

LATS2 expression differences in lung adenocarcinoma and lung squamous cell carcinoma analyzed using bioinformatics and experimental approaches

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Abstract. The present study aimed to investigate the role and expression of large tumor suppressor kinase 2 (LATS2) in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). The expression levels of LATS2 in LUAD and LUSC, as well as their association with clinical characteristics and patient survival, were analyzed using data obtained from The Cancer Genome Atlas. A total of 100 LATS2-related genes were screened to conduct Kyoto Encyclopedia of Genes and Genomes and Gene Ontology enrichment analyses. The association between LATS2 expression and immune cell infiltration, particularly CD4⁺ and CD8⁺ tumor-infiltrating lymphocytes (TILs), was analyzed. The role of LATS2 in tumor cells was validated by observing the changes in proliferation, apoptosis, migration and invasion of LUAD and LUSC cells following LATS2 overexpression. LATS2 expression was low in both LUAD and LUSC. In LUAD, high expression of LATS2 was associated with lymph node metastasis, distant metastasis and TNM stage and served as an independent risk factor for both overall survival and progression-free survival. Conversely, in LUSC, LATS2 exhibited a weak association with clinical characteristics and survival. In LUAD and LUSC, LATS2-related genes exhibited differences in their associated functional pathways and biological processes. In LUAD, LATS2 was positively associated with CD4⁺ TIL proportions and CD4⁺/CD8⁺ TIL proportions, while exhibiting a negative association with CD8⁺ TIL proportions. In LUSC, no such associations were observed. *In vitro* experiments demonstrated

that overexpression of LATS2 inhibited proliferation, migration and invasion, while promoting apoptosis, in both LUAD and LUSC cell lines, with notably stronger effects observed in LUAD cells. In conclusion, LATS2 exerts tumor-suppressive functions in both LUAD and LUSC. In LUAD, LATS2 is an independent risk factor for patient survival, possibly due to its close association with CD8⁺ TIL levels; however, this relationship is not pronounced in LUSC.

Introduction

Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the predominant subtypes of non-small cell lung cancer (NSCLC), accounting for ~85% of all lung cancer cases (1). LUAD and LUSC share similar mechanisms in terms of tumorigenesis and progression, and are often collectively analyzed as NSCLC. However, there are also numerous distinct gene expression patterns and developmental mechanisms observed between these two histological subtypes. For example, a previous study demonstrated distinct T-lymphocyte infiltration profiles and prognostic implications in LUAD compared with LUSC (2). Consequently, it is necessary to distinguish the pathological types of lung cancer when exploring the molecular biological mechanisms of the occurrence and development of lung cancer.

The Hippo signaling pathway serves a pivotal role in regulating cell proliferation, apoptosis, migration and organ size control (3). As a core kinase within this pathway, large tumor suppressor kinase 2 (LATS2) has been implicated in the pathogenesis of various malignancies (4). Previous studies have revealed that LATS2 inhibits the nuclear transcriptional activity of downstream effectors through phosphorylation, thereby modulating cell cycle progression, promoting apoptosis and suppressing cell migration and invasion (5,6). Consequently, reduced LATS2 expression has been observed in multiple types of cancer, including breast and esophageal cancer (7,8). Current investigations of LATS2 in lung cancer have predominantly focused on NSCLC as a collective entity. While some studies suggest that low LATS2 expression is associated with a poor prognosis (8,9), analysis using biological information databases, such as the Gene Expression

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Profiling Interactive Analysis database (10), has revealed that patients with LUAD with high LATS2 expression have worse survival time than those with lower expression (<http://gepia.cancer-pku.cn/detail.php?gene=LATS2>). However, the expression patterns, biological functions and underlying mechanisms of LATS2 across different lung cancer subtypes remain poorly characterized.

The advent of bioinformatics has revolutionized tumor research by enabling large-scale gene expression profiling using public databases. The Cancer Genome Atlas (TCGA), which houses extensive genomic data and clinical annotations from diverse tumor types, provides an invaluable resource for such investigations (11). Leveraging the existing bioinformatics tools such as TCGA, the present study aimed to comprehensively analyze LATS2 expression profiles, prognostic importance and subtype-specific differences in LUAD and LUSC. Furthermore, *in vitro* experiments were performed to investigate the impact of LATS2 modulation on the biological behaviors of LUAD and LUSC cell lines, thereby shedding light on the potential mechanisms underlying LATS2-mediated tumor suppression in lung cancer.

Materials and methods

Data acquisition. Raw RNA sequencing expression profile data of LUAD and LUSC samples were obtained from TCGA (<https://portal.gdc.cancer.gov>; data 43.0; release date: May 7, 2025). The LUAD dataset comprised 600 samples from 517 patients, including 59 normal samples and 541 tumor samples, while the LUSC dataset comprised 561 samples from 501 patients, including 51 normal samples and 510 tumor samples. The data were downloaded and cleaned using the R program (R 4.4.1; Posit Software, PBC) and were organized into expression matrices for LUAD and LUSC.

Differential expression analysis. Firstly, the Gene Set Cancer Analysis (GSCA) platform (12) was utilized to investigate LATS2 expression disparities between normal and tumor tissues in LUAD and LUSC. Secondly, independent samples t-tests and paired samples t-tests were employed to further compare LATS2 expression between normal and tumor tissues in LUAD and LUSC expression matrices. Finally, the Human Protein Atlas database (version 25.0; <https://www.proteinatlas.org/>) (13,14) was consulted to retrieve and compare the expression levels of LATS2 detected by immunohistochemistry in normal lung tissues, LUAD tissues and LUSC tissues.

Clinical relevance analysis. LATS2 expression levels were compared across subgroups categorized by tumor size (T; T0 vs. T1-4), lymph node metastasis status (N; N0 vs. N1-3), distant metastasis status (M; M0 vs. M1) and TNM stage (stage I/II vs. stage III/IV) in both LUAD and LUSC. Survival data and tumor recurrence information were compiled, and Kaplan-Meier analysis was performed to assess associations between LATS2 expression and overall survival (OS) and progression-free survival (PFS). Based on the clinical information in TCGA, univariate survival analyses were conducted for age, sex, smoking history, history of other malignancies, residual tumor, adjuvant therapy, TNM stage and LATS2 expression level (converted into binary variables based on the

median value). Subsequently, the variables with $P < 0.1$ were included in multivariate regression analysis to determine whether LATS2 served as an independent prognostic factor for patient survival. To avoid setting the P-value too strictly, which would result in a smaller number of variables that can be included, the P-value was relaxed to 0.1 after multiple trials. Survival risk forest plots were drawn based on the results.

Kyoto encyclopedia of genes and genomes (KEGG) and gene ontology (GO) enrichment analysis of LATS2-related genes. The R package ‘DESeq2’ (15) was employed to identify differentially expressed genes between normal and tumor tissues within the LUAD and LUSC expression matrices, with the fold change threshold set at >2 (absolute value) and the significance threshold set at <0.05 . The ‘cor.test’ function of package ‘psych’ (<https://cran.r-project.org/web/packages/psych/index.html>) was utilized to calculate correlations between LATS2 and each differentially expressed gene. Based on the correlation coefficient (R), the 100 genes (50 positive and 50 negative) with the strongest correlation with LATS2 were selected. The 100 genes were included in KEGG pathway analysis and GO functional enrichment analysis using the Metascape platform (version v3.5.20260201; <https://metascape.org/gp/index.html>) (16) to elucidate the primary biological functions and signaling pathways associated with LATS2-related genes in LUAD or LUSC.

Immune infiltration analysis. The correlation between the expression levels of LATS2 and 22 types of immune cells was analyzed using the CIBERSORT algorithm (17) in the R program, with the LM22 gene set as the reference. Furthermore, the GSCA database (<http://guolab.wchscu.cn/GSCA/>) (12) was used to analyze the correlations between LATS2 expression and CD4 and CD8⁺ T cell infiltration in patients with LUAD or LUSC online.

Human sample collection. Patients with LUAD or LUSC who underwent lung cancer radical surgery at the Second Affiliated Hospital of Zhengzhou University (Zhengzhou, China) between July 2022 and January 2023 were included. Inclusion criteria included the following: i) Age between 18-80 years; ii) diagnosed with primary LUAD or LUSC or TNM Stage I-IIIb; iii) underwent lung cancer radical surgery without prior systemic chemotherapy, radiotherapy or immunotherapy; and iv) Eastern Cooperative Oncology Group score 0-2 and expected survival >6 months. Exclusion criteria included the following: Patients with severe infectious diseases, immune system diseases, blood system diseases, active tuberculosis, heart, liver and kidney diseases and other organic disorders. A total of 42 lung cancer samples were collected, 20 LUAD and 22 LUSC, 26 men and 16 women, aged 63.4 ± 5.6 years. The present study was approved by the Ethics Committee of the Second Affiliated Hospital of Zhengzhou University (approval no. 2022243). Written informed consent for the acquisition and use of samples was obtained from patients or their legal guardians, and all experiments were carried out in accordance with The Declaration of Helsinki and guidelines and regulations of Zhengzhou University.

Cell transfection. The A549 and SK-MES-1 cells (Procell Life Science & Technology Co., Ltd.) were divided into BC

(blank control (BC), negative control (NC) and overexpression (OE) groups. All cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) containing dual antibodies (Gibco; Thermo Fisher Scientific, Inc.) and fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) at a temperature of 37°C, with 5% CO₂ concentration and 75% humidity. The NC and OE groups were transfected with empty lentivirus and LATS2-overexpression lentivirus (Guangzhou Ruibo Biotechnology Co., Ltd.), respectively. At 24 h before cell transfection, the cells were seeded at 1x10⁵ cells/well in a 6-well plate and then transferred to an antibiotic-free medium. At the beginning of transfection, the cells were washed and 1 ml serum-free medium was added. Then, 1.6 µg plasmid (2nd generation system; interim cell line, 293T provided by Procell Life Science & Technology Co., Ltd.; lentivirus:packaging plasmid:envelope plasmids=4:3:1; MOI=10) and 4 µl the transfection complex composed of Lipofectamine 3000 (Thermo Fisher Scientific, Inc.) were added. The cells were cultured at 37°C for 6 h, then the medium was changed to a complete medium containing serum, and the cells were further cultured for 72 h before the detection was performed.

Quantitative PCR (qPCR). Cells were collected 72 h post-transfection, and qPCR was conducted to determine the level of LATS2. The primer sequences for LATS2 were as follows: Forward, 5'-TGTAGACCGCGCCCCCT-3' and reverse, 5'-TCTTTCCTTCCATTTTGTAGTTCC-3'. GAPDH was used as the internal control, with the following primers: Forward, 5'-GGAGCGAGATCCCTCCAAAAT-3' and reverse, 5'-GGCTGTTGTCATACTTCTCATGG-3'. The qPCR reaction conditions were as follows: Initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C for 20 sec (with fluorescence detection). The relative gene expression levels were calculated using the 2^{-ΔΔC_q} method (18).

Western blot. At 72 h post-cell transfection, the culture medium was discarded. Total protein was extracted using RIPA lysis and extraction buffer [Thermo Fisher Scientific, Inc.; containing 25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS]. The protein concentration was determined using a BCA assay (Thermo Fisher Scientific, Inc.), and 30 µg total protein was loaded per lane. A 10% SDS-PAGE gel was prepared, and a 0.45 µm PVDF membrane (Beyotime Biotechnology) was transferred at a constant current of 300 mA for 90 min. The membrane was blocked with 5% BSA at room temperature for 2 h. Subsequently, the anti-LATS2 primary antibody (diluted 1:1,000; Abcam; cat. no. ab243657) or anti-GAPDH primary antibody (1:1,000; Abcam; cat. no. ab243657) was added and incubated overnight at 4°C. After washing, a goat anti-rabbit secondary antibody (1:2,000; Abcam; cat. no. ab6721) was added and incubated at room temperature for 1 h. The membrane was scanned after developing, and the bands were quantitatively analyzed using Image J software (version 1.8.0; National Institutes of Health). GAPDH was used as the internal reference for normalization.

MTT assay. The A549 or SK-MES-1 cells after 72 h of transfection were prepared into a suspension with RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) containing

10% fetal bovine serum and inoculated into 96-well plates (200 µl) at 5x10⁴/well. After 24, 48 and 72 h, 20 µl MTT solution was added to each well. The culture was terminated after 4 h incubation (37°C; 5% CO₂), centrifuged at 440 x g for 5 min at 4°C and the supernatant was discarded. After adding 150 µl DMSO and oscillating for 10 min, absorbance values were observed at 490 nm wavelength, and cell growth curves were drawn.

Flow cytometry. For cell apoptosis rate determination, cells were collected after transfection for 72 h and adjusted to 1x10⁵ cells/ml. In a 4°C dark environment, 5 µl AnnexinV-FITC was added to the cells for a 15 min staining period, followed by the addition of 5 µl PE staining period for 5 min. The stained cells were then analyzed using a flow cytometer (CytoFLEX; Beckman Coulter, Inc.) to determine the cell apoptosis rate.

Flow cytometry was also used to determine the proportions of CD4⁺ and CD8⁺ subsets of tumor-infiltrating lymphocytes (TILs) in lung cancer tissues: The tissues were thawed, minced and washed in RPMI-1640 medium containing 10% FBS, then digested in trypsin digestion solution (Gibco; Thermo Fisher Scientific, Inc.) at 37°C on a shaker for 4 h. FACS lysis solution (Gibco; Thermo Fisher Scientific, Inc.) was added to remove red blood cells. Ficoll-Paque PLUS separation solution (Cytiva) was added to collect mononuclear cells and prepare single-cell suspensions. A total of 1x10⁶ cells per tube were collected, and CD45, CD3, CD4, CD8 antibodies (BD Biosciences; cat. nos. 572781, 572799, 572785 and 572798, respectively) were added. The tubes were incubated in the dark at 4°C for 30 min and then analyzed via a flow cytometer (BD FACS Canto, BD Company) to determine the proportions of CD4⁺ and CD8⁺ cells and calculate the CD4⁺/CD8⁺ ratio. The gating path (BD FACS Diva 8.0.2; BD Biosciences) was set as follows: Lymphocyte population (FSC-A vs. SSC-A) → CD45⁺ → CD3⁺ → CD4⁺/CD8⁺.

Wound healing assay. Cells were collected 72 h post-transfection and seeded into 6-well plates at 5x10⁵ cells per well. After overnight incubation, a straight line was scratched across the cell monolayer perpendicularly using a pipette tip. The cells within the scratch were washed away. Subsequently, serum-free medium was added, and the cells were cultured at 37°C in a 5% CO₂ incubator. Images were captured (Zeiss LSM880) at 0 and 24 h. Lines were drawn along the cell edges on the images, and the distance between the two lines was defined as the scratch width (D). The average of three measurements was taken. The cell migration rate after 24 h was calculated using the formula: (D_{0h}-D_{24h})/D_{0h} x100%.

Transwell assay. The upper surface of the Transwell chambers was coated with diluted Matrigel at 37°C for 3 h, followed by the addition of 50 µl serum-free medium containing BSA. Cells collected 72 h after transfection were resuspended in serum-free medium (Gibco; Thermo Fisher Scientific, Inc.) and adjusted to 1x10⁵ cells/ml. A 100 µl aliquot of the cell suspension was added to the upper chamber of the Transwell insert, while 500 µl RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) was added to the lower chamber. After 24 h incubation, the Matrigel and the cells in the upper chamber were wiped away. The cells were then stained with

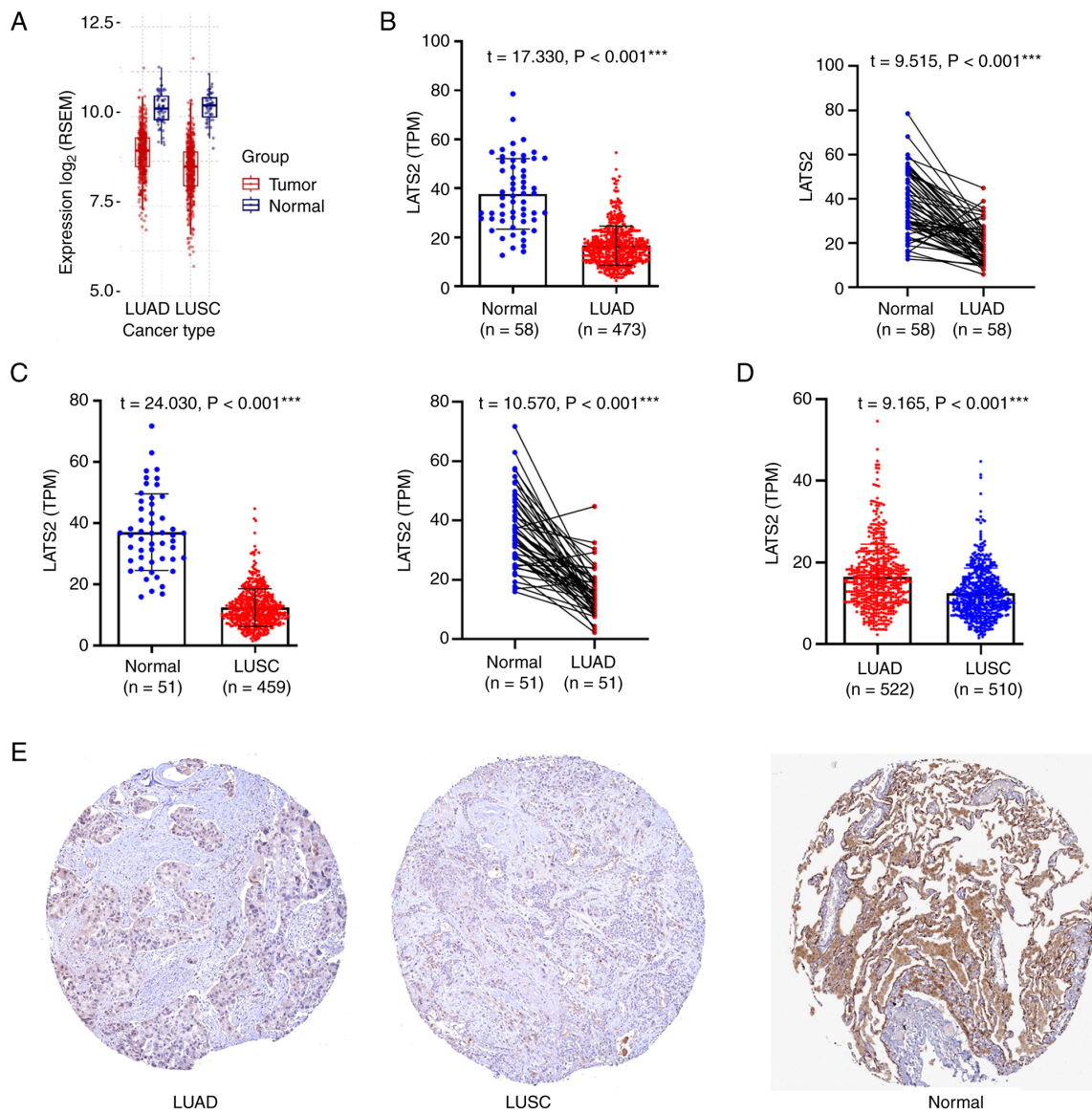


Figure 1. Comprehensive expression analysis of LATS2 in LUAD and LUSC. (A) Expression levels of LATS2 in LUAD and LUSC analyzed using the Gene Set Cancer Analysis platform. (B) Comparative analysis of LATS2 expression between tumor and normal tissues in LUAD using independent sample t-test and paired sample t-test. (C) Comparative analysis of LATS2 expression between tumor and normal tissues in LUSC using independent sample t-test and paired sample t-test. (D) Differential expression of LATS2 between LUAD and LUSC tumor tissues. (E) Immunohistochemical validation of LATS2 expression in LUAD, LUSC and normal lung tissues using the Human Protein Atlas database (antibody ID: 039191). All the data are presented as mean $\pm$ SD. *** $P < 0.001$. LATS2, large tumor suppressor kinase 2; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TPM, transcripts per million; RSEM, RNA-seq by expectation-maximization.

0.1% crystal violet at room temperature for 15 min and observed and images captured under a microscope (Olympus GX53). The average number of cells in five fields of view was calculated.

Statistical analysis. The statistical tools used in the present study included IBM SPSS 20.0 (IBM Corp.), GraphPad Prism 8 (Dotmatics) and R 4.4.1. Inter-group comparisons were conducted using unpaired sample t-tests or paired sample t-tests. Comparisons among multiple groups were conducted using ANOVA (parametric) or Kruskal-Wallis test (non-parametric), followed by a Student-Newman-Keuls post hoc test. The survival time between two groups was compared using Kaplan-Meier analysis, and differences were evaluated using a log-rank test (performed using SPSS). Two-step tests were

performed using R 4.4.1 and the 'survival' package (19) to compare differences for the survival curves with a late-stage crossover. Cox multivariate regression analysis was performed using SPSS, and the differences were examined using the log-rank method. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Low LATS2 expression in LUAD and LUSC. GSCA database analysis revealed that LATS2 expression was downregulated in LUAD and LUSC compared with normal tissues (Fig. 1A). Further analysis of TCGA-LUAD and TCGA-LUSC data also demonstrated that the expression levels of LATS2 in tumor tissues were significantly lower than those in normal tissues

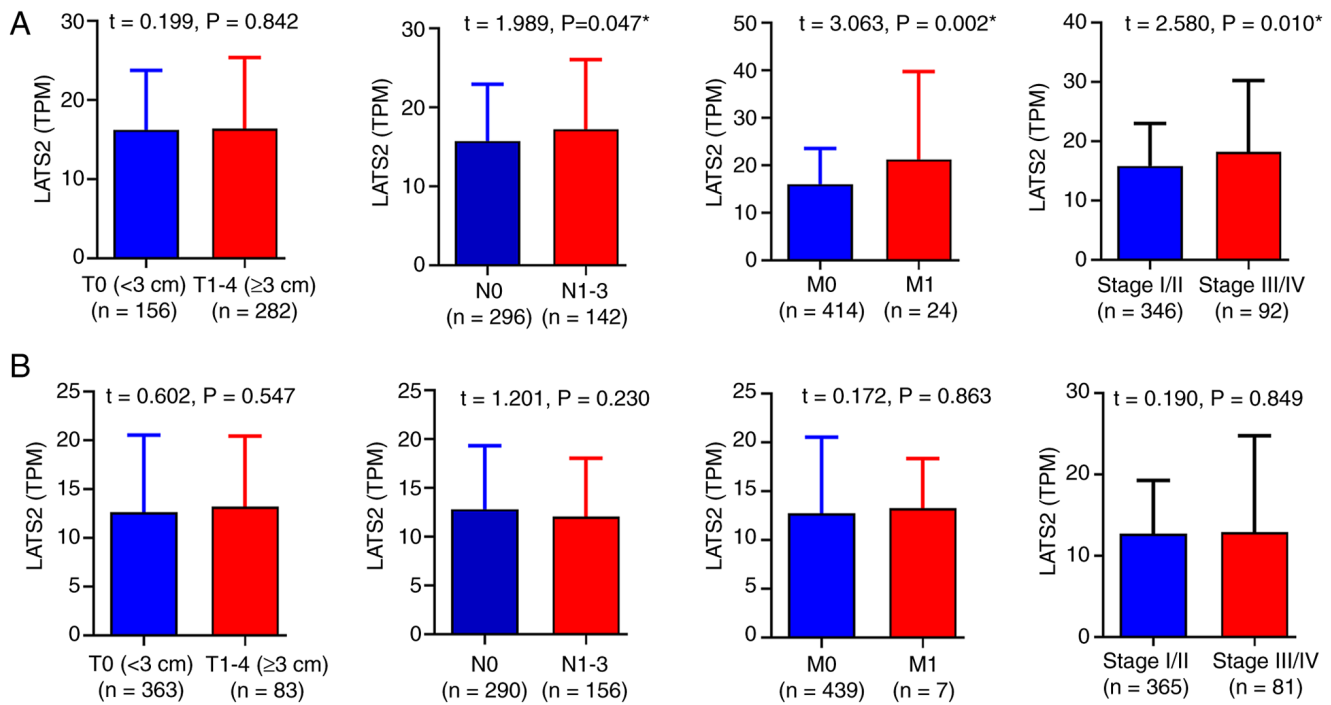


Figure 2. Correlation between LATS2 expression and clinical characteristics in LUAD and LUSC. (A) Stratified analysis of LATS2 expression across T, N and M stage and TNM stage in LUAD. (B) Stratified analysis of LATS2 expression across T stage, N stage, M stage and TNM stage in LUSC. All the data are presented as mean $\pm$ SD. * $P < 0.05$. LATS2, large tumor suppressor kinase 2; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; T, tumor size; N, lymph node metastasis; M, distant metastasis; TPM, transcripts per million.

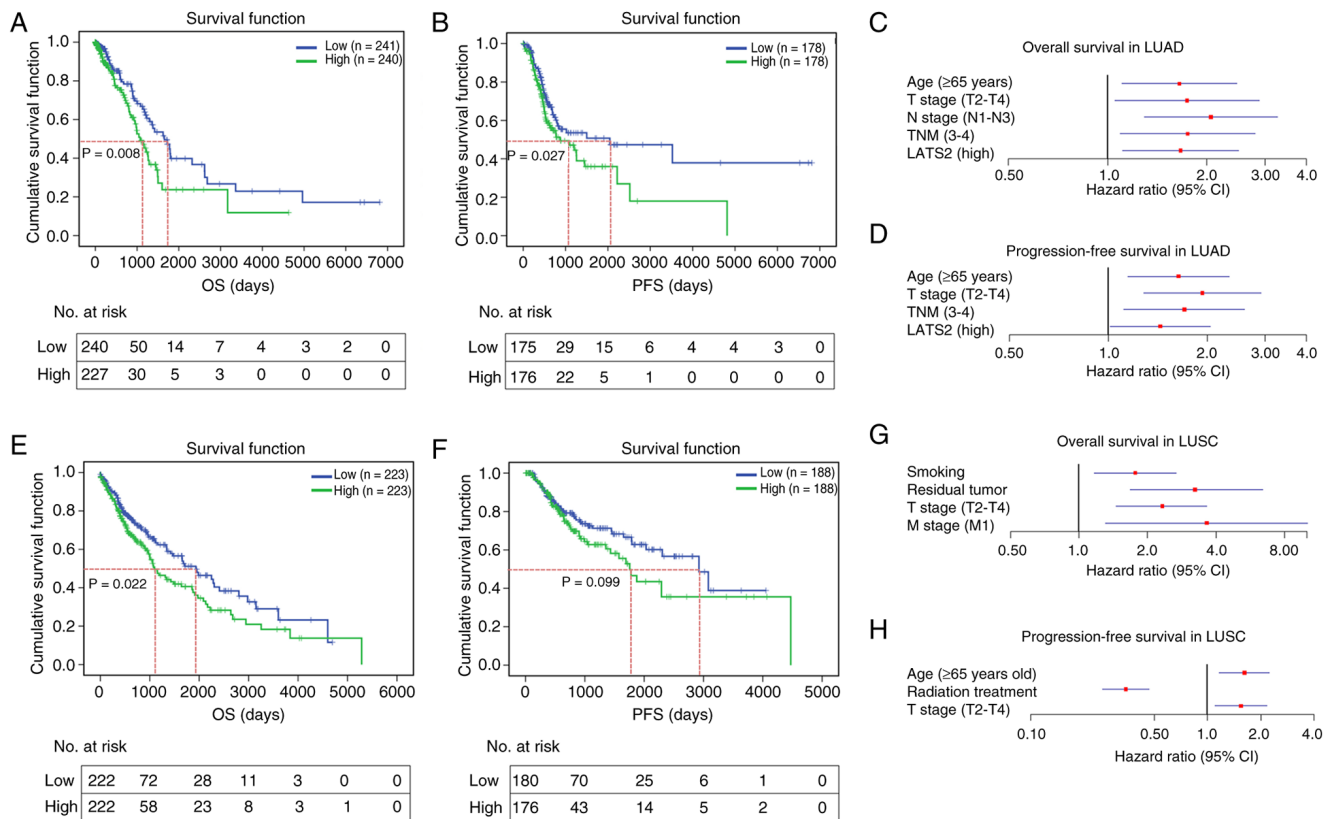


Figure 3. Prognostic significance of LATS2 expression in LUAD and LUSC survival analyses. Kaplan-Meier curves demonstrating association between LATS2 expression and (A) OS or (B) PFS in LUAD using log-rank test. Forest plots illustrating hazard ratios for (C) OS or (D) PFS in LUAD patients with LUAD. (E) Kaplan-Meier survival curves of LATS2 for OS in patients LUSC using the two-stage method. (F) Kaplan-Meier survival curves of LATS2 for OS or PFS in patients with LUSC, using the log-rank test. Forest plots illustrating hazard ratios for (G) OS or (H) PFS in patients with LUSC. OS, overall survival; PFA, progression-free survival; LATS2, large tumor suppressor kinase 2; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; T stage; tumor size; N, lymph node metastasis.

Table I. Multivariate COX regression analysis of OS and PFS in LUAD.

Survival	Variables	Univariate analyses		Multivariate analysis	
		P-value	HR (95% CI)	P-value	HR (95% CI)
OS	Age (≥ 65 years)	0.058	1.462 (0.987-2.166)	0.014	1.650 (1.106-2.461)
	Sex (male)	0.506	0.875 (0.590-1.297)	-	-
	Smoking	0.257	1.272 (0.839-1.929)	-	-
	History of malignant tumors	0.040	1.691 (1.023-2.793)	-	-
	Radiation treatment	0.015	1.851 (1.128-3.037)	-	-
	Pharmaceutical treatment	0.622	1.112 (0.730-1.694)	-	-
	Residual tumor	0.010	2.795 (1.537-5.083)	-	-
	T stage (T2-T4)	0.005	2.037 (1.247-3.329)	0.031	1.741 (1.053-2.877)
	N stage (N1-N3)	0.000	2.725 (1.843-4.028)	0.002	2.054 (1.292-3.266)
	M stage (M1)	0.100	1.733 (0.900-3.335)	-	-
	TNM stage (III/IV)	0.000	2.857 (1.911-4.272)	0.020	1.747 (1.091-2.798)
	LATS2 (high)	0.004	1.805 (1.213-2.687)	0.014	1.663 (1.109-2.496)
PFS	Age (≥ 65 years)	0.006	1.643 (1.153-2.341)	0.007	1.633 (1.144-2.330)
	Sex (male)	0.700	1.070 (0.757-1.513)	-	-
	Smoking	0.870	1.043 (0.633-1.716)	-	-
	History of malignant tumors	0.613	1.116 (0.729-1.708)	-	-
	Radiation treatment	0.596	1.097 (0.77-1.548)	-	-
	Pharmaceutical treatment	0.419	0.864 (0.60-1.232)	-	-
	Residual tumor	0.005	0.464 (0.270-0.798)	-	-
	T stage (T2-T4)	0.000	2.063 (1.384-3.074)	0.002	1.931 (1.283-2.908)
	N stage (N1-N3)	0.003	1.702 (1.199-2.415)	-	-
	M stage (M1)	0.142	1.772 (0.826-3.804)	-	-
	TNM stage (III/IV)	0.001	2.047 (1.358-3.085)	0.014	1.702 (1.115-2.597)
	LATS2 (high)	0.028	1.473 (1.043-2.080)	0.042	1.438 (1.014-2.040)

OS, overall survival; PFS, progression-free survival; LATS2, large tumor suppressor kinase 2; LUAD, lung adenocarcinoma; HR, hazard ratio.

(all $P < 0.05$; Fig. 1B and C). Furthermore, LATS2 expression in LUAD tumor tissues was higher than that in LUSC ($P < 0.05$; Fig. 1D). Immunohistochemical images obtained from the Human Protein Atlas database indicated that the proportion of LATS2-positive cells was markedly higher in normal lung tissues compared with LUAD and LUSC tumor tissues (Fig. 1E).

Association between LATS2 expression and clinical data. In patients with LUAD, the expression levels of LATS2 in the stage N1-N3, stage M1 and stage III/IV groups were significantly higher than those in the N0, M0 and stage I/II groups, respectively (all $P < 0.05$; Fig. 2A). By contrast, in patients with LUSC, there were no statistically significant differences in LATS2 expression levels between the T0 and T1-4 groups, N0 and N1-3 groups, M0 and M1 groups or stage I/II and stage III/IV groups (all $P > 0.05$; Fig. 2B).

Association between LATS2 expression and patient survival. The Kaplan-Meier curves demonstrated that both OS and PFS times were longer in the low LATS2 expression group compared with the high LATS2 expression group in LUAD (both $P < 0.05$; Fig. 3A and B). In LUSC, the OS time was longer in the low LATS2 expression group than in the high LATS2 expression group ($P < 0.05$; Fig. 3E), but there was no

significant difference in PFS between the two groups ($P > 0.05$; Fig. 3F). The results of univariate and multivariate Cox analyses demonstrated that in patients with LUAD, age (≥ 65 years), T stage (T2-T4), N stage (N1-N3), TNM stage (III/IV) and high LATS2 expression were independent risk factors for OS (Table I; Fig. 3C) and age (≥ 65 years), T stage (T2-T4), TNM stage (III/IV) and high LATS2 expression were independent risk factors for PFS (Table I; Fig. 3D). In patients with LUSC, LATS2 expression was not an independent influencing factor for OS or PFS (Table II; Fig. 3G and H).

Analysis of LATS2-related genes. The process of screening out genes related to LATS2 for GO and KEGG analysis is shown in Fig. 4A. The results of KEGG and GO enrichment analyses revealed that in LUAD, LATS2-related genes were mainly involved in biological processes such as 'cell morphogenesis', 'negative regulation of cellular component organization', 'cell-cell junction', 'histone modifying activity' and 'cadherin binding' (Fig. 4B), as well as pathways such as the 'Hippo signaling pathway (hsa04392)' and 'pathways in cancer (hsa05200)' (Fig. 4C). In LUSC, LATS2-related genes were primarily associated with functions such as 'positive regulation of cell adhesion', 'negative regulation of cell population proliferation', 'extracellular matrix', 'calcium ion binding'

Table II. Multivariate COX regression analysis of OS and PFS in lung squamous cell carcinoma.

Survival	Variables	Univariate analyses		Multivariate analysis	
		P-value	HR (95% CI)	P-value	HR (95% CI)
OS	Age (≥ 65 years)	0.049	1.381 (1.001-1.904)	0.004	1.615 (1.167-2.235)
	Sex (male)	0.500	0.891 (0.637-1.246)	-	-
	Smoking	0.146	1.272 (0.839-1.929)	-	-
	Radiation treatment	0.000	0.349 (0.259-0.470)	<0.001	0.345 (0.255-0.466)
	Pharmaceutical treatment	0.000	0.530 (0.391-0.718)	-	-
	Residual tumor	0.020	1.890 (1.105-3.235)	-	-
	T stage (T2-T4)	0.001	1.727 (1.237-2.411)	0.011	1.548 (1.104-2.170)
	N stage (N1-N3)	0.265	1.182 (0.881-1.585)	-	-
	M stage (M1)	0.115	2.227 (0.824-6.022)	-	-
	TNM stage (III/IV)	0.003	1.639 (1.180-2.276)	-	-
	LATS2 (high)	0.062	1.313 (0.986-1.748)	-	-
PFS	Age (≥ 65 years)	0.463	1.168 (0.771-1.769)	-	-
	Sex (male)	0.016	0.560 (0.350-0.897)	-	-
	Smoking	0.007	1.726 (1.163-2.561)	0.007	1.767 (1.170-2.669)
	Radiation treatment	0.002	0.462 (0.281-0.759)	-	-
	Pharmaceutical treatment	0.866	0.966 (0.645-1.446)	-	-
	Residual tumor	0.000	3.490 (1.813-6.717)	0.001	3.227 (1.676-6.407)
	T stage (T2-T4)	0.000	2.581 (1.663-4.007)	<0.001	2.320 (1.457-3.693)
	N stage (N1-N3)	0.050	1.469 (0.999-2.160)	-	-
	M stage (M1)	0.009	3.870 (1.411-10.617)	0.013	3.637 (1.309-10.103)
	TNM stage (III/IV)	0.000	2.164 (1.404-3.336)	-	-
	LATS2 (high)	0.104	1.374 (0.937-2.016)	-	-

OS, overall survival; PFS, progression-free survival; LATS2, large tumor suppressor kinase 2; LUSC; HR; hazard ratio; T, tumor; N, lymph node metastasis; M, distant metastasis.

and 'enzyme activator activity' (Fig. 4B), along with pathways such as 'ECM-receptor interaction (hsa04512)', 'MAPK signaling pathway (hsa04010)' and 'Th17 cell differentiation (hsa04659)' (Fig. 4C).

Immune infiltration analysis of LATS2. The results of CIBERSORT algorithm analysis in the R program revealed that the high LATS2 expression group exhibited significantly higher infiltration levels of immune cell subsets such as T cell CD4 memory resting, natural killer (NK) cell resting, monocytes and macrophages M2, whereas the levels of cell subsets such as plasma cells, T cells CD8, T cell regulatory, T cell follicular helper and NK cell activated were significantly lower (all $P < 0.05$; Fig. 5A).

In LUSC, the high LATS2 expression group exhibited significantly higher infiltration levels of immune cell subsets such as T cell CD4 memory resting, and macrophages M0 and M2, while the levels of cell subsets such as plasma cells, T cell CD4 memory activated and T cells follicular helper were significantly lower in the high LATS2 expression group (Fig. 5B). The results of the GSEA database analysis showed that LATS2 expression was not correlated with CD4⁺ T-cell infiltration (Fig. 5C) but was negatively correlated with CD8⁺ T-cell infiltration in LUAD (Fig. 5D). But in LUSC, LATS2 expression was positively correlated with the infiltration levels

of CD4⁺ T cells (Fig. 5E) but was not correlated with CD8⁺ T-cell infiltration (Fig. 5F).

The data from the 42 patients included in the present study revealed that the proportion of CD8⁺ cells in patients with LUAD was lower ($P = 0.041$) and the level of LATS2 was higher ($P = 0.042$) compared with patients with LUSC (Fig. 6A-C). Correlation analysis revealed that, in LUAD, LATS2 was positively correlated with the proportion of CD4⁺ TILs and the CD4⁺/CD8⁺ ratio and negatively correlated with the proportion of CD8⁺ TILs. In LUSC, LATS2 expression levels exhibited no correlation with CD4⁺ TIL proportions, CD8⁺ TILs or the CD4⁺/CD8⁺ ratio (Fig. 6D). Based on the aforementioned results, it can be concluded that LATS2 expression exhibited a negative correlation with CD8⁺ TILs in LUAD, while it exhibited a positive but weak correlation with CD4⁺ TILs in LUSC.

Effects of LATS2 overexpression on the biological behaviors of LUAD and LUSC cells. To further investigate the role of LATS2 in LUAD and LUSC, the expression levels of LATS2 in the Beas-2B lung epithelial, the A549 LUAD and the SK-MES-1 LUSC cell lines were assessed. The results demonstrated that compared with Beas-2B cells, the expression levels of LATS2 were significantly reduced in both A549 and SK-MES-1 cells ($P < 0.001$; Fig. 7A). After transfection with

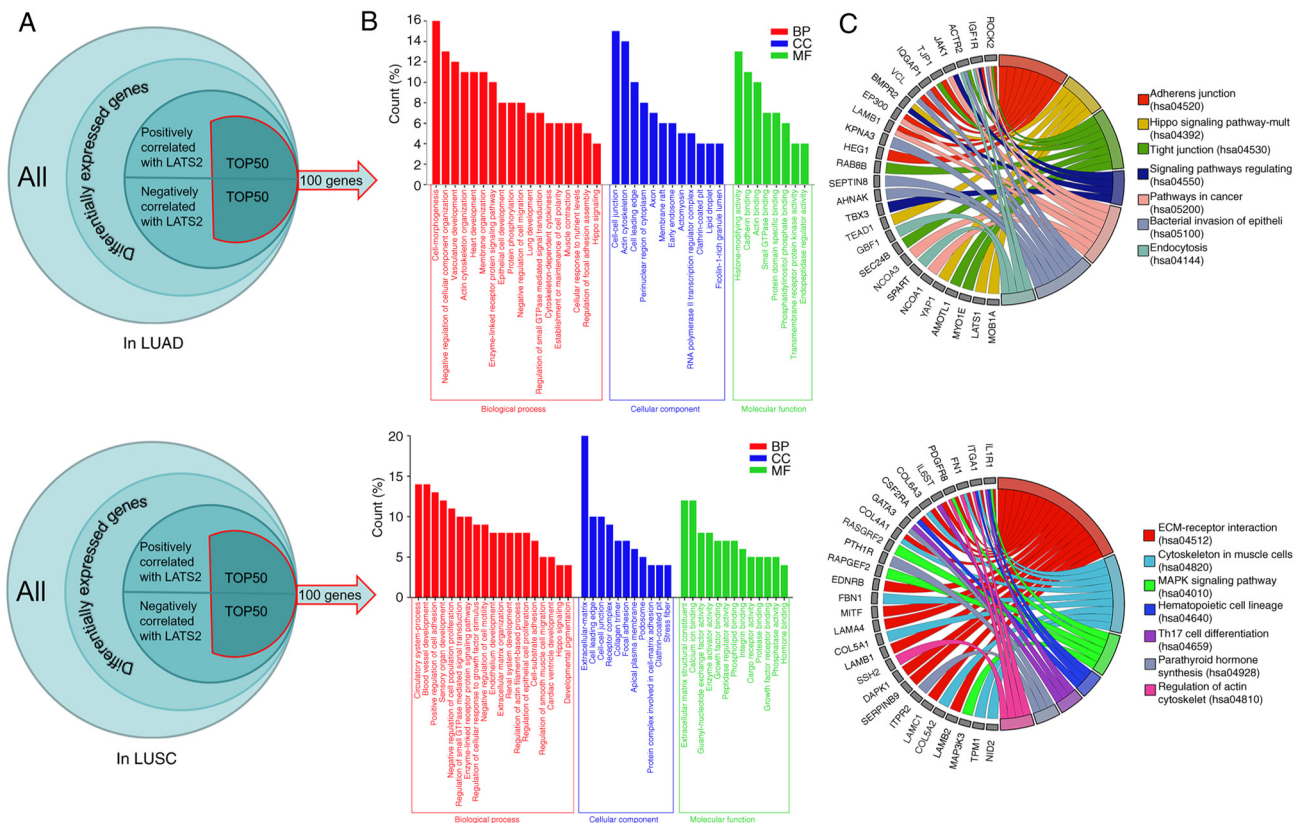


Figure 4. KEGG and GO analysis of LATS2-correlated genes. (A) Workflow for identifying LATS2-correlated genes prior to GO and KEGG enrichment analysis. (B) GO enrichment analysis revealing biological processes, cellular components and molecular functions associated with LATS2-associated genes in LUAD/LUSC. (C) KEGG pathway analysis identifying signaling pathways enriched in LATS2-correlated genes across LUAD/LUSC. LATS2, large tumor suppressor kinase 2; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function; ECM, extracellular matrix.

LATS2 OE or NC lentivirus (Fig. 7D), there was a significant increase in LATS2 expression in both A549 and SK-MES-1 cells in the OE group compared with that in the BC group (all $P < 0.05$; Fig. 7B), while no significant change was observed in the NC group ($P > 0.05$; Fig. 7B). The changes in proliferation, apoptosis, migration and invasion of A549 and SK-MES-1 cells were subsequently assessed. After overexpression of LATS2, the proliferative activity of A549 cells in the LATS2 OE group was markedly decreased ($P < 0.001$, Fig. 7C), the apoptosis rate was increased ($P < 0.001$, Fig. 7E) and both the migration rate (Fig. 8A, B and D) and the number of invading cells were significantly reduced (all $P < 0.001$; Fig. 8C and E). Similar changes were observed in SK-MES-1 cells. However, the SK-MES-1 OE group exhibited significantly higher proliferative activity, a lower apoptosis rate and a significantly higher migration rate and number of invading cells compared with those in the A549 OE group (all $P < 0.05$). The overall changes in proliferation, apoptosis, migration and invasion were more pronounced in A549 cells than in SK-MES-1 cells following LATS2 overexpression.

Discussion

The Hippo signaling pathway is a kinase cascade system that serves a core role in cell proliferation, apoptosis, organ

size and tissue regeneration (20), and has been demonstrated to be closely related to the occurrence and development of lung cancer. LATS2 is one of the core components of the Hippo signaling pathway (21,22). Studies have shown that the inactivation or functional deficiency of LATS1/2 in the Hippo signaling pathway can lead to the dephosphorylation and nuclear translocation of YAP/TAZ, which then binds to TEAD transcription factors, promoting the proliferation, invasion and metastasis of lung cancer cells (23,24). The present study aimed to systematically explore the tumor-suppressing role of LATS2 in lung cancer, its relevance for patient prognosis, and whether its expression and functional roles are the same in different pathological types of lung cancer. The results indicated that LATS2 was generally expressed at a low level in the tumors analyzed in the present study, including LUAD and LUSC, which is consistent with most previous studies (24,25). For instance, in one study, Malik *et al* (24) observed low LATS2 expression in 66.66% of NSCLC tumor tissues. Matsuda *et al* (25) confirmed that the positive expression rate of LATS2 was markedly lower in prostate cancer tissues than in non-cancerous tissues. However, Zhang *et al* (26) found that LATS2 was positively expressed in $>80\%$ of nasopharyngeal carcinomas. The authors also revealed that, among patients with LUAD, those with larger tumor sizes (≥ 3 cm), lymph node metastasis, distant metastasis and higher TNM stages

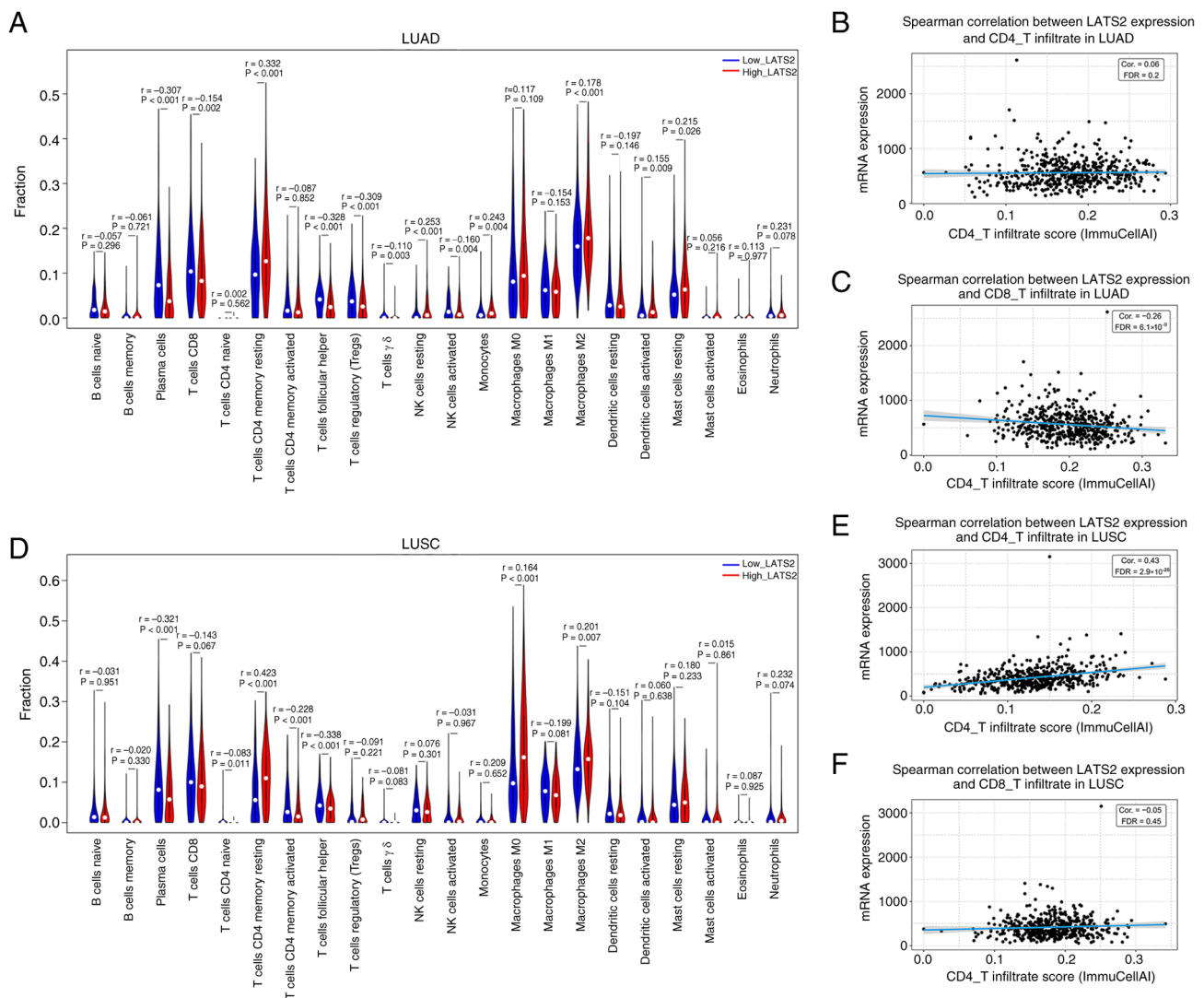


Figure 5. Immune infiltration landscape associated with LATS2 expression. (A and B) Differential immune cell infiltration profiles between LATS2 high/low expression groups using CIBERSORT algorithm in LUAD/LUSC. Correlation between LATS2 mRNA expression and (C) CD4⁺ or (D) CD8⁺ T cell infiltration in LUAD via the GSCA database. Correlation between LATS2 mRNA expression and (E) CD4⁺ T or (F) CD8⁺ T cell infiltration in LUSC via the GSCA database. NK, natural killer; LATS2, large tumor suppressor kinase 2; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; GSCA, Gene Set Cancer Analysis; FDR, false discovery rate; ImmuCellAI, immune cell abundance identifier.

had higher LATS2 expression levels. Furthermore, elevated LATS2 expression was an independent risk factor for OS and PFS in these patients. By contrast, in LUSC, LATS2 expression exhibited no significant association with clinical data; although patients in the low LATS2 expression group had a longer OS time, it was not an independent indicator. Therefore, the relationship between LATS2 and LUAD in the present study is inconsistent with that reported in existing studies. Matsuda *et al* (25) also confirmed that patients with prostate cancer with negative expression of LATS2 have a significantly higher risk of biochemical recurrence compared with those with positive expression. Sun *et al* (27) also demonstrated that LATS2 expression was negatively associated with OS in gastric cancer.

In the present study, the bioinformatics analysis based on TCGA data revealed that LATS2 was expressed at a low level in LUAD and LUSC, but was a risk factor for patient survival, raising doubts on the tumor suppressive role of LATS2. Therefore, *in vitro* basic experiments were conducted

to clarify this. The results demonstrated that LATS2 expression was notably lower in LUAD and LUSC cell lines than in normal lung epithelial cells. Overexpression of LATS2 notably inhibited the proliferation, migration and invasion of A549 and SK-MES-1 cells, and promoted apoptosis. Wu *et al* (9) found that patients with low LATS2 expression had a significantly shorter OS time than those with high LATS2 expression, making it an independent prognostic indicator for patients with NSCLC, and overexpression of LATS2 could inhibit the migration of A549 and H1299 cells. When comparing the present study with the study by Wu *et al* (9), the common conclusion is the observation that upregulation of LATS2 expression markedly inhibits malignant behaviors of tumor cells. The difference is that Wu *et al* (9) considered LATS2 to be positively associated with prognosis, while the present results demonstrated that high LATS2 expression was associated with worse survival. The main reasons for the differences in these conclusions may be sample size and histological subtypes. The study by Wu *et al* (9) had a relatively small sample size (73 cases)

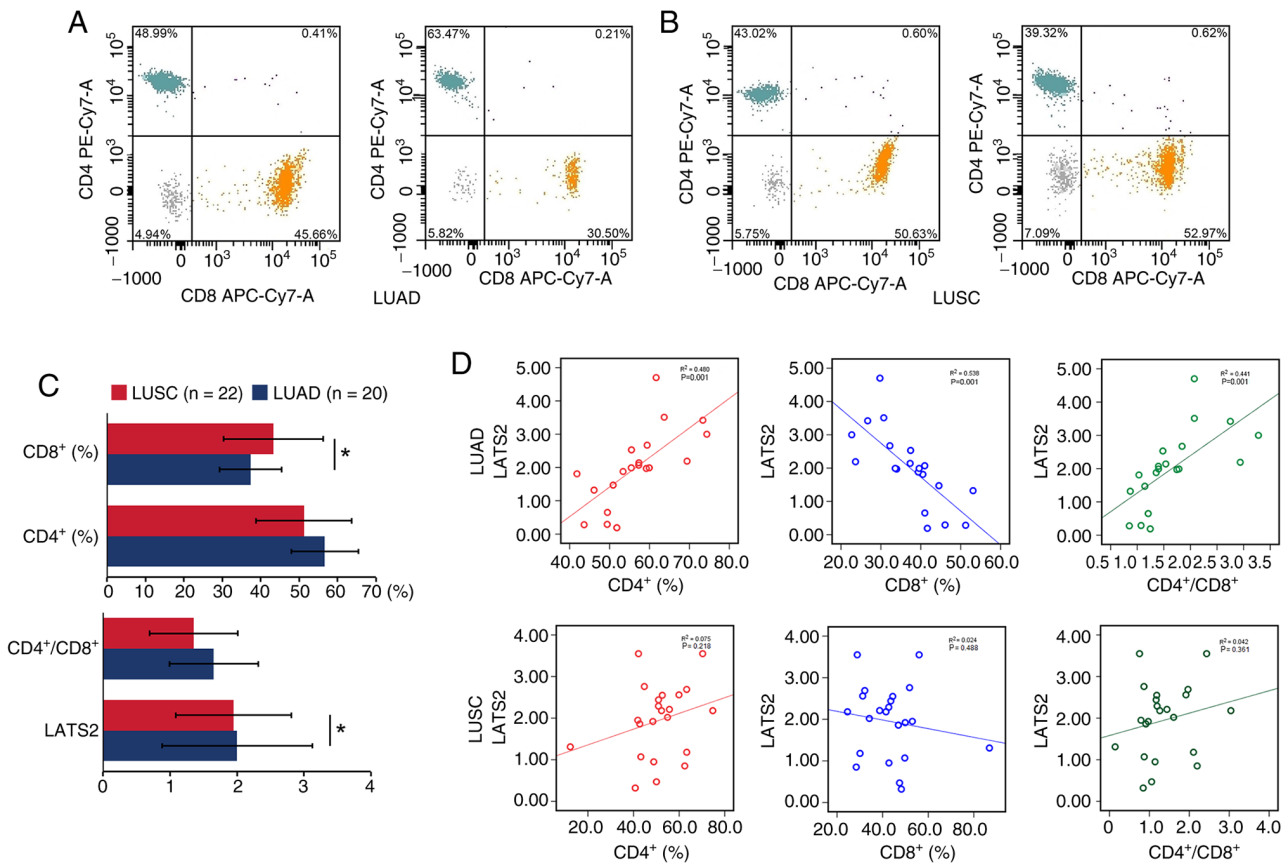


Figure 6. Correlation between the expression of LATS2 and the distribution of CD4⁺ and CD8⁺ TILs in LUAD/LUSC tissues. Distributions of CD4⁺ and CD8⁺ TILs in (A) LUAD or (B) LUSC tissues. (C) Comparative analysis of CD4⁺/CD8⁺ TIL proportion and LATS2 expression levels between LUAD and LUSC cohorts. (D) Multivariate correlation analysis of LATS2 expression with CD4⁺ TIL proportion, CD8⁺ TIL proportion and CD4⁺/CD8⁺ ratio in LUAD/LUSC. All data are presented as mean $\pm$ SD, all comparisons were conducted using independent samples t-test. *P<0.05. LATS2, large tumor suppressor kinase 2; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TILs, tumor-infiltrating lymphocytes.

across all NSCLC subtypes, while the present study used TCGA data for >500 LUAD or LUSC samples and focused exclusively on either LUAD or LUSC as distinct histological subtypes. The results of the *in vitro* experiments are generally consistent with existing cell biology research results in lung cancer cells (28,29), as well as hepatocellular carcinoma cells (30) and glioma cells (31). Therefore, the conclusion is that, although the low expression of LATS2 in lung cancer and its role as a risk factor for lung cancer appear contradictory, its inhibitory effect on the growth of lung cancer cells is clear. Thus, the mechanism by which its high expression becomes a risk factor requires further exploration.

It is worth noting that the present study also revealed that, compared with those in A549 cells, the effects of LATS2 overexpression on SK-MES-1 cells were less pronounced, suggesting that although LATS2 exerted tumor-suppressing effects in both LUAD and LUSC, there may be different molecular mechanisms or pathways involved. Other research results in the present study also supported the differences in the roles of LATS2 in LUAD and LUSC. GO analysis results showed that LATS2-related genes in LUAD were involved in the biological process of negative regulation of cell migration, consistent with the results of cell experiments showing that LATS2 overexpression could inhibit the migration of A549 cells. By contrast, in LUSC, LATS2-related genes were involved in the biological processes of positive regulation of

cell adhesion and negative regulation of cell motility, corresponding to the less pronounced inhibitory effect of LATS2 overexpression on cell migration and invasion. KEGG analysis results showed that LATS2-related genes in LUAD were involved in pathways in cancer, but no such involvement was found in LUSC.

For the analysis of immune infiltration, three methods were employed: GSEA online analysis, the CIBERSORT algorithm within the R program and actual clinical data analysis. Overall, in LUAD, LATS2 expression was negatively correlated with CD8⁺ TIL levels, whereas in LUSC, LATS2 expression was positively correlated with CD4⁺ TIL levels, however the correlation was relatively weak. CD8⁺ T cells serve important roles in the tumor immune microenvironment (32). After tumor occurrence, tumor cells, as foreign bodies, activate the immune system. Under normal circumstances, the levels of both CD4⁺ and CD8⁺ TILs increase (33); CD8⁺ T cells are the direct effector cells that mediate tumor killing (34), whereas CD4⁺ T cells generally assist in making the immune response as persistent as possible (35). However, given a certain level of T cells, an increase in CD4⁺ T cells indicates a decrease in CD8⁺ T cell levels. The association between CD4⁺ or CD8⁺ TILs and the prognosis of patients with cancer has previously been suggested. Giatromanolaki *et al* (36) reported that elevated ratios of CD4⁺ and CD8⁺ TILs in the tumor stroma predicted worse and positive prognoses, respectively, and that

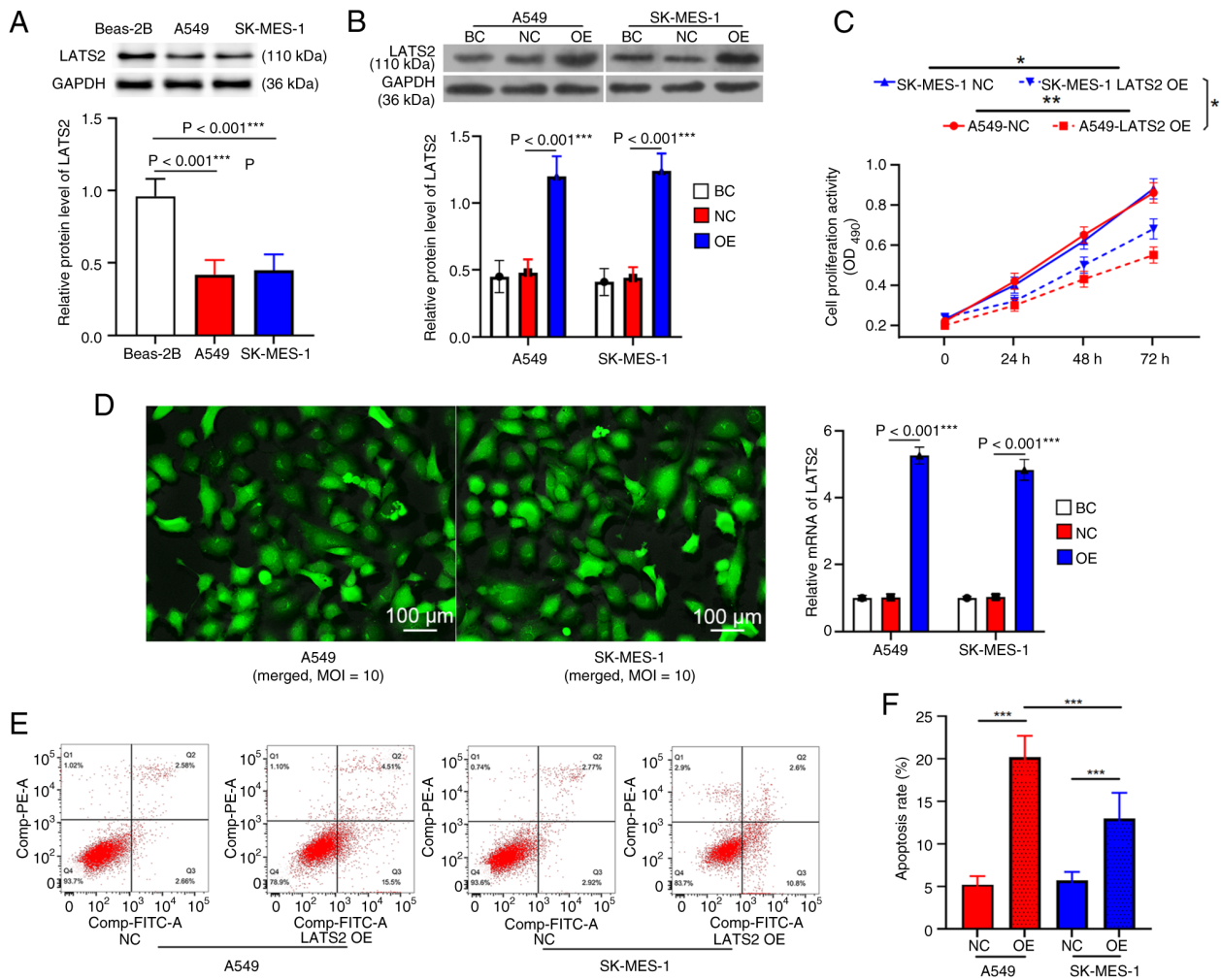


Figure 7. Functional impact of LATS2 upregulation on LUAD/LUSC cell viability and apoptosis. (A) Baseline LATS2 expression comparison in A549 (LUAD), SK-MES-1 (LUSC) and Beas-2B (normal bronchial) cell lines. (B) Western blot validation of LATS2 protein overexpression post-transfection. (C) Transfection efficiency confirmation via fluorescence labeling (MOI=10; transfection rate=90.5 and 90% in LUAD and LUSC, respectively). Scale bar, 100 μ m. (D) qPCR quantification of LATS2 mRNA induction post-transfection. (E) MTT assay evaluation of cell proliferation changes following LATS2 upregulation. (F) Flow cytometry analysis of apoptosis rate alterations after LATS2 upregulation. Data are presented as mean $\pm$ SD and all comparisons were conducted using independent samples t-test. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. LATS2, large tumor suppressor kinase 2; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; BC, blank control; NC, negative control; OE, overexpression; OD, optical density; MOI, multiplicity of infection; qPCR, quantitative PCR.

a higher CD4⁺/CD8⁺ ratio was an independent risk factor for worse survival. Tao and Xie (37) found that, compared with patients with low CD8⁺ T lymphocyte infiltration, those with high CD8⁺ T lymphocyte infiltration had longer disease-free survival and OS times. The results of the present study revealed that the correlation between LATS2 expression and the infiltration levels of CD8⁺ T cells was significantly stronger in LUAD than in LUSC. Therefore, it can be hypothesized that in LUAD, the positive association between high LATS2 expression and larger tumor volume, lymph node metastasis, distant metastasis and worse survival is due to LATS2 decreasing the levels of CD8⁺ TILs via pathways such as by activating YAP/TAZ to recruit regulatory T cells (immune-suppressive cells), thereby weakening the antitumor immune response, promoting tumor progression and metastasis, and thus, affecting the survival of patients. In LUSC, the association between LATS2 expression and immune infiltration is weaker, its inhibitory effect on cell migration and invasion is not as obvious as in LUAD and the prognostic significance is also relatively limited. To confirm

this conclusion, it is necessary to clarify the detailed underlying mechanism of the role of LATS2 in tumor immunity.

In summary, by integrating bioinformatics analysis and *in vitro* experiments, the present study demonstrated that LATS2 expression was generally low in both LUAD and LUSC. LATS2 served a tumor-suppressive role in cancer cells, and this effect was stronger in LUAD than LUSC. In LUAD, LATS2 served as an independent risk factor for both OS and PFS, likely due to its correlation with CD4⁺ and CD8⁺ TILs that modulate tumor immune processes. Conversely, in LUSC, LATS2 expression exhibited weak associations with patient survival outcomes and CD4⁺ and CD8⁺ TIL levels. These findings provide a theoretical basis for a deeper understanding of the role of LATS2 in lung cancer. The present study also has some limitations. For instance, due to budget constraints, *in vitro* LATS2 knock-down experiments were not performed, and the correlation between LATS2 expression levels and T cell exhaustion markers was not examined. In future research, these gaps

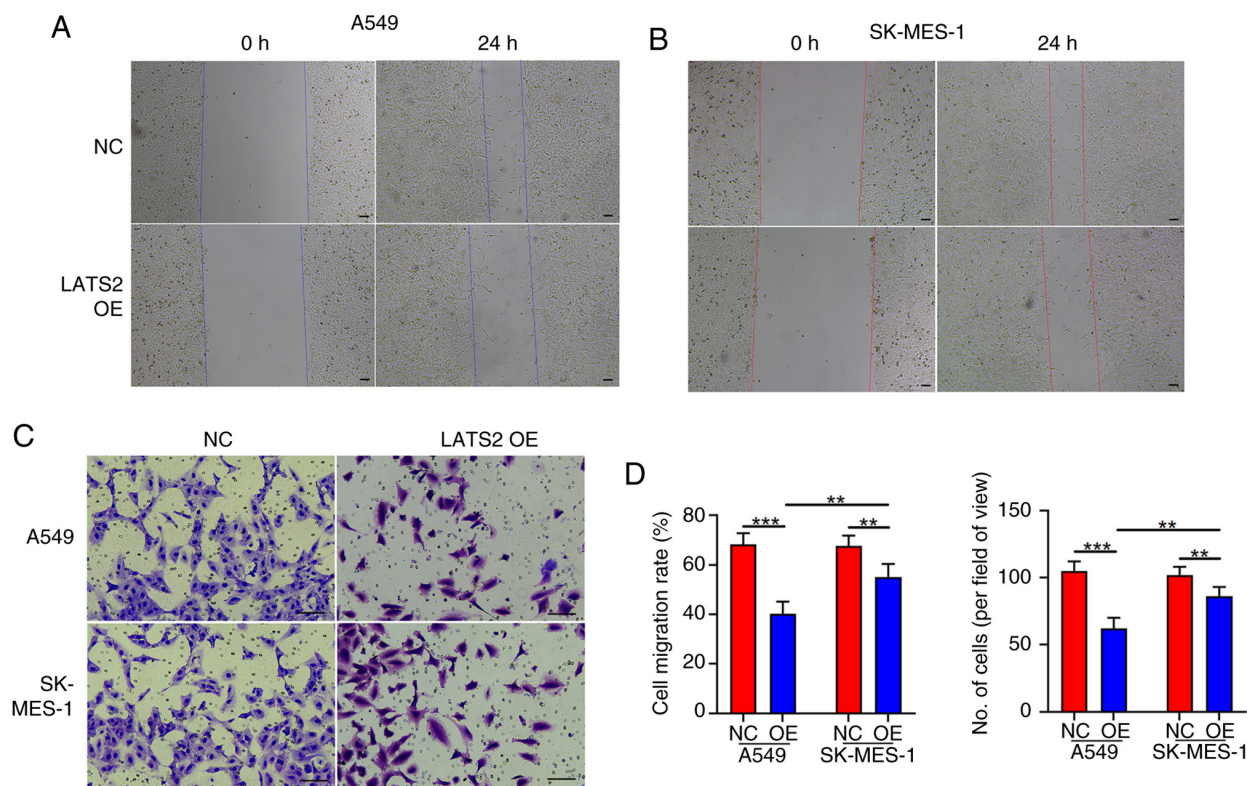


Figure 8. Influence of LATS2 overexpression on LUAD and LUSC cell migration and invasion. (A) Wound healing assay in A549 cells. (B) Wound healing assay in SK-MES-1 cell lines. (C) Transwell assay evaluating altered invasive capacity of A549 and SK-MES-1 cells. (D) Comparison of cell migration rates. (E) Comparison of number of invading cells. Scale bar, 100 μ m, ** P <0.01 and *** P <0.001. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; LATS2, large tumor suppressor kinase 2; BC, blank control; NC, negative control; OE, overexpression.

can be addressed by conducting LATS2 knockdown experiments in lung cancer cell lines with relatively high LATS2 expression, and investigating the *in vivo* correlation between LATS2 expression levels and genes such as programmed cell death protein-1 and cell immunoglobulin and mucin domain-containing protein-3 to further elucidate the mechanistic role of LATS2 in the development and progression of LUAD and LUSC. At the same time, relevant research should be performed to determine the critical points for moderate enhancement (such as gene therapy) and excessive expression (such as autonomous activation of tumor cells) of LATS2 to avoid the 'dose-effect' trap in treatment. Simultaneously, the establishment of subtype-specific immune microenvironment regulation networks should be explored for differences in the role of LATS2 in different subtypes to formulate more precise targeted treatment strategies.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XZ and ZZ designed the research approach, XZ was responsible for coordinating and supporting project resources; ZZ and YX conducted the foundational experiments, CZ oversaw clinical data acquisition; SZ performed the bioinformatics analysis and the visualization of data; CZ, ZZ and SZ performed all data analysis and result interpretation and confirm the authenticity of all the raw data. ZZ completed the drafting and language translation of the initial manuscript, and all authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Second Affiliated Hospital of Zhengzhou University (approval no. 2022243). Written informed consent for the acquisition and use of samples was obtained from patients or their legal guardians, and all experiments were carried out in accordance with The Declaration of Helsinki and guidelines and regulations of Zhengzhou University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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