Enhanced Expression of Prostate-specific Membrane Antigen Gene in Prostate Cancer as Revealed by in Situ Hybridization

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Abstract

Recently, the cDNA encoding a novel candidate for prostate cancer-specific antigen, named prostate-specific membrane antigen (PSM), was cloned from the LNCaP prostate cell line (R. S. Israeli, C. T. Powell, W. R. Fair, and W. D. H. Weston, Cancer Res., 53: 227-230, 1993). More recently, they also identified an alternatively spliced variant of PSM in normal prostate tissues (S. L. Su, I-P. Huang, W. R. Fair, C. T. Powell, and W. D. H. Weston, Cancer Res., 55: 1441-1443, 1995). The cDNA of this variant, named PSM', lacks 266 nucleotides present in PSM cDNA; so the transcripts derived from this particular nucleotide sequence can be regarded as PSM-specific transcripts. In this study, we investigated the expression of PSM-specific transcripts in 15 specimens of prostate cancer obtained by needle biopsy using in situ hybridization with a newly developed RNA probe. PSM-specific transcripts were detected in most of the carcinoma cells in all of the specimens examined, and the level of expression was higher in carcinoma cells from hormone-refractory patients than in the cells of those who showed a good response to hormonal therapy. In addition, increased expression of PSM-specific transcripts was also associated with an increased Gleason score. In the normal prostate, on the other hand, PSM-specific transcripts were limited to the basal cells of the prostate glands. These results clearly show that expression of PSM-specific transcripts is closely associated with malignant transformation of the prostate; thus, in situ hybridization for detection of the transcripts is useful for the diagnosis of prostate cancer.

Introduction

Prostate cancer most frequently occurs in men over 75 years old in Japan as well as in Western countries (1). For the detection and monitoring of prostate cancer, several tumor-associated antigens such as PSA2 and prostatic acid phosphatase are available as routine laboratory tests (2). Although such biomarkers are generally useful in detecting prostate cancer, some of these cancers actually lack them (3).

Heston’s group recently cloned cDNA encoding a novel candidate for a prostate cancer-associated antigen, named PSM, from the LNCaP prostate cell line (4). The deduced amino acid sequence of PSM reveals that it is a membrane-bound glycoprotein composed of 760 amino acids with a type II membrane topology. The nucleotide sequence from +1250 to +1700 revealed that PSM has a homology with human transferrin receptor (54% identity), but the role of PSM is not fully understood (4). More recently, Heston’s group cloned the cDNA for an alternatively spliced variant of PSM in normal prostate tissues (5). The cDNA of this variant, named PSM', lacks 266 nucleotides encoding part of the transmembrane domain of PSM; therefore, transcripts derived from this particular sequence can be regarded as PSM-specific (Fig. 1). The study by Heston’s group using the RNase protection assay provided evidence that PSM expression is predominant over that of PSM' in prostate cancer, whereas normal prostate expressed more PSM' than PSM (5). In addition, a high PSM:PSM' ratio was coupled with malignant transformation of the prostate gland. These results, taken together, strongly suggested that PSM-specific transcripts derived from the 266-nucleotide sequence lacking in PSM' cDNA are cancer-specific. The purpose of the present study was to test the hypothesis that PSM-specific transcripts are associated with malignant transformation of the prostate gland by using in situ hybridization. A RNA probe was designed to identify PSM-specific transcripts derived from the 266-nucleotide sequence of PSM cDNA, and the expression of PSM-specific transcripts was compared to that of PSA.

Materials and Methods

Patients. Needle biopsy prostate specimens from 15 patients with prostate cancer were obtained at Shinshu University Hospital and used in this study. The clinicopathological features of the patients are shown in Table 1. All specimens were reviewed and graded according to the system of Gleason (6).

Preparation of a PSM-specific Transcript Probe for in Situ Hybridization. Total cellular RNA was isolated from 5 × 10^6 LNCaP cells according to the acid guanidinium thiocyanate-chloroform extraction method using an Isogen kit (Nippon Gene, Tokyo, Japan; Ref. 9). Subsequently, double-stranded cDNA was synthesized by reverse transcription of the extracted RNA. Briefly, total RNA (1 μg) was reverse-transcribed with 0.25 μL of Moloney murine leukemia virus reverse transcriptase (200 units/μL) in a total volume of 20 μL containing 10× reverse transcription buffer, 150 μM of each deoxynucleotide triphosphate, 0.5 μg of oligo(dT), primer, 10 mM DTT, and 1 μL of RNase inhibitor, RNasin (10 units/μL; Promega, Madison, WI). The reaction was performed at 42°C for 1 h.

Using this double-stranded cDNA as a template, a PSM-specific nucleotide sequence surrounding the initiation methionine (nucleotides -101 to +37; the first nucleotide of the initiation codon is +1) was amplified by PCR (Fig. 1). The 5' and 3' primers were designed to be 5'-GCTCTAGAGATT-GAGAGACATTTAC-3' and 5'-GGGTTACACGCCGACTGTT-3', according to the published sequence (4). The XhoI and Asp718 sites are underlined. This amplified cDNA sequence was cloned into the XhoI and Asp718 sites of pGEM-3Zf(±) (Promega), and the resultant vector was used as a template for construction of the RNA probe. A digoxigenin-labeled antisense RNA probe was obtained using an XhoI-cut template and T7 RNA polymerase with a DIG RNA labeling kit (Boehringer Mannheim, Mannheim, Germany).

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2 The abbreviations used are: PSA, prostate-specific antigen; PSM, prostate-specific membrane antigen.
PSM cDNA

PSM' cDNA

RNA probe

ATG

TAA

+1

+119

+172

+2253

-261

-147

+1

AAA

AAA

ATG

TAA

-101

+37

-101

+37

-101

+37

-101

+37

Fig. 1. Diagram of PSM and PSM' cDNAs. The translation initiation site of PSM cDNA is denoted as +1. Both PSM and PSM' are generated through alternative splicing, and the nucleotides from -147 to +119 are lacking in PSM' cDNA. The RNA probe was designed to identify the nucleotide sequence from -101 to +37 of PSM cDNA, which is absent from PSM' cDNA. Box, open reading frame. TM, transmembrane region.

as described previously (10, 11). Similarly, a sense probe was prepared for negative control experiments by using an Asp718-cleave template and SP6 RNA polymerase with the same kit.

In Situ Hybridization of PSM-specific Transcripts. Tissue specimens were subjected to in situ hybridization to detect PSM-specific transcripts using a nonradioactive system (10, 11). After the tissue sections were deparaffinized in xylene, hydrated slides were immersed in 0.2 M HCl for 20 min and then digested with 100 μg/ml proteinase K at 37°C for 20 min, followed by postfixation with 4% paraphormaldehyde. Theses slides were rinsed with 2 mg/ml glycine and subsequently acetylated for 10 min in freshly prepared 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0). The hydrated slides were then deaffted with chloroform and air-dried. After prehybridization with 50% deionized formamide and 2× SSC for 1 h at 45°C, the slides were hybridized with 0.5 mg/ml antisense or sense probe in 50% deionized formamide, 2.5 mM EDTA (pH 8.0), 300 mM NaCl, 1X Denhardt’s solution, 10% dextran sulfate, and 1 mg/ml breuer’s yeast tRNA at 45°C for 16 h.

After hybridization, the slides were washed in 50% formamide and 2× SSC for 1 h at 45°C and digested with 10 mg/ml RNase A at 37°C for 30 min. After washing with 2× SSC and 50% formamide at 45°C for 1 h, 1X SSC and 50% formamide at 45°C for 1 h, and 1X SSC and 50% formamide at room temperature for 30 min, the sections were subjected to immunohistochemistry for detection of the hybridized probes using an alkaline phosphatase-conjugated anti-digoxigenin antibody (Boehringer Mannheim). The alkaline phosphatase reaction was visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium. A control study using the sense probe showed no specific reactivity. The intensity of the signal in the carcinoma cells was graded on a scale of - to +++ (-, no reactivity; +, weak reactivity; ++, moderate reactivity; ++++, strong reactivity).

Immunohistochemistry for PSA. Deparaffinized tissue slices were also subjected to immunohistochemical staining for the detection of PSA. A rabbit polyclonal antibody directed against human PSA was purchased from DAKO (Glostrup, Denmark), and immunohistochemical detection was performed by the indirect method followed by counterstaining with hematoxylin (12). A control experiment was done by omitting the primary antibody from the staining procedure, and no specific staining was found. The intensity of the staining in the carcinoma cells was graded on a scale of - to +++ (-, no reactivity; +, weak reactivity; ++, moderate reactivity; ++++, strong reactivity).

Results and Discussion

Expression of PSM-specific Transcripts in Prostate Cancer. We have successfully demonstrated PSM-specific transcripts in prostate tissues fixed with a standard fixative, 20% neutral formalin. The transcripts could be detected in most of the carcinoma cells from all 15 cancer specimens examined irrespective of the clinical stage, Gleason score, preoperative serum PSA value, and response to hormonal therapy, as shown in Table 1. Although the level of transcripts in the carcinoma cells varies among patients, transcription of the PSM gene was markedly increased in carcinoma cells compared to that of normal prostate glands (Fig. 2B). In normal prostate glands, PSM-specific transcripts were restricted to the basal cells (Fig. 2B, inset). As background activity, weak signals for the transcripts were also noted in the prostatic stromal cells. These results clearly show that the PSM-specific transcripts were closely associated with malignant transformation of the prostate. This finding is also consistent with the result of an immunohistochemical study by Lopes et al. (13) using monoclonal antibody 7E11-C5, which was originally used for molecular cloning of PSM cDNA. They showed that 7E11-C5 antibody reacted more strongly with all of the prostate carcinoma cells examined than it did with normal prostate tissue. The same study also revealed that immunostaining with 7E11-C5 could not distinguish basal cells from secretory cells in the normal prostate gland, and there was weak staining of both types of glandular epithelia. Taking into account that the antigenic determinant of 7E11-C5 is the first 6 amino acids from the amino-terminal of PSM (14), whereas the RNA probe we used contains the nucleotide sequence corresponding to these particular 6 amino acids, it may be possible that the PSM gene is transcribed in the basal cells but not in the secretory cells of the normal prostate gland, although the secretory cells express PSM.

Interestingly, the level of expression of PSM-specific transcripts was dependent on resistance to hormonal therapy as well as on the histological differentiation of the carcinoma. As shown in Table 1, carcinoma cells from hormone-refractory patients expressed higher levels of the transcripts (Fig. 2J) compared to cells from patients who showed a response to hormonal therapy (Fig. 2F). Recently, Israeli et al. (15) showed that the expression of PSM by LnCap cells was highest in steroid-depleted medium and was down-regulated in the presence of dihydrotestosterone and progesterone. Further study is required to investigate the transcriptional regulation of PSM by various hormones. In addition, increased expression of PSM-specific transcripts was found in poorly differentiated adenocarcinoma rather than in well-differentiated adenocarcinoma (Table 1). These results, taken together, indicate that enhanced expression of PSM-specific transcripts could be closely associated with the progression of prostate cancer.

Comparison of PSM-specific Transcripts and PSA. PSA is a distinct molecule present in the prostate gland and is also expressed in the vast majority of prostate cancers (2). The pattern of PSA expression was compared to that of PSM-specific transcripts in the present study. As reported previously (3) and confirmed here, PSA was most strongly expressed in the normal glandular epithelia (Fig. 2D). In prostate carcinomas, PSA was preferentially expressed in well-differentiated adenocarcinoma (Fig. 2H) rather than in poorly differentiated adenocarcinoma (Fig. 2L). In contrast to PSA, the expression of PSM-specific transcripts was more restricted to carcinoma cells (Fig. 2B) and thus was more specific for prostate cancer. Moreover, the PSM-specific transcripts and PSA were expressed in a reciprocal manner, as shown in Table 1. The carcinoma cells that abundantly expressed PSM-specific transcripts showed relatively weak PSA expression (Fig. 2, J and L) compared to those expressing low levels of PSM-specific transcripts (Fig. 2, F and H). In situ hybridization for PSM-specific transcripts could therefore be a useful tool for the diagnosis of prostate cancer.

In routine pathological examination of prostate cancer, it is sometimes difficult to diagnose adenocarcinoma with minimal cytological atypia (Fig. 2E), and it is also hard to recognize that the tumor is of prostatic origin when the carcinoma cells express little or no PSA (Fig. 2L). In such cases, in situ hybridization for PSM-specific transcripts could make an effective contribution to the diagnosis of prostate cancer.

In summary, the present study demonstrated the increased expression of PSM-specific transcripts in prostate cancer and the value of in
Table 1  Clinicopathological features and expression of the PSM-specific transcript and PSA

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"Staining intensity: +, weak; + +, moderate; + + +, strong.

*b Normal range is below 2.8 ng/ml.

'D, responded to hormonal therapy; ID, refractory to hormonal therapy.

situ hybridization for the transcripts in the histological diagnosis of prostate cancer.

Acknowledgments

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References

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