with inflammation (Figures 3-6). Twenty-seven of 46 (58.7%) cores of atrophy and 13/17 (76.5%) cores of inflammation showed negative or low levels of PTEN (both P values < 0.001).

Correlation of PTEN Expression with Clinicopathologic Parameters

Age and pre-operative PSA level did not differ between normal and reduced PTEN groups. Decreased PTEN expression was more frequent in cases with unfavorable prognostic features (Table 3). Of the 34 cases with low LI for PTEN, 72.7% showed extraprostatic extension, 29.4% seminal vesicle invasion, 61.8% positive surgical margins, 20.6% lymphovascular involvement and 9% lymph node metastasis. These ratios were 62.6%, 20%, 29.4%, 8.6% and 5.1%, respectively, in tumors with normal PTEN expression. The size of the cohort possibly was not sufficient for meaningful statistical figures. The only correlation that reached statistical significance was between reduced expression and positive surgical margins ($P = 0.007$). No association was found between PTEN expression and Gleason scores or PTEN expression and pathological stage.

Discussion

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a dual function enzyme with protein/lipid phosphatase activity, and its main substrate is phosphatidyl-inositol 3,4,5 triphosphate (PIP3), a direct product of phosphoinositol-3-kinase (10). Increase in PIP3 recruits serine-threonine kinase AKT to the membrane. AKT promotes cell survival and proliferation. The role of PTEN is to maintain low levels of PIP3.

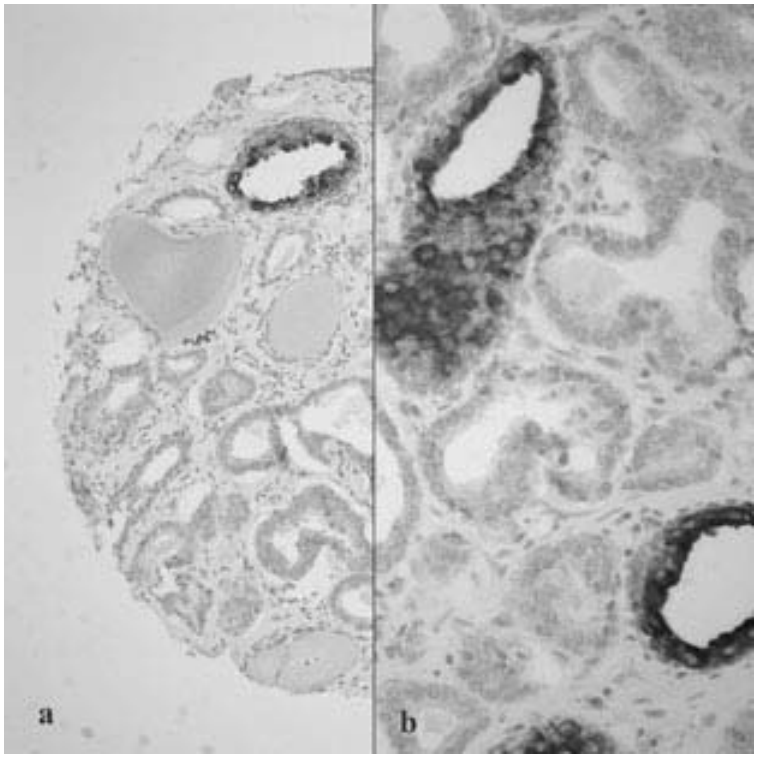

Figure 2. Markely reduced PTEN in prostatic adenocarcinoma. Normal glands display high staining intensity. (Immunohistochemistry, anti-PTEN Ab, ABC; *a:* × 200, *b:* × 400).

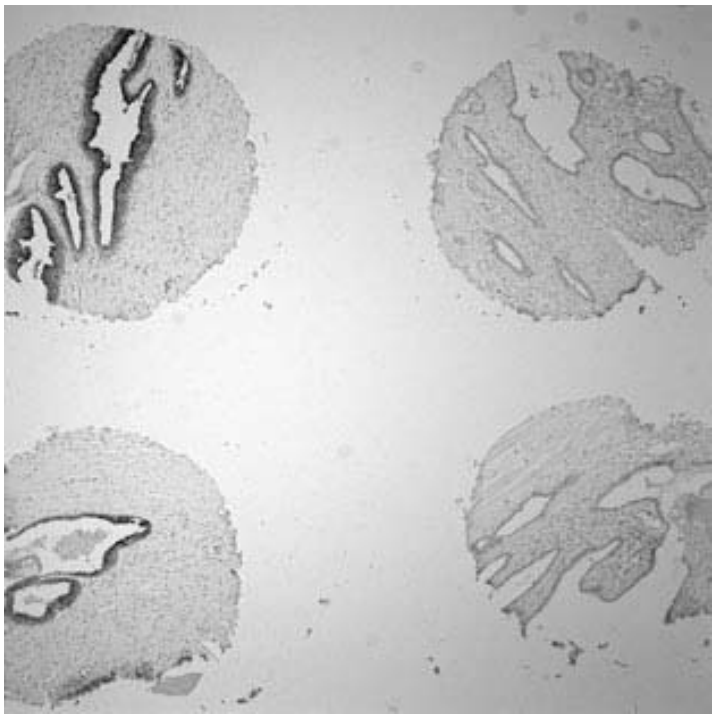

Figure 3. Two different cases in the array where normal and atrophic tissues from the same specimen are represented in the adjacent cores. No staining is evident in atrophy in either. (Immunohistochemistry, anti-PTEN Ab, ABC × 100).

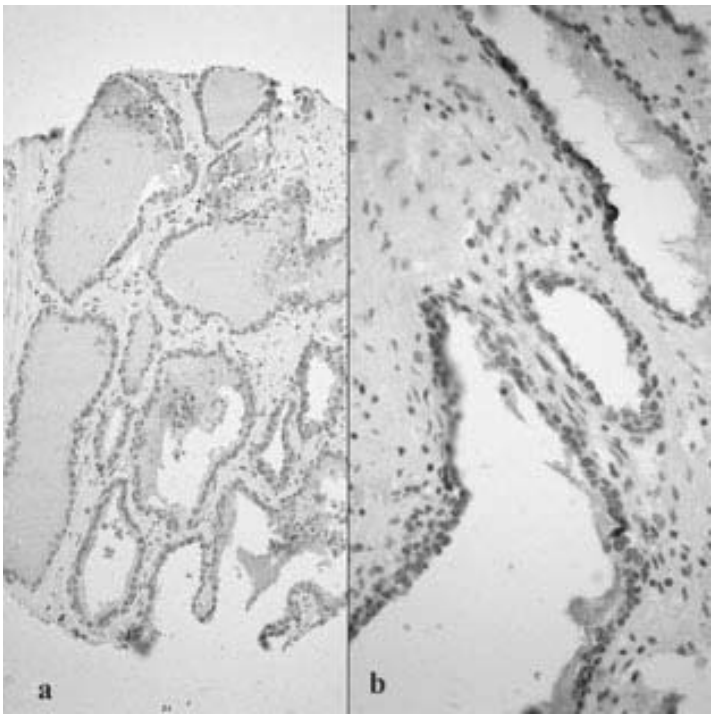

Figure 4. Atrophic glands with reduced PTEN in the form of low intensity or patchy staining. (Immunohistochemistry, anti-PTEN Ab, ABC; *a:* × 200; *b:* × 400).

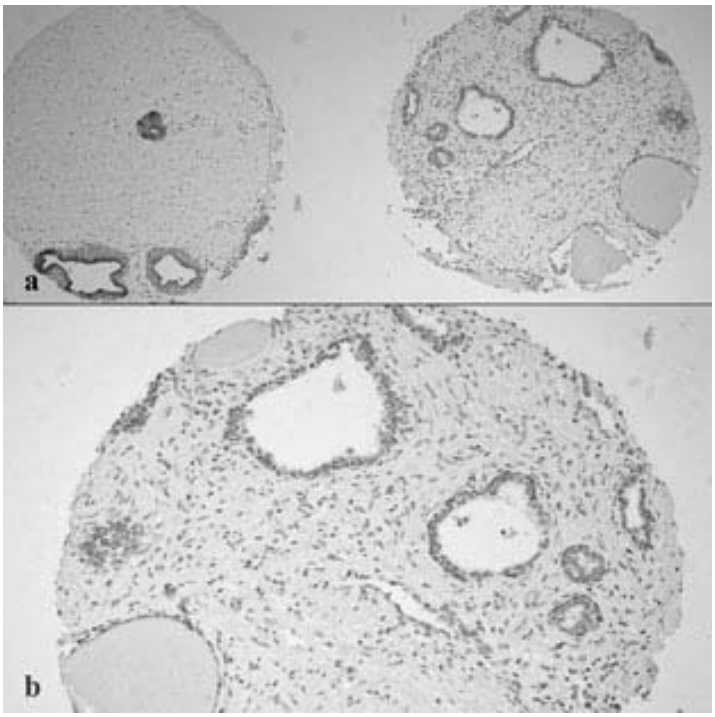

Figure 5. Two cores from the same case. Strong PTEN expression in normal glands when stroma is unremarkable (left core). Glands lose the expression when the stroma is inflamed (right core and lower picture). (Immunohistochemistry, anti-PTEN Ab, ABC; *a:* × 100; *b:* × 200).

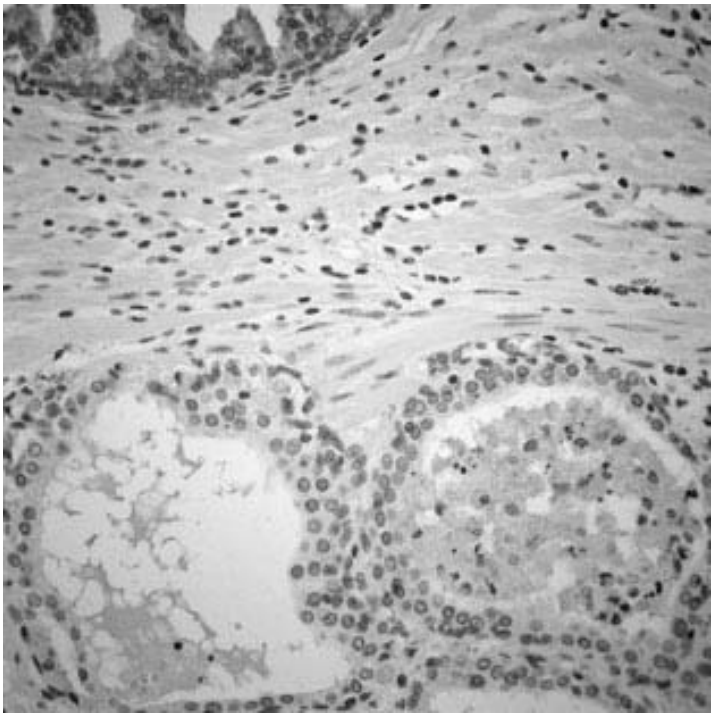

Figure 6. The inflammatory cells in the stroma and glandular lumina are associated with decreased PTEN expression (Immunohistochemistry, anti-PTEN Ab, ABC × 400).

Table 3. Distribution of cases according to prognostic factors and PTEN expression.

Variables		PTEN Expression		
		Decreased n (%)	Normal n (%)	Total
Gleason score	6	8 (47.1%)	9 (52.9%)	17
	7	17 (45.9%)	20 (54.1%)	37
	≥8	9 (60.0%)	6 (40.0%)	15
Extraprostatic extension	Absent	9 (40.9%)	13 (59.1%)	22
	Present	24 (52.2%)	22 (47.8%)	46
Seminal vesicle invasion	Absent	24 (46.1%)	28 (53.90%)	52
	Present	10 (58.9%)	7 (41.1%)	17
Surgical margins*	Positive	13 (35.1%)	24 (64.9%)	37
	Negative	21 (67.7%)	10 (32.3%)	31
Pathological stage	pT2	9 (40.9%)	13 (59.1%)	22
	pT3a	15 (50%)	15 (50%)	30
	pT3b	10 (58.8%)	7 (41.2%)	17
Lymph node metastasis	Absent	30 (48.4%)	32 (51.6%)	62
	Present	3 (60.0%)	2 (40.0%)	5
Lymphovascular invasion	Absent	27 (45.8%)	32 (54.2%)	59
	Present	7 (70.0%)	3 (30.0%)	10

n: number of case*s; * P = 0.007.*

Oncogenic effect of PTEN loss can be attributed to AKT activation. Additional PTEN-regulated pathways have also been identified as the genes associated with JNK activation (11).

PTEN mutations are among the most frequent genetic alterations detected in human prostate cancers. Our finding of reduced PTEN expression at the protein level in 34 (49%) of 69 primary prostate cancer specimens confirms that PTEN is one of the major genes in the prostatic malignancy. Its critical role has been evidenced by many studies. In PTEN heterozygous mice, precancerous lesions were found and when both alleles were deleted, cancer progression and metastasis occurred (12). PTEN negatively regulates prostatic basal cell proliferation (without blocking differentiation) and concomitant expansion of a prostate stem/progenitor-like subpopulation (13). Adenoviral vector-expressed PTEN strongly inhibits the growth of xenograft human prostate tumors in athymic mice, especially when combined with radiation therapy (14). Knocking down PTEN can convert the androgen-dependent Myc-CaP cell into androgen independence, suggesting intrinsic control of androgen responsiveness by PTEN (12). Androgen independence has been found to associate with activation of the phosphoinositide-3 kinase/Akt as well as Erk mitogen-activated protein kinase signaling pathways in the prostate epithelium (15). It has been shown that silencing Akt in PTEN-mutated prostate cancer cells enhances the antitumor effects of taxol (16).

Mutation frequencies of PTEN in prostate cancer differ among studies, largely because of differences in tumor grade and stage in the study populations. Loss of heterozygosity has been shown to occur in a range of 14% to 65% according to different reports (7,17-20). Immunohistochemical PTEN protein expression was weak in 24 of 58 primary prostate carcinomas (41.3%) and negative in 8 (13.8%) in the study of Fenic et al. (21). Halvorsen et al. (22) found lack of PTEN protein expression in 27% and McMenamin et al. (23) in 20% of cases. A 49% incidence of decreased PTEN expression at the protein level in primary prostate cancers in Turkish patients was similar to that observed in the German population in the study of Dreher et al. (24), in which they reported a 39% rate. These results suggest that PTEN is not a genetic factor contributing to the racial difference in prostate cancer incidence and that genetic pattern is similar in terms of its mutation frequency.

Loss of PTEN expression has been shown to correlate with unfavorable prognosis in primary prostate cancer. Patients with prostatic carcinoma who had PTEN mutation had significantly greater Gleason score, poorer prognosis, and higher rate of metastasis (23,25,26). In one study, loss of PTEN expression at first time diagnosis was 23%, while this was 59% in patients with lymph node metastasis, a finding demonstrating that loss of PTEN expression is an important factor in progression towards metastatic disease (27). Results in regard to PTEN loss as a predictor of disease progression are controversial. Some have stated that it acts as an independent indicator of biochemical recurrence after radical prostatectomy (26), whereas others cannot demonstrate this relation (25). In the series of Bedolla et al., PTEN was a predictor of the risk of biochemical recurrence if combined with pAkt while by itself it was not (28).

In our data set, we found that reduced PTEN was more frequent with worse prognostic factors. Rate of PTEN reduction was higher among the cases who showed extraprostatic extension, seminal vesicle involvement, positive surgical margins and lymphovascular invasion. However, size of our cohort was too small to give meaningful statistical results. The only statistical significance was obtained between surgical margin positivity and low PTEN level ($P = 0.007$). The loss of PTEN in multiple other tumor types has also been linked to advanced disease and poor patient outcome (29, 30).

One of our findings was the high frequency of reduced PTEN in atrophic prostatic glands. We observed PTEN loss at a rate of 58.7% in atrophy. This was even more apparent, reaching 76.5%, when there was associating chronic inflammation in stroma. These results evoke the question of the relationship between neoplastic transformation and inflammation/atrophy. In fact, about 20% of all human cancers are caused by chronic infection or chronic inflammatory states, including cancer of the liver, stomach, large intestine, biliary tree and urinary bladder. Cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) enzymes are overexpressed during inflammation and may contribute to multistage tumor development. It has been reported that PTEN is oxidized and inactivated during arachidonic acid metabolism in pancreatic cancer cell lines expressing COX-2 or 5-LOX (31). Oxidation of PTEN decreases its phosphatase activity, favoring elevated phosphatidylinositol 3,4,5-triphosphate

production and activation of AKT, which increases the risk for hypertrophic or neoplastic diseases. Molecular, histopathological, and epidemiological studies show that chronic inflammation might also be important in prostate carcinogenesis. The inflammation and following epithelial cellular injury may cause a loss of tolerance to normal prostatic antigens, resulting in a self-perpetuating autoimmune reaction. Chronic inflammatory infiltrates in the prostate are associated with focal epithelial atrophy, and atrophic epithelium displays many genetic alterations such as methylated GSTP1 in 6% (32), increases in chromosome 8 centromere signals (33, 34), loss of chromosome 8p (34, 35) and a gain of chromosome 8q24 (34), downregulated tumor suppressor genes p27 (36, 37), and NKX3.1 (38) in focal atrophy, and mutated p53 (39) and androgen receptor alleles (40) in post-atrophic hyperplasia. Transitions between atrophic epithelium and adenocarcinoma have been observed morphologically by several investigators (32, 41, 42). High rate of tumor suppressor PTEN protein reduction in our atrophic and inflamed prostatic tissues can be additional support for future studies that will target investigating the pathogenetic link between inflammatory atrophy and prostate carcinogenesis.

Our results indicate that PTEN loss is a common alteration in sporadic prostate cancer irrespective of different races, and it can be a universal genetic marker for predicting disease progression. Genetic studies in human tumors including documentation of the incidence and significance of various mutations are warranted since they will open the door for more effective disease treatment and patient management strategies.

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