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ORIGINAL RESEARCH

Decreased Expression of a Novel lncRNA FAM181A-AS1 is Associated with Poor Prognosis and Immune Infiltration in Lung Adenocarcinoma

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Background: There is no clear information regarding the role of FAM181A antisense RNA 1 (FAM181A-AS1) in lung adenocarcinoma (LUAD). We explored the relationship between FAM181A-AS1 and LUAD using bioinformatics analysis and experimental validation in this study.

Methods: Statistics and databases were used to evaluate the relationship between clinical features in LUAD patients and FAM181A-AS1 expression, prognostic factors, regulation network, and immune infiltration of FAM181A-AS1 in function. LUAD cell lines were tested for FAM181A-AS1 expression using qRT-PCR.

Results: FAM181A-AS1 showed significantly low expression in LUAD patients. Low FAM181A-AS1 expression predicted a poorer overall survival (OS) (HR: 0.66; 95% CI: 0.49–0.88; P=0.005) and disease specific survival (DSS) (HR: 0.64; 95% CI: 0.44–0.92; P=0.017) of LUAD patients. There was also an independent correlation between low FAM181A-AS1 expression (HR: 0.547; 95% CI: 0.350–0.857; P=0.008) and OS in LUAD patients. The FAM181A-AS1 high-expression phenotype was differentially enriched for M phase, cellular senescence, cell cycle checkpoints, chromatin modifying enzymes, ESR-mediated signaling, DNA repair, G2/M checkpoints, HCMV infection, and DNA double-strand break repair. A correlation was found between the expression of FAM181A-AS1 and immune infiltrating cells. A significant decrease in FAM181A-AS1 expression was observed in LUAD cell lines compared to Beas-2B.

Conclusion: There was a significant association between low FAM181A-AS1 expression in LUAD patients and poor survival and immune infiltration. The FAM181A-AS1 gene may provide a useful biomarker for LUAD prognosis and immunotherapy response.

Keywords: lung adenocarcinoma, FAM181A-AS1, prognosis, immune infiltration, biomarker

Introduction

Lung cancer is the leading cause of death worldwide, with approximately 2.1 million new cases and 1.8 million deaths in 2018.[1] These cases are mainly non-small cell lung cancer (NSCLC), divided into different histological categories, with lung adenocarcinoma (LUAD) being the most common subtype.[2] Despite significant advances in multimodal treatment strategies including immunotherapy, radiation therapy, and noninvasive surgical resection in recent decades, curative lung cancer outcomes remain suboptimal, with a five-year relative overall survival rate of approximately 18%.[3] There is a need to find more effective biomarkers for the diagnosis and prognosis prediction of LUAD patients.

Non-coding RNA (ncRNA) is an RNA molecule that is transcribed from the genome and does not encode a protein.[4] Long non-coding RNA (lncRNA) consists of RNAs over 200 nucleotides in length that do not contain open reading frames for translation into proteins.[5,6] LncRNAs account for more than 80% of ncRNAs and have a greater conservation

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rate than microRNAs.[7] A number of functions are regulated by lncRNAs, including cell growth, survival and differentiation, genomic imprinting, epigenetic and post-transcriptional regulation of gene expression, alternative splicing, chromatin modification, subcellular transport, and inflammatory mechanisms.[8,9] LUAD's growth and metastasis have been associated with dysregulated lncRNAs.[10–12] As a result, screening for lncRNAs clinically relevant to LUAD plays an important role in prognosis and treatment.

Some lncRNAs associated with LUAD prognosis were obtained by bioinformatics analysis and experimental validation.[13,14] The family with sequence similarity 181 (FAM181) is a gene family with two paralogous homologs (FAM181A and FAM181B) found in vertebrates.[15] LncRNA FAM181A antisense RNA 1 (FAM181A-AS1) is a potential prognostic biomarker and therapeutic target for patients with glioma.[16] FAM181A-AS1 spongy miR-129-5p enhances ZRANB2 expression to promote glioma development.[16] There has been no study to correlate FAM181A-AS1 with clinical characteristics of LUAD patients or immune infiltration.

In this study, the relationship between clinical features in LUAD patients and FAM181A-AS1 expression, prognostic factors, and regulation network, as well as immune infiltration, was assessed using statistics and databases. qRT-PCR was used to test the expression of FAM181A-AS1 in LUAD cell lines. The findings of this study may provide new directions for the development of prognosis and therapeutic strategies for LUAD patients.

Materials and Methods

Differential Expression of FAM181A-AS1

Baseline information. Software was R (version 3.6.3) (statistical analysis and visualization).[17,18] R package was Basic R package.[17] Molecule was FAM181A-AS1 [ENSG00000258584]. Subgroups were median.[17] Data was RNAseq data in level 3 HTSeq-FPKM format from TCGA (https://portal.gdc.cancer.gov/) LUAD project. Patient and clinical information in this study was obtained from the TCGA-LUAD database. The specific clinical information is shown in Table 1. RNAseq data in FPKM (Fragments Per Kilobase per Million) format were converted to TPM (transcripts per million reads) format and log2 transformed.

Table 1 Correlation Between FAM181A-AS1 Expression and Clinical Characteristics in LUAD

Characteristic	Overall	Low Expression of FAM181A-AS1	High Expression of FAM181A-AS1	p
n	535	267	268	
T stage, n (%)				0.616
T1	175 (32.9%)	83 (15.6%)	92 (17.3%)	
T2	289 (54.3%)	145 (27.3%)	144 (27.1%)	
T3	49 (9.2%)	25 (4.7%)	24 (4.5%)	
T4	19 (3.6%)	12 (2.3%)	7 (1.3%)	
N stage, n (%)				0.252
N0	348 (67.1%)	177 (34.1%)	171 (32.9%)	
N1	95 (18.3%)	42 (8.1%)	53 (10.2%)	
N2	74 (14.3%)	41 (7.9%)	33 (6.4%)	
N3	2 (0.4%)	2 (0.4%)	0 (0%)	
M stage, n (%)				0.979
M0	361 (93.5%)	182 (47.2%)	179 (46.4%)	
M1	25 (6.5%)	12 (3.1%)	13 (3.4%)	
Pathologic stage, n (%)				0.547
Stage I	294 (55.8%)	152 (28.8%)	142 (26.9%)	
Stage II	123 (23.3%)	55 (10.4%)	68 (12.9%)	
Stage III	84 (15.9%)	45 (8.5%)	39 (7.4%)	
Stage IV	26 (4.9%)	13 (2.5%)	13 (2.5%)	
Primary therapy outcome, n (%)				0.277
PD	71 (15.9%)	39 (8.7%)	32 (7.2%)	

(*Continued*)

Table 1 (Continued).

Characteristic	Overall	Low Expression of FAM181A-AS1	High Expression of FAM181A-AS1	p
SD	37 (8.3%)	16 (3.6%)	21 (4.7%)	
PR	6 (1.3%)	1 (0.2%)	5 (1.1%)	
CR	332 (74.4%)	164 (36.8%)	168 (37.7%)	
Race, n (%)				0.170
Asian	7 (1.5%)	4 (0.9%)	3 (0.6%)	
Black or African American	55 (11.8%)	21 (4.5%)	34 (7.3%)	
White	406 (86.8%)	209 (44.7%)	197 (42.1%)	
Gender, n (%)				0.894
Female	286 (53.5%)	144 (26.9%)	142 (26.5%)	
Male	249 (46.5%)	123 (23%)	126 (23.6%)	
Age, n (%)				0.427
<=65	255 (49.4%)	133 (25.8%)	122 (23.6%)	
>65	261 (50.6%)	126 (24.4%)	135 (26.2%)	
Residual tumor, n (%)				0.073
R0	355 (95.4%)	184 (49.5%)	171 (46%)	
R1	13 (3.5%)	5 (1.3%)	8 (2.2%)	
R2	4 (1.1%)	0 (0%)	4 (1.1%)	
Anatomic neoplasm subdivision, n (%)				1.000
Left	205 (39.4%)	102 (19.6%)	103 (19.8%)	
Right	315 (60.6%)	157 (30.2%)	158 (30.4%)	
Anatomic neoplasm subdivision2, n (%)				0.472
Central Lung	62 (32.8%)	29 (15.3%)	33 (17.5%)	
Peripheral Lung	127 (67.2%)	68 (36%)	59 (31.2%)	
Number_pack_years_smoked, n (%)				0.322
<40	188 (50.9%)	89 (24.1%)	99 (26.8%)	
≥40	181 (49.1%)	96 (26%)	85 (23%)	
Smoker, n (%)				0.510
No	75 (14.4%)	34 (6.5%)	41 (7.9%)	
Yes	446 (85.6%)	224 (43%)	222 (42.6%)	
Age, median (IQR)	66 (59, 72)	65 (59, 72)	66 (59, 73)	0.550

Unpaired Samples

The software used was R (version 3.6.3). It used ggplot2 [version 3.3.3] as its main package (for visualization).[17] Molecule was FAM181A-AS1. We used RNAseq data in level 3 HTSeq-FPKM format from the TCGA LUAD project. FPKM data was converted to TPM format and log2 transformed. Data was not filtered.

Paired Samples

The software used was R (version 3.6.3). It used ggplot2 [version 3.3.3] as its main package. Molecule was FAM181A-AS1. We used RNAseq data in level 3 HTSeq-FPKM format from the TCGA LUAD project. FPKM data was converted to TPM format and log2 transformed. Pairs of samples were retained in the data filtering condition.

ROC Analysis

The software used was R (version 3.6.3). It used pROC package [version 1.17.0.1] (for analysis) and ggplot2 package [version 3.3.3].[17] Molecule was FAM181A-AS1. Clinical variables were normal and tumor.[17] We used RNAseq data in level 3 HTSeq-FPKM format from the TCGA LUAD project. FPKM data was converted to TPM format and log2 transformed. Data was not filtered.

The Relationship Between FAM181A-AS1 and Clinical Characteristics

Clinical Relevance

The software used was R (version 3.6.3). It used ggplot2 [version 3.3.3] as its main package (for visualization). Molecule was FAM181A-AS1. Clinical variables were clinical features.[19] TNM staging classification for lung cancer (IASLC, 8th edition) was used.[20] We used RNAseq data in level 3 HTSeq-FPKM format from the TCGA LUAD project. FPKM data was converted to TPM format and log2 transformed. Clinical information was retained and controls/normal were removed (not all items have controls/normal).

Logistics Analysis

The software used was R (version 3.6.3). R package was mainly the basic package. Statistical method was a dichotomous logistic model.[19] Dependent variable was FAM181A-AS1. Dependent variable types were low and high dichotomous.[19] We used RNAseq data in level 3 HTSeq-FPKM format from the TCGA LUAD project. FPKM data was converted to TPM format and log2 transformed. Clinical information was retained and controls/normal were removed (not all items have controls/normal).

The Relationship Between FAM181A-AS1 and Prognosis

Kaplan–Meier Method

The software used was R (version 3.6.3). For visualization, we used the survminer package [version 0.4.9] and for statistical analysis, we used the survival package [version 3.2–10].[21] Molecule was FAM181A-AS1. Subgroups were 0–50 and 50–100.[21] Prognosis types were overall survival (OS), progression free survival (PFS), and disease specific survival (DSS).[21] DFS was defined as the time from surgical resection to local recurrence. We used RNAseq data in level 3 HTSeq-FPKM format from the TCGA LUAD project. FPKM data was converted to TPM format and log2 transformed. Table S1 was prognostic data from the reference.[22] Clinical information was retained and controls/normal were removed (not all items have controls/normal).

COX Regression

The software used was R (version 3.6.3). The survivor package was version 3.2–10. Statistical method was Cox regression module.[21] Prognosis type was OS. Included variables were clinical features and FAM181A-AS1. We used RNAseq data in level 3 HTSeq-FPKM format from the TCGA LUAD project. FPKM data was converted to TPM format and log2 transformed. Table S1 was prognostic data from the reference.[22] Clinical information was retained and controls/normal were removed (not all items have controls/normal).

Forest Plot

Software was R (version 3.6.3) and R package was ggplot2 package [version 3.3.3].[21]

Nomogram Plot

Software was R (version 3.6.3). R packages were rms package [version 6.2–0] and survival package [version 3.2–10].[21] Prognosis type was OS. Included variables were clinical features and FAM181A-AS1. We used RNAseq data in level 3 HTSeq-FPKM format from the TCGA LUAD project. Table S1 was prognostic data from the reference.[22] Clinical information was retained and controls/normal were removed (not all items have controls/normal).

Enrichment of FAM181A-AS1 Related Pathways

Single Gene Differential Analysis

The software used was R (version 3.6.3). R package was DESeq2 [version 1.26.0].[23,24] Molecule was FAM181A-AS1. Low-expression group was 0–50%.[25] High-expression group was 50–100%.[24] In this analysis, we used RNAseq data from TCGA LUAD project in level 3 HTSeq-counts format. We removed control and normal values (not all items have control and normal values).

GSEA Analysis

The software used was R (version 3.6.3). ClusterProfiler [version 3.14.3] was the main R package used for GSEA analysis.[24] A reference gene set for the Homo sapiens species was c2.cp.v7.2.symbols.gmt. The gene set database was MSigDB collections (database hyperlink) (with detailed descriptions for each gene set). Generally, a significant enrichment was considered when the False discovery rate (FDR) < 0.25 and p.adjust < 0.05.

Relationship Between FAM181A-AS1 Expression and Immune Infiltrating Cells

The software used was R (version 3.6.3). R package was GSVA package [version 1.34.0].[26] SsGSEA was used as the immune-infiltration algorithm (built-in to the GSVA package).[24] Molecule was FAM181A-AS1. Immune cells were aDC [activated DC], B cells, CD8 T cells, cytotoxic cells, DC, eosinophils, iDC [immature DC], macrophages, mast cells, neutrophils, NK CD56 bright cells, NK CD56 dim cells, NK cells, pDC [Plasmacytoid DC], T cells, T helper cells, Tcm [T central memory], Tem [T effector memory], Tfh [T follicular helper], Tgd [T gamma delta], Th1 cells; Th17 cells, Th2 cells, and Treg.[24] We used RNAseq data in level 3 HTSeq-FPKM format from the TCGA LUAD project. FPKM data was converted to TPM format and log2 transformed. Clinical information was retained and controls/normal were removed (not all items have controls/normal). Markers for 24 immune cells were obtained from the reference.[25]

QRT-PCR

Cells including BEAS-2B, A549, and PC9, were purchased from the Cell Bank of Chinese Academy of Sciences. DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin was used in the culture of BEAS-2B, A549, and PC9 cells at 37°C in 5% CO_2. The levels of FAM181A-AS1 in BEAS-2B, A549, and PC9 cell lines were assessed using QRT-PCR. Primers[27] used in this study were as follows: GAPDH forward: 5'-GAAGGTGAAGGTCGGAGTC−3', GAPDH reverse: 5'-GAAGATGGTGATGGGATTTC-3'; HMGA2-AS1 forward: 5'-GCAGCTTGTTTTCTGGGTGG-3', HMGA2-AS1 reverse: 5'-ACTTTGGGGGCAAAGTGTCA-3'.

Statistical Analysis

R (v.3.6.3) was used for all statistical analyses. Using Wilcoxon rank-sum, chi-square, and Fisher exact tests, we examined the association between clinical features and FAM181A-AS1. We considered statistically significant values below 0.05.

Results

Clinical Characteristics

The clinical information of 535 LUAD patients are collected in Table 1. There is a median age of 66 years, ranging from 59 to 72. The T stage included 175 patients (32.9%) in T1 stage, 289 patients (54.3%) in T2 stage, 49 patients (9.2%) in T3 stage, and 19 patients (3.6%) in T4 stage. The N stage included 348 patients (67.1%) in N0 stage, 95 patients (18.3%) in N1 stage, 74 patients (14.3%) in N2 stage, and 2 patients (0.4%) in N3 stage. The M stage included 361 patients (93.5%) in M0 stage and 25 patients (6.5%) in the M1 stage. The pathologic stage included 294 patients (55.8%) in Stage I, 123 patients (23.3%) in Stage II, 84 patients (15.9%) in Stage III, and 26 patients (4.9%) in Stage IV. There were 71 PD cases (15.9%), 37 SD cases (8.3%), PR cases (1.3%), and 332 CR cases (74.4%) among the results of the primary therapy. The Race included 7 Asian patients (1.5%), 55 Black or African American patients (11.8%), and 406 White patients (86.8%). There were 286 female patients (53.5%) and 249 male patients (46.5%). The age included 255 patients (<=65, 49.4%) and 261 patients (>65, 50.6%). Among the residual tumors, 355 R0 accounted for 95.4%, 13 R1 for 3.5%, and four R2 for 1.1%. The anatomic neoplasm subdivision included 205 left (39.4%) and 315 right (60.6%). The anatomic neoplasm subdivision2 included 62 central lung (32.8%) and 127 peripheral lung (60.6%). The number_pack_-years_smoked included 188 patients (<40, 50.9%) and 181 patients (≥40, 49.1%). The smokers included 75 No (14.4%) and 446 Yes (85.6%).

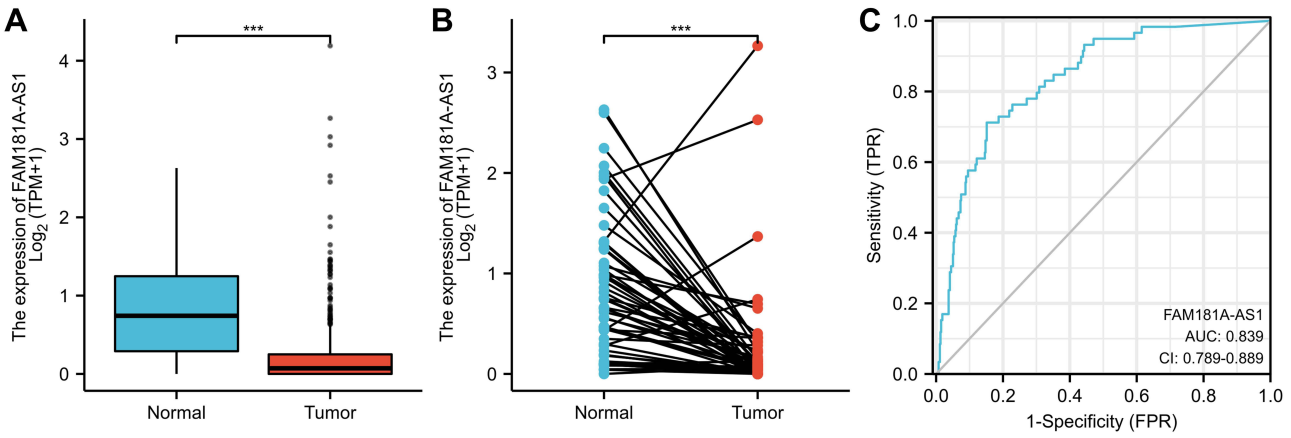

Figure 1 FAM181A-AS1 expression in LUAD is significantly lower than in normal or adjacent normal lung tissues. (**A**) Differential expression of FAM181A-AS1 in LUAD tissues and normal lung tissues. (**B**) Differential expression of FAM181A-AS1 in LUAD tissues and matched normal lung tissues. (**C**) Effectiveness of FAM181A-AS1 expression in distinguishing LUAD tissues from nontumor tissues (ROC curve). Significance markers: ***P<0.001.

Low Expression of FAM181A-AS1 in LUAD Patients

FAM181A-AS1 was expressed lower in LUAD tissues than in normal lung tissues (0.232±0.442 vs 0.853±0.679, P<0.001) (Figure 1A). FAM181A-AS1 was expressed lower in LUAD tissues than in matched normal lung tissues (0.255±0.566 vs 0.877±0.678, P<0.001) (Figure 1B). FAM181A-AS1 has an AUC of 0.839, suggesting that it could serve as a biomarker for distinguishing LUAD from nontumor lung tissue (Figure 1C). As shown in Tables 1 and 2, low expression of FAM181A-AS1 in LUAD patients did not correlate significantly with clinical features.

Role of FAM181A-AS1 in LUAD Patient Survival

A significant correlation was found between low FAM181A-AS1 expression and poor OS (HR: 0.66; 95% CI: 0.49–0.88; P=0.005) and DSS (HR: 0.64; 95% CI: 0.44–0.92; P=0.017) among LUAD patients (Figure 2). As shown in Table 3, univariate analysis of prognostic factors for OS with the Cox regression model indicated low expression levels of FAM181A-AS1 (HR: 0.658; 95% CI: 0.492–0.880; P=0.005) were associated with T stage (HR: 2.317; 95% CI: 1.591–3.375; P<0.011), N stage (HR: 2.601; 95% CI: 1.944–3.480; P<0.001), M stage (HR: 2.136; 95% CI: 1.248–3.653; P=0.006), pathologic stage (HR: 2.664; 95% CI: 1.960–3.621; P<0.001), primary therapy outcome (HR: 0.372; 95% CI: 0.265–0.521; P<0.001), and residual tumor (HR: 3.879; 95% CI: 2.169–6.936; P<0.001). A multivariate analysis showed that low expression of FAM181A-AS1 (HR: 0.547; 95% CI: 0.350–0.857; P=0.008), N stage (HR: 1.736; 95% CI: 1.056–2.852; P=0.03), primary therapy outcome (HR: 0.299; 95% CI:

Table 2 Correlation of FAM181A-AS1 Expression with Clinical Features of LUAD (Logistic Regression)

Characteristics	Total (N)	Odds Ratio (OR)	P value
T stage (T3&T4 vs T1&T2)	532	0.809 (0.483–1.347)	0.417
N stage (N1&N2&N3 vs N0)	519	1.047 (0.726–1.511)	0.805
M stage (M1 vs M0)	386	1.101 (0.487–2.512)	0.815
Pathologic stage (Stage III&Stage IV vs Stage I&Stage II)	527	0.884 (0.579–1.346)	0.565
Primary therapy outcome (CR vs PD&SD&PR)	446	0.989 (0.646–1.514)	0.960
Gender (Male vs Female)	535	1.039 (0.739–1.460)	0.826
Race (White vs Asian&Black or African American)	468	0.637 (0.366–1.091)	0.104
Age (>65 vs <=65)	516	1.168 (0.827–1.651)	0.378
Residual tumor (R1&R2 vs R0)	372	2.582 (0.937–8.256)	0.080
Anatomic neoplasm subdivision (Right vs Left)	520	0.997 (0.701–1.417)	0.985
Anatomic neoplasm subdivision2 (Peripheral Lung vs Central Lung)	189	0.762 (0.413–1.400)	0.383
Number_pack_years_smoked (≥40 vs <40)	369	0.796 (0.528–1.198)	0.274
Smoker (Yes vs No)	521	0.822 (0.501–1.341)	0.434

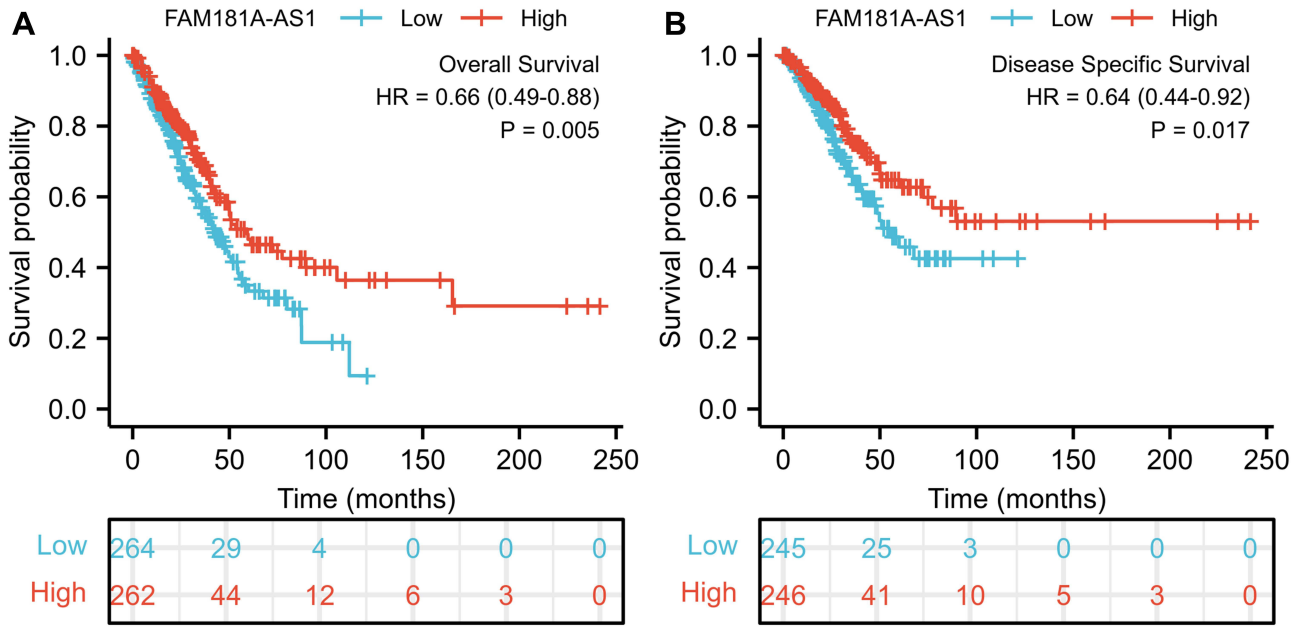

Figure 2 Low expression of FAM181A-AS1 is associated with poor OS and DSS in LUAD patients. (**A**) Kaplan–Meier curves and number at risk of OS in LUAD patients. (**B**) Kaplan–Meier curves and number at risk of DSS in LUAD patients.
Abbreviations: OS, overall survival; DSS, disease-specific survival.

0.182–0.491; P<0.001), and residual tumor (HR: 3.627; 95% CI: 1.404–9.368; P=0.008) were independently correlated with OS (Table 3 and Figure 3).

There is an association between decreased levels of FAM181A-AS1 and poor overall survival in the above analysis. The expression level of FAM181A-AS1 was combined with clinical variables to construct a nomogram for predicting survival probability for LUAD patients at 1-, 3-, and 5-years (Figure 4).

Table 3 Association Analysis of OS and Clinical Characteristics of LUAD Patients (Cox Regression)

Characteristics	Total (N)	Univariate Analysis		Multivariate Analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
T stage (T1&T2 vs T3&T4)	523	2.317 (1.591–3.375)	<0.001	1.967 (0.961–4.023)	0.064
N stage (N0 vs N1&N2&N3)	510	2.601 (1.944–3.480)	<0.001	1.736 (1.056–2.852)	0.03
M stage (M0 vs M1)	377	2.136 (1.248–3.653)	0.006	1.541 (0.576–4.126)	0.389
Pathologic stage (Stage I&Stage II vs Stage III&Stage IV)	518	2.664 (1.960–3.621)	<0.001	1.442 (0.730–2.849)	0.292
Primary therapy outcome (PD&SD&PR vs CR)	439	0.372 (0.265–0.521)	<0.001	0.299 (0.182–0.491)	<0.001
Gender (Female vs Male)	526	1.070 (0.803–1.426)	0.642		
Race (Asian&Black or African American vs White)	468	1.475 (0.902–2.411)	0.121		
Age (<=65 vs >65)	516	1.223 (0.916–1.635)	0.172		
Residual tumor (R0 vs R1&R2)	363	3.879 (2.169–6.936)	<0.001	3.627 (1.404–9.368)	0.008
Anatomic neoplasm subdivision (Left vs Right)	512	1.037 (0.770–1.397)	0.81		
Anatomic neoplasm subdivision2 (Central Lung vs Peripheral Lung)	182	0.913 (0.570–1.463)	0.706		
Number_pack_years_smoked (<40 vs ≥40)	363	1.073 (0.753–1.528)	0.697		
Smoker (No vs Yes)	512	0.894 (0.592–1.348)	0.591		
FAM181A-AS1 (Low vs High)	526	0.658 (0.492–0.880)	0.005	0.547 (0.350–0.857)	0.008

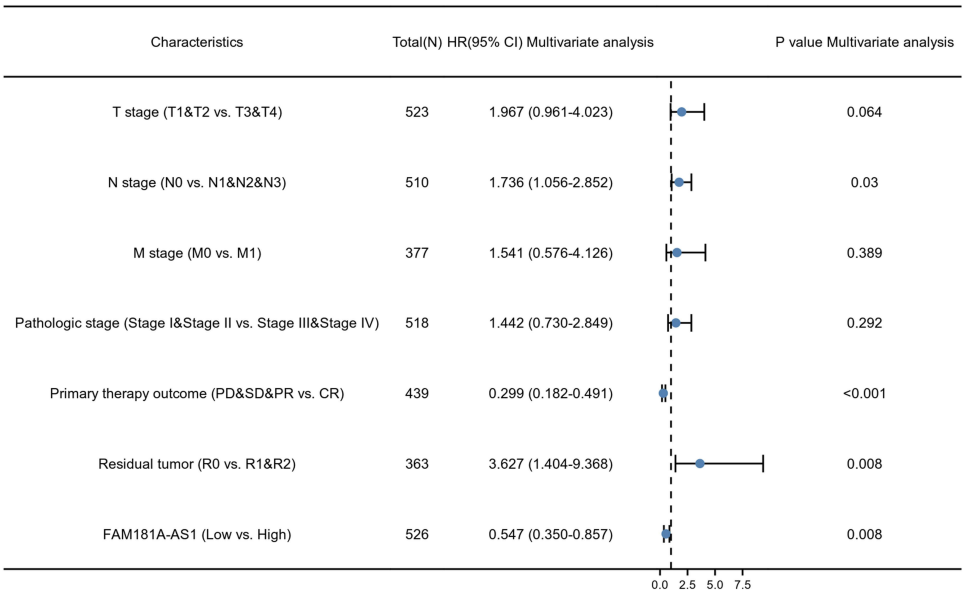

Figure 3 Forest plot of the multivariate Cox regression analysis for LUAD.

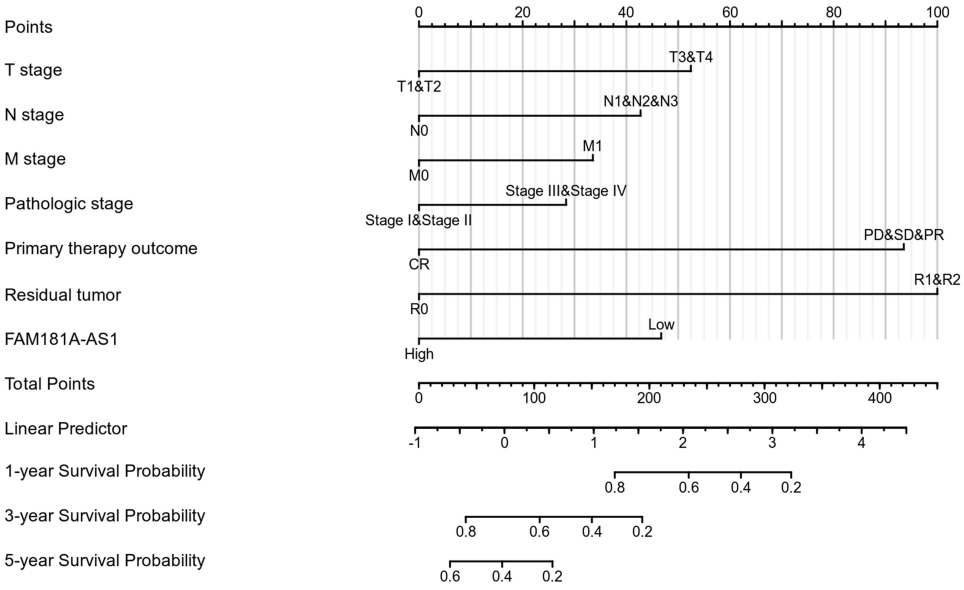

Figure 4 Nomogram for predicting the probability of patients with 1-, 3- and 5-year overall survival.

FAM181A-AS1-Related Pathways Based on GSEA

As shown in Table 4 and Figure 5, an analysis of GSEA identified 5 datasets with significant differential enrichment for FAM181A-AS1, including the M phase, cellular senescence, cell cycle checkpoints, chromatin modifying enzymes, ESR-mediated signaling, DNA repair, G2/M checkpoints, HCMV infection, and DNA double-strand break repair.

The Correlation Between FAM181A-AS1 Expression and Immune Infiltration

As shown in Figures 6 and 7, and Table 5, there was a negative correlation between FAM181A-AS1 expression and infiltration levels of Th2 cells (P=0.039) and a positive correlation between FAM181A-AS1 and aDC (P=0.004), B cells (P<0.001), CD8 T cells (P=0.018), cytotoxic cells (P<0.001), eosinophils (P<0.001), iDC (P<0.001), mast cells

Table 4 Gene Enrichment of FAM181A-AS1 High and Low Expression Groups (GSEA)

Description	NES	p.Adjust	q values
REACTOME_M_PHASE	−1.674	0.006	0.005
REACTOME_DNA_REPAIR	−1.617	0.006	0.005
REACTOME_CELL_CYCLE_CHECKPOINTS	−1.807	0.006	0.005
REACTOME_CHROMATIN_MODIFYING_ENZYMES	−1.699	0.006	0.005
REACTOME_ESR_MEDIATED_SIGNALING	−1.621	0.006	0.005
REACTOME_CELLULAR_SENESCENCE	−1.874	0.006	0.005
REACTOME_G2_M_CHECKPOINTS	−1.829	0.006	0.005
REACTOME_DNA_DOUBLE_STRAND_BREAK_REPAIR	−1.751	0.006	0.005
REACTOME_HCMV_INFECTION	−1.855	0.006	0.005

(P<0.001), neutrophils (P<0.001), NK CD56bright cells (P<0.001), NK cells (P<0.001), pDC (P<0.001), T cells (P=0.001), and TFH (P<0.001).

Cell Line Validation of FAM181A-AS1 Expression

There was a significant difference between A549 and Beas-2B (0.254±0.196 vs 0.893±0.174, P<0.05) when it came to FAM181A-AS1 expression (Figure 8). There was a significant difference between PC9 and Beas-2B (0.215±0.205 vs 0.893±0.174, P<0.05) when it came to FAM181A-AS1 expression (Figure 8). Based on these findings, FAM181A-AS1 expression was significantly higher in LUAD cells than in Beas-2B cells. The results showed that FAM181A-AS1 was significantly down-regulated in LUAD cell lines.

Discussion

LncRNAs could be used as new prognostic biomarkers for lung cancer. Expression of LncRNA AC079630.4 correlates with the progression and prognosis of lung cancer.[28] LncRNA CCDC144NL-AS1 can be used as a prognostic biomarker for NSCLC.[29] Overexpression of LncRNA-UCA1 is associated with progression and poor prognosis of lung adenocarcinoma.[30] Future research should investigate lncRNAs as biomarkers and therapeutic targets of LUAD.

According to the relevant literature we reviewed, there are fewer articles on the relationship between FAM181A-AS1 and clinical features of tumors, so the clinical significance and specific regulatory mechanisms of FAM181A-AS1 in tumors are unclear. FAM181A-AS1 enhances ZRANB2 expression by sponging miR-129-5p to promote gliomagenesis.[16] CNV of FAM181A-AS1 is associated with OS of thyroid cancer and is involved in cancer-related pathways.[31] FAM83A-AS1 promoted LUAD proliferation and stemness through the HIF-1α/glycolytic axis.[32] In this study, low FAM181A-AS1 expression predicted a poorer OS (HR: 0.66; 95% CI: 0.49–0.88; P=0.005) and DSS (HR: 0.64; 95% CI: 0.44–0.92; P=0.017) of LUAD patients. A significant correlation was found between low FAM181A-AS1 expression (HR: 0.547; 95% CI: 0.350–0.857; P=0.008) and OS in LUAD patients. FAM181A-AS1 was related to pathways including M Phase, cellular senescence, cell cycle checkpoints, chromatin modifying enzymes, ESR-mediated signaling, DNA repair, G2/M checkpoints, HCMV infection, and DNA double-strand break repair based on GESA.

According to the relevant literature we reviewed, there are fewer articles on FAM181A-AS1 and immunity in cancer, so the mechanism of FAM181A-AS1 and immune infiltration is unclear. New and emerging immunotherapies can be developed with a better understanding of immune infiltrating cells in LUAD. FAM181A-AS1 expression in LUAD was correlated with multiple immune infiltrating cells, which was another innovative aspect of this study. According to our findings, the expression of FAM181A-AS1 and the level of aDC, B cells, CD8 T cells, Cytotoxic cells, eosinophils, iDC, mast cells, neutrophils, NK CD56bright cells, NK cells, pDC, T cells, TFH, and Th2 cells were moderately correlated. These correlations may suggest potential mechanisms by which FAM181A-AS1 inhibits the function of Th2 cells, and promotes the function of aDC, B cells, CD8 T cells, cytotoxic cells, eosinophils, iDC, mast cells, neutrophils, NK CD56bright cells, NK cells, pDC, T cells, and TFH. FAM181A-AS1 may be a biomarker of immunotherapy response in LUAD.

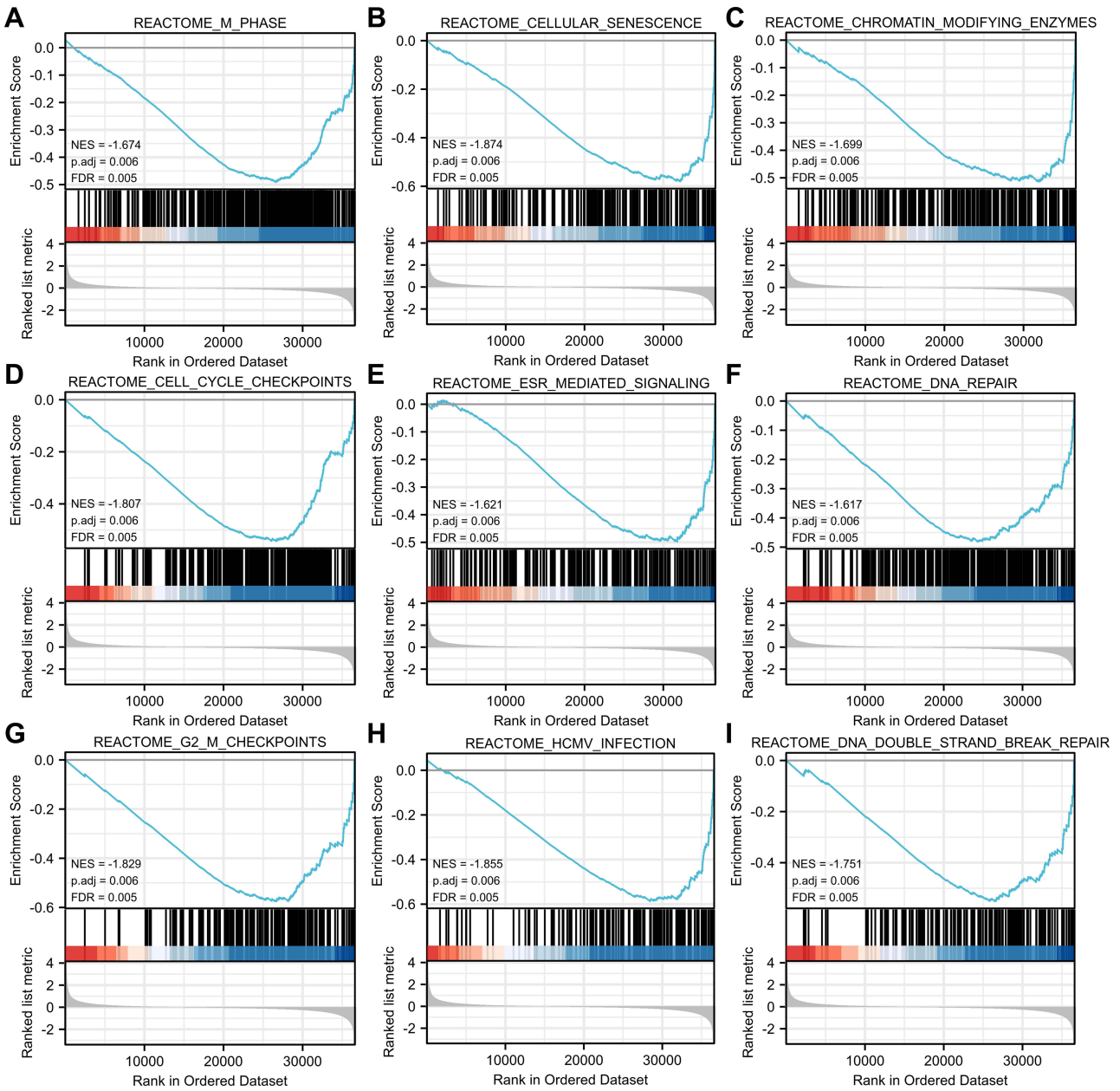

Figure 5 Enrichment plots from GSEA. (**A**) M Phase, (**B**) Cellular Senescence, (**C**) Chromatin modifying enzymes, (**D**) Cell Cycle Checkpoints, (**E**) ESR-mediated signaling, (**F**) DNA Repair, (**G**) G2/M Checkpoints, (**H**) HCMV Infection, (**I**) DNA Double-Strand Break Repair.
Abbreviations: NES, normalized es; FDR, false discovery rate.

LncRNAs are epigenetic regulators of gene expression that can influence gene regulation and are closely associated with the developmental process of many diseases. There is still a lack of efficient and universal prognostic markers for LUAD. In this study, we found that FAM181A-AS1 had clinical implications for prognosis and immune response in LUAD patients.

FAM181A-AS1 and LUAD are systematically investigated in this study despite some limitations. An analysis of TCGA RNA sequencing was performed for this study. It was not possible to demonstrate that FAM181A-AS1 is expressed at a protein level or to evaluate the direct mechanism through which FAM181A-AS1 is involved in LUAD. Several additional experiments need to be conducted in order to confirm the bioinformatics analysis. There is a need for further research regarding FAM181A-AS1-mediated LUAD's direct mechanism.

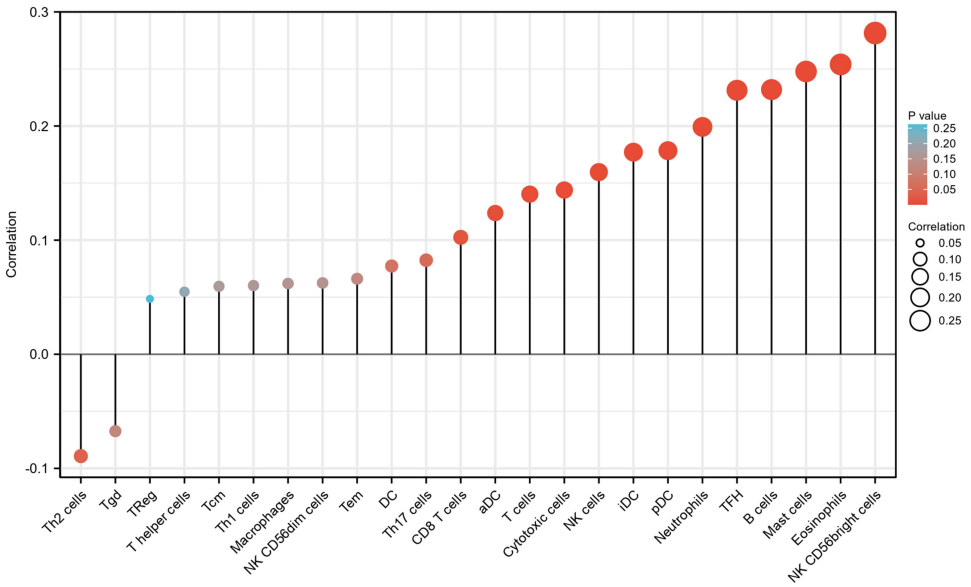

Figure 6 FAM181A-AS1 expression correlated with 24 immune cells in LUAD patients (lollipop chart).

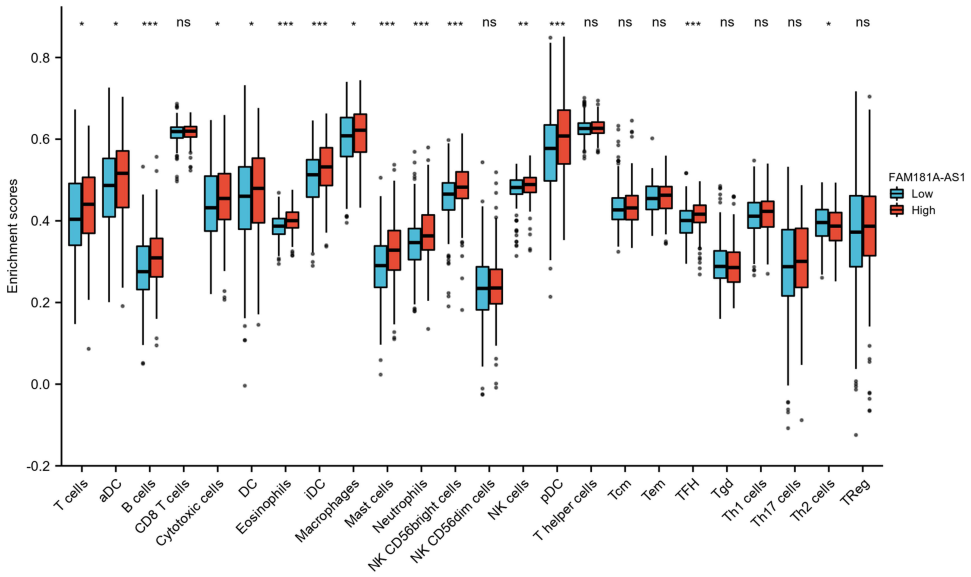

Figure 7 Expression of FAM181A-AS1 correlated with immune cells in LUAD patients (grouped comparison chart). ns, p≥0.05; *P<0.05; **P<0.01; ***P<0.001.

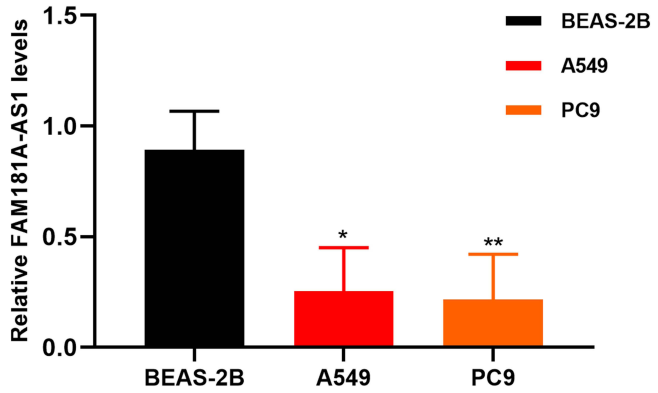

Figure 8 Expression of FAM181A-AS1 in A549, PC9, and Beas-2B cell lines. *P<0.05; **P<0.01.

Table 5 The Correlation Between FAM181A-AS1 Expression and Immune Cells Detected by Spearman Correlation Method

Gene Name	Cell Type	Correlation Coefficient (Spearman)	P value (Spearman)
FAM181A-AS1	aDC	0.124	0.004
FAM181A-AS1	B cells	0.232	<0.001
FAM181A-AS1	CD8 T cells	0.102	0.018
FAM181A-AS1	Cytotoxic cells	0.144	<0.001
FAM181A-AS1	DC	0.077	0.074
FAM181A-AS1	Eosinophils	0.254	<0.001
FAM181A-AS1	iDC	0.177	<0.001
FAM181A-AS1	Macrophages	0.062	0.152
FAM181A-AS1	Mast cells	0.248	<0.001
FAM181A-AS1	Neutrophils	0.199	<0.001
FAM181A-AS1	NK CD56bright cells	0.281	<0.001
FAM181A-AS1	NK CD56dim cells	0.062	0.149
FAM181A-AS1	NK cells	0.160	<0.001
FAM181A-AS1	pDC	0.178	<0.001
FAM181A-AS1	T cells	0.140	0.001
FAM181A-AS1	T helper cells	0.055	0.206
FAM181A-AS1	Tcm	0.060	0.169
FAM181A-AS1	Tem	0.066	0.126
FAM181A-AS1	TFH	0.231	<0.001
FAM181A-AS1	Tgd	−0.067	0.120
FAM181A-AS1	Th1 cells	0.060	0.164
FAM181A-AS1	Th17 cells	0.082	0.057
FAM181A-AS1	Th2 cells	−0.089	0.039
FAM181A-AS1	TReg	0.049	0.263

Conclusion

Low expression of FAM181A-AS1 was associated with poor OS and DSS in LUAD. FAM181A-AS1 might participate in the development of LUAD by pathways including M phase, cellular senescence, cell cycle checkpoints, chromatin modifying enzymes, ESR-mediated signaling, DNA repair, G2/M checkpoints, HCMV infection, and DNA double-strand break repair. There was a correlation between the expression of FAM181A-AS1 and immune infiltrating cells. A study conducted in the present study identified FAM181A-AS1 as a possible biomarker of prognosis and response to immunotherapy in LUAD.

Data Sharing Statement

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics Approval

This study was submitted to Medical Ethics Committee of the Second People's Hospital of Foshan for review and the ethics requirement was waived (Approval NO: [2022]01).

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Disclosure

The authors declare that they have no competing interests in this work.

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