PD-L1 and Diacylglycerol Kinase alpha (DGKa) Expression in NSCLC and Bladder Cancer

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Abstract

Diacylglycerol kinase alpha (DGKA) functions as lipid kinase to phosphorylate the lipid diacylglycerol (DAG). DGKA has been characterized to negative regulate the Ras-MAPK pathway in T lymphocytes. A 2018 study using crispr knockouts of DGKA revealed the anti-tumor activity of T-cells to increase in the presence of PD-L1 positive tumor cells. Little is known about the expression patterns of DGKA in human cancer. In our previous study we explore the tumor micro environment with a number of immune cell markers such as CD3, CD8A, CD20, CD68, TIM3 and LAG3 with PD-L1 expression in melanoma, NSCLC, and bladder cancer and found a few unique signatures for these cancers. In this study, we evaluate expression pattern of DGKA in various cancers by immunohistochemistry to elucidate the signature profile of DGKA expression with PD-L1.

Introduction

Current PD-L1 immune therapy targets T cells that are blocked by the PD-1–PD-L1 interaction between tumor and immune cells to reactivate T-cells activity (1). However total success of PDL1 therapy in preventing the progression of melanoma, non-small-cell lung cancer, bladder cancer, head and neck cancer, renal cell cancer are believed to be other factors in the tumor microenvironment. Numerous studies are ongoing to understand the role tumor microenvironment play in the T-cell regulation. Previously we have begun to look at the tumor microenvironment by evaluating the expression profile of immune cell markers CD3, CD8A, CD20, CD68, FOXP3, LAG3 and TIM3 and found that LAG3 and TIM3 produced unique expression profiles between the these three cancer types. This study continues screening possible markers by evaluating the same tumors for expression of DGKa. DGKa has been shown to participate in the up regulation of infiltrating T lymphocytes and believe to contribute to cytotoxic T cells activity. A 2018 study showed that crispr knockouts of DGKA revealed the anti-tumor activity of T-cells increase in the presence of PD-L1 positive tumor cells. Our study uses the traditional strategy IHC to investigate if DGKa expression pattern in these tissues where PD-L1 expression is known.

Table -1 Antibody Information and Dilution

Target	Clone	OriGene Cat #	Dilution
CD3e	UMAB54	UM500048	1:200dil
CD8A	UMAB241	UM800133	1:200dil
CD20	UMAB37	UM800001	1:200dil
CD68	UMAB150	UM800047	1:200dil
DGKa	OTI7B6	TA504048	1:100dil
FOXP3	UMAB248	UM800140	1:200dil
LAG3	OTI10E7	TA807146	1:250dil
TIM3	OTI5C8	TA812325	1:250dil
PD-L1	UMAB228	UM800120	1:200dil
PD-L1	OR-5H8	TA591003	1:100dil

Design & Methods

Rabbit PD-L1 Monoclonal Antibody Development

Rabbit recombinant monoclonal antibody platform was developed using B cells from peripheral blood. Briefly, B cells were isolated from the whole blood of rabbits immunized with PD-L1 peptides. Immune response positive cells were selected after they were cultured for 7-10 days. Rabbit IgG variable light and heavy chain were PCR amplified and cloned into vectors. Positive clones were sequenced. Both light and heavy chain were co- transfected into 293 cells for antibody expression. More than 10 positive PD-L1 clones, were first screened by immunocytochemistry and then immunohistochemistry. PD-L1 clone OR-5H8 for IHC works on both human and mouse tissues Figure 1.

Figure-1 Positive PD-L1 OR-5H8 On Human & Mouse Tissues

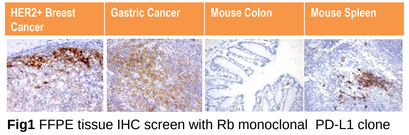


Fig1 FFPE tissue IHC screen with Rb monoclonal PD-L1 clone OR-5H8 on both human and mouse tissues.

Figure-2 PD-L1 On NSCLC

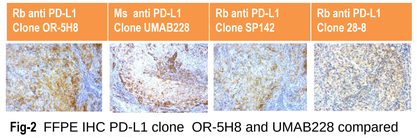


Fig-2 FFPE IHC PD-L1 clone OR-5H8 and UMAB228 compared to SP-142 & 28-8 FDA approved rabbit PD-L1 clones on NSCLC (lung), not shown are bladder cancer, and melanoma. Results all PD-L1 antibodies worked similarly using manual staining.

Immunohistochemistry:

Manual IHC staining of paraffin-embedded human and mouse tissues using anti PD-L1 rabbit mono antibodies clone SP142 [Spring Biosciences -Pleasanton, CA] , clone 28-8 [Abcam - Cambridge, MA], OR-5H8 & UMAB229, [C/N TA591004 & UM800120 OriGene Technologies-Rockville MD]. The immune marker antibodies from OriGene of CD3e, CD8A, CD20, CD68,DGKa, FOXP3, LAG3, and TIM3 are listed on the Table1 All antibodies required heat induced epitope retrieval HIER using OriGene-ACCEL Tris-EDTA buffer pH8.7 for clone OR-5H8 OriGene TEE pH9.0 for clone SP142; BioCare DIVA DeCloaker for clone 28-8 at 120C for 3 minutes in BioCare Decloaker chamber. PD-L1 clones SP142 and 28-8 were diluted 1:50; PD-L1 clone OR-5H8 were diluted 1:100 and incubated for 1hr at room temperature. OriGene Polink-1 a one step anti- rabbit polymer HRP detection (Cat# D13-100) was used except for clone 28-8 which used 2 step Rb Polymer Polink2Plus (D39) and DAB chromogen according to manufacture's protocol. The seven immune marker antibodies also required antigen retrieval.

Results

Figure-3: PD-L1 and DGKa NSCLC and Bladder Cancer

Fig-3 Shows various staining pattern PD-L1 and DGKa expression in NSCLC (lung cancer) and bladder cancer. Each row has one lung cancer and one bladder cancer case to represents an example of the staining patterns seen.

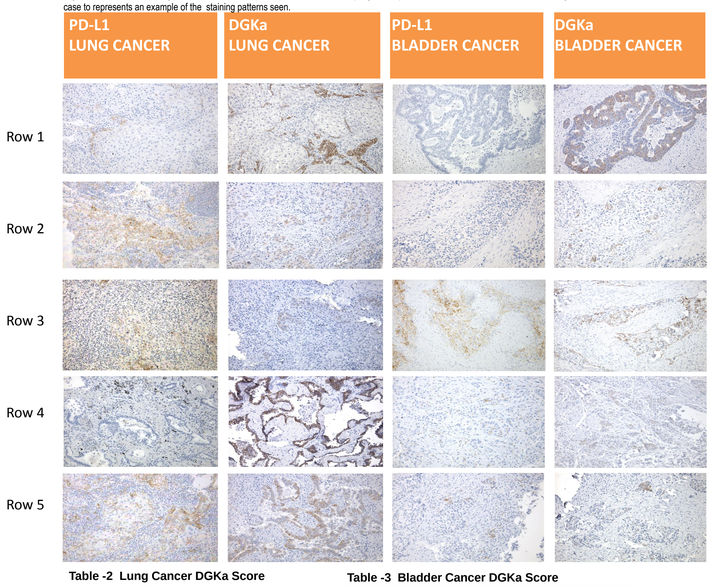


Table -2 Lung Cancer DGKa Score

Sample	Lung Cancer DGKa Score	Lung Cancer PD-L1 SCORE	ANY OVERLAP
1	3	IC Pos	+/-
2	3	Neg	-
3	3	Pos	+
4	Neg	pos	-
5	rare	pos	-
6	neg	Immune cell pos	–
7	neg	2-	_
8	neg	pos	-
9	3-	1-	+
10	neg	neg	-

Table -3 Bladder Cancer DGKa Score

Sample	Bladder Cancer DGKa Score	Bladder Cancer PD-L1 SCORE	ANY OVERLAP
1	Neg	pos	-
2	Neg	Neg	-
3	3	Neg	-
4	+/!	Foci pos	-
5	Neg	Neg	-
6	3+	3	+
7	Foci 3	Foci pos	+
8	Neg	Foci pos	+
9	3	-	-
10	Foci 3	1	+
11	Neg	3	-

Figure-4: PD-L1, CD3E, CD8A, CD20, CD68, FOXP3, LAG3 and TIM3 IHC Stain on NSCLC, Bladder Cancer, & Melanoma

Fig-4 Shows various staining pattern of immune cell markers with PD-L1 expression in NSCLC (lung) and bladder cancer

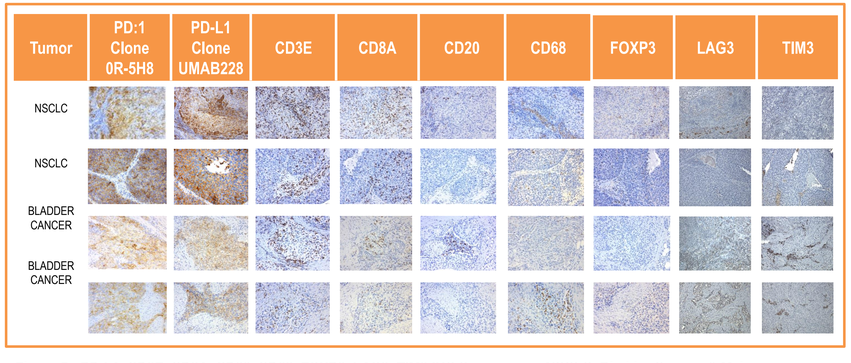
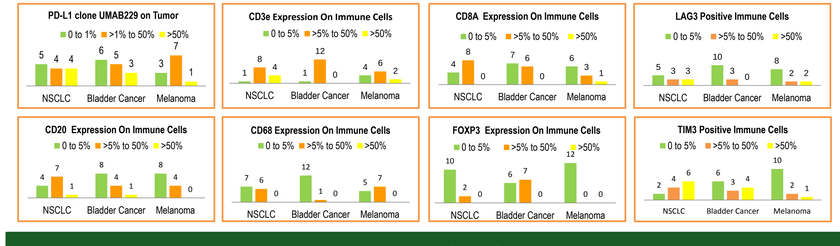


Figure 5: PD-L1, CD3E, CD8A, CD20, CD68, FOXP3, LAG3, TIM3 IHC Summary on NSCLC, Bladder Cancer, & Melanoma

(Score is based on immune cells present not stain on total tissue.)



Conclusion

1) DGKa clone OTI 7B6 stains mostly tumor cells and not immune cells in Bladder and Lung cancer. It has very little background and makes it a very good IHC antibody.
2) Staining pattern showed very little overlap between PD-L1 and DGKa in both the lung cancer and bladder tumor cells.
3) In data not presented 10 more cases of lung cancer where 6 tumors showed some DGKa positive tumor staining only 3 of those case showed overlap with PD-L1 staining.
4) Future goal is to review the stains