Original Article

Lymphocyte activation gene-3 is associated with programmed death-ligand 1 and programmed cell death protein 1 in small cell lung cancer

Hui Sun[1,2#], Jiawei Dai[3#], Lishu Zhao[1,2#^], Jun Zhu[1,2], Hao Wang[1,2], Peixin Chen[1,2], Hui Lu[3], Qiankun Chen[4], Zhemin Zhang[1,2]

[1]Department of Medical Oncology, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai, China; [2]School of Medicine, Tongji University, Shanghai, China; [3]SJTU-Yale Joint Center for Biostatistics and Data Science, Department of Bioinformatics and Biostatistics, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China; [4]Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China

Contributions: (I) Conception and design: Q Chen; Z Zhang; (II) Administrative support: Z Zhang; (III) Provision of study materials or patients: H Sun, J Dai; L Zhao; (IV) Collection and assembly of data: H Sun, J Dai; L Zhao; (V) Data analysis and interpretation: H Sun; J Dai; L Zhao; P Chen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Zhemin Zhang. Department of Medical Oncology, Shanghai Pulmonary Hospital, Tongji University Medical School Cancer Institute, Tongji University School of Medicine, Shanghai 200433, China. Email: zheminddoc@163.com; Qiankun Chen. Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200433, China. Email: drchen2016@126.com; Hui Lu. SJTU-Yale Joint Center for Biostatistics and Data Science, Department of Bioinformatics and Biostatistics, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China. Email: huilu@sjtu.edu.cn.

Background: In recent years, immunotherapy has achieved notable success in cancer treatment. Indeed, the novel immune checkpoint lymphocyte activation gene-3 (LAG3) has shown promising therapeutic efficacy in non-small cell lung cancer. However, it is unclear about the role of LAG3 in immunotherapy and survival in small cell lung cancer (SCLC).

Methods: The expression of LAG3 in SCLC was evaluated in four public datasets. The association of LAG3 with programmed death-ligand 1 (PD-L1), programmed cell death protein 1 (PD-1), and overall survival (OS) was investigated. The LAG3-related biological processes and pathways were identified by functional analyses.

Results: LAG3 expression was detected in SCLC tumor tissues. In the cBioPortal dataset with 81 clinical SCLC samples, LAG3 expression was markedly associated with PD-1 and PD-L1 expression (both P<0.050). In addition, Patients with high LAG3 expression had a trend toward a better OS (P=0.073). A similar survival trend was also observed in the GSE60052 dataset. Significantly, LAG3 expression was related to immune-related biological processes, such as immune response, antigen processing and presentation, and T cell co-stimulation (all P<0.001).

Conclusions: This study demonstrated that LAG3 is an important immune checkpoint that is closely associated with PD-1/PD-L1. LAG3 may be a promising novel immunotherapy target for SCLC.

Keywords: Lymphocyte activation gene-3 (LAG3), small cell lung cancer (SCLC), immunotherapy, programmed death-ligand 1 (PD-L1), programmed cell death protein 1 (PD-1)

Submitted Mar 22, 2021. Accepted for publication Sep 10, 2021.
doi: 10.21037/atm-21-4481
View this article at: https://dx.doi.org/10.21037/atm-21-4481

^ ORCID: 0000-0003-4728-9107.

Introduction

Lung cancer causes the highest morbidity and mortality amongst all malignancies worldwide (1,2). Approximately 10–15% of cases can be categorized as small cell lung cancer (SCLC) which is characterized by high growth fractions and high recurrence rates, resulting in poor prognosis (3-5). Although chemotherapy is the standard first-line treatment for SCLC (6), resistance to chemotherapy hinders long-term survival. Therefore, studies exploring alternative therapeutic strategies for the treatment of patients with SCLC are urgently needed.

Some tumor cells with less immunogenicity, such as SCLC, can escape immune elimination and develop into cancers, and this can be reversed by suppressing certain immune checkpoints (7-11). Indeed, some immune checkpoint inhibitors have demonstrated notable success in treating cancers (12,13). The programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) inhibitors are effective in treating non-small cell lung cancer (NSCLC) (14), and when combined with first-line chemotherapy, survival in SCLC patients was significantly increased (15-18).

Unfortunately, in some cases, insensitivity to PD-1/PD-L1 blockade can hinder its efficacy (19,20), and other immune inhibitory checkpoints are now at the forefront of research, such as lymphocyte activation gene-3 (LAG3) (21). LAG3, also known as cluster of differentiation 223 (CD223), is a surface molecule first identified in 1990 (22). It is expressed on the membrane of various immunocytes, including tumor-infiltrating lymphocytes (TILs), dendritic cells (DCs), T regulatory (Treg) cells, natural killer cells, B cells, and so on (23,24). As a member of the immunoglobulin superfamily, LAG3 is structurally similar to CD4, with approximately 20% homology shared at the DNA sequence (25). LAG3 shows a stronger affinity to human leukocyte antigen class II (HLA class II) expressed on antigen presenting cells (APCs) compared with CD4 and therefore inhibits the binding of HLA class II with TILs, hindering the anti-tumor response (26,27). In HLA-II-positive melanoma tumors, this may facilitate immune escape with bidirectional function (24). The presence of LAG3 serves as an essential marker of T cell exhaustion, promoting T-cell apoptosis and inhibiting their proliferation and activation. Furthermore, cytokine secretion is reduced and tolerance is increased (28,29). Elevated LAG3 expression has been observed on TILs of patients with various solid tumors, such as hepatocellular carcinoma and gastric carcinoma, as well as hematologic malignancies (30).

Reports have suggested that LAG3 co-functions with PD-L1 and PD-1 (12,31). *In vivo* experiments have shown that T cells may be activated if one of the pathways is blocked. The strategic blocking of both pathways resulted in an additive effect (32). Other studies have suggested that soluble LAG3 may be a potential anti-cancer vaccine (33). Thus, LAG3 may be a promising new immune checkpoint in cancer treatment. Additionally, combined inhibition of the LAG3 and PD-1 pathways may exert an additive therapeutic effect (34).

Our recent study found that similar to other types of cancers, some NSCLC patients showed LAG3-positive TILs. The expression of LAG3 could be predicted by PD-1 expression and was related to a poorer prognosis (7). However, there is a paucity of literature related to the expression of LAG3 in SCLC and how it affects survival in these patients. In this current study, four public datasets were accessed to investigate LAG3 expression in SCLC tissues (35-38). The relationship between LAG3 expression and survival, clinicopathological traits, and PD-L1 and PD-1 expression was investigated. Furthermore, functional analyses were performed to explore the LAG3-related biological processes and pathways. We present the following article in accordance with the REMARK reporting checklist (available at https://dx.doi.org/10.21037/atm-21-4481).

Methods

Acquisition of small cell lung cancer datasets

The original RNA sequencing (RNA-seq) data and clinical characteristics of SCLC patients were obtained from the cBioPortal database (https://www.cbioportal.org/study/summary?id=sclc_ucologne_2015) and the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) databases. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Datasets with less than 10 SCLC patients were excluded. Finally, 81 SCLC patients from the cBioPortal cohort (35), 79 SCLC patients from the GSE60052 cohort (36), 23 SCLC patients from the GSE43346 cohort (37), and 18 SCLC patients from the GSE149507 cohort (38) were enrolled for this study. The basic information of the enrolled datasets is summarized in *Table 1*.

Table 1 Basic information of the enrolled datasets

Datasets	Type	The number of SCLC samples	The number of NSCLC samples	The number of normal lung samples
cBioPortal	Clinical tissues	81	–	–
GSE60052	Clinical tissues	79	–	7
GSE43346	Clinical tissues	23	–	–
GSE149507	Clinical tissues	18	–	18

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

Identification of the differentially expressed genes (DEGs)

Based on the expression level of LAG3, the public cohort was equally divided into a high LAG3 expression group and a low LAG3 expression group. The limma R package was installed to search the DEGs. Following analysis, the DEGs between the high and low LAG3 expression groups were selected based on a 2-fold change and a P value of 0.05.

Functional analysis

For functional enrichment analysis, Gene Ontology (GO) (34,39,40) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (41,42) analyses were performed. The GO results contained three parts, namely, molecular function, cellular components, and biological processes. The open-source software RStudio version 4.0.3 was used to visualize the GO and KEGG results.

Statistical analysis

Comparison of LAG3 expression between two groups was conducted using *t*-tests. Pearson correlation analysis was applied to examine the relationship between LAG3 expression and PD-L1 and PD-1 expression. Linear analysis was used to evaluate the relationship between LAG3 expression and clinicopathological traits and PD-L1 and PD-1 expression. The Kaplan-Meier method was implemented to estimate survival curves, and the Cox regression model was used for correlation analysis on overall survival (OS) and clinical features, including age, gender, smoking status, staging of lung cancer, PD-1 expression, PD-L1 expression, and LAG3 expression. Variables with P<0.1 were regarded as potential predictive markers. Statistical significance was defined as P<0.05. All statistical tests were 2-sided. All statistical analyses were performed with the RStudio software (version 4.0.3; https://www.R-project.org).

Results

Characterization of LAG3 expression in small cell lung cancer tissues

LAG3 expression was detected in all SCLC tissues (*Figure 1*). In the GSE60052 cohort (36), the expression of LAG3 had no significant difference in 79 SCLC samples compared to normal lung tissues (n=7) (*Figure 1A*; P=0.23). However, in the GSE149507 cohort (38), overexpression of LAG3 was detected in all 18 SCLC patient samples compared with normal lung tissues (n=18) (*Figure 1B*; P=0.0011).

Correlation of LAG3 expression with PD-1 and PD-L1 expression

Correlation analyses were performed on the four clinical datasets (*Table 2*). In the cBioPortal cohort (35), there was a significant correlation between LAG3 expression and both PD-1 expression (P<0.001) and PD-L1 expression (P=0.011). In the GSE60052 cohort (36), LAG3 expression was statistically associated with PD-1 expression (P=0.017), but no significant correlation was detected between LAG3 and PD-L1 expression (P=0.501). On the contrary, there was a significant correlation between LAG3 expression and PD-L1 expression in the GSE43346 cohort (P<0.001) (37). In the GSE149507 cohort (38), there was no statistical relationship between LAG3 expression and PD-1 expression, nor PD-L1 expression. This negative result with the GSE149507 cohort may be due to the limited sample size of SCLC patients.

Univariate and multivariate linear analyses of LAG3 expression were applied to two datasets with more than 50 SCLC patients (Tables S1,S2). In the cBioPortal cohort (35), both PD-1 (P<0.001) and PD-L1 (P=0.049) had a certain significance in the prediction of LAG3 expression (Table S1). In the GSE60052 cohort (36), PD-L1 was also shown to be a potential factor in predicting LAG3 expression (P=0.060; Table S2).

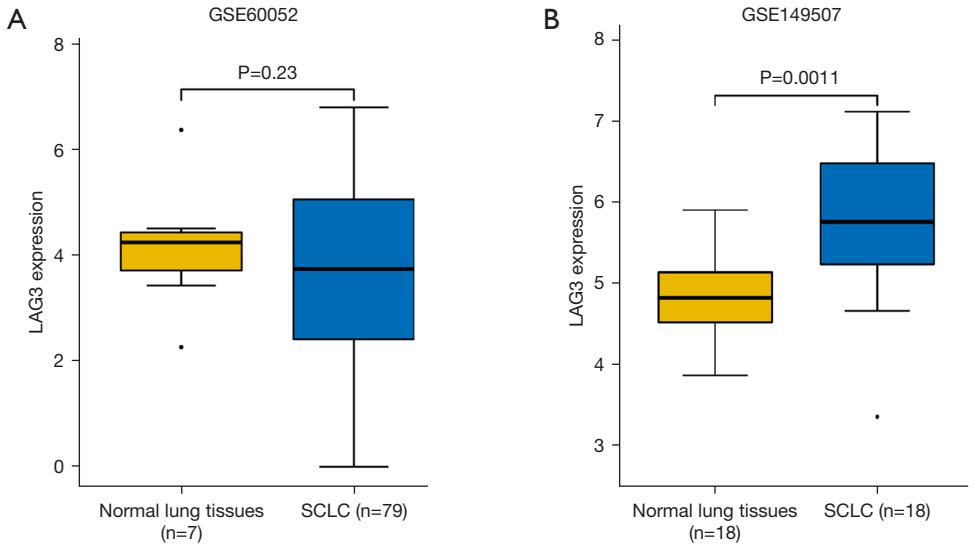

Figure 1 LAG3 expression in normal lung tissues versus SCLC tissues. (A) LAG3 expression in normal lung tissues versus SCLC tissues in the GSE60052 dataset; (B) LAG3 expression in normal lung tissues versus SCLC tissues in the GSE149507 dataset. LAG3, lymphocyte activation gene-3; SCLC, small cell lung cancer.

Table 2 Relationships between LAG3, PD-1, and PD-L1 in SCLC

Datasets	Variables	Correlation coefficient	P value
cBioPortal	PD-1	0.8599056	*<0.001*
	PD-L1	0.2800828	*0.011*
GSE60052	PD-1	0.267693	*0.017*
	PD-L1	0.07684498	0.501
GSE43346	PD-1	0.2580412	0.235
	PD-L1	0.6672631	*<0.001*
GSE149507	PD-1	−0.4078534	0.093
	PD-L1	−0.2673047	0.284

Statistically significant data were marked with italics. LAG3, lymphocyte activation gene 3; PD-1, programmed cell death protein 1; PD-L1, programmed cell death protein ligand 1; SCLC, small cell lung cancer.

Correlation of LAG3 expression and clinicopathologic features

The relationship between LAG3 expression and different clinicopathologic statuses (Figures S1-S3) was examined. In three datasets with available clinical information, LAG3 expression was down-regulated in stage III–IV patients and in patients with metastasis. However, in the cBioPortal cohort (35), LAG3 was not differentially expressed between different groups (both P>0.05). Similar negative results were obtained with the other two public cohorts, namely, the GSE60052 cohort (36) and the GSE149507 cohort (38). Univariate and multivariate linear analyses also suggested that clinical features failed to predict LAG3 expression (Tables S1,S2).

Survival analysis

Kaplan-Meier analysis of the cBioPortal cohort (35) revealed that patients with high LAG3 expression showed a trend toward better prognosis compared to patients with low LAG3 expression (P=0.073; *Figure 2A*). In the

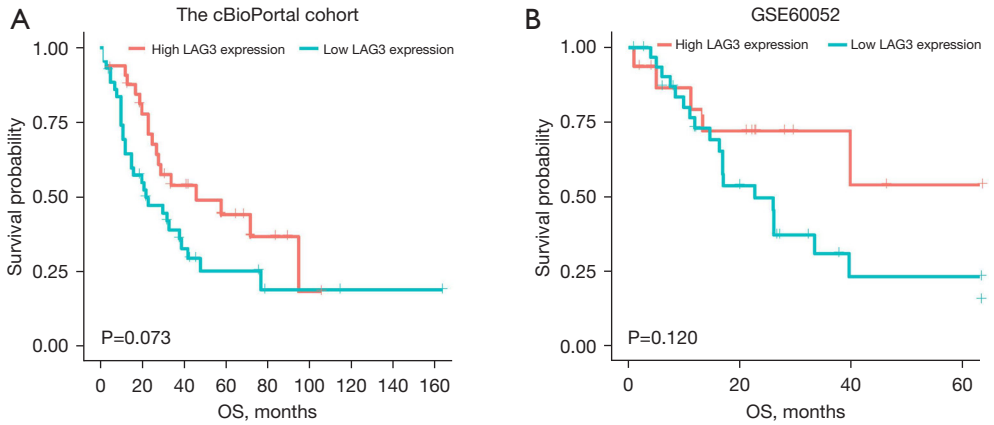

Figure 2 OS analysis in SCLC patients with different LAG3 expression levels. (A) OS analysis in SCLC patients with high versus low LAG3 expression in the cBioPortal cohort; (B) OS analysis in SCLC patients with high versus low LAG3 expression in the GSE60052 dataset. LAG3, lymphocyte activation gene-3; OS, overall survival; SCLC, small cell lung cancer.

GSE60052 cohort (36), a similar longer OS trend was observed in participants with high LAG3 expression compared to patients with low LAG3 expression (P=0.120; *Figure 2B*).

Cox regression analysis of OS

In the cBioPortal cohort (35), gender was the only predictive factor of OS [P=0.007; hazard ratio (HR) =0.329; 95% confidence interval (CI): 0.142 to 0.739; *Table 3*]. Univariate cox regression analyses revealed that lung cancer staging, tumor status, lymph node status, metastasis status, and PD-1 expression were potentially significant risk factors for OS (all P<0.1), but no significant association was found upon multivariate cox regression analyses (*Table 3*). In the GSE60052 cohort (36), low PD-1 expression was the only risk factor of OS (P=0.035; HR =2.570; 95% CI: 1.071 to 6.165; *Table 4*).

Identification of LAG3-related signaling pathways

The cBioPortal cohort was used to identify the LAG3-related GO terms and KEGG pathways as it had the largest number of SCLC patients (35). A total of 591 DEGs were found between patients with high and low LAG3 expression. GO analysis revealed that these LAG3-related genes were linked to several immune-related processes (*Figure 3* and *Table 5*). The top 10 LAG3-related biological processes were as follows: immune response (GO: 0006955; P<0.001), interferon-gamma-mediated signaling pathway (GO: 0060333; P<0.001); inflammatory response (GO: 0006954; P<0.001); type I interferon signaling pathway (GO: 0060337; P<0.001), defense response to virus (GO: 0051607; P<0.001), innate immune response (GO:0045087; P<0.001), regulation of immune response (GO: 0050776; P<0.001), antigen processing and presentation (GO: 0019882; P<0.001), response to virus (GO: 0009615; P<0.001), and T cell co-stimulation (GO: 0031295; P<0.001). The top 10 GO terms related to molecular function and cellular components between the high and low LAG3 expression groups are summarized in *Table 5*. KEGG analysis also confirmed the close relationship between LAG3 and immunity (*Figure 4* and *Table 6*). *Staphylococcus aureus* infection (hsa05150; P<0.001) was the most enriched KEGG pathway.

Discussion

LAG3 is a novel immune checkpoint but there is a paucity of literature related to the expression of LAG3 and its correlation with immune checkpoint PD-1/PD-L1 and patient survival in SCLC. This current study revealed the potential immunotherapeutic effects of LAG3 in patients with SCLC.

LAG3 serves as an essential marker of T cell exhaustion (28,29). The TILs are crucial components in the anti-tumor immune response and are directly related to the development of cancer (43). The function of CD4+ and CD8+ T cells, DCs, Tregs, and so on, is regulated by inhibitory and active receptors, and can significantly impact cancer immune

Table 3 Cox regression analysis for overall survival in the cBioPortal cohort

Variables	Univariate			Multivariate		
	HR	95% CI	P value	HR	95% CI	P value
Age (<65 *vs.* ≥65 y)	0.955	0.540–1.689	0.874	–	–	–
Sex (female *vs.* male)	0.295	0.132–0.661	*0.003*	0.329	0.142–0.739	*0.007*
Smoking status (no *vs.* yes)	0.410	0.056–2.996	0.380			
Stage (I–II *vs.* III–IV)	0.489	0.276–0.866	*0.014*	0.694	0.326–1.476	0.343
Tumor status (T1–2 *vs.* T3–4)	0.465	0.217–0.997	*0.049*	0.671	0.285–1.579	0.360
N status (N0 *vs.* N1–3)	0.615	0.332–1.139	0.122	–	–	–
Metastasis (M0 *vs.* M1)	0.469	0.235–0.936	*0.032*	0.552	0.247–1.233	0.147
PD-1 expression (low *vs.* high)	2.702	1.341–5.446	*0.005*	1.958	0.883–4.345	*0.098*
PD-L1 expression (low *vs.* high)	1.930	0.813–4.580	0.136	–	–	–
LAG-3 expression (low *vs.* high)	1.578	0.883–2.819	0.123	–	–	–

Data with P value less than 0.1 were marked with italics. CI, confidence interval; HR, hazard ratio; LAG3, lymphocyte activation gene 3; PD-1, programmed cell death protein 1; PD-L1, programmed cell death protein ligand 1.

Table 4 Cox regression analysis for overall survival in the GSE60052 cohort

Variables	Univariate			Multivariate		
	HR	95% CI	P value	HR	95% CI	P value
Age (<65y *vs.* ≥65 y)	2.528	0.753–8.487	0.134	–	–	–
Sex (female *vs.* male)	0.993	0.292–3.372	0.991	–	–	–
Smoking status (no *vs.* yes)	0.647	0.257–1.636	0.358	–	–	–
Stage (I–II *vs.* III–IV)	0.124	0.035–0.441	*0.001*	0.205	0.026–1.639	0.135
Tumor status (T1–2 *vs.* T3–4)	0.4262	0.180–1.011	*0.053*	0.717	0.294–1.746	0.463
N status (N0 *vs.* N1–3)	0.1749	0.049–0.630	*0.008*	0.643	0.063–6.563	0.710
Metastasis (M0 *vs.* M1)	1,243,399	0-Inf	0.999	–	–	–
PD-1 expression (low *vs.* high)	2.931	1.276–6.731	*0.011*	2.570	1.071–6.165	*0.035*
PD-L1 expression (low *vs.* high)	0.917	0.122–6.927	0.933	–	–	–
LAG-3 expression (low *vs.* high)	1.522	0.677–3.418	0.310	–	–	–

Data with P value less than 0.1 were marked with italics. CI, confidence interval; HR, hazard ratio; LAG3, lymphocyte activation gene 3; PD-1, programmed cell death protein 1; PD-L1, programmed cell death protein ligand 1.

escape (44). LAG3 blockade hinders the binding between LAG3 and HLA-II molecules, resulting in increased binding between HLA-II and TILs, thereby enhancing the anti-tumor response (45). High LAG3 expression has been observed on TILs in hematologic malignancies and various solid tumors, including hepatocellular carcinoma, gastric cancer, renal cell carcinomas, and ovarian cancer (30). In the current study, LAG3 gene expression was detected in SCLC tissues. Additionally, previous reports using antibody or knock-down experiments showed that blocking either the PD-1 or LAG3 pathway resulted in increased activation of TILs, which led to a prolonged survival (46).

In this study, LAG3 expression was shown to be statistically correlated with PD-1 and PD-L1 expression, similar to NSCLC. Immune escape pathways are closely associated with one another (47). LAG3 is co-expressed

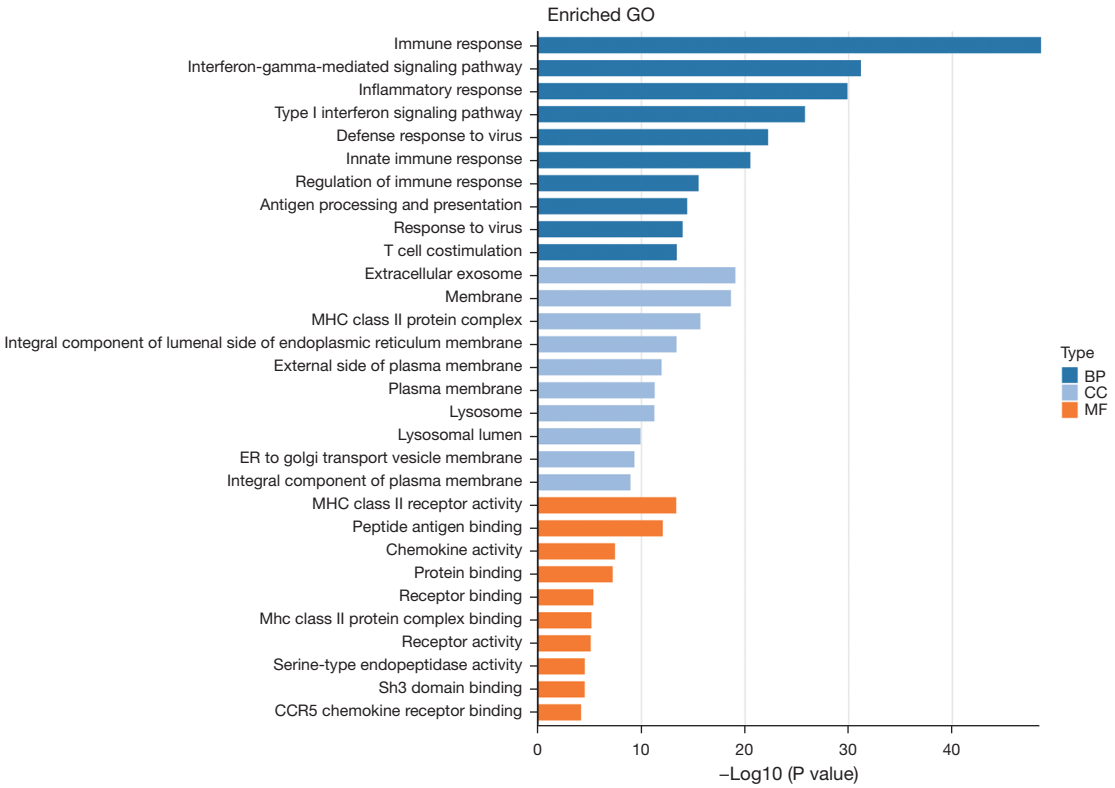

Figure 3 Enriched GO analysis of LAG3. LAG3, lymphocyte activation gene-3; GO, Gene Ontology; BP, biological process; CC, cell component; MF, molecular function.

with PD-1 on TILs and acts together to disrupt immune responses to cancer cells. This may partly explain why inhibition of the PD-1/PD-L1 pathway alone cannot lead to a notably improved prognosis in both NSCLC and SCLC. Previous researches showed that blocking both the LAG3 and PD-1 pathways resulted in superior therapeutic efficacy against cancers compared to blocking either pathway alone (32,46,48). In patients who present with upregulated LAG3 expression and are insensitive to PD-1 blocking treatment, the application of this combined strategy (anti-PD-1 and anti-LAG3) may improve prognosis (30,47), as demonstrated in such patients with melanoma (48).

This current study demonstrated that SCLC patients with high LAG3 expression had a trend toward a better OS. In our previous researches, we performed immunohistochemical staining on tumor tissues of NSCLC and SCLC patients, and we found that NSCLC patients with LAG3-negative TILs had longer survival (7), while SCLC patients with LAG3-negative TILs had no significance in survival versus those positive (49). Given the different impacts of the abovementioned checkpoints on survival of patients with NSCLC and SCLC, the immune mechanism was considered. The immune microenvironment and immunophenotypes of SCLC appear to be distinct from that of NSCLC, which may explain the discrepancies in the ICIs efficacy and survival in these two diseases (50). Firstly, different from NSCLC over-expressing PD-L1, PD-L1 expression in SCLC was relatively low but varied greatly in the majority studies (50). It may attribute to different staining antibodies or cut-off values for positivity, biopsied tissue types, and detection platforms (51). Secondly, SCLC had a significantly lower density of TILs and higher Treg cells compared with NSCLC (52). Thirdly, HLA class II-mediated antigen presentation plays a key role in activating anti-tumor immunity. However, HLA class II, the main ligand of LAG3, was rarely detected on SCLC tumor cells and HLA class II on TILs in SCLC was markedly lower than that in NSCLC (53). Therefore, all these immune factors may partly account for the different relationship between checkpoints and survival in NSCLC and SCLC and the reason for the poor efficacy of ICIs in SCLC.

In recent years, many researches have focused on

Table 5 The top 10 GO terms of each category between the high and low LAG3 expression groups

Categories	GO ID	Go terms	P value
Molecular function	GO:0032395	MHC class II receptor activity	4.45E-14
	GO:0042605	Peptide antigen binding	8.84E-13
	GO:0008009	Chemokine activity	3.60E-08
	GO:0005515	Protein binding	6.06E-08
	GO:0005102	Receptor binding	4.43E-06
	GO:0023026	MHC class II protein complex binding	6.76E-06
	GO:0004872	Receptor activity	7.97E-06
	GO:0004252	Serine-type endopeptidase activity	2.99E-05
	GO:0017124	SH3 domain binding	3.04E-05
	GO:0031730	CCR5 chemokine receptor binding	6.83E-05
Cellular components	GO:0070062	Extracellular exosome	8.66E-20
	GO:0016020	Membrane	2.34E-19
	GO:0042613	MHC class II protein complex	2.07E-16
	GO:0071556	Integral component of lumenal side of endoplasmic reticulum membrane	4.16E-14
	GO:0009897	External side of plasma membrane	1.15E-12
	GO:0005886	Plasma membrane	5.30E-12
	GO:0005764	Lysosome	5.67E-12
	GO:0043202	Lysosomal lumen	1.25E-10
	GO:0012507	ER to Golgi transport vesicle membrane	4.86E-10
	GO:0005887	Integral component of plasma membrane	1.15E-09
Biological processes	GO:0006955	Immune response	2.74E-49
	GO:0060333	Interferon-gamma-mediated signaling pathway	6.58E-32
	GO:0006954	Inflammatory response	1.31E-30
	GO:0060337	Type I interferon signaling pathway	1.66E-26
	GO:0051607	Defense response to virus	5.99E-23
	GO:0045087	Innate immune response	3.10E-21
	GO:0050776	Regulation of immune response	3.10E-16
	GO:0019882	Antigen processing and presentation	3.85E-15
	GO:0009615	Response to virus	1.06E-14
	GO:0031295	T cell costimulation	3.92E-14

LAG3, lymphocyte activation gene 3; GO, Gene Ontology.

immunotherapy as a novel approach to achieve a favorable prognosis for patients with SCLC. LAG3 is closely associated with PD-L1 and PD-1 in expression levels and function and maybe a target to potentiate the efficacy of PD-1/PD-L1 inhibitors in SCLC. In addition, autologous TILs after stimulated by interleukin-6 was infused into patients with anti-PD-1-resistant metastatic lung cancer and presented with general safety and clinical activity (54), which may

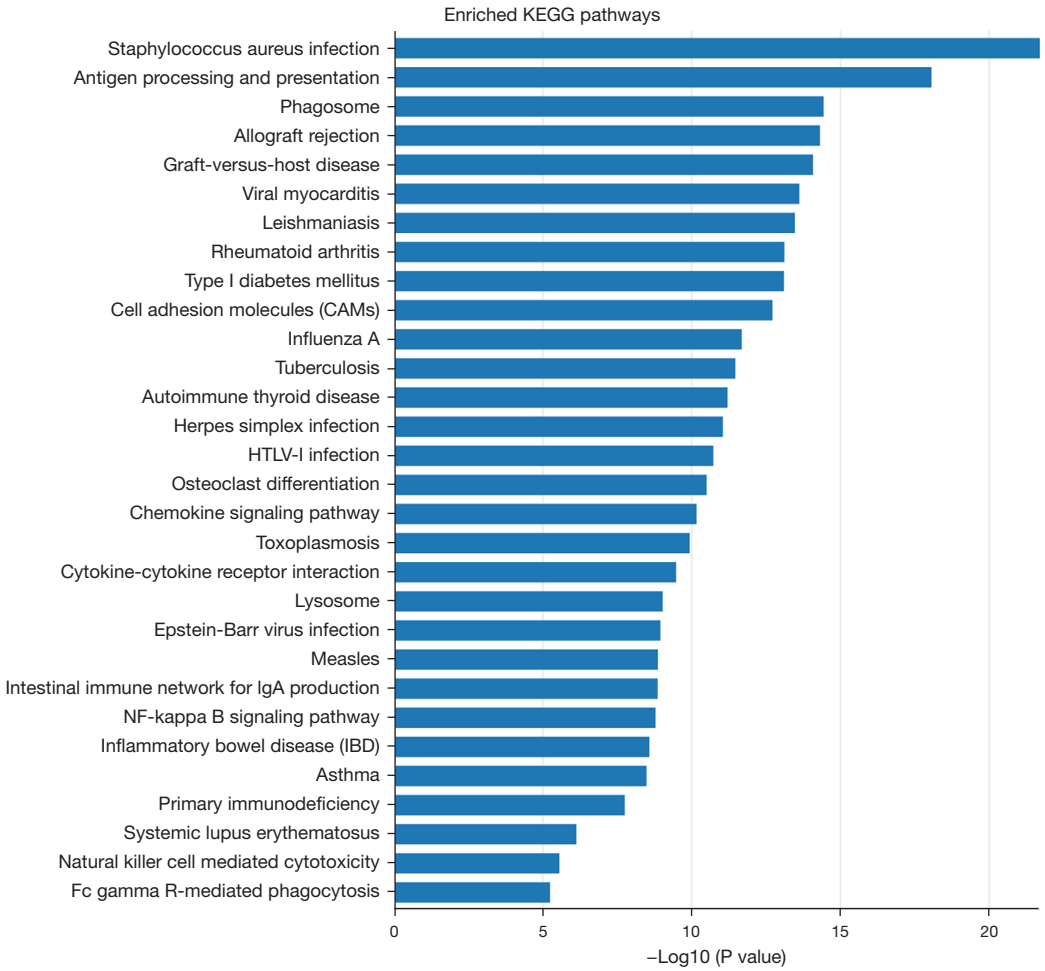

Figure 4 Enriched KEGG pathways analysis of LAG3. LAG3, lymphocyte activation gene-3; KEGG, Kyoto Encyclopedia of Genes and Genomes.

constitute a potential immunotherapy combination strategy for SCLC patients characterized by impaired antigen presentation and low-density TILs. Specifically, SCLC can be divided into four subtypes based on the dominant expression of four transcription factors: ASCL1 (SCLC-A), NeuroD1 (SCLC-N), YAP1 (SCLC-Y), and POU2F3 (SCLC-P) (55). This classification had important implications in the treatment, because SCLC-Y subtype presented with T-cell inflamed immunotype, high-expression interferon-γ-associated genes, and a better prognosis, predicting the potential population that may benefit from immunotherapy or combination therapy.

There were some limitations in this investigation. First, this study was performed retrospectively. Second, some clinical data and prognostic data were not available in some datasets. Third, the sample size was small, and more data from larger populations were needed to further verify these findings.

Conclusions

In conclusion, this study revealed the correlation of LAG3 with immune checkpoint PD-1/PD-L1 and patient survival, which indicated the potential immunotherapeutic effects of LAG3 in patients with SCLC. While there has been significant progress in understanding the function of LAG3 and its interaction with other immunomarkers, its precise role in the development of SCLC remains to be fully elucidated. Furthermore, the immune responses that occur during SCLC progression and the immune checkpoints that

Table 6 The top 10 KEGG enriched pathways of DEGs between the high and low LAG3 expression groups

KEGG ID	KEGG enriched pathways	P value
hsa05150	Staphylococcus aureus infection	1.98E-22
hsa04612	Antigen processing and presentation	8.68E-19
hsa04145	Phagosome	3.66E-15
hsa05330	Allograft rejection	4.87E-15
hsa05332	Graft-versus-host disease	8.29E-15
hsa05416	Viral myocarditis	2.39E-14
hsa05140	Leishmaniasis	3.37E-14
hsa05323	Rheumatoid arthritis	7.62E-14
hsa04940	Type I diabetes mellitus	7.88E-14
hsa04514	Cell adhesion molecules	1.92E-13

DEGs, differentially expressed genes; LAG3, lymphocyte activation gene 3; KEGG, Kyoto Encyclopedia of Genes and Genomes.

serve as key regulators in the anti-tumor responses remain to be investigated.

Acknowledgments

Funding: This study was supported by grants from the National Natural Science Foundation of China (81802255), the Clinical Research Project of Shanghai Pulmonary Hospital (FKLY20010 and FK18005), the Young Talents in Shanghai (2019 QNBJ), the 'Dream Tutor' Outstanding Young Talents Program (fkyq1901), the Key Discipline in 2019 (Oncology), the Project of Shanghai Municipal Science and Technology Commission, the Scientific Research Project of Shanghai Pulmonary Hospital (fkcx1903), the Shanghai Municipal Commission of Health and Family Planning (2017YQ050), the Innovation Training Project of SITP of Tongji University, the Key Projects of Leading Talent (19411950300), the Youth Project of Hospital Management Research Fund of Shanghai Hospital Association (Q1902037), National Key R&D Program of China (2018YFC0910500), and the Neil Shen's SJTU Medical Research Fund.

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://dx.doi.org/10.21037/atm-21-4481

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-4481). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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(English Language Editor: J. Jones)

Cite this article as: Sun H, Dai J, Zhao L, Zhu J, Wang H, Chen P, Lu H, Chen Q, Zhang Z. Lymphocyte activation gene-3 is associated with programmed death-ligand 1 and programmed cell death protein 1 in small cell lung cancer. Ann Transl Med 2021;9(18):1468. doi: 10.21037/atm-21-4481

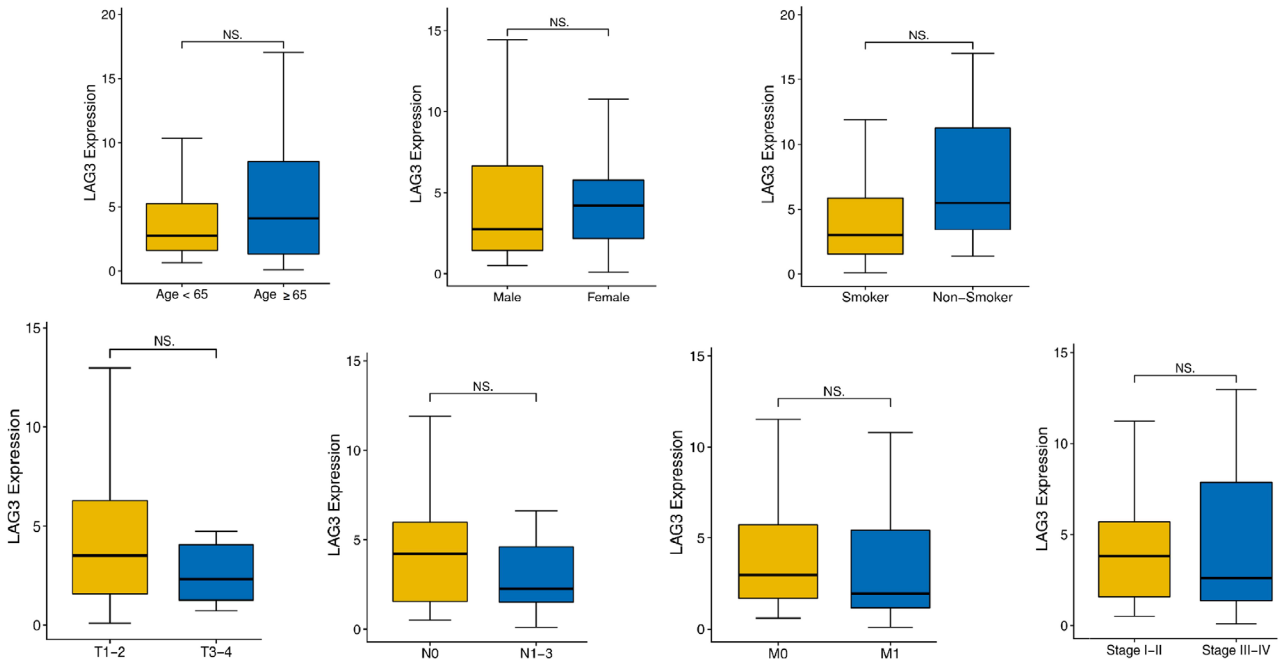

Figure S1 Correlation between LAG3 and clinical data in the cBioportal cohort. NS means no statistical significance.

https://dx.doi.org/10.21037/atm-21-4481

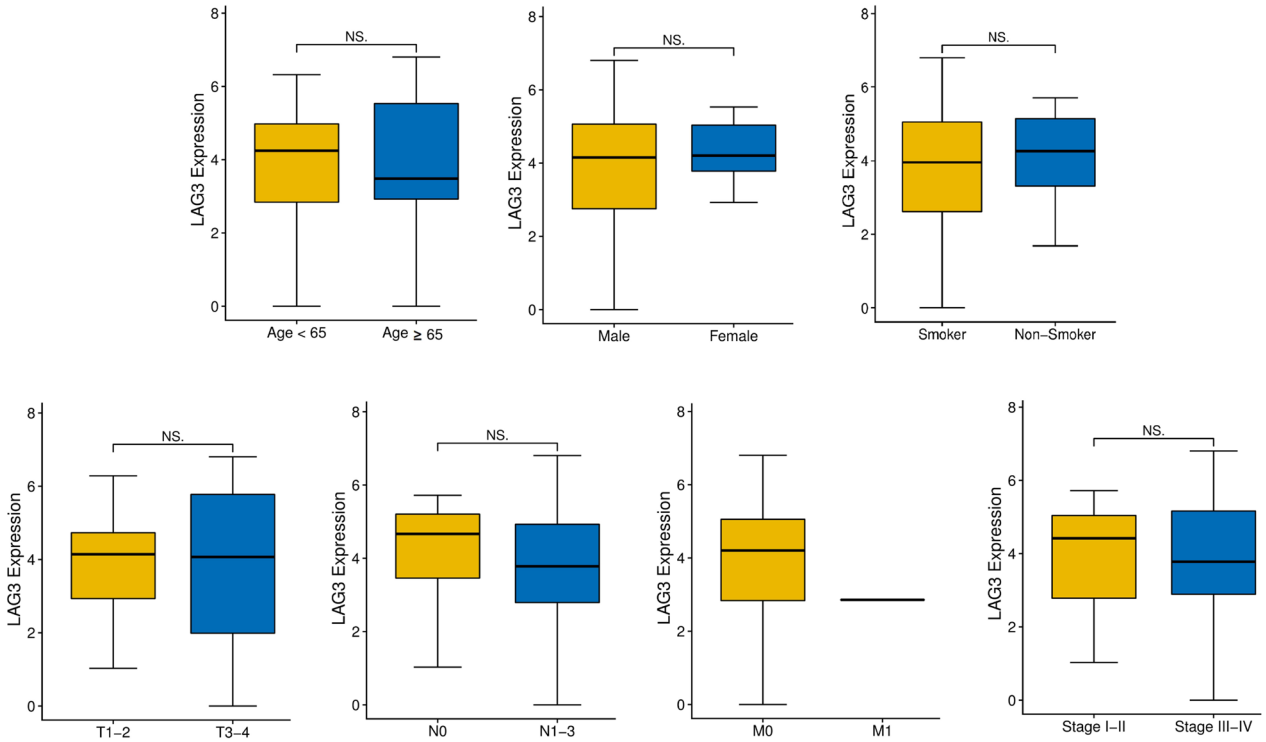

Figure S2 Correlation between LAG3 and clinical data in the GSE60052 cohort. NS means no statistical significance. LAG3, lymphocyte activation gene-3.

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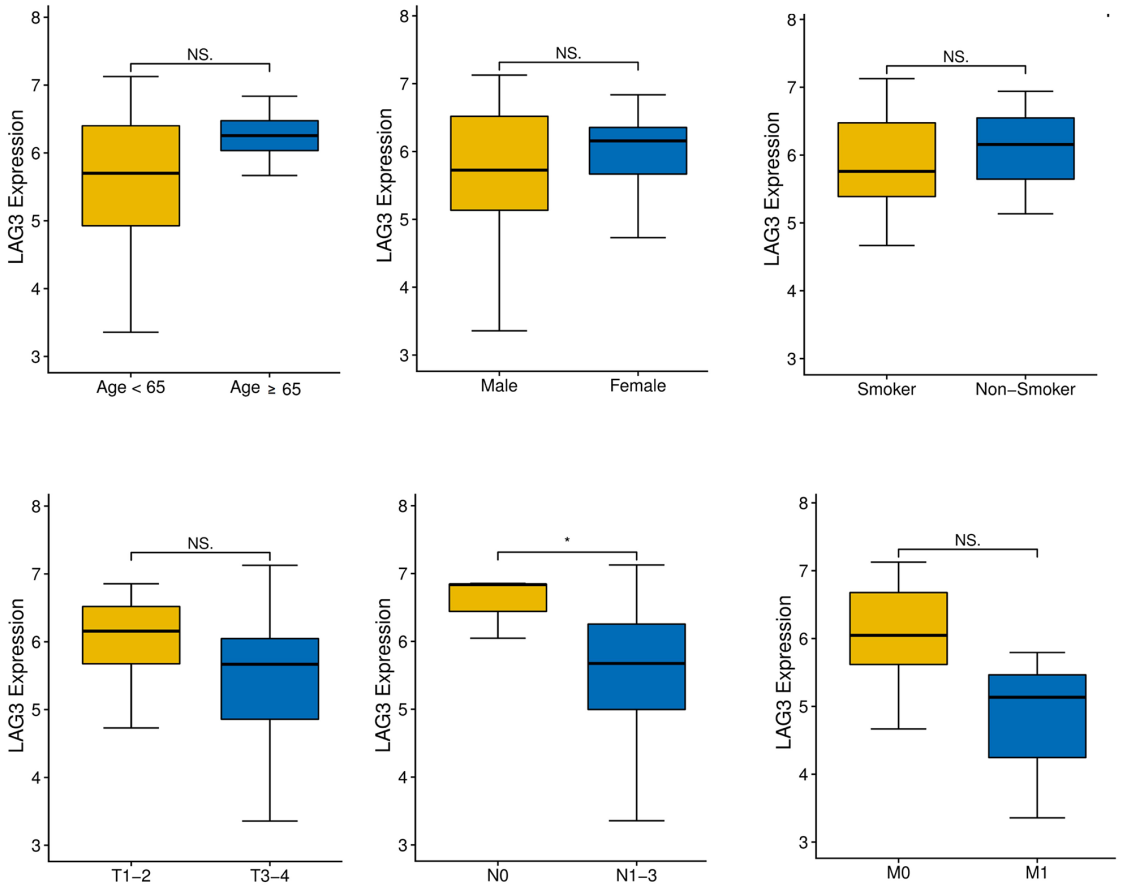

Figure S3 Correlation between LAG3 and clinical data in the GSE149507 cohort. "*" means P<0.05; NS means no statistical significance.

https://dx.doi.org/10.21037/atm-21-4481

Table S1 Liner analysis for LAG3 expression in the cBioPortal cohort

Variables	Univariate			Multivariate	
	Estimate	R square	P value	Estimate	P value
Age (<65 *vs.* ≥65 y)	-1.0914	0.01884	0.222		
Sex (female *vs.* male)	0.3660	0.001769	0.709		
Smoking status (non-smoker *vs.* smoker)	3.5942	0.0303	0.127		
Stage (I–II *vs.* III-IV)	0.2263	0.0007719	0.808		
Tumor status (T1-2 *vs.* T3-4)	-0.3991	0.008815	0.436		
N status (N0 *vs.* N1-3)	-0.3299	0.006899	0.491		
Metastasis (M0 *vs.* M1)	-0.7885	0.007022	0.484		
PD-1 expression	2.0994	0.7394	*<0.001*	2.04406	*<0.001*
PD-L1 expression	0.07531	0.07845	*0.011*	0.03089	*0.049*

Data with P value less than 0.1 were marked with italics. LAG3, lymphocyte activation gene 3; PD-1, programmed death 1; PD-L1, programmed death ligand 1.

Table S2 Liner analysis for LAG3 expression in the GSE60052 cohort

Variables	Univariate			Multivariate	
	Estimate	R square	P value	Estimate	P value
Age (<65 *vs.* ≥65 y)	-0.02317	3.353e-05	0.968		
Sex (female *vs.* male)	0.5363	0.008362	0.523		
Smoking status (non-smoker *vs.* smoker)	0.4980	0.01693	0.363		
Stage (I–II *vs.* III-IV)	0.2801	0.005555	0.603		
Tumor status (T1-2 *vs.* T3-4)	-0.09821	0.002019	0.757		
N status (N0 *vs.* N1-3)	-0.2878	0.01975	0.33		
Metastasis (M0 *vs.* M1)	-0.9452	0.005723	0.602		
PD-1 expression	0.2328	0.06086	*0.081*	0.1661	0.214
PD-L1 expression	0.3525	0.09948	*0.024*	0.3008	*0.060*

Data with P value less than 0.1 were marked with italics. LAG3, lymphocyte activation gene 3; PD-1, programmed death 1; PD-L1, programmed death ligand 1.

https://dx.doi.org/10.21037/atm-21-4481