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Original Article

Identification of cuproptosis-related long non-coding ribonucleic acid signature as a novel prognosis model for colon cancer

Rong Xu[1,2*], Xin Wu[3*], Ashuai Du[4], Qiangqiang Zhao[5#], He Huang[1,2#]

[1]NHC Key Laboratory of Carcinogenesis, Cancer Research Institute and School of Basic Medicine, Central South University, Changsha 410078, Hunan, China; [2]Department of Histology and Embryology, Xiangya School of Medicine, Central South University, Changsha 410013, Hunan, China; [3]Department of Orthopedics, The Third Xiangya Hospital, Central South University, Changsha 410013, Hunan, China; [4]Department of Cell Biology, School of Life Sciences, Central South University, Changsha 410013, Hunan, China; [5]Department of Hematology, The Qinghai Provincial People's Hospital, Xining 810007, Qinghai, China. [*]Equal contributors. [#]Co-corresponding authors.

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Abstract: Cuproptosis is a novel type of cell death that may play a vital role in preventing various types of cancer. Studies examining cuproptosis are limited, and the cuproptosis-related lncRNAs (long non-Coding ribonucleic acids) involved in the regulation of colon cancer remain unclear. This study aimed to identify the prognostic signature of cupronosis-related lncRNAs and explore their potential molecular functions in colon cancer. Data on the clinical correlation were obtained from The Cancer Genome Atlas (TCGA) database. The differentially expressed cuproptosis-related long non-coding ribonucleic acids (lncRNAs) were analyzed using the "limma" package. Then, the prognostic cuproptosis-related lncRNA signature (CupRLSig) was identified through univariate Cox and co-expression analyses, and a prognostic model was constructed based on CupRLSig using the least absolute shrinkage selection operator (LASSO) algorithm and Cox regression analysis. The Kaplan-Meier survival curve and receiver operating characteristic (ROC) curve were used for evaluating the model's capacity for prognosis prediction. In addition, the immune landscape, and drug sensitivity of CupRLSig were analyzed. Finally, the functions of AL512306.3 and ZEB1-AS1 were verified through in vitro experiments. The high- or low-risk groups were classified according to the risk score. The signature-based risk score showed a stronger ability to predict patient's survival compared with the traditional clinicopathological features. In addition, immune responses, such as inflammation-promoting response and T-cell co-inhibition, were significantly different between the two groups. Moreover, chemotherapy drugs or inhibitors, such as axitinib, cisplatin, doxorubicin, and elesclomol, may be considered as potential therapeutic drugs for patients in high-risk groups. Finally, inhibition of AL512306.3 and ZEB1-AS1 significantly suppressed the cell proliferation in colon cancer cells. These results provide novel insights into the pathogenesis of colon cancer and offer promising biomarkers with the potential to guide research on carcinogenesis and cancer treatment.

Keywords: Cuproptosis, lncRNAs, prognosis, colon cancer, risk score

Introduction

Colon cancer is a common digestive neoplasm with high incidence and mortality worldwide [1]. Recently, statistical studies reported more than 1.93 million new cases of colon cancer and 940,000 deaths [2]. Despite the rapid development of cancer screening methods, many patients have been diagnosed with advanced-stage disease with distant metastasis. However, therapeutic targets for patients with colon cancer remain scarce. Therefore, the development of diagnostic biomarkers and therapeutic targets is necessary for the treatment of colon cancer.

Copper (Cu) is an indispensable metal, which plays a central role in the formation of reactive oxygen species (ROS). The concentrations of copper in the cell are maintained at low levels owing to the active homeostatic mechanisms that prevent free copper from accumulating intracellularly [3, 4]. Since copper is considered a limiting factor in the process of cancer devel-

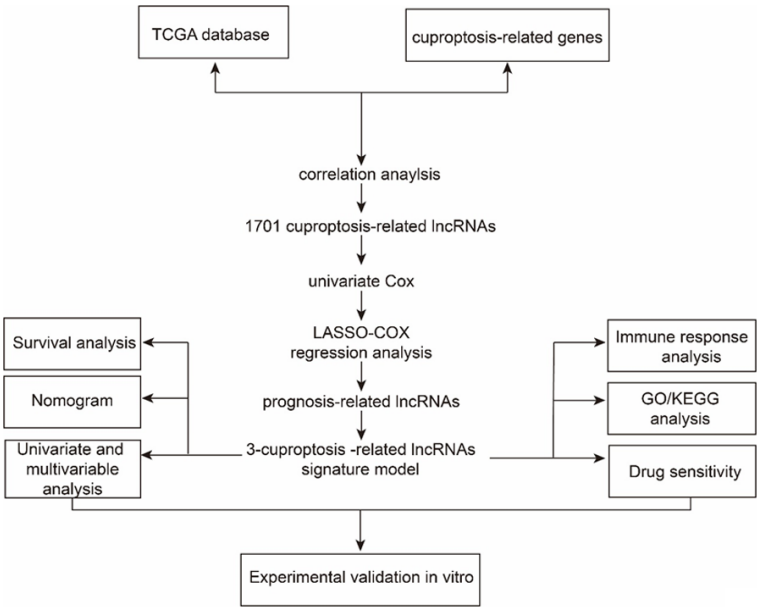

Figure 1. Flowchart of the study process. Cuproptosis-related genes (n = 19) and cuproptosis-related lncRNAs (n = 1,701) were obtained from The Cancer Genome Atlas database. Univariate Cox analysis was performed to screen for prognostic cuproptosis-related lncRNAs. Based on this analysis, a 3-cuproptosis-related lncRNA signature was constructed. Subsequently, Kyoto Encyclopedia of Genes and Genomes, Gene Ontology, immune-related analyses, and somatic mutation analyses, and drug sensitivity assays were used to identify the potential function of this signature. Finally, in vitro validation was conducted to explore the expression profiles and function of ZEB1-AS1 and AL512306.3.

opment, including growth, angiogenesis, and metastasis, copper-based cancer treatment has also attracted considerable attention [5, 6]. Recent studies have suggested that Cu can induce various forms of cell death through apoptosis, autophagy, ROS accumulation, proteasome inhibition, and antiangiogenesis [7]. The antiangiogenesis, antifibrotic, and anti-inflammatory effects of copper therapy have a potential impact in medicine [8].

Although several copper complexes have been evaluated based on their reactivity toward cancer cells, with some undergoing clinical trials to be developed as therapeutic agents [9]. However, the mechanism of Cu-induced cell death remains largely unknown. Although several cuproptosis-related genes have been discovered, cuproptosis-related therapeutic targets for colon cancer therapy need to be identified.

Long non-coding ribonucleic acids (lncRNAs) are a type of RNA with a length of more than 200 nt [10, 11]. Several ferroptosis-related lncRNAs have been reported in breast cancer, colon cancer, bladder cancer, and gastric cancer [4, 12-15]. However, only limited studies have reported the detection of cuproptosis-related lncRNAs in cancer. Identifying the key cuproptosis-related lncRNAs with prognostic significance in colon cancer may provide novel insights into the mechanism of cuproptosis.

In our study, we retrieved data from The Cancer Genome Atlas (TCGA) dataset, identified three differentially expressed cuproptosis-related lncRNAs, and developed a prognostic signature that was verified by the Gene Expression Omnibus (GEO) database. The mechanism of action of the cuproptosis-related lncRNA signature was further examined by performing Gene Ontology (GO)/Kyoto Encyclopedia of Genes and Genomes (KEGG), immune response, and drug sensitivity analyses. **Figure 1** shows a diagram of the study process. We selected two differentially expressed lncRNAs for functional analysis of colon cancer cells. Our findings may help further investigate the role of cuproptosis and serve as a basis for developing a therapeutic strategy for colon cancer.

Materials ad methods

Selection of cuproptosis-related lncRNAs

Data on the cuproptosis-related genes were obtained from previous literature [16-19]. The expression profile of lncRNAs was obtained from TCGA database through gene expression profiling. Then, the results of co-expression analysis of cuproptosis-related genes were used to determine the cuproptosis-related lncRNA profiles (threshold for coefficients = 0.40, $P < 0.01$).

Construction and validation of CupRLSig

LASSOCox regression and multi-factor Cox regression analyses were conducted using the "Glmnet" R package to analyze these prognos-

tic candidates. A 3-cuproptosis-related lncRNA signature was ultimately established. The formula for calculating the risk score is listed in the Table S1. TCGA cohort (n = 453) was randomly divided into training and testing cohorts. A cuproptosis-related lncRNA model was constructed using the training cohort, and the testing cohort was used to verify this model. We verified the prognosis of cuproptosis-related lncRNAs based on the colon adenocarcinoma (COAD) survival information in TCGA dataset ($P < 0.05$). The training and test cohorts were divided into high- and low-risk groups, based on the median risk value. The area under the receiver operating characteristic curve were used to evaluate the predictive capacity of this prognostic model. The "prcomp" package was used to perform principal component analysis (PCA) of the prognostic cuproptosis-related lncRNAs.

To verify the robustness of this signature, TCGA and GEO databases were used to validate the CupRLSig as described in previous studies [20-22]. The "Kaplan-Meier" method and "survival" package were used to perform survival analysis of the high-risk and low-risk group with the log-rank test in the GSE39582 (n = 579) data set.

Functional analysis

GO analysis was performed to identify the differentially expressed genes using the "GO plot" package. A *p*-value of less than 0.05 was used as the threshold for significantly enriched functional comments.

Independence of the cuproptosis-related lncRNA model

The Kaplan-Meier curves of the overall survival (OS) differences between the two groups were stratified by age, sex, tumor grade, or TNM stage to evaluate whether the prognostic mode of patients was an independent variable.

Drug sensitivity prediction

The "pRRophetic" R package was used to predict the half maximal inhibitory concentration (IC50) of certain chemotherapeutic drugs, which could be a promising lncRNA-based therapeutic approach for treating colon cancer patients.

Cell line culture

HCT116, SW480, HT29, and SW620 colon cancer cell lines and normal epithelial cells (NCM460) were purchased from the American Type Culture Collection (ATCC). The cells were cultured in Dulbecco's modified eagle medium with 10% fetal bovine serum (BI, Israel) in an incubator (37°C, 95% humidity, and 5% CO_2).

Quantitative reverse transcription polymerase chain reaction

Total RNA was extracted from cells using the Invitrogen™ TRIzol reagent (15596018, Invitrogen). Then, the extracted RNAs were reverse-transcribed into cDNA using a cDNA synthesis kit (K1622, Thermofisher Fisher Scientific). The gene expression level was measured with ABI 7500 using MonAmp™ SYBR® Green qPCR Mix (High ROX) (MQ10301S, Monad) and was calculated using the 2^{-DDCt} method. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control. The quantitative reverse transcription polymerase chain reaction primers were purchased from Tsingke Biotechnology (Tsingke, China). The primer sequences used are listed in Table S2.

Cell Counting Kit-8 assay and colony assay

During Cell Counting Kit-8 (CCK-8) assay, HCT116 and SW620 cells were cultured in 96-well plates at 3×10^4 cells/well. Approximately 10 μl of CCK-8 solution (A311-01, Vazyme) was added into each well, after incubating the cells for 2.5 hours; then, the optical density value was detected at 450 nm.

During colony formation assay, HCT116 and SW620 cells were seeded into 6-well plates at a density of 2×10^5 cells/well. The cells were cultured for 14 days. Then, the cells were fixed with methanol and stained with crystal violet for 20 min.

Statistical analyses

Image visualization and statistical analyses were performed using R (version 4.0.3). Spearman's correlation analysis was used to analyze the correlation between cuproptosis-related genes and lncRNAs. Survival analysis was performed using the Kaplan-Meier method. Chi-square test was used to analyze the dif-

ferences in the proportions of clinical features. The independent prognostic factors for OS were determined using univariate and multivariate Cox regression analyses. The prognostic model's accuracy for predicting OS was evaluated using ROC curve analysis. The Student's t-test was used to perform statistical analysis between the two groups.

Results

Identification of cuproptosis-related differentially expressed LncRNAs in COAD

The data for 459 COAD samples were obtained from TCGA database. Patients with no OS information were excluded, and 453 COAD samples were used in the study. Nineteen cuproptosis-related genes (mRNAs) were screened. The Sankey diagram presents the correlation between cuproptosis-related genes and cuproptosis-related lncRNAs (**Figure 2A**). A model for the evaluation of prognostic risk was established; the cvFIT output and lambda curves are shown in **Figure 2B** and **2C**. Forest plots showed that the three cuproptosis-related lncRNAs were also related to the OS of COAD patients (**Figure 2D**). The expression of all three lncRNAs correlated with patient's outcomes, indicating that they have a prognostic value for COAD. Pearson's correlation analysis was used to identify the specific lncRNAs associated with the patient's prognosis ($|R2| > 0.4$, $P < 0.05$) (**Figure 2E**).

Construction and validation of CupRLSig

To determine the prognostic scores of these cuproptosis-related lncRNAs, the acquired samples were randomly divided into the training and testing cohorts. We used a special formula (Table S1) to calculate the risk value and then assigned the sample from TCGA database into two groups based on the median risk scores. The clinical features of the high-risk and low-risk cohort are presented in **Table 1**. No significant differences were observed between the two datasets in terms of the clinical features ($P > 0.05$). As shown in **Figure 3A** and **3B**, the heatmap shows the expression levels of CupRLSig in these two cohorts. Visualization of the risk scores and OS status showed that the distribution of the samples from the above three cohorts (overall, training, and risk groups) was reasonable (**Figure 3C-F**). Kaplan-Meier survival analysis was performed to analyze the OS rate of COAD patients in TCGA and GSE39582 datasets, and results suggested that the OS rate in the low-risk cohorts was better than that in the high-risk cohorts (**Figure 4A-C**).

The OS differences between the low-risk and high-risk cohorts in TCGA datasets were analyzed, and these differences were stratified based on the universal clinicopathological features. The subgroups were divided by sex, age, tumor stage, and lymph node metastasis status; the OS of the low-risk cohorts continued to improve compared with that of the high-risk cohorts (Figure S1). Based on the above results, patients from the high-risk cohorts may have higher mortality rates compared with that of patients from the low-risk groups.

Verification of CupRLSig as an independent prognostic factor for COAD

Cox univariate and multivariate regression analyses were performed to assess the independent predictive potential of this signature. The risk value of this signature was related to the OS rates of patients ($P < 0.05$; **Figure 5A**). Moreover, results of the multivariate Cox regression analysis suggested that the CupRLSig could be used as an independent prognostic factor to predict the patient's OS ($P < 0.001$; **Figure 5B**). Furthermore, PCA was carried out to compare the low- and high-risk groups based on the expression profiles of cuproptosis-related genes, cuproptosis-related lncRNAs, and CupRLSig, including those of three cuproptosis-related lncRNAs. As shown in **Figure 5C-E**, the high- and low-risk groups could not be prominently distinguished based on the expression profiles of the cuproptosis-related gene or cuproptosis-related lncRNAs; hence, an ROC curve was constructed to validate that this signature had notable prognostic accuracy compared with other clinicopathological features (Figure S2A). The concordance index (C-index) was used to evaluate the prediction ability of the model and whether the predicted results were consistent with the actual results [23]. As shown in Figure S2B, the C-index of the risk score for predicting survival was statistically higher than those of age, gender, and TMN stage. Using CupRLSig, high- and low-risk patients could be effectively distin-

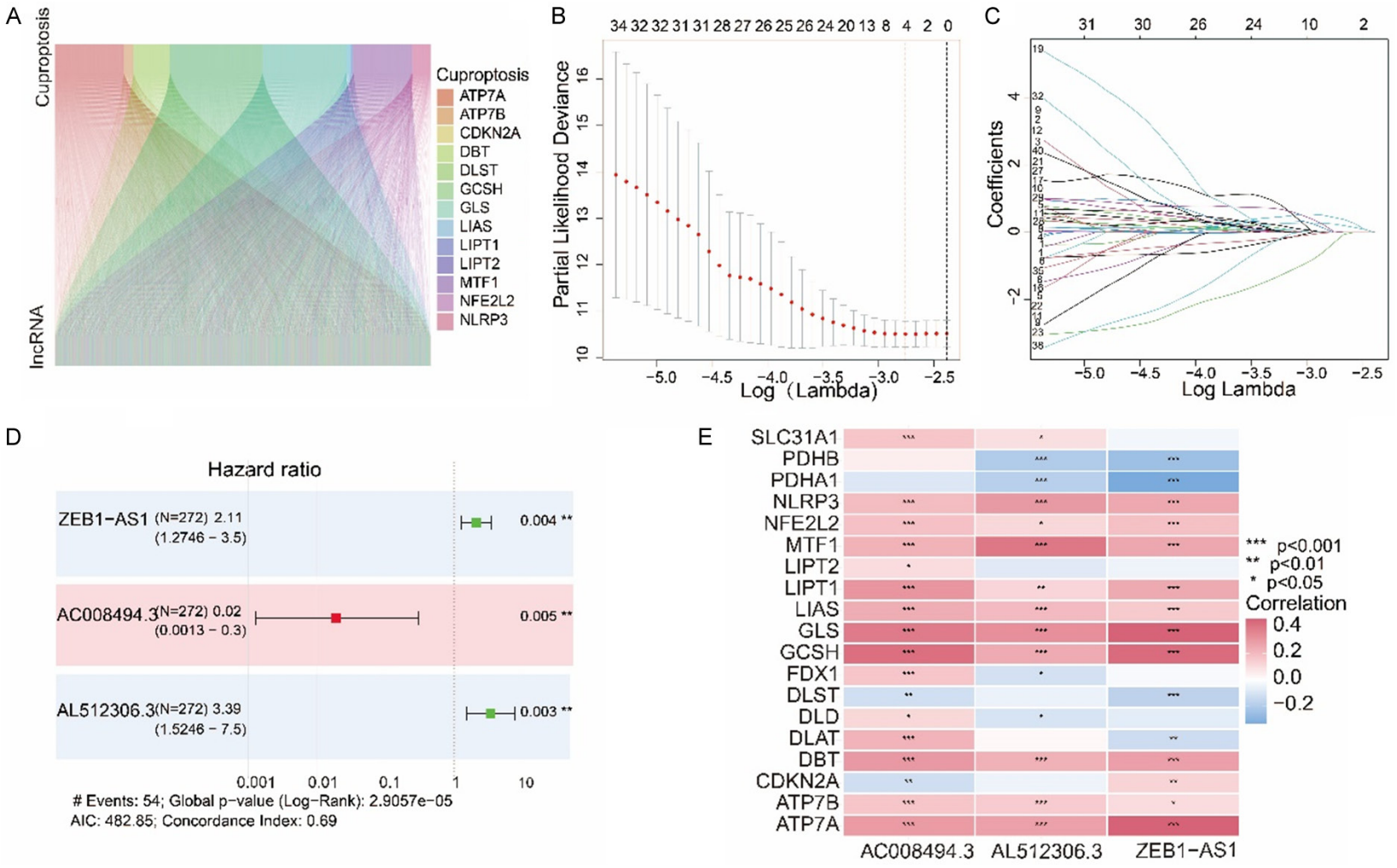

Figure 2. Prognostic analysis of differentially expressed cuproptosis-related lncRNAs and the construction of a co-expression network. A: Cuproptosis-related lncRNA co-expression network was visualized using a Sankey diagram. B-C: Cvfit and lambda curve analyses showing the least absolute shrinkage and selection operator regression were performed using the minimum criteria. D: Forest plots showing the results of the Cox univariate regression analysis of prognostic differentially expressed cuproptosis-related lncRNAs. E: Correlation between cuproptosis-related lncRNAs in TCGA cohort. Red represents positive correlation, while blue represents negative correlation; "*" indicates statistically significance.

Table 1. The clinical characteristics of colon cancer patients in total, train and test group

Covariates	Type	Test	Train	*P* value
Age	≤ 65	76 (41.99%)	112 (41.18%)	0.9406
Age	> 65	105 (58.01%)	160 (58.82%)	
Gender	FEMALE	86 (47.51%)	128 (47.06%)	1
Gender	MALE	95 (52.49%)	144 (52.94%)	
Stage	Stage I	24 (13.26%)	51 (18.75%)	0.2173
Stage	Stage II	72 (39.78%)	103 (37.87%)	
Stage	Stage III	58 (32.04%)	70 (25.74%)	
Stage	Stage IV	22 (12.15%)	42 (15.44%)	
Stage	unknow	5 (2.76%)	6 (2.21%)	
T	T1	5 (2.76%)	6 (2.21%)	0.6989
T	T2	27 (14.92%)	50 (18.38%)	
T	T3	124 (68.51%)	184 (67.65%)	
T	T4	25 (13.81%)	31 (11.4%)	
T	unknow	0 (0%)	1 (0.37%)	
M	M0	138 (76.24%)	194 (71.32%)	0.3501
M	M1	22 (12.15%)	42 (15.44%)	
M	unknow	21 (11.6%)	36 (13.24%)	
N	N0	102 (56.35%)	164 (60.29%)	0.6495
N	N1	43 (23.76%)	62 (22.79%)	
N	N2	36 (19.89%)	46 (16.91%)	

guished, further sustaining the accuracy of the model. These results suggest that CupRLSig can be used as an independent prognostic risk factor for patients with COAD.

Construction of a predictive nomogram

The predictive nomogram was used to compute the survival probability of these patients by adding the scores of several related factors determined on the score table. Compared with the ideal prediction model, the OS rates at 1 year, 3 years, and 5 years were accurately predicted (**Figure 5F**). The 1-year, 3-year, and 5-year nomograph calibration plots (**Figure 5G**) showed that the mortality rate estimated using the nomogram is close to the actual mortality rate. In addition, the area under the curve at 1 year, 3 years, and 5 years remained > 0.75 (**Figure 5H**). These results suggest that the nomogram is reliable for predicting the OS of patients with colon cancer.

Evaluation of immune response among high- and low-risk groups

The potential differences in biological functions and signaling pathways were investigated between the two risk groups. Results of the GO analysis suggested that various immune-related and cell proliferation-related biological processes were involved, such as the regulation of humoral immune response, growth factor activity, and B cell-mediated immunity (Figure S3A). Moreover, the immune-related and virus-infection-related pathways were also enriched (Figure S3B). In summary, the above results indicated that the risk score of CupRLSig was associated with tumor immunity and proliferation in colon cancer. To identify the relationship between CupRLSig and antitumor immunity in patients with COAD, we verified the immune response of patients. As shown in **Figure 6A**, inflammation-promoting response and T-cell co-inhibition showed significant differences between the two groups.

Drug sensitivity analysis

We further explored the differences in drug resistance potential between the two risk groups. The IC50 values for four representative drugs or inhibitors in the two groups and the correlation between IC50 and the risk score are shown in **Figure 6B-E**. The IC50 values of axitinib, cisplatin, doxorubicin, and elesclomol were negatively correlated with the risk score, which may be used as candidate drugs for the treatment of high-risk groups.

Expression of AP003392.4 and AC023157.2 in colon cancer cells

In this study, we detected the expression levels of AL512306.3 and ZEB1-ASA in CRC cell lines (HCT116, SW620, SW480, and HT29) and colonic epithelial cells (NCM460). The expression levels of both AL512306.3 and ZEB1-ASA were significantly upregulated in CRC cells (**Figure 7A** and **7B**). To further clarify the potential function of these two lncRNAs, we developed specific siRNAs to knockdown the expression of AL512306.3 and ZEB1-ASA in HCT116 and SW620 cells. The results of interference efficiency showed that si-2 and si-3 showed greater interference (**Figure 7C** and **7D**). Then, CCK-8 and colony formation assays were conducted to detect the effect of this interference on cell proliferation. As shown in **Figure 7E-H**,

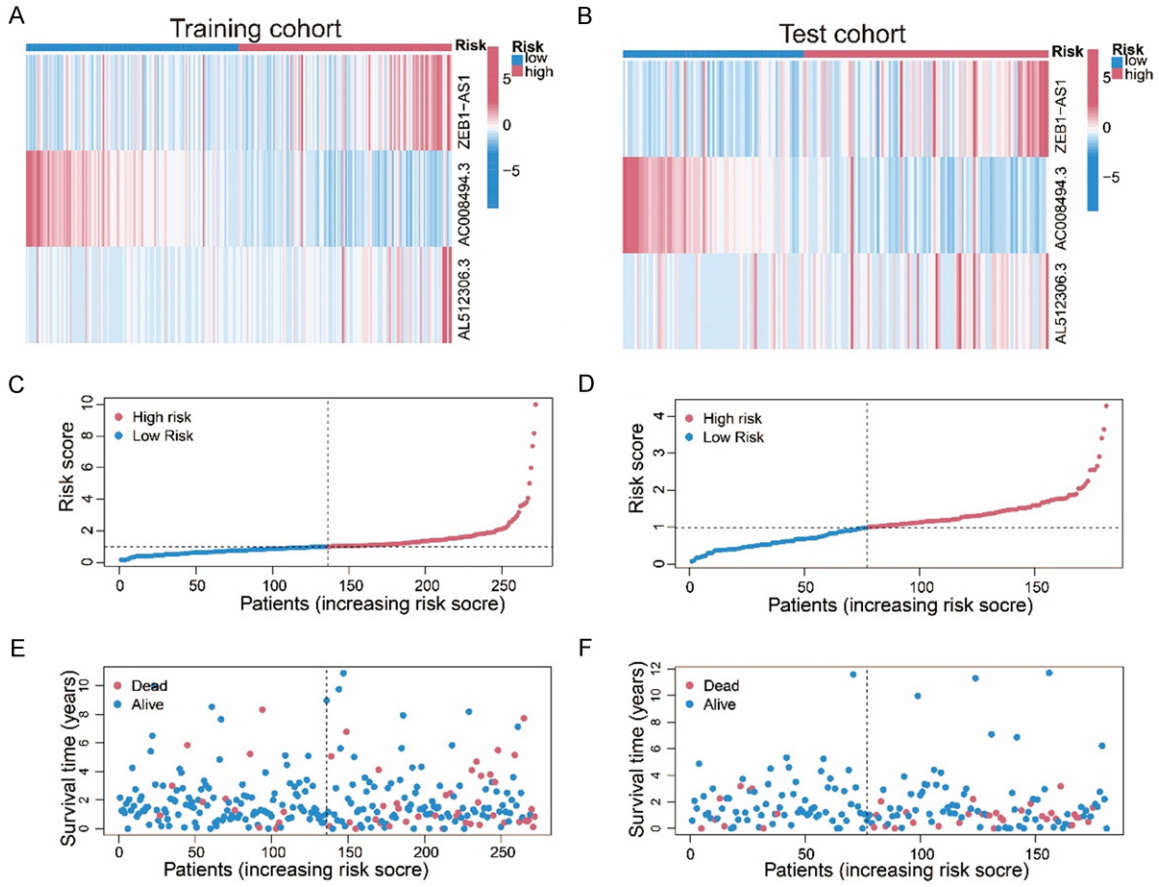

Figure 3. Construction and validation of the CupRLSig model in the training and test cohorts. A-B: Heatmap showing the expression of cuproptosis-related lncRNAs in high- and low-risk groups. C-F: Distribution of risk scores and distributions of overall survival status and risk score.

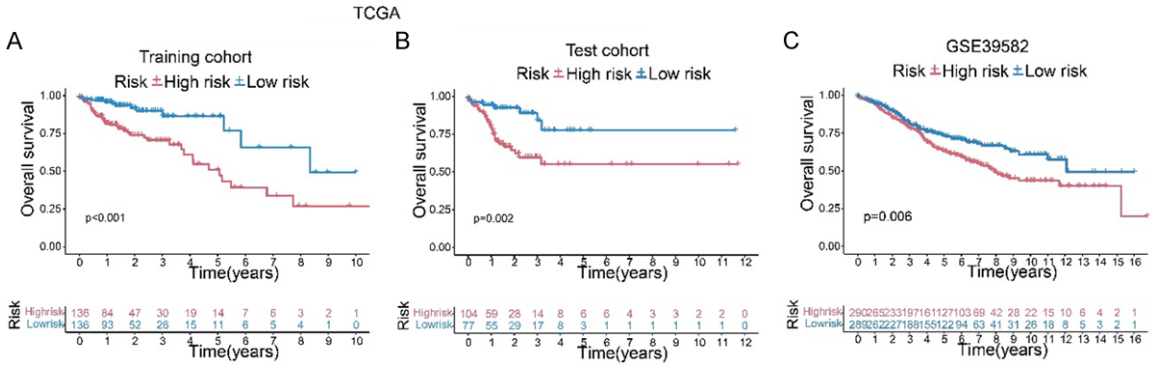

Figure 4. Validation of overall survival in TCGA and validation datasets. A-B: The Kaplan-Meier curves for survival status and survival time in TCGA data set. C: The Kaplan-Meier curves for survival status and survival time in GSE39582 data set.

knockdown of AL512306.3 and ZEB1-ASA significantly inhibited the cell proliferation.

Discussion

The functional roles of lncRNAs in cancer have been well studied [24]. The availability of open-source databases like TCGA and GEO, along with the popularity of sequencing technology makes it easier for researchers to obtain the expression profiles of lncRNAs in various human cancers. Prognostic models based on the expression profiles of lncRNAs have also been extensively studied to evaluate their prog-

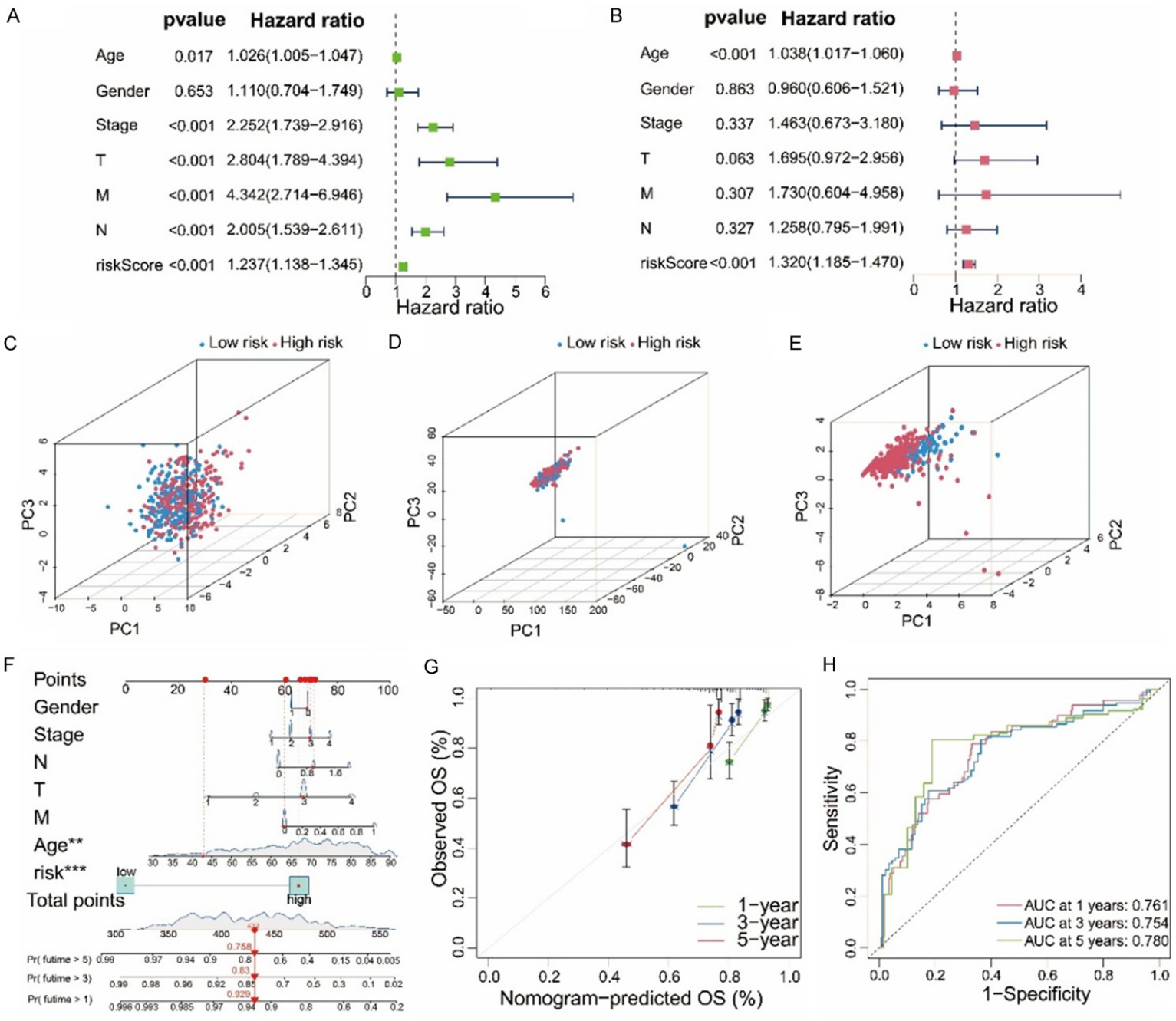
A
pvalue Hazard ratio
Age 0.017 1.026(1.005−1.047)
Gender 0.653 1.110(0.704−1.749)
Stage <0.001 2.252(1.739−2.916)
T <0.001 2.804(1.789−4.394)
M <0.001 4.342(2.714−6.946)
N <0.001 2.005(1.539−2.611)
riskScore <0.001 1.237(1.138−1.345)
Hazard ratio
B
pvalue Hazard ratio
Age <0.001 1.038(1.017−1.060)
Gender 0.863 0.960(0.606−1.521)
Stage 0.337 1.463(0.673−3.180)
T 0.063 1.695(0.972−2.956)
M 0.307 1.730(0.604−4.958)
N 0.327 1.258(0.795−1.991)
riskScore <0.001 1.320(1.185−1.470)
Hazard ratio
C
Low risk High risk
PC1
PC2
PC3
D
Low risk High risk
PC1
PC2
PC3
E
Low risk High risk
PC1
PC2
PC3
F
Points
Gender
Stage
N
T
M
Age**
risk***
Total points
low
high
Pr(futime > 5)
Pr(futime > 3)
Pr(futime > 1)
G
Observed OS (%)
Nomogram−predicted OS (%)
1−year
3−year
5−year
H
Sensitivity
1−Specificity
AUC at 1 years: 0.761
AUC at 3 years: 0.754
AUC at 5 years: 0.780

Figure 5. CupRLSig as an independent prognostic factor for overall survival. A-B: Results of univariate Cox regression analysis and multivariate Cox regression analysis of the OS of the 3-cuproptosis-related-lncRNA signature. C-E: Principal component analysis (PCA) of low-risk and high-risk groups based on the C cuproptosis-related genome, D cuproptosis-related lncRNAs, and E risk model including three cuproptosis-related lncRNAs. F: Nomogram for predicting the 1-year, 3-year, and 5-year overall survival rates of colon cancer patients. G: Calibration curve for evaluating the accuracy of the nomogram model. H: Receiver operating characteristic curve analysis of the clinical characteristics and 1-, 3-, and 5-year survival.

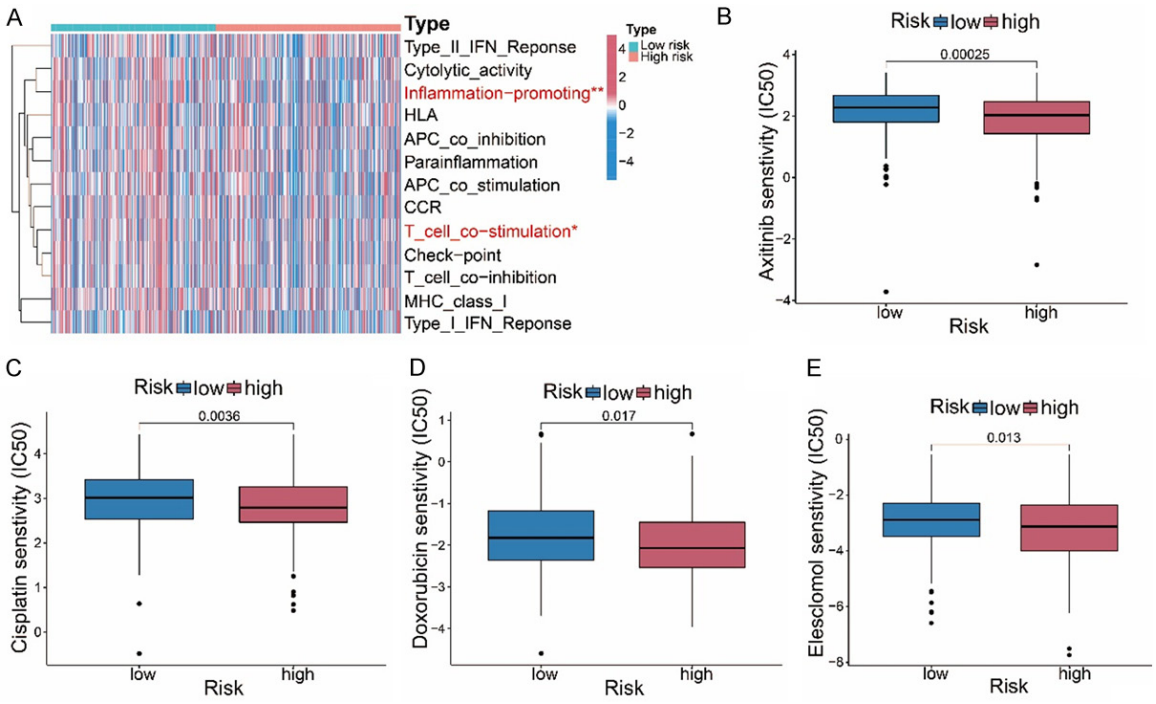

Figure 6. Immune response analysis and drug sensitive prediction in high-risk and low-risk groups. A: Heatmap showing the distribution of immune responses between high- and low-risk groups. B-E: Half maximal inhibitory concentration of axitinib, cisplatin, doxorubicin, and elesclomol in high- and low-risk groups.

nostic value in colon cancers [25, 26]. The mechanism of iron-induced cytotoxicity is well established [27, 28]. However, the function and mechanism of cooper-related cell death remain unclear. The identification of cuproptosis-related lncRNAs is crucial for identifying promising therapeutic targets and prognostic predictors of colon cancer. Studies on the role of cuproptosis-related lncRNAs in colon cancer are limited due to the small number of relevant studies on cuproptosis.

In this study, we explored the relationship between lncRNAs and cuproptosis-related genes in colon cancer and identified various cuproptosis-related lncRNAs. The relationship between the prognosis of patients and the expression of cuproptosis-related lncRNAs was analyzed, and a distinctive prognostic model based on three cuproptosis-related lncRNAs was established and verified using the GSE39582 dataset, which showed outstanding predictive power compared with the traditional TNM staging. Different risk groups were then divided based on this prognostic model, and the expression of cuproptosis-related lncRNAs was detected in these two groups. Functional enrichment analysis of differentially expressed genes showed that the immune-related pathways were significantly different between the high- and low-risk groups. Finally, we identified some potential drugs (axitinib, cisplatin, doxorubicin, and elesclomol) for treating high-risk group patients. Importantly, AL512306.3 and ZEB1-AS1 were identified as oncogene lncRNAs and may play an essential role in cell proliferation.

Cell death is an essential and finely regulated process, which is important for the removal of

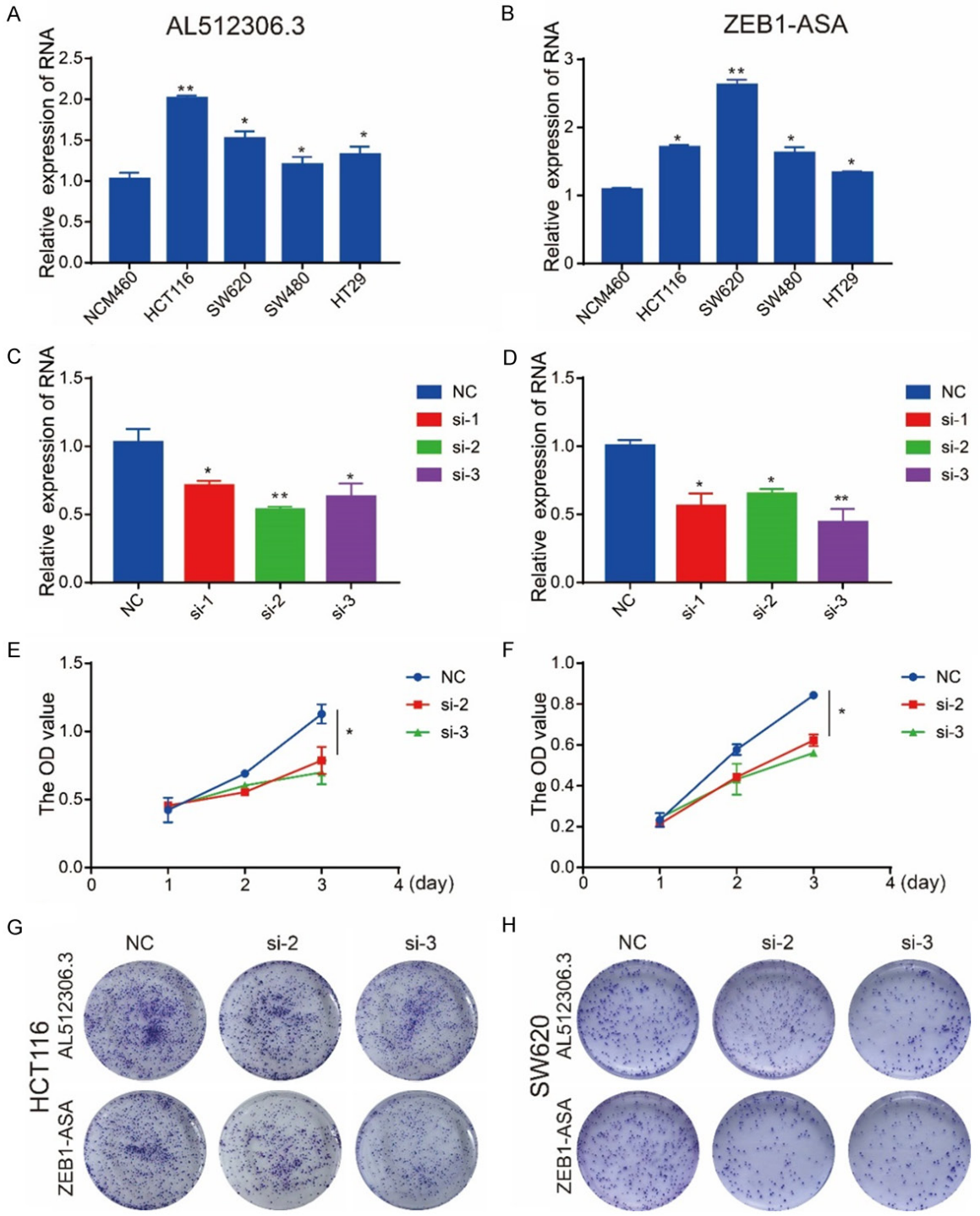

Figure 7. Expression and function of AL512306.3 and ZEB1-AS1 in colon cancer cells. A-B: Relative expression of AL512306.3 and ZEB1-AS1 in colon cancer cells. C-D: Interference efficiency of AL512306.3 and ZEB1-AS1 in colon cancer cells. E-F: Cell Counting Kit-8 assay detects the proliferation of colon cancer cells. G-H: Colony formation assay detects the colony formation ability of colon cancer cells.

damaged and redundant cells. Multiple forms of cell death have been identified, including apoptosis, necroptosis, autophagy, and ferroptosis [29, 30]. Previous studies investigated the correlation between abnormal copper levels in cells and cell death and identified several

genes related to cuproptosis. Copper death is a novel type of cell death, and the accumulation of copper and lipoacylated proteins in cells can promote their oligomerization and abnormal aggregation; on the other hand, they can reduce the level of Fe-S cluster proteins. Both lead to toxic protein stress reactions and finally induce cell death [16].

Copper is an essential factor for multiple functions of the immune system [31]. Functional enrichment analysis indicated that risk-related CupRLSig is mainly enriched in a multitude of immune-related functions and during cell proliferation. Immune response analysis indicated that inflammation-promoting response and T-cell co-inhibition differed significantly between the low- and high-risk groups. The inflammatory response is an essential constituent of the local tumor environment and may exist in tissues before the cancer cells burst and cancer metastasis occurs [32]. Colon cancer is regarded as an inflammation-associated cancer [33], and many proinflammatory signaling pathways are involved in the occurrence of inflammation in colon cancer. In addition, lncRNAs can participate in inflammation-promoting response [34]. For example, lncRNA FEZF1-AS1 activates the signal transducer and activator of transcription 3 (STAT3) signaling pathway and promotes the transformation of inflammation in various types of cancer [35]. AB073614 regulates epithelial to mesenchymal transition through the Janus kinase/STAT3 pathway in colon cancer [36]. HOX transcript antisense intergenic RNA activates NF-kB/TS signaling pathway in colon cancer [37]. Furthermore, we conducted a drug-sensitivity analysis of these two groups. Although the National Comprehensive Cancer Network (NCCN) guidelines recommended several chemotherapeutic drugs for colon cancer treatment [38], individual treatment based on the risk score obtained using a database analysis showed vital potential when combined with the results of our analysis. The smaller IC50 value in the high-risk groups means that patients required lower concentrations of drugs. Four chemotherapeutic drugs or inhibitors (axitinib, cisplatin, doxorubicin, and elesclomol) could be potential drugs for the treatment of high-risk patients. Axitinib, cisplatin, and doxorubicin are commonly used chemotherapeutic drugs that have been well studied in colon cancer treatment. Elesclomol is a stimulator of apoptosis, which has been used in clinical trials of several cancer treatments owing to its ability to destroy the actin cytoskeleton [39]. In addition, elesclomol was applied as combination treatment in melanoma patients [40]; the use of elesclomol combined with paclitaxel has shown good results in clinical trials [41]. In addition, one study reported that the mechanism of elesclomol in retarding colorectal cancer cells is by promoting the degradation of ATPase copper transporting alpha [42]. Elesclomol may have a better effect in the treatment of patients with colon cancer in high-risk groups, which indicates that elesclomol may be a potential anticancer drug for the individualized treatment of patients in high-risk groups. This finding indicates that using this signature may promote personalized treatment and encourage researchers to redesign clinical trials.

To confirm our prediction based on the bioinformatic results, two cuproptosis-related lncRNAs (AL512306.3 and ZEB1-AS1) were chosen for further analysis. ZEB1-AS1 was reported to be an oncogene in prostate cancer [43], hepatocellular carcinoma [44], pancreatic cancer [45], and colorectal cancer [46]. However, studies on the role of AL512306.3 in cancer are limited. Herein, we found that AL512306.3 and ZEB1-AS1 were more highly expressed in colon cancer cells than in normal epithelial cells. Furthermore, the interference of AL512306.3 and ZEB1-AS1 significantly inhibited the growth of cancer cells. These results indicate that AL512306.3 and ZEB1-AS1 act as unfavorable factors for the prognosis of patients with colon cancer.

Although a previous study on the CupRLSig of prognostic models for colon cancer has been published [47], some differences were observed between the two CupRLSig, which may be related to the different detection methods and samples from TCGA datasets, and/or tumor heterogeneity. The novel discoveries of the present study were as follows: (1) GEO database was used to validated the cuproptosis-related lncRNAs prognostic models. (2) The OS times of patients in the high-risk and low-risk groups differed in terms of distant metastasis status (M0-M1), lymph node metastasis status (N0-N2), disease stage (III-IV), and tumor stage (T3-4). This model more accurately distinguish-

es the prognosis of patients with different clinicopathological features and serves as a guide for cancer treatment and evaluating prognosis. (3) The high-risk and low-risk groups were divided based on the expression profiles of cuproptosis-related lncRNAs; elesclomol has a higher sensitivity in patients with colon cancer in high-risk groups and could be an effective drug for precise and individualized treatment.

In conclusion, we used three cuproptosis-related lncRNAs to build a prognosis model and analyzed the model from three perspectives: clinical parameters, GO and KEGG, and differentially expressed lncRNAs. The model exhibited a strong prognostic ability. In addition to devising a highly effective and robust nomogram to evaluate the colon cancer prognosis, we screened potential drugs for patients with colon cancer, which may contribute to individual therapy and predict the effect of treatment. We also identified AL512306.3 and ZEB1-AS1 as highly differentially expressed lncRNAs and oncogenes in colon cancer. Overall, these findings may help researchers to investigate the mechanism of cuproptosis and provide novel therapeutic strategies for future treatment.

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Disclosure of conflict of interest

None.

Address correspondence to: Qiangqiang Zhao, Department of Hematology, The Qinghai Provincial People's Hospital, Xining 810007, Qinghai, China. E-mail: zgzyws@163.com; He Huang, NHC Key Laboratory of Carcinogenesis, Cancer Research Institute and School of Basic Medicine, Central South University, Changsha 410078, Hunan, China. E-mail: huanghe@csu.edu.cn

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Table S1. The formula of the risk score

The formula of the risk score was followed:
RiskScore = 0.747644483×ExpZEB1-AS1 + -3.92554137651963×ExpAC008494.3 + 1.22136529854534 × ExpAL512306.3
(Note: Exp.... represent the expression of lncRNA in TCGA database)

Table S2. Primer sequence of lncRNAs

Name	Sequences	
ZEB1-AS1	Forward	TCCCTGCTAAGCTTCCTTCAGTGT
	Reverse	GACAGTGATCACTTTCATATCC
AL512306.3	Forward	TGCTTCCAACAAGTCTGCCA
	Reverse	CCACTGTGGACCCATCAAGG

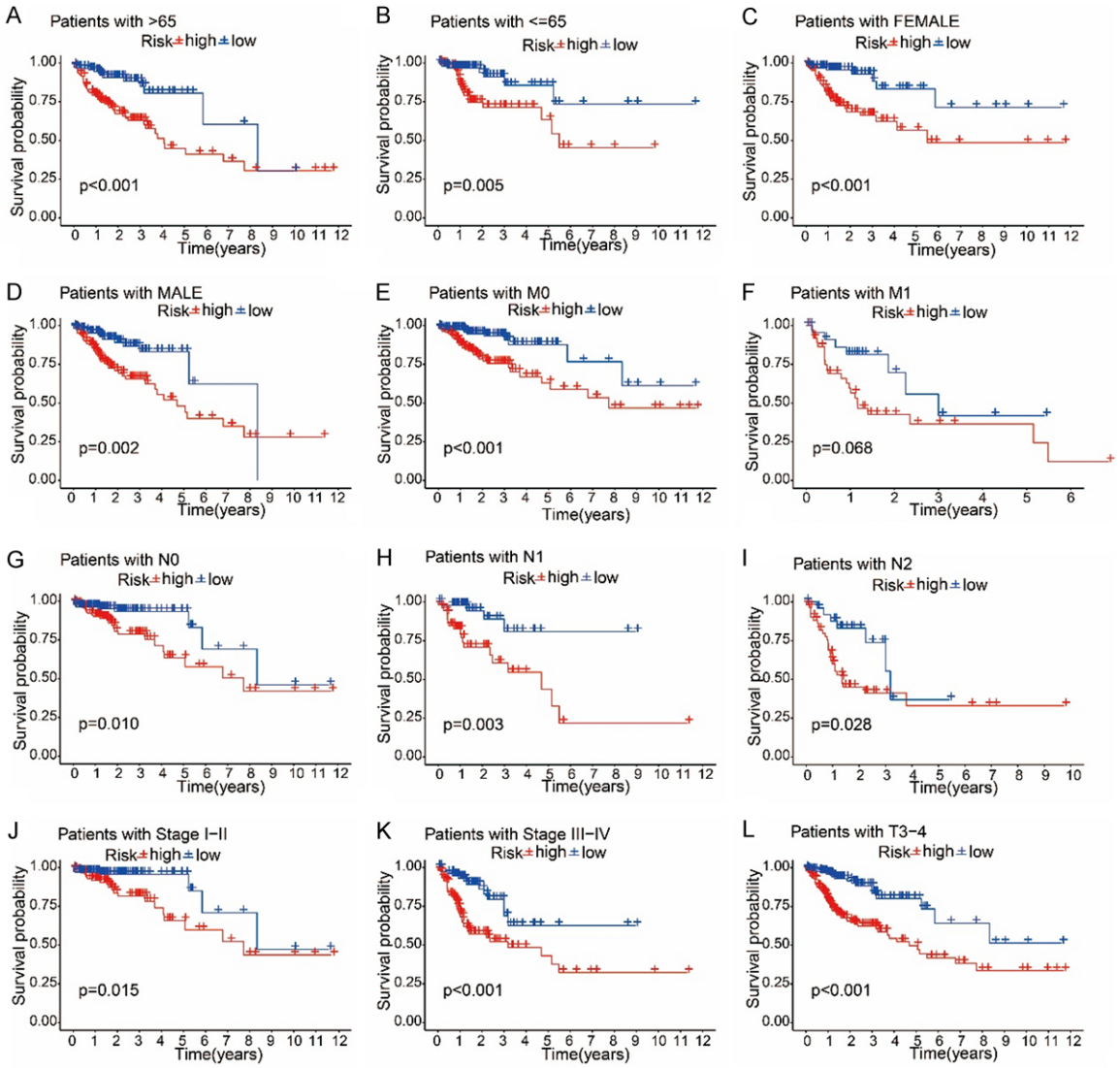

Figure S1. Kaplan-Meier curves of the overall survival differences stratified by gender (A-B), age (C-D), distant metastasis (E-F), lymph node metastasis (G-I), disease stage (J-K), and T stage (L) between the high- and low-risk groups in The Cancer Genome Atlas dataset.

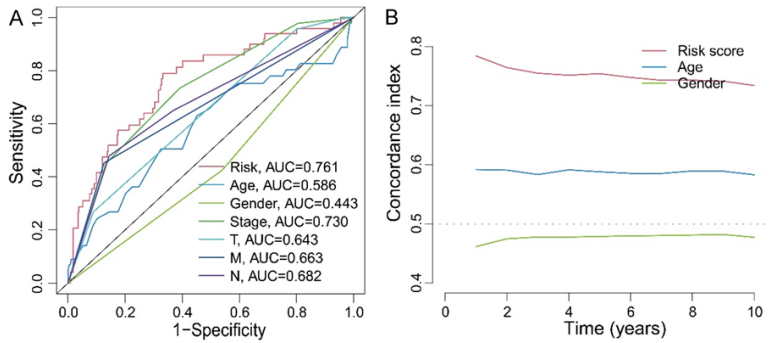

Figure S2. Verification of the predictive ability of CupRLSig. A: Overall survival comparison in receiver operating characteristic curves for clinicopathological features. B: Concordance index used to evaluate the prediction accuracy of the prognosis model.

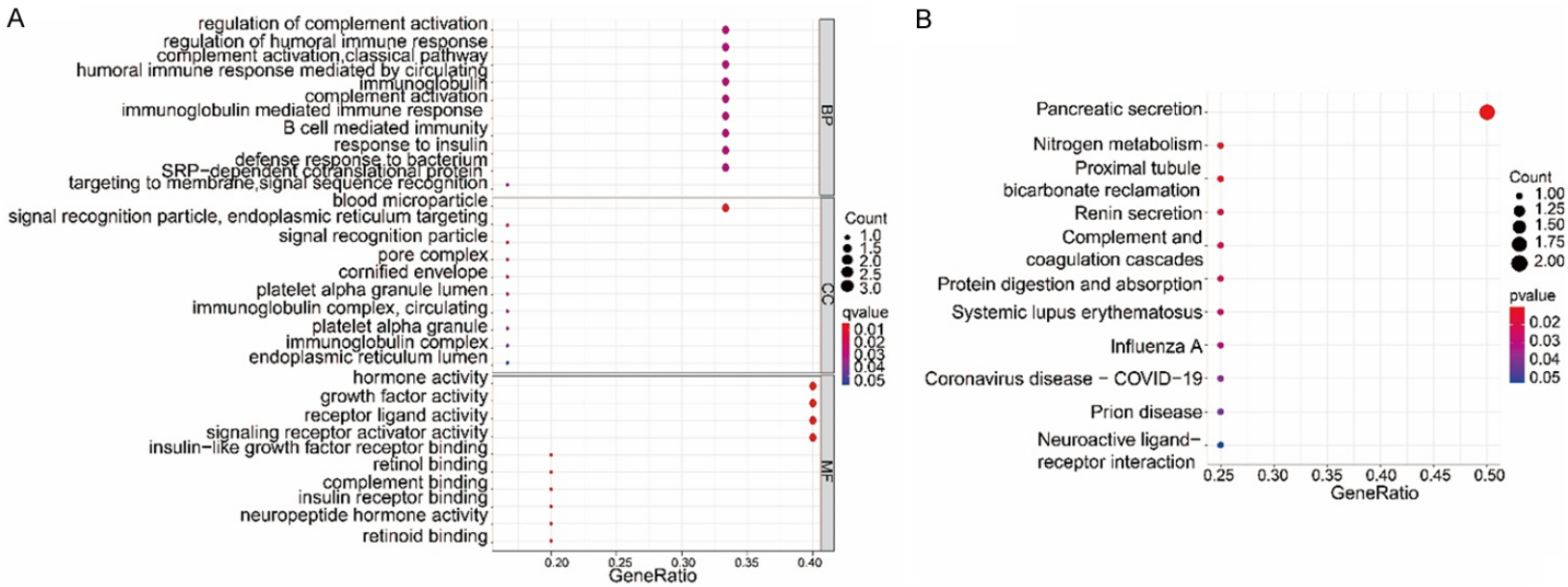

Figure S3. Biological functional and pathway enrichment analysis of high-risk group and low-risk group based on CupRLSig. A: Gene Ontology analysis showing that immune-related and cell proliferation biological processes were enriched. B: Kyoto Encyclopedia of Genes and Genomes analysis showing that many immune-related diseases and virus-infection-related diseases were enriched.