Pharmacogenomics and Personalized Medicine

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

# Cuproptosis-Related lncRNA Predict Prognosis and Immune Response of LUAD

Qianhui Zhou[1], Yi Liu[1], Yan Gao[1], Lingli Quan[1], Lin Wang[1], Hao Wang[2]

[1]Department of Respiratory and Critical Care Medicine, Zhuzhou Central Hospital, Zhuzhou, 412000, People's Republic of China; [2]Department of Urology, The First Affiliated Hospital, Hengyang Medical School, University of South China, HengYang, Hunan, 421005, People's Republic of China

Correspondence: Hao Wang, Email wanghao872013@163.com

**Background:** Lung cancer is the leading cause of cancer deaths worldwide, primarily due to lung adenocarcinoma (LUAD). However, the heterogeneity of programmed cell death results in varied prognostic and predictive outcomes. This study aimed to develop an LUAD evaluation marker based on cuproptosis-related lncRNAs.

**Methods:** First, transcriptome data and clinical data related to LUAD were downloaded from the Cancer Genome Atlas (TCGA), and cuproptosis-related genes were analyzed to identify cuproptosis-related lncRNAs. Univariate, LASSO, and multivariate Cox regression analyses were conducted to construct cuproptosis-associated lncRNA models. LUAD patients were categorized into high-risk and low-risk groups using prognostic risk values. Kaplan-Meier analysis, PCA, GSEA, and nomograms were employed to evaluate and validate the results.

**Results:** 7 cuproptosis-related lncRNAs were identified, and a risk model was created. High-risk tumors exhibited cuproptosis-related gene alterations in 95.54% of cases, while low-risk tumors showed alterations in 85.65% of cases, mainly involving TP53. The risk value outperformed other clinical variables and tumor mutation burden as a predictor of 1-, 3-, and 5-year overall survival. The cuproptosis-related lncRNA-based risk model demonstrated high validity for LUAD evaluation, potentially influencing individualized treatment approaches. Expression analysis of four candidate cuproptosis-related lncRNAs (AL606834.1, AL161431.1, AC007613.1, and LINC02835) in LUAD tissues and adjacent normal tissues revealed significantly higher expression levels of AL606834.1 and AL161431.1 in LUAD tissues, positively correlating with tumor stage, lymph node metastasis, and histopathological grade. Conversely, AC007613.1 and LINC02835 exhibited lower expression levels, negatively correlating with these factors. High expression of AL606834.1 and AL161431.1 indicated poor prognosis, while low expression of AC007613.1 and LINC02835 was associated with unfavorable outcomes. Univariate and multivariate analyses confirmed these lncRNAs as independent risk factors for LUAD prognosis.

**Conclusion:** The 4 cuproptosis-related (lncRNAsAL606834.1, AL161431.1, AC007613.1, and LINC02835) can accurately predict the prognosis of patients with LUAD and may provide new insights into clinical applications and immunotherapy.

**Keywords:** lung adenocarcinoma, tumor mutation load, cuproptosis, long noncoding RNA, immunotherapeutic response

## Introduction

Cancer of the lung, which includes adenocarcinoma, squamous cell carcinoma, small cell cancer of the lungs, and large cell cancer of the lungs,[1] is the most prevalent malignancy in China, if not the world.[2,3] Adenocarcinoma of the lung accounts for over 40% of all cases, making it the most prevalent kind.[4] Although the overall survival rate of patients with lung adenocarcinoma has increased as a consequence of surgery, radiation, chemotherapy, immunotherapy, and targeted therapy, the 5-year survival rate remains low.[5,6] Therefore, the treatment of LUAD should be improved through a rational treatment plan. Nowadays, tumor risk score prediction signatures are a non-invasive technique to identify patient survival. They can help to accurately predict prognosis and are gradually being used in clinical applications. Therefore, prognostic signatures should be developed urgently to predict the long-term survival of patients with LUAD.

Copper is essential to all living organisms and plays a dual, paradoxical role in the cell, which helps to maintain intracellular copper concentrations at very low levels via a homeostatic mechanism. Copper-induced cell death is

Received: 14 December 2023
Accepted: 18 June 2024
Published: 25 June 2024

mediated by iron-sulfur protein, when copper binds to acylated lipid products in the TCA cycle, leading to lipid acylated protein aggregation and loss of iron-sulfur cluster proteins. This results in proteotoxic stress and ultimately cell death, and this unique form of cell death is called cuproptosis[7] Cuproptosis is a sort of regulated cell death with a process that differs significantly from apoptosis, pyroptosis, necroptosis, and ferroptosis.[8] Recent research[9] indicates that cuproptosis-associated genes influence the clinical outcomes of clear cell renal cell carcinoma. Long noncoding RNAs have gained interest as a research focus during the last decade.[10] Among these are several tumor-associated studies, such as those on prostate, pancreatic, and colon cancers.[11–13] Multiple studies[14,15] have connected LncRNAs to the prevalence and progression of lung cancer. According to research,[15] copper may alter the biological process of non-small cell lung cancer through the lncRNA MALAT1, however the precise regulatory mechanism is unknown. Understanding the mechanism of cuproptosis-associated lncRNA in lung adenocarcinoma is advantageous for lung cancer prognostication and immunotherapy. In addition, the relationship between cuproptosis and lncRNAs remains controversial, and few research have shed light on the function of cuproptosis-associated lncRNAs in lung cancer prognosis.

We identified cuproptosis-associated lncRNAs utilizing Pearson's correlation analysis, screened 16,866 lncRNAs and 19 cuproptosis genes from the TCGA database, and constructed a novel predictive risk model for predicting the overall survival of lung adenocarcinoma patients. Then, we examined the association between immunotherapy responses and cuproptosis-associated lncRNA, and we developed a nomogram that predicts overall survival in cancer of lung adenocarcinoma patients.

# Materials and Methods

## Clinical Data and Extraction of Datasets

Lung adenocarcinoma patients' clinical data and cuproptosis-associated genes were extracted from the TCGA database (https://cancergenome.nih.gov/, accessed on June 13, 2022). We excluded patients with insufficient data from this research. Throughout the data analysis process, R is used (Version 4.1.1). This study has complied with the Declaration of Helsinki.

## Clinical Specimens

Total of 60 paired LUAD samples and adjacent normal renal specimens were collected from Zhuzhou Central Hospital between June 2010 and June 2012. Inclusion criteria for specimen collection: (1) Postoperative pathological examination confirmed LUAD; (2) The patients with neither radiotherapy nor chemotherapy; (3) Complete follow-up data were available; (4) The patients understood the purpose and requirements of the study, agreed to participate in the study and signed a written informed consent, which was reviewed and approved by the Ethics Committee of Zhuzhou Central Hospital (registered as NCT03438477 in ClinicalTrails.gov).

Total RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

RNA was isolated by TRIzol® reagent (Ambion; USA) from LUAD tissues according to the manufacturer's protocols. And cDNA was reversely transcribed by PrimeScript RT reagent kit (Takara, China). We conducted RT-qPCR on ABI 7500 RT PCR system using SYBR Premix Ex TaqII Kit (Takara, China). All quantifications were normalized to the level of glyceraldehyde phosphate dehydrogenase (GAPDH) in the reaction. The comparative threshold cycle (CT) method, which compares differences in CT values between common reference RNA and target gene RNA, was used to obtain the relative fold changes in gene expression. The expressions were calculated by $2-\Delta\Delta ct$ method. Each experiment was performed in triplicate and repeated three times.

## The Selection of lncRNAs and Genes Relevant to Cuproptosis

From the lncRNA and cuproptosis gene profiles of lung adenocarcinoma patients, we identified 19 cuproptosis genes, including NFE2L2, NLRP3, ATP7B, ATP7A, SLC31A1, FDX1, LIAS, LIPT1, LIPT2, DLD, DLAT, PDHA1, PDHB, MTF1, GLS, CDKN2A, DBT, GCSH, DLST. Utilizing Pearson correlation analysis, 2244 cnuproptosis-associated lncRNAs were identified (R more than 0.4 and p less than 0.001).

## The Process of Generating and Confirming the Risk Signature

To generate and verify the cuproptosis-associated lncRNA prognostic model, the whole collection of TCGA clinical data was randomly split into training and internal test sets. The sample data collected by Zhuzhou Central Hospital is used as an external validation set. Cuproptosis-associated lncRNAs and clinical data were paired, and univariate cox regression analysis was used to identify cuproptosis-associated lncRNAs having prognostic value.[16] The LASS regression model improves the accuracy and explanatory nature of predictions by selecting relevant variables and reducing the complexity of the model. LASSO regression is a widely used method for feature selection in high-dimensional data analysis, which can effectively select important features and avoid overfitting. Utilizing LASSO Cox regression analysis, we then selected 13 lncRNAs linked to cuproptosis.[17] The 13 cuproptosis-associated lncRNAs were investigated utilizing multivariate regression analysis, and a risk model for 7 cuproptosis-associated lncRNAs was established.[18] Each lncRNA had a coefficient that contributed differentially to prognosis, and risk values were generated utilizing the formula risk $\sum_{i=0}^{n} expri * coefi$ value= for each patient. We divided lung adenocarcinoma patients into high- and low-risk categories based on the median risk value.[19]

## Analysis of Gene Networks and Enrichment

We identified differentially expressed genes and regulatory pathways utilizing GO and KEGG analysis utilizing the R software clusterProfiler. The p value was used to define these analytic criteria, with p less than 0.05 being a highly enriched functional comment.[19]

## The Model Evaluation in Immunotherapeutic Therapy

The mutation data were analyzed and summarized utilizing the R package maftools. The TMB was computed utilizing altered tumor-specific genes.[20] We evaluated the probability of an immunotherapeutic response utilizing the TIDE algorithm.[16]

## PCA and Kaplan-Meier Analysis of Survival

PCA, an efficient method for dimensionality reduction, model identification, and categorying, was utilized to visualize high-dimensional data from the gene expression profiles, 19 cuproptosis genes, 7 cuproptosis-associated lncRNAs, and a risk model based on the expression patterns of the 7 cuproptosis-associated lncRNAs.[21] Utilizing Kaplan-Meier survival analysis, the R packages survMiner and survival tools were used to determine survival differences between high- and low-risk categories.[22]

## Self-Contained Model of the Cuproptosis-Associated lncRNA

The connection between risk value and clinical variables (gender, age, tumor stage, T stage, N stage, and history of tobacco use) and OS in patients with LUAD was studied utilizing univariate and multivariate Cox regression.[23]

## Analysis of Gene Networks and Enrichment of Cuproptosis-Associated Genes

We used the GENEMANIA website to do a gene network analysis in order to research the possible connections between these genes.[24] Additionally, we did pathway enrichment analysis on cuproptosis-related genes utilizing the website Metascape.[25] As references, Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were used. The enrichment research was conducted utilizing the clusterProfiler R program.[26] Multiple correction was performed utilizing the Benjamini-Hochberg technique, and a false discovery rate (FDR) less than 0.05 or below was deemed significant.

## Developing and Exhibiting a Projected Nomogram

The R package "regplot" was used to construct better regression nomograms of the cuproptosis-associated lncRNAs risk values and other clinical data (age, gender, risk value, tumor stage, T stage, N stage, and smoking history) for the 1-, 3-, and 5-year OS of LUAD patients.

## Statistical Analysis

SPSS 23.0 software was used for statistical analysis, and GraphPad Prism 8.0 software was used for analysis and mapping. All measurement data in the form of mean ±standard deviation(SD), according to two groups and multiple groups of measuring data comparison using Student's t-tests and one-way ANOVA. Using Pearson chi-square test to analyze the relationship between the expression levels of candidate cuproptosis-associated lncRNAs (AL606834.1, AL161431.1, AC007613.1, LINC02835) in tissue samples of LUAD patients and the clinical and pathological characteristics of LUAD patients. Cuproptosis-associated lncRNAs (AL606834.1, AL161431.1, AC007613.1, LINC02835) were used to analyze the prognosis of LUAD patients using Kaplan-Meier survival analysis and Cox proportional risk hazard model. $P<0.05$ was considered as significantly different.

# Results

## Extraction of lncRNAs Linked to Cuproptosis

Figure 1 depicts the whole flowchart for the building of the prognostic risk model and subsequent analysis. We began by searching the TCGA database for 16,866 lncRNAs and 19 cuproptosis genes. Utilizing Pearson correlation analysis, 2244 lncRNAs associated with cuproptosis were identified (R more than 0.40 and p less than 0.001). Figure 2A depicts the coexpression network between cuproptosis and lncRNA utilizing a Sankey diagram. Figure 2B shows the relationship between cuprotosis genes and involved in cuprotosis- process lncRNA in the TCGA dataset.

## Development and Validation of a Risk Model Utilizing lncRNAs Associated with Cuproptosis

Utilizing univariate regression analysis, we determined that 22 cuproptosis-associated genes out of 2244 cuproptosis-associated lncRNAs in the TCGA database were significantly associated with OS (Figure 3A). LASSO-penalized the

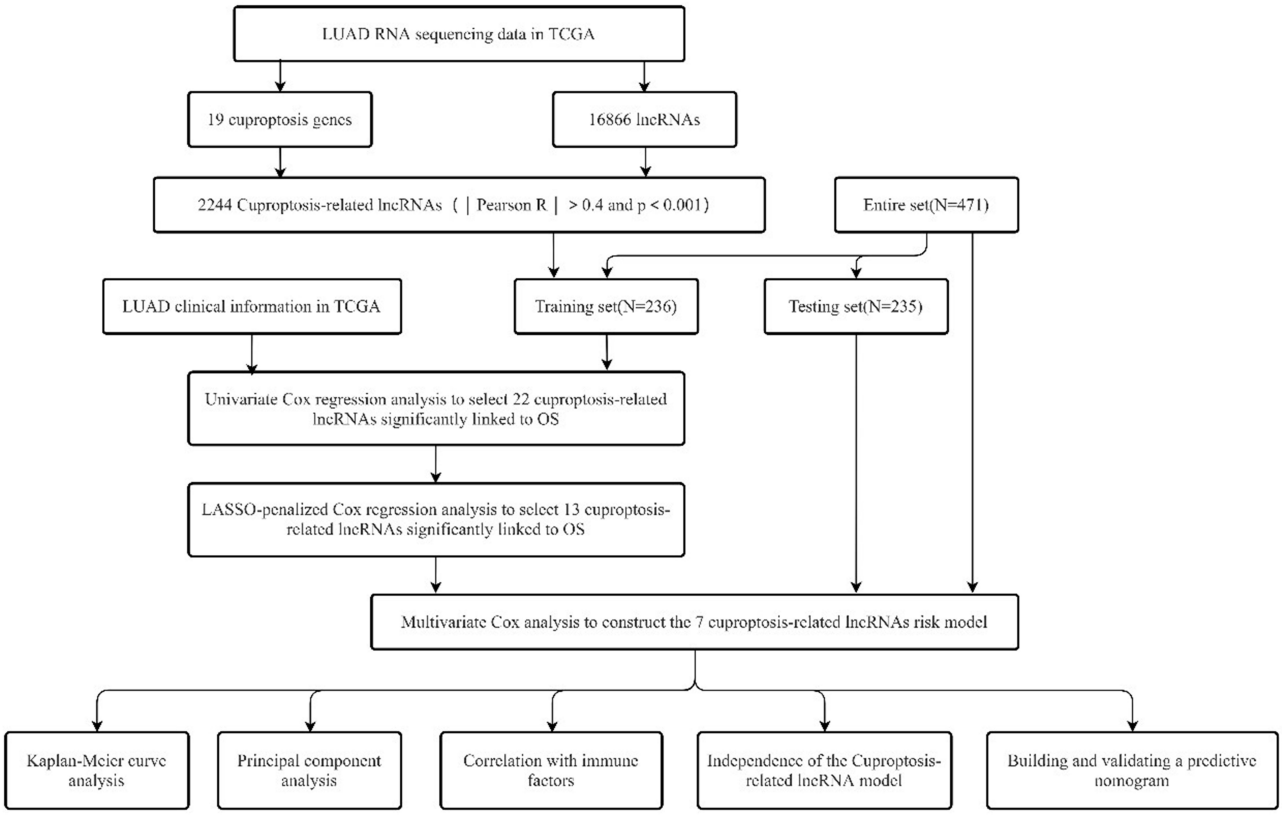


**Figure 1** Flow chart of this study.

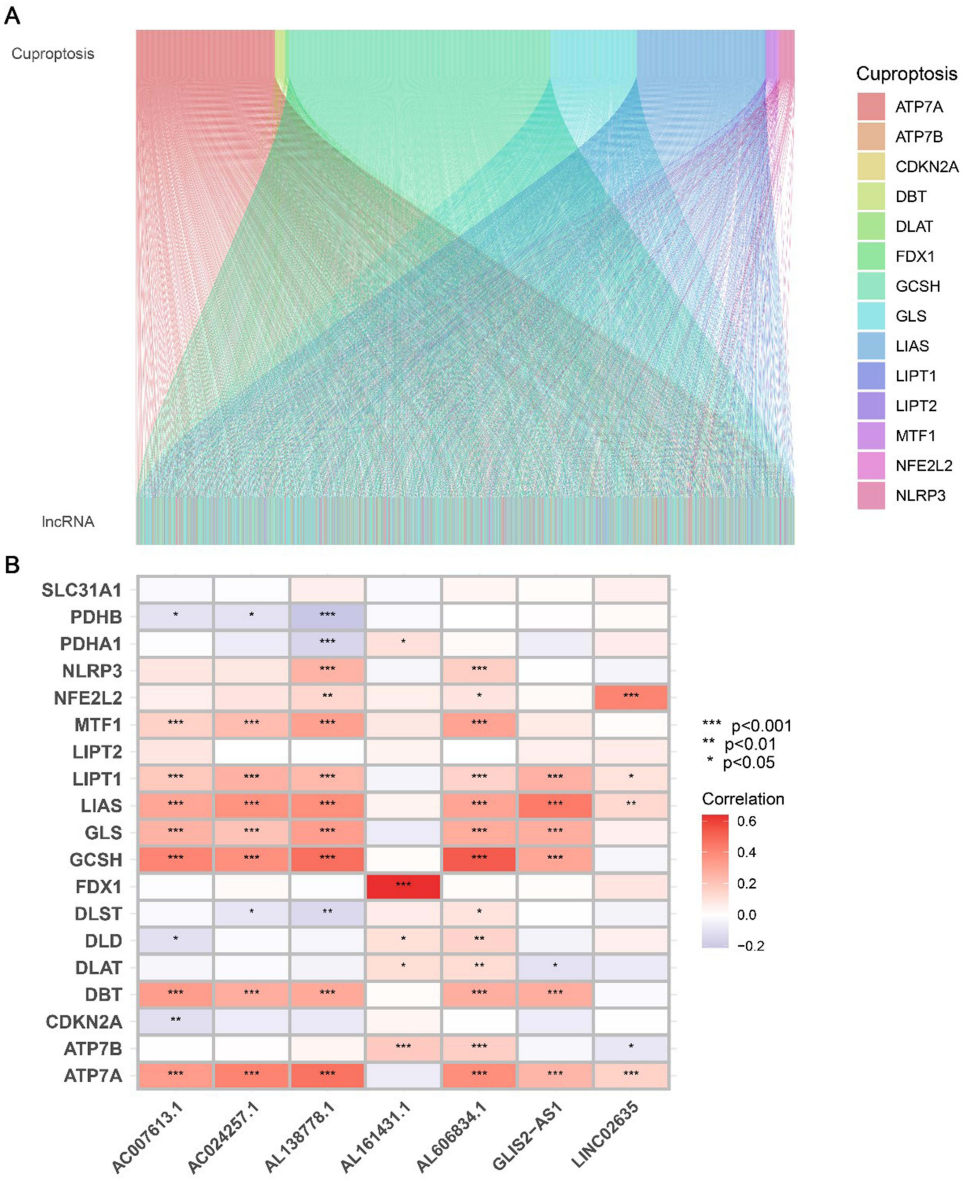


**Figure 2** Identification of cuproptosis-associated lncRNAs in LUAD patients.(**A**) Sankey relational diagram for 19 cuproptosis genes and cuproptosis-associated lncRNAs. (**B**) Heatmap for the correlations between 19 cuproptosis genes and the 7 prognostic cuproptosis-associated lncRNAs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

advantage of Cox analysis over conventional stepwise regression analysis is that it may assess all independent samples simultaneously, hence enhancing the stability of the model. Principal benefit of the lasso approach is that its ability to remove unimportant independent variables and reduce overfitting. To minimize data overfitting, we ran Lasso regression on the major screen variables for further screening analysis and provided the spectrum of Lasso regression coefficients. The subsequent multivariate analysis will concentrate on 13 cuproptosis-associated genes (Figure 3B and C). They were used to develop a risk model for LUAD patient prognosis risk assessment. In the training queue, 7 cuproptosis-associated lncRNAs separately associated with OS were predictive proteins. The median value was used to split patients in the training category into high-risk and low-risk categories based on LUAD patients prognostic risk values. The survival status, survival time, and risk value distributions for all lung adenocarcinomas are shown in Figure 4A and B. Clearly, death rates increase in proportion to risk values as patient risk rises. Figure 4C illustrates the relative expression criteria for each patient's 7 cuproptosis-associated lncRNAs. We found that OS was significantly shorter in the high-risk group than in the low-risk group in the training set, internal test set and external verification set training. (p less than 0.001). (Figure 4D.)

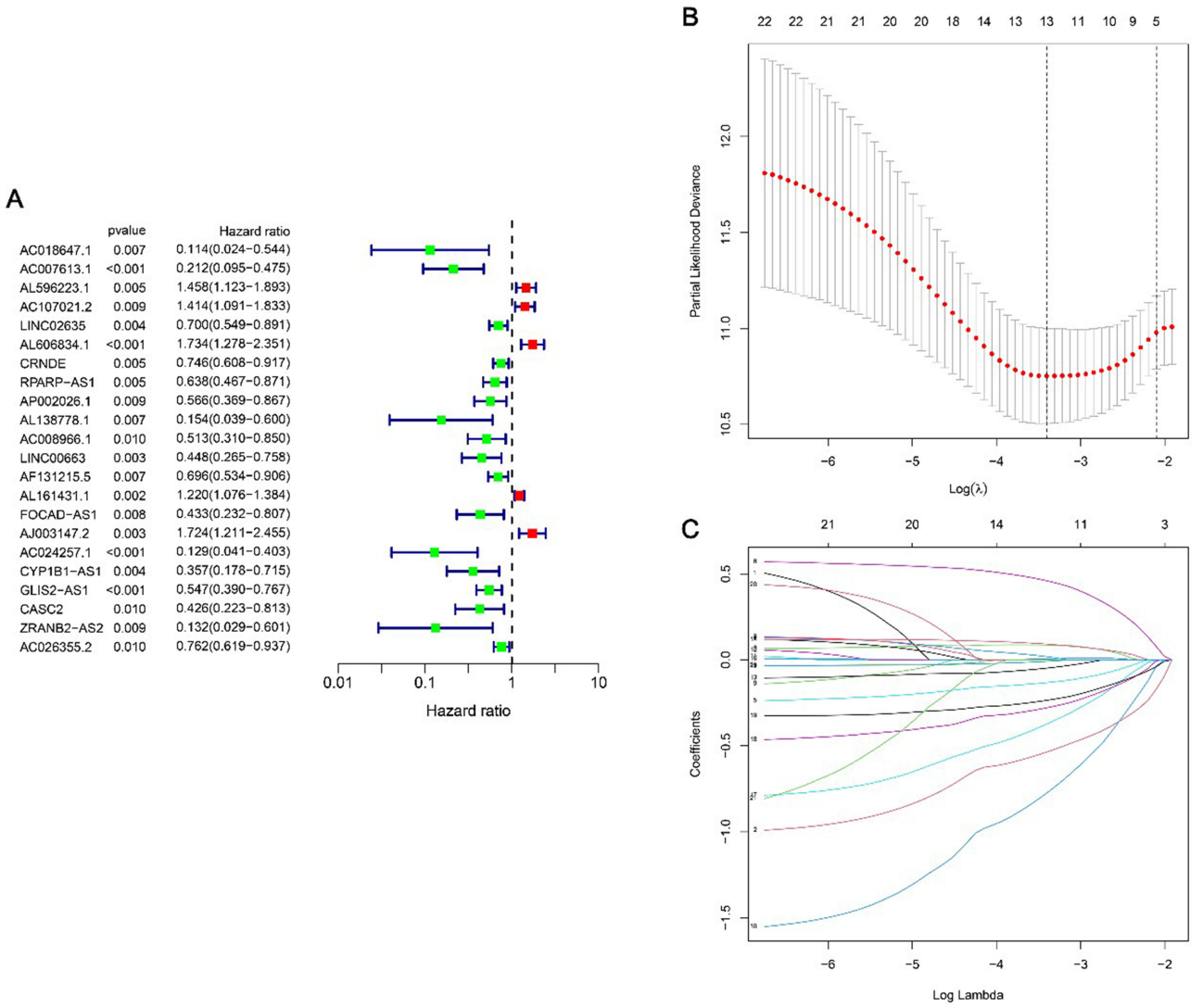


**Figure 3** Risk model for LUAD patients based on cuproptosis-associated lncRANs.(**A**) Univariate Cox regression analysis revealed that the selected lncRNAs significantly correlated with clinical prognosis. (**B**) The tuning parameters (log l) of OS-related proteins were selected to cross-verify the error curve. According to the minimal criterion and 1-se criterion, perpendicular imaginary lines were drawn at the optimal value. (**C**) The LASSO coefficient profile of 13 OS-related lncRNAs and perpendicular imaginary line were drawn at the value chosen by 10-fold cross-validation.

To evaluate the model's ability to predict outcomes, we computed the risk value for each patient in the testing set and the entire set, and then created separate plots for each category. Figure 5 illustrates the expression of cuproptosis-associated lncRNAs in the testing and entire sets (Figure 5A–C), together with the distribution of risk grades, survival status, and survival time. According to Kaplan-Meier analyses conducted on the testing set and the whole set, the OS of LUAD patients with greater risk ratings was inferior than that of patients with lower risk ratings (Figure 5D–H).

To more thoroughly evaluate the clinical effectiveness of the prognostic model, a Kaplan-Meier survival analysis was conducted on the clinical characteristics of the low-risk and high-risk categories in the TCGA full set. The OS of the low-risk category remained superior to that of the high-risk category across subcategories defined by age, gender, tumor stage, T stage, and N stage (Figure 6).

## Principal-Component Analysis Verifies the Cuproptosis-Associated lncRNA Model's Potential to Further Cluster

Utilizing data dimensionality reduction techniques like PCA, which transform high-dimensional data into low-dimensional data by extracting feature vectors, these attributes are shown on two- or three-dimensional graphs. PCA

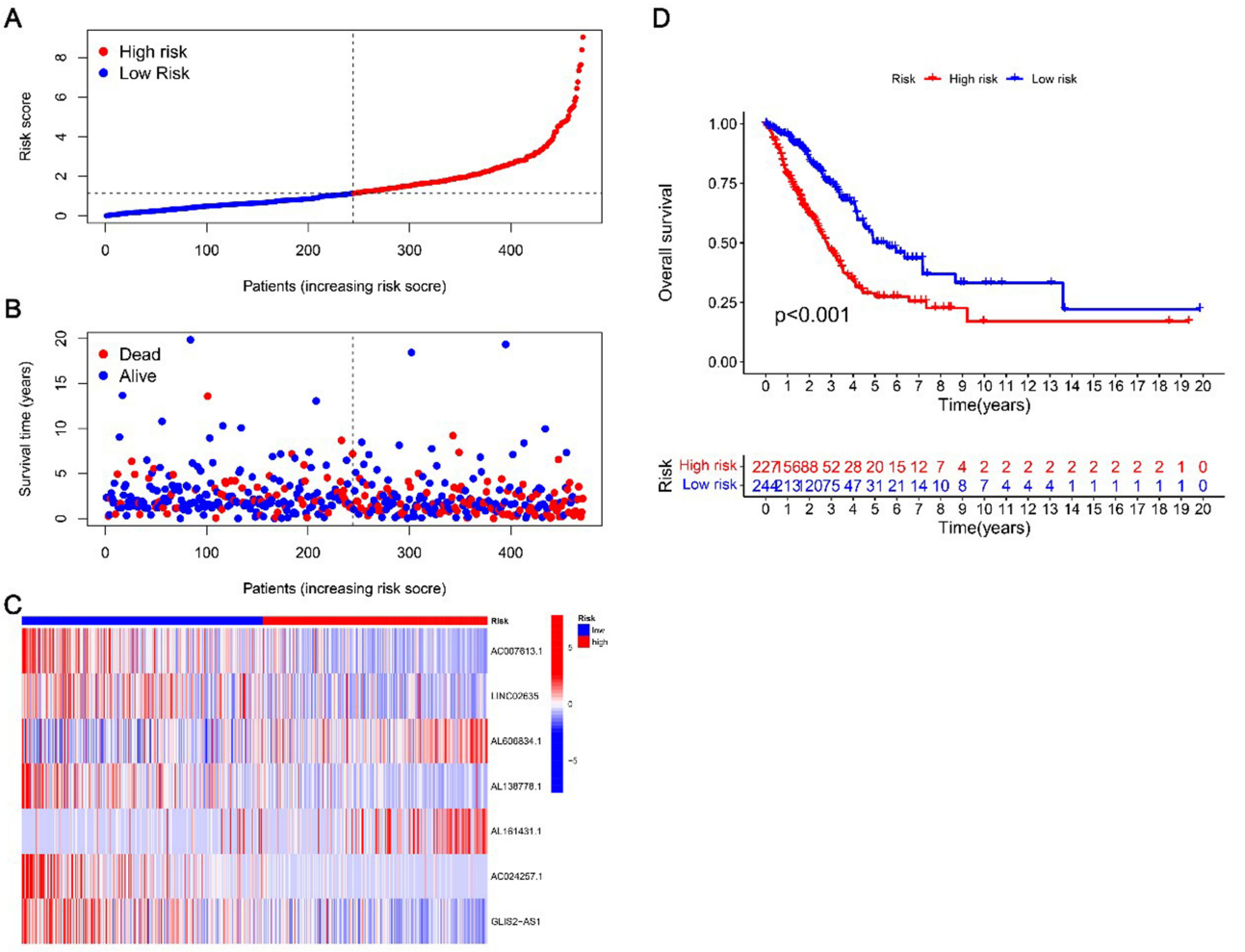


**Figure 4** Prognostic value of the risk patterns of the 7 cuproptosis-associated lncRNAs in the TCGA training set. (**A**) Distribution of cuproptosis-associated lncRNA model-based risk score. (**B**) Different patterns of survival status and survival time between the high- and low-risk groups. (**C**) Clustering analysis heatmap shows the expression standards of the 7 prognostic lncRNAs for each patient. (**D**) Kaplan-Meier survival curves of the OS of patients in the high- and low risk groups.

is often used to evaluate sample-to-sample variations in expression pattern datasets. Utilizing PCA, we compared the expression profiles of the entire gene, 19 cuproptosis genes, 2244 cuproptosis-associated lncRNAs, and a risk model based on the expression profiles of 7 cuproptosis-associated lncRNAs (Figure 7A–D). These four graphs illustrate the patterns of distribution between the high-risk and low-risk categories. The test findings indicate that the prognostic risk model can discriminate between categories with low and high risk.

## Functional Enrichment Analysis of Cuproptosis-Related lncRNA

Enhancing Gene set variation analysis was utilized to examine the biological processes and pathways of the cuproptosis-based model, as well as the KEGG pathway analysis, in order to comprehend the molecular mechanism behind the cuproptosis-based risk model (Figure 8A–D). Axoneme assembly, collage-containing extracellular matrix, motile cilium, axoneme, ciliary plasm, lamellar body, glycosaminoglycan binding, endopeptidase inhibitor activity, peptidase inhibitor activity, heparin binding, and secine-type endopeptidase inhibitor activity were the seven cuproptosis-associated lncRNAs that were most heavily involved in the GO analysis (Figure 8A). In the KEGG pathway enrichment analysis, the seven lncRNAs associated with cuproptosis were also significantly associated with endopeptidase activity regulation, proteolysis regulation, humoral immune response, cilium movement, endopeptidase activity regulation, peptidase activity regulation, hormone metabolic process, protein processing, cell killing, microtubule bundle formation, and leukocyte-mediated cytotoxicity (Figure 8B).

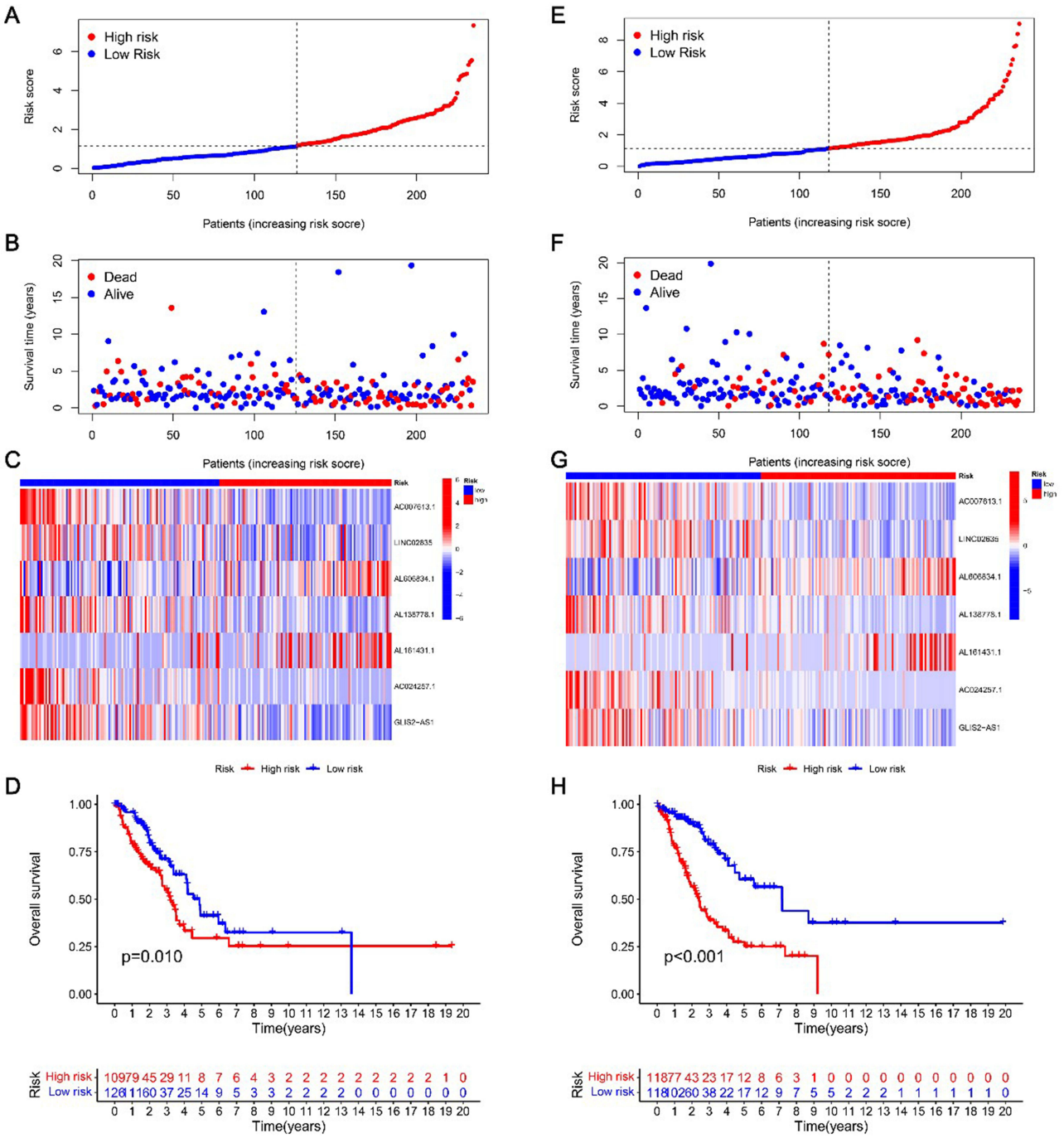


**Figure 5** Prognostic value of the risk model of the 7 cuproptosis-related lncRNAs in the TCGA testing and entire sets. (**A**) Distribution of cuproptosis -related lncRNA model-based risk score for the testing set. (**B**) Patterns of the survival time and survival status between the high- and low-risk groups for the testing set. (**C**) Clustering analysis heatmap shows the display levels of the 7 prognostic lncRNAs for each patient in the testing set. (**D**) Kaplan-Meier survival curves of the OS of patients in the high- and low-risk groups for the testing set. (**E**) Distribution of the cuproptosis-associated lncRNA model-based risk score for the entire set. (**F**) Patterns of the survival time and survival status between the high- and low-risk groups for the entire set. (**G**) Clustering analysis heatmap shows the expression levels of the 7 prognostic lncRNAs for each patient for the entire set. (**H**) Kaplan-Meier survival curves of OS of patients in the low- and high-risk groups for the entire set.

## Estimation of the Tumor Immune Microenvironment and Cancer Immunotherapy Response Using the Cuproptosis-Related lncRNA Model

Utilizing the cuproptosis-associated lncRNA model, estimate the tumor immune milieu and therapeutic response. We analyzed immunological markers in 471 cancer of the lungs patients. The immune-associated processes of patients in the

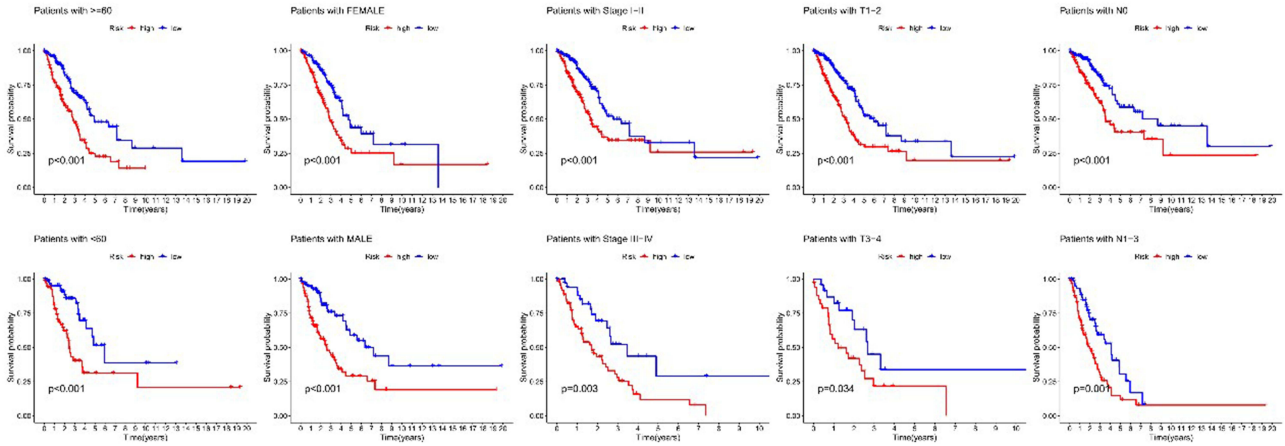


**Figure 6** Kaplan-Meier curves of OS differences stratified by gender, age, tumor grade, or TNM stage between the high- and low-risk groups in the TCGA entire set.

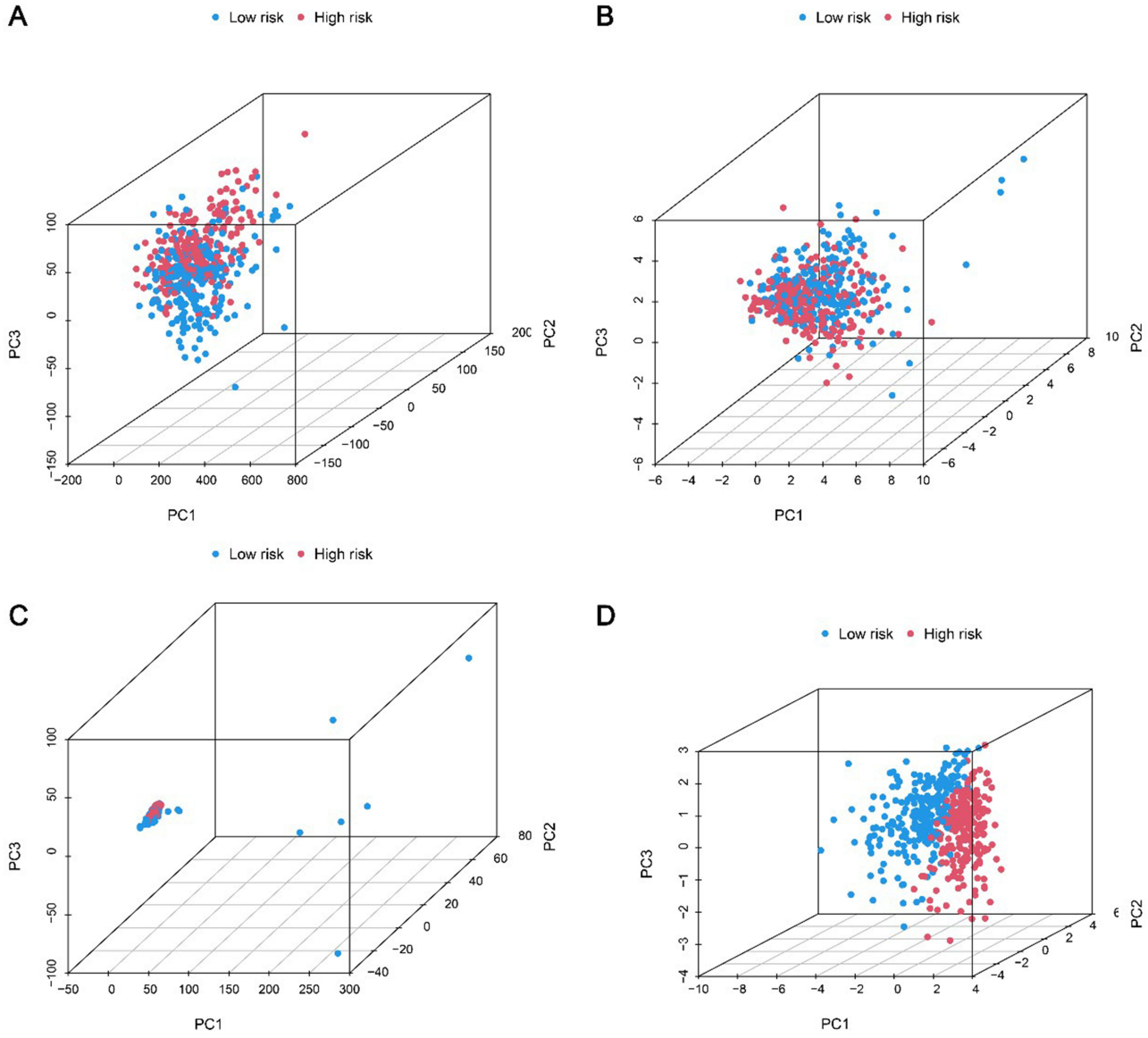


**Figure 7** Principal component analysis between the high- and low-risk groups based on cuproptosis-associated lncRNAs model.(**A**) entire gene expression profiles, (**B**) 19 cuproptosis genes, (**C**) 7 cuproptosis - associated lncRNAs, and (**D**) risk model based on the representation profiles of the 7 cuproptosis- associated lncRNAs in the TCGA entire set.

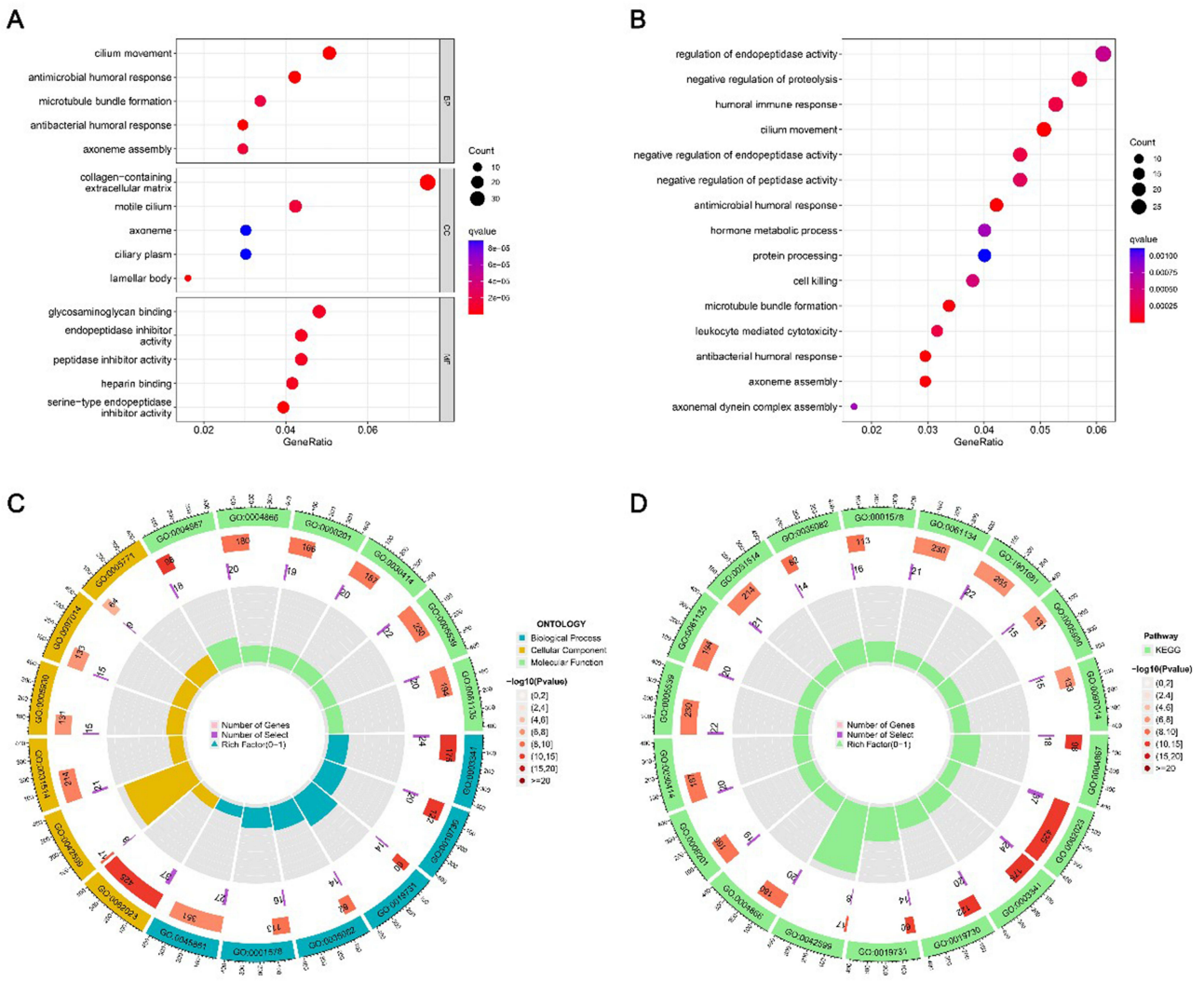


**Figure 8** Pathway enrichment analysis of cuproptosis- associated lncRNAs in LUAD patients of TCGA. (**A** and **C**) the enriched item in the gene ontology analysis; (**B** and **D**) the enriched item in the Kyoto Encyclopedia of Genes and Genomes analysis.

low- and high-risk categories vary significantly, according to studies (Figure 9A). 95.54% (214/224) of high-risk category tumor samples included cuproptosis-associated gene alterations, compared to 85.65% (203/237) of low-risk category tumor samples, with TP53 accounting for the bulk of occurrences (Figure 9B and C). This research examined the connection between the cuproptosis-associated lncRNA model and immunotherapy biomarkers. As anticipated, the cuproptosis-based classifier index indicated that the low-risk category responded less favorably to immunotherapy than the high-risk category, showing that it might be used to predict tumor immune dysfunction and exclusion (Figure 9D). We discriminated these categories further by generating TMB values in tumor patients utilizing TCGA somatic mutation data. TMB was strongly associated with the cuproptosis-based classifier index, as shown by the fact that TMB values were greater in the low risk category than in the high risk category (Figure 9E). Additionally, the research found that H-TMB patients had longer life spans (Figure 9F). Therefore, we compared the TMB and OS of patients in both categories to see if cuproptosis-associated lncRNAs might more correctly predict OS than TMB. The high-risk category had both high TMB values (H-TMB+high risk) and low TMB values (L-TMB+high risk), while the low-risk category had both high TMB values (H-TMB+low risk) and low TMB sores (H-TMB+low risk). (Please check Figure 9G.) Contrary to expectations, those with H-TMB+high risk fared better in terms of survival than those with L-TMB+high risk. The survival curves of patients with H-TMB and L-TMB in the low-risk category (H-TMB+low risk) were

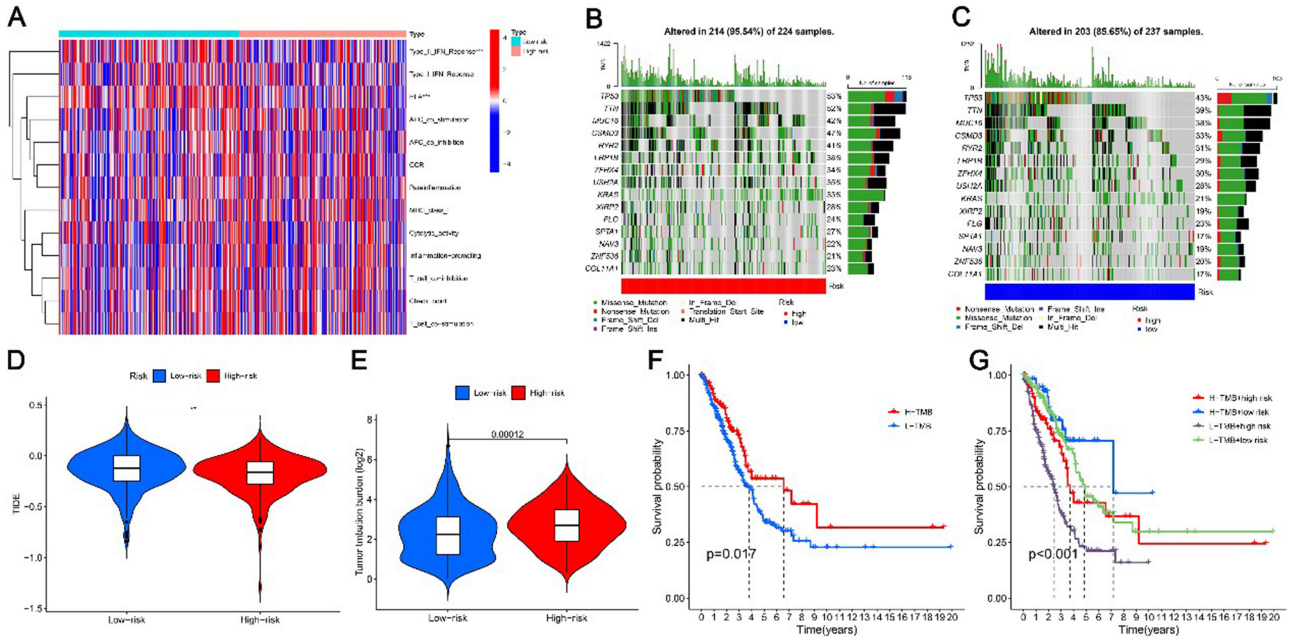


**Figure 9** Estimation of the tumor immune microenvironment and cancer immunotherapy response using the cuproptosis-associated lncRNA model in the TCGA entire set. (**A**) The indicated standards of the immunity index for each patient. (**B** and **C**) Waterfall plot displays mutation information of the genes with high mutation frequencies in the high-risk group (**B**) and low-risk group (**C**). (**D**) TIDE prediction difference in the high- and low-risk patients. (**E**) TMB difference in the high- and low-risk patients. (**F**) Kaplan-Meier curve analysis of OS is shown for patients of the entire set. (**G**) Kaplan-Meier curve analysis of OS is shown for patients classified according to the TMB and cuproptosis- associated lncRNA model.

comparable, demonstrating that TMB mutation status failed to differentiate the survival rate in the low-risk category. These results suggest that the cuproptosis-associated lncRNA model may be more prognostic than the TMB model.

## Evaluation of LUAD Clinical Characteristics and Cuproptosis-Associated lncRNAs Predictive Risk Model

Using univariate and multivariate Cox regression analyses, it was determined whether the risk model of 7cuproptosis-associated lncRNAs exhibited independent LUAD prediction value. In a univariate Cox regression analysis, the risk value's hazard ratio was 1.317 (95% confidence interval: 1.209–1.434) (P less than 0.001), but it was 1.253 in a multivariate Cox regression research (95% CI 1.144–1.372). (Figure 10A and B) The findings indicate that clinico-pathological variables such as gender, age, tumor stage, N stage, and M stage had no influence on the risk model of the seven cuproptosis-associated lncRNAs. The area under the ROC curve and risk value conformance index were generated to evaluate the independence and dependability of this risk model. Throughout time, the risk grade seemed to be able to predict the prognosis of LUAD more accurately than other clinical indicators, as shown by the fact that its c-index remained the highest. (Figure 10C) With AUCs of 0.744, 0.684, and 0.644 in 1, 3, and 5 years, respectively, the risk value demonstrated excellent predictive capacity (Figure 10D). The AUC of the risk grade was greater than the AUCs of the other clinicopathological parameters, suggesting that the predictive risk model of the 7 cuproptosis-associated lncRNAs for LUAD is rather accurate (Figure 10E).

The expression of candidate cuproptosis-associated lncRNAs AL606834.1, AL161431.1, AC007613.1, and LINC02835 in LUAD tissues and adjacent normal tissues, as well as the relationship between the expression of candidate cuproptosis-associated lncRNAs in LUAD tissues and clinicopathological characteristics of LUAD patients.

We selected 120 tissue samples (including 60 LUAD tissues and 60 normal adjacent tissues) to analyze the expression of candidate cuproptosis-associated lncRNAs AL606834.1, AL161431.1, AC007613.1, and LINC02835 in LUAD tissues by qRT-PCR. The results showed that the expression of candidate cuproptosis-associated lncRNAs AL606834.1 and AL161431.1 in LUAD tissues was significantly higher than that in normal adjacent tissues (Figure 11A–C). While the expression of candidate cuproptosis-associated lncRNAs AC007613.1, and LINC02835 in LUAD tissues was

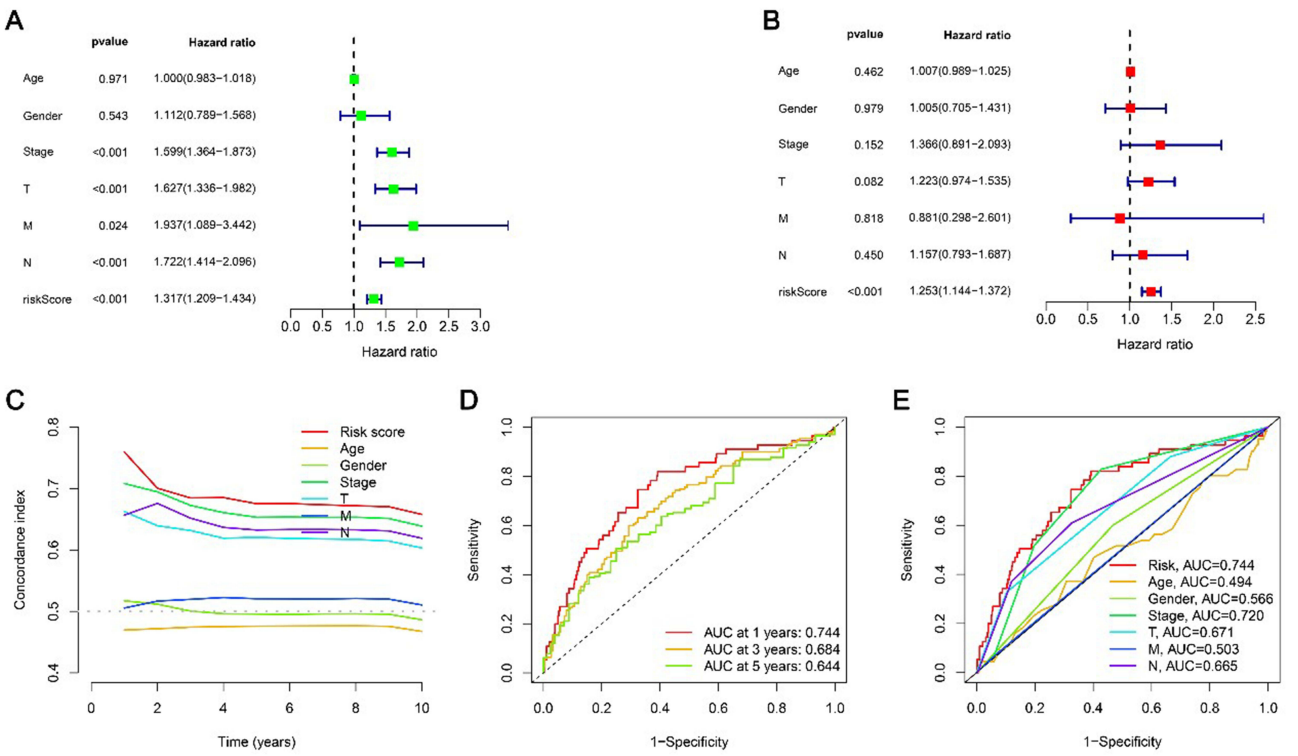


**Figure 10** Assessment of the prognostic risk model of the cuproptosis- associated lncRNAs and clinical features in LUAD in the TCGA entire set. (**A** and **B**) Univariate(**A**) and multivariate analyses(**B**) of the clinical characteristics and risk score with the OS. (**C**) Concordance indexes of the risk score and clinical characteristics. (**D**) ROC curves of nomogram in predicting 1-, 3-, and 5-years OS. (**E**) ROC curves of the clinical characteristics and risk score.

significantly lower than that in normal adjacent tissues (Figure 11D–G). To further investigate the correlation between candidate cuproptosis-associated lncRNAs AL606834.1, AL161431.1, AC007613.1, and LINC02835 expression and clinicopathological features of LUAD, the above samples were divided into high (above the mean) and low (below the mean) candidate cuproptosis-associated lncRNAs expression groups. Subsequently, the chi-square test was used to analyze the relationship between candidate cuproptosis-associated lncRNAs AL606834.1, AL161431.1, AC007613.1, and LINC02835 expression level and clinicopathological characteristics of LUAD patients, and the results showed that the expression level of candidate cuproptosis-associated lncRNAs AL606834.1 and AL161431.1 in LUAD tissues was significantly positively correlated with tumor stage, lymph node metastasis and histopathological grade of LUAD, while the expression level of candidate cuproptosis-associated lncRNAs AC007613.1, and LINC02835 in LUAD tissues was significantly negatively correlated with tumor stage, lymph node metastasis and histopathological grade of LUAD (Figure 11B, D, F, H and Tables 1–4).

## Validation of the Model

At the same time, we also used clinical samples for external validation, and found that the low-risk group was superior to the high-risk group in terms of age and other factors. We observed a trend toward longer survival in the low-risk group than in the high-risk group (Figure 12A–D). Overall, the model was applicable to LUAD patients with different clinical characteristics.

# Discussion

The most prevalent histological subtype of lung cancer is adenocarcinoma.[1,4] Several studies on the prevalence, progression, and therapy of lung adenocarcinoma are now being conducted.[27,28] Numerous investigations have shown that the clinical symptoms and prognosis of diverse subcategories of lung cancer vary. In recent years, there has been an increase in research on the predictive value of lncRNAs in cancer of the lungs patients' survival and immunotherapy response.

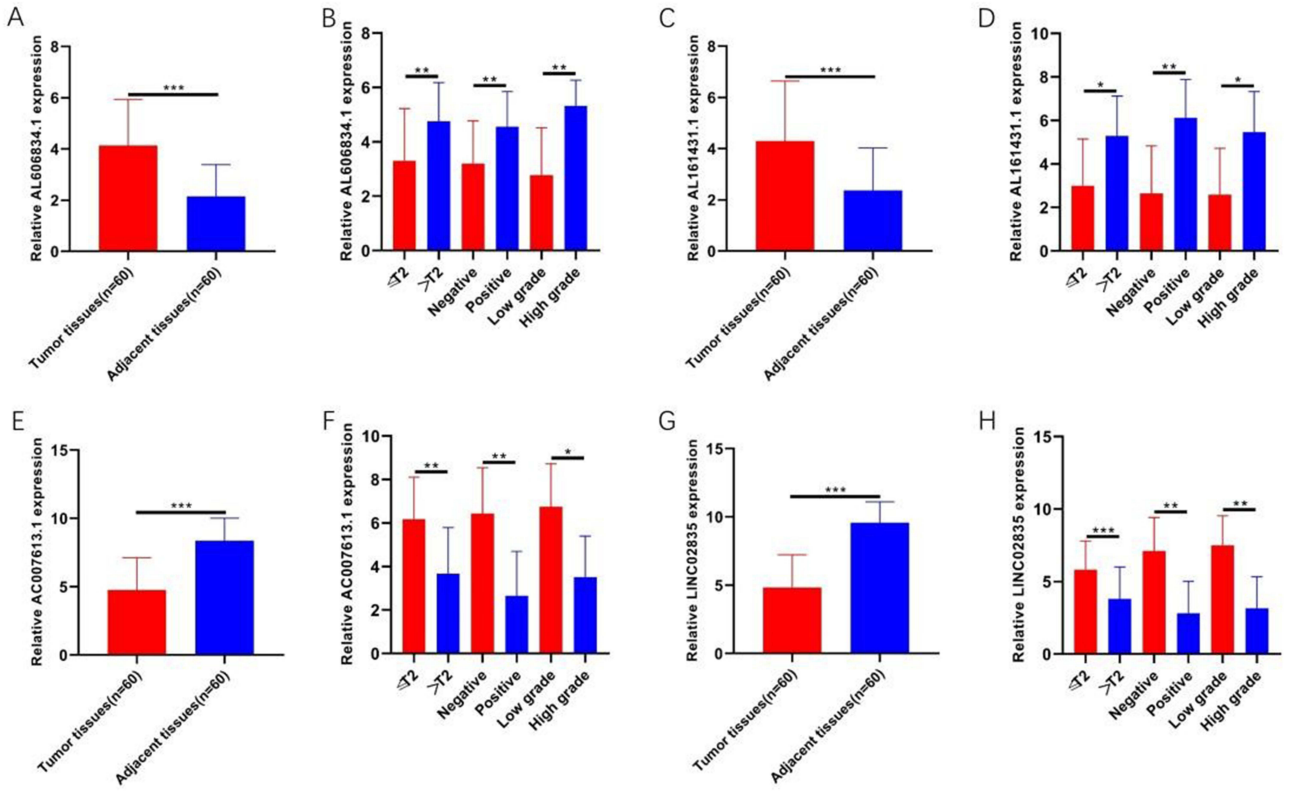


**Figure 11** The expression of candidate cuproptosis-associated lncRNAs and the relationship with clinicopathological characteristics of LUAD patients. The expression of candidate cuproptosis-associated lncRNAs AL606834.1(**A**), lncRNAs AL161431.1(**C**), lncRNAs AC007613.1(**E**), lncRNAs LINC02835(**G**) in LUAD tissues and adjacent normal tissues. The relationship between the expression of lncRNAs AL606834.1(**B**), lncRNAs AL161431.1(**D**), lncRNAs AC007613.1(**F**), lncRNAs LINC02835(**H**) in LUAD tissues and clinicopathological characteristics of LUAD patients. Two-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

LncRNAs are transcription factors that are longer than 200 nucleotides but lack the ability to encode proteins.[29] LncRNAs have a role in the beginning and progression of several diseases[30,31] and have the ability to specifically inhibit or stimulate gene expression. LncRNAs are likely to be employed as biomarkers for illness diagnosis and prognosis,

**Table 1** The Relationship Between AL606834.1 Expression Level in LUAD and Clinicopathological Features of LUAD Patients (n=60)

| Characteristics | AL606834.1 | | | |
|---|---|---|---|---|
| | Low No. Cases | High No. Cases | Chi-squared test | P-value |
| All patients | (n=25) | (n=35) | | |
| Gender | | | 0.49 | 0.484 |
| Male | 12 | 20 | | |
| Female | 13 | 15 | | |
| Age (years) | | | 0.433 | 0.511 |
| ≤60 | 10 | 17 | | |
| >60 | 15 | 18 | | |
| Tumor stage | | | 9.16 | 0.002 |
| ≤T2 | 17 | 10 | | |
| >T2 | 8 | 25 | | |
| Lymph-node metastasis | | | 8.297 | 0.004 |
| Negative | 18 | 12 | | |
| Positive | 7 | 23 | | |
| Pathologic grade | | | 6.898 | 0.009 |
| Low grade | 14 | 8 | | |
| High grade | 11 | 27 | | |

**Table 2** The Relationship Between AL161431.1 Expression Level in LUAD and Clinicopathological Features of LUAD Patients (n=60)

| Characteristics | AL161431.1 | | | |
|---|---|---|---|---|
| | Low No. Cases | High No. Cases | Chi-squared test | P-value |
| All patients | (n=23) | (n=37) | | |
| Gender | | | 0.455 | 0.5 |
| Male | 11 | 21 | | |
| Female | 12 | 16 | | |
| Age (years) | | | 0.004 | 0.951 |
| ≤60 | 12 | 19 | | |
| >60 | 11 | 18 | | |
| Tumor stage | | | 11.944 | 0.001 |
| ≤T2 | 16 | 9 | | |
| >T2 | 7 | 28 | | |
| Lymph-node metastasis | | | 11.125 | 0.001 |
| Negative | 17 | 11 | | |
| Positive | 6 | 26 | | |
| Pathologic grade | | | 8.511 | 0.004 |
| Low grade | 15 | 10 | | |
| High grade | 8 | 27 | | |

**Table 3** The Relationship Between AC007613.1 Expression Level in LUAD and Clinicopathological Features of LUAD Patients (n=60)

| Characteristics | AC007613.1 | | | |
|---|---|---|---|---|
| | Low No. Cases | High No. Cases | Chi-squared test | P-value |
| All patients | (n=20) | (n=40) | | |
| Gender | | | 0.134 | 0.714 |
| Male | 10 | 18 | | |
| Female | 10 | 22 | | |
| Age (years) | | | 0.033 | 0.855 |
| ≤60 | 11 | 21 | | |
| >60 | 9 | 19 | | |
| Tumor stage | | | 8.688 | 0.003 |
| ≤T2 | 6 | 28 | | |
| >T2 | 14 | 12 | | |
| Lymph-node metastasis | | | 7.033 | 0.008 |
| Negative | 8 | 30 | | |
| Positive | 12 | 10 | | |
| Pathologic grade | | | 11.868 | 0.001 |
| Low grade | 7 | 32 | | |
| High grade | 13 | 8 | | |

**Table 4** The Relationship Between LINC02835 Expression Level in LUAD and Clinicopathological Features of LUAD Patients (n=60)

| Characteristics | LINC02835 | | | |
|---|---|---|---|---|
| | Low No. Cases | High No. Cases | Chi-squared test | P-value |
| All patients | (n=22) | (n=38) | | |
| Gender | | | 0.463 | 0.496 |
| Male | 9 | 19 | | |

(*Continued*)

**Table 4** (Continued).

| Characteristics | LINC02835 | | | |
|---|---|---|---|---|
| | Low No. Cases | High No. Cases | Chi-squared test | P-value |
| Female | 13 | 19 | | |
| Age (years) | | | 0.021 | 0.886 |
| ≤60 | 10 | 18 | | |
| >60 | 12 | 20 | | |
| Tumor stage | | | 10.88 | 0.001 |
| ≤T2 | 8 | 30 | | |
| >T2 | 14 | 8 | | |
| Lymph-node metastasis | | | 10.048 | 0.002 |
| Negative | 7 | 28 | | |
| Positive | 15 | 10 | | |
| Pathologic grade | | | 10.371 | 0.001 |
| Low grade | 9 | 31 | | |
| High grade | 13 | 7 | | |

since several studies in recent years have shown their role in the development and survival of cancer patients. Copper is a vital trace element and cofactor for enzymes.

Throughout the whole animal kingdom.[32] Genetic variations in copper homeostasis cause illnesses with a high death rate. Copper concentrations in cells are a good indicator of cell viability. Cuproptosis is a new kind of programmed cell

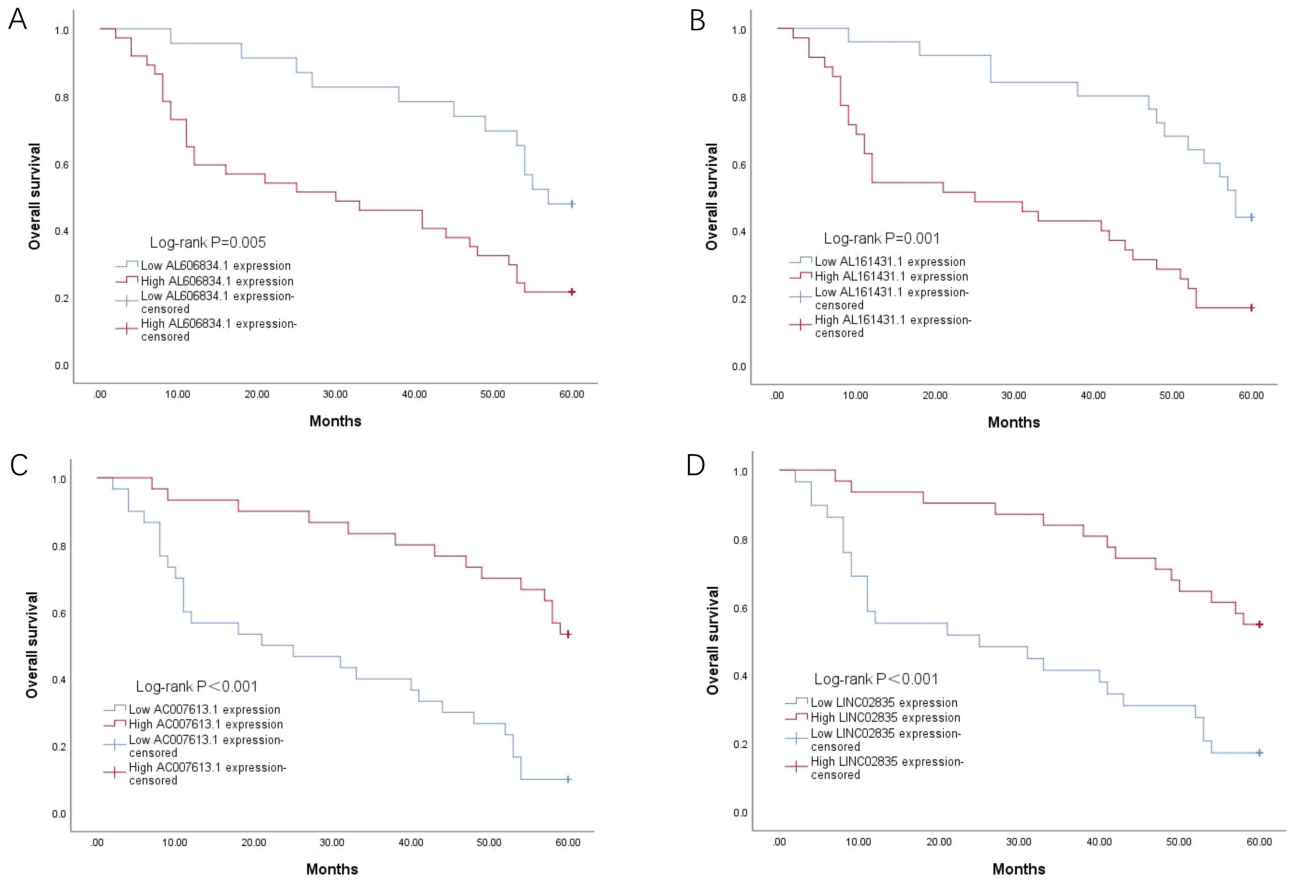


**Figure 12** Relationship between candidate cuproptosis-associated lncRNAs AL606834.1(**A**), lncRNAs AL161431.1(**B**), lncRNAs AC007613.1(**C**), lncRNAs LINC02835(**D**) expression and prognosis of patients with LUAD.

death in which excessive copper in the cellular matrix disrupts certain mitochondrial enzymes, induces protein lipoylation, and results in a unique mode of cell death.[8] Despite the fact that copper may influence the expression of a large number of genes in the body, no research has been undertaken to explain the molecular mechanism of copper-associated lncRNA in cancer of the lungs. We evaluated the functions of cuproptosis and lncRNA in cancer of the lungs and created a risk prediction model for cuproptosis-associated genes. Since the connection of cuproptosis and lncRNAs in LUAD piqued our interest, we wanted to construct an independent model based on cuproptosis-associated lncRNAs in this investigation.

Using the TCGA database, 2244 genes associated with cuproptosis were identified, and their predictive roles were investigated in this work. 13 cuproptosis-associated genes in LUAD patients were evaluated by univariate regression, and 7 of these genes were leveraged to create a cuproptosis-associated lncRNA model to predict patients' overall survival. According to one research,[33] AL606834.1 accelerates the progression of prostate cancer. AL606834.1, one of the predictive risk models of ferroptosis-associated lncRNA in LUAD, was verified in a second research.[21] Several additional studies[11,14,34] have demonstrated that immune-associated disorders are prevalent. Breast cancer, prostate cancer, non-small cell cancer of the lungs, endometrial cancer, and lung adenocarcinoma are affected by AL161431.1. Additionally, other cuproptosis-associated lncRNAs are the first reporter genes. On the basis of their median risk value, LUAD patients were then divided into high- and low-risk categories, with the high-risk category exhibiting markedly worse clinical outcomes. The cuproptosis-associated lncRNA model was identified as an autocephalous risk factor for OS by multivariable Cox regression analysis. According to ROC analysis, the system outperformed conventional clinical indications in LUAD survival prediction. Also, for the 1-year, 3-year, and 5-year OS, we produced a nomogram that demonstrates perfect agreement between observed and anticipated rates. The observed and anticipated 1-, 3-, and 5-year OS rates exhibited a high degree of concordance. The risk model was quite accurate, and this prediction model might aid in the identification of new biomarkers for further investigation. It was based on seven cuproptosis-associated lncRNAs that were separately linked to OS.

Mutational load refers to the number of somatic mutations remaining in the tumor genome after germline mutations have been eliminated.[35] Greater tumor immunogenicity is associated with a larger mutational load, indicating that PD-1/PD-L1 immune checkpoint inhibitor treatment will be more effective.[36,37] According to our results, the category at low risk had a greater TMB than the category at high risk. In addition, recent research has used the TIDE algorithm, which has been shown to accurately forecast the infiltration of immune cells into tumor tissue.[38,39] We found that the TIDE algorithm accurately predicted improved immunotherapy outcomes in high-risk patients. This work demonstrates that, when combined with previously disclosed data, this prediction model offers a viable immunological biomarker for tumor treatment.

Our findings provide novel insights into the molecular biological processes involving lncRNAs that are associated with cuproptosis in LUAD. In actual clinical practice, the prognosis of lung cancer patients is closely associated to tumor stage. Due to the variability of the human body, people with the same stage have different survival lengths. This suggests that the exact treatment of malignancies remains difficult. Consequently, more research on novel biological targets for diagnosis and treatment is essential. The discovery of cuproptosis-associated lncRNA expands the potential cuproptosis gene regulation of lncRNA and offers a novel way for predicting the prognosis of LUAD patients. In this work, we applied a range of methods to verify the model's prediction abilities.

According to the findings of this research, the prognostic risk model based on cuproptosis-associated lncRNA may predict the OS of lung adenocarcinoma consistently and is an independent prognostic signal for the disease. For more precise treatment, the number of digits may be used to classify LUAD patients into high-risk and low-risk categories. Our study constructed a cuprotosis related lncRNA tag for LUAD, providing new insights into survival prediction and clinical treatment outcomes for LUAD patients.

## Limitations

This study still has some limitations. Firstly, The TCGA dataset lacks detailed information such as smoking history and treatment. Secondly, the sample size of clinical specimens is relatively small. Moreover, we will continue to focus on and improve this limitation in the future.

## Data Sharing Statement

Datasets produced and/or studied during present research are usable in [https://portal.gdc.cancer.gov/].

## Acknowledgments

This project was funded and approved by the Hunan Provincial Natural Science Foundation (Provincial and Municipal Joint Fund) (#2022JJ2216).

## Disclosure

The authors declare no conflicts of interest in this work.

## References

1. Lortet-Tieulent J, Soerjomataram I, Ferlay J, et al. International trends in lung cancer incidence by histological subtype: adenocarcinoma stabilizing in men but still increasing in women. *Lung Cancer*. 2014;84(1):13–22. doi:10.1016/j.lungcan.2014.01.009
2. Wu F, Wang L, Zhou C. Lung cancer in China: current and prospect. *Curr Opin Oncol*. 2021;33(1):40–46. doi:10.1097/cco.0000000000000703
3. Travis WD, Brambilla E, Noguchi M, et al. International association for the study of lung cancer/American thoracic society/European respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol*. 2011;6(2):244–285. doi:10.1097/JTO.0b013e318206a221
4. Meza R, Meernik C, Jeon J, et al. Lung cancer incidence trends by gender, race and histology in the United States, 1973–2010. *PLoS One*. 2015;10(3):e0121323. doi:10.1371/journal.pone.0121323
5. Zhang C, Zhang J, Xu FP, et al. Genomic landscape and immune microenvironment features of preinvasive and early invasive lung adenocarcinoma. *J Thorac Oncol*. 2019;14(11):1912–1923. doi:10.1016/j.jtho.2019.07.031
6.. Jurisic V, Vukovic V, Obradovic J, et al. EGFR polymorphism and survival of NSCLC patients treated with TKIs: a systematic review and meta-analysis. *J Oncol*. 2020;2020(1):1973241. doi:10.1155/2020/1973241.
7. Wang F, Lin H, Su Q, Li C. Cuproptosis-related lncRNA predict prognosis and immune response of lung adenocarcinoma. *World J Surg Oncol*. 2022;20(1):275. doi:10.1186/s12957-022-02727-7
8. Bock FJ, Tait SWG. Mitochondria as multifaceted regulators of cell death. *Nat Rev Mol Cell Biol*. 2020;21(2):85–100. doi:10.1038/s41580-019-0173-8
9. Bian Z, Fan R, Xie L. A novel cuproptosis-related prognostic gene signature and validation of differential expression in clear cell renal cell carcinoma. *Genes*. 2022;13(5):851. doi:10.3390/genes13050851
10. Bridges MC, Daulagala AC, Kourtidis A. LNCcation: lncRNA localization and function. *J Cell Biol*. 2021;220(2). doi:10.1083/jcb.202009045
11. Ma G, Li G, Fan W, et al. The role of long noncoding RNA AL161431.1 in the development and progression of pancreatic cancer. *Front Oncol*. 2021:11666313. doi:10.3389/fonc.2021.666313.
12. Xia F, Yan Y, Shen C. A prognostic pyroptosis-related lncRNAs risk model correlates with the immune microenvironment in colon adenocarcinoma. *Front Cell Dev Biol*. 2021;9811734. doi:10.3389/fcell.2021.811734
13. Shang Z, Yu J, Sun L, et al. LncRNA PCAT1 activates AKT and NF-κB signaling in castration-resistant prostate cancer by regulating the PHLPP/FKBP51/IKKα complex. *Nucleic Acids Res*. 2019;47(8):4211–4225. doi:10.1093/nar/gkz108
14. Jiang H, Xu A, Li M, et al. Seven autophagy-related lncRNAs are associated with the tumor immune microenvironment in predicting survival risk of nonsmall cell lung cancer. *Brief Funct Genomics*. 2022;21(3):177–187. doi:10.1093/bfgp/elab043
15. Wang S, Wang T, Liu D, et al. LncRNA MALAT1 aggravates the progression of non-small cell lung cancer by stimulating the expression of COMMD8 via targeting miR-613. *Cancer Manag Res*. 2020:1210735–1210747. doi:10.2147/cmar.S263538.
16. Xu F, Zhan X, Zheng X, et al. A signature of immune-related gene pairs predicts oncologic outcomes and response to immunotherapy in lung adenocarcinoma. *Genomics*. 2020;112(6):4675–4683. doi:10.1016/j.ygeno.2020.08.014
17. Xu F, Lin H, He P, et al. A TP53-associated gene signature for prediction of prognosis and therapeutic responses in lung squamous cell carcinoma. *Oncoimmunology*. 2020;9(1):1731943. doi:10.1080/2162402x.2020.1731943
18. Xu F, He L, Zhan X, et al. DNA methylation-based lung adenocarcinoma subtypes can predict prognosis, recurrence, and immunotherapeutic implications. *Aging*. 2020;12(24):25275–25293. doi:10.18632/aging.104129
19. Hong W, Liang L, Gu Y, et al. Immune-related lncRNA to construct novel signature and predict the immune landscape of human hepatocellular carcinoma. *Mol Ther Nucleic Acids*. 2020:22937–22947. doi:10.1016/j.omtn.2020.10.002.
20. Wu Y, Zhang L, He S, et al. Identification of immune-related LncRNA for predicting prognosis and immunotherapeutic response in bladder cancer. *Aging*. 2020;12(22):23306–23325. doi:10.18632/aging.104115
21. Zheng Z, Zhang Q, Wu W, et al. Identification and validation of a ferroptosis-related long non-coding RNA signature for predicting the outcome of lung adenocarcinoma. *Front Genet*. 2021:12690509. doi:10.3389/fgene.2021.690509.
22. Cai Z, Wang C, Chen C, et al. Quality evaluation of lonicerae japonicae flos and lonicerae flos based on simultaneous determination of multiple bioactive constituents combined with multivariate statistical analysis. *Phytochem Anal*. 2021;32(2):129–140. doi:10.1002/pca.2882
23. Zhao X, Liu X, Cui L. Development of a five-protein signature for predicting the prognosis of head and neck squamous cell carcinoma. *Aging*. 2020;12(19):19740–19755. doi:10.18632/aging.104036
24. Franz M, Rodriguez H, Lopes C, et al. GeneMANIA update 2018. *Nucleic Acids Res*. 2018;46(W1):W60–w64. doi:10.1093/nar/gky311
25. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun*. 2019;10(1):1523. doi:10.1038/s41467-019-09234-6
26. Yu G, Wang LG, Han Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics*. 2012;16(5):284–287. doi:10.1089/omi.2011.0118

27. Su Z, Wang Y, Cao J, et al. Identification and validation of non-coding RNA-mediated high expression of IQGAP3 in poor prognosis of lung adenocarcinoma. *Gene Med*. 2024;26(1):e3664. doi:10.1002/jgm.3664
28. Chen H, Wang Y, Shao C, et al. Molecular subgroup establishment and signature creation of lncRNAs associated with acetylation in lung adenocarcinoma. *Aging*. 2024;16(2):1276–1297. doi:10.18632/aging.205407
29. Matsui M, Corey DR. Non-coding RNAs as drug targets. *Nat Rev Drug Discov*. 2017;16(3):167–179. doi:10.1038/nrd.2016.117
30. Ransohoff JD, Wei Y, Khavari PA. The functions and unique features of long intergenic non-coding RNA. *Nat Rev Mol Cell Biol*. 2018;19 (3):143–157. doi:10.1038/nrm.2017.104
31. Statello L, Guo CJ, Chen LL, et al. Gene regulation by long non-coding RNAs and its biological functions. *Nat Rev Mol Cell Biol*. 2021;22 (2):96–118. doi:10.1038/s41580-020-00315-9
32. Bao J, Xing Y, Feng C, et al. Acute and sub-chronic effects of copper on survival, respiratory metabolism, and metal accumulation in Cambaroides dauricus. *Sci Rep*. 2020;10(1):16700. doi:10.1038/s41598-020-73940-1
33. Zhu J, Huang Q, Peng X, et al. Identification of LncRNA prognostic signature associated with genomic instability in pancreatic adenocarcinoma. *Front Oncol*. 2022:12799475. doi:10.3389/fonc.2022.799475.
34. Gu ZR, Liu W. The LncRNA AL161431.1 targets miR-1252-5p and facilitates cellular proliferation and migration via MAPK signaling in endometrial carcinoma. *Eur Rev Med Pharmacol Sci*. 2020;24(5):2294–2302. doi:10.26355/eurrev_202003_20495
35. Ritterhouse LL. Tumor mutational burden. *Cancer Cytopathol*. 2019;127(12):735–736. doi:10.1002/cncy.22174
36. Chan TA, Yarchoan M, Jaffee E, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Ann Oncol*. 2019;30(1):44–56. doi:10.1093/annonc/mdy495
37. Jardim DL, Goodman A, de Melo Gagliato D, et al. The challenges of tumor mutational burden as an immunotherapy biomarker. *Cancer Cell*. 2021;39(2):154–173. doi:10.1016/j.ccell.2020.10.001
38. Peng Y, Liu C, Li M, et al. Identification of a prognostic and therapeutic immune signature associated with hepatocellular carcinoma. *Cancer Cell Int*. 2021;21(1):98. doi:10.1186/s12935-021-01792-4
39. Wang Q, Li M, Yang M, et al. Analysis of immune-related signatures of lung adenocarcinoma identified two distinct subtypes: implications for immune checkpoint blockade therapy. *Aging*. 2020;12(4):3312–3339. doi:10.18632/aging.102814