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Prostate Stem Cell Antigen: A Prospective Therapeutic and Diagnostic Target

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Abstract

The development of novel clinical tools to combat cancer is an intense field of research and recent efforts have been directed at the identification of proteins that may provide diagnostic, prognostic and/or therapeutic applications due to their restricted expression. To date, a number of protein candidates have emerged as potential clinical tools in the treatment of prostate cancer. Discovered over ten year ago, prostate stem cell antigen (PSCA) is a cell surface antigen that belongs to the Ly-6/Thy-1 family of glycosylphosphatidylinositol-anchored proteins. PSCA is highly overexpressed in human prostate cancer, with limited expression in normal tissues, making it an ideal target for both diagnosis and therapy. Several studies have now clearly correlated the expression of PSCA with relevant clinical benchmarks, such as Gleason score and metastasis, while others have demonstrated the efficacy of PSCA targeting in treatment through various modalities. The purpose of this review is to present the current body of knowledge about PSCA and its potential role in the treatment of human prostate cancer.

Keywords

prostate stem cell antigen; prostate cancer; immunotherapy

1. Introduction

Prostate cancer is the most commonly diagnosed nondermatological malignancy and the second leading cause of cancer-related death in American men [1]. Although there have been advances in the treatment of primary, localized prostate cancer with surgery and/or radiation therapy, therapeutic options for men with metastatic, hormone-refractory disease or for those who fail local treatment have made limited progress [2–4]. Considering that 10–20% of prostate cancer patients present with metastatic cancer and approximately 50% of patients will ultimately fail treatment for local disease [3], the need for novel strategies to combat prostate cancer is paramount.

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Conflicts of Interest Statement

None Declared

One potential promising strategy for this disease is immunotherapy. Prostate cancer immunotherapeutics use the patient's immune system to detect and kill cancer cells by targeting prostate cancer associated proteins expressed by tumor cells. To that end, prostate stem cell antigen (PSCA) represents an ideal choice for immunotherapy because it is highly expressed by a large proportion of human prostate tumors, including metastatic and hormone refractory cancers, but has limited expression in normal tissues [5–8].

This restricted expression profile of PSCA also makes it a potential diagnostic and prognostic biomarker for prostate cancer. Through rapid, cost-effective screening in the blood or analysis of expression levels on biopsies for example, PSCA may alert patients about the development of undiagnosed prostate cancer or provide clues concerning the aggressiveness and/or propensity to metastasize of established tumors. In this way, PSCA may help clinicians and researchers better identify patients that require additional interventions and offer a potential target for treatment through immunotherapy.

2. Prostate Stem Cell Antigen

The PSCA gene was originally identified through an analysis of genes up-regulated in the human prostate cancer LAPC-4 xenograft model [5]. The PSCA gene is located on chromosome 8q24.2 and encodes a 123 amino acid cell surface protein with 30% homology to stem cell antigen type 2 (SCA-2), an immature lymphocyte cell surface marker. It is because of this relatively weak homology that PSCA was named inaccurately since it is not a marker for a stem cell population nor is it exclusively expressed in the prostate. Like SCA-2, PSCA is a member of the Thy-1/Ly-6 family of glycosylphosphatidylinositol (GPI) anchored surface proteins. Additionally, a murine PSCA (mPSCA) homologue has been identified with 70% homology to human PSCA at the nucleotide and amino acid levels, which is located on chromosome 15 [5].

The function of PSCA in normal cellular processes or carcinogenesis is currently unknown, however PSCA homologues shed light on a variety of possible roles. It has been previously shown that GPI-anchored proteins, like PSCA, play a role in T cell activation [9]. Additionally, proteins in the Thy-1 family have been reported to function in T cell activation and proliferation, stem cell survival, and cytokine and growth factor responses [10]. Furthermore, the family of Ly-6 genes has been associated with carcinogenesis [11,12], cellular activation [13] and cell adhesion of tumor cells [14]. While these studies point to potential roles of PSCA, no definitive function has been attributed to the protein either in normal cellular processes or abnormal tumorigenesis. Interestingly, a recent study showed that PSCA homozygous knockout mice are viable, with both male and female mice fertile into adulthood with normal litter size and gender distribution [15]. This data suggests that PSCA in mice is not critical for either normal development or urogenital function. Furthermore in the same study, PSCA homozygous knockout mice did not have an increased incidence of cancer formation, even when they were further subjected to γ -radiation.

3. PSCA Expression in Normal Tissues

With any potential immunotherapeutic target it is necessary to assess the normal tissue expression. Ideal targets show overexpression on target cells with limited or no expression on normal tissues, thereby reducing the risk of autoimmunity as a side effect. PSCA messenger RNA (mRNA) expression in normal human tissues was found to be predominantly expressed in prostate, with a lower expression in placenta and a very low (1% of the level in prostate) amount in kidney and small intestine [5,16]. *In situ* hybridizations of normal human prostate sections found PSCA mRNA expression limited to the subjacent basal cells, with little or no staining of secretory luminal cells [5]. In contrast, a separate study demonstrated that PSCA expression was localized to the secretory epithelial cells, with no expression in the basal cells

or prostatic stroma [6]. This apparent disagreement in PSCA mRNA localization may be due in part to the relatively short half-life of mRNA in tissues combined with the sampling error inherent in small sectional biopsies within a larger tissue. To clarify this discrepancy, another study analyzed PSCA protein in the prostate, a much more stable form of expression, using monoclonal antibodies (mAbs) and found PSCA protein to be present in both the basal and secretory epithelial cell layers, along with the neuroendocrine cells of the prostate [7]. Additionally, in this study PSCA protein expression was demonstrated in the placenta, the bladder, the neuroendocrine cells of the stomach and colon, and weakly in the kidneys excluding the glomeruli.

Expression of the mouse PSCA homologue in normal tissues follows a similar pattern. In mouse fetal tissues, mPSCA mRNA expression was detected in the superficial epithelial lining of the urogenital sinus beginning at embryonic day 14 and maintained throughout fetal development [17]. Using a PSCA promoter driven green fluorescent protein (GFP) expression mouse model, GFP expression was detected at one week of age in most regions of the prostatic duct and increased dramatically over the following 4 weeks during puberty [18]. In the adult mouse tissues, mPSCA mRNA was limited to the urogenital tissues, specifically the prostate, urethra, bladder and kidney [17]. This expression was seen focally in the luminal cells of all three prostatic lobes, with no apparent expression in the basal cell population. By flow cytometry, mPSCA was detected on ~20% of normal adult prostate cells, decreasing as the mice aged [19]. In the PSCA-GFP model, as the mice aged GFP expression became further restricted to columnar cells in the distal regions of the prostatic ducts [18].

4. PSCA Expression in Cancer

In the initial study on PSCA expression in human prostate cancer, 102 of 126 (81%) primary human prostate tumors specimens along with 7 of 9 (78%) residual tumors removed after androgen ablation therapy stained strongly for PSCA using mRNA *in situ* hybridization [5]. Analysis by immunohistochemistry (IHC) using an anti-PSCA mAb demonstrated 105 of 112 (94%) prostate tumors positive for PSCA, of which 21% stained very strongly and 63% stained moderately [7]. Importantly, this study also revealed a significant correlation between PSCA expression and Gleason score, pathologic tumor stage and progression to androgen-independence. A later study also significantly correlated high PSCA intensity with seminal vesicle invasion and capsular involvement [8]. An additional study showed a higher percentage of metastatic prostate cancer cases staining positive for PSCA mRNA compared with nonmalignant prostate disease and organ-confined prostate cancer [6]. Specifically, prostate cancer metastases to bone marrow, lymph node and liver stained positively for PSCA expression, with bone marrow metastases staining with comparatively higher intensity [7,20].

To further explore PSCA as a putative prostate cancer immunotherapy target, several studies have detailed the mPSCA expression profile in mouse models that spontaneously generate prostate cancer. One well-known and well-characterized prostate cancer mouse model is the transgenic adenocarcinoma of the mouse prostate (TRAMP) model [21,22]. In the TRAMP model, the prostate specific probasin promoter directs the expression of the SV40 large T antigen in the prostatic epithelium. By 8 – 12 weeks of age, all male TRAMP mice develop prostatic intraepithelial neoplasia (PIN) and by 19–25 weeks all male mice develop prostate cancer. Analysis at different stages of prostatic neoplasia progression in the TRAMP model demonstrated a significant increase in mPSCA expression in foci of PIN and invasive prostate cancer compared to normal prostate [17,23]. mPSCA expression was positive on 24% and 73% of cells in the lateral prostates of 21- and 25-week-old TRAMP mice, respectively [19]. Additionally, mPSCA mRNA was highly up-regulated in metastatic samples from lymph node, liver and viscera of TRAMP mice [23]. Interestingly, when TRAMP mice were crossed with PSCA wild-type (+/+), PSCA heterozygous knockout (+/-) or PSCA homozygous knockout

($-/-$) mice, the resulting TRAMP+PSCA $+/-$ and TRAMP+PSCA $-/-$ mice had significantly increased metastases to the liver, kidney, lung and lymph nodes compared to TRAMP+PSCA $+/+$ [15]. The implications of this study may support the concept of tumor immunosurveillance of prostate tumors that overexpress PSCA. Specifically the immune system may be able to recognize the aberrant PSCA expression on the tumor cells and limit metastasis in PSCA wild-type mice, while PSCA knockout mice would lack this immunosurveillance target and therefore be more susceptible to distant metastasis. Another interpretation on this study may point back to the potential function of PSCA as a regulator of cell adhesion, like the PSCA homologue E48, a human GPI-anchored Ly-6 protein [14]. A loss of PSCA may cause a decrease in cell adhesion molecules that usually retain tumor cells at the primary site of carcinogenesis, thereby increasing the chance of metastasis formation.

In a separate prostate cancer mouse model, PTEN $+/-$ mice develop a broad range of spontaneous tumors, approximately 14% of which are prostate tumors, because of inactivation of one allele of the tumor suppressor gene *PTEN* [24]. Male PTEN $+/-$ mice develop PIN with near 100% penetrance but the latency for formation is approximately 10 months [25]. Analysis of prostates from 11–18-month-old PTEN $+/-$ mice revealed enlarged prostates with increased percentages of PSCA positive cells compared to wild-type mice of the same age [19].

Whether overexpression of PSCA in humans is causative of prostate carcinogenesis remains unknown. Studies have shown chromosome 8q to be commonly amplified in metastatic and recurrent prostate carcinoma and associated with a poor prognosis [26,27]. Additionally, PSCA is located close to the c-myc oncogene, and therefore PSCA expression may be increased incidentally with amplification of other genes shown to be up-regulated in prostate cancer [28,29]. These results also suggest that PSCA is a useful marker of MYC amplification on prostate cancer cells, allowing better characterization of each tumor [30].

Of interest, subsequent to the identification of PSCA as a prostate tumor associated protein, several other tumors were evaluated for PSCA expression including pancreatic adenocarcinoma [31–33], transitional cell carcinoma [34,35], renal cell carcinoma [36] and diffuse-type gastric cancer [37]. For pancreatic adenocarcinoma, overexpression of PSCA protein was established via IHC in 36 of 60 (60%) primary tumors analyzed, while adjacent normal pancreas had no expression in 59 of 60 samples [31]. Additional analysis of pancreatic cancer cell lines confirmed the cell surface expression of PSCA, making it a suitable target for immunotherapy [33]. When human transitional cell carcinoma (TCC) was studied, PSCA was expressed in a majority of TCC and a higher level of PSCA expression correlated with increasing tumor grade [35] and was an independent predictor of disease recurrence [34]. In addition, PSCA mRNA expression was increased in renal cell carcinoma and the gene expression level correlated with histological grade, pathological stage and prognosis [36]. Finally, in an interesting recent article, polymorphism of the PSCA gene was shown to influence susceptibility to diffuse-type gastric cancer [37].

5. PSCA in Diagnosis and Prognosis

The use of biomarkers for the detection and progression of prostate cancer has generated increasing interest since the development of prostate specific antigen (PSA) for prostate cancer screening. Unfortunately, over time it has become clear that the clinical application for PSA is not ideal for cancer detection and treatment, and therefore significant effort continues to be devoted to the discovery and development of novel biomarkers [38]. Due to increased PSCA expression in prostate cancer, particularly its correlation with Gleason score [7] and up-regulation in metastasis [20], it has become one of many investigated potentials. In the initial study using PSCA as a prostate cancer biomarker, a cost-effective and rapid reverse transcription-polymerase chain reaction (RT-PCR) screen from a milliliter of blood detected

PSCA mRNA in 13.8% of prostate cancer cases [39]. Notably, stage IV patients with positive PSCA mRNA correlated with a lower disease-free survival than those with negative PSCA mRNA, suggesting that PSCA may be promising for the molecular staging of prostate cancer. In a recent study [40], men with benign prostatic hyperplasia (BPH) treated with transurethral resection of the prostate (TURP) were screened for PSCA mRNA expression and subsequent prostate cancer. In total, the PSCA mRNA expression was not particularly strong in the BPH tissues, with 93 of 288 (32.3%) cases positive for PSCA. However, in the 93 patients with positive expression for PSCA mRNA, 23.7% subsequently developed clinical prostate cancer over the follow-up period versus only 1.0% of the 195 patients with negative expression for PSCA mRNA. Of note, the level of positive expression of PSCA mRNA was directly proportional to the Gleason score and clinical stage of the resulting prostate cancer.

6. PSCA as a Therapeutic Target

The ultimate goal in prostate cancer research is to discover, design and develop better diagnostic, prognostic and therapeutic options for patients. Due to the restricted pattern of expression, PSCA is an attractive candidate target protein for immunotherapy. Several immunotherapeutic strategies targeting PSCA have been explored, including monoclonal antibodies, antibodies conjugated to cytotoxins, genetically engineered T cells, PSCA vaccination and peptide-loaded dendritic cells (DC).

Using an orthotopic model of prostate cancer, where LAPC-9 human prostate cancer cells were injected directly into the prostates of SCID mice, anti-PSCA mAbs were explored for their efficacy in inhibiting tumor growth, decreasing metastasis formation and prolonging survival [41]. Because mice do not express PSA, the researchers were able to use PSA levels to track tumor growth in mice since the tumor cells injected were from human prostate cancers, which do express PSA. The researchers found that mice with low established tumor burden (PSA levels of 2–3 ng/ml) when treated with 1G8 mAb, an anti-PSCA antibody, showed a significantly prolonged survival, with an increased median survival of 32.5 days over PBS-treated mice. Mice with higher established tumor burden (PSA 64–78 ng/ml) treated with 1G8 again showed a significantly prolonged survival, with an increased median survival of 38.5 days above PBS-treated mice. To explore anti-PSCA mAb treatment in the prevention of metastasis formation, mice in both treated and PBS-treated groups were allowed to develop high tumor burdens (PSA levels > 300 ng/ml) to ensure sufficient numbers of lung metastasis. While PBS-treated mice had considerable lung metastases, the majority of 1G8 treated mice had no micrometastases and the remainder had only a few, demonstrating a significant inhibition of metastatic tumor formation with anti-PSCA mAb treatment. In a later publication [42], the mechanism of tumor growth inhibition by 1G8 mAb was evaluated *in vitro* and *in vivo*. *In vitro* experiments revealed similar abilities to kill prostate tumor cells between either 1G8 bivalent F(ab')₂ or whole antibody, while monovalent F(ab') had no effect, pointing to a direct mechanism of action that is not mediated by complement but that requires antigen cross-linking. Furthermore, the addition of a cell-permeable, broad-spectrum caspase inhibitor caused no loss of efficacy, suggesting that 1G8-induced cell killing is caspase independent. The ability of 1G8 whole antibody and 1G8 bivalent F(ab')₂ to prevent new tumor formation and inhibit growth of preexisting prostate cancer xenografts *in vivo* was equivalent and therefore consistent with the Fc-independent mechanism seen *in vitro*.

In a slightly different strategy, an anti-PSCA mAb conjugated to maytansinoid DM1, a potent antimicrotubule agent, was evaluated as a potential prostate cancer immunotherapy [6]. The antibodies used bind specifically to cell surface PSCA and are internalized via the caveolae pathway. When nude mice injected subcutaneously with PC3 cells expressing PSCA developed a tumor volume of 200 mm³, they were treated with anti-PSCA-DM1 and the tumor volumes

declined to the extent of complete tumor regression. Histological evaluation of the tumor inoculation site demonstrated the absence of residual tumor cells.

While they retain the potential to be useful therapeutic strategies, the use of antibodies in cancer therapy has been hampered by the limited penetration of solid tumors and the minimal half-life of the administered antibodies. In contrast, cell-mediated immunotherapies, such as those that focus on cytotoxic T lymphocytes (CTL), are able to effectively invade tumors and kill cancer cells without repeated administration, as demonstrated in a number of clinical trials [43,44]. In a recent study [45], genetically engineered T cells were generated to express a chimeric T cell receptor (TCR) recognizing PSCA. To accomplish this, a monoclonal antibody to PSCA was generated and its single-chain fragment was prepared and subsequently fused to the $\beta 2$ constant region of a TCR and to the CD3 ζ -signaling domain creating the chimeric α -PSCA- $\beta 2$ /CD3 ζ TCR. To determine the functionality of this chimeric TCR, a murine cytotoxic T cell line, MD.45, was transduced with the α -PSCA- $\beta 2$ /CD3 ζ TCR or an empty vector and mixed with either ^{51}Cr labeled PSCA-positive RT4 bladder carcinoma cells or PSCA-expressing HEK 293 cells. As shown by a chromium release assay, MD.45 cells expressing the α -PSCA- $\beta 2$ /CD3 ζ TCR efficiently lysed both PSCA positive cell lines, while two cell lines negative for PSCA were not lysed. While these results are encouraging for the feasibility of cell-mediated immunotherapies targeting PSCA, this study stopped short of testing the genetically engineered T cell *in vivo*, which is ultimately required before further conclusions can be made.

In a recent publication [46], a PSCA-based vaccine administered to prostate cancer prone TRAMP mice showed long term protection against prostate cancer development. As described above, the TRAMP mice develop *in situ* and invasive carcinomas of the prostate that progress through the natural course of human prostate cancer progression from PIN to androgen-independent disease. In this particular study, the prime-boost vaccination strategy employed consisted of first priming with mPSCA cDNA and then boosting with recombinant alphavirus replicon particles expressing mPSCA (mPSCA-VRP). This type of heterologous prime-boost vaccine regime has been shown previously to induce a better immune response than plasmid DNA alone [47]. Initial experiments using mice challenged with the TRAMP-C2 murine prostate tumor cell line resulted in 19 of 25 mice vaccinated with mPSCA DNA and boosted with mPSCA-VRP remaining tumor free for 52 days. This vaccination-induced protection was accompanied by a statistically significant increase in the number of tumor infiltrating CD4 $^{+}$ and CD8 $^{+}$ T cells, along with a statistically significant increase in the intratumoral levels of cytokines important in antitumoral immunity, such as IL-2, IFN- γ , and granulocyte macrophage colony-stimulating factor (GM-CSF). When CD4-deficient and CD8-deficient mice were used in a similar experiment, all mice developed tumors, indicating that both CD4 $^{+}$ and CD8 $^{+}$ T cells are essential for tumor immunity in this vaccination strategy. When the PSCA prime-boost vaccination was moved into the TRAMP mouse model, survival of vaccinated TRAMP mice was significantly prolonged over the control-vaccinated TRAMP group. Over 320 days, only 4 of 20 mPSCA-vaccinated TRAMP mice died, while 16 of 20 mice from the control-vaccinated TRAMP group died. Those tumors present in the vaccinated group had lower Gleason scores, increased numbers of infiltrating immune cells and increased intratumoral cytokines compared to control-vaccinated TRAMP mice. This specific immune response against mPSCA did not induce pathologic autoimmunity, as demonstrated by titers of rheumatoid factor or anti-DNA autoantibodies equivalent to control levels and no infiltration of inflammatory cells or tissue damage in organs where PSCA is normally expressed.

Moving into human clinical studies, in a preliminary clinical trial with three qualifying hormone-refractory prostate cancer patients, a vaccination strategy using autologous DC pulsed with multiple T cell epitopes from four different prostate-specific antigens was evaluated for safety, feasibility and cellular antitumor responses [48]. The four T cell epitopes

selected were prostate stem cell antigen (PSCA₁₄₋₂₂), prostatic acid phosphatase (PAP₂₉₉₋₃₀₇), prostate-specific membrane antigen (PSMA₄₋₁₂) and prostate-specific antigen (PSA₁₅₄₋₁₆₃). The vaccination strategy was well tolerated, resulted in significant CTL responses against all antigens tested and was associated clinically with an increase in PSA doubling time. A separate recent phase I/II trial was performed to assess the safety and efficacy of vaccination with PSCA peptide-loaded DC in twelve prostate cancer patients with hormone and chemotherapy refractory prostate cancer [49]. Five patients developed a positive delayed type hypersensitivity reaction to PSCA, defined as an induration of more than 10 mm in diameter 3 days after loaded DC injection. The median survival of patients with positive DTH responses was significantly superior to overall survival of the patients with negative DTH responses - 22 months versus 8 months. However, only one patient showed reduction in tumor mass with a complete regression of a retrovesical lymph node. Of note, there was no evidence of any immediate or delayed toxicity in any of the patients.

Keeping these clinical results in mind, it is of interest that while PSCA expression has been examined extensively in human prostate cancer, the effect of clinical treatment on PSCA expression has only recently been explored. One study found that PSCA mRNA *in situ* hybridization was positive in 67.3% of prostate cancer tumor cells before complete androgen ablation (CAA), while PSCA mRNA was positive in only 33.8% of tumor cells after CAA [50]. Interestingly, for those men who do not have a decrease in PSCA expression and instead show an increase in PSCA after CAA there is a significant increased risk for local recurrence or distant metastases on follow-up. In a similar study examining the effects of external beam radiotherapy (EBRT) on PSCA mRNA expression levels, the percent of cells positive for PSCA mRNA declined from 71.2% before EBRT to 30.7% after EBRT [51]. As with the previous study, men who instead had an increased percentage of cells positive for PSCA mRNA after EBRT had an increased risk of biochemical relapse or distant metastases on follow-up. Finally, after treatment with the chemotherapy agent flutamide, PSCA mRNA expression levels were significantly reduced compared to before treatment [52]. However, if PSCA mRNA expression was increased after treatment, patients had an increased relative risk of 4.33 to develop subsequent cancer.

As with all immunotherapeutic strategies, it is important to consider the possibility of inducing tumor escape by antigen specific immunotherapies. As the tumor escape theory maintains, when a population of genomically unstable, heterogeneous tumor cells are placed under the selective pressure of targeted immunotherapy, a “natural selection” may result in the survival and proliferation of tumor escape variants (reviewed in [53]). Many tumor escape mechanisms have been documented including loss of cell-surface MHC class I, loss of tumor antigens, lack of costimulation at the tumor site, tumor cell death receptor pathway defects, and immunosuppression through cytokines and regulatory T cells. Tumor escape has the potential to inhibit complete tumor regression in PSCA-directed immunotherapy just as it would with any cancer associated antigen. While PSCA-specific tumor escape has yet to be explored in human trials, mPSCA vaccination of TRAMP mice did show a decrease in mPSCA expressing cells in prostate tumor tissues compared to unvaccinated mice, suggesting the elimination of prostate tumor cells that express high levels of mPSCA [46]. However, the study also showed that mPSCA expressing cells still remain in the prostate tissue of vaccinated mice, therefore the complete loss of PSCA does not appear to be a tumor escape mechanism that would block the future potential of PSCA as a therapeutic target.

Obviously as therapeutic strategies with PSCA are further developed, the effects of multiple treatment regimes on PSCA expression and the challenges of prostate tumor escape mechanisms will need to be anticipated, evaluated and addressed in order to design effective future human clinical trials. Looking ahead, the alteration in PSCA expression after treatment points to a growing theme in immunotherapy – the need for vaccination in the preventative

setting. Recently, therapeutic cancer vaccinations have been administered at the earliest possible stage of carcinogenesis with significant improvements in survival (reviewed in [54]). Due to the fact that prostate adenocarcinoma can be detected at very early stages, therapeutic vaccination could be administered at the pre-malignant period, such as PIN, to arrest the tumor progression. In fact, PSCA might offer both prognostic information on tumor progression and the immunotherapeutic target to treat an individual patient's prostate cancer.

7. Conclusions and Future Directions

PSCA is a cell surface protein that has been demonstrated to be up-regulated in prostate cancer, along with several other tumors. Because of its limited expression in normal tissues, PSCA has significant potential as a diagnostic, prognostic and therapeutic tool in the management of prostate cancer patients. Through analysis of blood samples or biopsies, PSCA may allow clinicians and researchers to tailor treatments to best anticipate individual tumor characteristics and design appropriate therapies, possibly including immunotherapy. *In vitro* and *in vivo* studies have shown that PSCA immunotherapy can be not only safe but also quite effective in the treatment of prostate cancer. Animal models that recapitulate human prostatic disease have allowed PSCA to be validated as a therapeutic target in a multitude of approaches and clinical trials have proven the safety of PSCA immunotherapy, thereby giving hope for future successes.

The field is now primed to move forward with trials elucidating the efficacy of PSCA as a clinical tool. With animal models as the foundation, an appropriate vaccination strategy with PSCA as the immune target should be initiated in a human clinical trial. At the same time, PSCA should be further evaluated in a diagnostic and prognostic setting to better identify the relevant risks of a particular tumor in a given patient. There is even potential that PSCA vaccination could be initiated in a preventative setting, at first evidence of a pre-malignant lesion, thereby becoming part of a paradigm shift in tumor immunotherapy.

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Table 1

Clinical tools utilizing PSCA expression

Clinical Tool	Strategy	Species	Conclusion	Reference
Diagnostic	RT-PCR of blood sample	Human	PSCA mRNA detected in 13.8% of prostate cancers and expression correlates with lower disease-free survival	[39]
Prognostic	PSCA protein expression on tumor sections	Human	PSCA protein expression correlates with Gleason score, pathologic tumor stage, progression to androgen independence, seminal vesicle invasion and capsular involvement	[7,8]
	PSCA mRNA expression in BPH after TURP	Human	PSCA mRNA expression correlated with increased risk for development of prostate cancer	[40]
Therapeutic	PSCA mAbs	Mouse	PSCA mAbs inhibit tumor growth, decrease metastasis and prolong survival	[41]
	PSCA mAbs conjugated to cytotoxic agent	Mouse	Complete tumor regression	[6]
	Genetically engineered CTL	<i>In vitro</i> human cell lines	CTL targeting PSCA efficiently lyse target cells	[45]
	Vaccination with PSCA cDNA and PSCA-VRP	Mouse	Long-term protection in TRAMP mice without autoimmunity	[46]
	Vaccination with PSCA, PAP, PSMA, and PSA loaded DC	Human	Well tolerated, generates significant CTL responses, increased PSA doubling time	[48]
	Vaccination with PSCA loaded DC	Human	Increased median survival time of patients with positive DTH responses, 1 out of 12 patients showed reduction in tumor mass, PSCA vaccination is safe	[49]

Abbreviations: PSCA, prostate stem cell antigen, RT-PCR, reverse transcription-polymerase chain reaction, BPH, benign prostatic hyperplasia, TURP, transurethral resection of the prostate, mAbs, monoclonal antibodies, CTL, cytotoxic T cell, VRP, Venezuelan equine encephalitis virus replicon particles, TRAMP, transgenic adenocarcinoma mouse prostate cancer model, DC, dendritic cells, PAP, prostatic acid phosphatase, PSMA, prostate-specific membrane antigen, PSA, prostate-specific antigen, DTH, delayed type hypersensitivity