

Prostate Stem Cell Antigen (PSCA) Expression in Human Prostate Cancer Tissues: Implications for Prostate Carcinogenesis and Progression of Prostate Cancer

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Objective: Prostate stem cell antigen (PSCA) is a recently defined homolog of the Thy-1/Ly-6 family of glycosylphosphatidylinositol (GPI)-anchored cell surface antigens. The objective of the present study was to examine the expression status of PSCA protein and mRNA in clinical specimens of human prostate cancer (PCa) and to validate it as a potential molecular target for diagnosis and treatment of PCa.

Methods: Immunohistochemical (IHC) and *in situ* hybridization (ISH) analyses of PSCA expression were simultaneously performed on paraffin-embedded sections of 20 benign prostatic hyperplasia (BPH), 20 prostatic intraepithelial neoplasm (PIN) and 48 prostate cancer (PCa) tissues, including 9 androgen-independent prostate cancers. The level of PSCA expression was semiquantitatively scored by assessing both the percentage and intensity of PSCA-positive staining cells in the specimens. We then compared the PSCA expression between BPH, PIN and PCa tissues and analyzed the correlations of PSCA expression level with pathological grade, clinical stage and progression to androgen-independence in PCa.

Results: In BPH and low grade PIN, PSCA protein and mRNA staining were weak or negative and less intense and uniform than that observed in high grade PIN (HGPI) and PCa. Moderate to strong PSCA protein and mRNA expression were noted in 8 of 11 (72.7%) HGPI and in 40 of 48 (83.4%) PCa specimens examined by IHC and ISH analyses, and their statistical significance was compared with BPH (20%) and low-grade PIN (22.2%) specimens ($P < 0.05$). The expression level of PSCA increased with a higher Gleason grade, advanced stage and progression to androgen-independence ($P < 0.05$). In addition, IHC and ISH staining revealed a high degree of correlation between PSCA protein and mRNA overexpression.

Conclusions: Our data demonstrate that PSCA as a new cell surface marker is overexpressed in a majority of cases of human PCa. PSCA expression correlates positively with adverse tumor characteristics, such as increasing pathological grade (poor cell differentiation), worsening clinical stage and androgen-independence and speculatively with prostate carcinogenesis. PSCA may possess prognostic utility and may be a promising molecular target for diagnosis and treatment of PCa.

Key words: prostate – neoplasm – prostate stem cell antigen (PSCA)

INTRODUCTION

Prostate cancer (PCa) is the second leading cause of cancer-related deaths in American men and its incidence is on the rise in China. Despite the tremendous progress in the diagnosis and management of localized disease in the recent years, there continues to be a requirement for new diagnostic markers that can

accurately discriminate between indolent and aggressive variants of PCa. There also continues to be a requirement for the identification and characterization of potential new therapeutic targets on PCa cells. Current diagnostic and therapeutic modalities for recurrent and metastatic PCa have been limited by a lack of specific target antigens for PCa. Although a number of prostate-specific genes have been identified (i.e. prostate specific antigen, prostatic acid phosphatase and glandular kallikrein 2), a majority of these are secreted proteins which are not ideally suited for many immunological strategies. Therefore, the identification of new cell surface antigens

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Table 1. Correlation of PSCA expression with Gleason score

Gleason Score	Intensity × Frequency	
	0–6 (%)	9 (%)
2–4	5 (83)	1 (17)
5–7	19 (79)	5 (21)
8–10	5 (28)	3 (72)

is critical for the development of new diagnostic and therapeutic approaches in the management of PCa.

Reiter et al. (1) recently reported the identification of prostate stem cell antigen (PSCA), a cell surface antigen that is predominantly prostate specific. The PSCA gene encodes a 123 amino acid glycoprotein with 30% homology to stem cell antigen 2 (SCA-2). Similar to SCA-2, PSCA is also a member of the Thy-1/Ly-6 family and is anchored by a glycosylphosphatidylinositol (GPI) linkage. mRNA *in situ* hybridization (ISH) localizes PSCA expression in normal prostate to the basal cell epithelium, the putative stem cell compartment of prostatic epithelium, suggesting that PSCA may be a marker for prostate stem/progenitor cells.

In order to examine the expression status of PSCA protein and mRNA in human PCa and validate it as a potential diagnostic and therapeutic target for PCa, we used immunohistochemistry (IHC) and *in situ* hybridization (ISH) simultaneously and conducted PSCA protein and mRNA expression analyses in paraffin-embedded tissue specimens of benign prostatic hyperplasia (BPH, $n = 20$), prostate intraepithelial neoplasm (PIN, $n = 20$) and prostate cancer (PCa, $n = 48$). Furthermore, we evaluated the possible correlation between PSCA expression level and PCa tumorigenesis, grade, stage and progression to androgen-independence.

SUBJECTS AND METHODS

TISSUE SAMPLES

All the clinical tissue specimens in this study were obtained from 80 patients in the age group of 57–84 years, by prostatectomy, transurethral resection of prostate (TURP) or biopsies. The patients were classified as 20 cases of BPH, 20 cases of PIN and 40 cases of primary PCa, including nine patients with recurrent PCa and a history of androgen ablation therapy (orchiectomy and/or hormonal therapy), who were referred to as androgen-independent prostate cancer patients. Eight specimens harvested prior to androgen ablation treatment from the same androgen-independent PCa patients were referred to as androgen-dependent prostate cancer. Each tissue sample was cut into two parts, one was fixed in 10% formalin for IHC and the other was treated with 4% paraformaldehyde/0.1M PBS pH 7.4 in 0.1% DEPC for ISH analysis and then embedded in paraffin. All paraffin blocks were cut into 5-mm sections, mounted on glass slides in the usual manner and then examined. H&E-stained section of each PCa was evaluated and

Table 2. Correlation of PSCA expression with clinical stage

Tumor Stage	Intensity × Frequency	
	0–6 (%)	9 (%)
≤B	27 (67.5)	13 (32.5)
≥C	2 (25)	6 (75)

assigned a Gleason score by an experienced urological pathologist at our institution, based on the criteria for the Gleason score (2). The Gleason sums are summarized in Table 1. Clinical staging was performed according to Jewett–Whitmore–Prout staging system, as shown in Table 2. In the PIN category, we graded the specimens into two groups, i.e. low grade PIN (grade I–II) and high grade PIN (HGPIN, grade III) on the basis of relevant literature (3,4).

IMMUNOHISTOCHEMICAL (IHC) ANALYSIS

Briefly, tissue sections were deparaffinized, dehydrated and subjected to microwaving in 10 mmol/L citrate buffer with pH 6.0 (Boshide, Wuhan, China) in a 900-W oven for 5 min to induce epitope retrieval. The slides were allowed to cool at room temperature for 30 min. A primary mouse antibody specific to human PSCA (Boshide, Wuhan, China) with a 1:100 dilution was applied to the slides and incubated at room temperature for 2 h. Labeling was detected by sequentially adding biotinylated secondary antibodies and streptavidin-peroxidase, and color was developed using 3,3'-diaminobenzidine reaction. The sections were counterstained with hematoxylin. Substitution of the primary antibody with phosphate-buffered-saline (PBS) served as a negative control.

mRNA *IN SITU* HYBRIDIZATION (ISH)

Tissue sections with a thickness of 5 mm were deparaffinized and dehydrated, followed by digestion in pepsin solution (4 mg/ml in 3% citric acid) for 20 min at 37.5°C and were further processed for ISH. Digoxigenin-labeled sense and antisense human PSCA RNA probes (Boshide, Wuhan, China) were hybridized to the sections at 48°C overnight, followed by a highly stringent posthybridization wash in standard saline citrate (SSC). Subsequently, the slides were incubated with biotinylated mouse anti-digoxigenin antibody for 1 h at 37.5°C and streptavidin-peroxidase for 20 min at 37.5°C. After washing with PBS buffer, a drop of 3,3'-diaminobenzidine (DAB) was added and color was developed. Counterstaining was performed using hematoxylin. All slides were hybridized with PBS or the sense probe to substitute for the antisense probe as a negative control.

SCORING METHODS

In the present study, the same semi-quantitative scoring method was applied for PSCA protein staining by IHC and PSCA mRNA staining by ISH. Each slide was read and scored independently by two experienced urological pathologists,

using Olympus BX-41 light microscopes. This was done in a blinded fashion. The intensity of PSCA expression was graded on a scale of 0 to 3+ with 3 being the highest expression observed (0, no staining; 1+, mildly intense; 2+, moderately intense; 3+, severely intense). The staining density was quantified as the percentage of cells stained positive for PSCA, as follows: 0 = no staining; 1 = positive staining in <25% of the sample; 2 = positive staining in 25%–50% of the sample; 3 = positive staining in >50% of the sample. Intensity score (0–3+) was multiplied by the density score (0–3) to yield an overall score of 0–9 for each specimen (1,5), i.e. 0, negative expression; 1–2, weak expression; 3–6, moderate expression; 9, strong expression. In this manner, we were able to differentiate the specimens that may have had focal areas of increased staining from those that had diffuse areas of increased staining (6).

STATISTICAL ANALYSIS

The PSCA expression levels in BPH, PIN and PCa tissues were compared using the Chi-square test and Student's *t*-test. Univariate associations between PSCA expression and Gleason score, clinical stage and progression to androgen-independence were calculated using Fisher's Exact Test. For all the analyses, $P < 0.05$ was considered statistically significant.

RESULTS

PSCA EXPRESSION IN BPH

In general, PSCA protein and mRNA were weakly expressed in individual samples of BPH. Some areas of prostate expressed weak levels (composite score 1–2), whereas others were completely negative (composite score 0). Four cases (20%) of BPH showed moderate expression of PSCA protein and mRNA (composite score 4–6) by IHC and ISH. In two of twenty (10%) BPH specimens, PSCA mRNA expression was moderate (composite score 3–6), but PSCA protein expression was weak (composite score 2) in one and negative (composite score 0) in the other. PSCA expression was localized to the basal and secretory epithelial cells, and prostatic stroma showed almost negative staining for PSCA protein and mRNA in all cases examined.

PSCA EXPRESSION IN PIN

In this study, we detected weak or negative expression of PSCA protein and mRNA (scores ≤ 2) in seven of nine (77.8%) low grade PIN specimens and in two of eleven (18.2%) HGPIN specimens and moderate expression (score 3–6) in the other two low grade PIN specimens and in five of eleven (45.5%) HGPIN specimens. One HGPIN specimen with moderate PSCA mRNA expression (score 6) showed weak staining for PSCA protein (score 2) by IHC. Strong PSCA protein and mRNA expression (score 9) were detected in the remaining three of eleven (27.3%) HGPIN specimens. A statistically significant difference was observed between the PSCA protein and mRNA expression levels of HGPIN and BPH ($P < 0.05$),

but no statistical difference was observed for low grade PIN and BPH ($P > 0.05$).

PSCA EXPRESSION IN PCA

In order to determine if PSCA protein and mRNA can be detected in prostate cancer and if PSCA expression levels are higher in malignant glands compared with benign glands, 48 paraffin-embedded PCa specimens were analyzed by IHC and ISH. It was shown that 19 of 48 (39.6%) PCa samples stained very strongly for PSCA protein and mRNA with a score of 9 and the other 21 (43.8%) specimens displayed moderate staining with scores of 4–6 (Fig. 1). In addition, four specimens with moderate to strong PSCA mRNA expression (scores 4–9) had weak protein staining (score 2) by IHC analyses. Overall, PCa expressed a significantly higher level of PSCA protein and mRNA than any other specimen category in this study ($P < 0.05$). The result demonstrates that PSCA protein and mRNA are overexpressed in a majority of cases of human PCa.

CORRELATION OF PSCA EXPRESSION WITH GLEASON SCORE IN PCA

Using the semi-quantitative scoring method as described in Materials and Methods, we compared the expression level of PSCA protein and mRNA with Gleason grade for PCa, as shown in Table 1. Prostate adenocarcinomas were graded by Gleason score as 2–4 (well-differentiation), 5–7 (moderate-differentiation) and 8–10 (poor-differentiation) (7). In 72% prostate cancer cases with Gleason scores of 8–10, the exhibited staining for PSCA was very strong compared to 21% with Gleason scores of 5–7 and 17% with that of 2–4, demonstrating that poorly differentiated PCa had significantly stronger expression of PSCA protein and mRNA than moderately and well differentiated tumors ($P < 0.05$). As shown in Fig. 1, IHC and ISH analyses showed that PSCA protein and mRNA expression in several cases of poorly differentiated PCa were particularly prominent with more intense and uniform staining. The results indicate that PSCA expression increases significantly with higher tumor grade in human PCa.

CORRELATION OF PSCA EXPRESSION WITH CLINICAL STAGE IN PCA

The results regarding PSCA expression in each stage of PCa are shown in Table 2. Seventy-five percent of locally advanced and node positive cancers (i.e. C-D stages) expressed statistically high levels of PSCA versus 32.5% that were organ confined (i.e. A-B stages) ($P < 0.05$). The data demonstrate that PSCA expression increases significantly with advanced tumor stage in human PCa.

CORRELATION OF PSCA EXPRESSION WITH ANDROGEN-INDEPENDENT PROGRESSION OF PCA

All nine specimens of androgen-independent prostate cancers stained positive for PSCA protein and mRNA. Seven of eight (87.5%) androgen-independent PCa specimens were in the

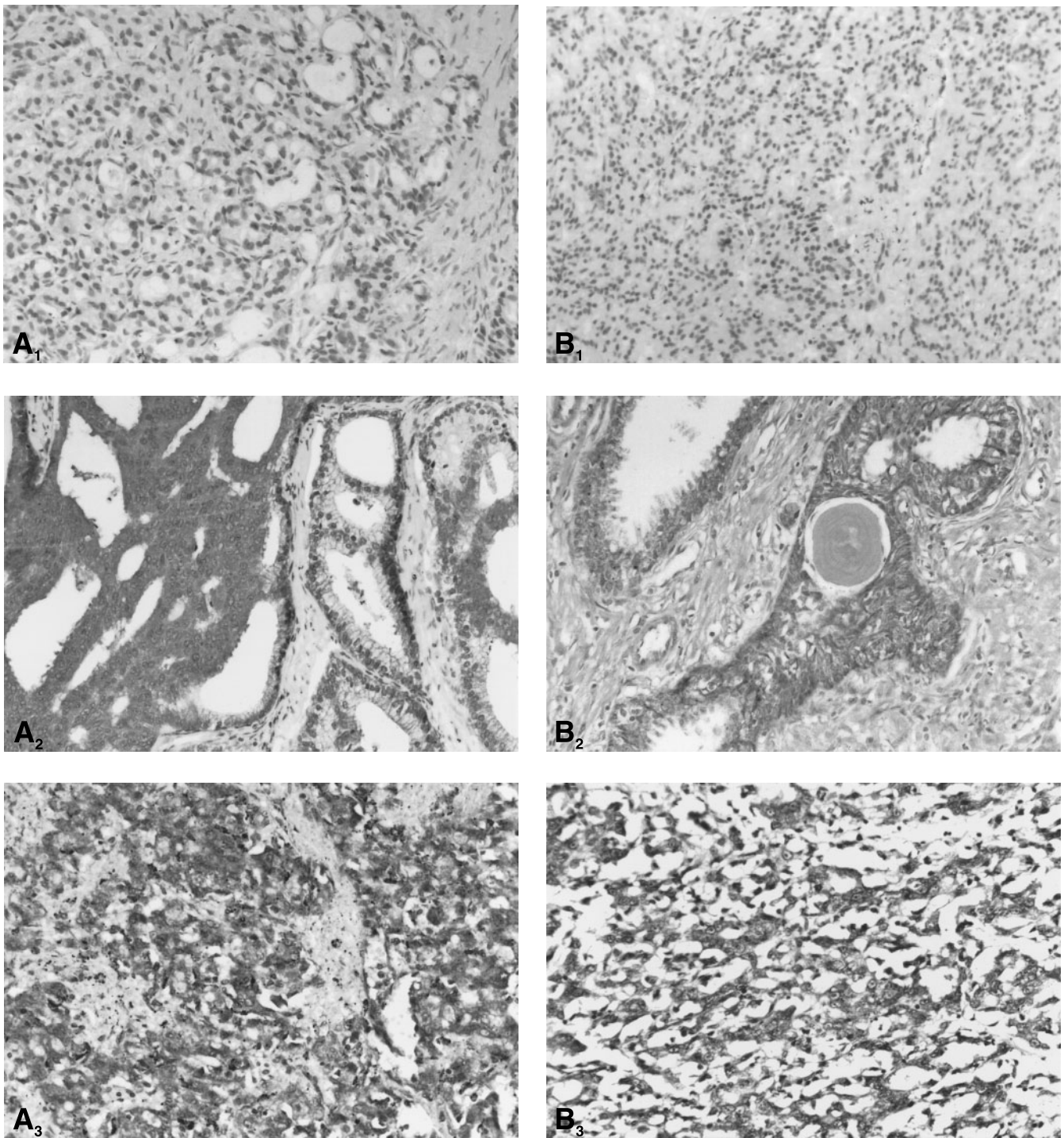


Figure 1. Representatives of PSCA IHC and ISH staining in PCa (A. IHC staining, B. ISH staining, $\times 200$ magnification). A₁, B₁: negative control for IHC and ISH. PBS replacing the primary antibody (A₁) and hybridization with a sense PSCA probe (B₁) does not show background staining. A₂, B₂: a moderately differentiated PCa (Gleason score = 3 + 3 = 6) with moderate staining (composite score = 6) in all malignant cells; A₂: IHC shows not only the cell surface but also apparent cytoplasmic staining of PSCA protein. A₃, B₃: a poorly differentiated PCa (Gleason score = 4 + 4 = 8) with very strong staining (composite score = 9) in all malignant cells.

strongest staining category (score 9) compared with three of eight (37.5%) androgen-dependent PCa specimens from the same patients ($P < 0.05$). The results demonstrate that PSCA expression increases significantly with progression to androgen-independence of human PCa.

It is evident from the above results that within a majority of human prostate cancers, the level of PSCA protein and mRNA expression correlates significantly with increasing grade, worsening stage and progression to androgen-independence.

CORRELATION BETWEEN PSCA IMMUNOSTAINING AND mRNA *IN SITU* HYBRIDIZATION

Of the 88 specimens surveyed, we compared the results of PSCA IHC staining with mRNA ISH analysis. Positive staining areas and their intensity and density scores evaluated by IHC were identical to those by ISH in 79 of 88 (89.8%) specimens (18 of 20 BPH, 19 of 20 PIN and 42 of 48 PCa). Importantly, 27 samples with PSCA mRNA composite scores of 0–2, 32 of 36 samples with scores of 3–6 and 22 of 24 samples with a score of 9 also showed PSCA protein expression scores of 0–2, 3–6 and 9, respectively. These data demonstrate that the results of PSCA immunostaining are consistent with those of mRNA ISH analysis. However, in five samples with overall PSCA mRNA scores of 3–6 and in two samples with a score of 9, a less or negative PSCA protein expression was observed (i.e. scores of 0–4), suggesting that this may reflect posttranscriptional modification of PSCA or that the epitopes recognized by PSCA antibody may be obscured in some cancers. Overall, our results show a strong correlation between PSCA protein and mRNA expression levels in human PCa.

DISCUSSION

PSCA is homologous to a group of cell surface proteins that indicate the earliest phase of hematopoietic development. PSCA mRNA expression is prostate-specific in normal male tissues and is highly up-regulated in both androgen-dependent and androgen-independent PCa xenografts (LAPC-4 tumors) (1). We hypothesize that PSCA may play a role in PCa tumorigenesis and progression and may serve as a target for PCa diagnosis and treatment. In this study, IHC and ISH showed that in general, PSCA protein and mRNA expression were either weak or absent in BPH and low grade PIN tissues. However, PSCA protein and mRNA are widely expressed in HGPIN, the putative precursor of invasive PCa, suggesting that up-regulation of PSCA is an early event in prostate carcinogenesis. Recently, Reiter et al. (1), using ISH analysis, reported that 97 of 118 (82%) HGPIN specimens stained strongly positive for PSCA mRNA. A similar finding was noted by Tran et al. in mouse PSCA (mPSCA) expression in mouse HGPIN tissues (8). These data suggest that PSCA may be a new marker associated with transformation of prostate cells and tumorigenesis.

We observed that PSCA protein and mRNA are highly expressed in a large percentage of human prostate cancer, including advanced, poorly differentiated, androgen-independent and metastatic cases. Fluorescence-activated cell sorting and confocal/immunofluorescent studies demonstrated cell surface expression of PSCA protein in PCa cells (9). Our IHC analysis of PSCA expression showed not only cell surface but also apparent cytoplasmic staining of PSCA protein in PCa specimens (Fig. 1). A possible explanation for this is that anti-PSCA antibody can recognize PSCA peptide precursors that reside in the cytoplasm and the other is that the positive staining that appears in the cytoplasm is actually due to the overly-

ing cell membrane (5). These data seem to indicate that PSCA is a novel cell surface marker for human PCa.

Our results show that elevated level of PSCA expression correlates with high grade (i.e. poor differentiation), increased tumor stage and progression to androgen-independence of PCa. These findings support the original IHC analyses by Gu et al. (9), who reported that PSCA protein was expressed in 94% of primary PCa and the intensity of PSCA protein expression increased with tumor grade, stage and progression to androgen-independence. It is suggested that PSCA overexpression may be an adverse predictor for recurrence, clinical progression or survival of PCa. Dubey et al. (10) reported that an elevated number of PSCA-positive cells correlate positively with the onset and development of prostate carcinoma over a long time span in the prostate of the TRAMP and PTEN +/- models compared with the normal prostate. When considered along with our present findings in which PSCA is overexpressed from HGPIN to almost frank carcinoma, it is reasonable and possible to use increased PSCA expression level or increased number of PSCA-positive cells in the prostate samples as a prognostic marker to predict the potential onset of this cancer. These data raise the possibility that PSCA may possess diagnostic utility or clinical prognostic value in human PCa.

The cause of PSCA overexpression in PCa is not known. A possible mechanism is that it may result from PSCA gene amplification. In humans, PSCA is located on chromosome 8q24.2 (1), which is often amplified in metastatic and recurrent PCa and considered to indicate a poor prognosis (11–13). Interestingly, PSCA is in close proximity to the c-myc oncogene, which is amplified in >20% of recurrent and metastatic prostate cancers (14,15). Reiter et al. (16) reported that PSCA and MYC gene copy numbers were co-amplified in 25% of the tumors (five of twenty), demonstrating that PSCA overexpression is associated with PSCA and MYC coamplification in PCa. Gu et al. (9) recently reported that among 102 specimens available for comparison of PSCA immunostaining with their previous mRNA ISH analysis, 92 specimens (90.2%) had identical positive areas of PSCA protein and mRNA expression. When considered along with our findings in which we detected moderate to strong expression of PSCA protein and mRNA in 34 of 40 (85%) PCa specimens examined simultaneously by IHC and ISH analyses, it was demonstrated that PSCA protein and mRNA overexpressed in human PCa, suggesting that PSCA protein overexpression may be partially due to PSCA mRNA overrepresentation.

The regulation mechanisms of human PSCA expression and its biological function are yet to be elucidated. PSCA expression may be regulated by multiple factors (16). Watabe et al. (17) reported that transcriptional control is a major component regulating PSCA expression levels. In addition, induction of PSCA expression may be regulated or mediated through cell-cell contact and protein kinase C (PKC) (18). Homologs of PSCA show diverse activities and are involved in carcinogenesis. Signaling through SCA-2 has been demonstrated to prevent apoptosis in immature thymocytes (19). Thy-1 is involved in T cell activation and transduces signals through src-like tyro-

sine kinases (20). Ly-6 genes have been implicated in both tumorigenesis and cell-cell adhesion (21–23). Cell-cell or cell-matrix interaction is critical for local tumor growth and spread to distal sites. Due to its restricted expression in basal cells of normal prostate and its homology to SCA-2, PSCA may play a role in stem/progenitor cell function, such as self-renewal (i.e. anti-apoptosis) and/or proliferation (1). When considered along with the results of the present study, we speculate that PSCA may play a role in tumorigenesis and clinical progression of PCa by affecting cell transformation and proliferation. From our results, it is also suggested that PSCA as a new cell surface antigen may have a number of potential uses in the diagnosis, therapy and clinical prognosis of human PCa. PSCA overexpression in prostate biopsies could be used to identify patients with a high risk of developing recurrent or metastatic disease and to discriminate cancer from normal glands in prostatectomy samples. Similarly, the detection of PSCA-overexpressing cells in bone marrow or peripheral blood may identify and predict metastatic progression better than current assays, which identify only PSA-positive or PSMA-positive prostate cells.

In summary, in this study we have shown that PSCA protein and mRNA are maintained in expression from HGPIN through all stages of PCa in a majority of cases which are probably associated with prostate carcinogenesis and correlate positively with high tumor grade (poor cell differentiation), advanced stage and androgen-independent progression. PSCA protein overexpression may be partially due to overrepresentation of PSCA mRNA. The results suggest that PSCA may be a promising molecular marker for the clinical prognosis of human PCa and a valuable target for diagnosis and therapy of this type of tumor.

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