

# Prognostic Significance of SPATS2L Methylation and mRNA Expression in Lung Adenocarcinoma

Siyuan Huai<sup>1,2#</sup>; Dan Li<sup>1#</sup>; Tiannan Ji<sup>3#</sup>; Wei Wei<sup>1\*</sup>

<sup>1</sup>Department of Radiotherapy, Senior Department of Oncology, the Fifth Medical Center of PLA General Hospital, Beijing, 100071, China.

<sup>2</sup>Medical School of Chinese PLA, Beijing, 100853, China.

<sup>3</sup>Department of Emergency, the Fifth Medical Center of PLA General Hospital, Beijing, 100071, China.

#These authors contributed equally to this work and share first authorship.

## Abstract

**Background:** Spermatogenesis-associated serine-rich 2-like gene (SPATS2L) is ubiquitously expressed in multiple tissues and involved in many diseases, while its role in Lung Adenocarcinoma (LUAD) remains elusive. The current study aimed to excavate the prognostic significance of SPATS2L methylation and mRNA expression for patients with LUAD.

**Research design and methods:** We analyzed the differential expression of SPATS2L mRNA in LUAD tissues and normal lung tissues, calculated the correlation between SPATS2L methylation sites and mRNA expression, and assessed the prognostic role of SPATS2L methylation and expression level for LUAD.

**Results:** SPATS2L mRNA expression was much higher in LUAD tissues ( $P < 0.0001$ ). SPATS2L mRNA expression was negatively regulated by SPATS2L methylation sites (Pearson:  $r = -0.57$ ,  $P < 0.0001$ ). Hypermethylation of SPATS2L prolonged the overall survival (OS) of LUAD. Hypermethylation [HR (95% CI) = 0.92(0.85, 1.00)] and low mRNA expression [HR (95% CI) = 1.20 (1.11, 1.03)] of SPATS2L were protective factors for LUAD OS.

**Conclusions:** The high methylation and low mRNA expression of SPATS2L were protective factors for LUAD OS. SPATS2L may play a crucial role in the development of lung adenocarcinoma and is a potential prognostic marker for LUAD, providing the possibility of improving the prognosis of LUAD patients.

**Keywords:** Lung adenocarcinoma; SPATS2L methylation; Biomarker; Prognosis; Immune infiltration.

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**Correspondance:** Wei Wei, Department of Radiotherapy, Senior Department of Oncology, the Fifth Medical Center of PLA General Hospital, Beijing, 100071, China. Email: dr\_weiwei528@163.com

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## Introduction

Lung Adenocarcinoma (LUAD) is the most common histological subtype of non-small cell lung cancer (NSCLC), accounting for about 40% of lung cancer [1]. With the rapid advances in diagnosis and treatment like surgery, radiotherapy, and molecular therapy, there is a remarkable improvement on clinical outcomes of patients with LUAD. However, the five-year Overall Survival (OS) of LUAD still remains lower than 20% [2]. There are proofs demonstrated that the exploration and application of molecular biomarkers can provide prognosis value for LUAD [3].

Spermatogenesis-Associated Serine-Rich 2-Like Gene (SPATS2L), which is ubiquitously expressed in multiple tissues [4], has the greatest number of GenBank accessions belonging to the lung with its function remaining elusive according to data collected by Ace View tool [5,6]. Whole genome association analysis revealed that SPATS2L is an important response gene to bronchodilators for asthma patients, and SPATS2L was reported to be associated with the therapy response of children with asthma [7,8]. Its over-expression is related to the carcinogenesis of glioma and hepatocellular carcinoma [6,9]. Another study discovered a relationship between the susceptibility loci of rs985256-SPATS2L and the susceptibility of Coal Workers' Pneumoconiosis (CWP) [10].

Based on the studies mentioned above, there seems to be a certain correlation between SPATS2L and the lungs. However, there is almost no research on the role of SPATS2L in the pathogenesis, development, and prognosis of lung adenocarcinoma. Therefore, we speculate that SPATS2L is a potential biomarker for lung adenocarcinoma. In this study, we first explored the differential expression of SPATS2L mRNA in LUAD tissues and normal lung tissues and analyzed the correlation between SPATS2L expression and SPATS2L DNA methylation based on The Cancer Genome Atlas (TCGA) LUAD dataset. The prognostic significance of SPATS2L expression and DNA methylation was also evaluated by meta-analysis. Tumor Immune Estimation Resource (TIMER) database was adopted to assess the potential correlation between SPATS2L and immune infiltration cells in LUAD. Then we analyzed the biological process of SPATS2L participating in LUAD through GO enrichment analysis. All of these works were intended to evaluate the prognosis significance of SPATS2L methylation and mRNA expression for LUAD patients and its latent value as a biomarker of LUAD.

## Patients and methods

### Mining data from public databases

GEPIA, an online gene expression profile interactive analysis website (<http://gepia.cancer-pku.cn/index.html>), was adopted to study the differential expression of SPATS2L mRNA in LUAD tissues and normal tissues. The clinical data, transcription, and methylation profiles of LUAD patients were collected from two articles [11,12], and the TCGA database (<https://www.cancer.gov/tcga>). Patients with complete clinical and transcriptional data were included. Additionally, we further verified the prognostic role of SPATS2L in LUAD by analyzing clinical data and SPATS2L mRNA expression data from 18 articles and TCGA database. A total of 2757 patients diagnosed with LUAD were brought into this analysis.

### Meta-analysis

A systematic search of PubMed, Web of Science, and Embase

databases was conducted to identify all published studies on the association between SPATS2L expression and the prognosis of LUAD. We used meta-analysis to analyze data from 1 database and 18 articles to assess the overall prognostic significance of SPATS2L expression for patients with LUAD. The consolidated HR and 95% CI were calculated to assess the correlation between SPATS2L expression and prognosis in patients with LUAD. The heterogeneity of data from 18 articles and a database was determined by the Q test ( $I^2$  statistics). If there's no significant heterogeneity ( $I^2 < 50\%$ ), a fixed-effects model would be performed. Otherwise, a random-effects model would be applied. The meta-analysis was accomplished by STATA 15.1 software.

### TIMER database analysis

The correlation between SPATS2L expression and the abundance of 7 immune cells (CD4+T cells, CD8+T cells, B cells, neutrophils, myeloid dendritic cells, macrophages, and monocytes) in LUAD was examined using the TIMER algorithm (<https://cistrome.shinyapps.io/timer/>), a robust website able to automatically analyze and visualize the correlation between immune infiltration levels and a range of variables.

### GO enrichment analysis

The Gene Ontology analysis was conducted based on the Gliovis database (<http://gliovis.bioinfo.cnio.es/>). The patients with lung adenocarcinoma in the Gliovis database were initially divided into high and low SPATS2L expression groups. The false discovery rate for selecting differentially expressed genes between the two groups was less than 0.05. Gene terms with  $|\log_{2}FC| \geq 1$  along with a  $P$  value less than 0.05 was considered significant. Then GO enrichment analysis and biological processes were chosen to explore the functional role of SPATS2L in lung adenocarcinoma.

### Statistics

Data was analyzed by the Medcalc program (version 19.4) and GraphPad Prism (version 6.0). Low and high SPATS2L expression groups were established based on the median SPATS2L mRNA expression values in different databases. Similarly, SPATS2L hypomethylation and hypermethylation groups were established based on the median value of SPATS2L DNA methylation in the TCGA-LUAD database. The relationship between SPATS2L expression or DNA methylation and a range of classification variables was analyzed using chi-square or Fisher exact tests. The difference between the continuous indices of the two groups with normal distribution was determined by Student's t-test, and the continuous indices with skewed distribution were tested by a nonparametric test. Spearman rank correlation coefficient and Pearson correlation coefficient were used to measure the correlation between SPATS2L expression and SPATS2L DNA methylation level. Kaplan Meier curve was used to evaluate the prognostic significance of SPATS2L expression and SPATS2L DNA methylation. The  $P$  values on both sides less than 0.05 were statistically significant.

## Results

### The prognostic value of SPATS2L methylation for LUAD

RNA-seq data from Kabbout\_2013 ( $P=0.011$ ), Okayama\_2012 ( $P=0.00062$ ), and TCGA ( $P<0.0001$ ) showed that SPATS2L mRNA was highly expressed in LUAD tissues than that in the normal lung

tissues (Figure 1A, B and C). As exhibited in the heatmap, SPATS2L was abundantly expressed in bronchus and lung tissues, and there was a strong correlation between SPATS2L and adenocarcinoma (Figure 1D). Besides, SPATS2L mRNA expression was negatively correlated ( $r=0.32$ ,  $P<0.0001$ ) with SPATS2L methylation level (Figure 1E). Figure 1F exhibits the distribution of 41 SPATS2L CpG islands. Figure 2A is the heatmap of methylation sites of SPATS2L, displaying hypomethylated and hypermethylated CpG islands. 8 SPATS2L methylation sites at which the methylation level was determined as most strongly correlated with SPATS2L mRNA expression were selected to assess the prognostic significance of SPATS2L methylation for patients with LUAD through Kaplan-Meier plots, manifesting that hypermethylation at 5 of 8 SPATS2L methylation sites was relevant to a longer survival time (Figure 2B, C, D, E, F, G, H and I). Based on the pooled HR (95% CI) of 0.92 (0.85, 1.00), we inferred that the high methylation level of SPATS2L methylation sites was a protective factor for LUAD OS (Figure 2J).

### Assessment of SPATS2L differential expression in subgroups based on TCGA database

The nonparametric test was applied to confirm the expression difference of SPATS2L mRNA in TCGA database-sourced populations grouped by gender (Figure 3A), IDH1 type (Figure 3B), MGMT methylation (Figure 3C), TP53 mutation status (Figure 3D), cancer stage (Figure 3E). On the whole, no close relationship was found between SPATS2L expression in LUAD tissues and gender ( $P=0.11$ ), IDH1 type ( $P=0.13$ ), and MGMT methylation level ( $P=0.45$ ). According to the large standard deviation gaps between groups, SPATS2L expression in LUAD was strongly correlated to TP53 mutation and individual cancer stage. Additionally, Kaplan-Meier curves visualized that low expression of SPATS2L in LUAD prolonged OS (Figure 3F).

### Meta-analysis of SPATS2L expression for patients OS with LUAD

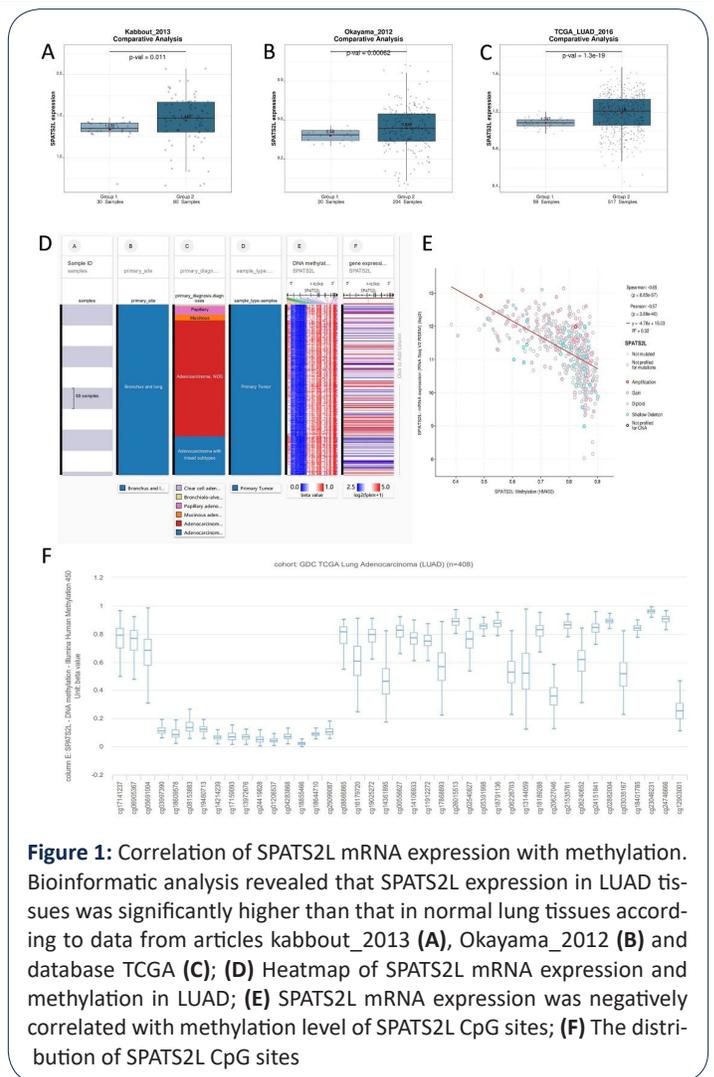
We performed meta-analysis using the data extracted from 18 articles and 1 database to observe the correlation of SPATS2L mRNA expression with the OS of LUAD patients (Figure 3G). With a pooled HR (95% CI) of 1.20 (1.11; 1.03), and no significant heterogeneity among 19 data sources ( $I^2=29\%$ ,  $P=0.12$ ), we concluded that low expression level of SPATS2L mRNA was a protective factor for LUAD OS.

### Correlation between SPATS2L mRNA expression and immune infiltration cells

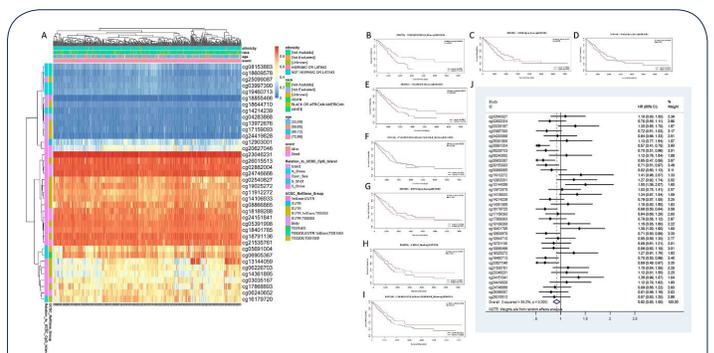
Spearman correlation analysis was used to evaluate the relationship of SPATS2L mRNA expression with immune cell infiltration by searching the TIMER database (Figure 4). It was illustrated that the relation between SPATS2L mRNA expression and immune infiltrating CD4+T cell ( $\rho=-0.002$ ,  $P=0.971$ ), CD8+T cell ( $\rho=0.355$ ,  $P<0.05$ ), neutrophil ( $\rho=0.377$ ,  $P<0.05$ ), B cell ( $\rho=-0.162$ ,  $P<0.05$ ), macrophage ( $\rho=0.314$ ,  $P<0.05$ ), myeloid dendritic cell ( $\rho=0.313$ ,  $P<0.05$ ), and monocyte ( $\rho=0.218$ ,  $P<0.05$ ) was not close.

### GO analysis of SPATS2L involved in cell function

Gene Ontology analysis based on Gliovis database was applied to explore the cell function in which SPATS2L participated. As displayed in Figure 5, SPATS2L mainly engaged in cell functions like gated channel activity, ligand-gated channel activity, ligand-

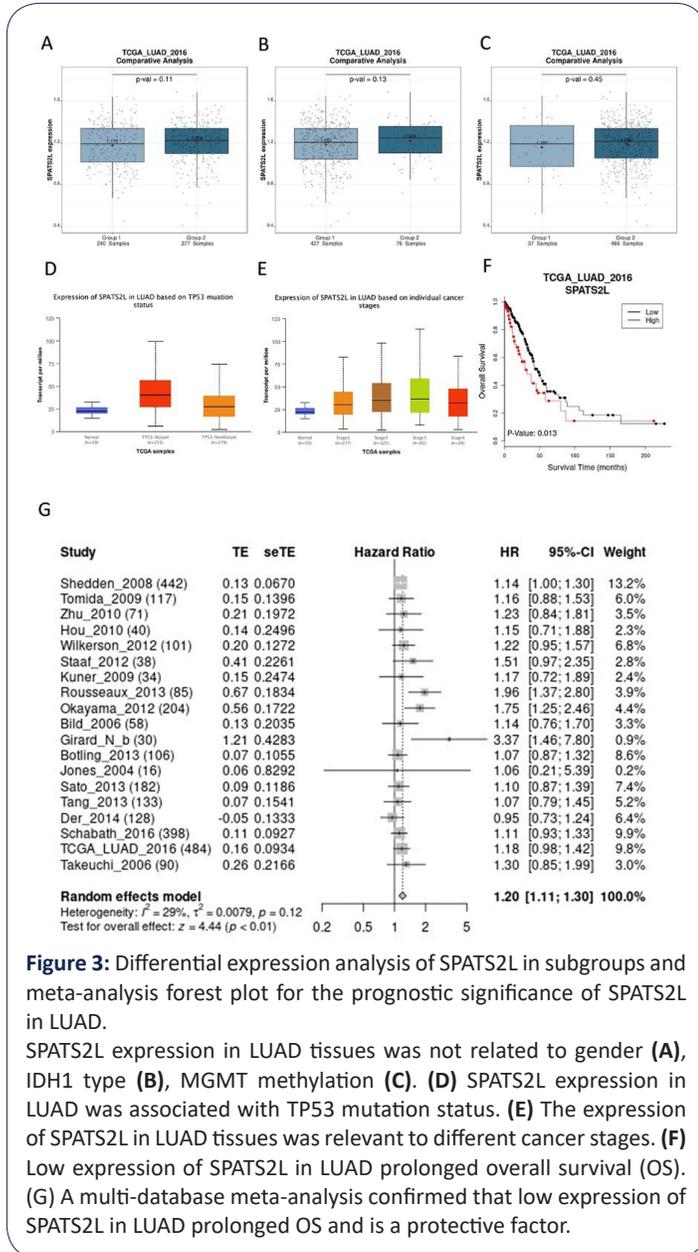


**Figure 1:** Correlation of SPATS2L mRNA expression with methylation. Bioinformatic analysis revealed that SPATS2L expression in LUAD tissues was significantly higher than that in normal lung tissues according to data from articles kabbout\_2013 (A), Okayama\_2012 (B) and database TCGA (C); (D) Heatmap of SPATS2L mRNA expression and methylation in LUAD; (E) SPATS2L mRNA expression was negatively correlated with methylation level of SPATS2L CpG sites; (F) The distribution of SPATS2L CpG sites



**Figure 2:** Heatmap of methylation sites of SPATS2L and the association between methylation level of 8 SPATS2L CpG sites and LUAD OS. (A) Heatmap of methylation sites of SPATS2L. Blue stands for hypomethylation. Red represents hypermethylation. (B,C,D,E,F,G,H,I) The association between methylation level of 8 SPATS2L methylation sites and LUAD OS. (J) Meta-analysis revealed that SPATS2L hypermethylation level was a protective factor for LUAD OS.

gated ion channel activity, channel regulator activity, extracellular ligand-gated ion channel activity, chloride transmembrane transporter activity. Previous studies revealed that low expression of Cystic Fibrosis Transmembrane Regulator (CFTR) was remarkably related to the development of non-small cell lung cancer and tumor invasion ability [13-15]. Hence, SPATS2L was assumed to affect LUAD development indirectly by regulating channel activity like chloride transmembrane transporter activity.

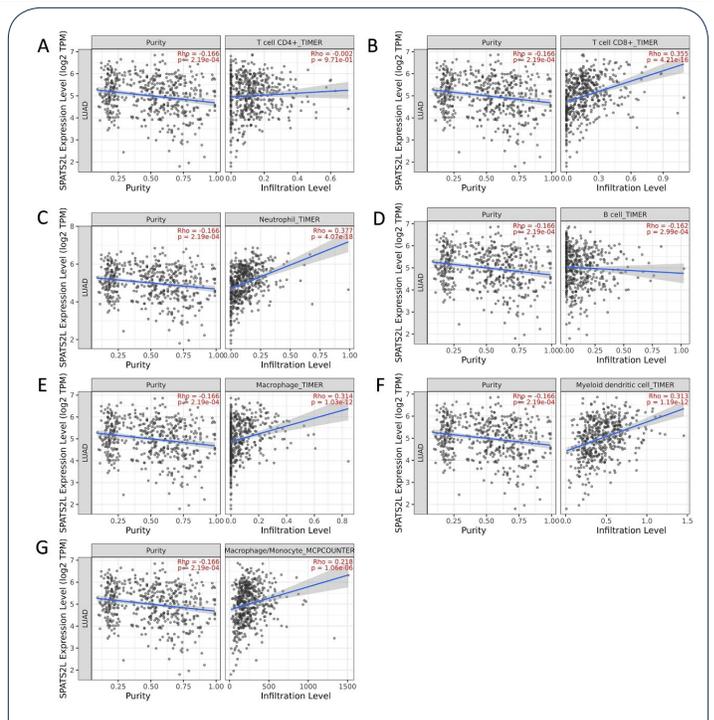


**Figure 3:** Differential expression analysis of SPATS2L in subgroups and meta-analysis forest plot for the prognostic significance of SPATS2L in LUAD.

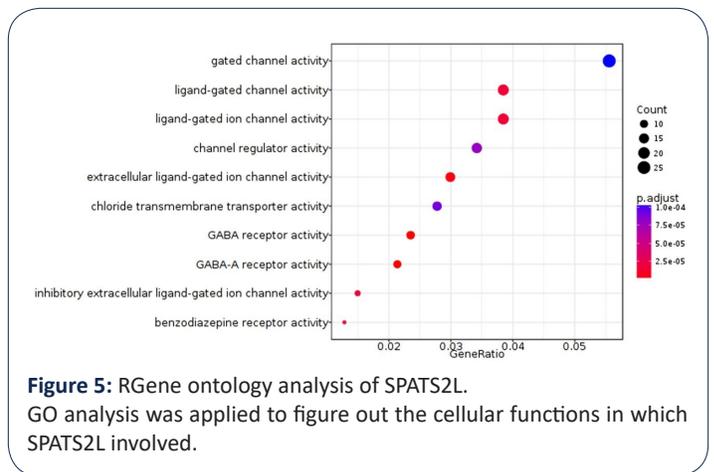
SPATS2L expression in LUAD tissues was not related to gender (A), IDH1 type (B), MGMT methylation (C). (D) SPATS2L expression in LUAD was associated with TP53 mutation status. (E) The expression of SPATS2L in LUAD tissues was relevant to different cancer stages. (F) Low expression of SPATS2L in LUAD prolonged overall survival (OS). (G) A multi-database meta-analysis confirmed that low expression of SPATS2L in LUAD prolonged OS and is a protective factor.

## Discussion

In recent years, lung cancer has become the most common malignant tumor, posing a serious threat to global human health, with an annual incidence rate of 7.5% [16]. As the major histological subtype of NSCLC, LUAD causes more than 10000 deaths worldwide each year [17]. Besides, the apparent heterogeneity of LUAD makes its diagnosis and treatment difficult [18]. A large number of literature studies have shown that environmental factors, as well as genetic and epigenetic factors, can affect the occurrence and development of lung adenocarcinoma [19-23]. Epigenetic studies of lung cancer illustrated that there are abnormal



**Figure 4:** Relationship between SPATS2L and immune infiltration cell. Analysis of the correlation between SPATS2L mRNA expression and immune cell infiltration including CD4+T cell (A), CD8+T cell (B), neutrophil (C), B cell (D), macrophage (E), myeloid dendritic cell (F), and monocyte (G), demonstrated that there was no apparent relationship between SPATS2L mRNA expression and immune infiltration cells.



**Figure 5:** RGene ontology analysis of SPATS2L. GO analysis was applied to figure out the cellular functions in which SPATS2L involved.

methylation states in various lung cancer patient samples, such as sputum [24], bronchoalveolar lavage [25], and cancer tissue [26]. Furthermore, many tumor related genes, including oncogenes and tumor suppressor genes, change their methylation status in the early stages of lung cancer [27]. DNA methylation can be used to track the recurrence of early lung adenocarcinoma (LUAD) after surgery [28]. Therefore, altered methylation status can be used in lung oncology to identify biomarkers to assist in tumor detection and predict cancer prognosis.

In this study, we investigated and validated the prognostic value of SPATS2L methylation in patients with LUAD. After analyzing the data obtained from TCGA, we found for the first time that the expression of SPATS2L in lung adenocarcinoma tissue was significantly higher than that in normal lung tissues, which was

verified by the data obtained from the other two articles. In addition, we statistically concluded that the mRNA level of SPATS2L was negatively correlated with the methylation level of CpG island. Among the 8 CpG islands most related to the mRNA expression level of SPATS2L, the hypermethylation level of 5 of them was related to a longer survival time. Meta-analysis results indicated that the hypermethylation of all CpG islands was a protective factor for the prognosis of LUAD. To better explain the relationship between SPATS2L methylation level and the prognosis of LUAD, we conducted a meta-analysis of LUAD patients' data from the TCGA database and 18 articles. The results indicated that a high SPATS2L methylation level was a protective factor for the prognosis of LUAD, that is, low SPATS2L expression prolonged the OS of LUAD patients. Likewise, SPATS2L expression is upregulated in patients with glioma, and its low expression is involved in a better OS [9]. For patients with acute myeloid leukemia, high expression of SPATS2L leads to a significantly lower survival rate than those with low expression [29]. Zhao et al. proposed a predictive model that combined SPATS2L and 9 other genes to evaluate the prognosis of acute myeloid leukemia, demonstrating an inferior 5-year OS in high-risk patients [30]. SPATS2L was also reported to be involved in mental disease [31], systemic lupus erythematosus [32], asthma [33], etc. Though studies on SPATS2L are comparatively fewer at present, it is clear that as an oncogene, upregulation of SPATS2L can lead to many diseases. While the specific role of SPATS2L in LUAD still needs to be deeply explored.

Immune cells infiltrated in the tumor microenvironment were proven to mediate the efficacy of immunotherapy, which is a promising target in drug development [34,35]. The increase in the proportion of effector cells in the immune microenvironment often indicates a better prognosis for patients [36,37]. This study analyzed the relationship between SPATS2L and LUAD immune infiltrating cells through the TIMER database. Unfortunately, no robust correlation was found between SPATS2L and immune infiltration cells.

GO enrichment analysis manifested that SPATS2L was closely related to the activity of various channels on the cell membrane, including the chlorine transport transporter. Coincidentally, the Crystal Fibrosis Transmembrane Regulator (CFTR) is a cAMP-dependent chloride ion channel that regulates the growth and proliferation of tumor cells in various cancers [38-41]. Studies have shown that the expression of CFTR is downregulated in lung cancer [13-15]. Downregulation of CFTR expression promotes EMT progression and metastasis of lung adenocarcinoma cell line A549 by upregulating the uPA/uPAR system. Inhibiting the functional activity of CFTR chloride channels also promotes the EMT process of A549 and increases the migration and invasion ability of lung cancer cells [15]. Therefore, SPATS2L may affect the development of lung adenocarcinoma by regulating the expression or function of CFTR. Given that high methylation of SPATS2L is a protective factor for LUAD, we speculate that low expression of SPATS2L could upregulate CFTR, thereby slowing down the migration and invasion ability of lung cancer cells. However, this conjecture needs further research to prove.

## Conclusions

In summary, the expression of SPATS2L was upregulated in LUAD. High methylation and low mRNA expression of SPATS2L

were associated with better OS of patients with LUAD. SPATS2L perhaps plays an important role in the occurrence and development of LUAD, and is a promising prognostic marker for LUAD, bringing more possibilities for improving the prognosis of patients.

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