Short Communication

The Expression of a Variant Prostate-specific Antigen in Human Prostate

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Abstract

Although a splicing variant of prostate specific antigen (PSA-v) mRNA has been described previously, whether its protein (PSA-v or PSA-related protein 1, i.e., PSA-RP1) is actually expressed in human prostate cells remains elusive. We report that PSA-v protein is expressed in prostatic epithelia of both cancerous and benign tissues. Also, secreted PSA-v can be detected in the medium of a prostate cancer (PCa) cell line. Consistently, PSA-v mRNA is exclusively expressed in benign luminal epithelia and cancer cells of the prostate by in situ hybridization. Northern analysis of a cohort of 51 pairs of RNA samples from microdissected tissues showed that PSA-v mRNA levels remained constant in both benign and cancerous tissues, whereas PSA levels declined in cancerous areas. Our result suggests that it would be feasible to develop proper immunohasays for PSA-v to test whether PSA-v could have some clinical utility.

Introduction

The PSA\(^1\) gene is located on human chromosome 19q13.3–13.4. It belongs to the human kallikrein gene family, which is composed of up to 15 members (1). The PSA gene contains five exons and four introns that code for a 261-amino acid prepro form of PSA in luminal epithelia of the prostate (2). According to the international nomenclature system, KLK3 is designated as the PSA gene, and hK3 is designated as the PSA protein (3). Mature PSA is a chymotrypsin-like serine protease glycoprotein containing 237 amino acids with 7% carbohydrate content (4, 5). Because serum PSA levels are often elevated (4 ng/ml) in patients with PCa, serum PSA tests have been used to screen for PCa among asymptomatic populations in the United States (5). Currently, PSA is the most sensitive biomarker for PCa (4–6). Previously, a PSA variant (PSA-v, also called PSA-RP1) cDNA has been cloned from a PCa cDNA library and characterized as representing a PSA-v mRNA that resulted from alternative splicing of the fourth intron of the PSA gene (7, 8). However, information regarding the expression of this PSA-v in human prostate is limited. In addition, it is not known whether the protein is expressed in human prostate cells, although recombinant PSA-v proteins can be expressed in heterologous eukaryotic and prokaryotic cell systems (7). In this investigation, we analyzed the expression of this PSA-v mRNA in a cohort of human prostate tissues with PCa, and for the first time, we detected PSA-v protein in human prostate cells by using a PSA-v-specific antibody.

Materials and Methods

Tissues. Fifty-one prostate tissue specimens containing PCa were retrieved from surgical resections at the Mayo Clinic with the Mayo Institutional Review Board-approved protocol. Patients who had received hormone therapy before prostatectomy were excluded from the study. Tissues were prepared either by freezing in liquid nitrogen or fixed in formalin fixative, followed by embedding in paraffin. Frozen sections (10-μm thick) were applied to microdissection under an anatomical microscope for total RNA extraction. Identification of cancer areas and adjacent benign areas was done on parallel sections stained with H&E by well-trained personnel. Paraffin sections were used for in situ hybridization (10-μm thick) and immunohistochemical (5-μm thick) analyses.

The average age of the patients included in this study was 64 years (range, 46–73 years). Preoperative serum PSA levels (determined by Tandem-R assay; Hybritech, San Diego, CA) ranged from 2.4 to 37.1 ng/ml with an average of 11.2 ± 7.9 ng/ml. The majority of the cases had a Gleason score of 6 or 7 (n = 47).

Probe Preparation. The PSA-specific oligonucleotide probe was synthesized at the Mayo Molecular Biology Core Facility and radiolabeled as described previously (9). It contained 77 bases (nucleotides 1159–1235) corresponding to the 3’-untranslated region in the fifth exon of the PSA gene. A PSA-v cDNA fragment (200 bp; nucleotides 4970–5169) according to Riegman et al. (8), residing in the alternative-splicing region in the fourth intron, was excised from a PSA-v cDNA/pGEM-7Zf+ construct by restriction enzymes SphI and KpnI and used as a probe specific for PSA-v. The sequence of these two probes only exists in each respective mRNA. Probes were labeled with \(^{32}\)PdCTP by random priming and used for Northern blot analysis. For in situ hybridization analysis, sense and antisense RNA probes corresponding to the same 200-bp cDNA fragment of PSA-v were prepared from the above cDNA construct linearized by SphI or KpnI and transcribed by Sp6 or T7 RNA polymerases, respectively. The probes were prepared following the manufacturer’s instructions (Promega) and labeled with digoxigenin (10).
Northern Blot Analysis. Total RNA was extracted by the guanidinium isothiocyanate method (11) from microdissected tissues described above. Equal amounts of RNA (20 μg/lane) were fractionated in the presence of ethidium bromide by denaturing gel electrophoresis and transferred to a Zeta-Probe membrane (Bio-Rad). The Zeta-Probe membrane were hybridized with the 32P-labeled PSA or PSA-v probe and washed as described previously (9). The films were autoradiographed at −70°C. The intensities of signals on the autoradiogram were estimated by densitometric scanning. The membranes were stripped of the radioactive probe by washing for 10 min in 0.5% SDS at 95°C. The blots were then reprobed with a 32P-labeled probe specific for glyceraldehyde-3-phosphate dehydrogenase, a housekeeping gene, for normalization of PSA and PSA-v mRNAs.

mRNA in Situ Hybridization. In situ hybridization was performed following a method described previously (12). In brief, tissue sections were microwaved in a 10 mM citric acid solution for 20 min before prehybridization to enhance probe hybridization. Tissue sections were hybridized with a digoxigenin-labeled antisense RNA probe specific for PSA-v (200 bp). A sense probe derived from the same region was used for hybridization to an adjacent section to confirm specificity of the observed signals. Slides were hybridized at 50°C for 16 h in a humid chamber and treated with RNase H to remove unhybridized, single-stranded probes. Hybridized probes were detected with a detection kit (DIG Nucleic Acid Detection kit; Boehringer-Mannheim). After the reaction, the slides were rinsed with 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA and counterstained with hematoxylin. Staining for PSA-v mRNA was regarded as positive if a distinct cytoplasmic staining was present, regardless of intensity.

Production of Polyclonal Peptide Antibody Specific for PSA-v. The PSA-v oligopeptide corresponding to amino acids 187–214 of the putative PSA-v (8) was synthesized and purified by high-performance liquid chromatography at the Protein Core Facility at Mayo Clinic. The peptide was conjugated with keyhole limpet hemocyanin and BSA for immunogens and assay reagents, respectively. Rabbits were immunized with KLH-PSA-v peptide for polyclonal antibody production. The antibody was first purified by protein G affinity column, followed by PSA-v 187–214 peptide-affinity column.

Western Blot Analysis. LNCaP cells, derived from a human prostate adenocarcinoma metastasis to a lymph node (13), were prepared following a method described previously (14). After 48 h, the cells were treated with 10 nM mibolerone. Supernatants were collected after 72 h of mibolerone treatment and were concentrated using a Vivaspin 20 (10,000 MWCO; Viva-science, UK) following the manufacturer’s instructions. Protein samples were subjected to electrophoresis on a NuPAGE 4–12% Bis-Tris Gel, transferred onto nitrocellulose membrane, followed by Western blot analysis as described previously (14). A Ponceau S stain was performed for total protein staining and followed by Western blot analysis as described previously (14).

Immunohistochemistry. Five-μm paraffin tissue sections were mounted on Superfrost/Plus glass slides (Fisher Scientific, Pittsburgh, PA). Immunohistochemical staining for PSA-v (1: 2,000 dilution) or PSA (polyclonal antibody; DAKO; 1:2,000 dilution) antibody was performed by the peroxidase-labeled streptavidin-biotin technique using a Histostain-SP kit according to the manufacturer’s instructions (Zymed, CA). A negative control slide was included in each experiment where the primary antibody was replaced by TBST.

Results

Analysis of PSA-v mRNA Expression. Northern blot analysis showed that PSA-v mRNA was detected as a specific band of around 1.9 kb, whereas PSA mRNA was detected as a band of 1.6 kb (Fig. 1A). The average expression level for the PSA-v mRNA from 51 patients was similar between cancer and benign areas (1.83 ± 1.67 versus 1.69 ± 1.60; P > 0.05, t test for paired samples; Fig. 1B). In contrast, the average expression for PSA mRNA was significantly lower in cancer areas than in benign areas (4.15 ± 4.55 versus 6.03 ± 9.02; P < 0.05, t test for paired samples; Fig. 1B). PSA-v mRNA levels in average were lower than those of PSA mRNA levels.

Our in situ hybridization results showed that the PSA-v mRNA was localized in the cytoplasm of both benign luminal epithelial and cancer cells in the prostate. No hybridizing signals were detected in stroma areas. The intensity of mRNA signals was similar between cancer cells and noncancerous cells (Fig. 2, A and C). No signal was detected on sections hybridized with sense probes (Fig. 2, B and D).

Anti-PSA-v Peptide Antibody. To test the specificity of the PSA-v antibody, 50 ng of PSA purified from seminal fluid, PSA-v peptide-BSA conjugates, and BSA were analyzed by Western blot analysis. Only PSA-v peptide-BSA conjugates were recognized, but not purified PSA and BSA, indicating the high specificity of the antibody (Fig. 3A). This was further confirmed by an enzyme-linked immunoassay in that the

![Image](https://example.com/image1.png)

**Fig. 1.** A, PSA-v mRNAs (~1.9 kb) and PSA mRNA (1.6 kb), detected by Northern blot analysis with the two specific cDNA probes, respectively, are shown from benign areas (b) and cancer areas (c) of prostate tissues from two representative patients. B, densitometric measurements of Northern blot analysis of PSA-v and PSA mRNAs in matched tissue specimens from 51 patients with PCa. Values were normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Data are presented as individual measurements (○) and average values (bars) of the group.
PSA-v peptide was specifically recognized by PSA-v peptide antibody (data not shown). Note that multiple high molecular forms of PSA-v peptide-BSA conjugates were detected (Fig. 3A) because of the nature of the conjugation reaction of PSA-v peptide with BSA \((M_r 67,000)\). Also, the PSA antibody can detect multiple cleaved PSA fragments (Fig. 3A) as described before (15, 16).

**PSA-v Protein Secreted by LNCaP Cells.** Concentrated spent medium from LNCaP cells in the presence (72 h) or absence of the synthetic androgen, mibolerone, were subjected to Western blot analysis. A specific band of \(M_r /H11011 30,000\) was detected with mibolerone-treated cells (Fig. 3B). A smaller amount of the protein was detected in the medium without the androgen. This observation suggests that PSA-v is subjected to androgen regulation.

**Expression of the PSA-v Protein in Benign and PCa Tissues.** Finally, we performed immunohistochemistry using the PSA-v antibody to detect PSA-v in prostate tissue specimens obtained from 10 patients. Specific signal for PSA-v protein was detected in the cytoplasm of benign luminal epithelia (Fig. 4A) and cancer cells of the prostate (Fig. 4B). Tissue sections from the same patients showed similar results when anti-PSA antibody was used for immunohistochemistry (Fig. 4, C and D). No signals were seen on negative control slides (Fig. 4, E and F).

**Discussion**

Previous studies have shown that the protein expression levels of some particular genes are not necessary in parallel to their mRNA levels (17–19). One such example was reported in a recent study that the protein of a prostate-specific gene, \(hPSE\), is translated only in normal prostate epithelial cells but is not found in PCa, although its mRNA is expressed in both types of prostate tissues. The regulatory mechanism of the expression patterns of the \(hPSE\) gene is not understood (20). Regarding PSA-v mRNA, there has been no report on whether its protein could be translated.

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**Fig. 2.** *In situ* hybridization of PSA-v mRNA is shown in normal (A) and cancer (C) of the prostate by an antisense probe. B and D are the adjacent sections corresponding to A and C, respectively, with a sense probe.

**Fig. 3.** A. Demonstration of the specificity of the PSA-v antibody by Western blot analysis. Lane 1, 50 ng of PSA-v peptide-BSA conjugate; Lane 2, 50 ng of purified PSA protein; Lane 3, 50 ng of BSA protein. The left panel was detected with the PSA-v antibody. The PSA-v antibody did not detect purified PSA and BSA protein. The right panel was analyzed with a PSA antibody. B, detection of secretory PSA-v from LNCaP cells by PSA-v antibody. Spent medium was collected from LNCaP cells with or without mibolerone \((Mib; 10 \text{ng})\) for 72 h and concentrated for Western blot analysis. Ponceau S protein staining shows equal amounts of total protein loaded.
under physiological or pathological conditions. It would be important to determine whether PSA-v protein is expressed in these tissues. In this study, we have found by use of a PSA-v-specific antibody that PSA-v protein is indeed expressed in normal luminal epithelial cells and in PCa cells (Fig. 4, A and B). This is, to the best of our knowledge, the first time that PSA-v protein has been detected in the prostate.

In this study, we also found that the average expression of PSA mRNA was significantly lower in cancer cells than in benign epithelium (Fig. 1B). It has been well documented that poorly differentiated and undifferentiated PCa cells sometimes express low levels of PSA protein (21–23). Our result for the relative expression of PSA mRNA between PCa and noncancerous cells is thus consistent with the expression of PSA protein. We hypothesize that the relative expression of PSA-v protein between PCa and benign epithelium may also correlate with the expression of PSA-v mRNA. Because the PSA-v mRNA expression level was found similar between PCa and benign epithelium (Fig. 1B), PSA-v protein levels in PCa cells may stay constant as compared with that in benign epithelium. PSA is the most useful biomarker in the detection and monitoring of PCa. However, many men with benign prostatic hyperplasia will undergoing a needle biopsy or additional testing because of false-positive PSA results. In addition, 25% of men with PCa have normal PSA levels (<4.0 ng/ml; Ref. 5). Further refinements of PSA evaluation are therefore needed to improve the specificity of PSA testing and thereby reduce the number of unnecessary prostatic biopsy (5). We are currently in the process of producing monoclonal antibodies for PSA-v and developing a serum PSA-v test. It remains to be seen whether serum PSA-v can provide additional information for PCa.

It is known that the expression of PSA is primarily modulated by androgen via the androgen receptor (4–6, 9). In this study, we demonstrated that PSA-v was up-regulated by androgens (Fig. 3B). However, our results showed differences in expression levels of PSA-v and PSA mRNAs in benign and malignant prostate tissues, indicating possible differences in the

![Fig. 4. Immunohistochemical stain of PSA-v protein with the anti-PSA-v antibody in A and B, with anti-PSA antibody in C and D, or without primary antibody as negative controls in E and F, respectively. A, C, and E, benign tissue sections; B, D, and F, cancerous sections.](image)
regulation of the two transcripts. Further elucidation of such differences in relation to pathogenesis and detection of prostatic diseases would be clinically interesting.

Tissue kallikreins and kallikrein-like proteins are a subgroup of closely related serine proteinases (5, 6). They are able to cleave specific precursor proteins at highly selective sites and generate the mature, biologically active proteins. Similar to other serine proteinases, there are 10 cysteine residues in mature PSA, predicting five disulfide bonds. Also, PSA protein has conserved active site residues HIS-41, ASP-96, and SER-189, which are common among serine proteinases (8). It is generally believed that the main biological role of PSA in semen is to digest the protein semenogelin I, so liquefying the seminal fluid and releasing sperm (5). The functional role of PSA, in particular, in connection to the pathogenesis and progression of PCa and other cancers has attracted a great deal of attention. Some reports (24) have suggested that PSA may act as a tumor suppressor, a negative regulator of cell growth, and an apoptotic molecule. However, most of reports have shown that PSA can proteolytically modulate the activity of insulin-like growth factor-binding protein-3, transforming growth factor-β, and cell surface receptors. This suggests that the enzymatic activity of PSA may be related to proliferation, migration, and metastasis of PCa cells (5). Compared with PSA, the deduced sequence of PSA-v lacks the serine at position 189, one of the three residues important for the proteolytic activity of kallikreins. It has thus been suggested that the enzymatic properties of the PSA-v could be significantly affected (8). However, the biological function of PSA-v remains under investigation at this time.

In conclusion, by using a specific probe and a specific antibody for PSA-v, we have shown the expression of PSA-v mRNA and protein in cancer cells and in benign luminal epithelia of the prostate. We have also revealed differences for the relative expression levels of PSA-v and PSA mRNAs in cancer and noncancerous tissue. Our data indicate the significance of further analyzing PSA-v in relation to the pathology and detection of PCa.

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References
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