SPAG5 Expression Correlated With Prognostic Implication and Immune Infiltration in Lung Adenocarcinoma

Jianjiang Xie (✉ xiejianjianggz@126.com)
South China University of Technology

Xie Xu
South China University of Technology

Huaping Zhou
Affiliated Cancer Hospital & Institute of Guangzhou Medical University

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Abstract

Background Sperm-associate antigen 5 (SPAG5) is a critical oncogene in several cancers. But the role of SPAG5A in lung adenocarcinoma (LUAD) remains unclear. Thus, the aims of our study are to explore the function and underlying mechanism of SPAG5 in LUAD.

Methods Expression of SPAG5 was determined using the Oncomine, TIMER, and GEPIA databases. Correlation of SPAG5 and survival was detected by GEPIA database, PrognoScan, Kaplan-Meier Plotter databases. And the association between SPAG5 and tumor malignant phenotypes were analyzed by the CancerSEA. Besides, the correlation between SPAG5 expression and tumor immune infiltration as well as immune checkpoints were analyzed by TIMER. the co-expression genes of SPAG5 were identified using STRING, and the mutation and biological function of SPAG5 and its co-expression genes were determined by cBioPortal and Metascape, respectively. Finally, the SPAG5 expression in LUAD samples was determined by tissues microarrays (TMA) and immunohistochemistry (IHC) analyses.

Results We found that upregulated SPAG5 associated with poor survival of LUAD patients. Besides, SPAG5 expression associated to B cell, CD4+ cell, CD8+ cell, macrophage, DC cell as well as CD274, CTLA4, GZMB, LAG3, PDCD1, TIGIT in LUAD. SPAG5 expression also associated with cell proliferation, cell cycle, DNA damage and repair, epithelial-mesenchymal transition (EMT), invasion, and stemness, inflammation in LUAD.

Conclusion Our finding indicated that SPAG5 acted as a crucial oncogene in LUAD, and correlated with unfavorable survival as well as tumor infiltration inflation.

Introduction

Lung cancer is a worldwide epidemic malignancy among both women and men, and terribly causes globally cancer-related deaths [1]. In China, the incidences and deaths of lung cancer emerge increasing rapidly in recent years, and reveal geographic and gender differences [2]. Lung adenocarcinoma (LUAD) comprises approximately over 40% of the lung cancer incidence, and showed systemic threat because of distant metastasis more frequently occurs in LUAD [3]. LUAD with overall survival less than 5 year because of the most aggressive and rapid metastasis [4]. For the past few years, immunotherapy has been highlighted very effective in lung cancer therapy, however, it also has previously failed in lung cancer [5, 6]. Programmed cell death protein 1/programmed cell death-ligand 1 monoclonal antibody (PD-1/PD-L1 mAb) has emerged therapeutic effective in LUAD [7]. Nevertheless, portion non-response to immunotherapy and reveal the immune-related toxicities of the immune checkpoint inhibitors [8–12]. Therefore, the terrible therapeutic results of immunotherapy are the new obstacles for clinical application of immune checkpoint inhibitors in LUAD. It is urgent to explore the leading causes contribute to fail of the immunotherapy in LUAD.

Sperm-associate antigen 5 (SPAG5) is a novel oncogene in several cancer types [13]. Increased SPAG5 has been observed in many cancers and correlates with unpleased prognosis and unfavorable
clinicopathological characteristics in breast cancer [14, 15]. High expression of SPAG5 implicates neoplastic growth, metastasis, chemoresistance and shorter survival time in a wide spectrum of cancers [16–19]. It also has been demonstrated that SPAG5 correlates with unfavorable prognosis and can be used as a therapeutic target in LUAD [20, 21]. Increasing evidences have indicated that SPAG5 exerts as an oncogene by regulating various signaling pathways to regulate tumorigenesis and progression and counteract the effects of many chemotherapies. Such as, SPAG5 involves in AKT/mTOR, WNT/β-catenin, PI3K/AKT signaling pathways to regulate tumor growth, apoptosis, metastasis [18, 22, 23]. There are little but strong evidences discover the connection between SPAG5 and immune cell infiltration in cancer. Previous study has implicated that SPAG5 is an alternative cancer vaccine target in several cancers and positively associates with CD8 T-cell infiltrating based on the comprehensive analyses of tumor immunity project [24]. In addition, SPAG5 has been observed significant correlation with CD8 T-cell infiltrating in breast cancer [14]. Despite of SPAG5 severs as an oncogene in LUAD, there are no evidences demonstrate the association between SPAG5 and immune cell infiltration in LUAD.

Therefore, in the present study, we detected the connection of SPAG5 with prognosis and immune infiltration in tumor samples of LUAD patients. Our data highlight the crucial role of SPAG5 in tumor initiation, progression, clinical outcome, and immune infiltration in LUAD.

**Methods**

**Oncomine database**

Oncomine database (http://www.oncomine.org) is a public platform to suppling microarray data download and mining for majority tumors [25]. Here, the expression levels of SPAG5 in pan-cancer were determined by Oncomine database analysis. The cut-off value set as fold change (FC) >1.5 and P value <0.05.

**TIMER database**

Tumor Immune Estimation Resource (TIMER) is a web platform incorporated 10,009 samples across 23 cancer types from The Cancer Genome Atlas (TCGA). TIMER2.0 can be used to estimate immune infiltration levels for TCGA or customer’s RNA-seq data, and explore the relationship between immune infiltration and other factors, such as genomic and transcriptome variation and clinical outcomes [26]. Here, we explored the expression levels of SPAG5 in various cancer types. In addition, the correlation analysis between SPAG5 expression and infiltrated immune cells, including gene markers of B cell, CD8+ cell, CD4+ cell, Macrophage, Neutrophil, and Dendritic cell. The gene expression level was exhibited with log2TPM. Moreover, we also explored the correlation among clinical outcome, immune infiltration, and SPAG5 expression. Correlation between immune checkpoints and immune cells were determined based on TIMER2.0.

**Function statue analysis**
CancerSEA (http://biocc.hrbmu.edu.cn/CancerSEA) is a specific database used to synthetically explore cancer cell function at the single-cell level across 41,900 cancer single cells from 25 cancer types. The cancer single-cell functional states of CancerSEA involved in the tumorigenesis, progression, and aggressive metastasis [27]. Here, we analyzed the functional state of SPAG5 in several cancer types based on CancerSEA with a threshold, correlation strength >0.3 and a false discovery rate (FDR) < 0.05.

GEPIA

Gene Expression Profiling Interactive Analysis (GEPIA) is a web tool, which processing RNA-seq data based on TCGA and Genotype-Tissue Expression (GTEx) projects, to comprehensively analyze gene expression at multiple levels [28]. Here, GEPIA validated the association between SPAG5 and clinical outcome.

Survival and prognostic value analysis

In the present study, TIMER, GEPIA, PrognoScan database (http://www.abren.net/PrognoScan/), Kaplan-Meier Plotter database (http://kmplot.com/analysis/) were performed to detect the correlation between SPAG5 expression and survival. PrognoScan database is a public platform for cancer microarray datasets, which was used to explore the correlation between gene expression and patient prognosis, including overall survival (OS) and disease-free survival (DFS) [29]. The cut-off value was adjusted to a Cox P value <0.05. Kaplan-Meier Plotter database is an online tool for exploring the relationship between gene expression and patient prognosis across majority cancer types based on GEO, EGA, TCGA and PubMed repositories RNA-seq data, microarray data, and clinical data [30]. The association between SPAG5 expression and OS, DFS, relapse-free survival (RSF), first progression (FP), post progression survival (PPS).

Construction of the PPI network

The protein-protein interaction (PPI) network was established based on SPAG5 using STRING database with the threshold, interaction score (median confidence) of 0.4.

Metascape

Metascape is a combination web portal for gene annotation and function enrichment [31]. In the present study, the function enrichment of SPAG5 and its co-expression genes were performed using Metascape with P<0.05.

cBioPortal database

The cBioPortal database (http://cbioportal.org) is a comprehensive web portal, is utilized to analyze and visualize the multidimensional cancer genomics database [32]. In this study, the genetic alterations of SPAG5 and its interacted genes were analyzed using cBioPortal based 432 LUAD samples in TCGA.

Construction of tissues microarrays (TMA) and immunohistochemistry (IHC)
TMA of 140 LUAD specimen were constructed by Department of Thoracic Surgery, Guangzhou First People's Hospital. And this study was approved by the Ethic Committee of Guangzhou First People's Hospital. The TMA was purchased from Xian Alenabio Biotech Co., Ltd. (Xian, China, Cat no:LC1401). Then, the SPAG5 expression was detected using IHC anti-SPAG5 antibody (HPA022008, Sigma-Aldrich, St. Louis, MO, USA). The SPAG5 expression was calculated based on the staining scope and intensity, and the staining scope and staining intensity were scored as following, staining scope, 1 (10%-25%), 2 (25%-50%), 3 (50%-75%), 4 (75%-100%). Staining intensity, 0 (negative), 1 (weakly positive, light yellow), 2 (moderately positive, yellow-brown), 3 (seriously positive, dark brown). Then, SPAG5 expression was calculated according staining scope x intensity. The high SPAG5 expression determined by the expression score more than 6, and the low SPAG5 expression determined by the expression score less than 6. The correlation between SPAG5 expression and clinicopathologic characteristics were analyzed by chi-square test, and P value < 0.05 was considered statistically significant.

Results

High expression of SPAG5 in tumors

We explored the role of SAPG5 in cancers based on the Oncomine database, we found the expression of SPAG5 upregulated in tumor compared with normal tissues of various cancer types, including bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gastric cancer, leukemia, liver cancer, lung cancer, lymphoma, ovarian cancer, sarcoma. Oppositely, SAPG5 downregulated in tumor than normal tissues in few cancer types, including breast cancer, kidney cancer, and leukemia (Figure 1A). Additionally, we also examined the expression of SPAG5 using TIMER2.0. As shown in Figure 1B, SPAG5 increasing in bladder urothelial cancer (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), and stomach adenocarcinoma (STAD) tumors compared with normal tissues, whereas SPAG5 decreasing in kidney chromophobe (KICH), kidney renal papillary cell carcinoma (KIRP), thyroid carcinoma (THCA), uterine corpus endometrial carcinoma (UCEC) tumors compared with normal tissues. Furthermore, we explored the mRNA expression of SPAG5 in NSCLC based on the TCGA and GTEx data. We found that SPAG5 upregulation in LUAD tissues and significantly expressed in different stages (Figure 1C-D). These results revealed that SPAG5 increasing in LUAD both in microarray data and RNA-seq data, it also indicated SPAG5 might act as an oncogene in LUAD.

Prognostic values of SPAG5 in LUAD

The correlation between expression of SPAG5 and clinical outcome was investigated using GEPIA based on TCGA and GTEx data. As shown in Figure 2A-B, OS curve indicated high expression of SPAG5 associated with poor survival of LUAD (HR=1.6, P=0.0012), whereas the expression of SPAG5 not
associated with DFS (HR=13, P=0.096). Using PrognoScan, GSE13213 and GSE31210 datasets, including 117 samples and 204 LUAD samples at different clinical stages, and indicated that high expression of SPAG5 associated with poor survival time (OS HR=1.54, Cox P=0.0025; OS HR=1.67, Cox P=0.057; RFS HR=1.91, Cox P=0.00065) (Figure 2C-E). These results suggested that SPAG5 acted as a potential risk factor for LUAD patients. Moreover, we validated the prognostic potential of SPAG5 using Kaplan-Meier Plotter database based on microarray data. As shown in Figure 2F-H, high expression of SPAG5 indicated the poor survival time of LUAD patients (OS HR=1.68, P=1E-15; FP HR=1.68, P=9.6E-08; PPS HR=1.56, P=0.00058). Our finding suggested the prognostic potential of SPAG5 in LUAD.

**SPAG5 upregulation associated with tumor malignant phenotypes**

To understand the function and underlying mechanism of SPAG5, we explored the functional state of SPAG5 in LUAD based on single-cell level in CancerSEA database. We found that SPAG5 expression associated with multiple tumor cancers, as shown in Figure 3, acute myelocytic leukemia, LUAD, melanoma, breast cancer, prostate cancer, etc. Of interest, SPAG5 expression positively associated with malignant phenotypes, including cell proliferation, cell cycle, DNA damage and repair, epithelial-mesenchymal transition (EMT), invasion, and stemness, whereas negative related to inflammation with the correlation coefficient>0.3 and P<0.05. These data reminded that SPAG5 expression associated the LUAD tumorigenesis and development.

**SPAG5 expression correlated with tumor immune infiltration in LUAD**

Emerge evidences indicate the landscape of tumor immune infiltration significant associates with outcome and survival of cancer. Here, we examined the relationship between SPAG5 and tumor immune infiltration in LUAD by TIMER. Our data discovered that SPAG5 expression positively related to B cell, CD4+ cell, CD8+ cell, macrophage M0/M1, whereas negatively correlated with macrophage M2 and DC cell (Figure 4A). Furthermore, we also investigated the correlation between SPAG5 expression and immune checkpoints in LUAD. And the results showed that SPAG5 significantly associated with CD274, CTLA4, GZMB, LAG3, PDCD1, and TIGIT in LUAD (Figure 4B). Our data suggested that SPAG5 exerted a key role in immune pathways of LUAD, and regulated the polarization of macrophage.

**PPI establishment and mutation of SPAG5 and its co-expression genes in LUAD**

We established a PPI network to analyze the interaction of SPAG5 and other proteins. And the network of SPAG5 along with co-expression proteins was displayed in Figure 5, ten hub genes, including KIF11, DLGAP5, KIF2C, BUB1, BUB1B, CCNB2, CDCA8, AURKB, CDC20, and AURKA, significantly interacted with SPAG5 in LUAD. We further investigated the mutation of SPAG5 and the ten genes using cBioPortal. And we observed nearly 2.3% (SPAG5), 1.3% (KIF11), 2.7% (DLGAP5), 1.5% (KIF2C), 0.6% (BUB1), 0.9% (BUB1B), 0.6% (CCNB2), 0.8% (CDCA8), 0.7% (AURKB), 1.7% (CDC20), 1.2% (AURKA) of LUAD sample emerged genetic alteration (Figure 6). The main genetic alterations of genes were amplification, deep deletion, and missense mutation.
**Function annotation of hub genes in LUAD**

We then enriched the function of hub genes using Metascape. And the results suggested that 11 hub genes significantly enriched in mitotic nuclear division, cell division, mitotic sister chromatid segregation, spindle organization, pid auraro B pathway, establishment of chromosome localization, microtubule-based movement (Figure 7A-D). Each MCODE component was enriched and data showed that SPAG5 and its co-expression genes mainly involved in molecular functions, including mitotic nuclear division, resolution of sister chromatid cohesion, and nuclear division (Table 1).

**Correlation of SPAG5 expression with PD-L1 expression and the clinicopathological characteristics of LUAD**

Finally, we observed the expression of SPAG5 in the LUAD tumor tissues, and we found relatively high expression of SPAG5 in advanced stage LUAD samples compared with early stage LUAD samples (Figure 8). Generally, we investigated relationship between the expression of SPAG5 and clinicopathological parameters in 140 LUAD samples (Figure 8), and we found SPAG5 expression associated with gender, clinical stages, tumor size, lymph node metastasis, and PD-L1 expression (Table 2). These results validated the previous predication according to the bioinformatics analyses.

**Discussion**

With the developing of next generation sequencing (NGS), a transformative technology, produces abundant and complex data in both genomics and omics [33]. Bioinformatics then widely used to biological research based on the NGS data, which used of the comprehensive analyze the biological data based on mathematics, statistics, and computational methods [34]. In recent years, numerous prognostic markers, therapeutic targets, regulating mechanisms of multiple tumors are identified by bioinformatics analysis [35–39]. Here, according the bioinformatics analysis, we explored the prognostic potential and correlation of SPAG5 and immune cell infiltration in LUAD. We demonstrated that SPAG5 acted as an oncogene in various cancer, especially in LUAD. Based on the microarray data and RNA-seq data from GEPIA, PrognoScan, and Kaplan-Meier Plotter database online databases, we found high SPAG5 expression associated with poor survival of LUAD patients. Our finding is supported the previous studies.

The underlying mechanisms analyses indicated SPAG5 involved in regulation of cell cycle, DNA damage/repair, EMT, invasion, proliferation, stemness and inflammation in LUAD. Previous study has found that SPAG5 promotes prostate cancer progression by inducing cell proliferation, migration, invasion [40]. SPAG5 also be found to promote lung cancer cell growth, migration, and invasion [41, 42]. There are firstly reported that SPAG5 expression associated to DNA damage/repair, stemness and inflammation in LUAD. We further explored the regulatory mechanism of SAPG5 in inflammation. Using TIMER, we found SPAG5 expression positively correlated with B cell, CD4 T cell, CD8 T cell, macrophage M0 and M1, but negatively associated to macrophage M2 and DC cell in LUAD. Previous researches demonstrate that SPAG5 expression associates with CD8+ cell infiltration in cancers [14, 24]. Our results consistence with previous studies. Although previous has revealed the correlation of SPAG5 expression
and CD8 T cell, macrophage, neutrophils, B cell and DC, there is firstly reported in LUAD. Of interest, we also found SAPG5 promoted antitumor phenotype, macrophage M1 polarization, and inhibited macrophage M2 polarization. It implicates SPAG5 might involve in therapeutic resistance by modulating macrophage M1 polarization. Immune cell infiltration, such as CD8 T cell and macrophage infiltration usually be controlled by different chemokine and chemokine receptors in different cancers [43], and immune checkpoint alteration [44]. The positive expression of PDCD1 (PD-1), CD274 (PD-L1) and CTLA4 with SPAG5 suggested the patients with high SPAG5 expression might benefit by anti-PD-1 and anti-CTLA4 agent treatment. Besides, SPAG5 expression also positively correlated with GZMB, LAG3 and TIGIT in LUAD. It has reported that GZMB expression correlates with cytotoxic activity ad tumor size reduction, it may be considered as a biomarker to predict the immune-therapeutic response of patients [45]. LAG3 and TIGIT has been introduced as the novel immune therapeutic targets with specialized function [46]. Our finding suggested that SPAG5 upregulation might act as a predicative indicator for benefit of immunotherapy.

In the present study, we also found the co-expression genes of SPAG5, including KIF11, DLGAP5, KIF2C, BUB1, BUB1B, CCNB2, CDCA8, AURKB, CDC20, and AURKA. They involved in several molecular functions, such as mitotic nuclear division, cell division, mitotic sister chromatid segregation, spindle organization, pid auraro B pathway, establishment of chromosome localization, microtubule-based movement. KIF11 [47], DLGAP5 [48], KIF2C [49], BUB1 [50], BUB1B [51], CCNB2 [52], CDCA8 [53], AURKB [54], CDC20 [55], AURKA [56] are found as vital oncogenes in some cancers, and most of the co-expression genes associate the mitosis [57–59]. The GO annotation analyses also demonstrated that SPAG5 and its co-expression genes involve in mitosis-related function.

**Conclusion**

Taken together, upregulated SPGA5 was relevant to poor survival of patients, and promoted immune cells, for example, B cell, CD4 T cell, CD8 T cell, macrophage, neutrophils, and DC infiltrated in LUAD. Moreover, SPAG5 expression induced macrophage M1 polarization, and significant correlated with CD274, CTLA4, GZMB, LAG3, PDCD1, and TIGIT expression. Therefore, SPGA5 may act as a potential prognostic biomarker, and a predicative indicator for response of immunotherapy in LUAD.

**Declarations**

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**Competing interest**

The authors have declared that no competing interest.

**Autor contributions**
Xu Xie performed the statistical analyses and written the draft. Zhou Huaping provided the methods for this study. Xie Jianjiang designed this study and revised the manuscript.

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### Tables

**Table 1** Function description of the corresponding component.

| MCODE     | GO         | Description                                      | Log10 (P) |
|------------|------------|--------------------------------------------------|-----------|
| MCODE1     | GO:0140014 | Mitotic nuclear division                          | -15.8     |
| MCODE1     | R-HSA-2500257 | Resolution of sister chromatid cohesion            | -15.6     |
| MCODE1     | GO:0000280 | Nuclear division                                  | -14.5     |

**Table 2** Correlation between SPAG5 expression and the clinicopathological characteristics of LUAD patients.
| Variable          | Overall (n=140) | High SPAG5 (n, %) | Low SPAG5 (n, %) | P value |
|-------------------|-----------------|-------------------|------------------|---------|
| Age, years        |                 |                   |                  | 0.8646  |
| ≤60               | 80              | 42 (52.50%)       | 38 (47.50%)      |         |
| >60               | 60              | 30 (50.00%)       | 30 (50.00%)      |         |
| Gender            |                 |                   |                  | 0.0054**|
| Male              | 100             | 33 (89.19%)       | 67 (65.05%)      |         |
| Female            | 40              | 4 (10.81%)        | 36 (34.95%)      |         |
| Stage             |                 |                   |                  | 0.0355* |
| +                 | 76              | 54 (71.05%)       | 22 (28.95%)      |         |
| ≥+                | 64              | 34 (53.13%)       | 30 (46.88%)      |         |
| Tumor T status    |                 |                   |                  | 0.0065* |
| T1+T2             | 103             | 53 (51.46%)       | 50 (48.54%)      |         |
| T3+T4             | 37              | 28 (75.68%)       | 9 (24.32%)       |         |
| Lymph node status |                 |                   |                  | 0.0281* |
| Negative          | 90              | 64 (71.11%)       | 26 (28.89%)      |         |
| Positive          | 50              | 24 (48.00%)       | 26 (52.00%)      |         |
| PD-L1 expression  |                 |                   |                  | <0.0001***|
| Low               | 97              | 31 (31.96%)       | 66 (68.04%)      |         |
| High              | 43              | 38 (88.37%)       | 5 (11.63%)       |         |

**Figures**

**Figure 1**

High expression of SPAG5 in tumors.

(A) Differentially expressed SPAG5 of multiple tumors in microarray datasets in the Oncomine database.

(B) Differentially expressed SPAG5 of multiple tumors in RNA-seq data from TCGA by TIMER.
(C) Differentially expressed SPAG5 of LUAD in RNA-seq data from TCGA and GTEx data in the GEPIA database.

(D) Differentially expressed SPAG5 at clinical stages in the GEPIA database.

*P<0.05, **P<0.01, ***P<0.001.

**Figure 2**

Prognostic values of SPAG5 in LUAD.

(A)-(B) Survival curves of OS and DFS of LUAD patients in the GEPIA database based on TCGA and GTEx data.

(C)-(E) Survival curves of OS and RFS of LUAD patients in the PrognoScan based on the GSE13213 and GSE31210 datasets.

(F)-(H) Survival curves of OS, FP, PPS of LUAD patients in the Kaplan-Meier Plotter database.

**Figure 3**

High expression of SPAG5 associated with tumor malignant phenotypes was measured in the single-cell level by the CancerSEA.

**Figure 4**

SPAG5 expression correlated with tumor immune infiltration in LUAD.

(A) Correlation between SPAG5 expression and tumor immune infiltration by the TIMER, X-axis is immune infiltration level, Y-axis is SPAG5 expression.

(B) Correlation between SPAG5 expression and immune checkpoints by the TIMER.

**Figure 5**

A PPI network of SPAG5 and its co-expression genes was constructed by STRING tool.
**Figure 6**

Mutation of SPAG5 and its co-expression genes in LUAD was evaluated by the cBioPortal.

**Figure 7**

Function annotation of hub genes in LUAD.

(A) Boxplot of enriched terms of SPAG5 and its co-expression genes in LUAD.

(B) Network of enriched terms of SPAG5 and its co-expression genes in LUAD, and colored by cluster ID. Each term is represented by a circle node, terms with a similarity score $> 0.3$ linked by an edge.

(C) Network of enriched terms of SPAG5 and its co-expression genes colored by $P$ value. The dark the color represented more statistically significant of the node.

(D) Network of the densely connected proteins was determined by MCODE algorithm, a unique color represented each MCODE network.

**Figure 8**

TMA and IHC analyses of the SPAG5 expression in 140 LUAD samples.