

Prostate Stem Cell Antigen: A Jekyll and Hyde Molecule?

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Abstract

Prostate stem cell antigen (PSCA) is a glycosylphosphatidylinositol (GPI)-anchored cell surface protein. Although PSCA is thought to be involved in intracellular signaling, much remains unknown about its physiological function and regulatory mechanism in normal and cancer cells. It is up-regulated in several major cancers including prostate, bladder, and pancreatic cancers. The expression of PSCA is positively correlated with advanced clinical stage and metastasis in prostate cancers and is also associated with malignant progression of premalignant prostate lesions. Therefore, PSCA has been proposed as a biomarker of diagnosis and prognosis, as well as a target of therapy for these cancers. In addition, PSCA has also shown clinical potential in immunotherapy as a prostate-specific antigen, which, when presented by dendritic cells, may elicit strong tumor-specific immunity. In contrast, PSCA is down-regulated in esophageal and gastric cancer and may have a tumor-suppressing function in the gastric epithelium. Recent exciting findings that genetic variations of *PSCA* conferred increased risks of gastric cancer and bladder cancer have opened up a new avenue of research about the pathological function of PSCA. PSCA seems to be a Jekyll and Hyde molecule that plays differential roles, tumor promoting or suppressing, depending on the cellular context. *Clin Cancer Res*; 16(14); 3533–8. ©2010 AACR.

Background

Prostate stem cell antigen (PSCA) is a small, glycosylphosphatidylinositol (GPI)-anchored cell surface protein belonging to the Thy-1/Ly-6 family. It shares 30% homology with stem cell antigen type 2 (SCA-2), a surface marker of immature lymphocytes (1). In human, the PSCA is expressed in the epithelial cells of prostate, urinary bladder, kidney, skin, esophagus, stomach, and placenta (1–4). Although it was originally designated as a “stem cell antigen” for similarity to SCA-2, PSCA is now known to be expressed mainly in differentiating cells rather than stem cells, which was shown by studies on prostate and gastric epithelial cells (5, 6). Other than the expression patterns, the physiological functions of the PSCA remain an enigma. PSCA knockout mice were viable and showed no gross abnormal phenotype (7). The Thy-1/Ly-6 family to which PSCA belongs does not seem to offer many clues, because the family members show a remarkable functional diversity ranging from T-cell activation (8) to apoptosis regulation in the nervous system (9).

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doi: 10.1158/1078-0432.CCR-09-3169

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Initially PSCA was identified and isolated as a tumor antigen overexpressed in prostate cancer (1), and subsequent investigations have revealed that it is also up-regulated in urinary bladder cancer, renal cell carcinoma, pancreatic cancer, hydatidiform mole, and ovarian mucinous tumor (10–14). Remarkably, it is down-regulated in esophageal and gastric cancers (2, 6).

Although little is known about the regulatory mechanism of PSCA expression, it is certain that androgen is involved in the PSCA regulation, at least in prostate epithelium, because an androgen responsive element was identified in its promoter region (15). Transgenic mice introduced with *PSCA* promoter-driven green fluorescent protein (GFP) constructs showed that the GFP expression was influenced by puberty, castration, and androgen restoration (16). In human, complete androgen ablation suppresses PSCA mRNA expression in human prostate carcinoma *in vivo* (17). In the bladder carcinoma cell line RT112, PSCA expression was stimulated by a culture dish surface that causes aggregation of cells, and by phorbol ester in a cycloheximide- and actinomycin-inhibitable manner, indicating that its expression is regulated by mechanisms related to the adhesion of epithelial cells and by some pathways involving protein kinase C and newly synthesized protein(s); see ref. 18. PSCA was recently reported to be down-regulated in telomerase-transduced urothelial cells (19), suggesting that PSCA may be regulated by some telomerase-related mechanism.

Although members of the GPI-anchor proteins have the GPI-moiety, a common feature for the family members, they have diverse structures and functions (20). In mammals, GPI-anchored proteins lacking a transmembrane domain are believed to be located in the lipid raft (Fig. 1),

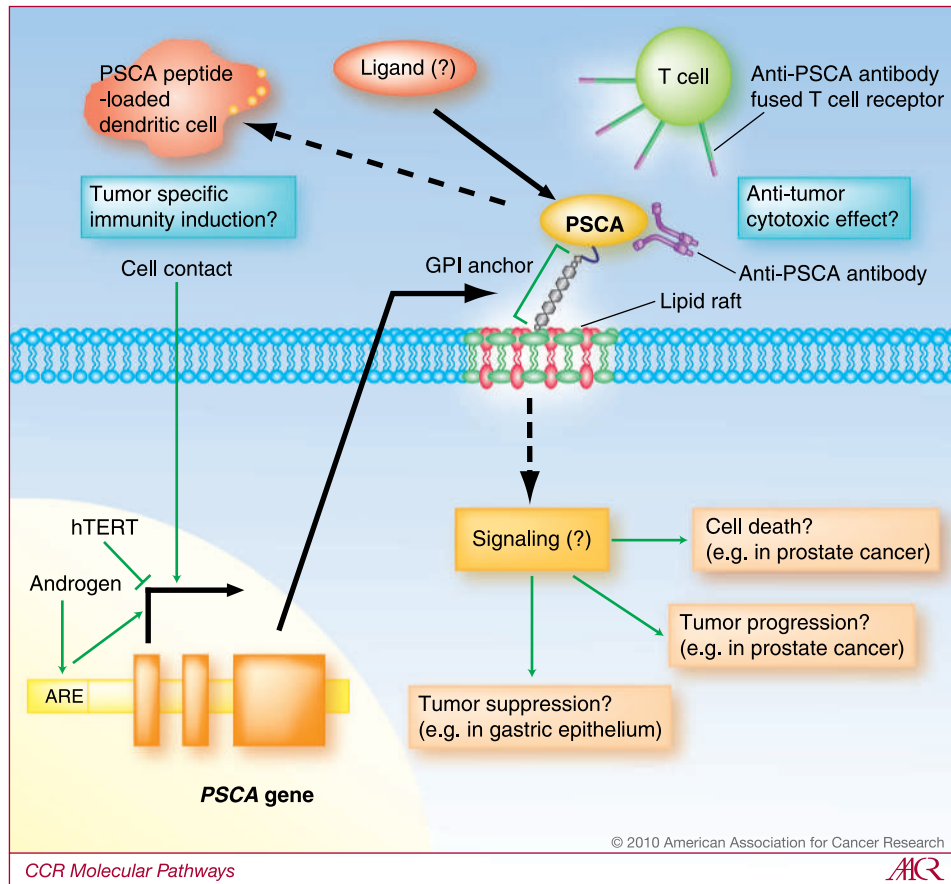


Fig. 1. Presumptive PSCA signaling pathway and PSCA-targeted immunotherapy. In the prostate epithelium, PSCA expression is induced by androgen through the binding of androgen-androgen receptor complex to androgen responsive element (ARE) located at the upstream of the gene. Its expression is also up-regulated by cell-cell contact *in vitro*. It was reported that introduction of human telomerase reverse transcriptase (hTERT) to urothelial cells suppresses PSCA expression. After translation, PSCA is sorted to endoplasmic reticulum, in which it is attached with GPI and transported to the outer surface of the cell membrane, where it is anchored to the outer lipid layer via fatty-acid chain of the GPI-moiety. As other GPI-anchored proteins, PSCA is believed to be located to a specialized microdomain, the so-called lipid raft, in the cell membrane. PSCA may play multiple roles in cell-death induction, tumor progression, and tumor suppression. However, the ligand of PSCA is not yet identified and the downstream signaling pathway and the physiological function are unknown. PSCA-targeted immunotherapy could be a new therapeutic option for hormone therapy-refractory prostate cancer. Possible strategies include direct cytotoxic effects by anti-PSCA antibody or by T cells with T-cell receptor genetically fused to anti-PSCA antibody, and induction of tumor specific immunity by DCs loaded with PSCA peptide.

which is still a somewhat hypothetical microdomain on the surface of the outer cell membrane; however, several pieces of biological evidence support its existence and propose that it is detergent-insoluble and enriched for sphingolipids and cholesterol (20). The structure of PSCA suggests at least two distinct mechanisms of its potential function. The first possibility is that PSCA may form a complex with another protein that has a transmembrane domain and intracellular domain to activate a downstream target. In this regard, it is interesting to note that on the basis of a protein motif scan, (http://myhits.isb-sib.ch/cgi-bin/motif_scan) PSCA contains an activin type I and II extracellular receptor domain, which binds to the transforming growth factor beta (TGF- β) superfamily of ligands and plays important roles in many cellular functions (21). Evolutionally, the Ly-6 family and activin receptor family are closely related and cluster together at the family level (22). It would be inter-

esting to test whether TGF- β family ligand binds to PSCA and whether there are transmembrane proteins on the cell surface that partner with PSCA and transmit signals. The second potential mechanism of PSCA function is through the cleavage of the GPI anchor by phospholipase C, which will release PSCA from membrane into secretion and act through a receptor-mediated signaling pathway. There have been no reports of any proteins that bind to PSCA and the identification of such proteins would shed significant light into the biologic function of PSCA.

A mouse monoclonal anti-PSCA antibody 1G8 inhibited tumor growth, prevented metastasis, and prolonged the survival of mice harboring inoculated human prostate cancer xenografts (23), which is probably through inducing caspase-independent cell death by cross-linking the PSCA proteins (24). On the other hand, PSCA was found to prevent the subpopulation of choroid cells in chicken

brain from cell death by modulating a signaling pathway involving $\alpha 7$ -containing nicotinic acetylcholine receptors (9). In addition, PSCA showed cell growth inhibition activity for a gastric cancer cell line without a significant induction of cell death (6). These paradoxical observations, together with the up- and down-regulation of PSCA in different human tissues, suggest that PSCA may be oncogenic for some epithelial cells and also be a tumor suppressor for others. This kind of Jekyll and Hyde molecule is not unprecedented. For example, in normal epithelium, Mucin 1 (MUC1) is protective against potentially tumorigenic environmental insults, but after significant epithelial damage that results in the loss of cell polarity and change in the membrane proteins' distribution, it becomes promotive for cancer cell growth and survival (25). Likewise, the Wilms' tumor 1 (WT1; ref. 26) and NOTCH gene (27) may function either as an oncogene or a tumor suppressor depending on the cellular context and the crosstalk with other cellular molecules and pathways.

In sum, although mechanistic details are unknown, the accumulated findings suggest that PSCA has a functional diversity depending on both tissue types and cell status, normal or malignant.

Clinical-Translational Advances

Clinical application of PSCA in prostate cancer

Because PSCA was originally isolated as a tumor antigen overexpressed in prostate cancer, investigation for its clinical application has mainly focused on prostate cancer. PSCA expression was detected in about 90% of primary prostate cancers, and the expression level is positively correlated with advanced clinical stage, invasion to seminal vesicle and prostate capsule, and progression to androgen-independence (1, 3, 28, 29). PSCA expression was also detected in the metastatic sites at bone, lymph node, and liver (65 to 100%, dependent on the organs and reports; refs. 3, 30). One study reported increased PSCA-gene copy number in 71% (five of seven cases) of prostate cancer with *MYC* gene amplification, and both genes were located in the same Chr. 8q amplicon, suggesting that gene amplification is the major cause of the overexpression of PSCA in prostate cancer (31). PSCA is approximately 15 Mb distal to *Myc* oncogene on 8q24, which is one of the most frequently amplified regions in human cancers (32). It is conceivable that increased copy number of PSCA is the major cause of the observed PSCA overexpression in some tumors. A study on 117 prostate biopsy specimens of prostate intraepithelial neoplasia (PIN) revealed that PSCA expression was higher in high-grade PIN (HGPN), a premalignant condition, than in low-grade PIN. Moreover, the expression levels were elevated in the PIN lesions that subsequently progressed to cancer compared with those that did not progress (33). This value of PSCA expression in predicting cancer progression was also observed in patients with benign prostatic hyperplasia (34, 35).

PSCA also seems to be a useful marker for a detection of metastasis and circulating tumor cells (CTC). More than 90% of lymph node and bone specimens with metastasis were positive for PSCA expression (36). PSCA expression was not detectable in peripheral blood samples of 71 non-malignant controls and 41 cases of prostate cancer confined to the organ, but detectable in 8 of 17 prostate cancers of extraprostate invasion (37). However, another study on blood samples showed a lower sensitivity for the castration-refractory prostate cancer cases (38). Combined use of other molecules, e.g., prostate-specific antigen (PSA), may overcome this limitation. The presence of PSCA transcripts in the peripheral blood was also a significant predictor of biochemical recurrence after radical prostatectomy in high-risk prostate cancer (39).

The PSCA expression also could be used as an index in the evaluation of therapeutic effect. After treatment of HGPIN with flutamide, an androgen receptor antagonist, 66 patients who showed reduction of PSCA mRNA in the prostate tissue did not develop cancer on follow-up, whereas 11 of 13 cases with increased PSCA expression levels developed cancer afterward (40). The reduction of PSCA expression in the prostate tissue was also shown in localized prostate cancer cases after external beam radiotherapy (41).

In addition to its potential applications as a diagnosis and prognosis biomarker, PSCA has been suggested as a therapeutic target. Current standard treatments for patients with localized prostate cancer are radiation, hormonal therapy, and radical prostatectomy. For patients with metastases, an androgen-ablation therapy is the first choice, which has shown a benefit in 70 to 80% of the cases (42). When the prostate cancer becomes refractory to the hormone therapy, PSCA gene-based strategies may offer a new therapeutic option.

The first step of mounting an immune response is the antigen presentation by dendritic cells (DC). Vaccination with tumor antigen-loaded DC is hypothesized to be a powerful therapeutic strategy to potentiate a tumor-specific immunity induction, and several studies have been conducted to identify a prostate cancer-specific tumor antigen suitable for the loading (43). Effect of PSCA peptide-loaded DC has already been evaluated in a phase I-II trial for patients with hormone- and chemotherapy-refractory prostate cancer (44). Of 12 patients entered into the trial, 5 patients developed delayed-type hypersensitivity reaction, indicating that the patients had obtained the tumor antigen-specific immunity. The patients tended to be free of disease progression and showed superior overall survival (median survival, 22 months) compared with the remaining patients (8 months). In another phase I-II study in advanced patients with hormone-refractory prostate cancer, DC loaded with a mixture of peptides from four prostate-specific antigens, PSCA, PSA, PSMA, and PAP, were shown to elicit strong cytotoxic T-cell response against all these tested tumor antigens. Clinically, the long-term DC vaccination was associated with an increase in PSA doubling time (45). In light of the recent approval of the first anticancer vaccine, Provenge

(Dendreon Corporation, Seattle, WA), against advanced prostate cancer by the U.S. Food and Drug Administration (FDA), the clinical potential of PSCA as a tumor antigen in immunotherapy is promising.

Some other strategies for immunotherapy involving the PSCA gene have been tested preclinically. Introduction of a PSCA-expression plasmid into mice harboring transplanted prostate cancer cells inhibited tumor growth via generating PSCA-specific CD8⁺ T-cell immune response (DNA vaccination; refs. 46–48). T-cell containing chimeric T-cell receptor (TCR), which was generated by fusing an anti-PSCA antibody single-chain fragment to the β -chain of TCR, showed potent cytotoxicity against PSCA-positive tumor cells (49).

Cytotoxic therapy targeting PSCA is another potential clinical application. As described before, anti-PSCA monoclonal antibody 1G8 exhibited tumor growth inhibition, metastasis prevention, and prolongation of the survival of mice inoculated with human prostate cancer xenografts (23, 24). Humanized 1G8 was generated by grafting complementarity determining regions to the anti-p185 4D5va (trastuzumab) framework and the radioiodinated antibody was shown to have specificity for PSCA. The localization of 1G8 to the prostate cancer xenograft was shown in mouse bodies with high-contrast micro positron emission tomography (microPET) imaging (50–52).

Clinical application in other cancers

PSCA has also been investigated as a biomarker for other cancers. In urinary bladder cancer, immunocytochemical study of PSCA was applied to voided urine specimens and the sensitivity of transitional cell carcinoma detection was 80% for PSCA immunocytochemistry alone compared with 46.7% for cytology alone. Combining cytology with PSCA staining increased the sensitivity to 84% without decreasing the specificity significantly, suggesting that immunocytochemical analysis of PSCA on voided urine samples may provide a simple and quantitative adjunct marker for cytological diagnosis of urothelial bladder cancer (53). On the other hand, despite its up-regulation in urinary bladder cancer over normal urothelium, PSCA had significantly higher expression in superficial than in invasive bladder cancer ($P < 0.001$), and there was a significant inverse relationship between PSCA expression and recurrence in superficial bladder cancer (54).

Pancreatic cancer has aberrantly up-regulated PSCA in nearly 60% of cases, whereas the gene is not expressed in normal pancreatic duct (12). PSCA was proposed as a specific biomarker of pancreatic adenocarcinoma cells in cytologic examination of fine-needle aspiration specimens (55) and as a biomarker for the detection of CTCs in peripheral blood of pancreatic cancer patients (56). Elevation of immunoglobulin G (IgG) reactive to PSCA-derived peptides was shown in 80% of pancreatic cancer, but only in 18% of subjects without the cancer (57). Radiolabeled and bioconjugated anti-PSCA antibodies have been tested for diagnostic imaging of pancreatic cancer (58, 59). As in prostate cancer, an anti-PSCA antibody 1G8 showed an in-

hibitory effect on tumor growth and progression in a mouse model of a pancreatic cancer xenograft (60). It should be noted, however, that PSCA may have a dual function, oncogenic and tumor suppressive, depending on the tissue types and malignant status of the cells. A close evaluation of a potential adverse effect on various organs may be required in the development of a PSCA-targeting systemic therapy.

PSCA as a cancer susceptibility gene: genome-wide association study

Although PSCA has been identified for more than a decade, the investigations have been largely superficial and have focused on its potential application as a biomarker and therapeutic target, as described above. However, several unexpected, exciting findings from a totally different field of research, genome-wide association studies (GWAS), have injected fresh life into the research of PSCA. We found a significant association of a functional single nucleotide polymorphism (SNP) in the PSCA gene rs2294008 with the risk of gastric and bladder cancers in two separate GWAS (6, 61). Two recent case control studies confirmed the association of this SNP with gastric and bladder cancer in the Chinese population (62, 63). The rs2294008 SNP is a missense SNP that alters the start codon of PSCA. In addition, *in vitro* reporter assays showed that the risk allele reduced the transcriptional activity of the PSCA promoter in both gastric and bladder cell lines (6, 61). Analysis of PSCA mRNA expression in 16 adjacent normal bladder tissues showed a significant correlation of the risk allele with low expression, consistent with *in vitro* data (63). These findings are congruous with the notion that PSCA may act as a tumor suppressor (6). No cases of somatic or germline mutations in the PSCA gene have been reported in any cancers to date. It is perplexing that the risk allele is the same and the reduced transcriptional activity is consistent in both gastric and bladder cancers, even though the PSCA gene is down-regulated in gastric cancer but up-regulated in bladder cancer. Nevertheless, these findings have opened up a new avenue of research about the pathological role of PSCA and created a new application of PSCA in cancer risk prediction.

Future directions

PSCA is up-regulated in prostate, urinary bladder, and pancreatic cancers and its clinical utility has been shown in the diagnosis and prognosis of these cancers. PSCA has also been investigated as a potential target for tumor-targeted immunity and for direct suppression by anti-PSCA antibody. Moreover, the PSCA germline genetic variation has been associated with the risk of specific cancers including gastric and urinary bladder cancers. However, it should be stressed that our knowledge of the physiological and pathological functions of PSCA is still quite limited. It seems that PSCA is a Jekyll and Hyde molecule with dual functions depending on the context of tissue specificity and pathophysiological conditions. There

are many challenges and opportunities ahead. The priority of research is to find the physiological functions of PSCA, its ligand, and its downstream signaling cascade. To determine the regulatory mechanisms of its expression in different normal and tumor tissues is essential to decipher its dual role of tumor promoting and suppressing functions. The functional consequence of the risk allele of rs2294008 on PSCA protein function and cellular trafficking and the biological mechanisms for the increased cancer risks associated with rs2294008 are a completely new territory. It will be of interest to know whether genetic variations in PSCA are associated with other cancers and whether there are somatic mutations of the PSCA gene in certain cancers. The clinical applications of PSCA need to be further vali-

dated and explored. The next decade of research on PSCA will undoubtedly be exciting and fruitful.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

J. Gu, NIH R01CA131335; T. Yoshida, The program for promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation; X. Wu, NIH U01CA127615, R01CA74880, and P50CA91846.

Received 03/29/2010; revised 04/25/2010; accepted 05/03/2010; published OnlineFirst 05/25/2010.

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