



## Evidence For A Prostate Cancer Associated Diagnostic Marker

### 1, PCADM-1: Immunohistochemistry And *In situ* Hybridization Studies

#### Authors

\*Akira Ohkia  
Youji Hu  
Min Wang  
Fernando U. Garcia  
\*\*Mark E. Stearns

#### Affiliations

**\*\*Drexel University**  
**College of Medicine**  
Department of Pathology and  
Laboratory Medicine  
Philadelphia, PA 19102

**\*Kurume University**  
**School of Medicine**  
Department of Surgery  
Asahi-machi, Kurume-shi, 830  
Japan

#### Running Title

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#### Key Words

- prostate cancer
- PCADM-1
- in situ hybridization
- immunohistochemistry

## Abbreviations

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EMSA:	electrophoretic mobility shift assay
HGPIN:	high grade prostatic intraepithelial neoplasm
BPH:	benign prostate hyperplasia
PCA:	prostate carcinoma
SV:	seminal vesicle
SM:	smooth muscle
GS:	Gleason sum
PSA:	prostate specific antigen
PCADM-1:	prostate cancer diagnostic marker-1

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## Abstract

We have identified a novel gene/protein associated with prostate cancer, termed prostate cancer diagnostic marker 1, or PCADM-1 (1). Immunological studies revealed that rabbit polyclonal antibodies generated against recombinant PCADM-1 specifically recognize the protein in crude protein extracts from a variety of prostate cancer cell lines (i.e. PC-3 ML, LNCaP, DU145, CPTX-1532 cells) and prostate cancer tissue. Combined immunolabeling and *in situ* hybridization studies demonstrated that PCADM-1 mRNA was expressed by the luminal epithelial cells of prostate cancer glands and was not expressed by HGPIN or HPV-MLC7 cells. Immunolabeling studies of 'tissue arrays' from biopsies of archival material (n=200 samples) confirmed that PCADM-1 was expressed by the luminal epithelial cells of prostate cancer. Finally, PCADM-1 was not expressed by other cancers or associated tissues. Taken together, the data suggest that PCADM-1 is a specific marker for human prostate cancer.

## Introduction

Currently there are greater than 400 tentative marker genes identified for prostate cancer (2). PSA is the only serum marker developed which is diagnostic for the disease, but the sensitivity and specificity ranges from about 43% to 65%, respectively, depending on the nature of the study (2). The widespread belief is that other prostate cancer specific genes might be identified which provides a more accurate diagnostic marker for the cancer. Several tentative examples of candidate markers are reviewed here. PSAM, a membrane bound PSA antigen, has been identified as specific for prostate cancer and may eventually improve the PSA assay (2). Su et al (3) reported that the product of a gene known as prostate carcinoma tumor antigen-1 (PCTA-1) was expressed in malignant cells, but not by normal or benign prostatic hyperplastic tissue. ELISAs of patient serum indicated the antigen was diagnostic for prostate cancer. Similarly, another gene, HPCA1, was reported to have a high linkage with prostate cancer when compared with a reference population from the National Cancer Database (4). We and others believe that further attempts to identify prostate cancer specific marker genes of prognostic or diagnostic utility are needed.

In a preceding paper (1), we cloned and partially characterized a gene encoding PCADM-1. Sequencing indicated PCADM-1 was ~99% homologous with human S2 ribosomal protein and chromosomal protein LLRep3. PCADM-1 exhibited 6 specific point mutations and the deduced amino acid sequence indicated five specific amino acid base substitutions distinguished mutant PCADM-1 from the human S2 ribosomal protein. In this paper, immunohistochemistry and *in situ* hybridization studies revealed that PCADM-1 was expressed by various prostate cancer cell lines and was specifically expressed by human prostate cancer luminal epithelial cells with a 100% sensitivity.

## Materials and Methods

**Cell Lines.** Malignant CPTX-1532 and normal NPTX 1532 human prostate cell lines were derived from the same human prostate tissue (5) (generously provided by Drs. Robert Bright and Susan Topalian, NIH- NCI, Bethesda, MD). These cells were immortalized with E6 and E7 transforming proteins of human papilloma virus serotype 16 and were maintained in Medium 154 (Cascade Biologics, Inc., Portland, OR) containing 1X Human Keratinocyte Growth Supplement 100X (Cascade Biologics, Inc., Portland, OR), 1X Antibiotic- Antimycotic 100X (GIBCO BRL, Grand Island, NY) and 5% fetal bovine serum (Biofluids, Rockville, MD). Human prostate cancer PC-3, LNCaP and DU145 cell lines were obtained from American Type Culture Collection, ATCC (Bethesda, MD). The bone metastasizing PC-3 ML subclones were selected from the parent PC-3 cell line by the Stearns laboratory (6). All these cell lines were cultured according to the directions of ATCC.

HPV-MLC7 cells (kindly provided by Dr. Donna Peehl, Dept. of Urology, Stanford Univ.) are benign prostatic hyperplasia (BPH) culture immortalized by human papilloma virus serotype 18 (7). A HGPIN cell line was established in our laboratory from human prostate tissue showing histological evidence of high grade prostatic intraepithelial neoplasia. They were immortalized by HPV-18 transfection. Both cell lines were maintained in Keratinocyte SFM (Gibco BRL, Grand Island, NY) containing 5 *ng/ml* epidermal growth factor (EGF), 50 *mg/ml* bovine pituitary extract, 5% fetal bovine serum (Biofluids, Rockville, MD), 100 units/ml of penicillin G sodium and 100 *ug/ml* streptomycin sulfate.

All cultures were routinely passaged by Trypsin-EDTA (Gibco BRL) detachment and cultured at 37°C in a humidified atmosphere of 95% air and 5% CO<sub>2</sub>.

**Preparation of Prostate Tissue.** Fresh prostates were obtained immediately following radical prostatectomy and pieces removed from sagittal dissections. Pieces approximately 1.5 *cm*<sup>2</sup> were dissected out and prepared for frozen sections. The sections were stained with Hematoxylin and Eosin to identify regions containing primarily stoma tissue, seminal vesicle (SV), or normal prostate, benign prostatic hyperplasia (BPH), high-grade prostatic intraepithelial neoplasia (HGPIN), and prostate cancer adenocarcinoma (PCA).

Tissue array blocks were prepared with biopsy cores from archival specimens. Each block contained cores from seminal vesicle, stroma, HGPIN, BPH, PCA (GS 4-8) plus localized neuronal metastases (n=~200 cores/block).

**Immunohistochemistry.** The protocol for immunolabeling frozen sections was a modification of the technique of Aoyaki *et al.* (8) and Tokunaga *et al.* (9). In brief, tissue was frozen with liquid nitrogen and embedded in Tissue-Tek (Sakura Finetek U.S.A., Torrance, CA) then was cut with a cryostat at 6  $\mu\text{m}$  and was placed on Superfrost/ Plus Microscope Slides (Fisher Scientific, Pittsburgh, PA). Frozen tissue sections (~10  $\mu\text{m}$  thick) were incubated for 20 min with 0.9% hydrogen peroxide in 100% methanol to inactivate any endogenous peroxidase. Sections were rinsed twice in 0.1M PBS, incubated with 1.5% normal goat serum for 60 min followed by rabbit polyclonal PCADM-1 antibody (1:20 dilution with 0.1 M PBS containing 1.5% normal goat serum) in a humidified chamber for 72 h at 4°C. Specimens were washed 3 times in 0.1M PBS and incubated in biotinylated goat anti-rabbit IgG (diluted 1:200 in PBS containing 1.5% normal goat serum) for 30 min at room temperature. After washing one time with PBS, the sections were developed using the Avidin-Biotin complex (ABC) Staining System (Santa Cruz Biotechnology, San Diego, CA). The sections were counterstained with hematoxylin-eosin. Control sections were immunolabeled with beta actin antibodies (Sigma Chemical, St. Louis, MO).

Tissue array sections were processed and immunolabeled according to methods previously described by our laboratory (10). The sections were routinely steamed for 30 min in a 10 mM citrate buffer (pH 8.0) to improve antigen retrieval and then labeled with primary antibodies (1:20 dilution for 1 hr) followed by biotinylated goat anti-rabbit IgG antibodies according to the protocol described for frozen sections.

**In situ Hybridization Studies.** The *in situ* hybridization protocol was a modification of the non-radioactive Digoxigenin technique of Wood *et al.* (11) and Miyajima *et al.* (12). In brief, a synthetic oligonucleotide probe with a complementary sequence to a coded region of human PCADM-1 mRNA (5'- CATCGGCAAGGCCCA-CACTGTCCG- 3') was synthesized and HPLC purified by

BioSource (BioSource International, Foster city, CA). Freshly cut frozen sections (~10 *um* thick) were fixed overnight in 4% paraformaldehyde in 0.1 M PBS, immersed in 20% sucrose at 4°C in PBS and placed on Superfrost/ Plus Microscope Slides (Fisher Scientific, Pittsburgh, PA). The sections were washed three times in 0.1 M PBS and incubated with 0.2 M hydrochloric acid for 20 min at room temperature to inactivate endogenous alkaline phosphatase, treated with 10 *µg/ml* Proteinase K (Roche Molecular Biochemicals, Indianapolis, IN) in 0.1M PBS for 15 min at room temperature, washed in PBS and then fixed in 4% paraformaldehyde in 0.1 M PBS. Fixed sections were treated with 2-*mg/ml* glycine (Sigma, St. Louis, MO) in PBS to neutralize aldehydes. The sections were equilibrated in prehybridization buffer (50% deionized formamide in 2X SSC). Hybridization was performed with the probes diluted in hybridization buffer (50% deionized formamide, 10 *mmol* Tris-HCl, pH 7.6, 200 *µg/ml* yeast transfer RNA (Sigma), 1 *mmol* EDTA, 600 *mmol* sodium chloride, 1X Denhardt's solution (Sigma), 10% Dextran sulfate (Sigma), 0.25% sodium dodecyl sulfate, and 20 *µg/ml* salmon sperm DNA). A Digoxigenin labeled PCADM-1 probe (3'-tailed with Digoxigenin deoxyuridine triphosphate (dUTP) (Roche Molecular Biochemicals, Princeton, N.J.)(10 *ng* probe/100 *µl*) was incubated with the tissue sections in hybridization buffer containing 50% formamide. Sections were incubated overnight in a humidified chamber at 40°C, and washed twice with 5 X SSC at 40°C. Then sections were washed twice for 15 min at 40°C in 50% formamide in 2 X SSC. Sections were placed in DIG 1 buffer (100 *mmol* Tris-HCl, pH 7.5) then DIG 2 buffer (1.5% blocking reagent in DIG 1) for 5 and 60 min, respectively at room temperature. Sections were then incubated in anti-Digoxigenin-antibody AP (Roche Molecular Biochemicals) in DIG 1 buffer for 60 min at 37°C. Sections were washed in DIG 1 buffer for 15 min and rinsed in DIG 3 buffer [100 *mmol/L* Tris-HCl (pH 9.5), 100 *mmol/L* sodium chloride, 50 *mmol/L* magnesium chloride] for 5 min at room temperature. Immunodetection of the Digoxigenin oligonucleotide was carried out using an antibody kit (Roche Molecular Biochemicals). The reaction was stopped in DIG 4 buffer [10 *mmol/L* Tris-HCl (pH 8.0), 1 *mmol/L* EDTA], and the sections were counterstained with methyl green.

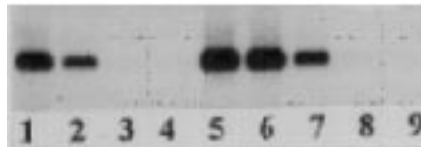
**Computer Assisted Analysis System (CASA.)** Computer Assisted Analysis System (CASA) was employed to measure anti-gen expression and compare the relative intensity of PCADM-1 expression in specific glandular regions of whole mount sections and tissue array cores according to methods of Fudge et al. (13) in our laboratory. The intensity of *in situ* hybridization and immuno-histochemical labeling was assigned values ranging from 0, +1, +2 and +3, with the overall intensity of labeling being (0) zero; (+1) 10%-25%; (+2) 26%-49% and (+3) 50% to 100%.

**Statistical Analysis.** The CASA data for each Gleason Sum were compared and evaluated relative to BPH by either the Student T test or Fisher's exact test with a p values level of significance set at  $p < 0.05$ .

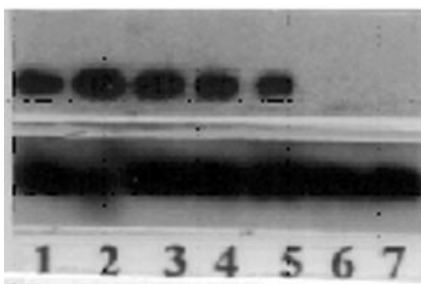
**Results**

**Western blots:** Western blots of crude whole cell extracts showed that the PCADM-1 antigen was expressed by a variety of human prostate tumor lines (PC-3ML, LNCaP, DU145, and CPTX-1532 cells), but not by BPH or HGPIN cells (figs. 1a-b.). In (fig. 1a, lanes 3 and 4) the antibodies were pre-absorbed with excess recombinant PCADM-1 and PC-3 ML protein, respectively, showing that the antibody specifically recognizes the 33 Kda PCADM-1 protein. Figure 1b shows that PCADM-1 is expressed in prostate tumors of Gleason score (GS) 4, 5 and 8. Figure 1c further shows that extracts from different regions of a GS 7 tumor consistently expressed elevated levels of PCADM-1 (fig. 1c, lanes 1-5), whereas the matching HGPIN and BPH tissue of the same prostate failed to express any detectable PCADM-1 antigen. Table 1 summarizes the observations from Western blotting of crude extracts from a variety of prostate tumor cell lines and non-malignant prostate cell lines, plus malignant and non-malignant prostate tissues. Basically, PCADM-1 was expressed by malignant cells and tumors, but not by benign, normal, HGPIN or fibroblast cells or by benign prostate tissue, seminal vesicle and stroma.

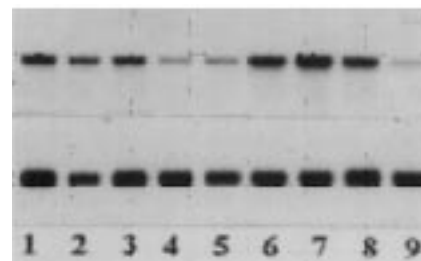
*Western blots with PCADM-1 antibodies (1:20 dilution at 0.01 ug/ml). Lanes were loaded with 10 ug protein/lane.*



**1A**



**1B**



**1C**

**Fig. 1a.** Blots with PCADM-1LG09 antibodies of crude protein extracts from (lane 1) recombinant clones expressing PCADM-1 protein; (lanes 2-4) PC-3 ML; (lane 5) DU145; (lane 6) LNCaP; (lane 7) CPTX-1532; (lane 8) BPH; and (lane 9) HGPIN cells. In (lanes 3 and 4) the antibodies were pre-absorbed with excess rPCADM-1 and PC-3 ML protein extracts, respectively.

**Fig. 1b.** Blots of extracts from: (lanes 1-3) GS 6; (lanes 4-5, 9) GS 4; and (lanes 6-8) GS 8 tumor tissues. Lower panel in figs. 1b, 1c shows beta-actin antibody blots.

**Fig. 1c.** Blots of extracts from (lanes 1-5) different regions of a GS 7 tumor; and matching (lane 6) HGPIN and (lane 7) BPH tissue of the same prostate.

**Table 1**

*Summary of immunolabeling results with cell lines & prostate tissue.*

<b>PCADM-1 (+)</b>	<b>PCADM-1 (-)</b>
PC-3 ML	BPH
LNCaP	HGPIN-1
DU145	HGPIN-2
CPTX-1532	NPTX-1532
HPCA-10a	WI38-fibroblasts
HPCA-10c	PCA-fibroblasts
<b>PCADM-1 (+)</b>	<b>PCADM-1 (-)</b>
PCA: GS 4-10	Stroma
HGPIN	
Seminal vesicle	

*Cells and tissues were either (+)positive or (-) negative for PCADM-1.*

**Table 2.**

*Summary of immunolabeling and in situ hybridization labeling studies with human prostate tissue specimens (n=149 total).*

<i>N = number of patient prostate specimens.</i>	<b>No. Prostates Assayed</b>	<b>Pathology</b>	<b>Gleason Sum</b>	<b>Immunolabeling</b>	<b>In Situ</b>
	n=5	Normal	0	0	0
	n=15	SV	0	0	0
	n=2	SV	0	+1	0
	n=20	BPH	0	0	0
	n=7	BPH	0	+1	0
	n=11	HGPIN		+1	+1
	n=1	HGPIN		+3	+3
	n=1	PCA	6	+1	+2
	n=2	PCA	6	+2	+2
	n=23	PCA	6	+3	+2
	n=1	PCA	7	+1	+2
	n=2	PCA	7	+2	+3
	n=18	PCA	7	+3	+2
	n=1	PCA	8	+1	+3
	n=23	PCA	8	+2	+2
	n=7	PCA	9	+3	+3
	n=10	PCA	10	+3	+3

*Note that the immunolabeling and in situ labeling was carried out on adjacent sections from each specimen.*

**Immunolabeling Studies** In Table 2, the data showed that the relative intensity of immunolabeling ranged from 0 to +3. The data showed that immunostaining was non-existent in normal prostate tissue specimens (n=5) and was very low or non-existent (i.e. ranging from 0 to +1) in BPH (n=27), SV (n=17) and HGPIN (n=12). Note that one HGPIN specimen showed positive labeling for PCADM-1 in a few glandular regions where the luminal epithelial cells exhibited abnormal nuclei and appeared malignant and invasive. In contrast to the BPH samples, the intensity of immunolabeling was higher in PCA specimens (n=88) (i.e. ranging from +1 to +3,  $p < 0.05$ ) with an increased intensity of labeling as a function of the Gleason Sum (GS). With tumor samples of GS 6 (n=26) the vast majority (n=23,  $p < 0.05$ ) were intensely positive (+3). Likewise, in GS 7 (n=21,  $p < 0.05$ ), GS 8 (n=24,  $p < 0.05$ ) and GS 9-10 (n=17,  $p < 0.05$ ) all the samples were intensely positive (+3) with the exception of a very few which were negative for PCADM-1. Note that all the cells in all the glands were immunolabeled in the prostates examined. Control studies showed that all the specimens were uniformly immunolabeled with beta actin antibodies.

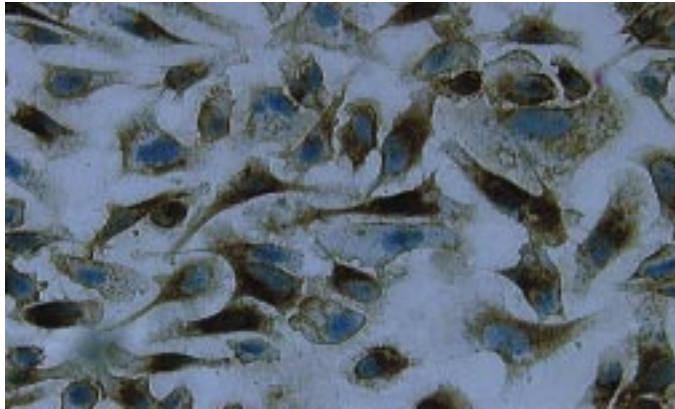
The *in situ* hybridization results closely correlated with the immunolabeling data and revealed that the PCADM-1 mRNA was not expressed in normal prostate, seminal vesicle or BPH tissue, but was weakly expressed in some HGPIN glands. In contrast to the BPH samples, the PCADM-1 mRNA was intensely expressed in the luminal epithelial cells of the PCA specimens, ranging from +2 to +3 ( $p < 0.05$ ). The intensity of labeling tended to increase with the GS and specimens of a GS 9-10 strongly expressed the PCADM-1 mRNA (i.e. +3) ( $p < 0.05$ ).

Figures 2-4 provide examples of the immunolabeling and *in situ* hybridization results observed for several human prostate cell lines and human prostate tissue. Figure 2A shows that the cytoplasm of PC-3 ML tumor cells was intensely labeled with the PCADM-1 antibody. Figures 2B-2C show that BPH and normal human prostate cells (NPTX-1532) were not labeled with the PCADM-1 antibody. Control studies with pre-immune serum or antibody pre-absorbed with excess recombinant antigen failed to label the PC-ML cells (data not shown). Figures 3A, 3B and 3C show that the PCADM-1 antibody failed to immunolabel the glands or stroma tissue in

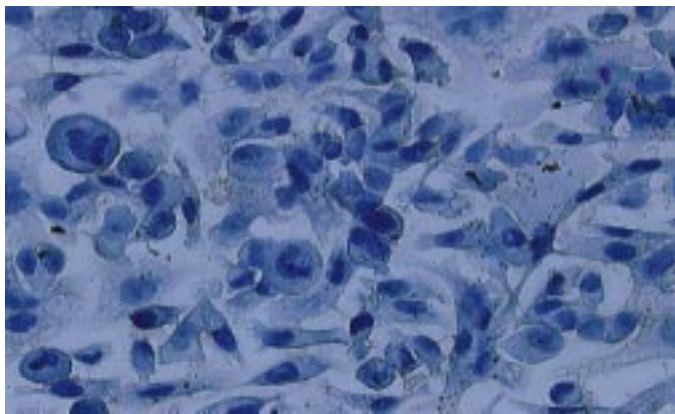
regions containing SV, BPH, and HGPIN, respectively. Note that occasional foci of positive labeling were sometimes observed in the HGPIN glands (fig. 3C). Figure 3D shows that all the luminal epithelial cells in glands of a high-grade cancer specimen were intensely labeled with PCADM-1 antibodies, but the antibody did not label the stroma compartment. Note that the pre-immune serum did not label the tissue. Comparative studies showed that the immunolabeling consistently coincided with the *in situ* hybridization results on adjacent sections (fig. 4). Figure 4 shows that SV and BPH tissues were not labeled with either PCADM-1 antibodies or by *in situ* hybridization with  $^{32}\text{P}$ -labeled anti-sense oligonucleotides (40 mer) specific for PCADM-1 mRNA. The HGPIN cells were immunolabeled in one region of the gland (arrows), but the *in situ* labeling was difficult to detect. In contrast, both the PCADM-1 antibodies and the *in situ* hybridization intensely labeled the luminal epithelial cells of the PCA GS 8 glands. Note that the stroma and blood vessel walls were uniformly negative in all the labeling studies. Controls with the  $^{32}\text{P}$ -labeled sense oligonucleotides (40 mer) failed to label the PCA glands.

The above results were strongly supported by immunolabeling studies of 'tissue array' sections containing 200 biopsy cores from a variety of prostate cancer tumors (n=108), stroma (n=20), BPH (n=50), SV (n=10) and neural metastases (n=12). Evaluation of these specimens clearly showed that PCADM-1 was expressed solely by the glandular epithelial of cancer specimens at an intensity of +1 to +3 in GS 2, GS 3 and GS 4 glands and PCADM-1 was not expressed by BPH, HGPIN, stroma, SV or by the other tissues or cells present in prostate tissue.

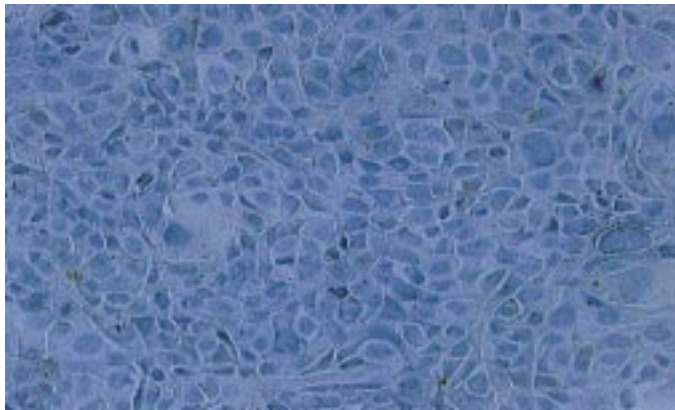
Finally, immunolabeling studies of a variety of other cancers and associated normal tissues (i.e. colorectal, gastric, breast, ovarian, lung, bladder, liver, and kidney) indicated PCADM-1 was uniquely expressed by prostate cancer cells and not by other cancers.



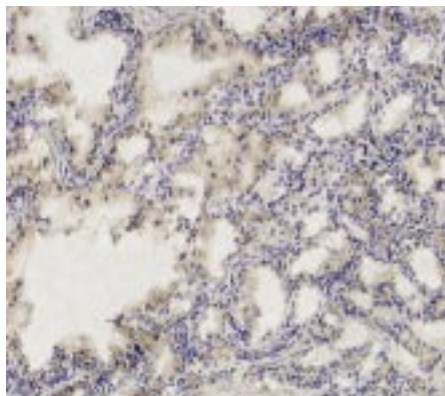
**Figure 2A.** Immunolabeling with PCADM-1 protein antibodies of PC-3 ML.



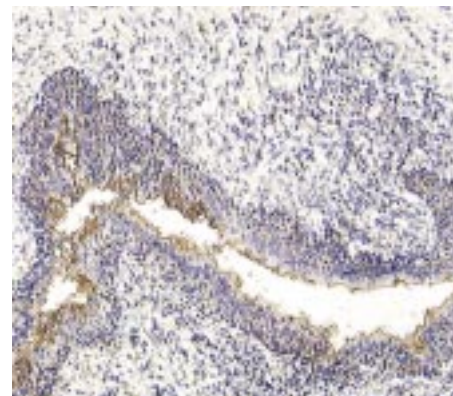
**Figure 2B.** Immunolabeling with PCADM-1 protein antibodies of HPV-MLC7 cells.



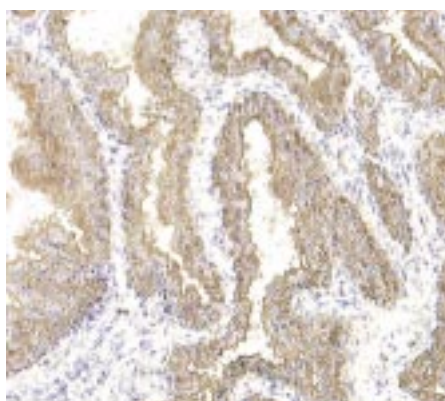
**Figure 2C.** Immunolabeling with PCADM-1 protein antibodies of NPTX-1532 normal prostate cells plus.



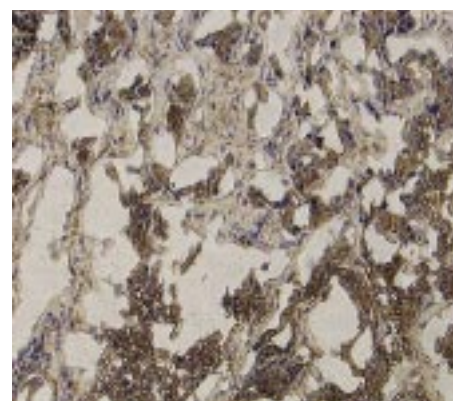
**Figure 3A.** Immunolabeling with PCADM-1 protein antibodies of SV.



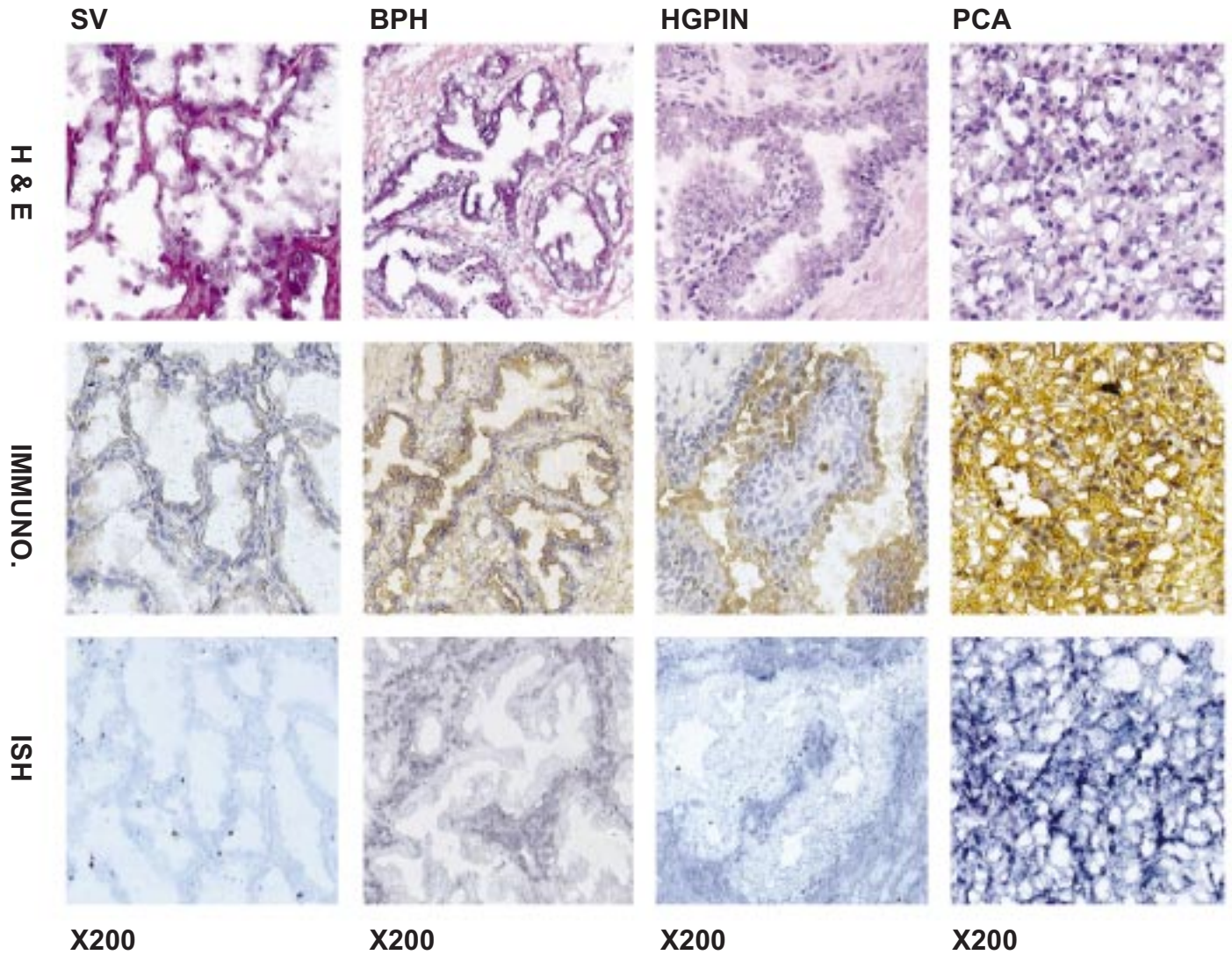
**Figure 3B.** Immunolabeling with PCADM-1 protein antibodies of BPH.



**Figure 3C.** Immunolabeling with PCADM-1 protein antibodies of HGPIN.



**Figure 3D.** Immunolabeling with PCADM-1 protein antibodies of GS 9 prostate glands.



**Figure 4.** Immunolabeling, in situ (ISH) and H@E labeling of adjacent sections from SV, BPH, HGPIN, and PCA glands. X axis shows the final magnification of the picture at 100X or 200X.

## Discussion

In the preceding paper, we identified a double stranded CACG-GATG sequence that binds a 33 Kda, 'S2-like' ribosomal protein present in nuclear extracts of prostate tumor cell lines and advanced human prostate cancer (1). The gene encoding the prostate cancer-specific binding protein was cloned. Sequencing and the deduced amino acid sequence revealed that the nucleic acid sequence was ~99% homologous with the human S2 ribosomal mRNA sequence and that the gene exhibited 6 specific base pair substitutions in the open reading frame. The amino acid sequence was 99% homologous with ribosomal chromosome protein S2 and LLRep3 and exhibited 5 amino acid substitutions in the n-terminal domain. Interestingly, 4 amino acid substitutions were observed in a putative DNA-binding heptamer region and the leucine zipper-like domain (e.g. **NNIGKAHTVRCKVTGR-CGSVLVRLIPAPRGTGIVSAPVPPKLLMMAGIDDCYTS**), suggesting these amino acid substitutions may account for a gain of function and the DNA binding properties of the mutated S2 protein.

In this paper, we have employed immunohistochemistry and *in situ* hybridization assays to show that PCADM-1 was specifically expressed by malignant PC-3 ML prostate cancer cell lines and by the luminal epithelial cells of human prostate cancer tissue. In comparison, PCADM-1 was not expressed in primary epithelial cell lines derived from stroma tissue, normal prostate, HGPIN or BPH glands. Combined immunolabeling and *in situ* hybridization studies of adjacent sections further demonstrated that PCADM-1 mRNA was expressed by the luminal epithelial cells of prostate cancer glands (n=28) and was not expressed by HGPIN or BPH tissue or cells. Taken together, the data suggest that PCADM-1 is a specific marker for human prostate cancer.

New biological markers are being explored which might improve detection of cancer as well as the ability to detect patients at high risk of recurrence for different cancers. Among antigens of potential benefit are the estrogen receptor (14), progesterone receptor (15), c-erbB-2 (16), CEA (17), p53 (18), MIB-1 antigen (19), bcl-2 (20) and Metalloproteinase (MPS-1) (21-23). Unfortunately, in most instances the biological properties of these markers and related importance as predictors of the disease state or behavior remains unclear (14-23). For example, recent work has identified

MPS, a multifunctional S27 ribosomal protein, as a marker for breast cancer as well as numerous other human cancers including prostate cancer (21-23). MPS is expressed at low levels in normal cells (21-23). Likewise, the S2-ribosomal or chromosomal protein was found to be elevated in head and neck cancer and barely detectable in normal tissue (24). We therefore suggest that both the 'S2-like' and MPS-1 or S27 ribosomal proteins are multifunctional nuclear 'zinc' finger proteins may be diagnostic for cancer.

In this respect, there are many reports showing a connection between over-expression of genes encoding ribosomal proteins and cancer (21-29). The implication is that these ribosomal proteins have additional functions distinct from their role as ribosomal proteins regulating protein synthesis (21-29). Interestingly, specific 'leucine zipper' sequence motifs are characteristic of numerous ribosomal proteins which allow binding to nucleic acids (23-29) and a possible role in regulating transcriptional and translational mechanisms. For example, the rat ribosomal protein S3a is identical to the product of the rat v-fos transformation effector gene (25). S3a is involved in initiation of protein synthesis and is also related to proteins involved in the regulation of growth and the cell cycle (25). Likewise, the rat ribosomal protein L10 is homologous to a DNA-binding protein and to a putative Wilm's tumor suppressor gene (26). The 'S2-like' ribosomal protein characterized here, binds a specific 8-mer sequence that is highly homologous to breakpoint cluster region consensus domains (1). We therefore speculate that the 5-point mutations identified in the heptamer domain or leucine zipper region of the 'S2-like' might influence the nucleic acid binding affinities of the protein and change the functional role of the protein as well. For example, 'S2-like' binding to breakpoint cluster regions might be involved in chromosomal translocations.

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**Notes**