Exploring the relationship between PSCA and PD-L1 by a comprehensive tumor immunohistochemical analysis

Poster #6445

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Abstract

Prostate Stem Cell Antigen (PSCA) is a well-established diagnostic marker in various cancers and a promising target for immunotherapy. Programmed Death-Ligand 1 (PD-L1) plays a crucial role in cancer immune regulation and is known to influence patient response to checkpoint inhibitors. The objective of this study is to elucidate the relationship between PSCA and PD-L1 in diverse cancer types and to provide valuable insights for the development of an effective immunotherapy strategy.

Immunohistochemical (IHC) and CytoSection analyses were performed on paraffin-embedded tissue sections obtained from 10 lung cancers, 10 prostate cancers, and 10 bladder cancers. Antibodies generated by Origene were utilized to detect the expression of PSCA and PD-L1 on the cell surface. The findings reveal a significant positive correlation between the expression of PSCA and PD-L1 in lung cancers, suggesting an underlying mechanism driving their co-expression in lung cancer study.

Understanding PSCA and PD-L1 coexpression in lung cancers has great potential for informing tailored immunotherapies. Future research should explore functional consequences within the tumor microenvironment, advancing precision immunotherapy for improved outcomes in these challenging malignancies.

Introduction

Prostate stem cell antigen (PSCA) is a cell surface protein that has been implicated in various cancers, particularly prostate cancer. It is overexpressed in a range of malignancies, including bladder, pancreatic, lung, and gastric cancers, making it a potential biomarker for cancer detection and prognosis. PSCA involvement in cancer pathogenesis extends beyond its role as a diagnostic marker; studies have shown that it promotes tumor growth, invasion, and metastasis through various signaling pathways. Consequently, PSCA represents a promising therapeutic target for cancer treatment, with ongoing research focused on developing targeted therapies to inhibit its activity and improve patient outcomes.

Programmed death-ligand 1 (PD-L1) is a key immune checkpoint protein that plays a crucial role in regulating the immune response and maintaining self-tolerance. In cancer, PD-L1 expression on tumor cells can inhibit the activity of cytotoxic T cells, allowing cancer cells to evade immune detection and destruction. Elevated PD-L1 expression is observed in various cancer types, including lung, breast, bladder, prostate, and melanoma, and is associated with poor prognosis and resistance to therapy. Consequently, PD-L1 has emerged as a major therapeutic target in cancer immunotherapy. Inhibitors of the PD-1/PD-L1 pathway, known as immune checkpoint inhibitors, have shown remarkable success in treating a subset of cancer patients by unleashing the anti-tumor immune response. Ongoing research continues to explore the role of PD-L1 in cancer progression and to identify novel strategies to target this pathway for more effective cancer treatment.

The coexpression of prostate stem cell antigen (PSCA) and programmed death-ligand 1 (PD-L1) in various cancers presents an intriguing area of investigation with significant implications for cancer biology and therapy. However, very few research shows the co-expression of PSCA and PD-L1 in difference cancers. This poster shows single and double staining of these two markers in lung cancers, bladder cancers, and prostate cancers. The results show that both of the two markers have high expression in lung, bladder, and prostate cancers. However, only lung cancers show high co-expression of PSCA and PD-L1. Therefore, PSCA and PD-L1 have emerged as a potential prognostic markers for some lung cancer patients. Target PSCA and PD-L1 immunotherapy may be applied for the treatment of lung cancers.

Design & Methods

Antibody Generation

Firstly, sequence analysis of the PSCA and PD-L1 proteins were performed to design peptide antigens based on different parameters related to peptide biophysics. 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 and PD-L1. 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. Third row and fourth row shows anti-PSCA and anti-PD-L1 staining respectively.

Immunocytochemistry

Manual immunohistochemical (IHC) staining was performed on paraffin-embedded tissues from lung cancers, bladder cancers, and prostate cancers using anti-PSCA antibodies and anti-PD-L1 antibodies.(Figure 5). All antibodies required heat induced epitope retrieval HIER using OriGene-Accel buffer (Cat# B22C-1L). For single staining, anti-PSCA and anti-PD-L1 were incubated for 1 hour at 1:300 in room temperature separately. OriGene's Polink-1 a one-step anti- rabbit polymer HRP detection (Cat# D13-100) was used for PSCA staining according to manufacturer's protocol. AP red chromogen DAB was applied for the detection of PD-L1 expression. Tissues were sourced from OriGene Technology's tissue collection. Pictures were captured by Olympus BX41 (Figure 5). Scoring was based on the percentage of positive cells and not the intensity. The scoring of PSCA and PD-L1 is shown in Table 1.

Western Blot

Western blot analysis was performed on lysates (15ug per lane) overexpressed in HEK293T cells transfected with an empty plasmid (PS100001, lane 1) or a human PSCA plasmid (RC209136, lane 2) using an anti-PSCA antibody (TA592754, diluted 1:3000). The expression of PD-L1 was observed in various cancer tissues through western blot analysis (Figure 3).

Results

Figure 1. Workflow of rabbit monoclonal antibody generation

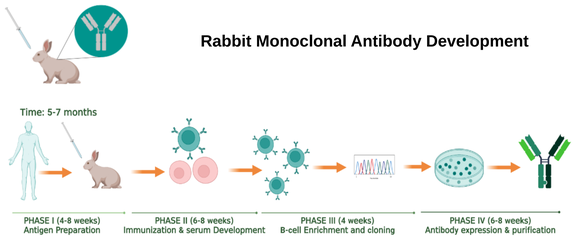

Figure 2. Development of CytoSections

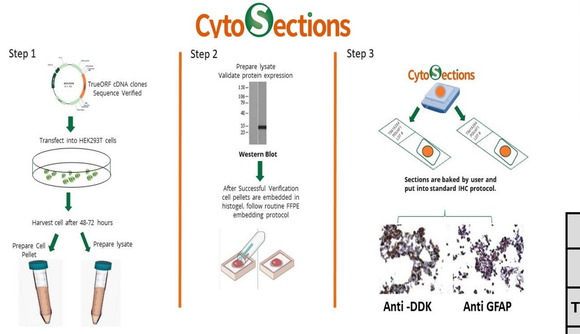

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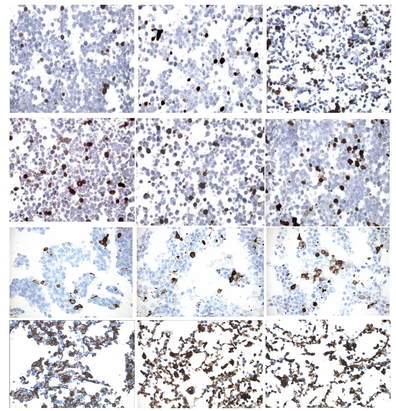
Figure 3. Anti-DDK and anti-PSCA in CytoSections



First row and second row: anti-DDK staining
Third row: anti-PSCA staining
Fourth row: anti-PD-L1 staining

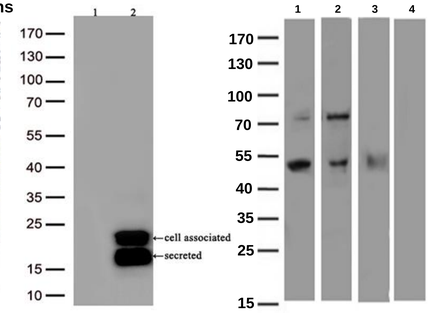


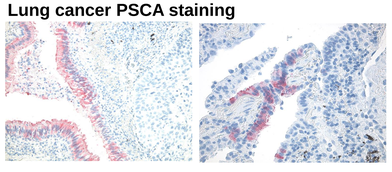
Figure 4. Western blot of PSCA and PD-L1 antibody quality control
PSCA antibody
Lane 1: empty plasmid;
Lane 2: human PSCA plasmid (RC209136, lane 2)
PD-L1 antibody
Lane 1: H2228; **Lane 2:** SK-MEL-28
Lane 3: HCC78; **Lane 4:** NCI-H460

	Single Staining					Co Expressions					
	PSCA in cancers			PD-L1 in cancers		Lung		Prostate		Bladder	
Tissue #	Lung	Bladder	Prostate	Lung	Bladder	PSCA	PD-L1	PSCA	PD-L1	PSCA	PD-L1
1	NG	0 (F+)	2+	3+	2+	2+	2+	2+	NG	2+	2+
2	NG	0 (F2+)	0 (F+)	3+	NG	NG	NG	NG	1+	2+	1+
3	0 (F+)	0 (F2+)	0	3+	0 (F+)	NG	1+	NG	1+	2+	NG
4	1+	2+	0 (F+)	0 (F+)	0 (F+)	2+	2+	NG	2+	2+	NG
5	0 (F+)	0 (F+)	3+	3+	1+	0(F+)	NG	2+	1+	2+	NG
6	NG	2+	2+	2+	3+	2+	NG	2+	NG	2+	NG
7	0 (F+)	NG	3+	0 (F+)	1+	NG	2+	2+	NG	NG	1+
8	1+	NG	2+	2+	1+	2+	NG	2+	0(F+)	NG	1+
9	NG	3+	NG	3+	2+	NG	2+	NG	NG	0(F+)	1+
10	0 (2F+)	3+	0 (F+)	NG	2+	0(F+)	1+	2+	NG	NG	2+

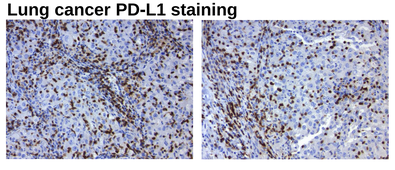
Table 1. Scoring of PSCA and PD-L1 staining in different cancers
NG is negative; 0(F+) is rare positive; 1+ is weak positive; 2+ is positive; 3+ is strong positive

Results

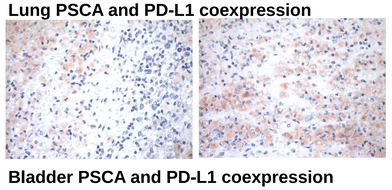
Lung cancer PSCA staining



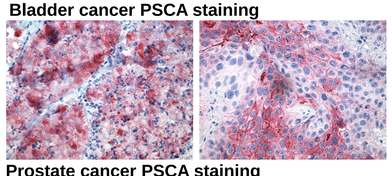
Lung cancer PD-L1 staining



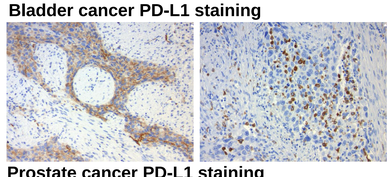
Lung PSCA and PD-L1 coexpression



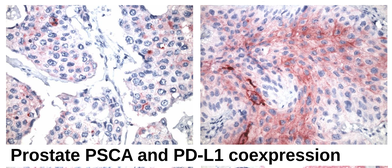
Bladder cancer PSCA staining



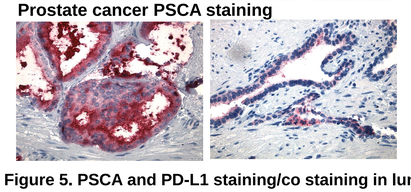
Bladder cancer PD-L1 staining



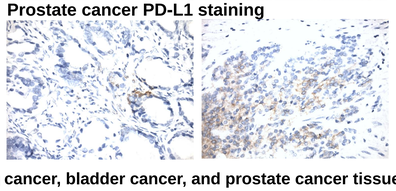
Bladder PSCA and PD-L1 coexpression



Prostate cancer PSCA staining



Prostate cancer PD-L1 staining



Prostate PSCA and PD-L1 coexpression

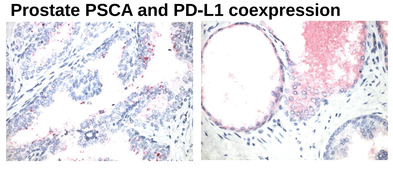


Figure 5. PSCA and PD-L1 staining/co staining in lung cancer, bladder cancer, and prostate cancer tissues

Conclusion and Discussion

- Specific monoclonal antibody for PSCA and PD-L1 were identified by western blot and CytoSections.
- Lung cancers show rarely positive PSCA.
- Bladder cancers and prostate cancers show higher expressions of PSCA.
- Bladder and lung cancers show high expression of PD-L1.
- The coexpression of PSCA and PD-L1 is significant in lung cancers.
- The coexpression PSCA and PD-L1 in prostate and bladder cancers is controversial.