



## Original Research

## PVR/TIGIT and PD-L1/PD-1 expression predicts survival and enlightens combined immunotherapy in lung squamous cell carcinoma

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## ABSTRACT

PVR/TIGIT and PD-L1/PD-1 axes play essential roles in tumor immune evasion and could be potential targets for combined immunotherapy. We aimed to evaluate the expression status of the above-mentioned immune markers in lung squamous cell carcinoma (LUSC), and investigate their survival impact and relevance with the immune microenvironment and clinicopathological features. We retrospectively collected specimens from 190 LUSC patients, who underwent pulmonary surgeries, and we performed immunohistochemistry assays of PVR, TIGIT, PD-L1, PD-1 and CD8. In our cohort, the positive rate of PVR was 85.8%, which was much higher than the positive rate of PD-L1 at 26.8%. A total of 32 (16.8%) patients demonstrated co-expression of PVR/PD-L1. High TIGIT density was correlated with positive PD-L1 expression, high PD-1 density, and high CD8 density (PD-L1,  $P=0.033$ ; PD-1,  $P<0.001$ ; CD8,  $P<0.001$ ), and positive PVR expression was correlated with positive PD-L1 expression ( $P=0.046$ ). High TIGIT density and high PVR/TIGIT expression were correlated with advanced TNM stage (TIGIT density,  $P=0.020$ ; PVR/TIGIT expression,  $P=0.041$ ). Patients with positive PVR expression, high TIGIT density, high PVR/TIGIT expression and PVR/PD-L1 co-expression exhibited a significantly worse prognosis (PVR,  $P=0.038$ ; TIGIT,  $P=0.027$ ; PVR/TIGIT,  $P=0.014$ ; PVR/PD-L1,  $P=0.018$ ). Multivariate analysis demonstrated that PVR/PD-L1 co-expression (Hazard ratio [HR], 1.756, 95% CI, 1.152-2.676,  $P=0.009$ ) was an independent prognostic factor in LUSC patients. In conclusion, we demonstrated the expression status of PVR/TIGIT and PD-L1/PD-1 in LUSC. PVR/PD-L1 co-expression was an independent prognostic factor in LUSC patients and may serve as a potential predictive biomarker for dual-targeting immunotherapy.

## Introduction

Lung cancer is the leading cause of cancer-related mortality, causing approximately 1.8 million deaths per year worldwide [1]. Lung squamous cell carcinoma (LUSC) accounts for 20-40% of lung cancers, and the 5-year survival rate is lower than 20% for advanced disease [2]. The

treatment of LUSC is still challenging as the patients with LUSC often have a history of smoking and a high incidence of comorbidities, such as chronic obstructive pulmonary disease and heart diseases. Besides LUSCs usually arise in the proximal bronchi, and are more likely to invade larger blood vessels, leading to increased difficulty and risk of surgeries [3,4]. Meanwhile, mutations of targetable genes, such as

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epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK), are rarely seen in LUSC [5].

Immune surveillance is essential for maintaining cellular homeostasis and preventing carcinogenesis [6]. Overexpression of immune checkpoint molecules in tumors can result in an immunosuppressive microenvironment that facilitates carcinogenesis [7]. Immune checkpoint blockade therapies have become an efficient treatment for a variety of tumors, and programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) inhibitors are the most commonly used inhibitors. PD-L1/PD-1 inhibitors have significantly improved the prognosis for non-small cell lung cancer (NSCLC) patients, however, only 30% of the patients could respond to the therapy [8,9]. As a result, it is essential to identify new immunotherapeutic targets and develop combined therapeutic strategies for NSCLC patients. T cell immunoreceptor with Ig and ITIM domains (TIGIT) is identified as an inhibitory receptor expressed on lymphocytes and has recently emerged as a new potential target of immunotherapy. TIGIT interacts with its major ligand poliovirus receptor (PVR), also called cluster of differentiation 155 (CD155), to exert its immunosuppressive effects: down-regulating NK and T cell proliferation and function [10,11]. PVR/TIGIT expression was elevated in a variety of cancer types, such as colon cancer, breast cancer, multiple myeloma, and pancreatic cancer, and a higher expression level correlated with higher rates of tumor metastases and tissue or lymph node invasion, and poorer survival [10,12]. However, limited studies have focused on PVR/TIGIT expression and its biological and clinical significance in LUSC remain limited.

The aim of this study is to evaluate the expression status of PVR/TIGIT and PD-L1/PD-1 in LUSC, and investigate their survival impact and relevance with the immune microenvironment and clinicopathological features. This study can serve as a useful reference for selecting beneficiaries for future dual-target immunotherapy in LUSC.

## Materials and methods

### Patients

A total of 190 patients diagnosed with LUSC were enrolled in this study. All patients underwent pulmonary surgeries in the Cancer Hospital, Chinese Academy of Medical Sciences from April 4th, 2006 to February 10th, 2013. If the clinical condition permitted, surgeries included sublobectomy, lobectomy or pneumonectomy, and all patients underwent lobe-specific or systematic lymph node dissection. We retrospectively obtained the clinicopathological characteristics and tumor tissues. The extracted clinical features included age, gender, smoking history, tumor differentiation, TNM staging, surgical procedures, completeness of resection, and overall survival (OS). The tumors were staged according to the 8<sup>th</sup> version of the American Joint Committee on Cancer staging system. All 190 patients were followed up until 20th September 2018 or until the date of death. We obtained the follow-up information from their medical records or by contacting their families. The study was approved by the ethics committee of the National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College.

### Evaluation of the tumor immune microenvironment

Immunohistochemistry (IHC) of PVR, TIGIT, PD-L1, PD-1 and CD8 was performed on tissue microarrays (TMAs). We obtained the tissues by surgery and embedded them in paraffin. Two 2-mm cores were taken from each sample to constitute the TMAs, and then 4- $\mu$ m thick TMA sections were manufactured. The TMAs were incubated with primary antibodies against PVR (D8A5G, CST), TIGIT (E5Y1W, CST), PD-L1 (28-8, Abcam), PD-1 (D4W2J, CST), and CD8 (D8A8Y, CST), and then with the secondary antibodies and 3, 3'-diaminobenzidine (DAB). The results of IHC were examined by two independent pathologists, who were blinded to the clinical information. Membranous tumor proportion score

(TPS) was applied to score PVR and PD-L1 expression. PVR and PD-L1 positivities were defined as TPS  $\geq$  10% and TPS  $\geq$  1%, respectively. PVR/PD-L1 co-expression was defined as PVR positivity and PD-L1 positivity. We counted the number of TIGIT-positive tumor-infiltrating lymphocytes (TILs), PD-1-positive TILs, and CD8-positive TILs in six high-power fields and calculated the average for each case. We divided the density of TIGIT-positive TILs, PD-1-positive TILs, and CD8-positive TILs into high and low groups, using the median count as the cut-off value.

### Statistical analysis

Pearson chi-square test was used to evaluate the correlation between the expression of immune markers and clinicopathological features. We used Wilcoxon test to compare the counts of TIGIT- and PD-1-positive lymphocytes between high CD8 density and low CD8 density groups. Linear regression and t test was used to evaluate the correlation between the counts of TIGIT-, PD-1- and CD8-positive lymphocytes. We defined OS as the time from initial surgery to death or the last follow-up. We evaluated survival of our cohort using the Kaplan–Meier curves, and used the log-rank test to determine significance. Using the Cox proportional hazard model, we performed multivariate analysis to assess the prognosis with adjustment for clinicopathological factors.  $P < 0.05$  was considered statistically significant. All statistical analyses were performed using SPSS version 26.

## Results

### Patients and clinicopathological characteristics

A total of 190 patients with LUSC were included in this study. All patients in our cohort underwent intent-to-treat surgeries, including sublobectomy, lobectomy, bilobectomy and pneumonectomy. The clinicopathological characteristics of the cohort are shown in Table 1. The median age of patients was 61 years (range 37–79 years). A total of 183 (96.3%) patients were male, and 176 (92.6%) were smokers. At diagnosis, 66.3% of these patients had regional lymph node metastasis, and 17.9%, 36.8% and 45.3% of patients were in stages I, II, and III, respectively.

### PVR and PD-L1 expression status in LUSC

We performed IHC analysis of PVR and PD-L1 in the cohort, and representative images and statistical results of IHC staining are shown in Fig. 1. A total of 163 (85.8%) cases were positive for PVR expression, and 51 (26.8%) cases were positive for PD-L1 expression. The positive rate of PVR was much higher than that of PD-L1, indicating that the PVR/TIGIT axis may play important roles in LUSC carcinogenesis. A total of 32 (16.8%) patients demonstrated co-expression of PVR/PD-L1, suggesting the applicability of future dual-target immunotherapy in these patients.

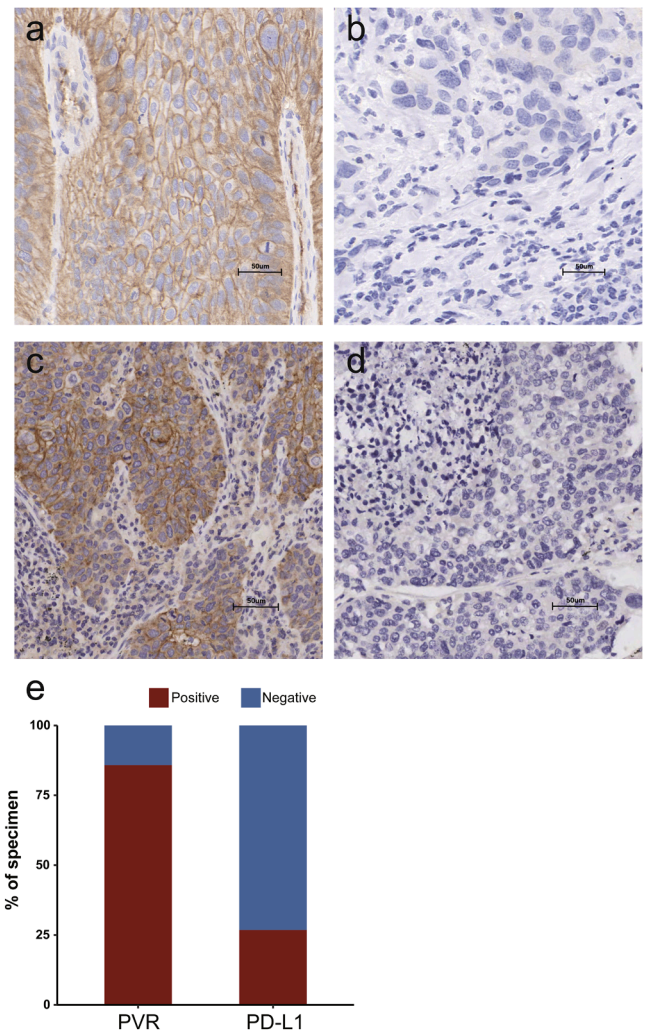
### Correlation between the expression of immune markers and clinicopathological features

In order to provide more details on LUSC immune microenvironment, we performed IHC analysis of TIGIT, PD-1, and CD8. Using the median count as the cut-off value, we divided the density of TIGIT-, PD-1-, and CD8-positive TILs into high and low groups. Representative images and statistical results of IHC staining are shown in Fig. 2. We further explored the expression correlation between these markers. As shown in Table 2, high TIGIT density was correlated with positive PD-L1 expression, high PD-1 density and high CD8 density (PD-L1,  $P=0.033$ ; PD-1,  $P<0.001$ ; CD8,  $P<0.001$ ), and positive PVR expression was correlated with positive PD-L1 expression ( $P=0.046$ ), suggesting the synergistic effects of PD-L1/PD-1 and PVR/TIGIT axes on LUSC

**Table 1**  
The clinicopathological characteristics and univariate and multivariate analyses for overall survival of the LUSC cohort.

Variable		N (%)	Univariate analysis		Multivariate analysis	
			HR (95% CI)	P value	HR (95% CI)	P value
Age (years)	<60	88 (46.3)	0.743 (0.510-1.083)	0.122	0.655 (0.445-0.963)	0.031
	≥60	102 (53.7)				
Gender	Male	183 (96.3)	1.435 (0.455-4.521)	0.537	0.907 (0.257-3.203)	0.880
	Female	7 (3.7)				
Smoking history	Smokers	176 (92.6)	1.174 (0.546-2.524)	0.681	1.160 (0.497-2.710)	0.731
	Non-smokers	14 (7.4)				
T	1-2	124 (65.3)	0.364 (0.250-0.531)	<0.001	0.472 (0.315-0.707)	<0.001
	3-4	66 (34.7)				
N	0-1	131 (68.9)	0.412 (0.282-0.602)	<0.001	0.426 (0.285-0.637)	<0.001
	2	59 (33.1)				
TNM stage	I-II	104 (54.7)	0.344 (0.235-0.504)	<0.001		
	III	86 (45.3)				
PVR	Positive	163 (85.8)	1.938 (1.037-3.621)	0.038		
	Negative	27 (14.2)				
TIGIT	High density	95 (50.0)	1.524 (1.048-2.216)	0.027		
	Low density	95 (50.0)				
PD-L1	Positive	51 (26.8)	1.504 (1.007-2.248)	0.046		
	Negative	139 (73.2)				
PD-1	High density	98 (51.6)	1.545 (1.059-2.253)	0.024		
	Low density	92 (48.4)				
CD8	High density	94 (49.5)	0.641 (0.440-0.933)	0.020	0.650 (0.437-0.966)	0.033
	Low density	96 (50.5)				
PVR/TIGIT Expression	High	84 (44.2)	1.592 (1.097-2.311)	0.014		
	Low	106 (55.8)				
PVR/PD-L1 Co-expression	Others	32 (16.8)	1.633 (1.088-2.449)	0.018	1.756 (1.152-2.676)	0.009
	Others	158 (83.2)				

PVR, PVR cell adhesion molecule; TIGIT, T cell immunoreceptor with Ig and ITIM domains; PD-L1, programmed cell death ligand 1; PD-1, programmed cell death 1; CD8, cluster of differentiation 8.



**Fig. 1.** Results of immunohistochemical staining for PVR and PD-L1. Representative images of positive membrane staining for PVR (a), negative membrane staining for PVR (b), positive membrane staining for PD-L1 (c), and negative membrane staining for PD-L1 (d). (e) Bar-graph showed the proportions of PVR-positive and PD-L1-positive patients. PVR and PD-L1 positivities were defined as membranous tumor proportion score (TPS) ≥ 10% and TPS ≥ 1%, respectively. Scale bar: 50µm. PVR, PVR cell adhesion molecule; PD-L1, programmed cell death ligand 1.

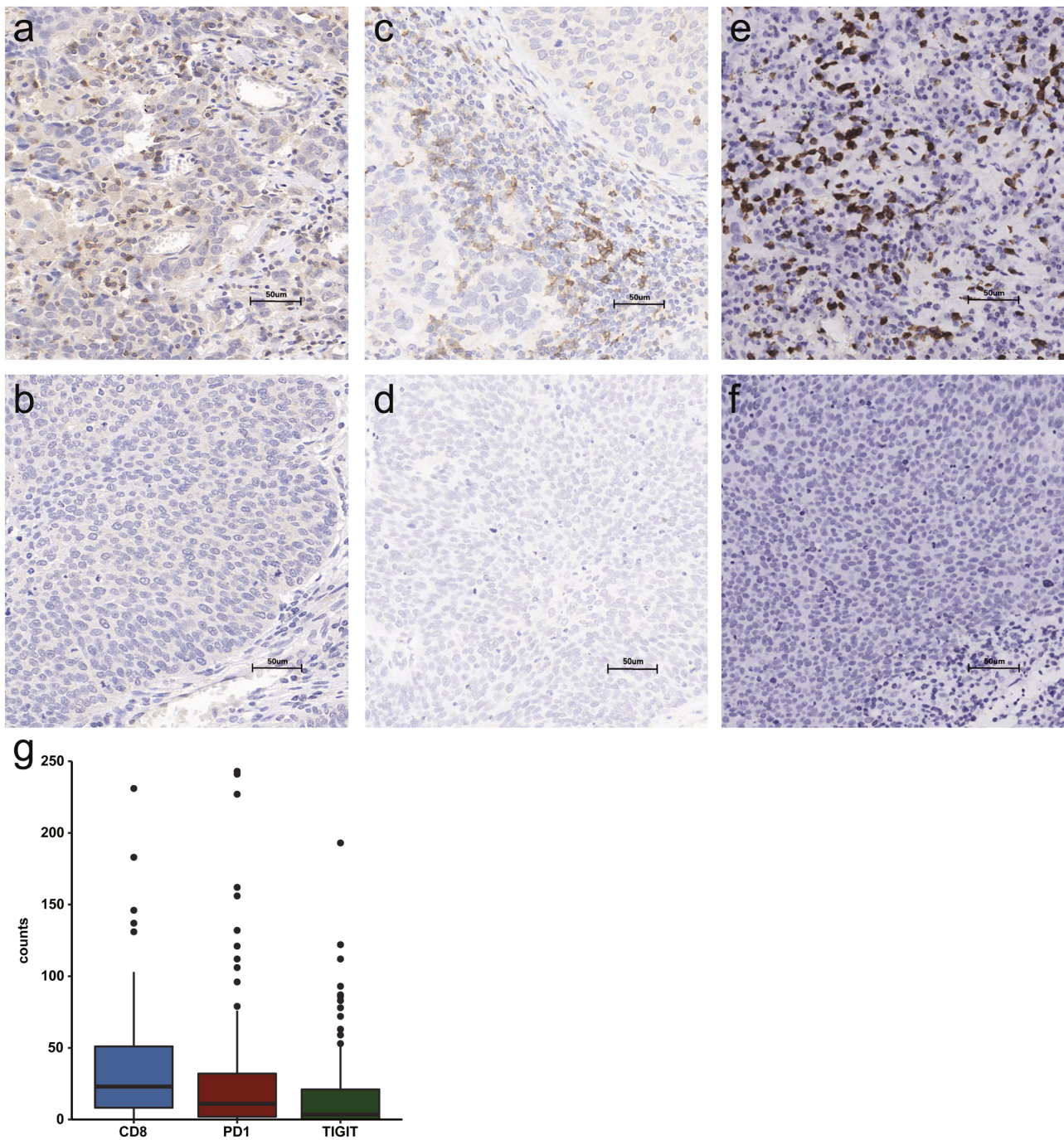
carcinogenesis. As demonstrated in Fig. 3, TIGIT- and PD-1-positive TIL counts were positively correlated with CD8-positive TIL counts (TIGIT,  $P < 0.001$ ; PD-1,  $P < 0.001$ ), and high CD8 density group had significantly more TIGIT- and PD-1-positive TIL counts than low CD8 density group (TIGIT,  $P < 0.001$ ; PD-1,  $P < 0.001$ ).

Further, we explored the relationship between PVR/TIGIT expression and clinicopathological features. As demonstrated in Table 3, high TIGIT density and high PVR/TIGIT expression were correlated with advanced TNM stage (TIGIT density,  $P = 0.020$ ; PVR/TIGIT expression,  $P = 0.041$ ), indicating that PVR/TIGIT played important roles in LUSC progression.

**Survival analysis**

We performed follow-up of all patients, and 112 (58.9%) patients had died at the time of data extraction. The median OS of all patients was 65.0 months (range 2.0–146.0 months) and the 5-year survival rate was 51.6% (stage I, 76.5%; stage II, 65.7%; stage III, 30.2%). Using the Kaplan–Meier curve, we performed survival analysis and found that





**Fig. 2.** Results of immunohistochemical staining for TIGIT, PD-1, and CD8. Representative images of high density of TIGIT-positive lymphocyte infiltration (a), low density of TIGIT-positive lymphocyte infiltration (b), high density of PD-1-positive lymphocyte infiltration (c), low density of PD-1-positive lymphocyte infiltration (d), high density of CD8-positive lymphocyte infiltration (e), and low density of CD8-positive lymphocyte infiltration (f). (g) Boxplots showed the counts of CD8-, PD-1-, and TIGIT-positive lymphocytes. Center line, median; box limits, upper and lower quartiles; whiskers,  $1.5 \times$  interquartile range; points, outliers. Scale bar: 50 μm. TIGIT, T cell immunoreceptor with Ig and ITIM domains; PD-1, programmed cell death 1; CD8, cluster of differentiation 8.

patients with positive PVR expression, high TIGIT density, high PVR/TIGIT expression, and PVR/PD-L1 co-expression exhibited a significantly worse prognosis (PVR,  $P=0.038$ ; TIGIT,  $P=0.027$ ; PVR/TIGIT,  $P=0.014$ ; PVR/PD-L1,  $P=0.018$ ) (Fig. 4). In addition, patients with advanced T stage, N stage, and TNM stage were associated with poor prognosis (T stage:  $P < 0.001$ ; N stage:  $P < 0.001$ ; TNM stage:  $P < 0.001$ ) (Table 1). In multivariate analysis, we identified that age (HR, 0.655, 95% CI, 0.445-0.963,  $P=0.031$ ), T stage (HR, 0.472, 95% CI, 0.315-0.707,  $P < 0.001$ ), N stage (HR, 0.426, 95% CI, 0.285-0.637,  $P < 0.001$ ), CD8 density (HR, 0.650, 95% CI, 0.437-0.966,  $P=0.033$ )

and PVR/PD-L1 co-expression (HR, 1.756, 95% CI, 1.152-2.676,  $P=0.009$ ) were independent prognostic factors in LUSC patients (Table 1).

## Discussion

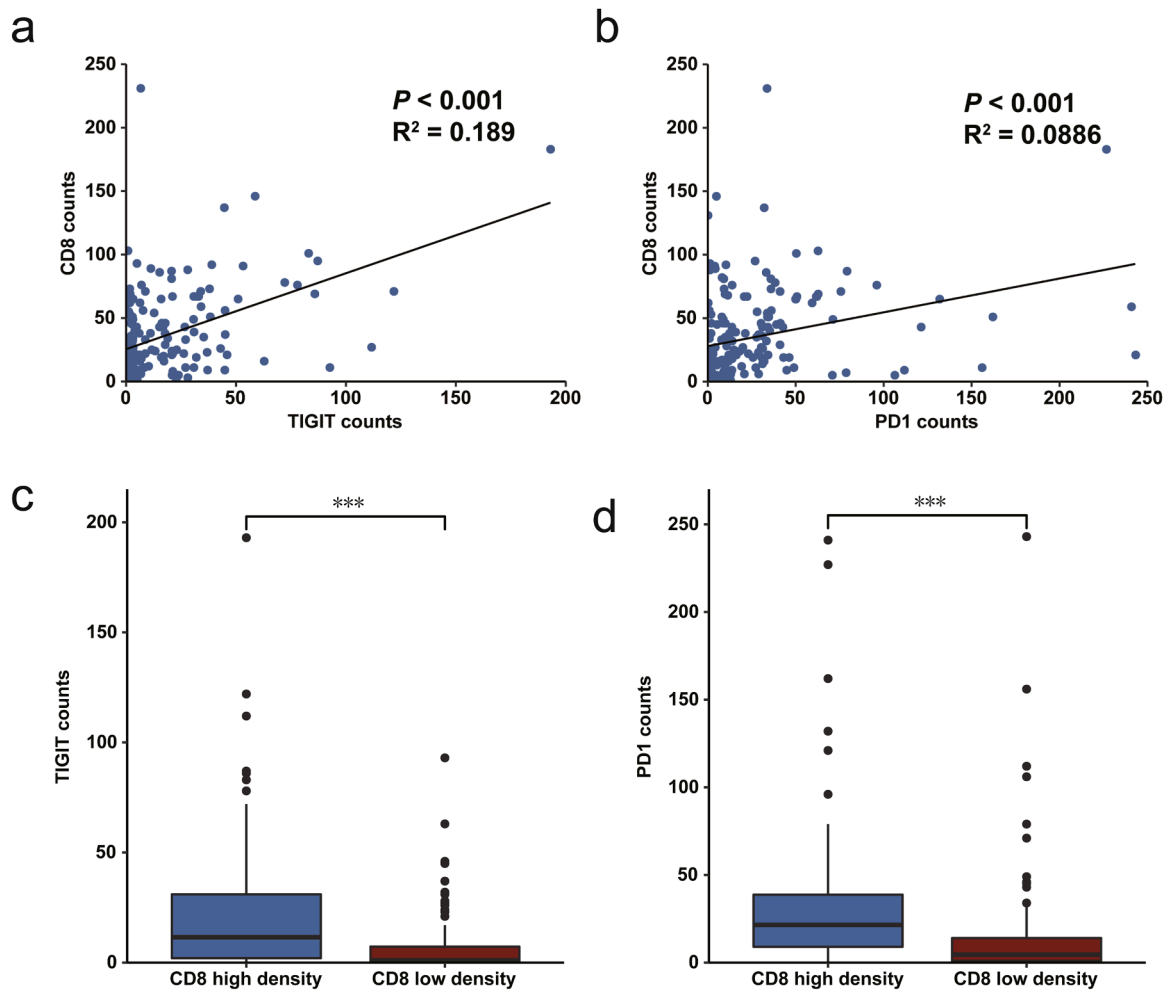
PD-L1/PD-1 inhibitors have been widely used in the treatment of advanced LUSC, however, only 30% of patients could response to the therapy [13,14]. As a result, it is essential to identify new immunotherapeutic targets for LUSC patients. The PVR/TIGIT axis, a novel



**Table 2**  
The expression correlation between immune markers.

Factors	PD-1			PD-L1			CD8			PVR			
	High density	Low density	<i>P</i>	Positive	Negative	<i>P</i>	High density	Low density	<i>P</i>	Positive	Negative	<i>P</i>	
TIGIT	High density	69	26	<0.001	32	63	0.033	60	35	<0.001	83	12	0.533
	Low density	29	66		19	76		34	61		80	15	
PVR	Positive	86	77	0.423	48	115	0.046	80	83	0.790			
	Negative	12	15		3	24		14	13				

Pearson chi-square test was used to evaluate the correlation among immune markers. PVR, PVR cell adhesion molecule; TIGIT, T cell immunoreceptor with Ig and ITIM domains; PD-L1, programmed cell death ligand 1; PD-1, programmed cell death 1; CD8, cluster of differentiation 8.



**Fig. 3.** Association between the expression status of TIGIT/PD-1 and CD8. Scatter plots showed that CD8-positive TIL counts were correlated with TIGIT-positive TIL counts (a) and PD-1-positive TIL counts (b). Boxplots showed that high CD8 density group had significantly more TIGIT-positive TIL counts (c) and PD-1-positive TIL counts (d). \*\*\*,  $P < 0.001$ . t test was used to determine *P* values in scatter plots. Wilcoxon test was used to determine *P* values in boxplots. Center line, median; box limits, upper and lower quartiles; whiskers,  $1.5 \times$  interquartile range; points, outliers. TIGIT, T cell immunoreceptor with Ig and ITIM domains; PD-1, programmed cell death 1; CD8, cluster of differentiation 8.

immune checkpoint pathway, has gained increasing attention in cancer immunotherapy. Previous studies have shown that elevated TIGIT expression on CD8<sup>+</sup>T cells and Tregs is correlated with poor survival [11,15–18]. However, studies focusing on PVR/TIGIT in LUSC have been rarely performed. In this study, we investigated the PVR/PD-L1 expression status and its prognostic value in LUSC. In our cohort, all patients underwent pulmonary surgeries, and the 5-year survival rate was 68.4% (stage I, 76.5%; stage II, 65.7%; stage III, 30.2%). The positive rate of PVR expression was 85.8%, which was much higher than that of PD-L1 (26.8%), indicating that the PVR/TIGIT axis may play

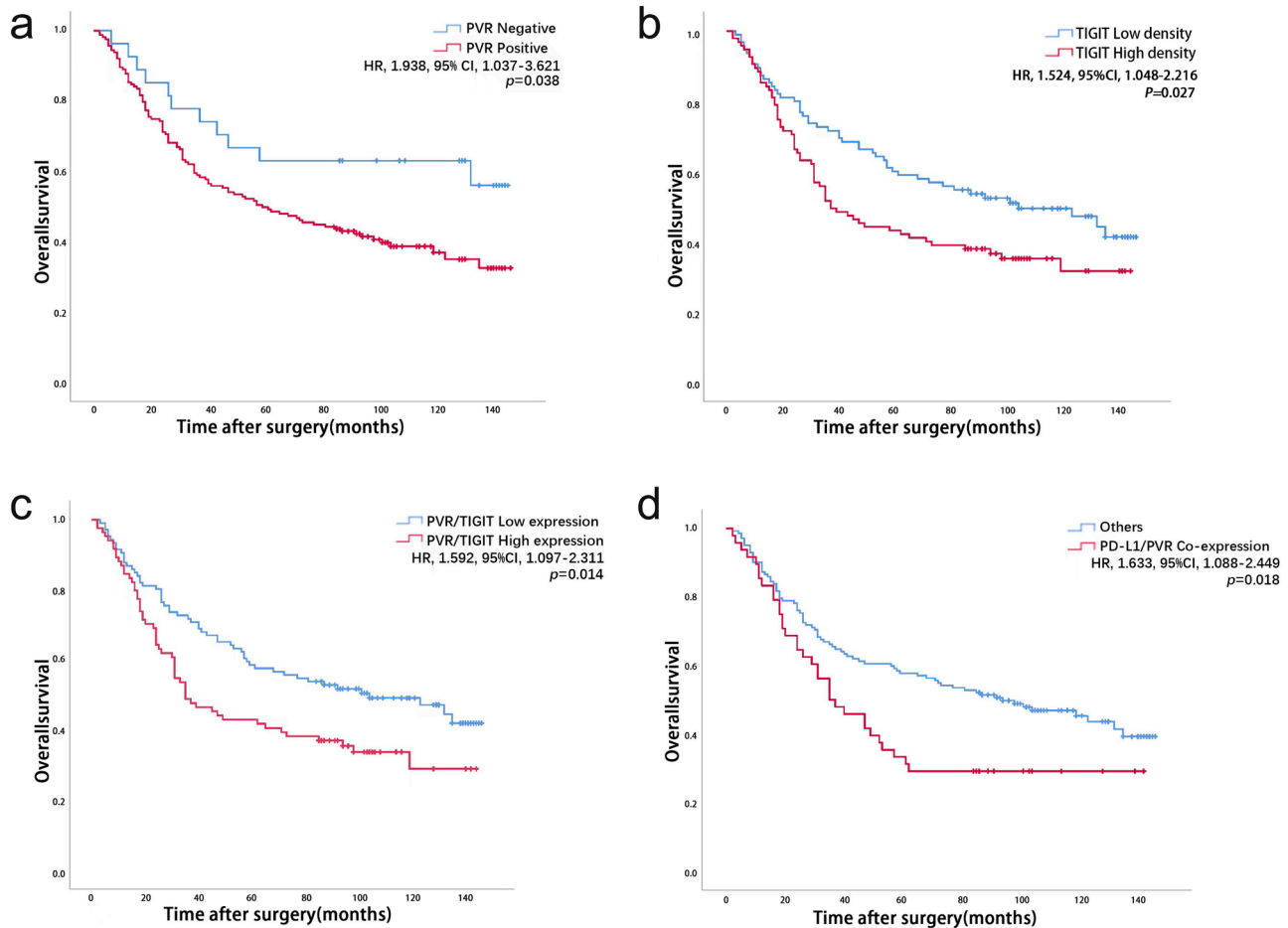
important roles in immune escape of LUSC. Survival analysis showed that patients with high PVR/TIGIT expression, PVR/PD-L1 co-expression, and low CD8 density exhibited a significantly worse prognosis, which was consistent with previous studies in other cancer types [15, 19].

TIGIT belongs to the CD28 family, and it is expressed on several important immune cell types, such as CD8<sup>+</sup> T cells, CD4<sup>+</sup> T cells, regulatory T cells (Tregs) and natural killer (NK) cells [20]. Guillerey et al. demonstrated that CD8<sup>+</sup> T cells expressed higher levels of TIGIT than CD4<sup>+</sup> T cells and NK cells in myeloma patients [12]. Sun et al. showed

**Table 3**  
The relationship between PVR/TIGIT expression and clinicopathological features.

Factors	Age			Gender			Smoking history			TNM			
	<60	≥60	P	F	M	P	S	N	P	I/ II	III	P	
TIGIT	High density	47	48	0.383	3	92	0.700	90	5	0.267	44	51	0.020
	Low density	41	54		4	91		86	9		60	35	
PVR	Positive	71	92	0.061	5	158	0.268	152	11	0.422	89	74	0.926
	Negative	17	10		2	25		24	3		15	12	
PVR/TIGIT	High expression	38	46	0.791	3	81	0.941	79	5	0.506	39	45	0.041
	Low expression	50	56		4	102		97	9		65	41	

Pearson chi-square test was used. PVR, PVR cell adhesion molecule; TIGIT, T cell immunoreceptor with Ig and ITIM domains; F, Female; M, Male; S, Smokers; N, Non-smokers.



**Fig. 4.** Kaplan–Meier curves demonstrating overall survival of LUSC patients according to PVR expression (a), TIGIT density (b), PVR/TIGIT expression (c), and PVR/PD-L1 co-expression (d). PVR, PVR cell adhesion molecule; TIGIT, T cell immunoreceptor with Ig and ITIM domains; PD-L1, programmed cell death ligand 1.

that high TIGIT expression was correlated with high degree of malignancy in lung adenocarcinoma (LUAD) [19]. In our LUSC cohort, the expression level of TIGIT was closely related to tumor staging, which was consistent with the previous study [19]. CD8 is a co-receptor for T cell receptor, and it plays core roles in cell-mediated immune attack [21]. Several studies have shown that TIGIT could be expressed on CD8<sup>+</sup> T cells, which explains the correlation of TIGIT and CD8 density in our cohort, and the expression of TIGIT is related to a decrease in tumor necrosis factor- $\alpha$  production by CD8<sup>+</sup> T cells, resulting in reduced killing ability and decreased proliferation [12,18]. Zhang et al. confirmed that TIGIT deficiency protected mice from lung metastasis, indicating the important roles of TIGIT in tumor progression [22]. Multiple studies have shown that genetic knockout or antibody inhibition of TIGIT can enhance the anti-tumor effects of NK cells and CD8<sup>+</sup> T cells [18,22, 23].

He et al. found that TIGIT expression on CD8<sup>+</sup> T cells inhibited glucose uptake and reduced the metabolic activity of cells. However, the effects could be reversed by adding glucose or blocking the PVR/TIGIT pathway [6]. In summary, TIGIT was essential for several biological procedures, and it played important roles in immune escape and progression of tumors, which was the deep reason for the prognostic value of TIGIT in our LUSC cohort.

PVR is the main ligand of TIGIT, and it binds to TIGIT with high affinity [10]. Through PVR knockdown in fibrosarcoma and glioblastoma cells, Kevin et al. revealed the role of PVR in cell migration and dispersal [24]. The loss of PVR decreased tumor growth, inhibited tumor metastasis, and enhanced sensitivity to immunotherapy [25]. Previous studies found that PVR was more highly and widely expressed in tumors than in normal tissues, and patients with high expression level of PVR



exhibited a significantly worse OS [19]. The same phenomenon was observed in our study: a large proportion (85.8%) of LUSC patients were positive for PVR expression, and the PVR positive group had a significantly poorer prognosis. However, there was no correlation between the expression level of PVR and tumor staging in our cohort, which was not consistent with the study by Wu et al. [25]. This inconsistency might be due to discrepancies in sample size, proportion of TNM stages at diagnosis and definition of PVR positivity.

Several studies have shown that blockade of the PVR/TIGIT axis had profound effects in multiple malignant tumor types, highlighting that the PVR/TIGIT axis could be a potential target for immune checkpoint therapy [26]. However, previous studies reported that TIGIT blockade monotherapy was not potent enough, and combined dual-targeting immunotherapy, inhibiting PD-1 and TIGIT simultaneously, induced a durable, protective anti-tumor immune response, resulting in complete regression and long-term OS in both glioblastoma and colon carcinoma [27]. Chauvin et al. demonstrated that blockade of TIGIT and PD-1 greatly enhanced the anti-tumor effects of tumor CD8<sup>+</sup> T cells from melanoma patients [11]. Using mouse models of subcutaneous tumors, researchers demonstrated that TIGIT blockade monotherapy was insufficient to suppress tumor growth, which could be overcome by combination with anti-PD-L1/PD-1 therapy [10]. In melanoma, TIGIT blockade could promote proliferation of CD8<sup>+</sup> T cells and increase cytokine production, and the effect could be magnified by combination with PD-L1/PD-1 therapy [11]. Previous study found that PVR is commonly expressed in TIL negative tumors, suggesting that targeting the PVR/TIGIT axis and PD-L1/PD-1 blockade therapy might have synergistic effects [19]. In our study, PVR/PD-L1 co-expression was an independent prognostic factor in LUSC patients. Moreover, high TIGIT density was correlated with positive PD-L1 expression and high PD-1 density, and high PVR expression was correlated with positive PD-L1 expression, indicating the applicability of future dual-target immunotherapy in LUSC.

While immune checkpoint blockade therapy has improved the prognosis of LUSC patients, it may disrupt immune homeostasis and cause autoimmunity, resulting in immune-related adverse events (irAEs) [28,29]. irAEs include fatigue, diarrhea, rash, and inflammatory diseases, such as pneumonitis, hepatitis, hypothyroidism, and colitis, and severe cases can result in immune-related deaths. According to recent studies, the incidences of severe irAEs (grade  $\geq 3$ ) in patients receiving CTLA-4 inhibitor, PD-1 inhibitor, and CTLA-4/PD-1 dual-target therapy were 20% to 30%, 10% to 15%, and 55%, respectively. CTLA4-deficient mice may develop severe autoimmune or lymphoproliferative syndrome, which does not occur in TIGIT-deficient mice, suggesting that TIGIT blockade could be relatively safer [10,28]. Nowadays, clinical trials targeting PVR/TIGIT combined with anti-PD-L1/PD-1 therapy, chemotherapy, or radiation therapy in multiple cancer types, such as triple negative breast cancer (NCT03564782), advanced gliomas (NCT02986178, NCT01491893, NCT03043391), and advanced or metastatic solid malignancies (ASP8374), are underway, and bispecific antibodies or multispecific antibodies as next-generation therapeutic agents have promoted multiple candidates into ongoing clinical trials [26,29,30]. In our cohort, 85.8% of cases were positive for PVR expression, and 26.8% of cases were positive for PD-L1 expression. Moreover, 16.8% of patients demonstrated co-expression of PVR/PD-L1, which was an independent prognostic factor for LUSC, indicating the therapeutic potential of PVR/TIGIT and PD-L1/PD-1 dual-target immunotherapy in LUSC patients.

In conclusion, we demonstrated the expression status of PVR/TIGIT and PD-L1/PD-1 in LUSC. High TIGIT density was correlated with positive PD-L1 expression, high PD-1 density and high CD8 density, and positive PVR expression was correlated with positive PD-L1 expression. High TIGIT density and high PVR/TIGIT expression were correlated with advanced TNM stage. PVR/PD-L1 co-expression was an independent prognostic factor in LUSC patients and may serve as a potential predictive biomarker for future dual-targeting immunotherapy. Future

prospective trials are needed for further exploration.

#### Author contributions statement

Zhenlin Yang, Shugeng Gao, Jie He: study design. Yue Peng, Jiachen Xu, He Tian: IHC experiment. Lin Li: IHC evaluation. Yue Peng, Ping Chen, Guangyu Bai, Lei Liu: data acquisition. Yue Peng, Zhenlin Yang, Qingyuan Cai, Zhenshan Zhao: statistical analysis. He Tian, Yue Peng, Zhenlin Yang, Jiachen Xu: drafting the manuscript.

#### Compliance with ethical standards

All the procedures performed in the study involving human participants were in accordance with the ethical standards of the ethics committee of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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