Prediction of prognosis, immune escape and drug sensitivity of lung adenocarcinoma based on Cuproptosis-related LncRNA

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Abstract
Background: Lung Adenocarcinoma (LUAD) is the leading cause of death from lung cancer. Cuproptosis is the latest discovered way of programmed cell death, and Cuproptosis-Related Gene (CRG) is associated with the risk of LUAD. At present, there are few research of LUAD and Cuproptosis focuses on Long non-coding RNA (LncRNA). As genomics advances, LncRNA emerges as a potential target for understanding tumor progression and prognosis, offering prospects for biological targeted therapy. Therefore, this study provides new biomarkers and therapeutic targets for LUAD from the perspective of LncRNA. Methods: Gene expression, clinical outcome and gene mutation data of LUAD patients were downloaded from TCGA database. Spearman correlation was used to analyze the correlation between LncRNA and CRG. Univariate Cox, multivariate Cox and LASSO Cox regression analysis were used to construct a prognostic model of Cuproptosis-LncRNAs. GO and KEGG enrichment and immune function analysis were performed on differentially expressed genes between different risk groups. Then, immune escape analysis was performed on LUAD patients with different TIDE score. Finally, drug sensitivity analysis was performed on these differentially expressed genes. Results: A total of 2244 Cuproptosis-LncRNAs were found. Through the application of univariate Cox regression analysis, multivariate Cox regression analysis, and LASSO Cox regression analysis, a prognostic model was developed, integrating 15 Cuproptosis-LncRNAs to assess the risk of mortality. Following that, the model underwent assessment through risk score analysis, Kaplan-Meier survival analysis, risk distribution, and evaluation of survival outcomes. The results revealed an AUC value of 0.755 for the model, surpassing the AUC of other clinical pathological features. The results of KEGG analysis showed that the differentially expressed genes in different model groups were mainly involved in Amoebiasis, Fat digestion and absorption, and other signaling pathways. The results of TMB showed that the prognostic model of TMB combined with risk score could well evaluate the prognosis of patients. The TIDE scores did not exhibit a notable distinction between the two risk models. Analysis of drug sensitivity revealed that individuals in the low-risk category demonstrated greater responsiveness to 5-Fluorouracil, Axitinib, Bexarotene, and other drugs compared to those in the high-risk group. Conclusion: Our research offers a valuable reference for predicting the prognosis of LUAD, contributing to a better understanding of the future elucidation of the process and mechanism of Cuproptosis-LncRNAs in LUAD.

Keywords: lung adenocarcinoma; cuproptosis; LncRNA; prognostic signature; programmed cell death
Introduction

Lung cancer (LC) ranks as the second most prevalent malignant tumor globally, carrying the highest rates of both morbidity and mortality [1]. Research findings indicate a 5-year overall survival of only 19.8% for lung cancer patients in China [2]. The majority of LC cases are classified as non-small cell lung cancer (NSCLC), with small cell lung cancer (SCLC) following closely. Additionally, approximately 3% of cases present with histological unknown characteristics [3]. Due to the high prevalence and low survival rate of NSCLC, it has always been the focus and frontier of LC diagnosis and treatment research [4]. NSCLC can be divided into three types: squamous cell carcinoma (SCC), lung adenocarcinoma (LUAD) and large cell carcinoma (LCC). LUAD is the main pathological subtype of NSCLC and one of the main causes of LC-related death [5]. LUAD is prone to early metastasis, so its prognosis is poor [6]. Hence, there is an immediate need to identify novel biomarkers and therapeutic targets.

Programmed cell death is a widespread phenomenon in organismal development, characterized by a well-orderly demise of cells dictated by genetic factors [7]. It includes apoptosis, pyroptosis, ferroptosis, autophagy and the latest discovery of Cuproptosis [7, 8]. Current research suggests a correlation between LUAD and programmed cell death, making the modulation of programmed cell death a potential therapeutic target and a future direction in LUAD treatment [9]. Cuproptosis, a recently identified form of programmed cell death, primarily occurs through the direct binding of copper to the acylated components of the tricarboxylic acid cycle. This process leads to the aggregation of acylated proteins, subsequent loss of Fe-S cluster proteins, and the induction of protein toxic stress, ultimately resulting in cell death [8]. Yun et al. identified Cuproptosis-Related Genes (CLGs), namely SLC31A1, FDX1, and ATP7B, associating them with the risk of LC [10]. At present, the research on LUAD and Cuproptosis mainly focuses on the genetic level [11], and there are few studies on Long non-coding RNA (LncRNA).

LncRNA, a class of RNA molecules exceeding 200 nucleotides in length, plays a direct role in promoting the proliferation, invasion, and metastasis of LUAD cells, along with fostering angiogenesis, thereby facilitating LUAD cell invasion and metastasis [12]. As genomics advances, LncRNA emerges as a potential target for understanding tumor progression and prognosis, offering prospects for biological targeted therapy [13]. Despite numerous studies on LncRNA and LUAD, the specific involvement of Cuproptosis-related LncRNAs (CRLs) in predicting prognosis and treatment response in LUAD remains incompletely explored. This study comprehensively assessed the role of CRLs in LUAD by integrating data from the TCGA database. Furthermore, we developed a risk scoring model for LUAD based on CRLs information. The prognostic performance of the risk score (RS) was compared with other clinical biomarkers. Additionally, we compared the biological characteristics, immune landscape, matrix activity, and genomic integrity between low-risk (LRS) and high-risk (HRS) populations. Ultimately, a model capable of predicting the prognosis and drug sensitivity of LUAD was constructed.

Information and methodology

Data acquisition and collation

Data on gene expression, clinical parameters, gene mutation GM, and Tumor Mutational Burden (TMB) for LUAD patients were extracted from The Cancer Genome Atlas (TCGA) database, accessible via the Genomic Data Commons (GDC) official website (https://portal.gdc.cancer.gov/corti). The specific project chosen was TCGA-LUAD. TCGA-LUAD gene expression data comprised 481 cases and 555 samples, clinical data encompassed 486 cases and 486 samples, and TMB data included 524 cases and 579 samples. Patient demographics, including age, gender, tumor stage, type, stage (T), lymph node metastasis (N), and overall survival status, were compiled. All statistical analyses and visualizations in this study were conducted using R 4.0 and strawberry-perl - 5.32.1.1 [14–16].

Identification of Cuproptosis-related LncRNAs

Based on a review of the literature, we compiled a list of 19 Cuproptosis regulatory genes (NFE2L2, NLRP3, ATP7B, ATP7A, SLC31A1, FDX1, LIAS, LIFT1, LIFT2, DLD, DLAT, PDHA1, PDHB, MTFL1, GLS, CDKN2A, DTB, GCSH, DLST) [8, 17]. Subsequently, we conducted co-expression correlation analysis on the LncRNA expression profiles and CRGs using the limma package, applying criteria of [R] > 0.4 and P < 0.001 [14]. Additionally, leveraging LncRNA annotation files obtained from the common code website, we retrieved the expression data for 16,876 LncRNAs from the TCGA database. Spearman correlation analysis was employed to examine the relationship between LncRNAs and CRGs. CRLs were defined as LncRNAs significantly associated with at least one regulatory factor ([Spearman correlation] ≥ 0.1 and P < 0.05) [15].

Construction and analysis of Cuproptosis-related risk model

The clinical data were collated to obtain the survival data of LUAD. By combining survival data and gene expression data for RS analysis, the data of genes and LncRNAs with different risks were obtained. Prognostically relevant LncRNAs were identified through univariate Cox regression analysis (P < 0.001) [16]. Next, candidate CRLs were identified through the least absolute shrinkage and selection operator (LASSO) analysis. Lasso-Cox regression analysis was employed to select genes for constructing the model. The regularization path, computed using survival function and LASSO, determined the lambda (logarithmic scale) for the lasso or elastic-net penalty. This algorithm, applicable to various regression models, is efficient and leverages the sparsity of the input matrix X. Parameters for LASSO regression included a p-value set at 0.05, 1000 cycles, and 1000 random stimuli for each process. The frequency of each pair in the LASSO regression model was recorded over 1000 repetitions. Pairs with a frequency exceeding 100 were chosen for Cox proportional hazard regression analysis and model generation. Area under the curve (AUC) values were computed, and curves were plotted until the maximum AUC value represented the optimal model, as indicated by the peak of the curve [18].

Following this, by combining survival data of CRLs and LUAD patients, we obtained risk data, divided into 1:1 training and testing sets. Employing packages such as ‘survival’, ‘caret’, ‘glmnet’, ‘survminer’, and ‘timeROC’ [15, 19, 20], multivariate Cox regression analysis was conducted on the training set to construct the predictive model for LUAD patients. Predicted RS from the model categorized patients into high and LRS groups based on the median RS, calculated using the formula: RS = -5 x (exp(x) x β) [16, 21]. Model accuracy validation involved applying the same formula to calculate RS for the validation set and the entire dataset. Subsequent analyses included Kaplan-Meier survival analysis, risk distribution, and survival outcomes, with samples grouped into LRS or HRS categories based on median RS. Scatter plots were generated for visualizing the association between RS and survival status. Kaplan-Meier analysis displayed survival differences between LRS and HRS groups [22].

To assess the clinical utility of the model, a chi-square test analyzed its relationship with clinical pathological features, visually presented through graphs with annotations (< 0.001, * < 0.01, + < 0.05). The Wilcoxon signed-rank test calculated risk score differences for clinical pathological features between groups, visualized using box plots. Univariate and multivariate Cox regression analyses between RS and clinical pathological features were conducted, illustrated through a forest plot [23]. Additionally, an independent prognosis analysis assessed whether the RS could predict prognosis independently of other traits. ROC curves, Concordance Index (CI), and column charts provided a comprehensive evaluation of the entire model.

Analysis of gene expression and function in different risk groups

Through the integration of RS data, gene expression data, and CRGs, we acquired expression data for CRGs, CRLs, all genes, and LncRNAs within distinct risk groups. Employing the ‘scatterplot3d’ package, Principal Component Analysis (PCA) was implemented on the dataset to contrast the profiles of patients in high and LRS groups across
 option was selected [30]. The detailed workflow is illustrated in Figure 1.

Data availability statement
Requests for additional data may be granted upon reasonable request by contacting the author.

Results

Identification of CRLs

The expression data of TCGA-LUAD were downloaded from the GDC website, including 481 cases and 555 samples. The clinical data of TCGA-LUAD were downloaded from the GDC website, including 486 cases and 486 samples. Based on the lncRNA annotation files downloaded from the common code website, the expression of 16876 LncRNAs was retrieved from the TCGA database. By reading the literature, 19 genes related to Cuproptosis were found. Setting (spearman correlation) ≥ 0.1 and P < 0.05, 2244 CRLs were found.

Construction and validation of CRLs prognostic model in LUAD patients

Univariate Cox regression analysis was employed to identify CRLs associated with disease prognosis from a pool of 2244 CRLs within the TCGA training set. The findings revealed that 236 CRLs exhibited a correlation with overall survival (OS) in the training set. Employing LASSO Cox regression analysis, a condensed estimation method for variable reduction, led to the identification of 81 CRLs (Figure 2A–2C). Subsequent integration of survival data for CRLs and LUAD patients enabled the exploration of the relationship between CRLs and prognosis. Using the GLNET package in R, a Lasso-Cox regression analysis was conducted on the 81 CRLs to pinpoint the optimal prognostic genes. The coefficients obtained from Cox multivariate regression analyses for these selected prognostic genes were extracted to compute the RS for each patient. Ultimately, 15 CRLs were incorporated, as detailed in Table 1. The RS was determined through the specified formula: RS = (AC090826.1 * 1.831206503) + (AC10 6882.1 * -1.242354618) + (AC107021.2 * 0.628477889) + (LINCO 2785 * -1.70768419) + (TRMT2B-AS1 * -0.7654264) + (CYP1B1- AS1 * -0.647481052) + (AC185291.9 * -1.079704207) + (AC0263 55.2 * -0.295158687) + (AC90018.2 * 0.508682466) + (AC0847 81.1 * 2.597724733) + (AC010999.2 * -1.518129388) + (AC186 53.4 * 2.012349794) + (AC234775.2 * -0.572578384) + (MIR443 S-2HG * 0.840069995) + (AC092279.1 * -0.491097777).

Survival results and multivariate test

Utilizing a correlation heatmap, we displayed the correlation between the 15 CRLs and differentially expressed CRGs. The results indicate that these 15 CRLs are associated with at least one or multiple CRGs (Figure 3A). According to the predicted RS from the prognostic model for LUAD patients, we stratified LUAD data in the training set into high and LRS groups. The results revealed a significantly longer survival time for the two groups in the training dataset (P < 0.001) (Figure 3B). Significant differences in the expression of the 15 CRLs survival time were observed between the two groups (Figure 3C). Substantial variations in RS were evident among patients in different groups (Figure 3D). Additionally, distinct survival outcomes were observed among patients in different RS groups, with HRS group patients exhibiting shorter survival times and higher mortality rates, as illustrated in Figure 3E. To validate the accuracy of the established signature, we employed a unified formula for RS calculation on the validation set and the entire dataset. Kaplan-Meier survival analysis, risk distribution analysis, and survival outcome analysis were conducted, yielding results entirely consistent with those from the training set. This consistency suggests the stability of the RS prediction model we constructed (Figure 3F–3M).

Results of independent prognostic analysis

Both single and multi-factor Cox regression analyses demonstrate that the RS derived from the 15 CRLs serves as an independent prognostic
factor for LUAD. In the single-factor analysis, variations were observed in stage and RS, along with age and gender. The single-factor Cox regression revealed an HR of 1.032 for the RS, accompanied by a 95% CI ranging from 1.017 to 1.071 (P < 0.001) (Figure 4A). The multi-factor analysis identified differences in age, stage, and RS. The multi-factor Cox regression displayed an HR of 1.038 for the RS, with a 95% CI spanning from 1.021 to 1.055 (P < 0.001) (Figure 4B). The area under the curve (AUC) for the RS stood at 0.755, surpassing the AUC for other clinical-pathological features, signifying the credibility and reliability of the prognostic signature constituted by the 15 CRLs (Figure 4C). Furthermore, the AUC for the CRLs signature concerning 1-, 3-, and 5-year survival rates were 0.755, 0.745, and 0.748, respectively (Figure 4D). The CI, representing the consistency between predicted and actual results, indicated that the CI value for the RS exceeded 0.7, whereas the CI values for other clinical-pathological features were below 0.7. This suggests that the prognostic evaluation based on the 15 CRLs for LUAD surpasses that of other clinical-pathological features (Figure 4E).

Clinicopathological features and PCA results of low and high-risk group

Through the creation of a prognostic model, patients can receive scores based on their age, gender, and clinical tumor stage, enabling the prediction of their 1-3-year survival rates. Figure 5A presents a robust and accurate mixed nomogram incorporating both clinicopathological features and novel prognostic signatures. The forest plot, encompassing clinical variables and RS, indicates that the selected patients exhibit 1-year, 3-year, and 5-year overall survival probabilities of 0.964, 0.87, and 0.72, respectively. This underscores the potential applicability of the prognostic signature in the clinical management of LUAD patients. Utilizing the established prognostic model, we further explored disparities in tumor staging between HRS and LRS groups. The outcomes revealed that, across various stages of LUAD patients, those in the HRS group exhibited significantly lower overall survival than their counterparts in the LRS group (Figure 5B, Figure 5C). Principal Component Analysis (PCA) was conducted based on the entire gene expression profile, CRGs, CRLs, and LncRNAs within the prognostic model. The results demonstrated a relatively dispersed distribution of HRS and LRS LUAD patients in the whole gene expression profile, CRGs, and CRLs (Figure 5D–5F). However, in the case of CRLs within the prognostic model, the distribution was concentrated and easily distinguishable (Figure 5G).

Figure 1 Workflow diagram
Table 1 Results of multivariate Cox regression analysis

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Figure 2 Construction and validation of CRLs prognostic model in LUAD patients. (A) Lasso Cox analysis of CRLs associated with prognosis. (B) Lambda diagram of Lasso Cox analysis of CRLs associated with prognosis; Figure 2C. Forest diagram of Lasso Cox analysis of CRLs associated with prognosis.
Figure 3 Survival results and multivariate test. (A) A correlation heat map illustrating the relationships between CRLs and differentially expressed CRGs. (B) Survival analysis for LUAD patients is presented, categorizing them into distinct RS groups within the training set. (C) The expression profiles of CRLs in LUAD patients across various RS groups in the training set. (D) A depiction of the RS assigned to different groups of LUAD patients in the training set. (E) The survival outcomes of LUAD patients within these different RS groups in the training set. (F) Survival analysis is extended to LUAD patients in different RS groups within the test set. (G, H) Portray the expression profiles of CRLs and the corresponding RS for LUAD patients in various RS groups within the test set. (I) Highlights the comprehensive survival analysis conducted across various groups of LUAD patients. (J) Focuses on the survival outcomes within different RS groups. (K, L) The expression profiles of CRLs and the associated RS for LUAD patients, respectively, across various RS groups. (M) Provides a comprehensive overview of the survival outcomes for LUAD patients across various RS groups.
Figure 4 Results of independent prognostic analysis. (A) showcases the results of univariate Cox regression analysis examining the relationship between the RS and traits of LUAD patients. (B) The findings of multivariate Cox regression analysis, exploring the association between the RS and LUAD patient traits, are presented. (C) AUC results of RS. (D) AUC results of the signature of CRLs for 1-, 3-, 5-year survival rates. (E) Comparison of CRLs and other traits in evaluating the prognosis of LUAD.
Figure 5 Clinicopathological features and PCA results of low and high-risk group. (A) displays a nomogram incorporating age, gender, tumor clinical stage, and RS. (B) The survival analysis results for stage 1-2 LUAD patients across different RS groups are presented. (C) provides the survival analysis outcomes for stage 3-4 LUAD patients in varying RS groups. (D–F) illustrate the distribution of LUAD patients in different RS groups within the entire gene expression profile, CRGs, and CRLs, respectively. (G) focuses on the distribution of CRLs in different RS groups of LUAD patients within the prognostic model.
GO and KEGG enrichment analysis of differentially expressed risk genes

Integrating expression data with RS allowed us to categorize gene expression in distinct RS groups. Through the comparison of gene expression variations in these groups, we identified 318 genes exhibiting differential expression between HRS and LRS groups. GO enrichment analysis of these genes highlighted their association with 128 GO terms, primarily involving processes such as negative regulation of proteolysis, negative regulation of endopeptidase activity, and digestive tract development in BP. In terms of CC, these genes were implicated in collagen-containing extracellular matrix, apical plasma membrane, and vesicle lumen. Additionally, MF analysis revealed involvement in glycosaminoglycan binding, sulfur compound binding, and heparin binding (Figure 6A, Figure 6B). Furthermore, KEGG enrichment analysis demonstrated that the 318 differentially expressed genes between HRS and LRS groups were predominantly associated with pathways such as Amoebiasis, Fat digestion and absorption, Hematopoietic cell lineage, ECM-receptor interaction, and the Wnt signaling pathway, among others (Figure 6C, Figure 6D).

Immune function analysis and gene mutation analysis

By integrating data on RS, gene expressions, and immune genes, we examined the differential expression of immune genes between HRS and LRS. Immune functional analysis of these differentially expressed genes revealed variations in various immune functions between the HRS and LRS. Notably, significant differences were observed in inflammation pathways, including Type II IFN response, HLA, and MHC class I ($P < 0.05$) (Figure 7A). Tumor mutation burden (TMB) data for TCGA-LUAD, consisting of 524 cases and 579 samples, were obtained and analyzed. Merging TMB information with RS files allowed us to discern the TMB disparities between HRS and LRS. Analysis of mutation frequencies revealed higher rates for TP53, TTN, MUC16, CSMD3, RYR2, LRPIP1, ZFHX4, FLG, SPTA1, and COL11A1 in the HRS, while USH2A, KRRS, XIPR2, NAV3, and ZNF536 exhibited lower rates compared to the LRS (Figure 7B, Figure 7C). However, no significant overall difference in tumor mutation burden between HRS and LRS was observed ($P = 0.56$) (Figure 8A). Based on TMB status, patients were categorized into High TMB group (H-TMB) and Low TMB group (L-TMB). Survival analysis indicated that patients in the H-TMB exhibited potentially higher survival rates than those in the L-TMB, with a statistically significant difference ($P = 0.017$). Combining TMB and RS grouping information generated four groups: High TMB high-risk group (H-TMB-H-risk), Low TMB high-risk group (L-TMB-H-risk), High TMB low-risk group (H-TMB-L-risk), and Low TMB low-risk group (L-TMB-L-risk). Subsequent survival analysis revealed significant differences in survival probability among the four groups ($P < 0.001$). Notably, the H-TMB-L-risk group demonstrated the highest survival probability, followed by the L-TMB-L-risk group (Figure 8B, Figure 8C).

Figure 6 GO and KEGG Enrichment Analysis of Differentially Expressed Risk Genes. (A) The ring diagram of GO enrichment analysis. (B) The bar chart of GO enrichment analysis. (C) Ring diagram of KEGG analysis. (D) Bar graph of KEGG analysis.
Figure 7 Immune function analysis and gene mutation analysis. (A) Immune function analysis. (B) Gene mutation rate in HRS. (C) Gene mutation rate in LRS.

Figure 8 Results of Immune Function. (A) illustrates the comparison of TMB in LUAD patients across various RS groups. (B) The survival probability of LUAD patients is depicted based on different TMB levels. (C) presents the survival probability of LUAD patients considering both different RS groups and TMB levels.
**Immune function and drug sensitivity analysis**

The TIDE score is an assessment tool for evaluating the immune escape status of cancer patients, providing insights into whether patients are suitable for immune therapy from an immune escape perspective. By merging the TIDE score file with the RS file for immune function analysis, the results revealed no significant difference in TIDE scores between the two groups (Figure 9A), suggesting that the effectiveness of immune therapy may be relatively consistent among LUAD patients in two groups. Drug sensitivity analysis is a method used to assess the effectiveness of drug treatment. Sensitivity analysis on patients from different risk groups showed that patients in the low-risk group had higher drug sensitivity to 5-Fluorouracil, Axitinib, Bexarotene, Doxorubicin, Bortezomib, Etopophine B, Bosutinib, Cisplatin, Etoposide, Ispinesib Mesylate, and Lenalidomide than those in the HRS (Figure 9B–9L).

**External dataset validation**

Using the external KM Plotter database, we systematically analyzed the prognostic value of 81 CRLs. Upon investigation, data for 72 CRLs were found to be lacking in the database, and only data for 9 CRLs were retrieved. The results indicated a strong correlation between the expression of CYP1B1-AS1, ADAMTS9-AS2, ADPGK-AS1, CRNDE, GLIS2-AS1, LANCL1-AS1, LINC01960, LY86-AS1, TAPAOAP1-AS1 and the OS of LUAD patients (P < 0.05), as shown in Figure 10A–10J. Among these 9 CRLs, CYP1B1-AS1 was part of the RS calculation equation and demonstrated significant correlation with OS (HR = 0.58 (0.43–0.79), P = 0.00056) (Figure 10A). Our data align closely with the OS analysis results from the external database.

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**Figure 9 Results of Drug Sensitivity Analysis.** (A) presents a comparison of TIDE scores among LUAD patients within various RS groups. (B–L) The sensitivity of LUAD patients in different RS groups is assessed concerning various therapeutic agents, including 5-Fluorouracil, Axitinib, Bexarotene, Bortezomib, Bosutinib, Cisplatin, Doxorubicin, Etopophine B, Etoposide, Ispinesib Mesylate, and Lenalidomide, respectively.
Discussion

LC is a malignant tumor with significantly increased morbidity and mortality [1]. NSCLC is one of the main types of LC, accounting for about 85% of all cases [31]. LUAD accounts for the highest proportion in NSCLC, about 40% [31]. Although surgical resection can improve the symptoms of early LUAD patients to some extent, it may lead to recurrence or metastasis of LUAD. Therefore, further understanding the occurrence and development mechanism of LUAD and finding a breakthrough in treatment is an important source of ideas for finding new LUAD treatment methods. Advancements in high-throughput sequencing technology have unveiled the pivotal regulatory role of lncRNA in numerous biological processes. Increasing evidence suggests that lncRNA may significantly influence the prognosis of LUAD patients and contribute to drug resistance in treatment [32, 33]. The identification of Cuproptosis has spurred research on drug development targeting the Cuproptosis pathway [34]. This development opens up new avenues for the prognosis evaluation and treatment of LUAD [8]. As of January 10, 2023, there were 8 papers published in PubMed on the evaluation of LUAD by CRLs [35–43]. Among them, Wang carried out experiments to verify [39], Gao’s research focused on m6A-related LncRNA [41], and Chen’s research did not have permission to obtain the full text [42]. These studies have established a prognostic model of CRLs, and some studies have evaluated the relationship between CRLs and immune function [35–40]. However, no sensitivity analysis of immune escape and potential intervention drugs has been carried out [35–40]. And Wang Z included only 8 CRGs, and Wang S included only 10 CRGs [37, 40]. Therefore, the development of this study is extremely necessary.

Utilizing bioinformatics analysis, we delineated the expression patterns of 16,876 LncRNAs and 19 CRGs. Upon integrating these two datasets, a total of 2,244 CRLs were identified. Employing univariate Cox regression analysis, multivariate Cox regression analysis, and LASSO Cox regression analysis, we pinpointed 15 CRGs to establish a prognostic model for evaluating the risk of mortality. The number of CRLs in this study is higher than Mo X’s 7 and Wang Z’s 11, and lower
than Wang F’s 37 [35–37]. The key CRLs identified in predicting the prognosis of LUAD include: AC090826.1, AC106882.1, AC107021.2, LINC02785, TRMT2B-AS1, CYP1B1-AS1, AC185291.9, AC026355.2, AC090018.4, AC084781.1, AC101099.2, AC180653.4, AC234775.2, MIR4435-2HG, AC092279.1. Among these 15 LncRNAs, AC107021.2, CYP1B1-AS1, AC206355.2, AC101099.2, MIR4435-2HG, AC092279.1, and others have previously been found to predict the long-term prognosis of LUAD [44–49]. External dataset analysis also supports the association of CYP1B1-AS1 with OS in LUAD patients. Due to the lack of data for other LncRNAs in the external dataset, it is not clear how other LncRNAs may affect the OS of LUAD patients. This study is the first to conduct a comprehensive analysis of the mechanism of action of the 15 identified LncRNAs. Among these, MIR4435-2HG has a relatively clear relationship with cancer occurrence. Previous studies have shown that MIR4435-2HG acts as an oncogene in various cancers [50]. Its expression levels are abnormally elevated in a range of cancers, including LUAD [50]. Moreover, MIR4435-2HG is associated with poor prognosis in various cancers [50]. It also affects tumor proliferation, invasion, and apoptosis [50]. Subsequent to conducting RS analysis, Kaplan-Meier survival analysis, risk distribution assessment, and survival results analysis, we thoroughly evaluated the efficacy of our model. The outcomes demonstrated the model’s effectiveness in differentiating between high and LRS, affirming the success of our prognostic model. The one-year AUC value of the RS reached 0.755, surpassing the AUC for other clinical-pathological features. This indicates the practicability of our model for prognostic assessment in LUAD patients. The PCA results depicted a concentrated and easily distinguishable clustering of HRS and LRS LUAD patients based on the CRLs within the prognostic model. Notably, this outcome surpasses the findings of Wang F [35].

By integrating expression data and RS grouping information, we identified differentially expressed genes across distinct grouping categories. These genes predominantly participated in signaling pathways such as Amoebiasis, Fat digestion and absorption, Hematopoietic cell lineage, ECM-receptor interaction, and the Wnt signaling pathway. While the relationship between Amoebiasis and LUAD remains unexplored, previous studies have investigated the connections between Fat digestion and absorption, Hematopoietic cell lineage, ECM-receptor interaction, Wnt signaling pathway, and LUAD [43, 51–53]. Notably, the Wnt signaling pathway, recognized as a complex protein–protein interaction network, primarily functions in embryonic development and cancer, and is also implicated in normal physiological processes in adult animals [54]. Studies have shown that these signaling pathways also play a crucial role in the regulation of LC stem cells [55]. Zha et al. discovered that the heightened expression of neuron-specific enolase fosters the epithelial-mesenchymal transition, invasion, and metastasis of lung cancer by incessantly activating the Wnt/β-catenin signaling pathway. This activation leads to an upregulation in the expression of downstream genes such as c-Myc and Snail [56]. Similarly, Liao et al. identified that the oncogene cold-inducible RNA binding protein continuously activates the Wnt/β-catenin signaling pathway, fostering the expression of downstream genes including c-Myc, COX-2, CCND1, MMP7, VEGFA, and CD44. This activation, in turn, promotes the proliferation and invasion of NSCLC [57]. These findings align with some of the outcomes of the present study, suggesting that these pathways could serve as potential novel targets for immunotherapy in lung adenocarcinoma.

Immunotherapy drugs have now changed the treatment of many cancers, especially LUAD [58]. How to determine whether LUAD patients are suitable for immunotherapy has always been the focus of research. Numerous studies have employed TMB as a predictive biomarker for gauging the response to immunotherapy [59]. TMB quantifies the number of mutations per megabase carried by tumor cells [59]. In this study, TMB data were systematically analyzed, and a prognostic model integrating TMB with OS was devised. The LUAD patients were categorized into four groups: H-TMB-H-risk, L-TMB-H-risk, H-TMB-L-risk, and L-TMB-L-risk for survival analysis. The findings revealed that the prognostic model combining TMB with RS effectively assessed the prognosis of patients. Notably, this study did not assess the efficacy of immunotherapy in LUAD patients with H-TMB. Nevertheless, the data presented suggest that the survival rates of LUAD patients with H-TMB might be notably higher than those with L-TMB. This observation provides a novel perspective for immunotherapy approaches in the context of LUAD patients.

TIDE score is an evaluation tool to evaluate the immune escape of tumor patients, which can evaluate whether tumor patients are suitable for immunotherapy from the perspective of immune escape [60]. The data presented in this paper indicate that there is no substantial difference in TIDE scores between RS models based on CRLs. Drug sensitivity analysis, employed to assess the responsiveness to drug treatment, revealed that patients in the LRS exhibited greater sensitivity to 5-Fluorouracil, Axitinib, Bexarotene, Bortezomib, Bosutinib, Cisplatin, Doxorubicin, Epothilone B, Etoposide, Ipinisib Mesylate, and Lenalidomide compared to those in the HRS. The efficacy of 5-Fluorouracil, Axitinib, Bexarotene, Bortezomib, Bosutinib, Cisplatin, Doxorubicin and Etoposide in the treatment of LUAD has also been supported by experimental data [61–68].

**Limitations**

There are some limitations in this paper. First of all, all data acquisition is from the TCGA database, and there is a lack of cross-verification to display the real clinical data. Despite utilizing external databases for cross-validation, we acknowledge that there were no corresponding animal or cell experiments conducted to enhance the credibility of the relationship between these LncRNAs and LUAD. The data sample size obtained by the database is small, including only 555 samples of expression data, only 486 samples of clinical data, and only 579 samples of TMB data. This study constructed a prognostic model of CRLs, but did not compare this model with other prognostic models, and could not further evaluate the advantages of this prognostic model. Although this study selected genes related to the risk of death, and studied the molecular functions and KEGG pathways that may be involved in these genes, no experimental research was conducted to further verify and refine the specific molecular mechanism.

**Conclusion**

In summary, our investigation serves as a reference for predicting the prognosis of LUAD patients, contributing valuable insights for the future exploration of the roles and mechanisms of CRLs in LUAD. While our results have been validated through grouping, it is imperative to conduct further verification to validate the prognostic markers developed in this study.

**References**


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