ELSEVIER

Urologic Oncology: Seminars and Original Investigations 22 (2004) 307–312

UROLOGIC ONCOLOGY

Original article

# The assessment of PTEN tumor suppressor gene in combination with Gleason scoring and serum PSA to evaluate progression of prostate carcinoma

Ismail Turker Koksal[a,c], Ercument Dirice[a,b], Duygu Yasar[a,b], Ahter D. Sanlioglu[a,b], Akif Ciftcioglu[d], Kemal H. Gulkesen[e], Nidai O. Ozes[b], Mehmet Baykara[a,c], Guven Luleci[a,b], Salih Sanlioglu[a,b,*]

[a] *The Human Gene Therapy Unit, Akdeniz University, Faculty of Medicine, Antalya 07070, Turkey*
[b] *Department of Medical Biology and Genetics, Akdeniz University, Faculty of Medicine, Antalya 07070, Turkey*
[c] *Department of Urology, Akdeniz University, Faculty of Medicine, Antalya 07070, Turkey*
[d] *Department of Pathology, Akdeniz University, Faculty of Medicine, Antalya 07070, Turkey*
[e] *Department of Biostatistics, Akdeniz University, Faculty of Medicine, Antalya 07070, Turkey*

Received 24 July 2003; received in revised form 14 October 2003; accepted 26 January 2004

**Abstract**

*Objective:* The purpose of the study was to determine if the tumor suppressor gene phosphate and tensin homolog (PTEN) (mutated in multiple advanced cancers 1) in combination with Gleason scoring and serum prostate specific antigen (PSA) could be employed to better predict the progression of prostate carcinoma. *Materials and Methods:* The study group consisted of 43 patients with benign prostate hyperplasia (BPH), 15 with organ confined prostate carcinoma (OCPCa), and 18 with advanced prostate carcinoma (APCa). Prostate tissue samples were obtained from radical prostatectomy, transurethral resection, and TRUS guided trans-rectal needle biopsy and then evaluated for biomarker expression. The clinical stage was assessed according to tumor node metastasis classification and grade according to Gleason system. Serum PSA was measured by conventional techniques and Western blotting analysis was used to determine PTEN expression in the primary tissue. Multivariate analysis was performed to analyze whether these markers could individually predict the progression of prostate carcinoma. *Results:* APCa patients displayed higher Gleason scores and serum PSA levels. But much lower PTEN expression was detected in prostate of APCa patients compared to patients with BPH or OCPCa. Hormone refractory (HR) and hormone sensitive (HS) APCa cases did not yield any significant differences in terms of Gleason scoring, serum PSA and PTEN expression. PSA levels were significantly higher in patients with OCPCa or APCa compared to patients with BPH. *Conclusion:* Our results suggested that both PTEN and serum PSA appeared to be useful as independent markers to depict the nature of tumor behavior as benign or malign. In addition, PTEN also appeared to be useful as an independent marker to predict the progression of prostate carcinoma. 

*Keywords:* PTEN; Prostate carcinoma; Gleason system; PSA

## 1. Introduction

Prostate cancer is the second leading cause of death in men from cancer following lung carcinoma with an annual mortality rate of 38,000 [1]. Several hundred clinical studies using experimental or approved chemotherapeutics failed to improve survival rates of patients with prostate cancer. Because prostate cancer is a heterogeneous disease and genetically unstable, treating patients with prostate cancer still remains as a formidable task [2]. In addition, the molecular mechanism responsible for the onset of the disease is poorly understood. Luckily, earlier detection of the prostate cancer has been associated with an improved outcome. Thus, the detection of the prostate cancer at an earlier stage remains as the most realistic chance for therapy [3].

Different molecular screening methods have currently been employed to follow up the progression of prostate cancer, but the most effective method satisfying our need is yet to be established [4,5]. The most commonly used screening assays are based on the detection of up-regulated

* Corresponding author. Tel.: +90-242-227-4343; fax: +90-242-227-4482.
*E-mail address:* sanlioglu@akdeniz.edu.tr (S. Sanlioglu).

1078-1439/$ – see front matter 
doi:10.1016/j.urolonc.2004.01.009

prostate specific markers such as prostate specific antigen (PSA). Currently prostate specific antigen, when it is used in conjunction with other markers such as Gleason system or tumor node metastasis (TNM) staging is considered to be a valuable tool to evaluate the progression of prostate cancer [6,7]. In addition, a number of potential biomarkers that are altered in the primary neoplasm have been identified that may be useful for predicting the biological activity of localized prostate cancer. Some of these markers include cell adhesion molecules and tumor suppressor genes [8,9]. Therefore, addition of biomarkers could potentially improve the predictive value of such a biomarker profile [10].

PTEN/MMAC1 (phosphatase and tensin homolog/mutated in multiple advanced cancers 1) is a tumor suppressor gene [11] that is deregulated in a wide range of human tumors including carcinomas of the lung, breast and kidney. More recently PTEN tumor suppressor gene has been shown to be down regulated in a high percentage of advanced prostate cancers [12,13]. In this study, we investigate the potential utility of PTEN as a prospective biomarker to determine its value in addition to Gleason system and serum PSA.

## 2. Materials and methods

### *2.1. Clinical assessment of patients with prostate cancer*

There were 15 patients with OCPCa, 18 with APCa and 43 with BPH enrolled in our study, which was conducted at the Urology Clinic of the University Hospital. Prostate tissue was obtained from patients undergoing radical prostatectomy for clinically organ-confined disease. Other tissues were obtained through transurethral resection of prostate (TURP) from patients with BPH and some patients with advanced disease because of infra-vesical obstruction. For tissue confirmation and diagnosis, patients with advanced prostate carcinoma had a trans-rectal ultrasonography (TRUS) guided biopsy. Patients with advanced prostate cancer were categorized into two subgroups, one subgroup was hormone sensitive ($n = 6$) and the other was hormone resistant ($n = 12$). The criteria for hormone sensitivity status of prostate carcinoma were assessed as previously described [14]. Clinical and pathological stages were assigned according to TNM prostate cancer pathologic staging system [15].

### *2.2. Histological grading of prostate tissue and the specimen processing*

The processing of prostate tissue samples was performed routinely as previously described [16]. In brief, each was weighed, measured in three dimensions, inked, and fixed in 10% neutral buffered formalin. After fixation, the apex and the base were amputated at a thickness of 4 to 5 mm, and serially sectioned at 3-mm intervals in the vertical parasagittal plane. The remaining prostate was serially sectioned at 5-mm intervals using a knife perpendicular to the long axis of the gland from the apex of the prostate to the tip of the seminal vesicles. Needle biopsy procedures were performed using a Siemens Sonoline Prima (Siemens, Issaquah, WA) console an Endo-PII biplaner 5 to 7.5 Mhz endocavitary probe. Biopsies were obtained using a spring-loaded biopsy gun with an 18-gauge core biopsy needle. All patients had a designated Gleason score based on the specimens obtained through the radical prostatectomy and TRUS guided needle biopsy.

### *2.3. Western blotting analysis*

Following radical prostatectomy, pathologic examination was performed on the surgically dissected prostate. After the confirmation of tumor presence, tumor tissue was excised from the prostate and treated as follows. Prostate tumor, BPH and control specimens were homogenized in Triton X-100 buffer with 500 mM HEPES pH:7.0, 150 mM NaCl, 10% glycerol, 1 mM EDTA, and 1.2% Triton X-100. SDS-PAGE, blotting and developing procedures of the Western blotting assay were performed according to the protocol published previously [17]. A polyclonal antibody raised against PTEN (Santa Cruz, Cat# SC-9145) was utilized to detect PTEN expression in the prostate tissue. As an internal control, GAPDH expression was detected using GAPDH antibody (Biodesign, Cat# H86504) to normalize PTEN expression in each blot. Densitometric analysis was performed using Scion Image software (NIH). Control samples were surgically obtained from the normal part of the prostate tissue from 10 separate patients with OCPCa. The average of this densitometric analysis was used to compare and find the percent reduction in PTEN expression in patients with BHP, OCPCa and APCa.

### *2.4. Statistical analysis*

SPSS for Windows 10.0 software was used for statistical analysis. Normality of the groups was tested by Shapiro-Wilk method. Student *t*-test or Mann–Whitney U-test were used for the comparison of the groups. Multivariate analysis was performed by binary logistic regression analysis. The error bars for all data points in all figures portray the standard error of the mean ($\pm$SEM; *marks $P < 0.05$).

## 3. Results

### *3.1. Staging of the human prostate tissue and the classification of patients*

Prostate tissue was obtained surgically from 43 patients with BPH, 15 with OCPCa and 18 with APCa as described in Section 2. TNM staging was used for the clinical and pathological staging of the prostate cancer (data not shown).

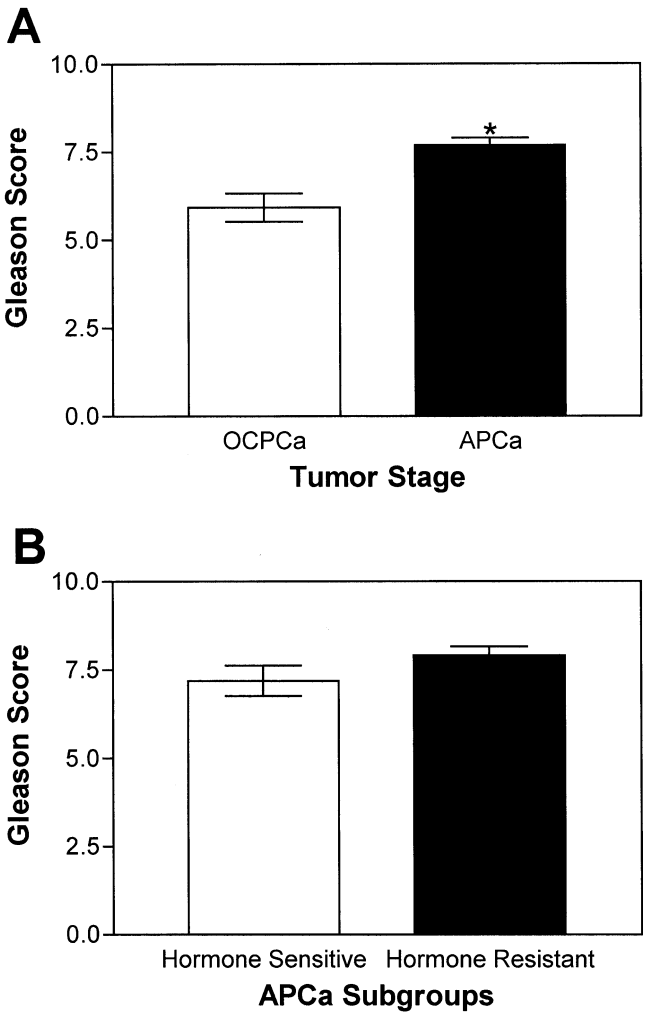


Fig. 1. Histo-pathologic analysis of the human prostate tissue. The comparison of Gleason scores of OCPCa ($n$ = 15) and APCa ($n$ = 18) is given in Panel A. Panel B depicts the mean Gleason scores for hormone sensitive ($n$ = 6) and hormone resistant ($n$ = 12) advanced prostate carcinoma patients.

All of the OCPCa cases were pT2. There were 16 of the APCa patients staged as **c**T3aM1b and two as cT4aM1b. In addition, one patient had lymph node metastasis and assigned as cT3aN+M1b.

### *3.2. Histo-pathologic examination of the prostate carcinoma*

Prostate tissues obtained from patients with OCPCa or APCa were graded according to Gleason system as shown in Fig. 1A. The mean Gleason score for OCPCa samples ($n$ = 15) was 5.93 ± 0.4 compared to 7.8 ± 0.2 for APCa ($n$ = 18). Moreover, APCa patients were categorized into two different subgroups based on the sensitivity to hormone therapy. No significant variation was found based on the Gleason scoring between hormone sensitive and hormone resistant prostate carcinoma samples (Fig. 1B).

### *3.3. Alterations in the serum PSA levels and its connection to the staging of the prostate carcinoma*

Analysis of serum PSA level as a prognostic factor for the progression of prostate cancer illustrated that the mean PSA level of patients with BPH was approximately 8.5 ± 1.9 ng/mL. On the other hand, patients with OCPCa (13.8 ± 3.1 ng/mL) or APCa (94 ± 32.6 ng/mL) both displayed significantly higher levels of serum PSA. Detailed analysis of the subcategories within APCa patients as shown in Fig. 2B portrayed that despite the fact that serum PSA levels (101±38 ng/mL) were much higher in the hormone resistant group compared to that of hormone sensitive group (15.2 ± 4.4 ng/mL), the difference was not statistically significant.

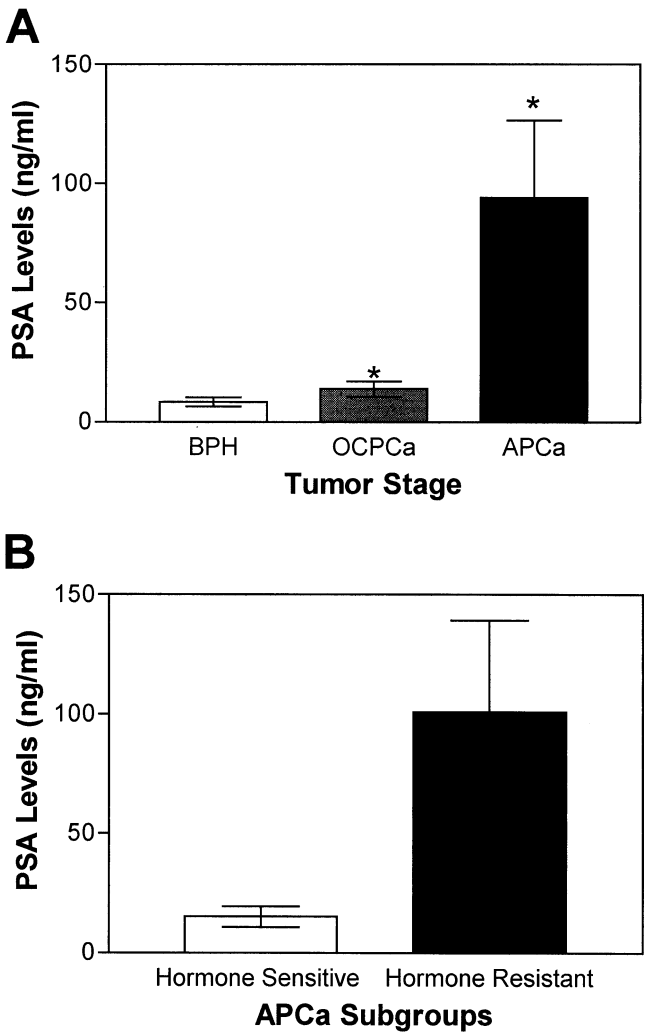


Fig. 2. Quantitative analysis of the serum prostate specific antigen. The mean PSA values for BPH ($n$ = 43), OCPCa ($n$ = 15) and APCa ($n$ = 18) samples are provided in Panel A. Panel B illustrates the mean PSA levels for the advanced prostate carcinoma subgroups.

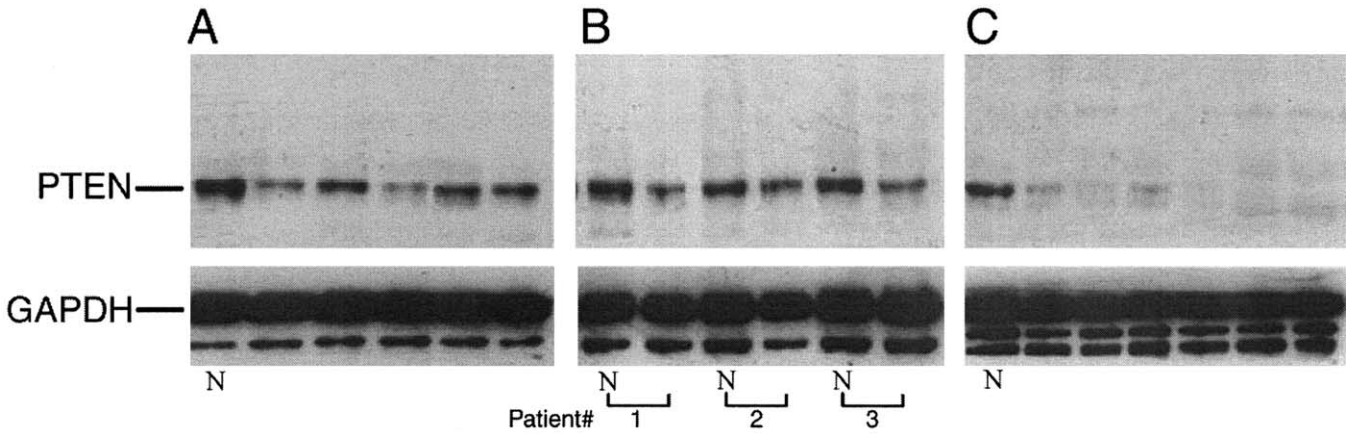


Fig. 3. A representative Western blotting analysis for patients with BPH (Panel A), OCPa (Panel B) and APCa (Panel C). Upper panels represent PTEN expression profiles. Bottom panels indicate GAPDH levels used as an internal control. N marks control samples obtained from the normal part of the prostate tissue in patients with OCPCa and loaded at least once for each gel. Control and the tumor part of the prostate tissues obtained from three different individuals with OCPCa are given in Panel B for comparison purposes.

### *3.4. Analysis of PTEN expression in patients with BPH, OCPCa and APCa*

Prostate samples were analyzed by Western blotting to determine the level of PTEN expression as described in Section 2. A representative image of such an analysis is placed in Fig. 3. The decrease in the level of PTEN expression did not vary much between BPH (Fig. 3A) and OCPCa samples (Fig. 3B). On the contrary, APCa samples manifested a drastic reduction in the level of PTEN expression greater than 86% (Fig. 3C). Densitometric analysis of Western blotting results exhibited no statistically important divergence between BPH and OCPCa patients in terms of PTEN expression but only APCa patients unveiled such a disparity (Fig. 4A). Furthermore, the percent decrease in PTEN expression was similar between hormone sensitive and hormone resistant groups in advanced prostate carcinoma samples (Fig. 4B).

### *3.5. Pathologic value of PTEN for the staging of prostate carcinoma*

To evaluate the importance of PTEN as a pathologic marker for the evaluation of prostate carcinoma, univariate and multivariate statistical analysis were performed as shown in Table 1 and Table 2. Cases were categorized into two subgroups based on the nature of tumor, one as benign (BPH) and another as malign (patients with OCPCa or APCa). Univariate analysis result indicated that there appeared to be a significant rise in PSA levels and a significant drop in PTEN expression in prostate of patients with malign tumors compared to those patients with benign tumors. In addition, multivariate analysis result suggested that both PTEN ($P = 0.025$) and PSA ($P = 0.036$) could be used as an independent marker to predict the nature of tumor whether it is a benign or a malign tumor (Table 1). Com-

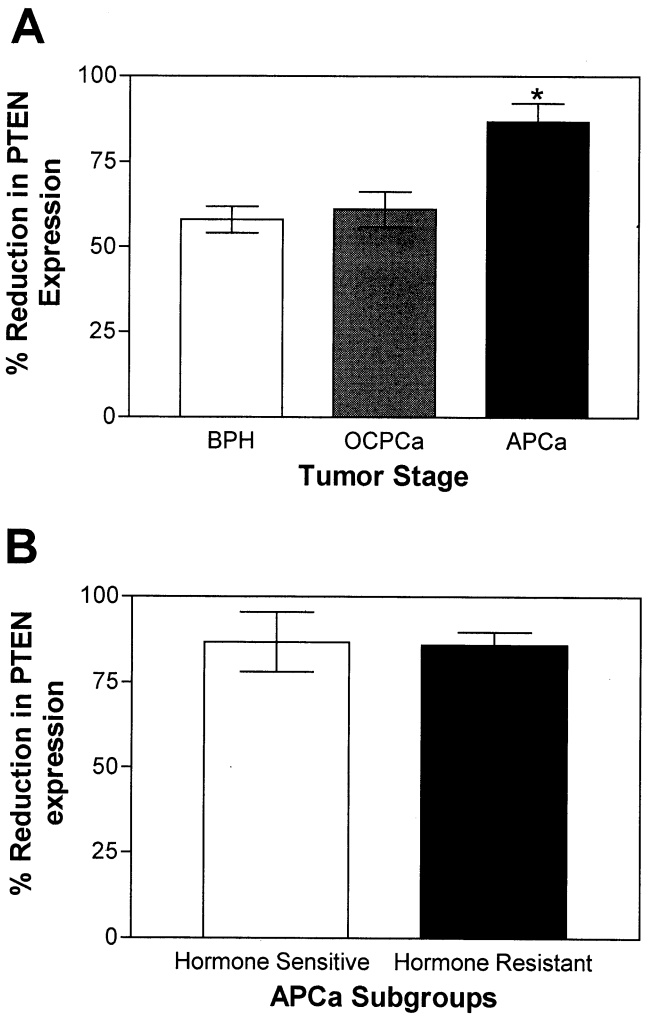


Fig. 4. PTEN expression profiles of patients with BPH ($n = 43$), OCPCa ($n = 15$) and APCa ($n = 18$). The reduction in PTEN expression is indicated as percent decrease on Y-axis in Panel A. The mean percent decrease for hormone sensitive and hormone resistant groups of advanced prostate carcinoma cases is provided in Panel B.

Table 1
Comparison of malign and benign cases (mean-95 % confidence interval)

| | PTEN | PSA |
|---|---|---|
| BPH ($n$ = 43) | 58 (50–66) | 8.5 (4.6–12.3) |
| Malign ($n$ = 33) | 75 (69–82) | 57.5 (19.1–95.8) |
| $P$ (Univariate) | 0.003 | 0.001 |
| $P$ (Multivariate) | 0.025 | 0.036 |

parison of the local and advanced prostate carcinoma samples showed that all the markers tested such as PTEN, PSA and Gleason score, appeared to be valuable parameters to follow up the progression of prostate carcinoma when they are used in combination (Table 2). However, according to the multivariate analysis result, only PTEN ($P$ = 0.032) appeared to be useful as an independent marker to predict the progression of prostate carcinoma from localized form to an advanced stage (Table 2).

## 4. Discussion

Recent studies suggest that screening for prostate cancer is identifying more patients with localized disease who are now potential candidates for curative local therapy. However, despite earlier detection and operative intervention, attempts for treating localized prostate cancer using multiple treatment modalities including radical surgery, radiation therapy, chemotherapy or any of these in combination fail in a significant number of patients. The ability to better predict individual patients who will have disease extension at the time of radical surgery or subsequently develop local recurrence or metastatic disease is an area of great interest for treatment planning. Elucidating the molecular pathways in prostate cancer is also leading to new treatment strategies and they include immunotherapy, gene therapy and conventional drug therapy directed at cell signaling pathways associated with the process of carcinogenesis. Some of these include attacking signaling pathways associated with proliferation, apoptosis, cell adhesion, angiogenesis, tumor invasion and metastasis [18]. For these reasons, tumor suppressor gene, PTEN, became a heavy area of investigation to determine its role during the progression of prostate cancer [19,20]. In this study we evaluate the potential utility of a tumor suppressor gene as a pathologic marker to predict the local extension of prostate cancer in combination with Gleason system, PSA and the clinical stage at the time of initial diagnosis.

Seventy-six patients with prostate ailment were admitted to the Urology Clinic of Akdeniz University, Faculty of Medicine. In addition to the serum PSA level, the level of PTEN in prostate tissues was assayed using Western blotting to determine the correlation of the biomarker level with histological grade compared to benign tissues. This would allow us to determine if there is a possible link between PTEN expression and the progression of prostate cancer. The rationale behind this comparison is the previous documented finding that there is a tight correlation between histological grade and disease progression [21]. Our study also demonstrated that there is a statistically important difference between Gleason scores of patients with APCa and those of OCPCa (Fig. 1A). Despite the observed difference, multivariate analysis result indicated that Gleason scoring alone ($P$ = 0.134) did not seem to be a good parameter to distinguish patients with OCPCa from patients with APCa (Table 2). In addition, the mean Gleason scores of hormone sensitive and hormone resistant APCa groups did not deviate much to generate a statistically vital divergence (Fig. 1B). This finding somewhat challenges a recent report, which suggested high Gleason scores exclusively correlating with the androgen independent progression of prostate cancer [22].

As a second prognostic marker, serum PSA levels were also analyzed in these patients with prostate cancer. Much higher PSA values were observed in patients with OCPCa or APCa compared to those with BPH (Fig. 2A). These results are also supported by a previous report indicating a correlation between serum PSA levels and the clinical stage of prostate carcinoma [23]. Interestingly, based on our results PSA appeared to be a good independent marker to predict the nature of tumor to figure out whether the patient have a benign or a malign tumor (Table 1). However, PSA by itself was not useful to confirm whether patients had a localized or an advanced form of prostate carcinoma (Table 2). Because of this reason, preoperative PSA screening alone to predict the behavior of prostate carcinoma in terms of cancer progression appears to be somewhat inadequate. No significant difference was found between HS and HR group in terms of serum PSA levels either (Fig. 2B).

On top of all these changes observed with Gleason score and the PSA level, the percent reduction in PTEN expression (86.8%) correlated well with the advanced stage of prostate carcinoma while this reduction remained about the same in patients with BPH (58%) and those of OCPCa (61%). Therefore, there appeared to be an inverse correlation between PTEN expression and the progression of prostate cancer. PTEN was originally identified as a tumor suppressor gene with a dual function. It is interesting to speculate how this tumor suppressor gene's dual function contributes to the progression of prostate cancer. The lipid phosphatase activity of PTEN is essential for the regulation

Table 2
Comparison of local and advanced malign cases (mean-95 % confidence interval)

| | PTEN | PSA | Gleason |
|---|---|---|---|
| Local ($n$ = 15) | 61 (52–71) | 13.8 (7.2–20.5) | 5.9 (5.0–6.8) |
| Advanced ($n$ = 18) | 87 (83–91) | 93.8 (25.7–161.9) | 7.8 (7.3–8.3) |
| $P$ (Univariate) | 0.001 | 0.015 | 0.002 |
| $P$ (Multivariate) | 0.032 | 0.862 | 0.134 |

of cell growth and apoptosis and its action is mediated through PI3K pathways. However, it's the tyrosine phosphatase activity, which is imperative for the cell adhesion, migration and invasion. Interestingly, a recent molecular study suggested that PTEN might be associated with tumor cell invasion [24]. In another study, Hwang et al. suggested that PTEN possibly controls the formation of metastasis in an animal metastatic lung model [25]. For the reasons mentioned above, pathologic value of PTEN for the purpose of predicting the nature of prostate carcinoma was evaluated in detail. Interestingly, PTEN exhibited a great promise to be used as an independent marker to predict the tumor behavior whether it is a benign or a malign tumor (Table 1). On top of that, our results also suggest that PTEN alone can be classified as an independent marker to follow up the progression of prostate carcinoma from a localized form to an advanced stage (Table 2).

## 5. Conclusion

The results presented here suggest that compared to the currently known prognostic markers such as PSA and Gleason system, PTEN appeared to be an independent parameter both to predict the nature of prostate tumor behavior and also further classify it whether it is an organ confined form or an advanced form. Combining PTEN in a biomarker profile with Gleason score and PSA will certainly be useful to develop new treatment strategies for the prevention or progression of prostate cancer.

## Acknowledgments

This work is supported by Akdeniz University Scientific Research Project Administration Division Grant to Dr. Salih Sanlioglu.

## References

[1] Yeung F, Chung LW. Molecular basis of co-targeting prostate tumor and stroma. J Cell Biochem Suppl 2002;38:65–72.
[2] Devi GR. Prostate cancer: status of current treatments and emerging antisense-based therapies. Curr Opin Mol Ther 2002;4:138–48.
[3] Perrotti M, Rabbani F, Farkas A, Ward WS, Cummings KB. Trends in poorly differentiated prostate cancer 1973 to 1994: observations from the Surveillance, Epidemiology and End Results database. J Urol 1998;160:811–5.
[4] Ross JS, Sheehan CE, Fisher HA, Kauffman RA, Dolen EM, Kallakury BV. Prognostic markers in prostate cancer. Expert Rev Mol Diagn 2002;2:129–42.
[5] Ross JS, Sheehan CE, Dolen EM, Kallakury BV. Morphologic and molecular prognostic markers in prostate cancer. Adv Anat Pathol 2002;9:115–28.
[6] Koksal IT, Ozcan F, Kadioglu TC, Esen T, Kilicaslan I, Tunc M. Discrepancy between Gleason scores of biopsy and radical prostatectomy specimens. Eur Urol 2000;37:670–4.
[7] Schroder FH, Hermanek P, Denis L, Fair WR, Gospodarowicz MK, Pavone-Macaluso M. The TNM classification of prostate cancer. Prostate Suppl 1992;4:129–38.
[8] Koksal IT, Ozcan F, Kilicaslan I, Tefekli A. Expression of E-cadherin in prostate cancer in formalin-fixed, paraffin-embedded tissues: correlation with pathological features. Pathology 2002;34:233–8.
[9] Qian J, Hirasawa K, Bostwick DG, et al. Loss of p53 and c-myc overrepresentation in stage T(2-3)N(1-3)M(0) prostate cancer are potential markers for cancer progression. Mod Pathol 2002;15:35–44.
[10] Partin AW, Mangold LA, Lamm DM, Walsh PC, Epstein JI, Pearson JD. Contemporary update of prostate cancer staging nomograms (Partin Tables) for the new millennium. Urology 2001;58:843–8.
[11] Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997;15:356–62.
[12] Whang YE, Wu X, Suzuki H, et al. Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. Proc Natl Acad Sci USA 1998;95:5246–50.
[13] McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. Cancer Res 1999;59:4291–6.
[14] Goodin S, Rao KV, DiPaola RS. State-of-the-art treatment of metastatic hormone-refractory prostate cancer. Oncologist 2002;7:360–70.
[15] Bostwick DG. Staging prostate cancer–1997: current methods and limitations. Eur Urol 1997;32:2–14.
[16] Bostwick DG, Myers RP, Oesterling JE. Staging of prostate cancer. Semin Surg Oncol 1994;10:60–72.
[17] Sanlioglu S, Williams CM, Samavati L, et al. Lipopolysaccharide induces Rac1-dependent reactive oxygen species formation and coordinates tumor necrosis factor-alpha secretion through IKK regulation of NF-kappa B. J Biol Chem 2001;276:30188–98.
[18] Morris MJ, Scher HI. Novel therapies for the treatment of prostate cancer: current clinical trials and development strategies. Surg Oncol 2002;11:13–23.
[19] Fernandez M, Eng C. The expanding role of PTEN in neoplasia: a molecule for all seasons? Commentary re: M.A. Davies, et al., Adenoviral-mediated expression of MMAC/PTEN inhibits proliferation and metastasis of human prostate cancer cells. Clin Cancer Res 8:1904-1914, 2002. Clin Cancer Res 2002;8:1695-8.
[20] Davies MA, Kim SJ, Parikh NU, Dong Z, Bucana CD, Gallick GE. Adenoviral-mediated Expression of MMAC/PTEN Inhibits Proliferation and Metastasis of Human Prostate Cancer Cells. Clin Cancer Res 2002;8:1904–14.
[21] Fowler JE Jr, Bigler SA, White PC, Duncan WL. Hormone therapy for locally advanced prostate cancer. J Urol 2002;168:546–9.
[22] Benaim EA, Pace CM, Roehrborn CG. Gleason score predicts androgen independent progression after androgen deprivation therapy. Eur Urol 2002;42:12–7.
[23] Xess A, Singh M, Raghwendra KH, Sharma HP, Shahi SK. Prostate specific antigen as tumor marker: relationship with histologic grading. Indian J Pathol Microbiol 2001;44:261–4.
[24] Tamura M, Gu J, Takino T, Yamada KM. Tumor suppressor PTEN inhibition of cell invasion, migration, and growth: differential involvement of focal adhesion kinase and p130Cas. Cancer Res 1999; 59:442–9.
[25] Hwang PH, Yi HK, Kim DS, Nam SY, Kim JS, Lee DY. Suppression of tumorigenicity and metastasis in B16F10 cells by PTEN/MMAC1/TEP1 gene. Cancer Lett 2001;172:83–91.