Expression and Significance of PVR-TIGIT/CD226, An Immune Checkpoint Axis in Small Cell Lung Cancer

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ABSTRACT

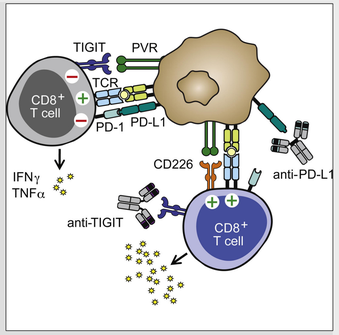
Background: Although immunotherapy has renewed hopes for the treatment of SCLC, the “classical” co-inhibitory PD-1/PD-L1 pathway is not very common in SCLC. The poliovirus receptor (PVR, also called CD155) is an immune checkpoint molecule on various tumor cells. It has been reported to mediate activation of T cells via CD226 or inhibition of T cells by binding to TIGIT. TIGIT competes with CD226 for binding to PVR, and exhibits stronger affinity for PVR. PVR is expressed at low levels in a number of cell types of epithelial origin and is overexpressed in various carcinomas with epithelial and neurological origins. Recently we have found that PVR was highly expressed in SCLC cell lines. The further illustration of the expression and significance of PVR-TIGIT/CD226 axis in SCLC will help us to better understand the immunology of SCLC and may lead to a novel therapeutic strategy to combine checkpoint blocking agents for success in SCLC immunotherapy.

Material and Methods: Gene expression data for tumor tissues was derived from the TCGA database (www.cbioportal.org). Immunohistochemistry (IHC) was performed to evaluate PVR protein expression in a 41 SCLC cell line TMA and a SCLC cohort from 77 patients with clinical data. The data was generated using both the H-score and tumor proportion score (TPS) systems. mRNA expression levels of PVR, PD-L1, PD-1 were evaluated by mRNAseq with a targeted panel provided by HTG molecular in a 32 primary SCLC resected cohorts. The detection of TIGIT and CD226 protein expression were optimized in tonsil tissue by singleplex IHC staining. Multiplex bright field IHC staining was employed to demonstrate the protein expression of PVR/TIGIT in situ.

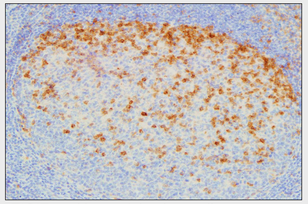
Results: In a TMA 41 SCLC cell lines, two cell lines were not evaluable due to the insufficient cells. Thirty-seven cell lines (95%, 37/39) demonstrated staining for PVR with 4 cell lines (10.3%, 4/39) showed strong staining (H-score ≥ 270). PVR had a higher expression level in cell lines established from pre-treated SCLC patients than those established from treatment-naïve patients (P=0.037). However, there was no significant difference in PVR expression between suspension cultured cell lines and adherent cells. In the SCLC patient cohort, PVR was found to be expressed predominantly on the membrane of tumor cells, with minimal expression observed on the immune cells in the tumor microenvironment. The PVR expression in the SCLC patient cohort is 82% with a H-score cutoff of ≥ 50. SCLC patients who had higher PVR expression showed poorer prognosis, however the difference was not statistically significant (p=0.05).Higher PVR expression was found in advanced stage (P=0.0073) and male patients (P=0.04). With a TPS score system (cutoff of ≥ 50%) the prevalence of PVR expression is 79% in the SCLC cohort. Using the TPS system, higher PVR expression was associated with poor prognosis and advanced stage (P=0.046, and P=0.045). We also found that PVR expression as evaluated with the TPS score system was correlated with large tumor diameter (P=0.043) and old age of patients (P=0.046).

INTRODUCTION

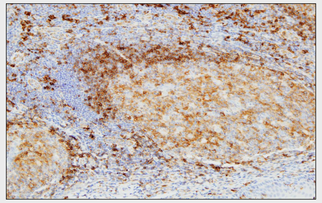
Although immunotherapy has renewed hopes for the treatment of SCLC, patients demonstrated an overall response to anti-PD-1/PD-L1 mono-therapy of around 10% response in unselected SCLC patients in recent clinical trials. Several mechanisms may contribute to these results, one of them may be the low prevalence of PD-1/PD-L1expression in SCLC. The human Poliovirus Receptor (CD155) has been reported to mediate activation of T cells via CD226 or inhibition of T cells by binding to TIGIT. TIGIT



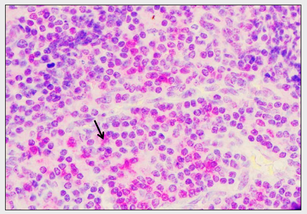
competes with CD226 for binding to PVR, and exhibits stronger affinity for PVR, which is similar to the CTLA4-CD28-B7 axis. Recently, we found that PVR is highly expressed in SCLC cell lines. Determining expression of PVR and its related receptors TIGIT and CD226 expression in SCLC could shine a light on the axis’ potential role and inform novel therapeutic strategy to combine different checkpoint blocking agents for success in SCLC immunotherapy.



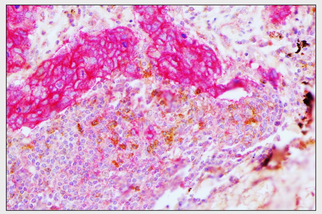
TIGIT positive staining in tonsil



CD226 positive staining in tonsil



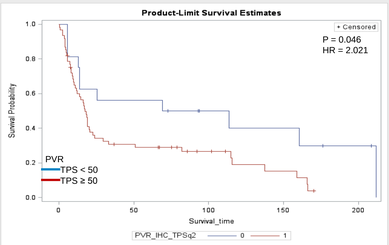
TIGIT positive staining in SCLC by red, the arrow points to the TIGIT staining on TILs

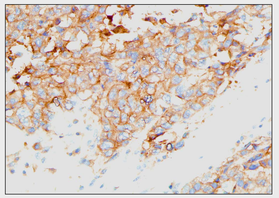


PVR (red) and TIGIT (brown) dual IHC staining in SCLC.

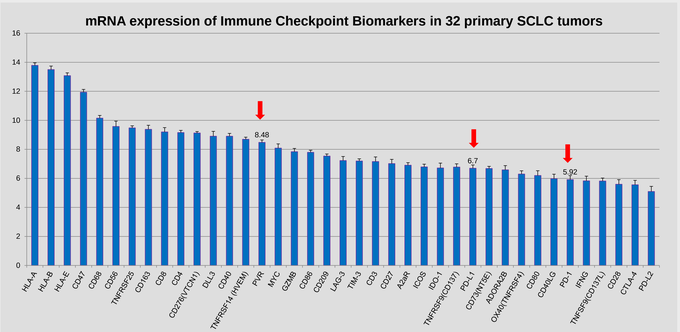
RESULTS

Parameter	PVR H-score threshold ≥ 50			PVR TPS threshold ≥ 50%		
	PVR negative (n = 14)	PVR positive (n = 63)	p-value	PVR negative (n = 16)	PVR positive (n = 61)	p-value
Age, years mean ± SD	54.7 ± 8.2	59.2 ± 8.7	0.077	54.5 ± 7.71	59.4 ± 8.8	0.046
Gender, n (%) female male	7 (12.3) 50 (87.7)	7 (35) 13 (65)	0.04	7 (43.7) 9 (56.3)	13 (21.3) 48 (78.7)	0.107
Stage, n (%) I II III	9 (64.3) 0 (0) 5 (35.7)	16 (25.8) 19 (30.7) 27(43.5)	0.0073	9 (56.3) 1 (6.3) 6 (37.4)	16 (26.7) 18 (30.0) 26 (43.3)	0.045
Tumor size mean ± SD	4.04 ± 1.23	5.01 ± 2.97	0.061	4.03 ± 1.15	5.04 ± 3.01	0.043

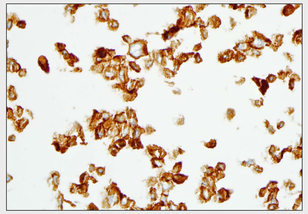




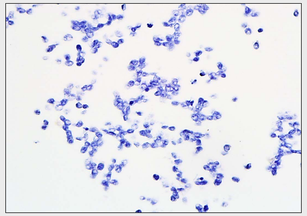
PVR positive membranous expression in SCLC, H-score 250



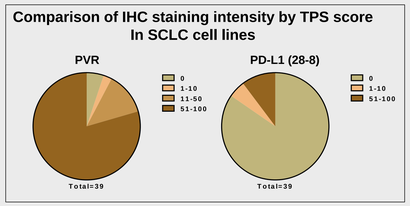
Genomic data from the Oncology Biomarker panel using the HTG EdgeSeq Platform



PVR strong membranous staining on H211 SCLC cell line, H-score 300, 40x



PD-L1 negative staining on H211 SCLC cell line, TPS 0, 40x



Biomarker	Positive Cell Lines
PVR H-score cutoff ≥ 50	82.1% (32/39)
PD-L1 TPS cutoff ≥ 1%	16.7% (8/48)

CONCLUSIONS

- PVR is broadly expressed in SCLC cell lines and tumor tissues.
- High PVR expression was associated with poor prognosis, advanced tumor stage and large tumor size.
- The IHC assay to evaluate the protein expression of TIGIT and CD226 has been optimized, and further research on the significance of PVR-TIGIT/CD226 in SCLC is ongoing.
- PVR expression may represent an important targeted immune checkpoint in lung cancer. The blockade of TIGIT pathway may represent a potent therapeutic strategy to combine different checkpoint blocking agents for greater success in SCLC therapy.

ACKNOWLEDGEMENTS

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