

An Autophagy-Related Long Non-Coding RNA Prognostic Signature for Patients with Lung Squamous Carcinoma Based on Bioinformatics Analysis

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Purpose: Lung cancer is the most common and deadly cancer type affecting humans. Although huge progress has been made on early diagnosis and precision treatment, the overall 5 year survival rate remains low. In this study, we constructed an autophagy-related long non-coding RNA (lncRNA) prognostic signature for guiding clinical practice.

Methods: From The Cancer Genome Atlas, we retrieved mRNA and lncRNA expression matrices of patients with lung squamous carcinoma. We then established a prognostic risk model using Lasso regression and multivariate Cox regression. The model generated a risk score to differentiate high- and low-risk groups. An ROC curve and nomogram were used to visualize the predictive ability of the current signatures. Finally, we used Gene Set Enrichment Analysis to determine gene ontology and pathway enrichment.

Results: After screening 1248 autophagy-related lncRNAs, we selected seven lncRNAs (LUCAT1, AC022150.2, AL035425.3, AC138976.2, AC106786.1, GPRC5D-AS1 and AP006545.2) for our signature. Univariate (hazard ratio [HR] = 2.147, 95% confidence interval [CI]: 1.681–2.743, $P < 0.001$) and multivariate (HR = 2.096, 95% CI: 1.652–2.658, $P < 0.001$) Cox regression analyses revealed that the risk score is an independent predictive factor for LUSC patients. Further, areas under the receiver operating characteristic curve were 0.622, 0.699, and 0.721, respectively, for the 1 year, 3 year, and 5 year risk scores—indicating a reliable model. Selected lncRNAs were primarily enriched in autophagy, metabolism, MAPK pathway, and JAK/STAT pathway. Further drug sensitivity analysis revealed that low-risk patients were more sensitive to Cisplatin, Docetaxel, Vinblastine, and Vinorelbine. Finally, a multi-omics analysis found that lncRNA-linked proteins IKBKB and SQSTM1 were expressed at low levels and significantly correlated in tumor samples, compared with normal tissue.

Conclusion: Our prognostic model successfully predicted patient prognosis in lung cancer.

Keywords: non-small cell lung cancer, cancer therapy, non-coding RNA, cancer prognosis, The Cancer Genome Atlas

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Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. In the United States alone, newly diagnosed patients exceeded 2 million a year. Non-small cell lung cancer (NSCLC), including lung adenocarcinoma and lung squamous carcinoma (LUSC), accounts for nearly 80% of all new cases.¹

Novel treatment options such as targeted therapy and immune therapy have dramatically improved prognosis of lung adenocarcinoma. However, little progress has been made with respect to LUSC treatment, with most patients still subjected to traditional platinum-based chemotherapy (eg, gemcitabine and pemetrexed).² One way to address this issue is the development of a new prognostic model that can help physicians formulate patient-specific treatment.

Autophagy is a protein degradation pathway that is vital to maintaining whole-body homeostasis in eukaryotes. This pathway plays a critical part in various disorders, including heart disease and neurological malfunction.^{3,4} Recent studies indicate the involvement of autophagy in tumor occurrence, maintenance, and progression; indeed, autophagy is a tumor suppressor mechanism that enhances the chemotherapy response.^{5,6} Several cancer-related pathways, including PI3K/AKT/mTOR, p53/DRAM, JAK-STAT, and AMPK/CaMKK exhibit a crosstalk with autophagy. Thus, several researchers have suggested that key molecules in autophagy may serve as therapeutic targets or biomarkers for lung cancer.⁷ For example, Xie et al found that the increased frequency of three single nucleotide polymorphisms in the autophagy gene *ATG10* was significantly correlated with overall survival, while the elevated expression of *ATG10* enhanced tumor proliferation and migration.⁸

In addition to autophagy-related molecules or pathways, long non-coding RNAs (lncRNAs) represent another promising biomarker candidate for cancer. Most of the human genome is transcribed into ncRNA, with lncRNAs (>200 nucleotide-long) being the most extensively studied due to their involvement in tumorigenesis, cell cycle, apoptosis, and chemo-resistance.^{9–12} The expression of certain lncRNAs has been linked to cancers. Moreover, a five-lncRNA signature was demonstrated to act as an independent survival predictor in NSCLC. Promising findings are available for LUSC as well, with lncRNAs SFTA1P and LINC00519 being associated with the occurrence of LUSC, suggesting that they have the potential to be new diagnostic/prognostic biomarkers.¹³ Nevertheless, none of the studies have investigated whether autophagy-related lncRNAs (ARlncRNAs) can be used to establish a risk model that predicts LUSC prognosis. Therefore, in this study, we employed mRNA and lncRNA expression profiles derived from The Cancer

Genome Atlas (TCGA) data to generate a prognostic model for LUSC patients.

Materials and Methods

Raw Data Acquisition and Initial Analysis

Clinical data of patients with LUSC, along with their mRNA and lncRNA expression profiles, were downloaded from TCGA (<https://portal.gdc.cancer.gov/>). A Perl script was then used to extract survival time, age, sex, tumor stage, and TNM stage; these data were merged into a single file. After initial screening, patients with a short follow-up time (<30 days) were excluded, yielding a final patient count of 502.

Screening ARlncRNAs

Autophagy-related genes (File S1) were obtained from the Human Autophagy Database (HADb, <http://autophagy.lu/clustering/index.html>), filtered using expression matrices, and log2-transformed. Pearson's correlations were run to remove lncRNAs with correlation coefficients $|R2| > 0.3$ and $P < 0.001$.

Identification of ARlncRNA Signature for Lung Adenocarcinoma

After initial screening, Cox regression was used to determine if the remaining ARlncRNAs were significantly correlated with prognosis. A prognostic risk score was then constructed using a least absolute shrinkage and selection operator (Lasso) regression, as follows:

$$\begin{aligned} \text{risk score} = & \text{coef}(\text{lncRNA1}) \times \text{expr}(\text{lncRNA1}) \\ & + \text{coef}(\text{lncRNA2}) \times \text{expr}(\text{lncRNA2}) \\ & + \dots + \text{coef}(\text{lncRNA}_n) \times \text{expr}(\text{lncRNA}_n), \end{aligned}$$

with $\text{coef}(\text{lncRNA}_n)$ being lncRNA coefficients and $\text{expr}(\text{lncRNA}_n)$ being lncRNA expression.

All included samples were then allocated into a high-risk group and a low-risk group based on the median risk score.

Independent Survival Analysis of the Signature for LUSC Patients

To test the prognostic model, the relationship between patient survival with clinicopathological factors and risk score was assessed using univariate and multivariate Cox regression analyses. Receiver operating characteristic (ROC) curves were generated using the

“survivalROC” package in R. A nomogram was generated to evaluate the efficacy of our prognostic signature to predict survival. The index of concordance (C-index) was also calculated to determine the accuracy with which our signature could predict the survival.

Drug Sensitivity Analysis

The gene expression profile of included patients and drug sensitivity data from various cancer cell lines were incorporated in a statistical model generated under “pRRophetic” package from R software.⁴³

Functional Analysis

Gene Set Enrichment Analysis (GSEA, <http://software.broadinstitute.org/gsea/index.jsp>) calculates whether a set of genes differs significantly between two groups. The analysis was conducted with risk score as the phenotype to identify differentially expressed genes, along with their enriched Gene Ontology (GO) terms and KEGG pathways.

Multi-Omics Analysis of Associated Genes

Gene Expression Profiling Interactive Analysis (GEPIA, <http://gepia.cancer-pku.cn/>) was used to investigate the differences in mRNA expression between cancer tissues and normal tissues.¹⁴ Additionally, differential protein expression between normal tissue and tumors was investigated using the Human Protein Atlas (HPA), an open source database of immunohistochemistry results.

Statistical Analysis

All analyses were performed in R Studio (version 1.1.453). The “limma” package in R was used to differentiate survival-related lncRNAs. The lncRNA-mRNA co-expression network was established and visualized using Cytoscape and the Sankey diagram, respectively. The “survival” package in R was used to perform the Kaplan–Meier analysis; P-values were derived using Log rank tests. Univariate and multivariate Cox regression analyses were employed to assess the relationship between risk score and clinicopathological parameters. The “rms” package in R was used to generate the nomograms. Model fit was determined using area

under the ROC curve (AUC); AUC > 0.60 was deemed a good fit (reliable predictive ability). Significance was set at $P < 0.05$ (two-tailed).

Results

Establishment of a Co-Expression Network

We obtained 14,142 lncRNAs from patient expression profiles. The HADb yielded 223 autophagy-related genes (File S1), and our TCGA-LUSC expression matrix extracted 210 genes. Using a cut-off of $|R^2| > 0.3$ and $P < 0.001$, we constructed a co-expression network between autophagy-related genes and correlated lncRNAs. We then filtered out 1248 ARlncRNAs for further analysis.

Development of a Prognostic Risk Model Using ARlncRNAs

Using univariate Cox regression analyses, we identified 27 ARlncRNAs that were significantly correlated with patient survival in LUSC (Supplementary Table 1). Of these, 21 were favorable factors (hazard ratio, HR < 1) and 6 were harmful. Lasso regression analysis identified 18 lncRNAs that were the most highly correlated with patient survival in LUSC (Figures 1A and B).

Multivariate Cox regression analysis further revealed that seven lncRNAs, ie, LUCAT1, AC022150.2, AL035425.3, AC138976.2, AC106786.1, GPRC5D-AS1, and AP006545.2, can constitute a prognostic signature for patient survival in LUSC (Figure 1C, Table 1). Kaplan–Meier analyses revealed that LUCAT1 and AC022150.2 were negatively correlated with the survival of LUSC patients while the remainder were positively correlated (Figure 2). We then established a co-expression network between these ARlncRNAs and autophagy-related genes (Figures 3A and B, Table 2). Multivariate Cox regression analyses allowed us to generate risk scores and allocate patients into high- and low-risk groups based on the following formula: Risk Score = $(0.1182 \times \text{LUCAT1 expression}) + (0.2566 \times \text{AC022150.2 expression}) + (-0.03645 \times \text{AL035425.3 expression}) + (-0.1442 \times \text{AL138976.2 expression}) \times \text{AC106786.1 expression} + (-0.1133 \times \text{GPRC5D-AS1 expression}) + (-0.5401 \times \text{AP006545.2 expression})$.

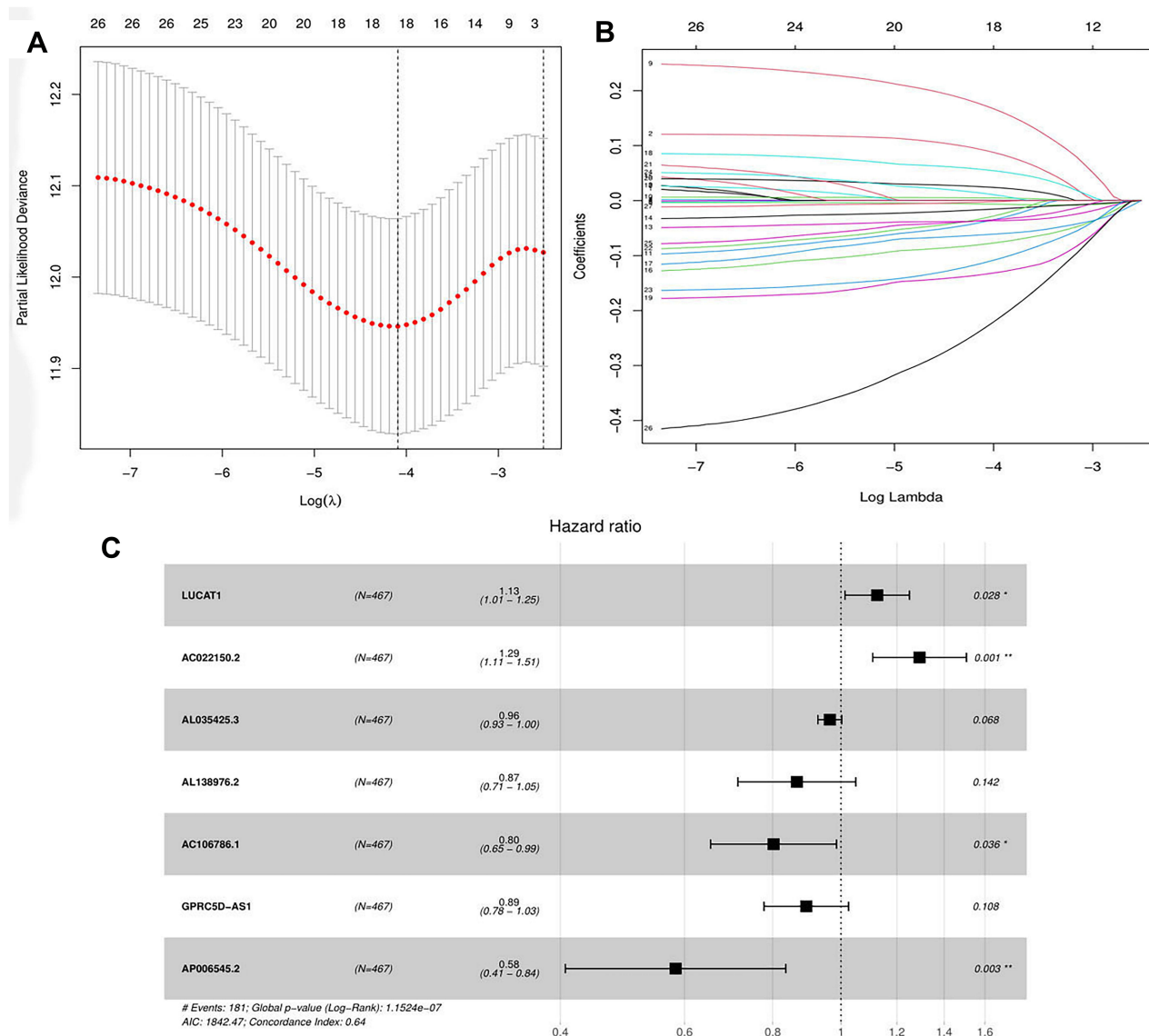


Figure 1 Selection of lncRNA using Lasso regression. **(A)** Lasso coefficient of the five included lncRNAs. **(B)** Profiles of Lasso coefficients. **(C)** Univariate analysis of included lncRNAs from the samples. Abbreviations: * $P < 0.05$; ** $P < 0.01$.

Prognostic Value of the Established Signature

The risk score cut-off was 1.138; this resulted in 234 low-risk patients and 233 high-risk patients. The high-

risk group had poorer survival outcomes than the low-risk group ($P < 0.001$, Kaplan-Meier; **Figure 4E**). For the time-dependent ROC curve, the 1-year, 3-year,

Table 1 Survival Analysis of the Seven lncRNAs Obtained Using TCGA-LUSC Data

lncRNA	Coefficient	HR	HR.95L	HR.95H
LUCAT1	0.11817468	1.125440686	1.013060473	1.250287393
AC022150.2	0.256563385	1.292480687	1.109025419	1.506283171
AL035425.3	-0.036452079	0.964204298	0.927236749	1.002645689
AL138976.2	-0.144229192	0.865689316	0.714085896	1.049478776
AC106786.1	-0.220237542	0.802328189	0.653175957	0.985539219
GPRC5D-AS1	-0.113321588	0.89286348	0.777694467	1.025087908
AP006545.2	-0.540079131	0.582702141	0.40657098	0.835135318

Abbreviations: lncRNA, long non-coding RNA; TCGA, The Cancer Genome Atlas database; HR, hazard ratio; LUSC, lung squamous carcinoma.

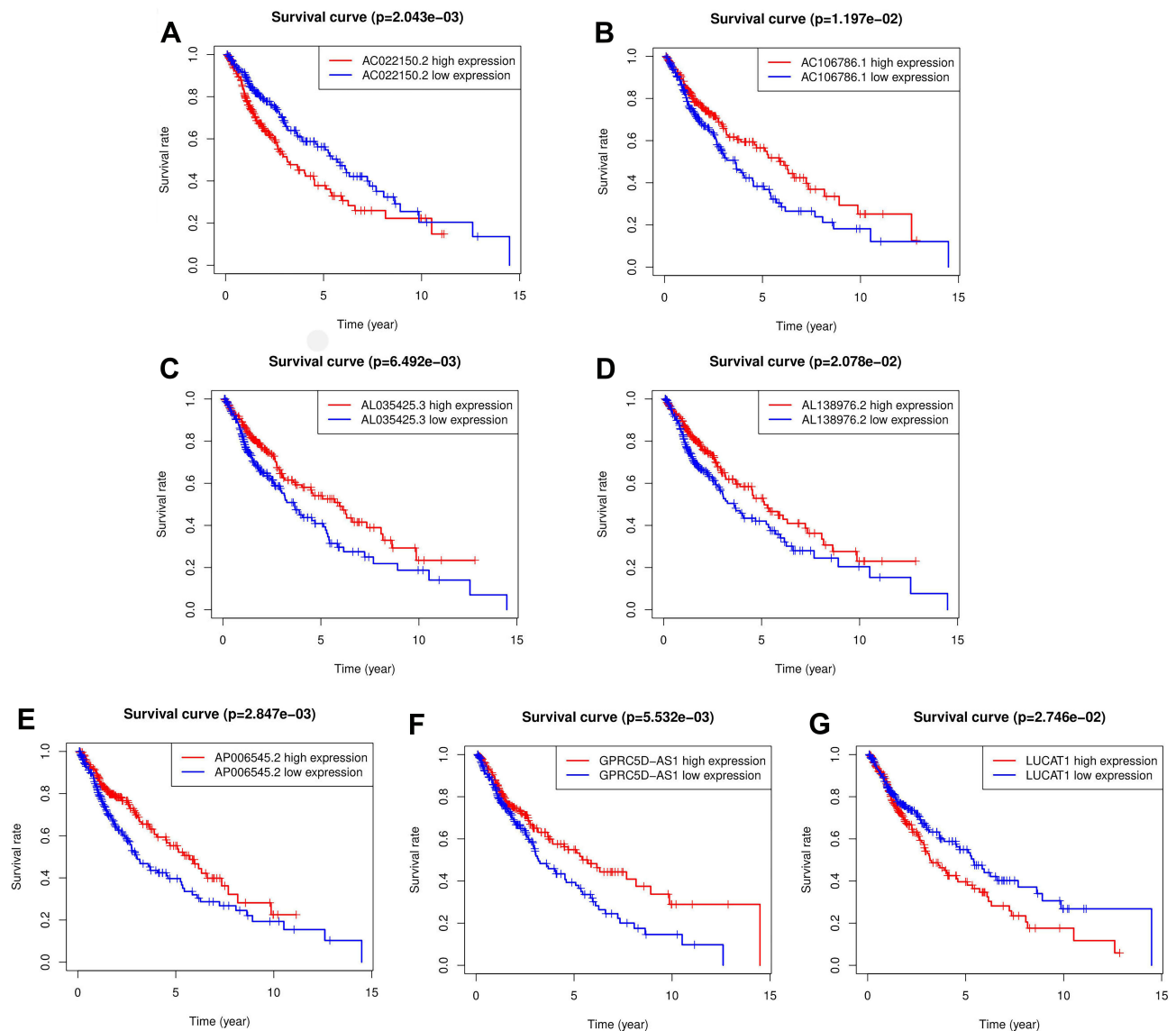


Figure 2 The Kaplan-Meier survival analysis of seven included lncRNAs. LUCAT1, AC022150.2, AL035425.3, AL138976.2, AC106786.1, GPRC5D-AS1 and AP006545.2 were independent protective factors for lung squamous carcinoma. (A) Survival analysis of AC022150.2 in LUSC patients; (B) survival analysis of AC106786.1 in LUSC patients; (C) survival analysis of AL035425.3 in LUSC patients; (D) survival analysis of AL138976.2 in LUSC patients; (E) survival analysis of AP006545.2 in LUSC patients; (F) survival analysis of GPRC5D-AS1 in LUSC patients; (G) survival analysis of LUCAT1 in LUSC patients.

5-year, and 7-year AUC were 0.622, 0.699, 0.721, and 0.733, respectively. These values indicate that this signature reliably predicted the patient survival (Figures 4A–D). Next, the risk score and survival distribution between high- and low-risk groups indicated that the former had a poorer probability of survival (Figures 5A and C). A heatmap illustrating lncRNA expression patterns in high- and low-risk patients also indicated that LUCAT1 and AC022150.2 were risk factors (Figure 5B).

Clinical Value of ARlncRNA Signature

Univariate Cox regression analyses revealed that age (HR = 1.020, 95% CI: 1.000–1.041, $P < 0.05$), tumor stage (HR = 1.311, 95% CI: 1.084–1.586, $P = 0.005$), tumor size (HR = 1.312, 95% CI: 1.065–1.615, $P = 0.011$), and the risk score (HR = 2.147, 95% CI: 1.681–2.743, $P < 0.001$) were significantly correlated with patient survival (Table 3, Figure 6A). Multivariate Cox regression analyses—with clinical factors (age, sex, tumor size and stage) as covariates of the risk

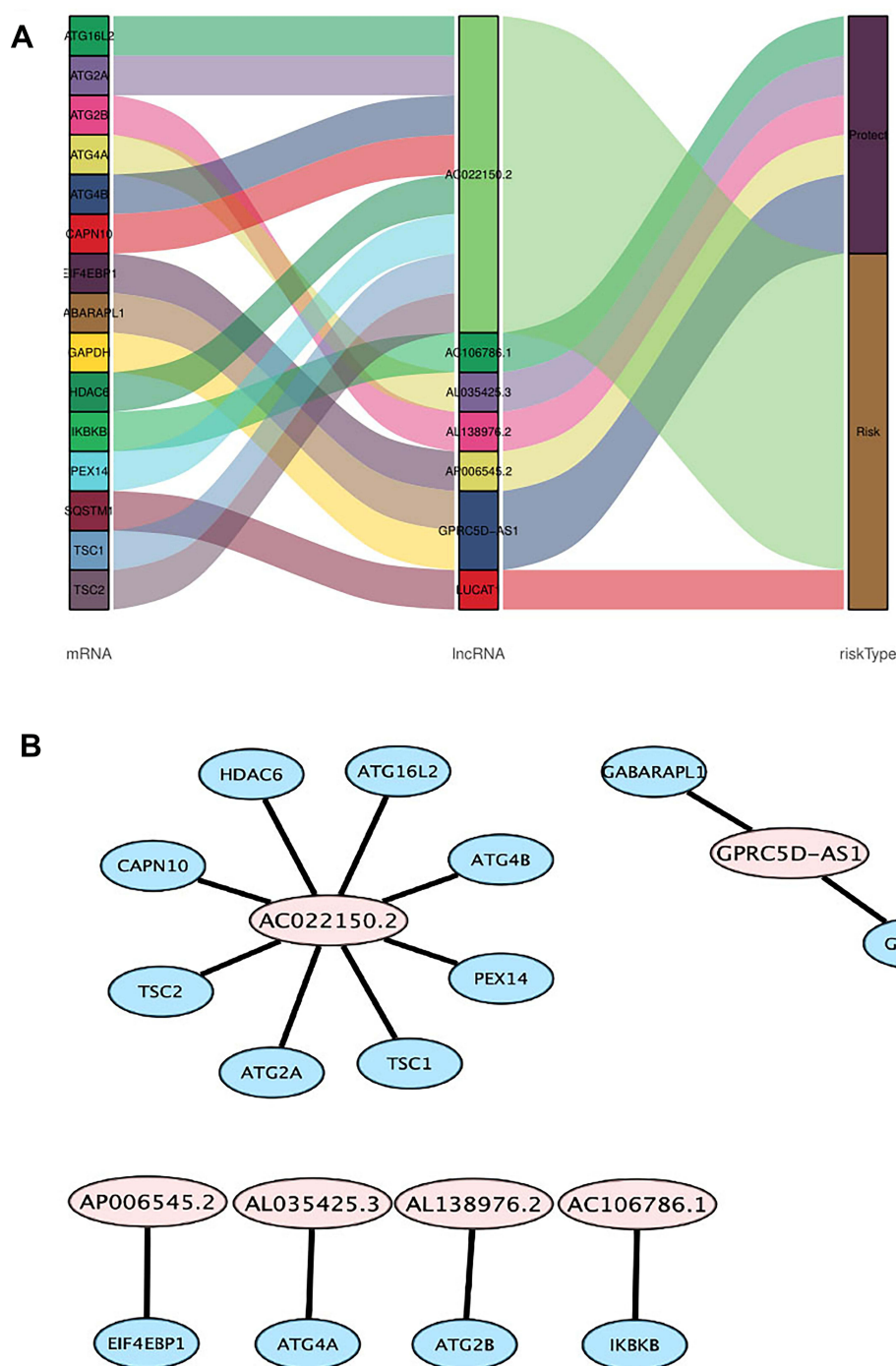


Figure 3 The co-expression network of autophagy-related lncRNA-mRNA and Sankey diagram. **(A)** mRNA–autophagy-related lncRNAs–risk type relationship showed in Sankey diagram. **(B)** The co-expression network visualized using Cytoscape 3.7.2 software.

score—identified only age (HR = 1.027, 95% CI: 1.005–1.050, $P = 0.014$) and risk score (HR = 2.096, 95% CI: 1.652–2.658, $P < 0.001$) as being significantly correlated with the overall survival (Table 4,

Figure 6B). An ROC curve also indicated that risk score had the highest AUC value (0.720) among all clinicopathological parameters (Figure 6C). A detailed stratification of the clinicopathological characteristics

Table 2 Correlation Coefficients of Genes Associated with the Seven lncRNAs Obtained Using TCGA-LUSC Data

Gene	LncRNA	Coefficient	P-value
EIF4EBP1	AP006545.2	0.59908892	3.19E-50
SQSTM1	LUCAT1	0.33459949	1.35E-14
CAPN10	AC022150.2	0.39999618	1.04E-20
TSC2	AC022150.2	0.38645259	2.50E-19
ATG4B	AC022150.2	0.38439657	4.01E-19
HDAC6	AC022150.2	0.38252256	6.14E-19
TSC1	AC022150.2	0.32524468	7.83E-14
ATG2A	AC022150.2	0.32348368	1.08E-13
ATG16L2	AC022150.2	0.31424867	5.73E-13
PEX14	AC022150.2	0.30305019	4.01E-12
ATG4A	AL035425.3	0.48301972	1.05E-30
ATG2B	AL138976.2	0.36429129	3.36E-17
IKBKB	AC106786.1	0.39751821	1.88E-20
GAPDH	GPRC5D-AS1	0.33683065	8.78E-15

Abbreviations: lncRNA, long non-coding RNA; TCGA, The Cancer Genome Atlas database; LUSC, lung squamous carcinoma.

did not reveal any significant correlations with the risk score (Table 5).

Establishment of a Nomogram Prediction Model

A nomogram incorporates multiple clinicopathological characteristics to quantify an individual's prognosis. Here, we employed a nomogram as a rating system to predict the 1-year, 3-year, and 5-year survival probability (Figure 7A). Calibration curves of these three durations matched well, with a C-index of 0.657 (95% CI: 0.633–0.681), indicating that the predictions of the nomogram are reliable (Figures 7B–D).

Drug Sensitivity Analysis of ARlncRNAs

To further explore the usage of our signature in clinical setting, we applied drug sensitivity analysis using “pRRophetic” package in software R to predict the IC50 of the conventional chemotherapy agent between high-risk and low-risk group. We found that low-risk group patients are more sensitive in treating with Cisplatin, Docetaxel, Vinblastine, and Vinorelbine (Figure 8A, B, E and F), indicating a better drug response in low-risk group. As for Gemcitabine and Paclitaxel, there were no significant difference been found (Figures 8C and D).

Functional Analysis

The KEGG pathway analysis identified 51 gene sets in the high-risk group that were significantly associated (P

< 0.01) with cancer-related pathways, ie, JAK/STAT signaling, MAPK signaling, cytokine receptor, chemokine signaling, focal adhesion, and cell adhesion (Figures 9A and B, Supplementary Table 2). In the low-risk group, enriched genes were mainly associated with RNA synthesis, RNA degradation, and neurodegenerative disease (eg, Parkinson's, Huntington's, and Alzheimer's) pathways. Gene Ontology analysis showed that the high-risk group (Figure 9C) was significantly associated with immune response ($NES = 2.08$, $P < 0.0001$), epithelial cell proliferation ($NES = 2.21$, $P < 0.0001$), and endothelial cell proliferation ($NES = 2.69$, $P < 0.0001$). Enriched GO terms in the low-risk group (Figure 9D) were RNA methylation ($NES = -2.23$, $P < 0.0001$) and protein mannosylation ($NES = -1.97$, $P < 0.0001$) pathways. Most of the enriched GO terms and KEGG pathways were closely related to the occurrence and development of lung adenocarcinoma, indicating that the five lncRNAs are involved in functions related to lung cancer.

Multi-Omics Validation of the lncRNA Signature and Correlated Genes

Correlation analysis (Supplementary Table 3) revealed that the genes EIF4EBP1 (AP006545.2), ATG4A (AL035425.3), CAPN10 (AC022150.2), IKBKB (AC106786.1), ATG2B (AL138976.2), GAPDH (GPRC5D-AS1), and SQSTM1 (LUCAT1) were most highly correlated with selected lncRNAs (in parentheses) (Table 2). Because only AC106786.1 and LUCAT1 were available in GEPIA, we explored the mRNA and protein expression of these two pairs (IKBKB-AC106786.1 and SQSTM1-LUCAT1), along with their clinical significance.

GEPIA showed that the transcript levels of IKBKB were significantly lower in LUSC patients, whereas the transcript levels of AC106786.1 were slightly higher (Figures 10A and B). Both SQSTM1 and LUCAT1 were expressed at low levels in tumor tissues (Figures 10C and D). Survival analysis revealed that low LUCAT1 expression was associated with favorable patient outcomes (Figures 10E–H). Spearman correlations confirmed that IKBKB-AC106786.1 and SQSTM1-LUCAT1 exhibited a significant association only in tumor samples (and not in normal tissues) (Figures 11A–D).

We then analyzed the protein expression of these molecules using the HPA database. IKBKB protein was

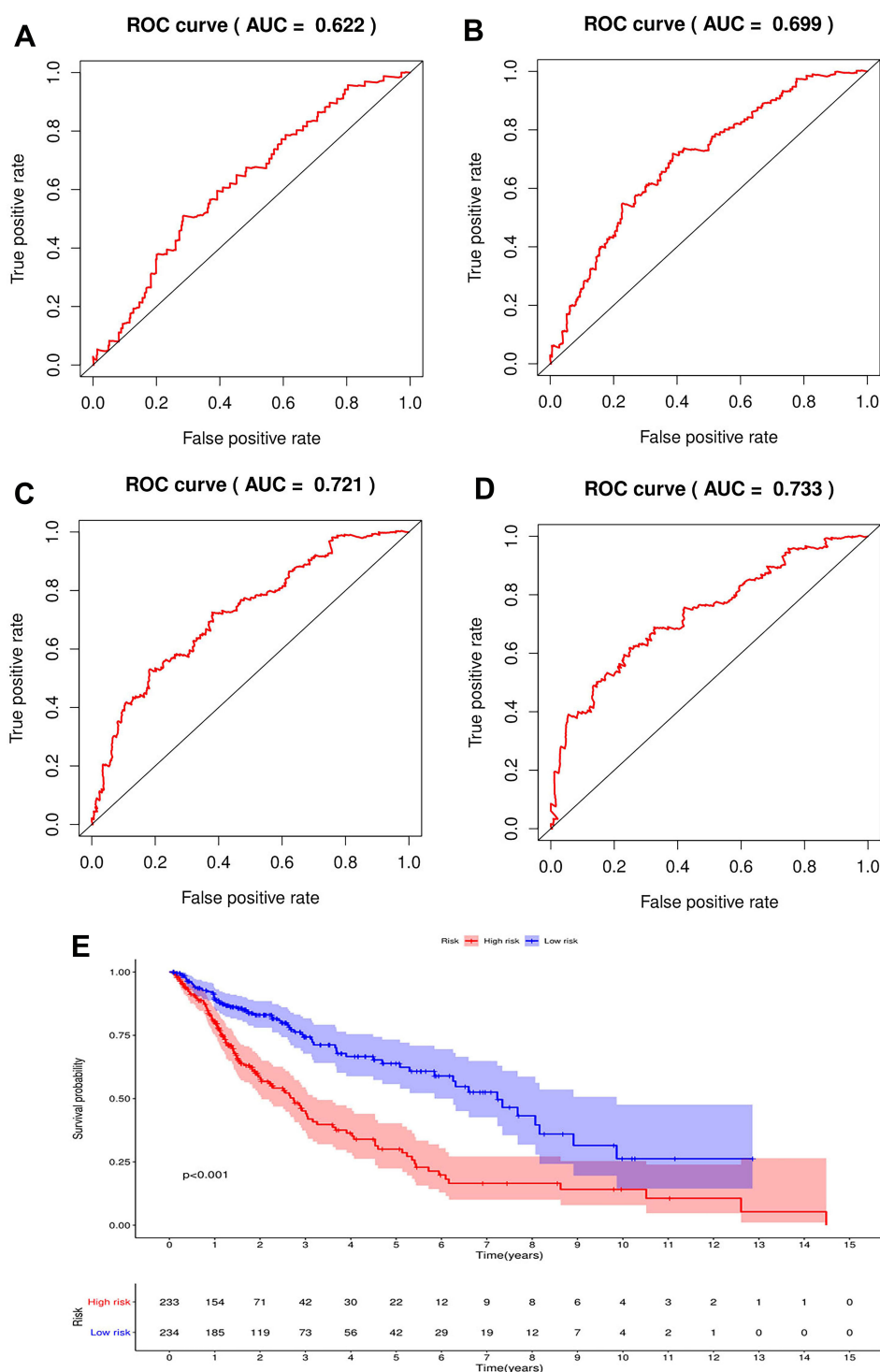


Figure 4 The prognostic indicators of the five autophagy-related lncRNAs signature. (A) 1-year survival ROC curve for LUSC patients. (B) 3-year survival ROC curve for LUSC patients. (C) 5-year survival ROC curve for LUSC patients. (D) 7-year survival ROC curve for LUSC patients. (E) Kaplan-Meier survival curve of the high-risk and low-risk groups for LUSC patients.

expressed at low levels in tumor samples, consistent with the mRNA expression data. However, in tumor samples the expression of SQSTM1 at the protein level was higher than that at the mRNA level (Figures 11E–H).

Discussion

The treatment of patients with LUSC is still limited to traditional chemotherapy despite recent progress in treatments for various lung cancers. Cancer research

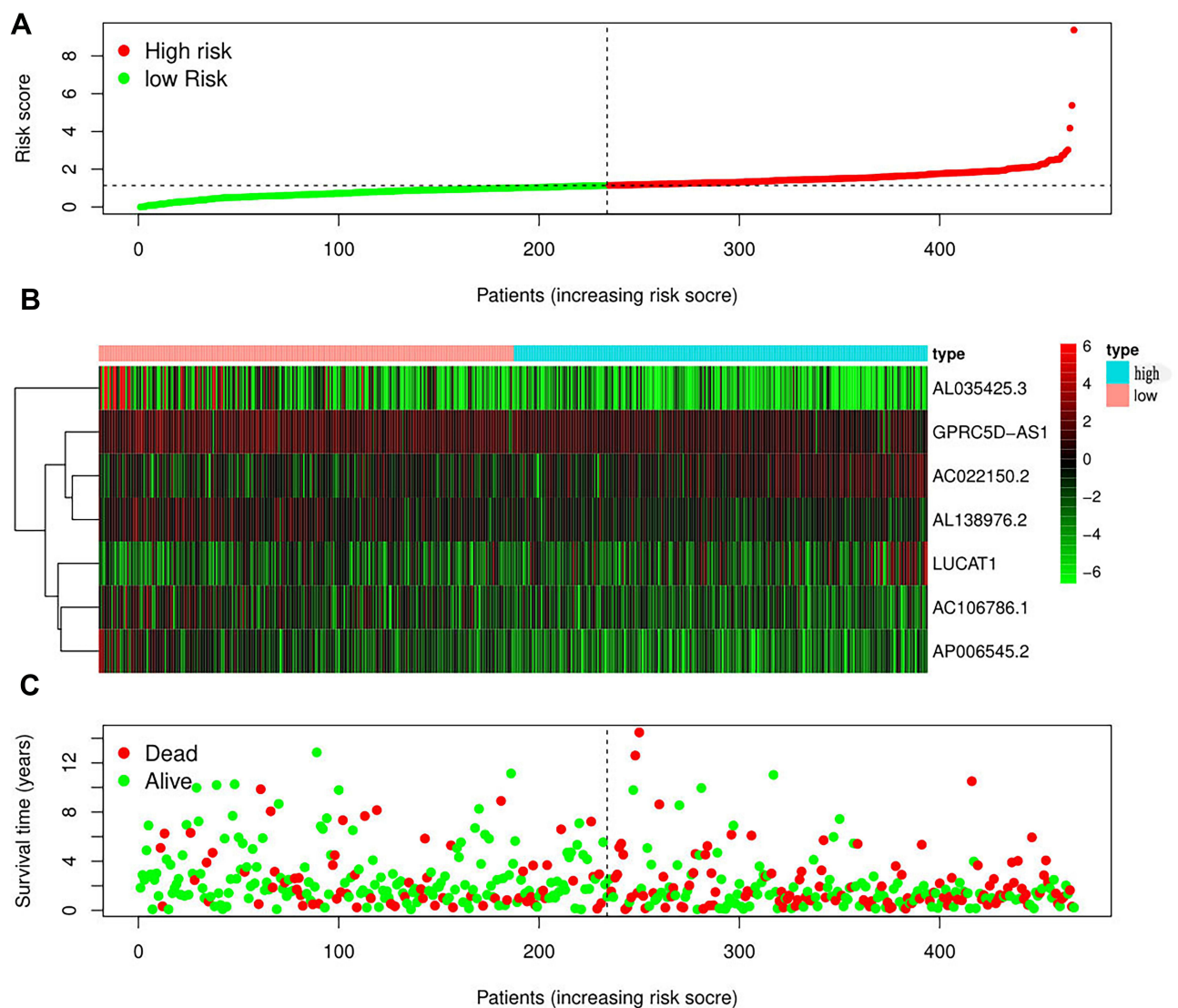


Figure 5 The analysis of the risk score from the generated risk model. (A) Expression profiles of lncRNAs in high and low risk groups; (B) the risk curve of each sample in high and low risk group; (C) the survival plot of each sample based on the risk score.

has focused on autophagy as it can enhance the efficacy of the existing treatment and even reverse drug resistance, owing to its role in stress response and cell death.^{15,16} Certain autophagy proteins are significantly

correlated with the survival of cancer patients; for instance, high ATG10 and LC3A expression is linked to poor survival outcomes.^{8,17} Because of these associations, researchers have employed autophagy genes

Table 3 Univariate Cox Regression Analysis to Identify a Correlation Between the Clinicopathological Characteristics and Risk Scores

Variable	B	SE	z	HR	HR.95L	HR.95H	P-value
Age	0.0202676	0.0103367	1.9607414	1.0204744	1.0000080	1.0413597	0.0499092
Sex	0.2477650	0.1998197	1.2399429	1.2811588	0.8659965	1.8953516	0.2149965
Stage	0.2707820	0.0970016	2.7915227	1.3109893	1.0840050	1.5855028	0.0052461
T	0.2712086	0.1062424	2.5527353	1.3115487	1.0650028	1.6151694	0.0106881
M	0.6764471	0.5857954	1.1547497	1.9668772	0.6239464	6.2002215	0.2481929
N	0.2034908	0.1123744	1.8108286	1.2256739	0.9833807	1.5276653	0.0701674
Risk Score	0.7643015	0.1248087	6.1237847	2.1474937	1.6814911	2.7426428	9.14E-10

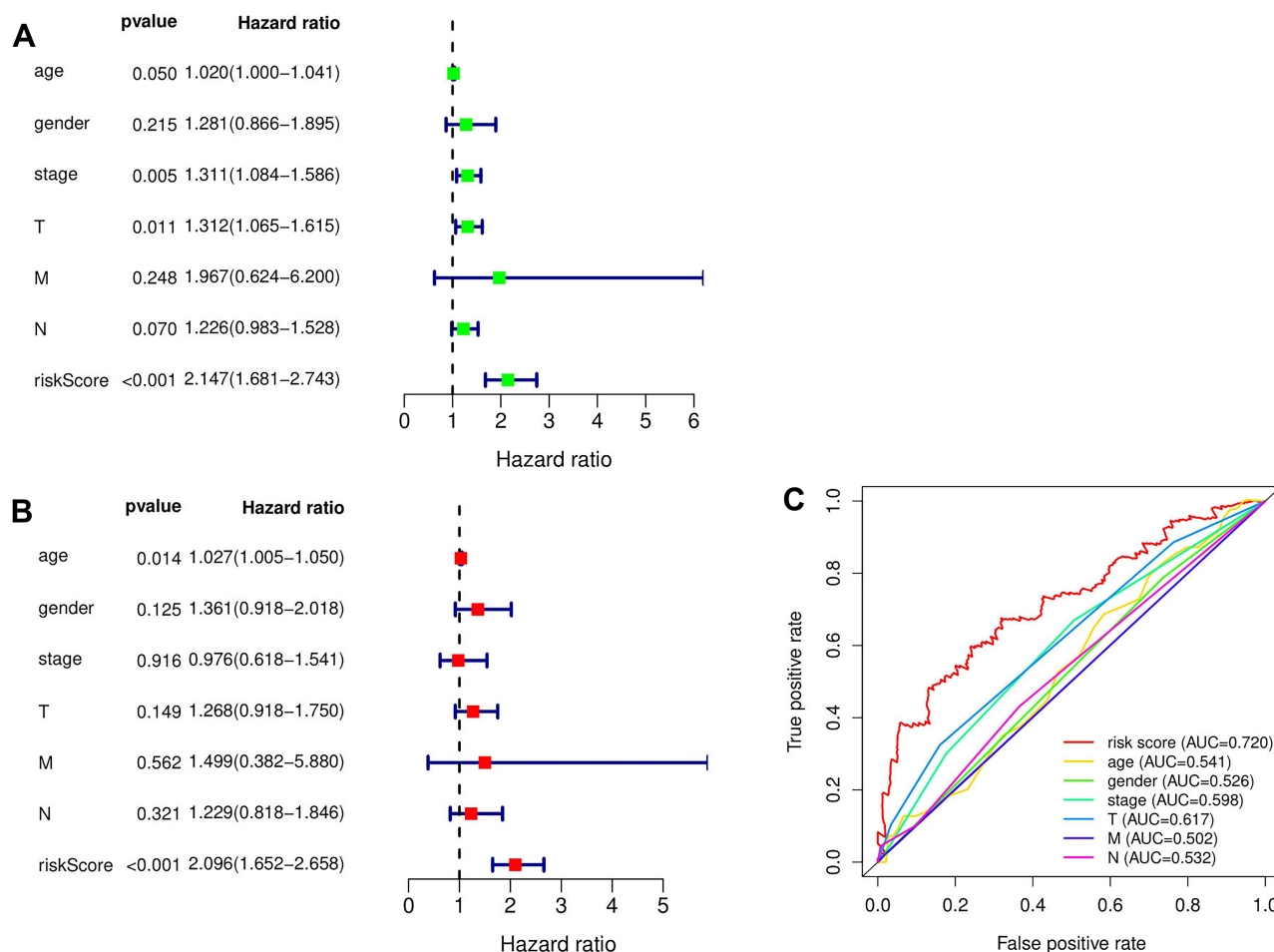


Figure 6 The evaluation of the constructed signature's prognostic credibility in LUSC patients. (**A** and **B**) The univariate and multivariate Cox regression analysis of risk score and clinicopathological characteristics. (**C**) The integrated ROC curves of risk score and clinical features.

to construct prognostic signatures that can predict the overall survival with some reliability.^{18,19} Similarly, lncRNAs have received attention for their involvement in tumorigenesis, suggesting a role as biomarkers in cancer diagnosis and prognosis.²⁰ For example, a lncRNA-based predictive model was recently developed for lung adenocarcinoma with potential for future clinical use. These advances led

us to investigate whether specific ARlncRNA signatures could be used to predict the prognosis of patients with LUSC.

We initially filtered out 1248 lncRNAs that significantly correlated with 210 autophagy-related genes from an LUSC cohort in TCGA. Using Lasso and multivariate Cox regressions, we established an independent prognostic model of seven ARlncRNAs to

Table 4 Multivariate Cox Regression Analysis to Identify a Correlation Between Clinicopathological Characteristics and Risk Scores

Variable	B	SE	z	HR	HR.95L	HR.95H	p-value
Age	0.02687767	0.01099368	2.44483005	1.02724213	1.00534469	1.04961652	0.01449204
Sex	0.30834498	0.20096401	1.53432934	1.36117049	0.91801895	2.01824276	0.12494865
Stage	-0.02465649	0.23322151	-0.10572134	0.97564500	0.61769390	1.54102729	0.91580345
T	0.23712498	0.16440633	1.44231053	1.26759954	0.91841538	1.74954451	0.14921479
M	0.40448973	0.69746694	0.57994108	1.49853764	0.38192908	5.87966510	0.56195435
N	0.20613540	0.20765636	0.99267556	1.22891959	0.81802400	1.84620908	0.32086809
Risk Score	0.73993423	0.12128895	6.10059081	2.09579766	1.65237271	2.65821858	1.06E-09

Table 5 Relationship Between Clinicopathological Characteristics and Risk Score

Clinical	n	Mean	SD	t	p-value
Age					
>60	300	1.18	0.582	1.83645746	0.068
≤60	87	1.067	0.481		
Sex					
Male	99	1.186	0.526	0.65832409	0.511
Female	288	1.144	0.575		
Stage					
I–II	312	1.144	0.541	−0.6779731	0.499
III–IV	75	1.199	0.647		
T					
1–2	316	1.133	0.563	−1.5924177	0.114
3–4	71	1.25	0.556		
M					
0	381	1.156	0.566	0.38776339	0.713
I	6	1.105	0.311		
N					
0	242	1.157	0.541	0.08511214	0.932
1–3	145	1.152	0.599		

predict patient survival and calculated a risk score for each patient. Of the lncRNAs included in our model, LUCAT1 is the most extensively studied, with numerous studies demonstrating its vital role in various cancers, such as gastric cancer,²¹ esophageal cancer,²² liver cancer,²³ and lung cancer.²⁴ Consistent with our findings, LUCAT1 is known to be expressed at significantly higher levels in lung tumors than in the paracancerous tissue and is a negative indicator of prognosis.²⁴ Overexpression of LUCAT1 may inhibit apoptosis and enhance cisplatin resistance development via IGF-2.²⁵ This mechanism likely explains why LUSC patients with LUCAT1 overexpression have a poorer prognosis, given that the conventional treatment for this cancer is platinum-based. Although no cancer-related studies have been conducted on AC106786.1, it was correlated with the autophagy gene IKBKB, which plays a negative role in inhibiting cisplatin-induced apoptosis in lung adenocarcinoma.²⁶ Likewise, EIF4EBP1—the gene correlated with AP006545.2, an under-researched lncRNA—plays a role in several cancers via the mTOR signaling

pathway.²⁷ EIF4EBP1 is also a key gene for predicting the prognosis of patients with lung cancer.¹⁹

Autophagy-related (ATG) proteins play a dual role in cancer, ie, they can inhibit as well as accelerate cancer progression. Even though little is known about the lncRNAs AC022150.2, AL035425.3, AC138976.2, and GPRC5D-AS1, they were all associated with ATGs in our study. Of the 16 known mammalian ATGs, ATG2, ATG4, ATG8, and ATG16 are most correlated with cancer in our established signature. ATG2 is an essential part of the ATG9/ATG12-WIPI complex that facilitates ATG9 recruitment to expand autophagosomes.⁴ ATG2B is associated with oncogenesis, and a frameshift mutation affecting this protein is implicated in the development of gastric and colorectal cancer.²⁸ ATG4 activates LC3 by cleaving LC3-I to produce LC3-II.²⁹ Enhanced ATG4 expression is positively correlated with the occurrence and progression of colorectal cancer,³⁰ as well as with increased susceptibility to ovarian and lung cancer.^{31,32} As ATG4 can function as an alternative to anti-lysosomal treatment, there is widespread interest in developing drugs

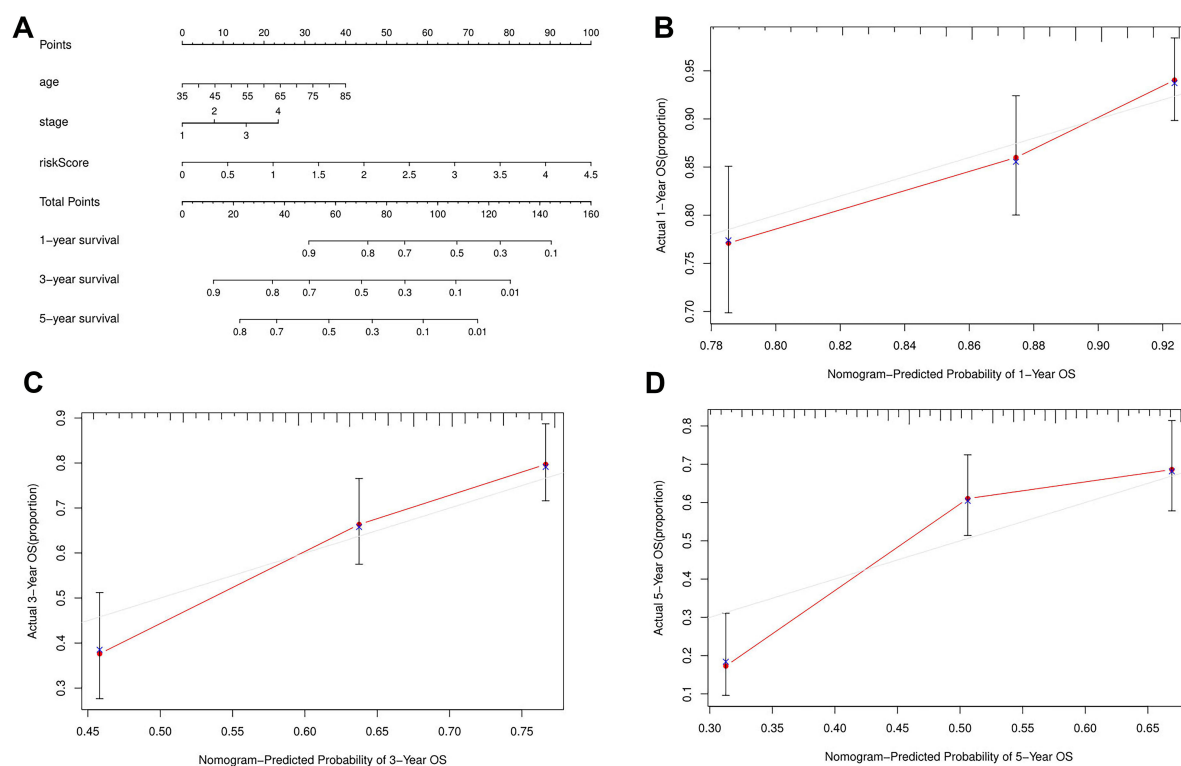


Figure 7 Nomogram prediction model in LUSC patients. **(A)** The nomogram of 1-year, 3-year or 5-year survival predictability based on risk score, age and TNM stage. **(B–D)** The calibration plots for predicting 1-year, 3-year or 5-year survival.

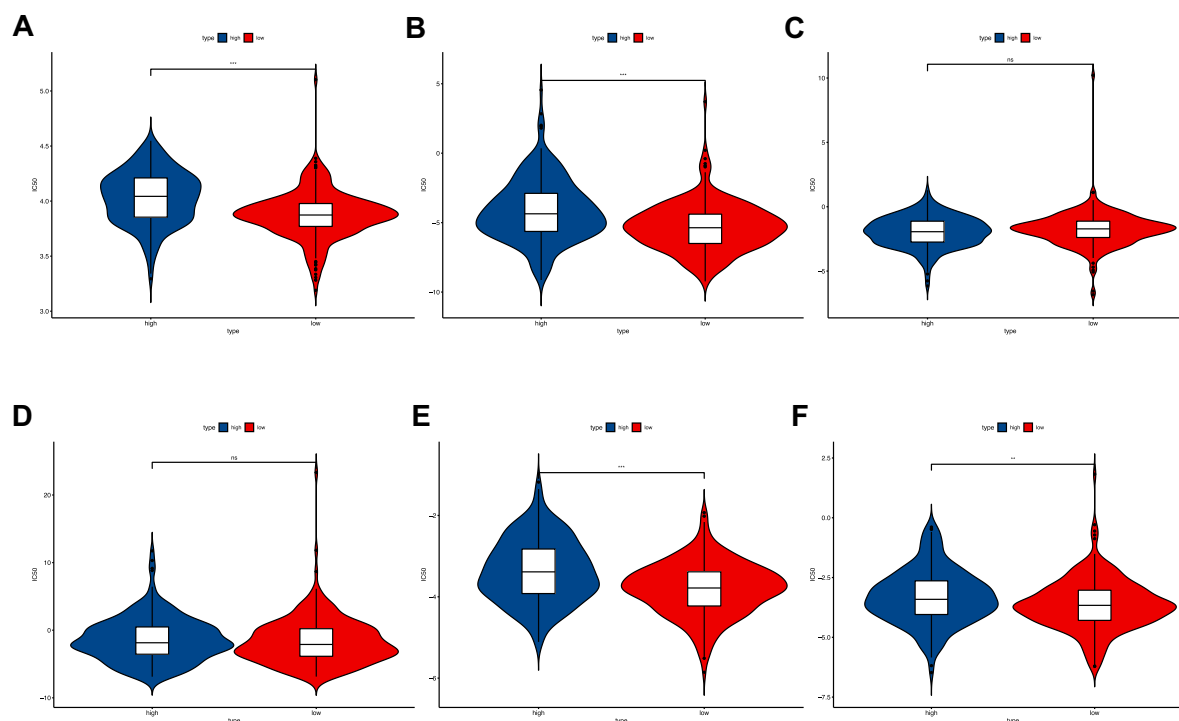


Figure 8 Drug sensitivity analysis between high-risk and low-risk group. **(A)** The IC50 of Cisplatin between the two groups; **(B)** The IC50 of Docetaxel between the two groups; **(C)** The IC50 of Gemcitabine between the two groups; **(D)** The IC50 of Paclitaxel between the two groups; **(E)** The IC50 of Vinblastine between the two groups; **(F)** The IC50 of Vinorelbine between the two groups. ** $P < 0.01$; *** $P < 0.001$. **Abbreviation:** ns, not significant.

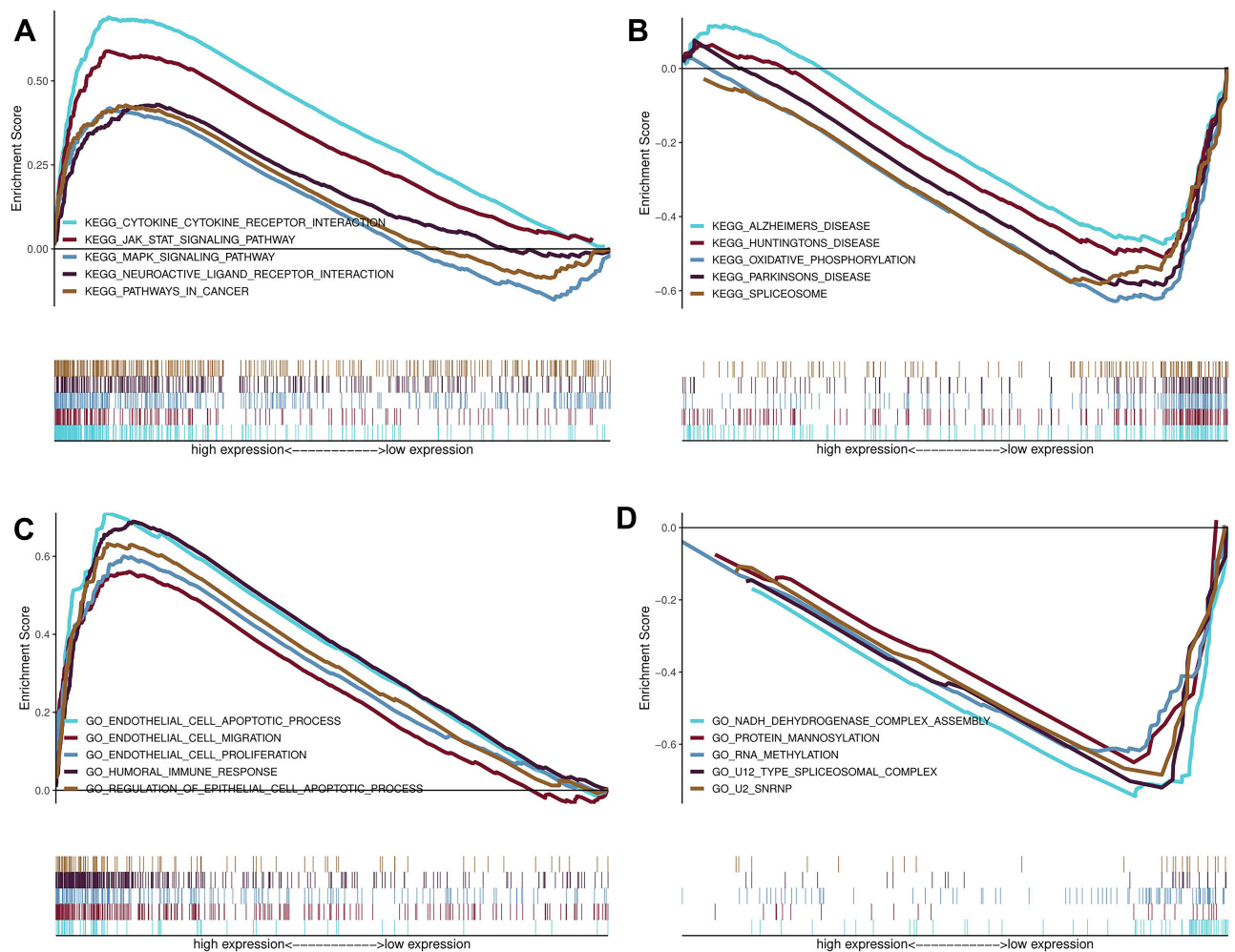


Figure 9 The functional enrichment analysis based on autophagy-related lncRNAs. (A and B) Upregulated KEGG pathway in high risk group and low risk group; (C and D) Upregulated Gene Ontology terms in high risk group and low risk group.

targeting ATG4B, ie, generation of several ATG4 inhibitors that have a potential clinical value.^{30,33,34} Finally, the ATG8 family member, GABARAPL1 is involved in cellular trafficking and cancer, in addition to autophagy. High GABARAPL1 expression is correlated with longer survival time in renal, prostate, breast, and liver cancers.^{35–37} In terms of mechanism, GABARAPL1 upregulation appears to induce cell arrest via the P53 pathway, thereby decreasing cell viability, proliferation, and migration.³⁸

With respect to clinical significance, the high AUC values of our seven ARlncRNAs across 1, 3, and 5 years suggests that the model reliably predicted patient survival. Subsequent multivariate Cox analyses confirmed that the risk score generated using our model can be used as an independent prognostic indicator. Overall, our results

suggest that the lncRNA signature has the potential to predict patient survival.

Functional enrichment analysis showed that the most significantly enriched KEGG pathways were MAPK and JAK/STAT signaling pathways, both known to be implicated in various cancers. In lung cancer, the MAPK axis crosstalks with some lncRNAs to promote cancer cell invasion and drug resistance.^{39,40} Additionally, STAT3 overexpression is observed in nearly 55% of the patients with lung cancer and most lung cancer cell lines.⁴¹ Furthermore, JAK1/STAT1 activation is positively correlated with cancer stage, especially with nodal metastasis.⁴² Beyond this, few studies have investigated the relationships between lncRNAs and various signaling pathways, a topic that we intend to pursue in future work.

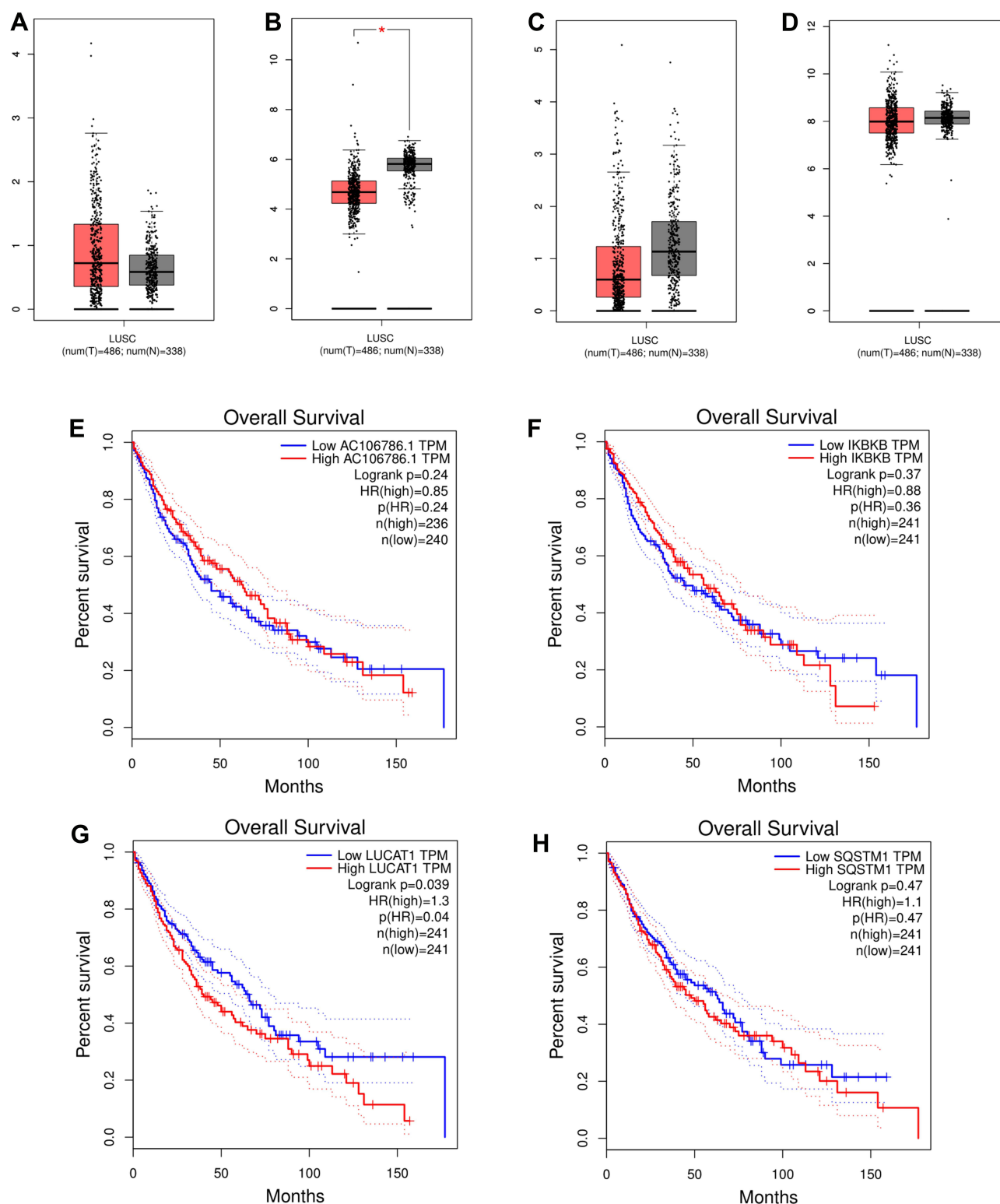


Figure 10 Validation of IKBKB-AC106786.1 and SQSTM1-LUCAT1 in GEPIA database. **(A)** The mRNA expression levels of AC106786.1; **(B)** The mRNA expression levels of IKBKB; **(C)** The mRNA expression levels of LUCAT1; **(D)** The mRNA expression levels of SQSTM1; **(E)** Kaplan-Meier survival analysis of AC106786.1; **(F)** Kaplan-Meier survival analysis of IKBKB; **(G)** Kaplan-Meier survival analysis of LUCAT1; **(H)** Kaplan-Meier survival analysis of SQSTM1.

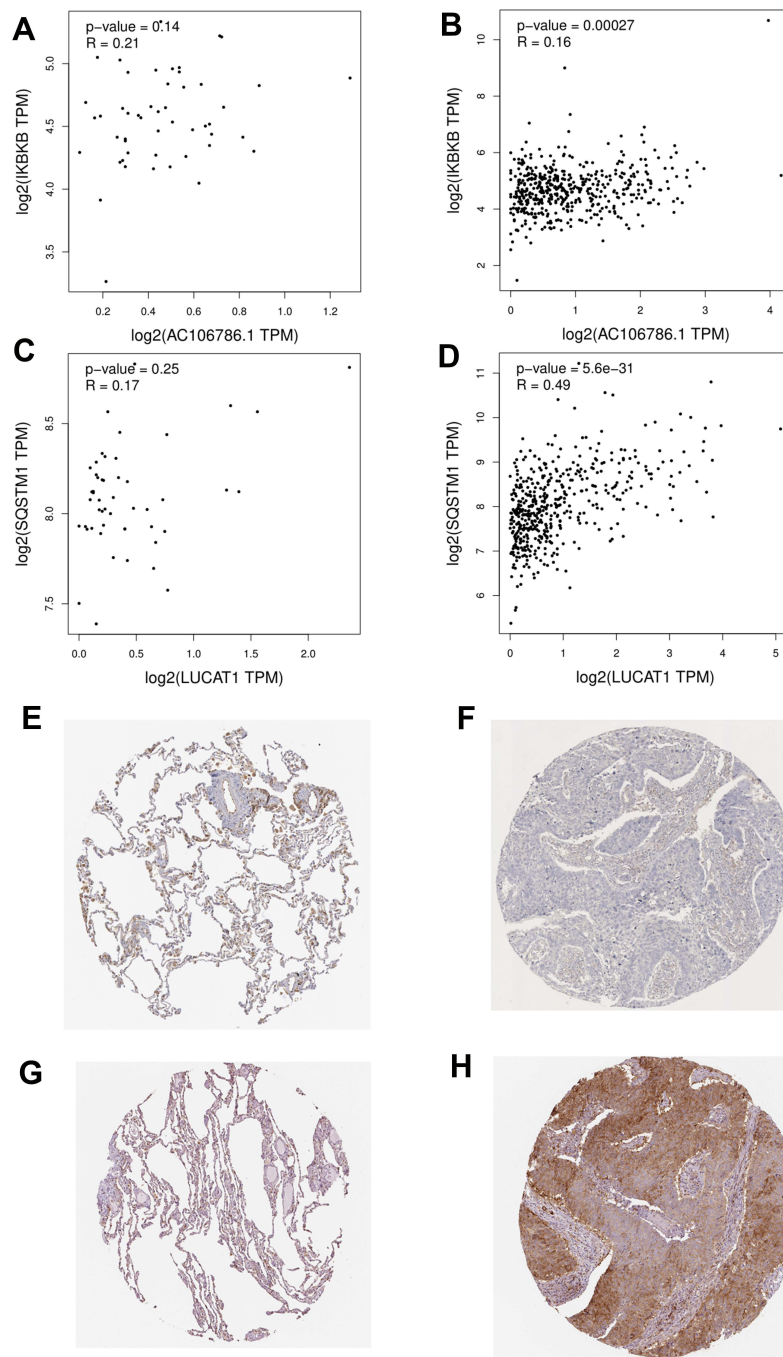


Figure 11 Correlation test in GEPIA and protein expression in HPA. (A) Spearman correlation tests for IKBKB-AC106786.1 in tumor sample; (B) Spearman correlation tests for IKBKB-AC106786.1 in normal lung sample; (C) Spearman correlation tests for SQSTM1-LUCAT1 in tumor sample; (D) Spearman correlation tests for SQSTM1-LUCAT1 in normal lung sample; (E) the expression level of IKBKB in normal lung sample; (F) the expression level of IKBKB in tumor sample; (G) the expression level of SQSTM1 in normal lung sample; (H) the expression level of SQSTM1 in tumor sample.

Our study has several limitations. First, the small number of patients included means that our results may not be extrapolatable to a larger population. Second, the established prognostic model has not been tested, and a prospective

study is needed to verify its predictive efficacy. Third, we did not detect lncRNA expression in tumor samples or identify potential molecular mechanisms, highlighting the need for additional basic biological experiments.

Conclusions

In conclusion, our study provided a comprehensive analysis of ARlncRNAs in LUSC. We constructed a co-expression network that points to a potential mechanism underlying the effects of the seven ARlncRNAs. The lncRNA-based prognostic signature was significantly correlated with patient survival, and the derived risk score was an independent predictive factor for LUSC progression. Moreover, genes associated with the seven ARlncRNAs were potential therapeutic targets in LUSC. Overall, we believe that the identified lncRNAs have clinical value as diagnostic or prognostic tools, and as candidates for drug development.

Disclosure

The authors report no conflicts of interest in this work.

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