PSCA Expressions in Cancers: A Potential Prognostic Marker and Target for Immunotherapy

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Abstract

Prostate stem cell antigen (PSCA) is a protein expressed in various cancers and has been proposed as a prognostic marker for certain cancer types. PSCA is also being studied as a potential target for cancer immunotherapy, including CAR-T cell therapy. Promising results have been reported in preclinical and clinical studies, suggesting the potential of PSCA-targeted immunotherapy to improve patient outcomes in different cancers. However, more research is needed to optimize this approach and evaluate the clinical significance of PSCA as a prognostic markers.

Introduction

Prostate stem cell antigen (PSCA) is a cell surface protein that is expressed in various cancer types, including prostate, bladder, pancreatic, and gastric cancers. The expression of PSCA is associated with the aggressiveness of the cancer and a poor prognosis for patients. In prostate cancer, for example, high PSCA expression is associated with a higher risk of recurrence, metastasis, and cancer-specific mortality. Similarly, in bladder cancer, high PSCA expression is associated with a higher risk of lymph node metastasis and advanced-stage disease. Therefore, PSCA has emerged as a potential prognostic marker for cancer patients.

In addition to its potential use as a prognostic marker, PSCA has also been investigated as a potential target for cancer immunotherapy. Several preclinical and clinical studies have demonstrated promising results for PSCA-targeted immunotherapy, including chimeric antigen receptor (CAR) T cell therapy, in patients with advanced solid tumors expressing PSCA. However, several challenges remain in the development of PSCA-targeted immunotherapy, including the identification of suitable patient populations, optimization of targeting agents, and reduction of off-target effects.

The potential of PSCA as a prognostic marker and target for cancer immunotherapy has generated significant interest among researchers and clinicians. Continued efforts in this field may lead to the development of more effective and targeted therapies for patients with PSCA-expressing cancers, ultimately increasing the survival rate and quality of patient life.

Based on the importance of PSCA in clinical prognosis, developing PSCA antibody which has good expression in different cancers is significant. In this poster, we will show production of PSCA antibody, quality control, and its expression in CytoSections and various cancer tissues.

Design & Methods

Antibody Generation

Firstly, sequence analysis of the PSCA protein was performed to design peptide antigens based on homology and immunogenicity. Purified antigen were immunized into the rabbits. Monoclonal antibodies were developed using a rabbit monoclonal antibody technology platform as briefly described. Immunized rabbits' peripheral blood was collected, and ELISA was used to screen for the secretion of monoclonal antibodies that recognized PSCA. PCR amplification of rabbit antibody gene from positive cells was obtained then cloned into a vector, and its functionality to produce an antibody was confirmed. Unique sequence of the recombinant rabbit monoclonal antibody that met the requirements was transfected, and expression was carried out in mammalian cells. The recombinant rabbit monoclonal antibody was then produced, purified and screened by ELISA and IHC immunohistochemistry assays . Figure 1 shows the whole process of antibody generation.

CytoSections

CytoSections offer a verified, reproducible and renewable source of positive/negative controls where the expression of the target biomarker is confirmed for accuracy & specificity by an immunoassay. The whole process of making CytoSections is demonstrated in Figure 2. In Figure 3, successful anti-DDK staining in CytoSections is shown in the first two rows.

Manual IHC staining of paraffin-embedded CytoSections using anti PSCA antibodies. All antibodies required heat induced epitope retrieval HIER using OriGene-Citrate pH6.0 (Cat# B05C-100) buffer for PSCA antibodies. OriGene's Polink-1 a one-step anti- mouse polymer HRP detection (Cat# D12-100) and DAB chromogen was used according to manufacturer's protocol. Scoring was based on the percentage of positive cells and not the intensity.

Immunocytochemistry

Manual IHC staining of paraffin-embedded breast cancer, colon cancer, lung cancer, bladder cancer, and prostate cancer tissues using anti PSCA antibodies (Figure 5). All antibodies required heat induced epitope retrieval HIER using OriGene-Accel buffer (Cat# B22C-1L). Anti-PSCA was incubated for 1 hour at 1:300 in room temperature. OriGene's Polink-1 a one-step anti- rabbit polymer HRP detection (Cat# D13-100) and DAB chromogen was used according to manufacturer's protocol. Tissues were sourced from OriGene Technology's tissue collection. Scoring was based on the percentage of positive cells and not the intensity.

Western Blot

Western blot analysis of overexpressed lysates(15ug per lane) from HEK293T cells transfected with empty plasmid (PS100001, lane 1) , human PSCA plasmid (RC209136, lane 2) using anti-PSCA antibody TA592754(1:3000).

Conclusion

- Specific monoclonal antibody for PSCA was identified by western blot and CytoSections
- Lung cancers show rarely positive
- PSCA expression in breast cancer is controversial
- Bladder cancers and prostate cancers show higher expression of PSCA

Results

Figure 1

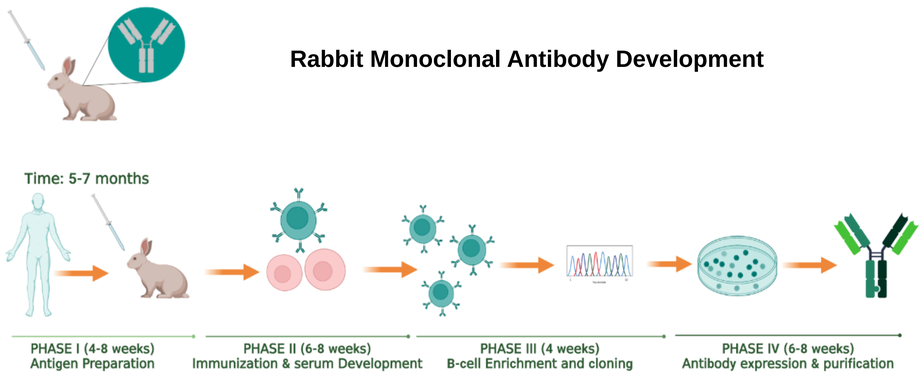


Figure 2

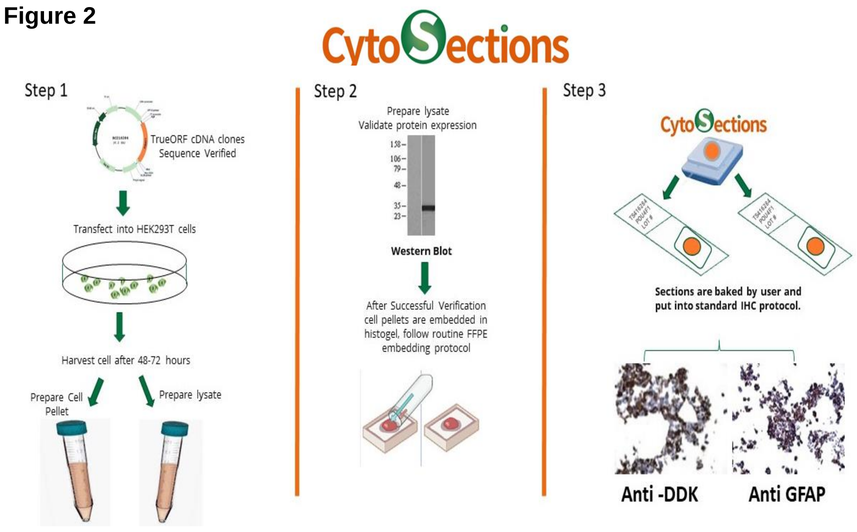


Figure 3. Anti-DDK and anti-PSCA in CytoSection

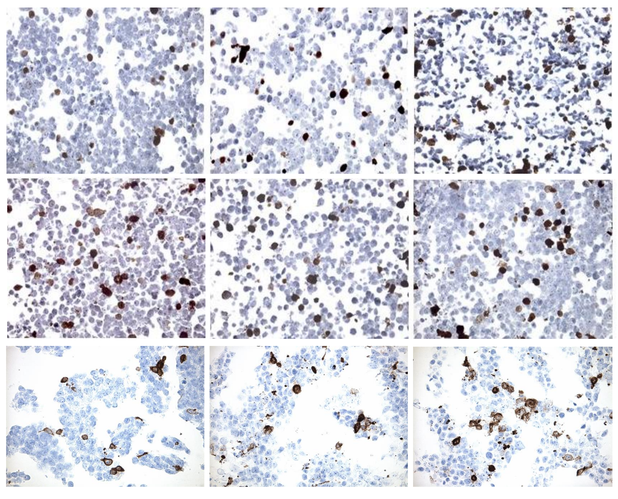


Figure 4

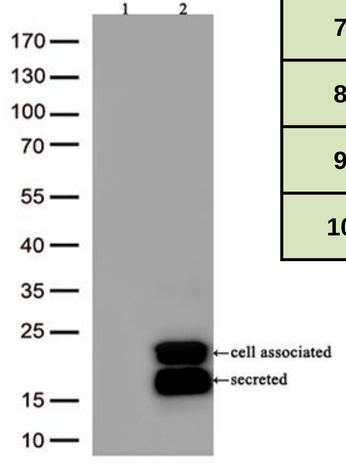
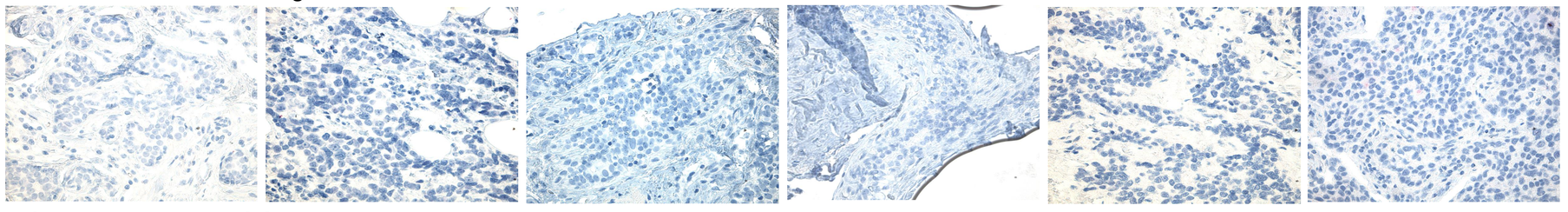


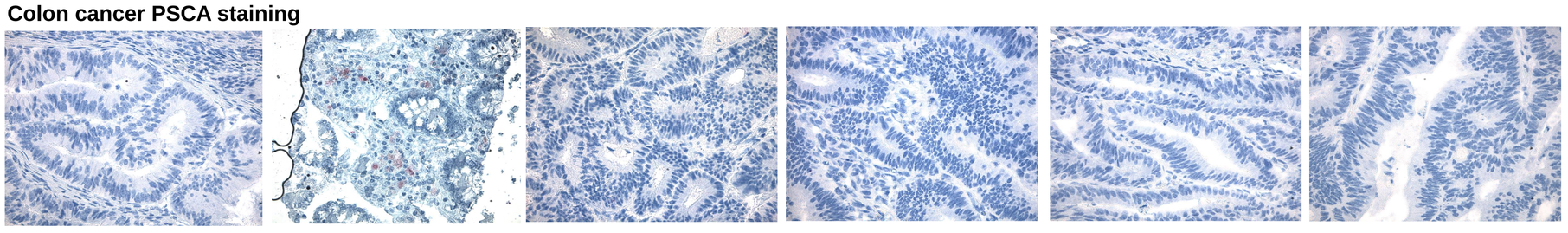
Figure 4. Western blot PSCA antibody quality control
Lane 1: empty plasmid;
Lane 2: human PSCA plasmid (RC209136, lane 2)

Figure 5

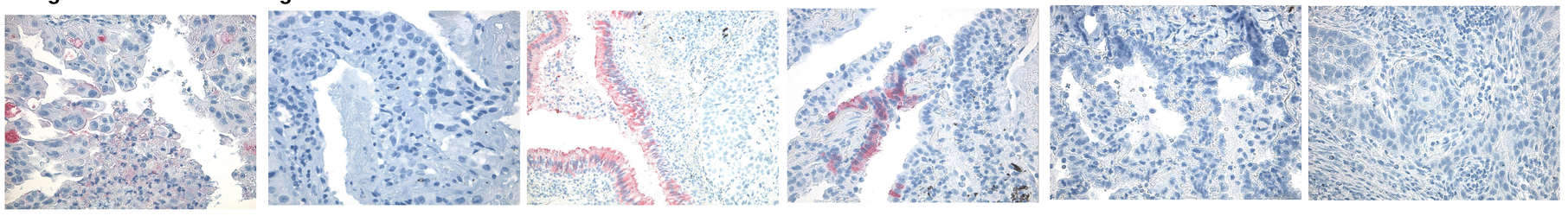
Breast cancer PSCA staining



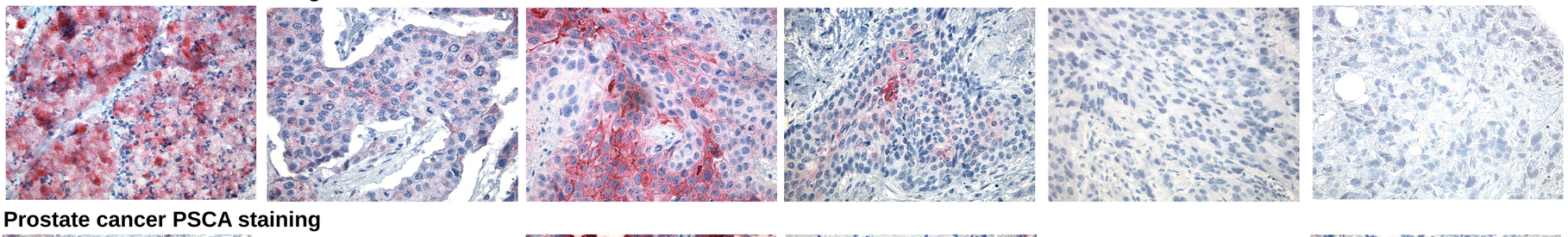
Colon cancer PSCA staining



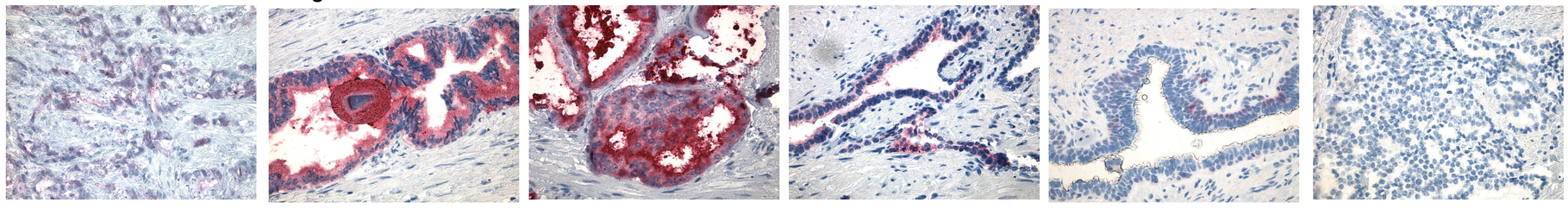
Lung cancer PSCA staining



Bladder cancer PSCA staining



Prostate cancer PSCA staining



Customer Project Service

- **Comprehensive Integrated Service:** antigen production, antibody engineering, development, purification, validation, and **large-scale production (mg to Kgs)**
- **Experienced:** 25+ years' experience serving pharma, biotech, and academia, developed 15,000+ mouse and rabbit monoclonal antibodies
- **Customer-Centric:** Ph.D. level project managers providing timely and expert guidance
- **GMP Manufacturing:** Large scale (gms to Kgs) manufacturing available, ISL13485-2016 certified.

•Phase I: Antigen for immunization (4-8 weeks)
•Phase II: Two monthly reports, including the ELISA results of serum testing (6-8 weeks)
•Phase III: Culture supernatants from a minimum of 10 antigen reactive clones along with the ELISA results (4 weeks)
•Phase IV: 2 mg of purified antibodies per clone from three selected clones, frozen hybridomas (6-8 weeks)

Table 1. Scoring of PSCA staining in different cancers

Tissue #	Breast Cancer	Colon Cancer	Lung Cancer	Bladder Cancer	Prostate Cancer
1	NEGATIVE	0 (F+)	NEGATIVE	0 (F+)	2+
2	NEGATIVE	NEGATIVE	NEGATIVE	0 (F2+)	0 (F+)
3	NEGATIVE	NEGATIVE	0 (F+)	0 (F2+)	0
4	0 (F+)	NEGATIVE	1	2+	0 (F+)
5	0 (F+)	NEGATIVE	0 (F+)	0 (F+)	3
6	NEGATIVE	NEGATIVE	NEGATIVE	2+	2
7	NEGATIVE	NEGATIVE	0 (F+)	NEGATIVE	3
8	NEGATIVE	0 (F+)	1	NEGATIVE	2
9	NEGATIVE	NEGATIVE	NEGATIVE	3+	NEGATIVE
10	NEGATIVE	NEGATIVE	0 (2F+)	3+	0 (F+)