[CANCER RESEARCH 59, 4291–4296, September 1, 1999]

# Loss of PTEN Expression in Paraffin-embedded Primary Prostate Cancer Correlates with High Gleason Score and Advanced Stage[1]

**Máirín E. McMenamin, Peggy Soung, Sauni Perera, Irving Kaplan, Massimo Loda,[2] and William R. Sellers[2]**

*Departments of Pathology [M. E. M.] and Radiation Oncology [I. K.], Beth Israel Deaconess Medical Center, Department of Pathology, Brigham and Women's Hospital [M. L.], and the Department of Adult Oncology and the Lank Center for Genitourinary Oncology, Dana-Farber Cancer Institute [P. S., S. P., M. L., W. R. S.], Harvard Medical School, Boston, Massachusetts 02115*

## ABSTRACT

**The tumor suppressor gene PTEN/MMAC-1/TEP-1 (referred to hereafter as PTEN) maps to chromosome 10q23 and encodes a dual specificity phosphatase. The PTEN protein negatively regulates cell migration and cell survival and induces a $G_1$ cell cycle block via negative regulation of the phosphatidylinositol 3′-kinase/protein kinase B/Akt signaling pathway. *PTEN* is frequently mutated or deleted in both prostate cancer cell lines and primary prostate cancers. A murine polyclonal antiserum was raised against a glutathione *S*-transferase fusion polypeptide of the COOH terminus of PTEN. Archival paraffin tissue sections from 109 cases of resected prostate cancer were immunostained with the antiserum, using DU145 and PC-3 cells as positive and negative controls, respectively. PTEN expression was seen in the secretory cells. Cases were considered positive when granular cytoplasmic staining was seen in all tumor cells, mixed when areas of both positive and negative tumor cell clones were seen, and negative when adjacent benign prostate tissue but not tumor tissue showed positive staining. Seventeen cases (15.6%) of prostate cancer were positive, 70 cases (64.2%) were mixed, and 22 cases (20.2%) were negative. Total absence of PTEN expression correlated with the Gleason score ($P = 0.0081$) and correlated more significantly with a Gleason score of 7 or higher ($P = 0.0004$) and with advanced pathological stage (American Joint Committee on Cancer stages T3b and T4; $P = 0.0078$). Thus, loss of PTEN protein is correlated with pathological markers of poor prognosis in prostate cancer.**

## INTRODUCTION

*PTEN* is a tumor suppressor gene that maps to the 10q23.3 interval (1–3). The protein product, PTEN, shares homology with the cytoskeletal protein tensin and the secretory vesicle protein auxilin and also with dual specificity phosphatases. Indeed, recombinant PTEN exhibits activity against both phosphotyrosine- and phosphothreonine-containing protein substrates (4). Overexpression of PTEN suppresses tumor colony formation in certain cell lines and can suppress tumor formation in nude mice (5–7). PTEN overexpression can also negatively regulate cellular adhesion and cell mobility on fibronectin-coated plates (8). This activity may result from PTEN-mediated dephosphorylation of focal adhesion kinase. PTEN may also alter mitogen-activated protein kinase signaling (9).

PTEN can also act as a lipid phosphatase. Specifically, PTEN can dephosphorylate phosphotidylinositol 3,4,5-trisphosphate and phosphotidylinositol 3,4-trisphosphate, which are both direct products of PI3K[3] activity (10). We and others have shown that PTEN can inhibit cell cycle progression and induce a $G_1$ arrest. This function appears to require the lipid phosphatase activity of PTEN, resulting in the negative regulation of the PI3K/Akt signaling pathway (11–13). A significant increase in the level of the cell cycle kinase inhibitor $p27^{KIP1}$ occurs with concomitant decreases in $G_1$ cyclin-dependent kinase activity upon the introduction of PTEN into human glioblastoma U87MG cells, suggesting that p27 may be a target of the PTEN cell cycle arrest pathway (13). In keeping with these data, heterozygous loss of the murine *PTEN* gene (*mPTEN*) leads to an increase in the mitotic index and the Ki-67 staining index in the murine prostate (7). In addition, PTEN negatively regulates Akt-dependent cell survival (14–17). Akt is one of the key regulatory molecules involved in the protection of cells against apoptosis. These data support the idea that PTEN negatively regulates cell growth and/or proliferation through its ability to act as an *in vivo* phosphoinositide 3-phosphatase, thus negatively regulating the PI3K/Akt signaling pathway.

Germ-line mutations of *PTEN* have been detected in cases of Cowden disease and Bannayan-Zonana syndrome, two related hamartoma syndromes (18–20). Patients with Cowden disease have an elevated risk of various cancers, including breast and thyroid cancer. Alterations of the second *PTEN* allele have been demonstrated in gastrointestinal polyps in patients with Cowden disease (21).

Somatic alterations of *PTEN* are common in certain cell lines and in primary tumors including gliomas (22–25), melanoma (26, 27), and thyroid (28) and endometrial cancers (29, 30). On the other hand, somatic alterations are rare in breast (31) and renal cancer (32) and were not detected in a series of squamous carcinomas from the head and neck (33). *PTEN* mutations and allele loss at 10q23 appear to be a late event in glioblastoma, melanoma, and prostate cancer (22–26, 34). In contrast, *PTEN* alterations are more common in benign tumors than in malignant thyroid tumors (28) and also occur in a proportion of cases of endometrial hyperplasia, a precursor of endometrial carcinoma (35), suggesting that the genetic alteration may occur at an early stage in these tumors.

Prostate cancer is the most prevalent form of cancer in men in the Western world and is the second most common cause of male cancer deaths in the United States (36). Pathological stage and Gleason grade are important predictors of prognosis in patients with primary prostate cancer who undergo radical prostatectomy. Prostate cancer, however, is a remarkably heterogeneous disease. Distinguishing tumors associated with a poor outcome at the time of radical prostatectomy is problematic. The molecular mechanisms of prostate carcinogenesis remain poorly understood. LOH of 10q has been reported to occur in prostate cancer with a high frequency (30–60%; Refs. 37 and 38), and two distinct, commonly deleted regions have been identified at 10q22–q24 and 10q25, respectively, implying the presence of putative tumor suppressor genes at these loci (38). Homozygous deletions and somatic mutations of *PTEN* have been identified in prostate cell lines and tumor specimens (1, 2, 34, 39–45). Marked heterogeneity of *PTEN* alterations has been observed in metastatic prostate cancer tissues (43). Loss of PTEN expression is more frequently detected in xenografts of cell lines (34). *PTEN* may be inactivated by mechanisms other than gene deletion and mutations, including promoter methylation or translational modification (34). However, other groups failed

Received 3/11/99; accepted 7/2/99.

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[1] Supported in part by grants from CaP CURE (to M. L. and W. R. S.) and the United States Army Medical Research and Material Command-Prostate Cancer Research Program (USAMRMC) (to W. R. S.). Part of this work was presented at the United States and Canadian Academy of Pathology meeting held in Boston, Massachusetts in 1998.

[2] To whom requests for reprints should be addressed. Phone: (617) 632-5261; Fax: (617) 632-5417; E-mail: william_sellers@dfci.harvard.edu (W. R. S.). E-mail: massimo_loda@dfci.harvard.edu (M. L.).

[3] The abbreviations used are: PI3K, phosphatidylinositol 3′-kinase; LOH, loss of heterozygosity.; GST, glutathione *S*-transferase; HA, hemagglutinin; PIN, prostatic intraepithelial neoplasia.



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to detect *PTEN* methylation in prostate, bladder, and renal cell cancer with LOH of 10q when a PCR-based assay was used (32, 40).

In this study, we wanted to assess the extent of loss of the PTEN protein in prostate cancer using immunohistochemistry. We analyzed the pattern of immunohistochemical staining in 109 cases of paraffin-embedded resected prostate cancer using a murine polyclonal antibody to PTEN. Detection of PTEN protein was correlated with the Gleason score and the pathological stage of the tumor, known prognosticators in prostate cancer.

## MATERIALS AND METHODS

**Tissue Specimens.** We used cases from a prostate database consisting of 128 paraffin-embedded prostate cancers that had been collected in the Department of Pathology, Beth Israel Deaconess Medical Center, West Campus, dating from 1990–1997. This database has been described previously (46–49). Nineteen cases were transurethral resection specimens. We used the remaining 109 radical prostatectomy specimens for this study. Follow-up data were available on 69 cases in the database, with a mean patient follow-up of 19.84 months. The pathological tumor (T) stage (American Joint Committee on Cancer; Ref. 50) and Gleason score were available in each case. Five of the 109 cases had been treated with preoperative total androgen ablation. For each case, a representative paraffin block was selected that contained both tumor and benign prostate tissue.

**Processing of Cell Lines and Cell Blocks.** DU145, PC-3, and LNCaP prostate cancer cell lines were obtained from the American Type Culture Collection. The U2-OS cell line was a generous gift of W. G. Kaelin (Dana-Farber Cancer Institute). DU145 cells contain one wild-type *PTEN* allele and a second variant allele (*M134L*). PC-3 cells have sustained a homozygous deletion of *PTEN*. LNCaP cells have a deletion of one allele and a mutation of the other *PTEN* allele, and the genetic state of *PTEN* has not been characterized in U2-OS cells. LNCaP and PC-3 cells were grown in RPMI 1640 supplemented with D-glucose, HEPES buffer, L-glutamine, $PP_i$, penicillin, streptomycin, and 10% fetal bovine serum. DU145 cells were maintained in DMEM supplemented with penicillin, streptomycin, and 10% fetal bovine serum, and U2-OS cells were maintained in DMEM supplemented with penicillin, streptomycin, and 10% fetal clone (HyClone). All cells were grown on P100 tissue culture dishes at 37°C. LNCaP and PC-3 cells were grown in a 5% $CO_2$ atmosphere, and DU145 and U2-OS cells were grown in a 10% $CO_2$ atmosphere. Cell pellets were created from DU145 and PC-3 cells, fixed in 10% formalin overnight, and then processed in the regular manner for pathology specimens to produce paraffin cell blocks.

**Plasmids.** A cDNA fragment of the *PTEN* gene encoding amino residues 239–403 was amplified by PCR using primers WRSO-56 (5′-GACTGGATCCATGTACTTTGAGTTCCCTCAGCC-3′) and WRSO-57 (5′-CGCGGAATTCTCAGACTTTTGTAATTTGTGTATGC-3′) from a cDNA library derived from human embryonic kidney 293 cells (51). The resulting PCR fragment was isolated, restricted with *Bam*HI and *Eco*RI, and ligated to similarly restricted pSG5L to produce pSG5L-PTEN (239–403). This cDNA was confirmed by sequencing. The insert from this plasmid was excised and ligated to *Bam*HI/*Eco*RI-restricted pGEX2T vector to produce pGEX2T-PTEN (239–403) plasmid.

**Antibodies.** Recombinant GST-PTEN (239–403) was produced in *Escherichia coli* and affinity-purified on glutathione-Sepharose beads by conventional methods (52). Mice were inoculated with 100 μg of GST-PTEN (239–403) mixed with Freund's complete adjuvant. Two weeks later, the mice received a subsequent boost of 100 μg of the purified protein in Freund's incomplete adjuvant. Immune sera (M1) was obtained by orbital sinus puncture.

***In Vitro* Translation, Immunoprecipitation, and Immunoblotting.** Full-length HA-tagged PTEN protein (HA-PTEN) was produced *in vitro* by coupled transcription and translation of the pSG5L-PTEN plasmid using the TnT kit (Promega, Madison, WI). Cell extracts were prepared in the following manner. Cells grown on P100 plates were washed twice with PBS and then lysed on the plate in 500 μl of TNN buffer [150 mM NaCl, 50 mM Tris (pH 7.4), and 0.5% NP40] at 4°C for 20 min. Collected extracts were then cleared by centrifugation at 14,000 rpm for 15 min. Immunoprecipitations of *in vitro* translated products were carried out at 4°C in NET-N buffer [120 mM NaCl, 10 mM EDTA (pH 8.0), 100 M Tris (pH 7.4), and 0.5% NP40] along with 5 μl of *in vitro* translated PTEN in 250 μl of NET-N. One μl of antiserum was used per immunoprecipitation experiment. Immune complexes were captured on protein A-Sepharose beads (30 μl of 1:1 beads), washed five times with NET-N, and boiled in 1× Laemmli sample buffer. Whole cell extracts or immunoprecipitates were separated by vertical gel electrophoresis on 7.5% gels. Proteins were transferred to Sequi-blot polyvinylidene difluoride membrane (Bio-Rad) by wet transfer in Towbin's buffer for 6–16 h. Immunoblots were blocked in TBS + 4% milk. M1 was used at a concentration of 1:10,000 in TBS + 4% milk. Alkaline phosphatase-conjugated goat antimouse antibody was the secondary antibody.

**Immunohistochemistry.** Five-μm sections were cut from the selected paraffin blocks of prostate tumor and the DU145 and PC-3 cell blocks, mounted on charged glass slides, baked at 60°C for 60 min, deparaffinized, and rehydrated through graded alcohol rinses. Slides were immersed in 10 mM/liter citrate buffer (pH 6.0; Biogenex, San Ramon, CA) and microwaved in a 750 W oven inside a pressure cooker for 30 min. The slides were cooled at room temperature for 15 min and rinsed in tap water. A 1:2000 dilution of M1, the PTEN murine polyclonal antiserum, was applied for 32 min at 37°C. An automated processor (Ventana ES; Ventana Medical Systems, Tuscon, AZ) was used to incubate the slides in blocker (10% normal goat serum and 10% normal horse serum in Ventana diluent) for 8 min, followed by an incubation in secondary antibody conjugated to an avidin-biotin peroxidase complex (antirabbit and antimouse). Finally, 3,3′-diaminobenzidine was used as a substrate to detect bound antibody complex. The slides were counterstained with hematoxylin. Standardization of the incubation and development times allowed an accurate comparison of expression levels in all cases.

**Analysis of Immunohistochemical Staining.** Positive cases were defined by the presence of granular, crisp cytoplasmic staining, as seen in the DU145 positive control samples. The cases were initially divided into three groups: (*a*) positive (the entire tumor showed staining); (*b*) mixed (both positive and negative cells/glands were present); and (*c*) negative (no staining was seen in the represented tumor). The grading of PTEN expression was performed without knowledge of the Gleason score or pathological stage. The presence of positive staining in PIN was noted. The cases were then divided into those that showed positive staining (positive and mixed groups) and those with a total absence of staining (negative group).

**Statistical Analysis.** We tested for associations between PTEN expression and Gleason score or pathological stage of disease using the Mann-Whitney nonparametric *U* test, the $c^2$ test, or Fisher's exact test, as appropriate. All calculations were performed using StatView 4.5 software (Abacus Concepts, Inc., Berkeley, CA).

## RESULTS

**Characterization of the Antibody.** A murine polyclonal antiserum (M1) was raised against a protein chimera encoding GST and PTEN amino acid residues 237–403. HA-PTEN was produced by *in vitro* translation and subjected to immunoprecipitation with M1. Purified anti-HA antibody and the M1 preimmune sera served as positive and negative controls, respectively. Both the M1 antiserum and the anti-HA antibody immunoprecipitated HA-PTEN, whereas the nonimmune serum did not (Fig. 1, *left panel*). To determine whether M1 might specifically recognize the endogenous PTEN protein, whole cell protein extracts were prepared from U2-OS osteosarcoma cells and DU145 and PC-3 prostate carcinoma cells, separated by electrophoresis, and subjected to immunoblotting with M1 antiserum (Fig. 1, *right panel*). M1 recognized a protein species of approximately $M_r$ 58,000 that is present in DU145 and U2-OS cells but is absent in PC-3 cells. PC-3 cells have sustained a biallelic deletion of the *PTEN* gene, whereas DU145 contains a wild-type *PTEN* allele and an allele harboring a missense change at codon 134 (M134L). This protein species migrates slightly faster than the *in vitro* translated HA-PTEN. Taken together, these data indicate that the recognized protein is endogenous PTEN.

**PTEN Expression in Human Prostate Cancer Cell Lines.** Next we asked whether M1 was capable of recognizing PTEN by immu-



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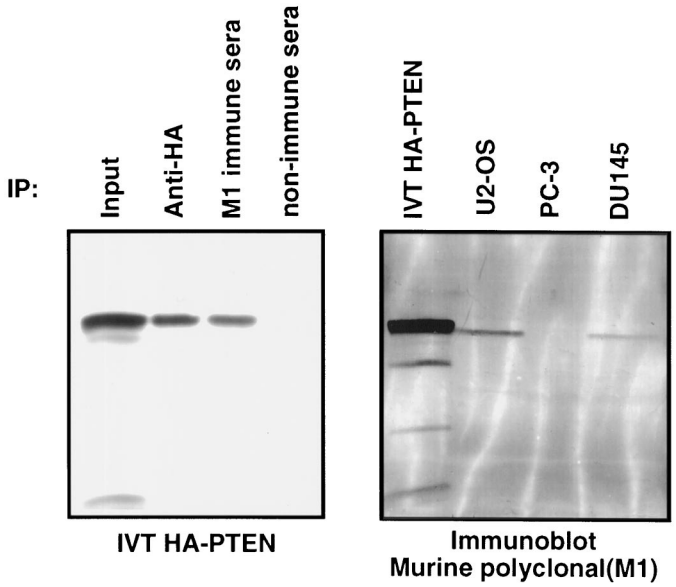


Fig. 1. Immunoprecipitation of *in vitro* translated HA-PTEN with the murine polyclonal antisera M1. HA-PTEN was produced by *in vitro* translation and immunoprecipitated with either anti-HA antibody, murine polyclonal antisera M1, or nonimmune murine antisera, as indicated (*left panel*). The input is 5 $\mu$l of *in vitro* translated HA-PTEN loaded in sample buffer. Immunoblot detection of endogenous PTEN was performed using murine polyclonal antisera M1.Whole cell extracts (150 $\mu$g) prepared from U2-OS cells, PC-3 cells, and DU145 cells (as indicated) and HA-PTEN produced by *in vitro* translation were separated by gel electrophoresis, transferred to polyvinylidene difluoride membrane, and immunoblotted with murine polyclonal antisera M1 (*right panel*).

nohistochemistry. Cell blocks were prepared from DU145 and PC-3 cells that were grown in culture. M1 was then used to detect PTEN in 5-$\mu$m sections from these paraffin blocks by immunohistochemical means. Strong positive granular cytoplasmic staining was detected in DU145 cells, but PC-3 cells were negative (Fig. 2, *A* and *B*). Thus, sections of cell blocks of DU145 and PC-3 cell lines served as positive and negative controls, respectively. Preimmune serum also served as a negative control.

**Patient Databases and Tumor Characteristics.** The mean patient age at the time of surgery was 65.2 $\pm$ 8.4 years, with an age range of 40–86 years. The Gleason score of the tumors ranged from 4–9 with the following frequency: (*a*) Gleason score = 4, 4 tumors; (*b*) Gleason score = 5, 9 tumors; (*c*) Gleason score = 6, 17 tumors; (*d*) Gleason score = 7, 58 tumors; (*e*) Gleason score = 8, 12 tumors; and (*f*) Gleason score = 9, 9 tumors. The median Gleason score was 7. The cases were then subdivided into two groups: (*a*) those with a Gleason score $<$ 7 (30 cases); and (*b*) those with a Gleason score $\geq$ 7 (79 cases; Table 1). The cases were divided into two groups: (*a*) those with either organ-confined disease or disease extends through into the prostate capsule (T1–T3a; 83 cases); and (*b*) those with seminal vesicle involvement or metastases to the lymph nodes (T3b and T4; 26 cases; Table 2).

**PTEN Expression in Human Prostate Tissue.** Benign prostate epithelium showed positive staining for PTEN with granular cytoplasmic staining observed in the prostatic secretory cells. PIN was present in the selected slides in 58 cases, and all cases showed positive staining (Fig. 3, *A1* and *A2*). The cases were initially divided into three groups: (*a*) positive (the entire tumor showed staining); (*b*) mixed (both positive and negative cells/glands were present); and (*c*) negative (no staining was seen in the represented tumor). Heterogeneous staining of the tumors was present. Seventeen cases (15.6%) were positive (Fig. 3, *B1* and *B2*). Seventy cases (64.2%) showed a mixed staining pattern. Specifically, there were areas of tumor that stained positively, whereas other areas of tumor showed negative staining (Fig. 3, *C1* and *C2*). The remaining 22 tumors (20.2%) were negative (Fig. 3, *D1* and *D2*). The cases were subsequently divided into those that showed positive staining (positive and mixed groups) and those with a total absence of staining (negative group). The results of PTEN expression in each group were compared.

**Correlation of PTEN Expression with Gleason Score and Pathological Stage.** Loss of PTEN expression correlated significantly with increasing Gleason score ($P$ = 0.0081), and when cases were divided into those with a Gleason score $<$ 7 and those with a Gleason score $\geq$ 7, the correlation with a Gleason score $\geq$ 7 was highly significant ($P$ = 0.0004). Loss of PTEN expression also correlated with advanced disease (pathological tumor stage T3c and T4; $P$ = 0.0078). PTEN expression was seen in two of the five tumors where patients had undergone preoperative total androgen ablation.

Follow-up for the cohort was too short to give meaningful survival figures because only four deaths had occurred in the study group.

## DISCUSSION

In this study, a murine polyclonal antiserum (M1) was raised against a protein chimera encoding GST and PTEN amino acid residues 237–403. Using this antiserum, the expression of the PTEN protein was determined by immunohistochemistry in 109 prostate cancers of varying grade and pathological stage. Whereas PTEN was expressed in all cases of PIN, the presumed precursor lesion of

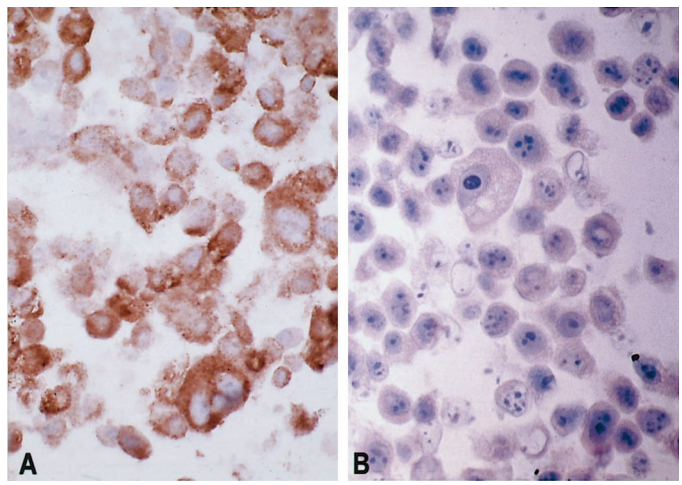


Fig. 2. PTEN expression in prostate cancer cell lines. Sections of cell blocks prepared from (*A*) DU145 cells (which contain one wild-type *PTEN* allele) or (*B*) PC-3 cells (which show a homozygous deletion of *PTEN*) were stained with M1 antiserum as described in "Materials and Methods." $\times$400.

Table 1 *Observed frequencies for PTEN expression and Gleason score*

| | PTEN expression[a] | | |
|---|---|---|---|
| Gleason score | Positive (%) | Mixed (%) | Negative (%) |
| $<$7 | 10 (9.2) | 20 (18.3) | 0 (0) |
| $\geq$7 | 7 (6.4) | 50 (45.9) | 22 (20.2) |

[a] Negative group *versus* positive plus mixed groups, $P$ = 0.0004.

Table 2 *Observed frequencies for PTEN expression and pathological stage*

| | PTEN expression[a] | | |
|---|---|---|---|
| Pathological stage | Positive (%) | Mixed (%) | Negative (%) |
| T1–T3a | 14 (12.8) | 57 (52.3) | 12 (11.0) |
| T3b and T4 | 3 (2.8) | 13 (11.9) | 10 (9.2) |

[a] Negative group *versus* positive plus mixed groups, $P$ = 0.0078.



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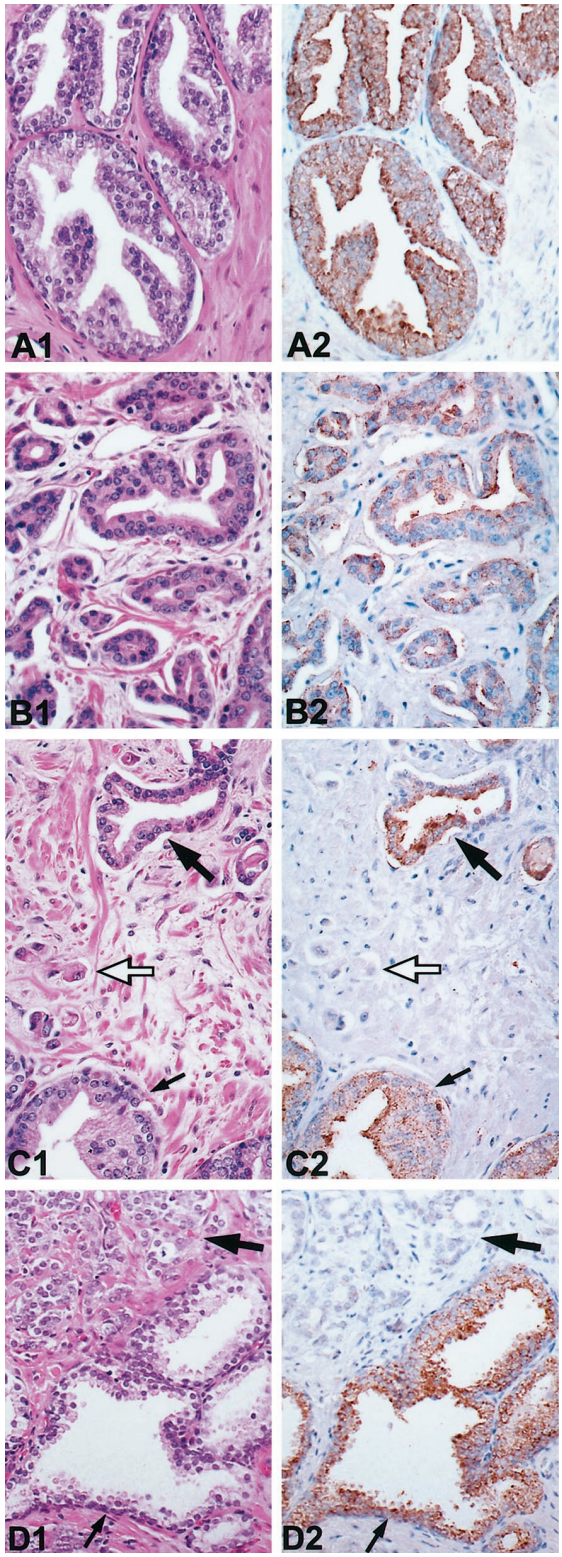


Fig. 3. PTEN expression in prostate tissue. *A*, PIN. *A1*, H&E-stained section of PIN. *A2*, positive staining for PTEN in PIN. *B*, prostate cancer (Gleason score, 3 + 3 = 6). *B1*, H&E-stained section. *B2*, positive staining for PTEN in prostate cancer. *C*, an example of heterogeneous expression of PTEN in a case of prostate cancer (Gleason score, 3 + 5 = 8). *C1*, prostate cancer. Gleason grade 3 cancer (*large black arrow*), Gleason grade 5 cancer (*open arrow*), and PIN (*short black arrow*). H&E-stained section. *C2*, Gleason grade 3 prostate cancer stains positively for PTEN (see *large black arrow*). In contrast, Gleason grade 5 prostatic carcinoma is negative for PTEN expression (*open arrow*). PIN shows positive staining for PTEN (*short black arrow*). *D*, benign prostate acinus with surrounding prostate cancer (Gleason score, 4 + 3 = 7). *D1*, H&E-stained section. *Small arrow*, benign prostatic glandular epithelium; *large arrow*, prostatic adenocarcinoma). *D2*, positive staining for PTEN in benign prostate tissue and absence of staining for PTEN in prostate cancer. ×200. All tissue sections were processed as described in "Materials and Methods."

prostate cancer (53), total loss of expression of PTEN was found in 20.2% of the prostate cancers. In an additional 70 of 109 cases (64.2%), there was a mixed pattern of staining, with areas of tumor with positive staining and other areas that were negative for PTEN. A mixed pattern of staining for PTEN was also recently found in glioma (54). Finally, 15.6% of tumors appeared to have homogenous positive staining for PTEN.

In this data set, complete PTEN loss was found to correlate significantly with the presence of high-stage disease (pathological stage T3b and T4; $P = 0.0078$). Indeed, *PTEN* mutations and allele loss at 10q23 have been reported to occur as a late event in most, albeit not all, tumors, including prostate cancers (22–26, 28, 34, 35). We also found that loss of PTEN expression correlated significantly with increasing grade of prostate cancer, *i.e.*, Gleason score ($P = 0.0081$). When cases were divided into those with a Gleason score $< 7$ or $\geq 7$, loss of PTEN expression correlated significantly with a Gleason score $\geq 7$ ($P = 0.0004$). A cut point between Gleason score 6 and 7 has previously been recommended when compression of the Gleason score is required (55). Similarly, *PTEN* is altered in high-grade gliomas, but not in low-grade gliomas (22, 23). On the other hand, *PTEN* alteration occurs in all three grades of endometrial cancer (29, 30), and mutation of a germ-line *PTEN* allele predisposes carriers to breast and thyroid cancer in humans and to a number of malignancies including prostate cancer in mice (7, 18, 20, 56). Thus, PTEN appears to play a role in the initiation of certain tumors, including a murine form of prostate cancer, and may play a role in the progression of other tumors such as gliomas and prostate cancer. Although seemingly paradoxical, the role of PTEN loss as an initiating event *versus* its role as an agent of progression might arise from fundamental differences between tissues with respect to the order of addition of various oncogenic events. For example, the human adult male prostate epithelial cell might not tolerate loss of PTEN unless the loss was first preceded by a permissive mutational event. On the other hand, this paradox, at least with respect to the prostate, might simply reflect upon our current ability, or lack thereof, to detect certain PTEN mutational events. Indeed, in our data set, the vast majority (85%) of tumors had a portion of the tumor in which PTEN staining was absent, in keeping with the marked heterogeneity of *PTEN* alterations that has been reported previously in metastatic prostate cancer samples (43). If it is the PTEN-null portion of the tumor that is destined to become the predominant metastatic clone, then the apparent lack of PTEN mutations in such organ-confined tumors might simply result from a lack of detection by conventional methodologies.

PTEN alterations have also been described in prostate cancer cell lines, xenografts, and tumors (1, 2, 34, 39–44). The true number of inactivating events is likely to be greater because the presence of sequence mutations in promoter/regulatory regions was not excluded by these studies. Of interest, there has been no evidence of *PTEN* promoter methylation in prostate cancers or bladder and renal cancers with 10q LOH using a DNA-based assay (32, 40). However, in certain prostate cancer cells, *PTEN* mRNA was restored after treatment with the demethylating agent 5-azadeoxycytidine (34). It is possible that methylation of a transcription factor for *PTEN* may play a role in the regulation of the gene.

Although we did not assess the genetic status of *PTEN* in our cases, loss of expression as assessed by immunohistochemistry might reflect a majority of the possible mechanisms resulting in PTEN inactivation. These would include direct inactivation by homozygous deletion, nonsense mutation, certain internal deletions, and promoter methylation or indirect inactivation such as loss of a PTEN-directed transcription factor or posttranscriptional modification, such as that which occurs with cdc25, another dual specificity phosphatase (57). Mis-



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sense mutations, which do not grossly destabilize the protein product, would not be accounted for by immunohistochemistry.

PTEN appears to function, at least in part, by acting to brake cell cycle progression (11–13). We and others (11, 12) have previously demonstrated that this function appears to require PTEN lipid phosphatase activity, suggesting that cell cycle regulation may result from inhibition of the PI3K pathway. We further demonstrated that activated forms but not wild-type forms of the proto-oncogene Akt were capable of overriding a PTEN-mediated cell cycle block, raising the possibility that Akt might be an important downstream target of PTEN with respect to cell cycle progression (12). Similar conclusions have been reached with respect to the function of PTEN as a regulator of apoptosis or cell survival (14–17, 58). These data, taken together, suggest the possibility that targeted inhibition of the PI3K/Akt pathway might be of therapeutic value in patients with prostate cancer. We and others (12, 59) have also shown that cell lines and tumors in which PTEN is lost have elevated levels of activated Akt. Thus, loss of immunohistochemical detection of PTEN might predict for the presence of activated Akt and, in turn, might become useful as a factor predictive of success for therapies directed against this pathway. In general, this type of predictive factor, such as the estrogen receptor, which can predict for the efficacy of a given therapy, such as tamoxifen, has great clinical utility because it directly impacts treatment decisions.

Our results support the candidacy of PTEN as a tumor suppressor gene in prostate cancer progression. Indeed, loss of PTEN expression may be an important negative prognostic indicator. We are currently working on the development of well-characterized rabbit polyclonal or murine monoclonal antibodies that would provide unlimited amounts of antibody capable of reacting with formalin-fixed tissue. It is possible that immunohistochemistry may be the optimal method for evaluating the functional status of *PTEN* because it would detect a loss of PTEN induced by a majority of the mechanisms through which gene products are inactivated.

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# Loss of PTEN Expression in Paraffin-embedded Primary Prostate Cancer Correlates with High Gleason Score and Advanced Stage

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*Cancer Res* 1999;59:4291-4296.

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