

Role of *PTEN* Gene in Progression of Prostate Cancer

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Introduction: The aim of this study was to clarify the role of *PTEN* gene in progression of prostate cancer.

Materials and Methods: A total of 51 formalin-fixed paraffin-embedded specimens of prostate cancer were analyzed for *PTEN* mutations. Tissue microdissection and polymerase chain reaction/single-strand conformation polymorphism methods were used. Clinical and pathologic data of the patients were reviewed with regard to *PTEN* mutation.

Results: The Gleason score (GS) was less than 7 in 29 (56.8%), 7 in 11 (21.6%), and greater than 7 in 11 (21.6%). Tumor stage was IIa, IIb, IIc, and IV in 14 (27.4%), 4 (7.8%), 21 (41.2%), and 12 (23.6%) patients, respectively. Eleven of 12 stage IV tumors had metastases at the time of presentation. Six of 51 cases (11.6%) showed mutation in *PTEN* which had involved exons 1, 2, and 5. Two of these cases had localized and the others had advanced prostate cancer. One case of the tumors with *PTEN* mutation had a GS of 7 and 5 had GSs greater than 7. Patients with a positive mutation of *PTEN* had a significantly greater GS ($P < .001$), lower survival rate ($P = .001$), higher tendency to metastasis ($P = .002$), and higher prostate-specific antigen ($P = .03$). Cox proportional hazard model showed that only GS was significantly correlated with mortality ($P = .03$).

Conclusion: Patients with prostate cancer who had *PTEN* mutation had also a significantly greater GS, poorer prognosis, and higher rate of metastasis. However, this mutation cannot predict the prognosis and the GS is a more precise factor.

Keywords: prostatic neoplasms, *PTEN*, mutations, Gleason score, prostate-specific antigen, Iran

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INTRODUCTION

Prostate adenocarcinoma is one of the most commonly diagnosed malignancies affecting the men in both the United States and Europe.⁽¹⁾ The prognostic factors in patients with prostate cancer who undergo radical prostatectomy are pathological stage and Gleason score (GS).⁽²⁾ Prostate cancer is a heterogeneous disease and identifying factors associated with a poor outcome at the time of radical prostatectomy is challenging.⁽²⁾ The molecular mechanisms of prostate

carcinogenesis are unknown. *PTEN/MMAC1* is a tumor suppressor gene located on 10q23.^(3,4) *PTEN* that encodes a dual-specificity phosphatase is a tumor suppressor gene whose inactivation has been associated with many different types of cancers including glioma, melanoma, and carcinoma of the endometrium, kidney, breast, lung, upper respiratory tract, and prostate.⁽⁵⁾ The tumor suppressor activity of *PTEN* is thought to be primarily due to its ability to dephosphorylate phosphoproteins

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or phospholipids and negatively regulate the activity of the phosphatidylinositol 3-kinase pathway. It is shown that expression of *PTEN* can inhibit cell cycle progression, induce a G1 arrest, inhibit cell migration, and induce cell cycle arrest and apoptosis.⁽⁶⁻⁸⁾

Loss of *PTEN* activity as a tumor suppressor gene enhances cell proliferation and tumor angiogenesis and decreases apoptosis.⁽⁹⁾ Mutations in *PTEN* have often been detected in metastases of prostate cancer; however, lower rates of mutations have been found in localized tumors (0 to 20% in different studies).⁽²⁾

The rate of *PTEN* mutations in prostate cancer has not been adequately studied in Asia. One study based on 32 Chinese men with prostate cancer showed a 16% rate of *PTEN* mutations.⁽⁵⁾ We analyzed prostate cancers of 51 Iranian patients to scrutinize the role of *PTEN* mutations in tumor progression.

MATERIALS AND METHODS

Tumor Specimens

This study was performed in accordance with the declaration of Helsinki and subsequent revisions and approved by ethics committee at Tehran University of Medical Sciences. We used 51 paraffin-embedded prostate cancer specimens archived in the departments of pathology of Day and Sina hospitals between 1997 and 2005. Twelve specimens were of patients who had undergone transurethral resection and the remaining had been obtained by radical prostatectomy. The prostatectomy procedures had been performed by 3 surgeons over a period of 8 years. All specimens were collected from the archived paraffin blocks used for routine diagnosis of cancer. Follow-up data were available for all of the cases in the database with a mean patient follow-up period of 48 months. The initial values of prostate-specific antigen (PSA) and GS were recorded. For every case, a representative paraffin block was selected that contained both tumor and benign prostate tissue.

DNA Extraction

Formalin-fixed paraffin samples were cut into 10- μ m sections. The sections were pulverized under liquid nitrogen condition using microdismembrator (B Braun Melsungen AG, Melsungen, Germany). Of each sample, 0.1 g of pulverized tissue powder was resuspended in 1 mL of xylene and left for 15 minutes at 55°C. The suspension was then centrifuged at 14 000 g for 5 minutes. The pellet was suspended in 0.1 mL of xylene and processed as above for the second time. The resulted sediment was mixed with 100% ethanol and processed with xylene lysis buffer (Tris, sodium dodecyl sulfate, ethylenediamine tetraacetic acid [EDTA]). A lysis buffer containing 300 μ g/mL of proteinase-k was added to the pellet, mixed and incubated at 55°C for an overnight period. The DNA was extracted following the use of phenol-chloroform procedure, then dissolved in TE buffer (Tris-HCl and EDTA) and stored at 4°C.

Polymerase Chain Reaction Analysis

For polymerase chain reaction (PCR) application (Genius, Boehringer-Mannheim, Indianapolis, USA), increasing concentrations of extracted DNA of each specimen was tested to find out the optimum dose that resulted in good amplicon product. Each primer pair of the selected exons was used for mutation detection of *PTEN/MMAC1* following the PCR for the single-strand conformation polymorphism (PCR-SSCP). The PCR protocol was carried out as outlined in Table 1, and primers used for each *PTEN* exon were as follows:

PTEN 1F 5'-AGTCGCTGCAACCATCCA

PTEN 1R 5'-GATATTTGCAAGCATAACAAA

PTEN 2F 5'-GTTTGATTGCCATATTTTCAG

PTEN 2R 5'-GGCTTAGAAATCTTTTCTAAATG

PTEN 5F 5'-GCAACATTTCTAAAGTTACCTACTTG

PTEN 5R 5'-CATATCATTACACCAGTTTCG

Table 1. Polymerase Chain Reaction Protocol

Exon	Denaturation		Annealing		Extension	
	Temperature, °C	Times	Temperature, °C	Times	Temperature, °C	Times
<i>PTEN</i> exon 1	95	40	60	45	72	60
<i>PTEN</i> exon 2	95	30	60	45	72	60
<i>PTEN</i> exon 5	95	40	56	60	72	60
<i>PTEN</i> exon 8	95	40	58	45	72	60

PTEN 8F 5'-CATTATAAAGATTCAGGCAATG

PTEN 8R 5'-GACAGTAAGATACAGTCTATC

Any shift in the pattern of single-strand migration in the gel electrophoresis was considered as mutated exon. The PCR product was mixed with an SSCP denaturing buffer (98% formamide, 20 mM EDTA, 0.05% xylene cynol, 0.05% bromophenol blue, 0.05 M NaOH) and heated at 98°C for 8 minutes. The heated mixture was subsequently loaded on polyacrylamide gel and electrophoresed at 250 V for 6 to 8 hours at room temperature. The electrophoresed gel was processed, stained, and developed using Silver staining method.

Statistical Analyses

The *t* test and Mann-Whitney *U* test were used to compare the PSA and GS between the patients with and without *PTEN* mutation, respectively. The chi-square test was used to evaluate metastasis in the patients with and without *PTEN* mutation. To analyze survival of the patients and the prognostic variables, Kaplan-Meier method, log-rank test, and Cox proportional hazards regression model were used. Data analyses were performed by the SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA). A value of *P* less than .05 was considered significant.

RESULTS

Fifty-one formalin-fixed paraffin-embedded prostate cancer specimens of the patients were used in this study. Radical prostatectomy had been performed in 39 localized prostate cancers and transurethral resection of prostate plus adjuvant therapy in 12 advanced cancer cases. The mean age of the patients was 69.1 ± 7.9 years (range, 57 to 82 years). Of the patients, 29 (56.8%), 11 (21.6%), and 11 (21.6%) had GSs less than 7, equal to 7, and greater than 7, respectively. Twenty-eight of 39 localized prostate cancers (71.8%) had GSs of less than 7, while 9 out of 12 advanced prostate cancers (75.0%) had GSs greater than 7. Preoperative serum PSA level was less than 4 ng/mL in 9 patients with localized prostate cancer (23.1%), greater than 10 ng/mL in 3 (7.7%), and between 4 ng/mL and 10 ng/mL in 27 (69.2%). In advanced cancer cases, all of the patients had a preoperative PSA level greater than 10 ng/mL. There was a correlation between the PSA level and the GS

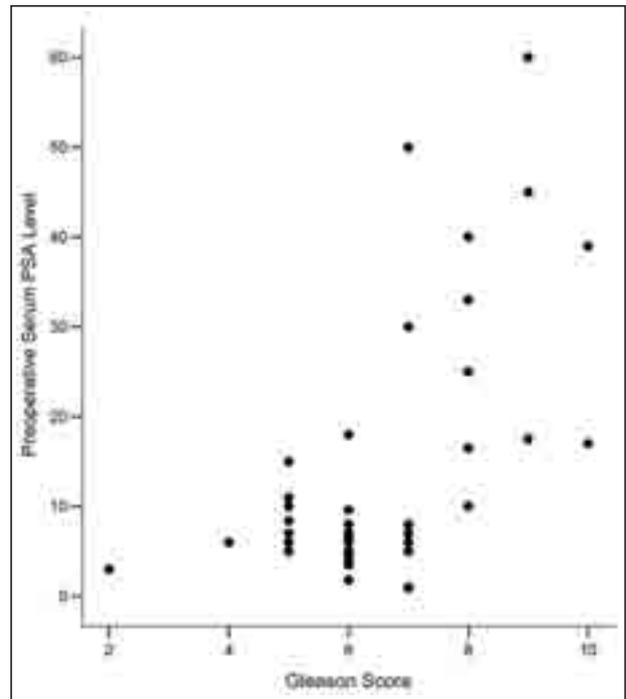


Figure 1. The correlation of preoperative serum PSA level with Gleason score.

(*P* = .001; Figure 1). Eleven of 12 advanced prostate cancers had metastases at the time of presentation. The prostate cancer stage was IIa in 14 patients with radical prostatectomy (35.9%), IIb in 4 (10.3%), and IIc in 21 (53.8%).

The PCR-SSCP analyses of the specimens revealed band shifts in 6 tumors (2 in exon 1, 1 in exon 2, and 3 in exon 5) which indicated the existence of possible sequence alterations within these sites. Two of these cases had localized and the others had advanced prostate cancer. One case of the tumors with *PTEN* mutation had a GS of 7 and 5 had GSs greater than 7 (Table 2). All of the tumors with a positive mutation of *PTEN* and advanced prostate cancer were associated with metastasis, whereas 1 of the tumors with a positive mutation and localized prostate cancer was metastatic. During the follow-up,

Table 2. Frequency of *PTEN* Gene Mutation in Different Gleason Score Categories

Gleason Score	PTEN		Total
	Negative	Positive	
< 7	29 (100.0)	0	29
7	10 (90.9)	1 (9.1)	11
> 7	6 (54.6)	5 (45.4)	11
Total	45 (88.2)	6 (11.8)	51

5 of 6 patients with *PTEN* mutation had died as a result of metastases.

Patients with a positive mutation of *PTEN* had a significantly greater GS ($P < .001$), lower survival rate ($P = .001$; Figure 2), and higher tendency to metastasis ($P = .002$). The mean PSA value was 21.42 ± 14.60 ng/mL in the mutation-positive patients and 11.25 ± 12.93 ng/mL in the mutation-negative ones ($P = .03$; Figure 3). Cox proportional hazard model was used and the variables including age, GS, *PTEN* mutation, and PSA value entered in the model (Table 3). Only GS was significantly correlated with mortality ($P = .03$; Figure 4).

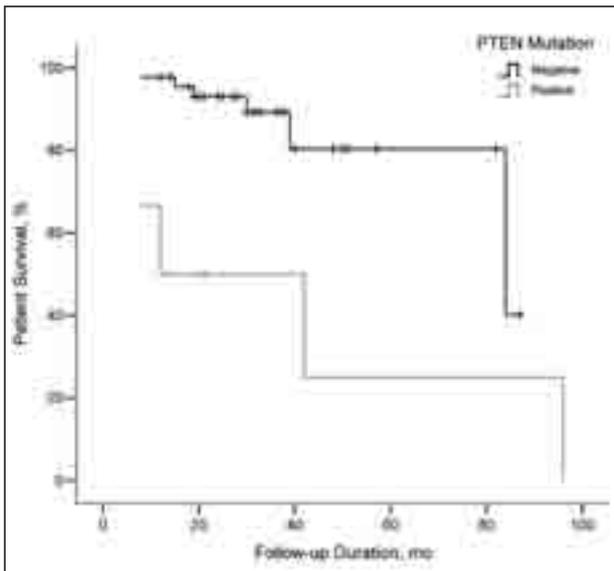


Figure 2. Patients with positive mutation had worse prognoses.

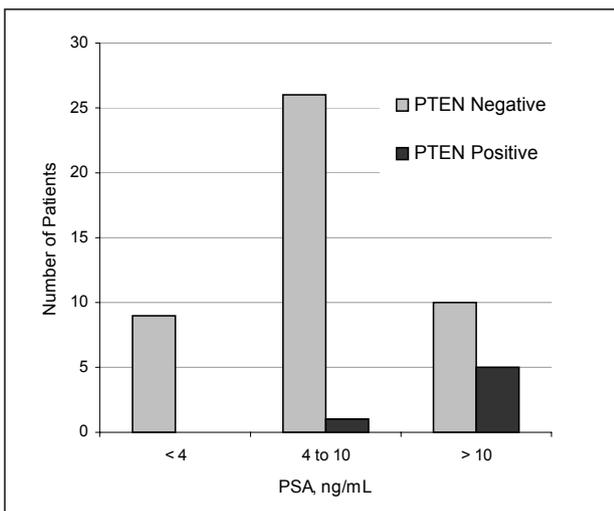


Figure 3. The frequency of *PTEN* gene mutation in each preoperative PSA level category.

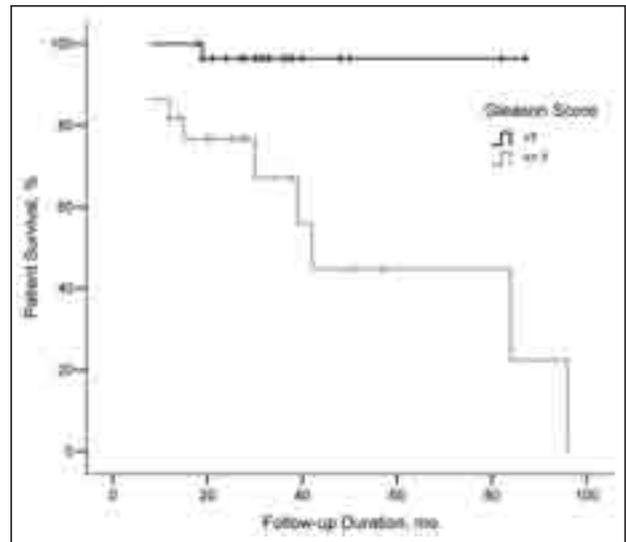


Figure 4. High grade tumors have worse prognosis.

DISCUSSION

Prostate cancer is the most common form of malignancy in men in the western countries and is the second most common cause of cancer deaths in the United States.⁽¹⁾ Quantitative and structural genetic alterations can cause the development and progression of prostate cancer. Detection of these genes will be a key role for improving the treatment of prostate cancer. The frequency of *PTEN* inactivation coincide with the progression of prostate cancer.⁽¹⁾ The *PTEN* tumor suppressor gene is frequently inactivated in human tumors including glioma, melanoma, and carcinoma of the endometrium, kidney, breast, lung, upper respiratory tract, and prostate.⁽¹⁰⁾ Our finding of *PTEN* mutations in 6 of 51 prostate cancer specimens, 5 of which being high grade, confirms that *PTEN* is a major gene in progression of prostate cancers.

The frequency of *PTEN* mutation in prostate cancer differs between studies published to date, most probably because of differences in tumors' grade and stage in the study populations. In one study of 37 tumors with 20 (54.1%) high-grade and 17 (45.9%) low-grade tumors, 5 cases had *PTEN* mutations, 4 of which were high-grade tumors.⁽¹¹⁾ In another study of 45 prostate cancers that were mainly low grade (67%), no *PTEN* mutations were found.⁽¹²⁾ In a study on 32 cases of prostate cancer (70% with a GS of 8 to 10), *PTEN* mutations were detected in 5 (15.6%).⁽⁵⁾ Summarizing 5 studies on *PTEN* in prostate cancer, 51 of 192 high-grade tumors (26.6%) showed

Table 3. Cox Regression Hazard Model for Survival of Patients With Prostate Cancer

Variable	Hazard Ratio	Standard Error	z	P	95% Confidence Interval	
Age	1.068	0.048	1.46	0.14	0.977	1.167
PSA	1.007	0.026	0.27	0.79	0.956	1.060
GS	2.347	0.915	2.19	0.03	1.092	5.041
PTEN	1.699	1.698	0.53	0.60	0.239	12.051

Table 4. Summary of 5 Studies on PTEN Mutations and Gleason Scores in Prostate Cancer

Authors	Patients		High-grade Tumor		Low-grade Tumor	
	Total	PTEN Mutation	Total	PTEN Mutation	Total	PTEN Mutation
McMenamin and colleagues ⁽²⁾	109	17 (15.6)	79	17 (21.5)	30	0
Dong and colleagues ⁽⁵⁾	38	7 (18.4)	27	6 (22.2)	12	1 (8.3)
Gray and colleagues ⁽¹¹⁾	37	5 (13.5)	20	4 (20.0)	17	1 (5.9)
Orikasa and colleagues ⁽¹²⁾	45	0	15	0	30	0
Leube and colleagues ⁽¹³⁾	57	22 (38.6)	51	21 (41.1)	6	1 (16.7)

mutations in the *PTEN*, while only 3 of 95 low-grade cases (3.2%) showed mutations (Table 4).^(2,5,11-13) We found *PTEN* mutations in 6 of 51 (11.8%) Iranian patients; Five of the 6 cases with mutations were high-grade tumors and the patients died as a result of metastasis. These studies indicate that *PTEN* mutations occur more often in tumors with greater GSs.

Orikasa and associates examined 45 primary prostate cancer specimens. Loss of heterozygosity at the *PTEN* locus was observed in 2 out of 18 tumors (11.1%). However, no mutations were observed in any of the primary prostate cancers. These data propose that mutation of the *PTEN* gene does not play an important role in prostate carcinogenesis of Japanese patients.⁽¹²⁾ In another study, the *PTEN* appeared to be the most commonly mutated gene in metastases of prostate cancer occurring in at least 1 metastatic site in 12 of 19 (63%) patients with multiple metastases.⁽¹⁴⁾ Mutations of *PTEN* in localized prostate cancers have been detected at lower rates (2.5% to 5%).⁽¹⁵⁻¹⁷⁾ These results show a role for the *PTEN* in the progression of prostate cancer. In our study, the variables which showed correlation with mortality in univariate analyses (PSA and *PTEN*), did not correlate with mortality in multivariate analysis. Only GS was significantly correlated with mortality ($P = .03$).

CONCLUSION

Patients with prostate cancer who had *PTEN* mutation had also a significantly greater GS, poorer prognosis, and higher rate of metastasis. The increase

in the GS was associated with *PTEN* gene mutation and increase in the mortality. The same condition exists about the PSA value. As a result, in multivariate analysis, only GS was significantly correlated with mortality.

CONFLICT OF INTEREST

None declared.

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