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TIGIT/CD47 dual high expression predicts prognosis and is associated with immunotherapy response in lung squamous cell carcinoma

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Abstract

Background: Recent studies indicated that T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) and cluster of differentiation 47 (CD47) have emerged as new potential immunotherapy targets. However, the roles of TIGIT and CD47 in lung squamous cell carcinoma (LUSC) have not been fully illustrated.

Methods: The specimens and clinicopathological information from 190 LUSC patients who underwent surgeries in our center were retrospectively collected. Immunohistochemical staining for TIGIT and CD47 was conducted. Transcriptional and clinical data of 479 LUSC were downloaded from The Cancer Genome Atlas (TCGA).

Results: In the TCGA LUSC cohort, 142 (29.6%) cases were TIGIT/CD47 dual high expression at RNA level. The expression levels of TIGIT and CD47 were significantly correlated ($p < 0.001$). The proportions of patients with high TIGIT expression ($p = 0.001$) and high TIGIT/CD47 dual high expression ($p = 0.049$) were both higher in female cases. Advanced TNM stage ($p = 0.006$) and TIGIT/CD47 dual high expression ($p = 0.047$) were independent prognostic factors for LUSC. In the 190 LUSC cohort of our center, 75 (39.5%) cases were TIGIT/CD47 dual high expression at protein level. Cross-table analysis showed a correlation between TIGIT and CD47 expression. Older age ($p = 0.001$), advanced TNM stage ($p < 0.001$) and TIGIT/CD47 dual high expression ($p = 0.046$) were independent prognostic factors in our cohort.

Conclusion: We found that TIGIT and CD47 dual high expression was associated with poor prognosis in LUSC. We speculated that patients with dual high expression of CD47/TIGIT might be suitable for new target immunotherapy in the future.

KEYWORDS
CD47, immunotherapy, LUSC, NSCLC, TIGIT

INTRODUCTION

Despite updated treatment, lung cancer is still one of the most malignant diseases worldwide. GLOBOCAN reported 2.21 million new lung cancer patients and 1.8 million deaths worldwide in 2020.[1] Lung cancer brings a huge medical burden every year. A survey by the National Cancer Center shows that China had 828 100 new cases of lung cancer in 2015, with an annual incidence of 59.89/100 000, and approximately 657 000 lung cancer deaths, with an annual mortality rate of 47.51/100 000.[2] In patients with lung cancer, radical surgery is the primary treatment for early and partial locally advanced non-small cell lung cancer (NSCLC). Although surgery can provide optimal local control, 30%–55% of patients tend to experience disease recurrence; with a more advanced stage, the risk of recurrence increases rapidly.[3] However, advanced-stage patients generally receive chemotherapy and radiotherapy, and the 5-year survival rate is only about 5%.[4]

In recent years, immune checkpoint inhibitor (ICI) therapies in tumor treatment have become increasingly prominent and markedly shifted the therapeutic paradigm of various tumors. Currently, PD-1/PD-L1 (programmed cell death 1/programmed cell death 1 ligand 1) inhibitors are the most widely used for immunotherapy. The 5-year overall survival (OS) follow-up results of the KEYNOTE-024 study released at the annual meeting of the European Society for Medical Oncology (ESMO) was shown to be nearly two times higher in the pembrolizumab group than in the chemotherapy group (31.9% vs. 16.3%).[5] However, the response rate of PD-1/PD-L1 inhibitors to NSCLC was only 20%.[6] Many patients still have dismal prognoses, hence the importance of finding potential immune checkpoints.

The immune system plays a vital role in the antitumor response, but cancers can still progress by evading immune surveillance. The T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) is a coinhibitory receptor. Studies have found that TIGIT is highly expressed in colon cancer, multiple myeloma, breast cancer, prostate cancer, and so on. It is closely related to prognosis.[7,8] Targeted TIGIT antibodies can effectively restore T cell function and exert an antitumor effect.[9] Studies on TIGIT are rare in lung squamous cell carcinoma (LUSC). Cluster of differentiation 47 (CD47) is a glycoprotein widely expressed on cell surface, plays an essential role in various cellular functions such as regulating adhesion, proliferation, axon extension, fusion, migration, phagocytosis, and apoptosis.[10,11] It can block the phagocytosis of macrophages, as well as mediate the migration of neutrophils and the activation and diffusion of platelets by binding to signal-regulatory proteins (SIRP α).[11] CD47 inhibitors have been used in clinical trials in ovarian cancer, colorectal cancer, and leukemia;[12–14] however, lung cancer-related studies remain limited.

In the current study, we aimed to investigate the expression status of TIGIT and CD47 in LUSC at RNA and protein level, explore the relationship among TIGIT and CD47 expression status and clinicopathological features, and find potential therapeutic targets for LUSC.

METHODS

Clinical characteristics

The transcriptional and clinical data of LUSC was downloaded from The Cancer Genome Atlas (TCGA), including age, gender, stage, transcriptome data and so on. The expression data of TIGIT and CD47 and the survival data were extracted ($n = 479$). Based on the median transcriptional level, we divided the expression of TIGIT and CD47 into high and low groups. Patients with high TIGIT and CD47 expression were grouped into dual high expression subgroup. Data pertaining to 190 patients with LUSC who underwent thoracic surgery at the Cancer Hospital, Chinese Academy of Medical Sciences from April 2006 to February 2011 were collected retrospectively. Lobectomy or pneumonectomy and systematic lymph node dissection were conducted in all patients. The pathological staging criterion of the study was in accordance with the eighth edition of TNM Staging of Lung Cancer. Data on the gender, age, smoking history, mode of operation, postoperative pathological report and staging were collected retrospectively in the medical history system. Formalin fixed paraffin-embedded (FFPE) specimen of tumor tissues were retrieved from the department of pathology of our center. We defined the OS as the time from the date of the operation to the date of last follow-up or death. The last follow-up time of this study was September 20, 2018.

Tumor immune microenvironment

TIGIT and CD47 were detected by immunohistochemical staining on tissue microarrays (TMAs). The numbers of TIGIT-positive tumor-infiltrating lymphocytes (TILs) in six high-power visual fields were counted, and the average number was used to measure TIGIT positive TILs density. Using the median average number as the cutoff value, the density of TIGIT-positive TILs was divided into high-density (TIGIT high expression) and low-density (TIGIT low expression) groups. The CD47 expression at protein level was evaluated by recording the percentage of membranous stained tumor cells: a percentage less than 5% was defined as low expression, whereas a percentage ≥5% was defined as high expression. Patients with CD47 high expression and TIGIT high expression were grouped into a TIGIT and CD47 dual high expression subgroup.

Statistical analysis

The data were statistically analyzed using SPSS version 26.0. The total survival curve was generated using the Kaplan–Meier

method. The significance of differences among the survival curves for each group were calculated using the log-rank test. The Cox's regression model was used for multifactor analysis. Chi-square test was used for the analysis of categorical variables. Spearman's correlation was used to analyze the correlation between continuous variables. $p < 0.05$ was considered statistically significant.

RESULTS

Patient and clinicopathological characteristics

Data on 479 LUSC patients who had undergone surgeries were extracted from the TCGA database. The clinicopathological characteristics are listed in Table 1. The median age was 68 years (range: 39–85 years). The patients included 353 males (73.7%), and the cases were classified into different stages: stage I, 234 cases (48.9%); stage II, 156 cases (32.5%); stage III, 83 cases (17.3%); and stage IV, 6 cases (1.3%). TIGIT and CD47 dual high expression was observed in 142 cases (29.6%) in TCGA cohort. The clinicopathological characteristics of the 190 LUSC patients who underwent surgery in our center are shown in Table 2. The median age of the patients was 61 years (range: 37–79 years), and 183 (96.3%) of the patients were males. A total of 176 (92.6%) cases had a history of smoking, and 95 (50.0%) had poorly differentiated tumors. The average maximum diameter of the lesions was 4.99 cm (range: 1.8–12 cm). 25 (13.2%), 99 (52.1%), 45 (23.7%) and 21 (11.1%) cases were pathologically staged in T1, T2, T3 and T4,

TABLE 1 Univariate and multivariate analyses in 479 LUSC cases downloaded from TCGA

		Univariate analysis		Multivariate analysis	
Variable	***N* %**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Age (years)					
<65	166 (34.7)	0.791 (0.577–1.083)	0.144	0.758 (0.551–1.043)	0.089
≥65	313 (65.3)				
Gender					
Male	353 (73.7)	1.072 (0.768–1.497)	0.682	1.130 (0.806–1.584)	0.478
Female	126 (26.3)				
T					
1–2	389 (81.4)	0.578 (0.414–0.808)	0.001		
3–4	90 (18.6)				
N					
0–1	435 (90.8)	0.737 (0.472–1.151)	0.180		
2–3	44 (9.2)				
TNM stage					
1–2	390 (81.4)	0.625 (0.448–0.870)	0.005	0.624 (0.446–0.872)	0.006
3–4	89 (18.6)				
PD-L1					
High expression	239 (49.9)	0.988 (0.740–1.320)	0.937	1.021 (0.757–1.377)	0.892
Low expression	240 (50.1)				
PD-1					
High expression	239 (49.9)	0.983 (0.738–1.310)	0.908	1.124 (0.798–1.582)	0.503
Low expression	240 (50.1)				
TIGIT					
High expression	240 (50.1)	1.029 (0.771–1.373)	0.847		
Low expression	239 (49.9)				
CD47					
High expression	240 (50.1)	1.402 (1.050–1.872)	0.022		
Low expression	239 (49.9)				
TIGIT/CD47					
Dual high expression	142 (29.6)	1.360 (1.001–1.847)	0.049	1.446 (1.006–2.079)	0.047
Others	337 (70.4)				

Abbreviations: CD47, cluster of differentiation 47; CI, confidence interval; LUSC, lung squamous cell carcinoma; PD1, programmed cell death 1; TCGA, The Cancer Genome Atlas; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains.

TABLE 2 Univariate and multivariate analyses in 190 LUSC cases from our center

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	0.527 (0.356–0.781)	0.001
≥60	102 (53.7)				
Gender					
Male	183 (96.3)	1.435 (0.455–4.521)	0.537	0.749 (0.210–2.669)	0.656
Female	7 (3.7)				
Smoking history					
Smokers	176 (92.6)	0.582 (0.396–1.830)	0.681	0.923 (0.397–2.150)	0.854
Nonsmokers	14 (7.4)				
T					
1–2	124 (65.3)	0.364 (0.250–0.531)	<0.001		
3–4	66 (34.7)				
N					
0–1	131 (68.9)	0.421 (0.282–0.602)	<0.001		
2	59 (33.1)				
TNM stage					
1–2	104 (54.7)	0.344 (0.235–0.504)	<0.001	0.298 (0.200–0.444)	<0.001
3	86 (45.3)				
TIGIT					
High expression	95 (50.0)	1.524 (1.048–2.216)	0.027		
Low expression	95 (50.0)				
CD47					
High expression	124 (65.3)	1.866 (1.232–2.826)	0.003		
Low expression	66 (34.7)				
TIGIT/CD47					
Dual high expression	75 (39.5)	1.561 (1.074–2.270)	0.020	1.468 (1.007–2.141)	0.046
Others	115 (60.5)				

Abbreviations: CD47, cluster of differentiation 47; CI, confidence interval; LUSC, lung squamous cell carcinoma; PD1, programmed cell death 1; TCGA, The Cancer Genome Atlas; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains; TNM stage, tumour-node-metastasis stage.

respectively. A total of 126 (66.3%) cases had regional lymph node metastasis. Only 34 cases (17.9%) were categorized as stage I, and 70 (36.8%) and 86 (45.3%) cases were stage II and stage III, respectively. Immunohistochemical staining (IHC) for TIGIT and CD47 (Figure 1) revealed that 124 cases (65.3%) were CD47 high expression and 75 (39.5%) cases were TIGIT/CD47 dual high expression.

Association between TIGIT/CD47 expression and clinicopathological characteristics

We extracted data from TCGA database and explored the association between TIGIT/CD47 expression and clinicopathological features. Spearman correlation analysis between the expression levels of CD47 and TIGIT in the TCGA cohort was performed, and we found that CD47 and TIGIT expressions were significantly correlated ($p = 0.00058$, $\rho = 0.16$) (Figure 2). Meanwhile, the expression of TIGIT and CD47 at RNA level were related ($p < 0.001$), as determined by cross-table analysis (Table 3). The proportions of patients with high TIGIT expression and high TIGIT/CD47 dual expression were both higher in female cases (TIGIT, $p = 0.001$; TIGIT/CD47 dual high expression, $p = 0.049$) (Table 3). In addition, we performed cross-table analysis on 190 LUSC patients of our cohort. As demonstrated in Table 4, we found a correlation between TIGIT and CD47 expression ($p < 0.001$), and TIGIT high expression was closely related to advanced staging ($p = 0.020$). In addition, TIGIT and CD47 dual high expression was associated with poor differentiation ($p = 0.012$) (Table 4).

Analysis of survival

In the LUSC cohort from the TCGA database, the median follow-up period was 18.3 months (0.1–176.2 months). Survival analyses were conducted utilizing Kaplan–Meier

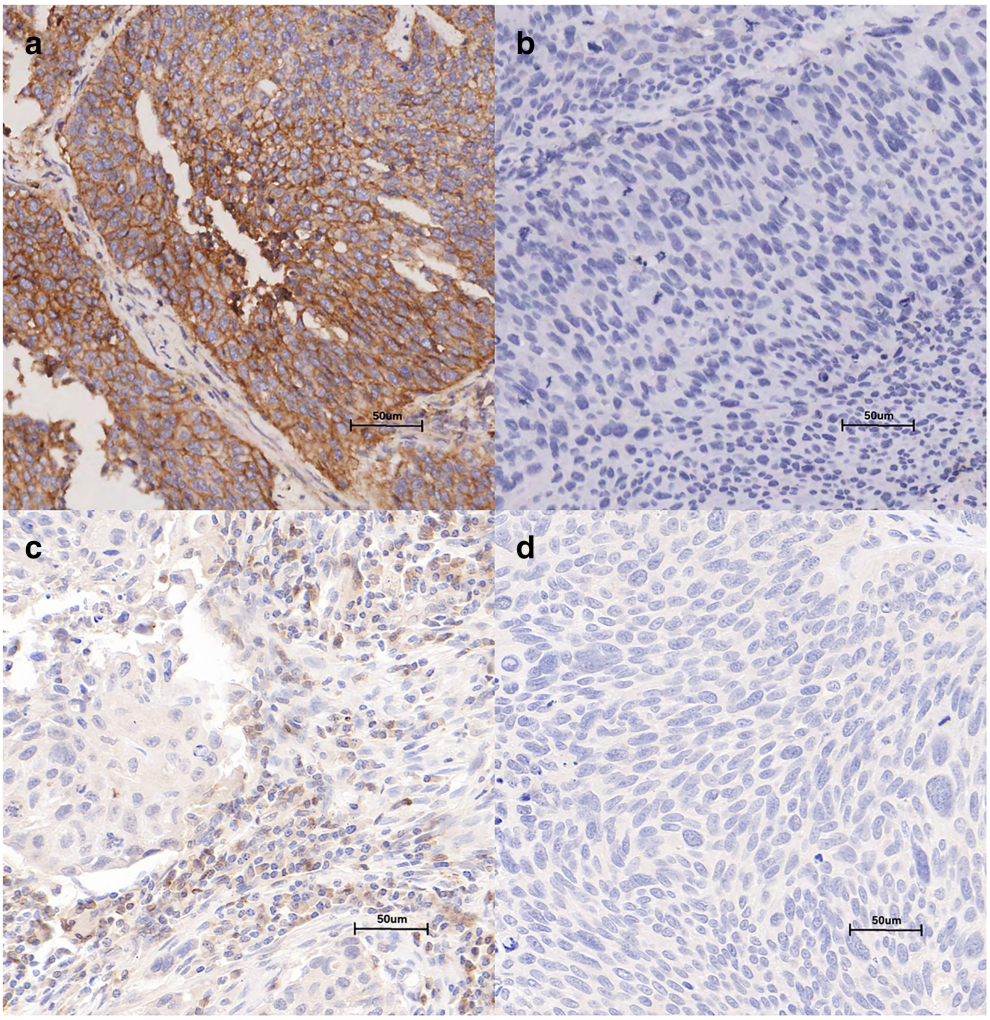

FIGURE 1 Representative IHC images of TIGIT and CD47 expression. (a) CD47 high expression. (b) CD47 low expression. (c) TIGIT high expression. (d) TIGIT low expression. Scale bar: 50 μm. CD47, cluster of differentiation 47; IHC, immunohistochemistry; LUSC, lung squamous cell carcinoma; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains

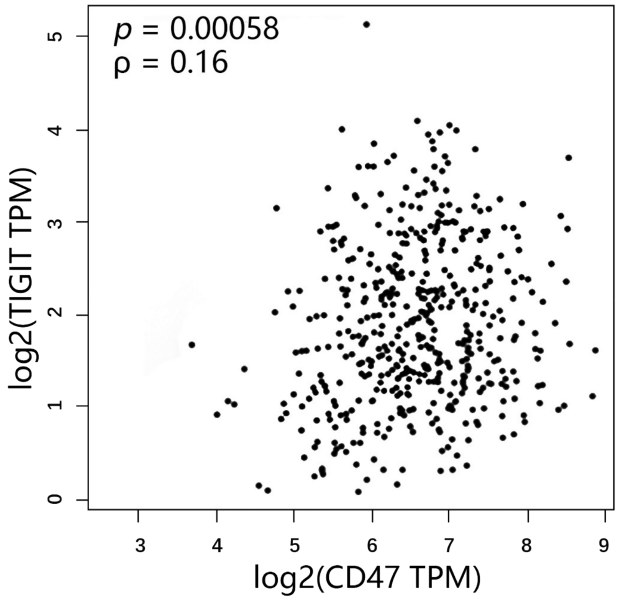

FIGURE 2 The scatter plot demonstrates the correlation between CD47 and TIGIT expression levels in TCGA cohort. CD47, cluster of differentiation 47; TCGA, The Cancer Genome Atlas; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains

curves. Univariate analysis indicated that advanced T stage, advanced TNM stage, CD47 high expression and TIGIT/CD47 dual high expression (Figure 3) were associated with shorter OS (T stage, $p = 0.001$; TNM stage, $p = 0.005$; CD47 expression, $p = 0.022$; TIGIT/CD47 dual expression, $p = 0.049$). Multivariate analysis demonstrated that advanced TNM stage (HR 0.624, 95% CI: 0.446–03872, $p = 0.006$) and TIGIT/CD47 dual high expression (HR 1.446, 95% CI: 1.006–2.079, $p = 0.047$) were independent prognostic factors for LUSC patients.

In the LUSC cohort of our hospital, the 5-year OS rate was 68.4%, and the median survival time was 65 months. In the univariate analysis, advanced T stage, advanced N stage, advanced TNM stage, TIGIT high expression, CD47 high expression and TIGIT/CD47 dual high expression (Figure 4) were associated with shorter OS (T stage, $p < 0.001$; N stage, $p < 0.001$; TNM stage, $p < 0.001$; CD47 expression, $p = 0.003$; TIGIT high expression, $p = 0.027$; TIGIT/CD47 dual high expression: $p = 0.020$). In the multivariate analysis, older age, advanced TNM stage and TIGIT/CD47 dual high expression were independent prognostic factors for LUSC patients (age: HR 0.527, 95% CI: 0.356–0.781, $p = 0.001$; TNM stage: HR 0.298, 95% CI: 0.200–0.444,

TABLE 3 Correlation between patient clinicopathological characteristics and TIGIT/CD47 expression in 479 LUSC cases from TCGA

	CD47			TIGIT			TIGIT/CD47		
Factors	**High**	**Low**	***p*-value**	**High**	**Low**	***p*-value**	**High**	**Others**	***p*-value**
Age									
<60	79	87	0.462	83	83	0.973	49	117	0.965
≥60	160	153		156	157		93	220	
Gender									
M	173	180	0.516	160	193	0.001	96	257	0.049
F	66	60		79	47		46	80	
TNM									
I/II	199	191	0.300	195	195	0.924	115	275	0.874
III/IV	40	49		44	45		27	62	
TIGIT									
High	142	97	<0.001						
Low	97	143							

Abbreviations: CD47, cluster of differentiation 47; LUSC, lung squamous cell carcinoma; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains; TCGA, The Cancer Genome Atlas.

TABLE 4 Correlation between patient clinicopathological characteristics and TIGIT/CD47 expression in 190 LUSC cases from our center

	CD47			TIGIT			TIGIT/CD47		
Factors	**High**	**Low**	***p*-value**	**High**	**Low**	***p*-value**	**High**	**Others**	***p*-value**
Age									
<60	57	31	0.895	47	41	0.383	38	50	0.331
≥60	67	35		48	54		37	65	
Gender									
M	120	63	0.646	92	91	0.700	73	110	0.548
F	4	3		3	4		2	5	
Smoking history									
Yes	113	63	0.277	90	86	0.267	70	106	0.765
Never	11	3		5	9		5	9	
Differentiation									
Poor	68	27	0.067	51	44	0.310	46	49	0.012
Others	56	39		44	51		29	66	
TNM									
I/II	64	40	0.236	44	60	0.020	35	69	0.071
III	60	26		51	35		40	46	
TIGIT									
High	75	20	<0.001						
Low	49	46							

Abbreviations: CD47, cluster of differentiation 47; LUSC, lung squamous cell carcinoma; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains.

$p < 0.001$; TIGIT/CD47 dual high expression: HR 1.468, 95% CI: 1.007–2.141, $p = 0.046$).

DISCUSSION

Therapeutic PD-1/PD-L1 antibodies are widely used in advanced NSCLC patients, and in a previous study only 20% responded to therapy,[6] indicating that PD-1/PD-L1 monotherapy was not potent enough. Novel immune checkpoints for NSCLC are warranted. Our study revealed that the upregulation of TIGIT and CD47 expression in LUSC was associated with poor survival. We first extracted the transcriptional and clinical data of 479 LUSC patients from the TCGA database. Data analysis showed that the expression levels of CD47 and TIGIT were correlated ($p < 0.001$), and TIGIT/CD47 dual high expression was associated with poor prognosis ($p = 0.047$). Subsequently, we performed

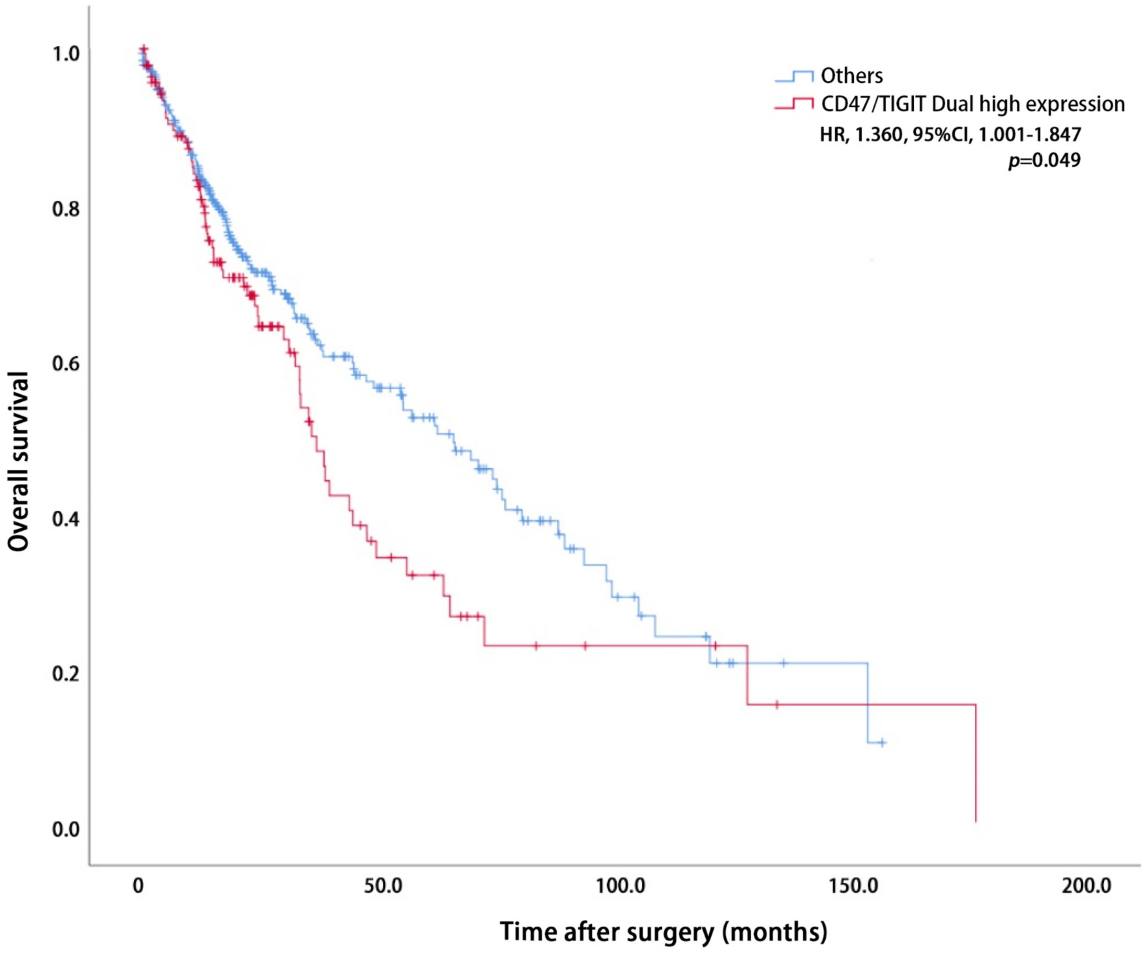

FIGURE 3 Kaplan–Meier curves for overall survival of 479 LUSC cases from TCGA categorized by TIGIT/CD47 dual expression status. CD47, cluster of differentiation 47; LUSC, lung squamous cell carcinoma; TGA, The Cancer Genome Atlas; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains

immunohistochemistry on our LUSC TMAs, and found that CD47 expression was related to TIGIT expression ($p < 0.001$). In addition, TIGIT and CD47 dual high expression was associated with poor prognosis in our cohort ($p = 0.046$). Our results illustrated the potentiality of novel immune checkpoint inhibitors application in LUSC based on immune microenvironment characteristics.

CD47 is a glycosylated protein, widely expressed on the surface of normal cells, especially on leukocytes and red blood cells.[15] Tumor-associated macrophages are widely-spread immune cells in solid tumors.[16,17] Meanwhile, in several studies, tumor cells expressed CD47 on their surface, which could bind to SIRP α expressed on the surface of macrophages and send a "do not eat me" signal, thereby promoting immune escape of tumor cells.[16,18,19] CD47 were found to be highly expressed in colon cancer, breast cancer, hepatocellular carcinoma, leukemia, lymphoma, and small cell lung cancer, and also related to short OS and increased risk of recurrence and metastasis.[20] Several CD47 inhibitors, such as AK117, AO-176, CC-90002, CPO107, Hu5F9-G4, DSP107, JMT601 (CPO107), ALX148, HX009 and SRF231, have entered clinical trials for non-Hodgkin's lymphoma, leukemia and solid tumors.[14,16] In mouse models of myeloma, lymphoma and leukemia, treatment with CD47 inhibitors could promote macrophage phagocytosis and initiate T cell immunity, and CD47 inhibitor showed synergistic effects with rituximab therapy.[10] Zhang et al. treated nude mice with transplanted NSCLC with VEGFR inhibitor monotherapy, or in combination with the SIRPα inhibitor, and found that the tumor burden increased after 2–3 weeks of treatment with VEGFR inhibitor monotherapy, whereas combined therapy suppressed tumor growth and reduced the tumor burden.[21] CD47 high expression (the proportion of membranous staining tumor cells ≥5%) was found in 65.3% of the patients in our cohort, which is consistent with other NSCLC studies (range 53%–84%).[22,23] We confirmed that CD47 high expression at RNA and protein level was associated with poor prognosis, suggesting that CD47 might be a potential immunotherapeutic target for LUSC.

We found that CD47 expression at RNA level in the TCGA database was closely related to that of TIGIT. Subsequently, immunohistochemical verification was conducted on our TMAs, which confirmed that CD47 expression was closely related to TIGIT expression at protein level. This

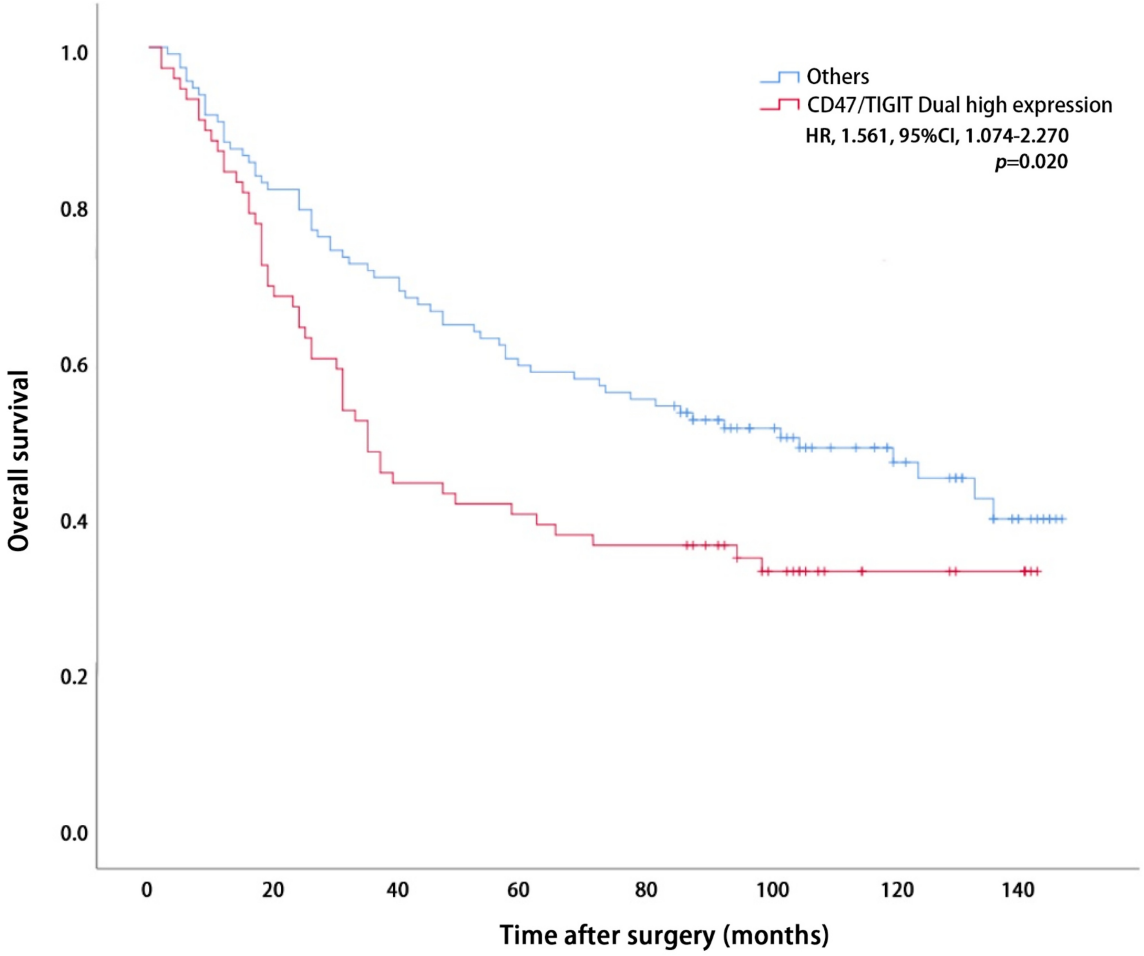

FIGURE 4 Kaplan–Meier curves for 190 LUSC cases from our center categorized by TIGIT/CD47 dual expression status. CD47, cluster of differentiation 47; LUSC, lung squamous cell carcinoma; TGA, The Cancer Genome Atlas; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains

phenomenon has not been observed in other studies, and we speculated that the reason might be that they played synergistic roles in the development of tumors. Various studies have shown that tumor cells expressing CD47 on their surface could protect them from phagocytosis by macrophages, indicating that CD47 blockade could promote the phagocytosis of tumor cells mediated by macrophages and dendritic cells. Mittruecker et al. reported that macrophages could deliver tumor neoantigens to T cells through MHC-II molecules, thus activating $CD8^+$ T cells and promoting T cell killing of tumors, which was confirmed by blocking CD47 in several mouse models.[10,24] Liu et al. demonstrated that T cell-mediated immune response plays an important role in CD47 blockade therapy in mouse models.[11,25] In addition, depletion of $CD8^+$ T cells reduced the therapeutic effect of mouse CD47 antibody. Mouse models treated with anti-CD47 therapy had significantly more antigen-specific $CD8^+$ T cells infiltrating in their tumors and formed a T cell-mediated immune memory to protect them from tumor reattack.[11,26] All these results suggest that T cells are essential for the antitumor effects of CD47 blockade, and that CD47 might play a synergistic role with multiple T cell-associated immune checkpoints. Recently, TIGIT has emerged as an essential checkpoint in cancer development; it belongs to the immunoglobulin superfamily, and is overexpressed on tumor-infiltrating T cells as $CD4^+$ T cells, $CD8^+$ T cells, regulatory T cells, and natural killer (NK) cells.[27] TIGIT participates in the inhibition of antitumor immune responses at multiple stages of the cancer immune cycle. Normally, NK cells kill tumor cells and promote the release of tumor antigens, which are taken up by dendritic cells (DCs) and presented to T cells. However, it has previously been determined that TIGIT could inhibit the tumor killing of NK cells and the antigen presentation of DCs by interacting with CD155 expressed on DCs, thus weakening the antitumor effects of T cells.[7] In summary, CD47 and TIGIT could therefore play synergistic roles in tumor immunity.

We observed that TIGIT high expression was associated with shorter OS, consistent with other studies. In addition, TIGIT was reported to be overexpressed on lymphoid cells in NSCLC, breast cancer, colon adenocarcinoma, melanoma,

acute myelocytic leukemia (AML) and multiple myeloma. TIGIT interacted with its ligand CD155 to induce immunosuppression.[8,28–31] Several studies have shown that TIGIT inhibition or gene knockout can enhance the killing of NK cells and the antitumor activity of $CD8^+$ T cells.[32,33] Zhang et al. injected B16 melanoma cells into mouse models, and found that mouse models with TIGIT-deficiency developed lower incidence of lung metastasis and had prolonged OS, confirming that blocking TIGIT could reverse NK cell depletion and inhibit tumor growth.[33] TIGIT expression is related to a decrease in tumor necrosis factor-α production by $CD8^+$ T cells. A recent study demonstrated that TIGIT high expression was correlated with $CD8^+$ T cell exhaustion and immune functional impairment. However, their functional defects could be reversed by TIGIT knockout.[30] TIGIT high expression has also previously been associated with a higher degree of malignancy, which was consistent with our finding: TIGIT high expression was closely related to advanced staging.[28,34] Analysis of the TCGA data showed that the proportion of patients with high TIGIT expression was higher in female cases, which was similar to the conclusion of a LUAD study.[34] However, the phenomenon was not observed in the LUSC cohort of our center, probably because only 3.7% of cases in our cohort were female.

The data in TCGA and our center showed that TIGIT and CD47 dual high expression at RNA and protein level was closely associated with poor prognosis. As bispecific antibodies or multispecific antibodies have become the next generation of therapeutic drugs, we believe that the combined use of TIGIT and CD47 inhibitors could be potential therapeutic strategies for LUSC in the future. Owing to the high expression of CD47 on red blood cells (RBCs), and according to the results of some clinical trials, the main risk of CD47 inhibitors is anemia. In their toxicokinetic study, Liu et al. suggested that a low start-up dose and a high maintenance dose could help HU5F9-G4 reach the level of therapeutic dose, only slightly affecting RBCs.[16,35] Meanwhile, autoimmunity or hematopoietic dysplasia was not observed in TIGIT-deficient mice, while CTLA4-deficient mice could develop severe autoimmune or lymphoproliferative syndrome. TIGIT-deficient mice exhibited a milder autoimmune phenotype, thus indicating that TIGIT inhibitor was relatively safe.[7] Based on previous studies, the safety of the two inhibitors administered alone was acceptable, we speculated that treatment with combined TIGIT and CD47 inhibitors might be relatively safe. However, this argument lacks evidence and needs to be further verified.

There were also several limitations of the study. The main point was that although we made a prediction for the efficacy of immunotherapy with new targets for LUSC, we did not include any treated patient. Therefore, we could not draw any strong conclusions about efficacy prediction. In addition, we described the association between the expression of CD47 and TIGIT, and tried to explain the underlying mechanism by literature review; however, we could not provide direct evidence as no in vivo and in vitro experiments were performed.

In conclusion, we explored the roles of TIGIT and CD47 in LUSC patients and proved that TIGIT and CD47 dual high expression was associated with poor prognosis. We speculated that patients with dual high expression of CD47/TIGIT might be suitable for new targeted immunotherapy in the future.

CONFLICT OF INTEREST

No conflict of interest was declared.

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