A Systematic Pan-Cancer Analysis of SPATS2L, a Potential New Immunological and Prognostic Biomarker

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Abstract

SPATS2L (Spermatogenesis-associated serine-rich 2-like) is an intranucleolar stress-response protein involved in chromosomal organization, ribosomal biogenesis, and translational control. Although there is mounting evidence that SPATS2L was involved in the carcinogenesis of some cancers, no thorough pan-cancer investigation of SPATS2L is available so far. Based on multiple databases, including TCGA, GTEx, CCLE, cBioPortal, TIMER2, ImmuCellAI, GDSC, and Reactome, we analyzed the expression, prognosis, DNA methylation, tumor mutational burden (TMB), microsatellite instability (MSI), immune cell infiltration, drug sensitivity, and clinicopathological and prognostic relevance of SPATS2L in pan-cancer including 33 types of cancers. SPATS2L expression was dramatically increased in a variety of malignancies, while it was low in ACC, KICH, and LAML as indicated by multiple databases and confirmed by immunohistochemistry assays. Importantly, SPATS2L has been found to have prognostic and clinicopathological importance in several malignancies. SPATS2L expression was also linked to TMB and MSI in 9 types of cancers, and there was a link between SPATS2L expression and DNA methylation in 28 types of cancers. SPATS2L was also found to be highly linked with immune cell infiltration, ICP expression, stromal score, immune score, and ESTIMATE score in various malignancies, demonstrating their regulatory roles on the TME. Consistently, the results of GSEA and GSVA analyses revealed a substantial link between SPATS2L and certain cellular immunological responses. Finally, SPATS2L was found to be strongly linked to 173 anti-tumor drugs. This study indicated that SPATS2L might be a potential cancer biomarker for the prognosis and immunotherapeutic response. SPATS2L expression in cancers may be involved in the regulation of the tumor immune microenvironment and drug sensitivity, which may be a new-targeted molecule for developing anti-tumor drugs and immunotherapy.

Introduction

Cancer has ranked as the leading cause of death and the most critical barrier to increasing life expectancy in every country of the world[1]. As an alternative to classical anticancer therapies, cancer immunotherapy, especially immune checkpoint blocking therapy, has been developed in recent years to reactivate the adaptive and innate immune systems and to create a solid anti-tumor immune response[2; 3]. The clinical practice of immune checkpoint inhibitors (ICIs), such as cytotoxic T-lymphocyte-associated protein 4 (CTLA 4), programmed death 1 (PD-1), and programmed death-ligand 1 (PD-L1), represents a breakthrough in immunotherapy[4; 5; 6]. However, since only a limited proportion of patients in certain types of cancer responded well to existing immunotherapy, finding new broader expressed immune checkpoints by performing pan-cancer expression analysis and evaluating their correlations with clinical prognosis will be necessary for improving the response rate of immunotherapy[7].

Spermatogenesis-associated serine-rich 2-like gene (SPATS2L) is an intranucleolar stress-response protein involved in ribosomal biogenesis and translational control in response to oxidative stress[8]. Research has shown that SPATS2L has vital roles in psoriasis and lymphoproliferative-type disorders and is involved in numerous cell growth and cell proliferation pathways that contribute to the etiology of these diseases[9; 10]. Meanwhile, reports have reflected that SPATS2L can increase glucose uptake by
regulating the protein expression of β2-adrenergic receptors[11]. In addition, SPATS2L is strongly associated with bronchodilator response in asthma [12] and is also shown to interact with skeletal muscle differentiation and response to TNF-α[13]. Recently, accumulating evidence has suggested that SPATS2L also impacts the occurrence and development of tumors. SPATS2L is ubiquitously expressed in multiple tissues, and its overexpression is related to the carcinogenesis of glioma and hepatocellular carcinoma[14; 15]. SPATS2L can alter cell proliferation and metastasis, promote tumor growth, and enhance cisplatin resistance in laryngeal squamous cell carcinoma[16]. However, the role of SPATS2L across cancers remains unclear. There are no completed studies on the prognostic value of SPATS2L across cancers.

Given the crucial role of SPATS2L in tumorigenesis, we used multiple databases, including TCGA, Clinical Proteomic Tumor Analysis Consortium (CPTAC), Cancer Cell Line Encyclopedia (CCLE), Genotype Tissue-Expression (GTEx), cBioPortal, and GEO databases, to analyze SPATS2L expression and their relationship with patient prognoses. In addition, we explored the potential correlations between SPATS2L expression and microsatellite instability (MSI), tumor mutational burden (TMB), DNA methylation, and immune infiltration, which suggests that SPATS2L is a potential prognostic biomarker. Further, we conducted co-expression analyses of immune-related genes with SPATS2L and enrichment analysis to study the biological functions of SPATS2L in tumors. Our results indicated that SPATS2L influenced the prognosis of cancers and probably via affecting infiltrating immune cells, MSI, and TMB. This study may provide insight into further research on SPATS2L.

**Methods**

**Data acquisition, processing and Differential Expression Analysis**

The TCGA, GTEx, CCLE, and clinical data were obtained from the UCSC Xena database (https://xenabrowser.net/datapages/). The cBioPortal database (https://www.cbioportal.org/) was used to download DNA mutation and methylation data. The IC50 values and gene expression profiles for the respective cell lines were obtained from the Genomics of Drug Sensitivity in Cancer (GDSC) database (https://www.cancerrxgene.org/).

**Survival Analysis and Relationship with Clinical Stage**

To examine the relationship between SPATS2L expression and patient prognosis, we extracted information on overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) for each sample downloaded from TCGA. Survival analyses were performed using Kaplan–Meier curves and log-rank tests (p < 0.05). Kaplan-Meier survival analysis was performed using the R packages “survival” and “survminer,” and univariate Cox regression analysis was performed using the R packages “survival” and “forestplot” to estimate SPATS2L’s prognosis-predictive value. Additionally, correlation analyses between SPATS2L expression and various pathological stages
were performed using the R packages "ggplot2" and "ggpubr"; a significance level of p < 0.05 was considered.

**Association analysis of SPATS2L With Genetic Alteration, DNA Methylation, Tumor Mutation Burden and Mismatch Repair Gene Expression**

SPATS2L genetic alteration features were retrieved from the cBioPortal web module "TCGA Pan-Cancer Atlas Studies" (https://www.cbioportal.org/). The "Cancer Types Summary" module was used to determine the mutation frequency, mutation type, and copy number alteration of the SPATS2L gene across all tumors in TCGA. cBioPortal (http://www.cbioportal.org/) was used to download methylation HM450 data. We used Kaplan-Meier survival analysis to determine the relationship between SPATS2L methylation and prognosis (OS, DSS, DFI, and PFI). TMB scores were downloaded in R using the TCGA biolinks package, calculated with a Perl script, and corrected by dividing by the exon length. MANTIS was used to estimate MSI across 33 cancer types using data from the TCGA.

**SPATS2L-Related Immune Infiltration Analysis**

The ESTIMATE (Estimation of Stromal and Immune cells in Malignant Tumor tissues Using Expression Data) algorithm was used to define the Stromal and Immune scores. The relationship between SPATS2L expression and these two scores was evaluated using the R software package "estimate" in relation to the degree of immune infiltration. Additionally, we used Immune Cell Abundance Identifier (ImmuCellAI) to determine the infiltration scores of 24 immune cells (http://bioinfo.life.hust.edu.cn/web/ImmuCellAI/). Moreover, the TIMER2 database was used to examine associations between SPATS2L and tumor stromal cells, tumor-infiltrating immune cells. We used the R-package "limma" to analyze the co-expression of SPATS2L and immune-related genes, including those encoding immune activation, immunosuppressive, major histocompatibility complex proteins (MHC), and immune checkpoint genes.

**GSEA and GSVA**

Biological functions of SPATS2L in tumors were investigated using Gene Set Enrichment Analysis (GSEA) and the Gene Set Variation Analysis (GSVA). The GSEA official website (https://www.gsea-msigdb.org/gsea/downloads.jsp) was used to download the Gene Ontology (GO), Reactome, and Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets. The functional analysis was carried out with the help of the R package "clusterprofiler." The GSVA gene set was obtained from the Molecular Signatures Database (MSigDB) collection of signature gene sets (v7.4; April 2021; https://www.gsea-msigdb.org/gsea/msigdb/index.jsp). For each tumor, GSVA scores were generated, and then the correlation between gene expression and pathway scores was calculated. We summarized the correlation results for 33 tumors and created a correlation heat map.

**Patients and Clinical Specimens**

All the biospecimens are provided by Pathology department, the 8th medical center of Chinese PLA general hospital. Written informed consents were obtained from all subjects, and normalized ethnic audit has been proceeded.
Immunohistochemistry (IHC)

Antibody recognizing SPATS2L (Proteintech, 16938-1-AP) was purchased from Proteintech. After fixation, decalcification, dehydration, transparency, paraffin embedding, dewaxing and dehydration, human slides were infiltrated in the preheated cell transparent liquid including PBS, Triton and 30% Hydrogen Peroxide (H₂O₂) for 30 min. Then, human slides were incubated in citric acid buffer for 15 min for antigen retrieval. After incubating with 0.3% H₂O₂ and blocking with 5% goat serum, the slides were incubated with rabbit polyclonal antibody against SPATS2L (1:100, Proteintech, USA) overnight. Results were visualized by 3,3′-diaminobenzidine tetrahydrochloride (DBA) staining. Subsequently, the slides were redyed with hematoxylin for 5 min. After washed, dehydrated, transparent and fixed with a gel, the microscope was utilized to detect the immune complexes. All experiments were performed in triplicate. Immunohistochemical staining was evaluated by two independent pathologists, without knowledge of the clinicopathological information of these patients. SPATS2L expression levels were evaluated by integrating the percentage of positive cells and staining intensity. The scoring criteria were as follows: (1) percentage of positive cells: no staining (score 0), 0%-25% (score 1), 26%-50% (score 2), 51%-75% (score 3), and 76%-100% (score 4); (2) staining intensity, negative (score 0), weak positive (score 1), positive (score 2), and strong positive (score 3). The final histochemistry score was produced by the product of the percentage of positive cells and the score of staining intensity.

Statistical Analysis

All the expression profiles were analyzed using Student’s t-test. To compare normal and cancerous tissue, two sets of t-tests were used; a P value less than 0.05 indicated statistical significance. The Kaplan-Meier method, log-rank test, and univariate Cox regression model were used for survival analysis. The correlation between the two variables was determined using Spearman’s or Pearson’s analysis; P values less than 0.05 were considered statistically significant. R software was used to conduct all statistical analyses (Version 4.1.1).

Results

Differential Expression of SPATS2L Between Tumor and Normal Tissue Samples

We began by conducting a thorough analysis of SPATS2L mRNA expression in normal tissues using the GTEx database. SPATS2L expression was lowest in the blood, whereas the majority of other normal tissues expressed high levels (Fig. 1A). Figure 1B shows the relative SPATS2L expression levels in 30 different types of cancer cell lines extracted from the CCLE database. As a result, the expression of SPATS2L was highly heterogeneous across the majority of cancer cell lines, which may reflect its influence on cancer cells’ malignant behaviors.
Further, we performed a pan-cancer expression profile analysis using datasets from the TCGA and GTEx databases in order to compare the expression profiles of tumor and normal tissue. SPATS2L expression was significantly increased in 23 TCGA tumors compared to normal tissues, including BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, OV, PAAD, READ, SKCM, STAD, THCA, THYM and UCS (Fig. 1C). In contrast, SPATS2L levels were decreased in three tumors: ACC, KICH and LAML; however, there was no significant difference in SPATS2L levels between PCPG, PRAD, SARC, TGCT, UCEC and non-tumor tissues, likely because of the limited number of normal tissue samples (for example, there were only data from three normal tissue samples in the PCPG and two normal tissue samples in the SARC dataset).

Immunohistochemical analyses to investigate SPATS2L expression was performed to confirm the results from TCGA and GTEx databases. We found that the SPATS2L protein is mainly located in the epithelial cells of glands and tumor-infiltrating lymphocytes. The expressions were significantly higher in the tumor tissues of LUAD, COAD, PAAD, and READ and lower in KIHC (Fig. 2).

**Clinicopathological Stages and Prognostic Value of SPATS2L in Various Cancers**

The TCGA cohort was used to investigate the relationship between SPATS2L expression and multiple cancers' clinical parameters (Fig. 3). Figures 3A–G shows that there was some effect on the high expression level of SPATS2L in seven different types of cancer at several stages, including COAD, KICH, LIHC, LUAD, LUSC, PAAD, and THCA.

Then, we conducted a survival analysis using OS, DSS, DFI, and PFI. SPATS2L expression was associated with a poorer overall survival (OS) prognosis in LGG (p < 0.001), LAML (p < 0.001), PAAD (p < 0.001), LIHC (p = 0.004), LUAD (p = 0.005), ACC (p = 0.005), GBM (p = 0.006) and KICH (p = 0.014) (Fig. 4A). Additionally, Kaplan-Meier survival analysis revealed a correlation between high SPATS2L expression and poor OS in patients with ACC, BRCA, LGG, DLBC, HNSC, GBM, LUAD, KICH, LAML, LIHC, MESO, PAAD, READ, SARC, TGCT, and UCEC, while high SPATS2L expression was associated with longer survival times in patients with ESCA and KIRC (Fig. 4B-Q). Moreover, we investigated the association between SPATS2L expression and DSS in cancer patients. SPATS2L expression was associated with a reduction in DSS in ten different types of cancer, including LGG (p = 0.001), PAAD (p = 0.001), LIHC (p = 0.002), KICH (p = 0.005), GBM (p = 0.012), ACC (p = 0.013), PRAD (p = 0.016), KIRC (p = 0.028), LUSC (p = 0.041), and LUAD (p = 0.047) (Fig. 5A). Increased SPATS2L expression was associated with a lower DSS in patients with ACC, BRCA, DLBC, GBM, HNSC, KICH, LGG, LIHC, LUAD, LUSC, MESO, PAAD, PRAD, READ, SARC, STAD, TGCT, UCEC, and UVM, according to Kaplan-Meier analysis. Besides that, low SPATS2L expression was associated with a worse DSS prognosis for BLCA, ESCA, and KIRC (Fig. 5B-W). Increased SPATS2L expression was found to be a risk factor for PAAD (p = 0.001), CESC (p = 0.004), and LUSC (p = 0.021) using Cox regression analysis. SPATS2L expression, on the other hand, had an inverse relationship with
prognosis in patients with READ (p = 0.039) and UCS (p = 0.042). (Fig. 6A). Increased SPATS2L
expression was associated with a poor prognosis in thirteen different types of cancer, including ACC,
CESC, COAD, KICH, STAD, UCEC, KIRP, LIHC, LUAD, LUSC, PAAD, and SARC (Fig. 6B-M). Besides, we
examined the relationship between SPATS2L expression and PFI and discovered that SPATS2L
expression was associated with PFI in patients with LGG (p < 0.001), PAAD (p < 0.001), LUSC (p < 0.001),
LIHC (p = 0.014), ACC (p = 0.019), GBM (p = 0.025), UVM (p = 0.025), KICH (p = 0.039) and HNSC (p = 0.043)
(Fig. 7A). Increased SPATS2L expression was associated with an unfavorable PFI in ACC, BRCA, GBM,
HNSC, KICH, LGG, LIHC, LUAD, LUSC, PAAD, READ, SARC, STAD, THCA, UCEC, and UVM, as determined by
Kaplan-Meier PFI curves (Fig. 7B-Q). The results indicated that SPATS2L expression is differently
correlated with the survival prognosis of patients with a variety of cancers.

Pan-Cancer Analysis of the Genetic Alteration and Methylation Level of SPATS2L.

Mutation directly affects gene expression and activation in mammalian cells[17]. Hence, we used the
cBioPortal (TCGA, Pan-Cancer Atlas) database to investigate the pan-cancer genetic alteration features of
SPATS2L. Across the 32 types of cancer, the mutation frequency was the highest (5.6%). The “mutation”
was the dominant type in the UCEC, SKCM, COAD, READ, and LGG (Fig. 8A).

We also investigated the DNA methylation of SPATS2L using the cBioPortal data set. A significant
negative correlation between gene expression and methylation was observed in 28 tumors. However, no
differences were observed between READ and UVM tissues and matched normal tissues (Fig. 8B). The
three strongest positive correlations (UCS, THCA, and LGG) are presented in Fig. 8C. Moreover, we
examined the possible relationship between SPATS2L promoter methylation and the prognosis of
patients with various types of cancer. As illustrated in Fig. 8D, SPATS2L methylation was a protective
factor for OS in patients with LGG, PAAD, and THYM. In patients with LGG, methylation of SPATS2L was a
protective factor for PFI and DSS. Moreover, PRAD patients with high methylation in SPATS2L had worse
DFI than patients with low alterations, but a high SPATS2L methylation level was protective for patient
DFI in TGCT.

The Relationships Between SPATS2L Expression and Tumor Mutation Burden and Microsatellite Instability Event

Tumor mutation burden (TMB) and Microsatellite instability (MSI) are two novel immunotherapy
response biomarkers[18; 19]. Subsequently, we examined the association between SPATS2L expression
and TMB, finding a significant association between them around CESC, HNSC, LAML, PRAD, and SKCM
(Fig. 9A). Hence, we discovered that SPATS2L expression was positively associated with MSI in BRCA
and TGCT, but negatively in CESC, DLBC, HNSC, PRAD, SKCM, or UCS (Fig. 9B).

Relationship Between SPATS2L Expression and the Tumor Microenvironment
Tumor microenvironment (TME) has a vital role in tumor occurrence and the prognosis of cancer patients\[20; 21\]. Hence, estimating the association between the TME and SPATS2L expression in a pan-cancer dataset is critical. The ESTIMATE algorithm was used to calculate stromal and immune scores for 33 different types of tumor tissues; we then examined the relationships between these scores and SPATS2L expression. SPATS2L levels were found to be significantly correlated with stromal and immune scores, as well as with estimate scores (Fig. 10A). The typical results in DLBC, LGG, and BRCA are depicted in Fig. 10B, C, and D. It suggested that SPATS2L was highly involved in immune infiltration in the above tumors.

**Relationship Between SPATS2L Expression Levels and Degree of Tumor Immune Cell Infiltration**

Tumor-infiltrating lymphocytes (TILs) can be used as independent predictors of sentinel lymph node status and cancer survivors\[22\]. We next examined the relationship between SPATS2L expression levels and the degree of immune cell infiltration across a variety of TIMER2-derived cancer types. Our data demonstrate that the abundance of infiltrating immune cells was significantly associated with SPATS2L expression in most types of cancer (Fig. 11A): cancer-associated fibroblast cells in 29 types, macrophages in 32 types, CD4 + T cells in 32 types, CD8 + T cells in 32 types, B cells in 31 types, neutrophils in 29 types, and dendritic cells in 30 types. In particular, fibroblast cell infiltration was positively correlated with high SPATS2L expression in 26 out of 32 types of cancer, except UVM, UCEC, KIRP, KIRC, ESCA, and CHOL (p < 0.05).

We also used the ImmuCellAI database to determine the correlations between SPATS2L expression and immune cell subgroup infiltration. We discovered that SPATS2L expression was significantly correlated with 24 subgroups of immune cells in 32 types of cancer. (Fig. 11B). SPATS2L expression was most strongly associated with iTreg, nTreg, and macrophage cells in these various cancers. SPATS2L expression, on the other hand, was negatively correlated with B cell (except in LIHC), Tgd cell (except in KIRC), and CD8 + T cell (except in LGG) counts. Furthermore, in MESO, only two types of immunocytes were associated with SPATS2L levels, whereas in other cancers, at least three immunocytes were associated with SPATS2L levels.

Furthermore, we systematically analyzed the relationships between SPATS2L expression and three major types of immune modulators in 33 tumors. Notably, we observed a positive correlation between the expression of SPATS2L and the majority of immunoinhibitors, immunostimulators, and MHC molecules in all types of tumors except CHOL, TGCT, and UCS (p < 0.05) (Fig. 11C, D, E).

Additionally, we discovered a strong correlation between SPATS2L and checkpoint members, including CD274 (PD-L1), CTLA4, PDCD1, TIGIT, and LAG3 (Fig. 12). Interestingly, there were distinctly positive associations between SPATS2L expression and checkpoint members in the majority of tumor types, but was negatively correlated with CHOL, UCS, and TGCT, implying that SPATS2L may regulate the immune response in these cancer types.
Association Between SPATS2L and Specific Cellular Immune Responses

To elucidate the molecular mechanisms underlying SPATS2L regulation in a variety of tumors, we used GSEA and GSVA. The functional annotations for SPATS2L in the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) are shown in Figs. 12A and B, respectively. Consistent with the aforementioned findings, the data indicate that SPATS2L positively regulates immunological processes in BLCA, CESC, DLBC, ESCA, GBM, HNSC, KICH, KIRC, LAML, LGG, LUSC, OV, PRAD, READ, and STAD, including myeloid activation, neutrophil activation, natural killer cell-mediated cytotoxicity, and immune regulation and signaling pathways (Figs. 13A). Similarly, GSEA identified multiple immune functional gene sets enriched in LGG, LUSC, PAAD, PRAD, SARC, SKCM, TGCT, and UCEC, including those involved in T cell receptor signaling pathways, Fc gamma R-mediated phagocytosis, and chemokine signaling pathways. SPATS2L, on the other hand, is predicted to act negatively on Th1 and Th2 cell differentiation, the B cell receptor signaling pathway, and primary immunodeficiency in CHOL (Figs. 13B). The GSEA results for Reactome terms suggested that several immune functional gene sets, including class I MHC mediated antigen processing and presentation, cytokine signaling in the immune system, the adaptive immune system, and the innate immune system, were enriched in 26 out of 33 types of cancer, except UCS, TGCT, SKCM, PCPG, LIHC and CHOL (Supplementary Figure S1).

Additionally, we used GSVA to investigate the biological significance of SPATS2L expression in 33 types of tumors. The top 20 signaling pathways positively influenced by SPATS2L were predominantly immune-related pathways, including TGF-β signaling, PI3K-AKT mito signaling, Notch signaling, TNF-α signaling via NF-κB, KRAS signaling up, mTORC1 signaling, and interferon-α response. In contrast, SPATS2L expression was negatively correlated with oxidative phosphorylation, KRAS signaling DN, DNA repair, and Myc targets V2 (Fig. 14).

Drug sensitivity analysis

To investigate the correlation between SPATS2L and chemotherapy or targeted therapy, we employed Spearman’s correlation analysis to evaluate drug sensitivity and calculate the correlation between SPATS2L and drug sensitivity across cancer cell lines based on data from GDSC. We identified that SPATS2L was significantly associated with sensitivity to 173 drugs, including five negatively correlated and 168 that were positively correlated (Supplementary Table S1). Intriguingly, high SPATS2L expression was related to high IC50 in multiple cell lines, further supporting that SPATS2L may play a role in chemotherapy and targeted drug therapy resistance.

Discussion

SPATS2L has been shown to influence the occurrence and progression of hepatocellular carcinoma and glioma cancers in previous studies[14; 15]. However, little is known regarding its role in other cancer types. Our findings indicate that the SPATS2L gene is highly expressed in 23 different types of cancer,
which is consistent with previous findings in glioma and liver cancer, indicating that this gene primarily functions as an oncogene in tumor progression. Cox proportional hazards modeling and Kaplan-Meier survival analysis revealed that increased SPATS2L expression was associated with a poor prognosis in certain types of cancer. In addition, elevated SPATS2L expression indicated clinicopathological advances in seven cancers. Further, SPATS2L expression was closely associated with DNA methylation, MSI, and TMB, serving as a prognostic biomarker. Using ESTIMATE, TIMER2, ImmuCellAI, and TCGA, we found that SPATS2L overexpression was associated with immune scores, immune infiltration, and immune checkpoint expression. Therefore, SPATS2L may be critical for cancer prognosis and tumor immunity.

TMB level is frequently regarded as a significant biomarker associated with treatment response in a large number of ICI-treated tumors[23; 24; 25]. High TMB enhanced the probability of cancer cells being recognized by the immune system[26; 27]. Earlier research has established that elevated TMB levels can be used as a biomarker to predict ICI response in a variety of cancer types, including [28], melanoma[29], and bladder cancer[30]. MSI is also an essential biomarker for tumor immunotherapy as a surrogate for neoantigens with immunogenicity[31]. High-frequency MSI in cervical squamous cell carcinoma and adrenocortical carcinoma is a crucial predictor of tumor development[32; 33]. In our analysis, SPATS2L expression is highly related with TMB in 5 cancer types and with MSI in 8 cancer types, suggesting that high SPATS2L expression might have a poorer prognosis from immunotherapy for patients in which SPATS2L expression is negatively correlated with TMB and/or MSI.

The TME is primarily composed of stromal and immune cells that regulate tumor growth via the secretion of signaling molecules and components of the extracellular matrix (ECM)[34]. TME features have emerged as the most promising biomarker for predicting immunotherapy responses and evaluating clinical outcomes[35]. Our findings indicate that SPATS2L is required for cancer immunity, as evidenced by the association between SPATS2L level and both stromal and immune cell content in the ESTIMATE analyses.

Immune cell infiltration is intimately linked to the occurrence and development of tumors and has a significant impact on clinical outcomes[36; 37]. The current study examined the correlation between SPATS2L levels and TMEs using two distinct methods, including the TIMER2 database and immune cell infiltration data from the ImmuCellAI database. Tumor-associated macrophages (TAMs) are a critical component of the tumor microenvironment, playing a role in tumor genesis, growth, invasion, and metastasis[38]. SPATS2L expression was found to be positively correlated with macrophage cells, particularly M2-like macrophages, implying that SPATS2L may play a role in macrophage polarization. Cancer-associated fibroblasts (CAFs) actively participate in cancer progression through complex interactions with other cell types in the tumor microenvironment[39]. In addition to producing extracellular matrix components that contribute to tumor matrix structure and function, CAFs also produce secretory factors, exosomes, and metabolites that influence tumor angiogenesis, immunology, and metabolism[40]. Notably, we observed a positive correlation between CAFs infiltration levels and SPATS2L. B cells have an effect on tumors' ability to resist immune attacks. Our analysis revealed a negative correlation between B cells and SPATS2L expression. These findings suggest that SPATS2L plays a critical role in tumor
immune evasion and plays an oncogenic role in most tumor types. Further, our results unraveled the co-expression of SPATS2L with immune-related genes, such as immunoinhibitors, immunostimulators, and MHC molecules. We also found that SPATS2L was positively correlated with immune checkpoint members in pan-cancer, such as CD274, TIGIT, CTLA4, LAG3, and PDCD1. Finally, through GSEA and GSVA of SPATS2L, we found that it was linked to pathways involved in immune regulation, especially immunoregulatory interactions between myeloid cell activation, B cell receptor signaling, and adaptive immune system. All of these findings indicate that SPATS2L plays a critical role in regulating immune infiltration of tumor cells, influencing the tumor immune microenvironment, and providing a novel drug target for tumor immunotherapy.

In conclusion, our first pan-cancer analysis of SPATS2L revealed that it has an oncogenic effect and may serve as a prognostic indicator for patients. Moreover, SPATS2L expression was contributed to immune cell infiltration and tumor immunosuppressive microenvironment across various cancer types, providing a potential target for more precise and personalized immunotherapy.

**Abbreviations**

ACC, Adrenocortical carcinoma; BLCA, Bladder Urothelial Carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangiocarcinoma; COAD, Colon adenocarcinoma; DLBC, Lymphoid neoplasm diffuse large B-cell lymphoma ; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LAML, Acute Myeloid Leukemia; LIHC, Liver hepatocellular carcinoma; LGG, Lower Grade Glioma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular Germ Cell Tumor; THYM, Thymoma; THCA, Thyroid carcinoma; UCS, Uterine Carcinosarcoma; UCEC, Uterine Corpus Endometrial Carcinoma; UVM, Uveal Melanoma; COADREAD, colorectal adenocarcinoma.

**Declarations**

**Ethical Approval:** This study was performed according to the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Chinese PLA General Hospital (Approval No. S2021-105-02).

**Author’s Contributions:** XLX, LX and YC conceived the study. YC and QL performed the data analysis, YC, YWY and QL drafted the manuscript and helped with the validation, XJW, HZL, ZWG helped with the validation. CY helped the revision. All authors read and approved the submitted version.

**Competing interests:** No conflicts of interest have been declared
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Availability of data and materials:

Publicly available datasets were analyzed in this study. This data can be found here: All the data sets used in this study were publicly available at cBioPortal (https://www.cbioportal.org), TIMER2.0 (http://timer.comp-genomics.org/), and TCGA database (https://xenabrowser.net or https://portal.gdc.cancer.gov/).

References


**Supplementary Figure And Table**

Supplementary Figure S1 and Table S1 are not available with this version

**Figures**
Figure 1

SPATS2L expression in different types of tissue or cancer. SPATS2L expression in (A) 31 types of tissue; (B) 30 types of cancer cell lines; (C) Comparison of SPATS2L expression between tumor and normal samples. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 2

Immunohistochemistry analysis of the expression of SPATS2L in tumor tissues. We detected the SPATS2L protein expressions in LUAD, COAD, PAAD, READ, and KICH. Typical results of one pair of samples (A); (B) The results were then quantitated. H score= the percentage of positive cells×the score of staining intensity. Data represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.
Figure 3

Correlations between the SPATS2L expression and the main clinicopathological stages including stage I, stage II, stage III, and stage IV of COAD, KICH, LIHC, LUAD, LUSC, PAAD, and THCA were investigated based on the TCGA data. Log2 (TPM+1) was used for log scale. *p < 0.05, **p < 0.01, and ***p < 0.001.
Figure 4

Association between SPATS2L expression and overall survival time in days (OS). (A) Univariate Cox regression analysis of overall survival in 33 types of tumor. (B–Q) Kaplan-Meier analysis of the association between SPATS2L expression and OS. HR, hazard ratio; CI, confidence interval.
Figure 5

Association between SPATS2L expression and disease-specific survival (DSS). (A) Univariate Cox regression analysis of DSS in 33 types of tumor. (B–W) Kaplan-Meier analysis of the association between SPATS2L expression and DSS. HR, hazard ratio; CI, confidence interval.
Figure 6

Association between SPATS2L expression and disease-free interval (DFI). (A) Univariate Cox regression analysis of DFI in 33 types of tumor. (B–M) Kaplan-Meier analysis of the association between SPATS2L expression and DFI. HR, hazard ratio; CI, confidence interval.
Figure 7

Association between SPATS2L expression and disease-free interval (PFI). (A) Univariate Cox regression analysis of PFI in 33 types of tumor. (B–Q) Kaplan-Meier analysis of the association between SPATS2L expression and PFI. HR, hazard ratio; CI, confidence interval.

Figure 8

Genetic alternations of SPATS2L in pan-cancer. (A) Alteration frequency with the mutation type of SPATS2L in human pan-cancer using cBioportal database. (B) The association between SPATS2L expression and DNA methylation in human pan-cancer. (C) Associations of SPATS2L expression with the DNA methylation in patients with UCS, THCA, and LGG. (D) Association between DNA methylation of SPATS2L and clinical survival.
Figure 9

Correlation of SPATS2L expression with tumor mutation burden (TMB) and microsatellite instability (MSI) in multiple cancer types. (A) Correlation between TMB and SPATS2L expression. (B) Correlation between MSI and SPATS2L expression. Spearman's correlation coefficients are shown above the bar graphs. (Spearman Correlation test, *p < 0.05, **p < 0.01, and ***p < 0.001, P<0.05 was considered statistically significant).
Figure 10

Correlation of SPATS2L expression with tumor immune microenvironment. (A) The negative or positive association between SPATS2L expression and stromal score, immune score, and estimate score. (B) Correlation between SPATS2L and immune scores in DLBC, LGG, and BRCA. (C) Correlation between SPATS2L and stromal scores in DLBC, LGG, and BRCA. (D) Correlation between SPATS2L and estimate scores in DLBC, LGG, and BRCA.
Figure 11

The SPATS2L expression correlated with immune infiltration. (A) The SPATS2L expression significantly correlated with the infiltration levels of various immune cells in the TIMER2 database. (B) Correlation between SPATS2L expression and different immune cells from ImmuCellAI database. (C, D, E) Co-expression of SPATS2L and immune-related genes. Red represents positive correlation, blue represents negative correlation, and the darker the color, the stronger the correlation. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Figure 12

Circle plot of the correlations between SPATS2L expression and TIGIT, CD274, LAG3 and PDCD1 in (A) BRCA, (B) DLBC, (C) CHOL, (D) UCS, (E) TGCT, and (F) THYM using the TCGA database.
Figure 13

GSEA of SPATS2L in TCGA pan-cancer. (A) GO functional annotation of SPATS2L in various cancers. (B) KEGG pathway analysis of SPATS2L in multiple cancers.
Figure 14

GSVA of SPATS2L in TCGA pan-cancer. The heatmap shows the correlation between SPATS2L expression and GSVA scores of 50 Hallmark pathways in pan cancer. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary1DrugsensitivityanalysisofSPATS2L.csv