Heliyon 9 (2023) e19035

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Prediction model of clinical prognosis and immunotherapy efficacy of gastric cancer based on level of expression of cuproptosis-related genes

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ARTICLE INFO

Keywords:
Cuproptosis
LncRNA prognostic model
Gastric carcinoma
Tumor microenvironment

ABSTRACT

Background: Gastric cancer is one of the most common malignancies in the world and ranks fourth among cancer-related causes of death. Gastric adenocarcinoma is the most common pathological type of gastric cancer; usually, this tumor is associated with distant metastasis upon first diagnosis and has a poor prognosis. Cuproptosis is a novel mechanism of cell death induced by copper, and is closely related to tumor progression, prognosis and immune response. However, the role of cuproptosis-related genes (CRGs) in the tumor microenvironment (TME) of gastric cancer has yet to be elucidated.

Methods: Gastric adenocarcinoma data were downloaded from the Cancer Genome Atlas (TCGA) database. Through bioinformatics analysis, a risk scoring model was constructed from cuproptosis gene-related lncRNA. Then, we investigated the relationship between prognosis and the TIME of gastric cancer according to clinical characteristics and risk score.

Results: Validation of the model showed that the overall survival (OS) of the high-risk group was significantly lower than that of the low-risk group ($P < 0.001$) and that the risk score was an independent predictor of prognosis ($P < 0.001$). The new model was significantly correlated with the prognosis and TIME of patients with gastric cancer, including immune cell infiltration, tumor mutation burden (TMB) score, targeted drug sensitivity, and immune checkpoint gene expression. In addition, a prognostic nomogram was established based on the risk score (AC008915.2, AC011005.4, AC023511.1, AC139792.1, AL355312.2, LINC01094 and LINC02476).

Conclusion: Our analysis revealed that the prognostic model of cuproptosis-related genes could effectively predict the prognosis of patients with gastric cancer and comprehensively establish the relationship between cuproptosis genes and tumor immunity. This may provide a new strategy for the precise treatment of gastric cancer.

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https://doi.org/10.1016/j.heliyon.2023.e19035
Received 8 September 2022; Received in revised form 29 July 2023; Accepted 7 August 2023
Available online 9 August 2023

1. Introduction

Globally, gastric carcinoma ranks fourth among the leading causes of cancer-related deaths, making it a considerable threat to global health. Of all gastric malignancies, gastric adenocarcinoma is the most common pathological type. Typically, upon initial diagnosis, this tumor exhibits a propensity for metastasis to distant sites, resulting in an unfavorable prognosis. Previous studies have shown that gene mutations and the immune microenvironment play a crucial role in the occurrence and development of gastric cancer. Traditional chemotherapy, targeted therapy and immune-related therapy can effectively improve the prognosis of gastric cancer. However, gastric cancer is a highly heterogeneous malignancy. Many individuals with gastric cancer fail to benefit from targeted therapy or immunotherapy, according to relevant studies. Therefore, it is critical that we identify the population of patients that might benefit from these treatments to optimize survival.

Research has shown that tumor cells' behavior, proliferation, and intercellular communication are influenced not only by their individual characteristics but also by their microenvironment [1–3]. Tumor microenvironment, including immune cells, extracellular matrix molecules, cytokines and tumor-associated macrophages, plays an important role in tumor progression [4]. The tumor immunomicroenvironment (TIME) has been proven to be a dynamic population, and previous studies have shown that the TIME plays a key role in the occurrence and metastasis of colorectal cancer [5,6]. Intrinsic tumor factors such as PD-L1 expression and tumor mutation burden have attracted extensive research attention. However, since tumors exist in a dynamic microenvironment [7], the problem of immunotherapy resistance becomes more complex.

Related studies have shown that copper plays an important role in mitochondrial respiration, iron absorption, antioxidant, detoxification, and other biological processes, and is an essential trace element in cell metabolism. Copper homeostasis is very important for all domains of life [8]; A copper imbalance is associated with a variety of diseases, including Alzheimer's disease, metabolic syndrome, and cancer [9]. Previously, researchers believed that copper was only a tissue metabolic cofactor. However, new studies have shown that copper acts as a dynamic signal and an allosteric regulator [10]. Evidence has shown that copper-dependent phosphodiesterase 3B (PDE3B), mitogen-activated protein kinase1 (MEK1), MEK2, as well as ULK1 and ULK2, play an important role in cell autophagy [11]. Copper has been found to play a critical role in the pathogenesis of malignant tumors by stimulating cellular proliferation and angiogenesis. The binding of copper ions to angiopoietin is a key factor in angiogenesis. In addition, copper induction does not only cause oxidative stress, it can also lead to DNA damage or modifications of molecular structures to activate oncogenes. Previous studies have demonstrated that cuproptosis is mediated by the direct binding of copper to lipoproteins in the tricarboxylic acid cycle, resulting in proteotoxic reactions and subsequent mitochondrial dysfunction [4]. However, abnormalities in the function of the TCA cycle involves a variety of pathological processes. In the tricarboxylic acid cycle and cellular respiration, copper facilitates the binding of fatty acid acylated proteins by coordinating with their carboxylate groups; this leads to the aggregation of fatty acid acylated proteins, the loss of ferrous sulfur cluster protein and the induction of HSP70, intracellular oxidation poisoning, and finally cell death. Furthermore, the generation of reactive oxygen species can activate the apoptotic signaling pathway and induce apoptotic mechanisms to produce anti-tumor effects. However, further research is needed to better explain the mechanism of interaction between cuproptosis and TIME.

In this study, we focused on 19 cuproptosis-related genes (CRGs) in gastric cancer. All samples were divided into high and low groups based on the expression profiles of the CRGs. Then we constructed a clinical prediction model for cuproptosis-related genes. According to the risk score and clinical predictors, the relationship between prognosis and the TIME of patients with gastric cancer was investigated by multivariate analysis. To the best of our knowledge, few studies have investigated the relationship between cuproptosis-related genes and gastric cancer. The purpose of this study was to evaluate the relationship between cuproptosis-related genes and the prognosis, immune microenvironment, immune checkpoint, targeted therapy, and immunotherapy of gastric cancer patients. Our results provide a new strategy for the effective treatment of gastric cancer.

2. Materials and methods

2.1. Data source and preprocessing and tissue samples

A total of 343 TCGA-gastric adenocarcinoma (STAD) specimens and 30 normal tissue samples were acquired from the TCGA database (HTTPS://portal.gdc.cancer.gov/) (Clinical data and RNA Sequencing Data (RNA-SEQ) released on March 15, 2022). Survival information related to the selected samples was complete, and mRNA expression data were in Fragments Per Kilobase of exon model per Million mapped fragments (FPKM) format. We collated a range of clinical data, including overall survival time, age, gender, tumor grade, and tumor stage. Whole exome/genome sequencing (WXS/WGS) somatic mutation data for gastric adenocarcinoma were downloaded to GDC TCGA-STAD on the UCSC Xena server [12]. The MuTect2 algorithm confers high confidence on somatic variants and is used to identify additional germline mutations [13]. The R package "mafTools" was used to plot Oncoplot in descending order of mutations [14]. Analysis of the package was performed using R software (version 4.0.3). The use of tissue samples was approved by the Ethics Committee of the Second Affiliated Hospital of Guangxi Medical University. The cancer tissues and normal tissues of 5 patients with gastric cancer was selected for PCR experiments, and the samples were confirmed as gastric cancer by pathologists.

2.2. The expression levels of CRGs and the identification of cuproptosis-related lncRNAs in gastric cancer

We used Strawberry Perl software (HTTPS://strawberryperl.com/) to divide transcriptomic data into two parts: lncRNAs and

mRNAs. Subsequently, the levels of lncRNAs associated with cuproptosis were determined through gene co-expression analysis of cuproptosis-related genes. The limma R package was then used to extract the expression matrix data of 19 cuproptosis-related genes after cleaning the data with CorFilter set to 0.4 and pvalueFilter set to 0.001, respectively. Finally, 255 lncRNAs related to cuproptosis were verified by co-expression analysis. After combining the expression matrix and survival information, we performed univariate Cox regression analysis with the survival R packet filtering condition set to a pFilter of 0.01 to identify seven prognostic lncRNAs and draw the corresponding forest map. Subsequently, we used the Limma R package to compare the differential expression of prognostic lncRNAs between tumor and normal specimens. (*P < 0.05, **P < 0.01, ***P < 0.001).

2.3. Gene network and enrichment analysis of CRGs

We analyzed the potential interactions of cuproptosis-related genes by GENEMANI and performed gene network analysis [15]. The R package "clusterProfiler" was utilized to conduct Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses [16]. The Benjamini-Hochberg method was used for multiple correction to obtain false discovery rate (FDR). $P < 0.05$ was statistically significant.

2.4. Construction and validation of a prognostic model for cuproptosis-related lncRNAs

We randomly divided the samples into a training group and validation group with a 1:1 ratio. The training group was subsequently used to analyze the CRG_score. Finally, we found that seven lncRNAs were co-expressed in cuproptosis gene-related lncRNAs and performed Lasso regression analysis. The GlMNet R package was then used to select the optimal model for predicting performance. The risk score formula is as follows:

$$Risk\ score = \sum_{i=1}^{n} Coef_i * x_i$$

Coef-i is the risk coefficient and X-i is the expression level of each lncRNA. Patients were divided into a high-risk group and a low-risk group according to the median risk score. We used Survival, SurvMiner and timeROC in the R Package for survival analysis and to evaluate the model with a total dataset, the training set and the test set, respectively. The number of groups was set as 1, and the data were randomly grouped to build a model. By comparing the survival time and survival status of each sample, we could perform univariate Cox analysis to obtain meaningful lncRNAs. The COX model was constructed using lncRNAs that had been selected by LASSO regression. Kaplan-Meier survival curves were drawn using "GGsurvplot" in the R package to compare OS or PFS between the high-risk and low-risk groups. The "survivalROC" tool in R package was used to calculate and draw ROCs. In addition, we identified potential differences between subgroups stratified by age, gender, tumor grade and tumor stage. The "Regplot" tool in R package was used to construct an enhanced regression nomogram of CRG scores and other clinical covariates for patients with gastric cancer. Calibration curves for CRGs scores and other clinical covariates in patients with gastric cancer were estimated using 1000 bootstrapping to determine predictive bias-corrected estimates compared to observations analyzed using the "rms" tool in R package.

2.5. Correlations between immune infiltration and drug sensitivity in gastric cancer

Next, we explored the independent predictive value of the model and its applicability to the clinical characteristics of the cohort, as well as the correlation between the risk score and clinicopathological factors and antitumor drug sensitivity. The LIMMA, GGPLOT2, GGPUBR and ggExtra tools in the R package were used to detect correlations between immune checkpoint and risk score. The high-risk group exhibited a significantly elevated immune score in comparison to the low-risk group. Next, according to the risk score, we investigated potential relationships with genes of immune checkpoint (PDCD1, CTLA4, LAG3 CD274, TIGIT, HAVCR2). By calculating the burden created by tumor mutation, the difference in survival between the high-risk group and low-risk group was identified according to the risk score and the tumor mutation burden. In addition, the IC50 of an antineoplastic drug (the lower the IC50, the higher the sensitivity to the drug) was predicted using the prediction model to identify a more effective subset of drug treatments.

2.6. qRT-PCR

Total RNA samples were extracted from gastric cancer tissues and corresponding paracancerous normal tissues using TRIzol reagent (Wuhan servicebio technology CO.,LTD, G3013) according to the manufacturer's instructions. Total RNA was reverse transcribed into cDNA using the reverse transcription reagent SweScript All-in-One RT SuperMix for qPCR (Wuhan servicebio technology CO.,LTD, G3337). Finally, 2 × SYBR Green qPCR Master Mix (Wuhan servicebio technology CO.,LTD, G3320) was used for qRT-PCR experiment to detect genes expression level.

2.7. Statistical analysis

The R survival package was used for survival analysis. The survival rate of each group was hypothesized by the log-rank test. The comparison between two sets of samples was conducted using Wilcoxon test. When comparing multiple sets of samples, Kruskal Wallis test was used. The Kaplan-Meier method was used to generate a survival curve for the patients. The frequency of gastric cancer

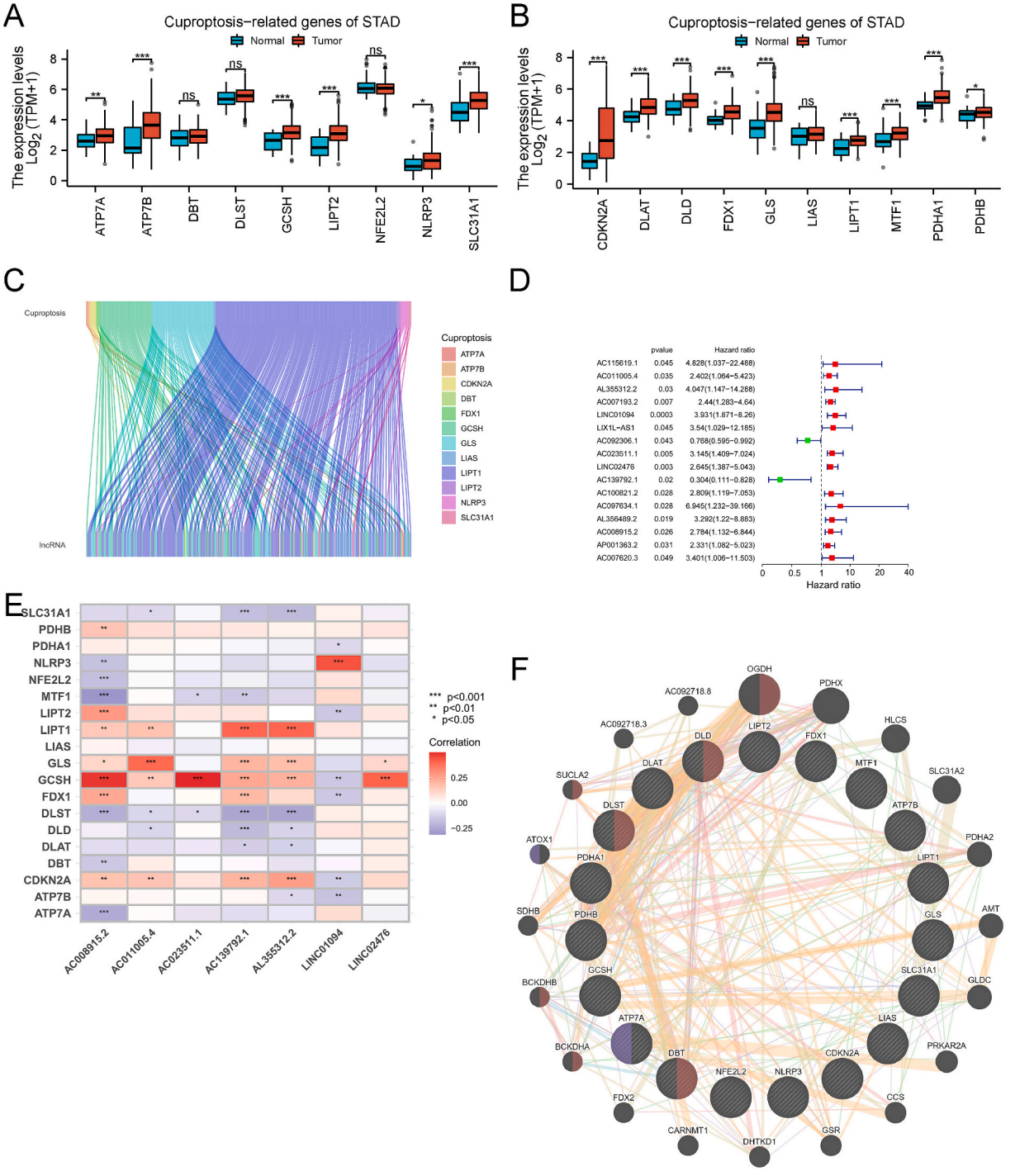

Fig. 1. Differential expression and genetic alterations of cuproptosis-related lncRNAs in gastric cancer patients: (A,B) Differential expression of cuproptosis-related genes in gastric cancer patients. (C) Sankey diagram of cuproptosis-related lncRNAs in gastric cancer (D) Univariate forest map of lncRNA for cuproptosis-related lncRNAs in gastric cancer (E)Heat Map of cuproptosis-related lncRNAs co-expression in gastric cancer (F) Protein-Protein Interaction of cuproptosis-related genes.

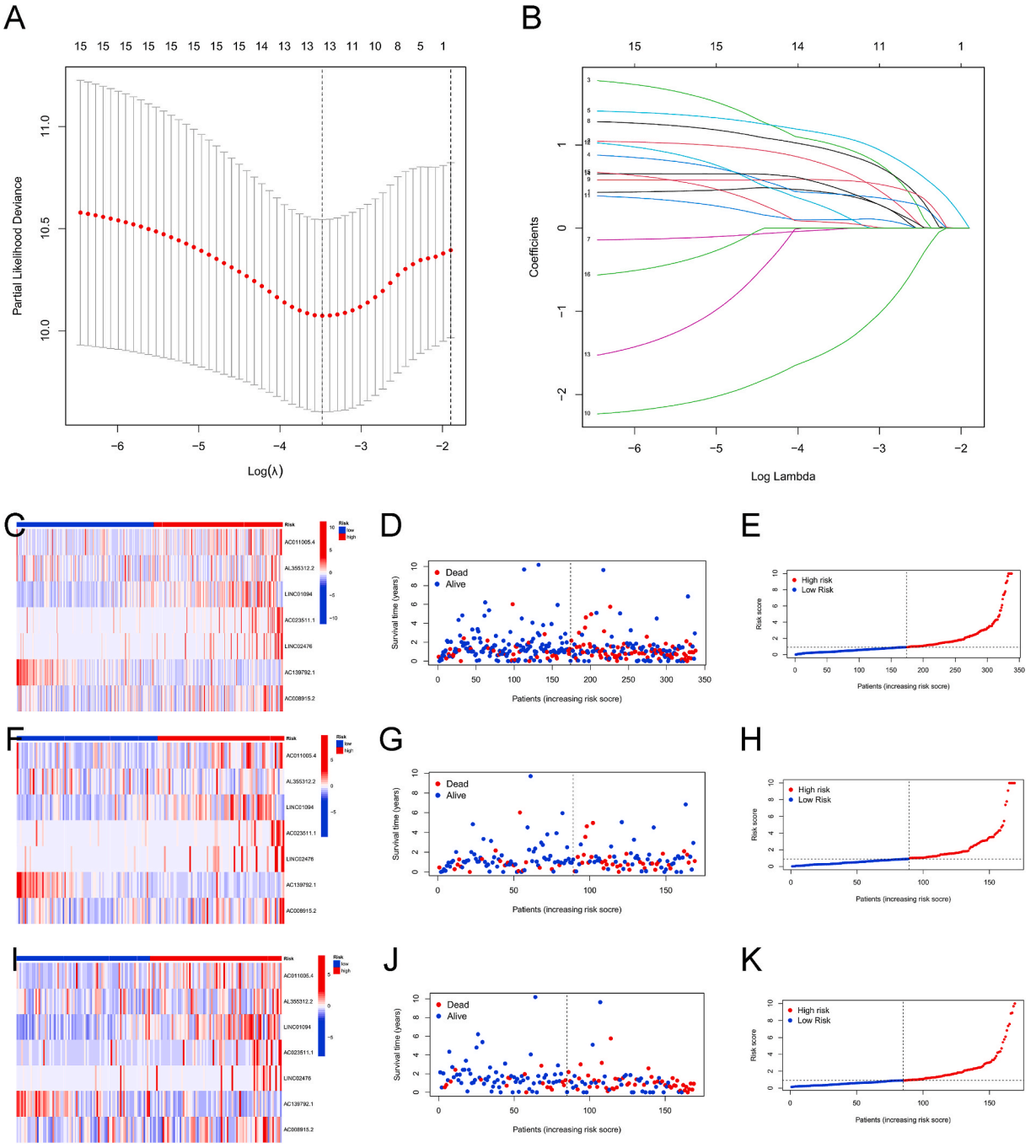

Fig. 2. Construction of the prognosis risk prediction model: (A, B) The least absolute shrinkage and selection operator (LASSO) regression was performed with the minimum criteria. (E, H, K) Exhibition of Cuproptosis-Related lncRNAs prediction model based on risk score of the train, test, and entire sets, respectively. (D,G,J) The survival status between the high-and low-risk group in the train, test, and entire sets, respectively. (C.F,I) cuproptosis-related lncRNAs heat map between the high-and low-risk group in the train, test, and entire sets, respectively. The asterisks represented the statistical p value (*P < 0.05; **P < 0.01; ***P < 0.001).

mutations was analyzed using the Chi-squared test, while the correlation coefficient was calculated through Spearman analysis. All statistical analyses were performed using R version 4.1.0. A significance level of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Acquisition of genetic alterations of cuproptosis-related lncRNAs in gastric cancer

We downloaded transcriptomic data relating to gastric adenocarcinoma from the TCGA database and then divided the mRNA expression matrix into mRNA and lncRNA components. After calculating the differential expression of cuproptosis genes in gastric cancer, we extracted the expression of 19 cuproptosis-related genes from mRNA expression data, and finally obtained 255 lncRNAs related to cuproptosis by co-expression analysis. We used the "GGplot2" and "GGalluvi1" tools in the R package to draw Sankey maps to determine the co-expression relationships between cuproptosis genes and related lncRNAs, and the results were visualized in Fig. 1 (A–F). By reading the expression files of cuproptosis-related lncRNAs and processing the data, the expression levels of lncRNAs in the risk model were extracted, and a forest map of single-factor cuproptosis-related lncRNAs was constructed. Finally, the potential interactions of cuproptosis-related genes were analyzed by GENEMANI (http://genemania.org).

3.2. Prognosis-related lncRNAs were screened by univariate Cox regression analysis and prognostic signature of cuproptosis-related genes in gastric cancer

The univariate Cox significance filtering standard was set to 0.05 (Cox Pfilter: 0.05), and 255 lncRNAs associated with cuproptosis

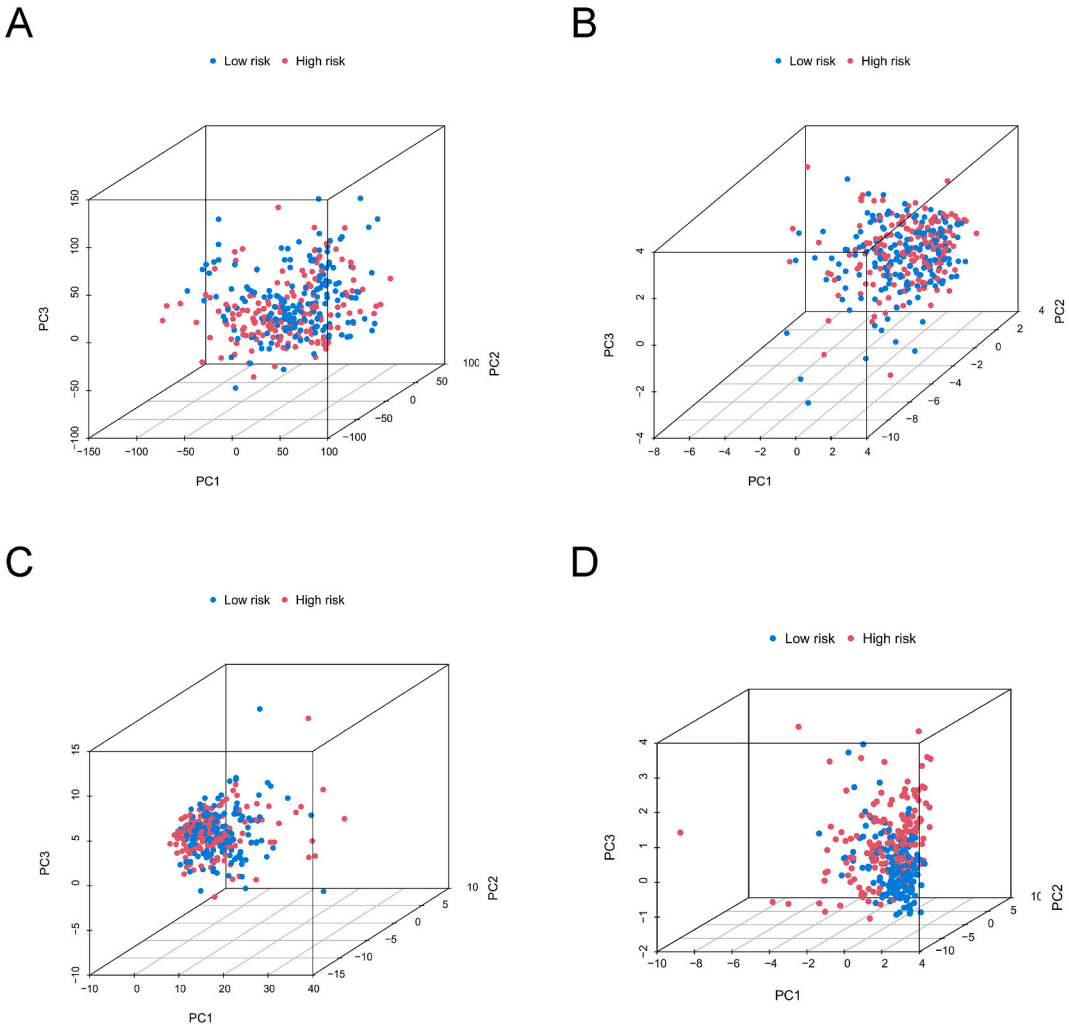

Fig. 3. Principal component analysis of lncRNA involved in model construction (A) Cuproptosis-Related genes (B) Cuproptosis-Related lncRNAs, (C) all gene sets, (D) model lncRNA.

were extracted for univariate Cox regression analysis. A total of 16 lncRNAs with prognostic significance were detected (Hazard Ratio, HR > 1, AC115619.1, AC011005.4, AL355312.2, AC007193.2, LINC01094, LIX1L−AS1, AC092306.1, AC023511.1, LINC02476, AC139792.1, AC100821.2, AC097634.1, AL356489.2 AC008915.2, AP001363.2 AC007620.3) (P < 0.01) (Fig. 1 D). Differential analysis confirmed that the expression of each lncRNA differed significantly between tumors and normal specimens. By setting the number of groups as 1, the data were randomly grouped to construct a prediction model. By comparing the survival time and survival status of each sample, univariate Cox analysis was performed to obtain meaningful lncRNAs. Finally, a LASSO regression model was

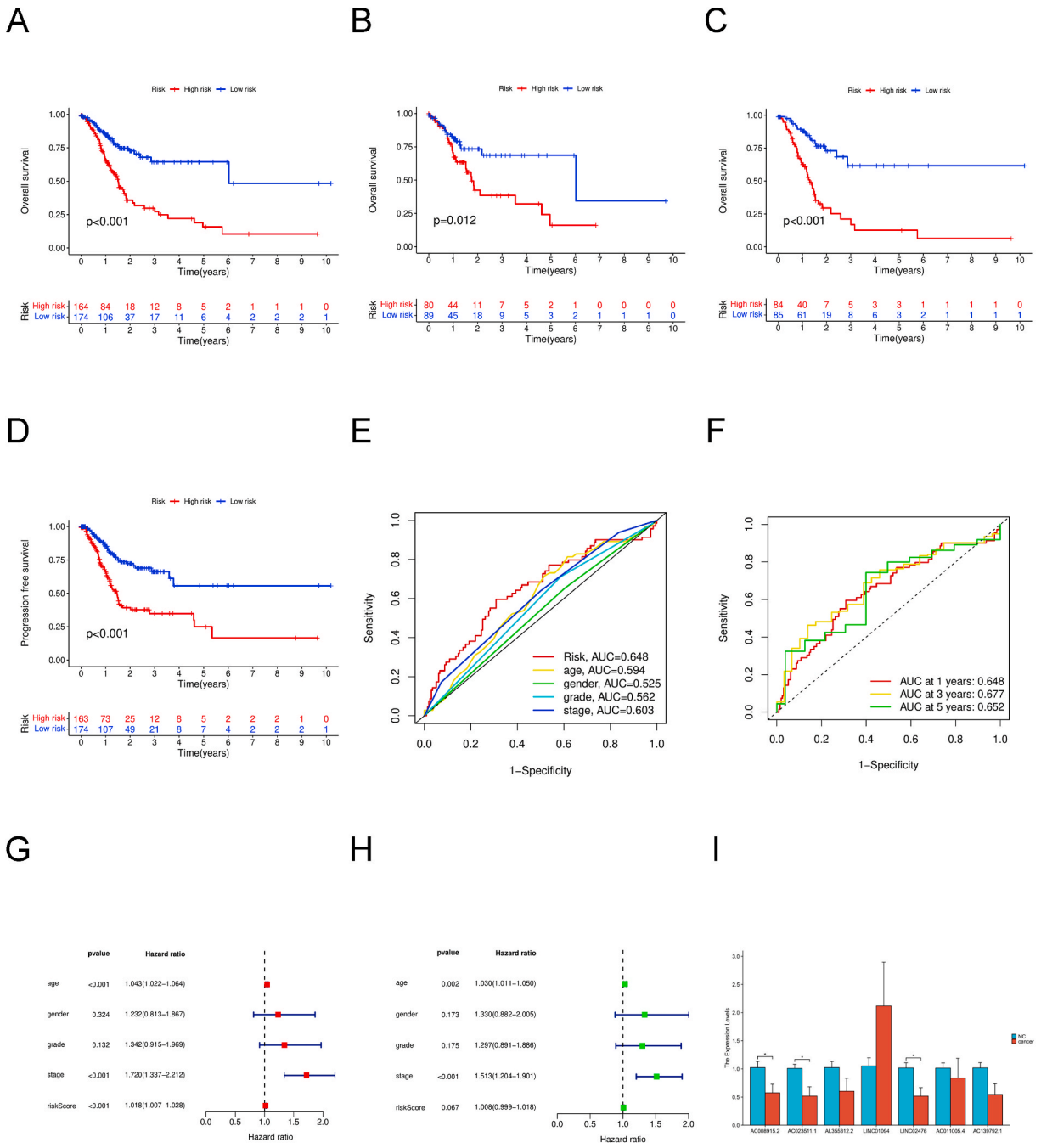

Fig. 4. Prognosis value of the risk prediction model in the train, test, and entire sets: (A–D) Kaplan–Meier survival curves of survival probability of patients and PFS between low-and high-risk groups in the train, test, and entire sets, respectively. (E,F) ROC curves to predict the sensitivity and specificity of 1-, 3-, and 5-year survival according to the CRG_score in the train, test, and entire sets, respectively. (G,H) Age, gender, risk score, tumor stage univariate and multivariate regression analysis of clinical factors and risk score with OS. (I) qRT-PCR was performed to detect the expression level of seven lncRNAs.

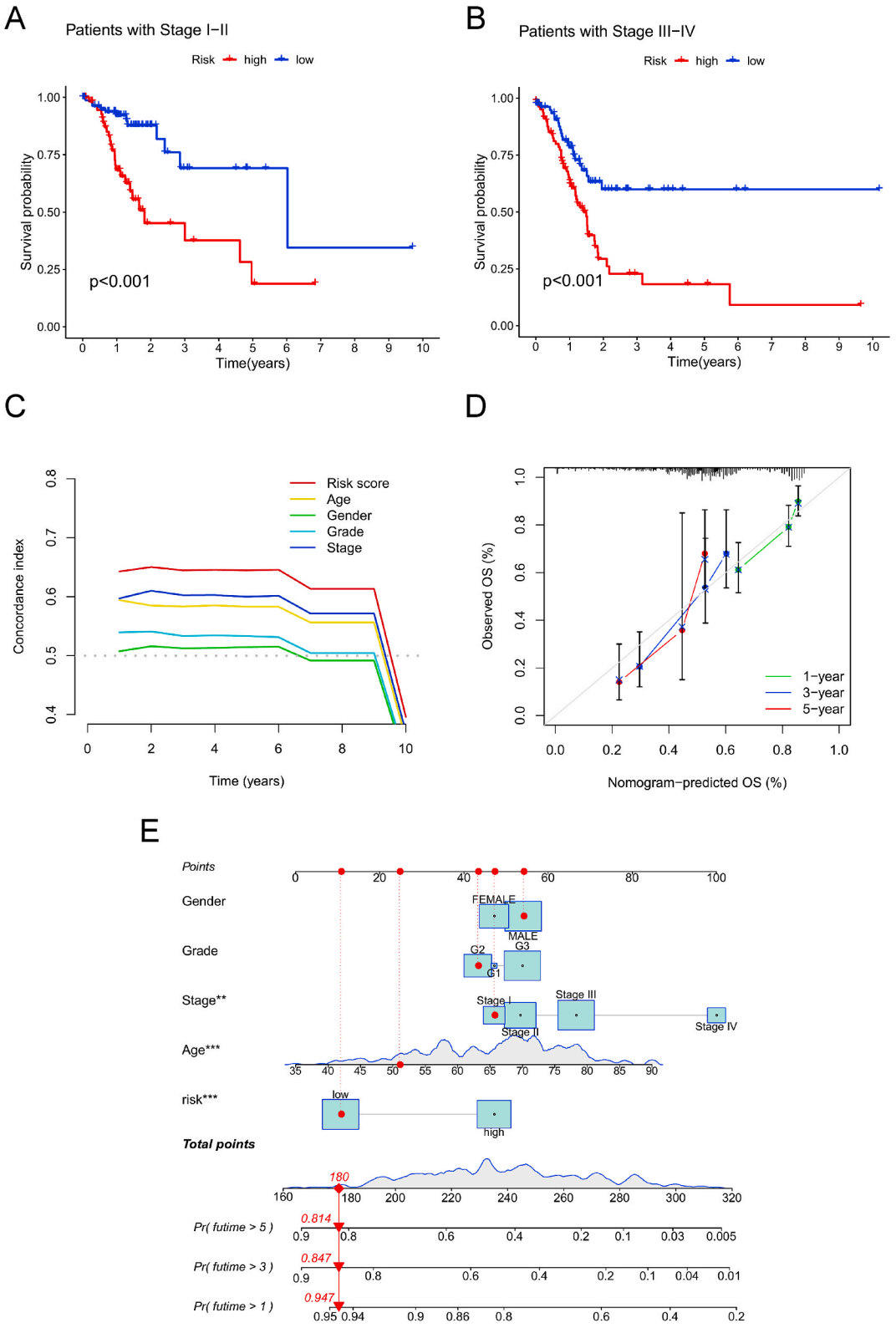

Fig. 5. Construction and Evaluation of Nomogram Based on CRG_score: (A,B)the OS of the high and low risk groups of stage I-II and III-IV patients respectively (C)The c-index curve of the model (D)Calibration curves of the nomogram (E) Nomogram for predicting the 1-, 3-, and 5-year OS of gastric cancer patients.

performed with a single factor meaningful expression file, and the point with minimum error was identified by cross validation. Finally, we constructed a COX model. According to the overall risk curve, we plotted median risk values for the training group and validation group and divided the samples into a high-risk group and a low-risk group. We found that the risk of death increased significantly with increasing risk scores. According to the risk heat map, we also intuitively identified the lncRNAs co-expressed in cuproptosis: AC008915.2, AC011005.4, AC023511.1, AC139792.1, AL355312.2, LINC01094 and LINC02476. The expression of AC011005.4 lncRNA was significantly increased, further indicating that these seven lncRNAs were associated with the high mortality of copper. However, the expression of AC139792.1 decreased as the risk score increased, thus indicating that these are lncRNAs associated with a low risk of copper outbreak, and the results were visualized in Fig. 2 (A – K).

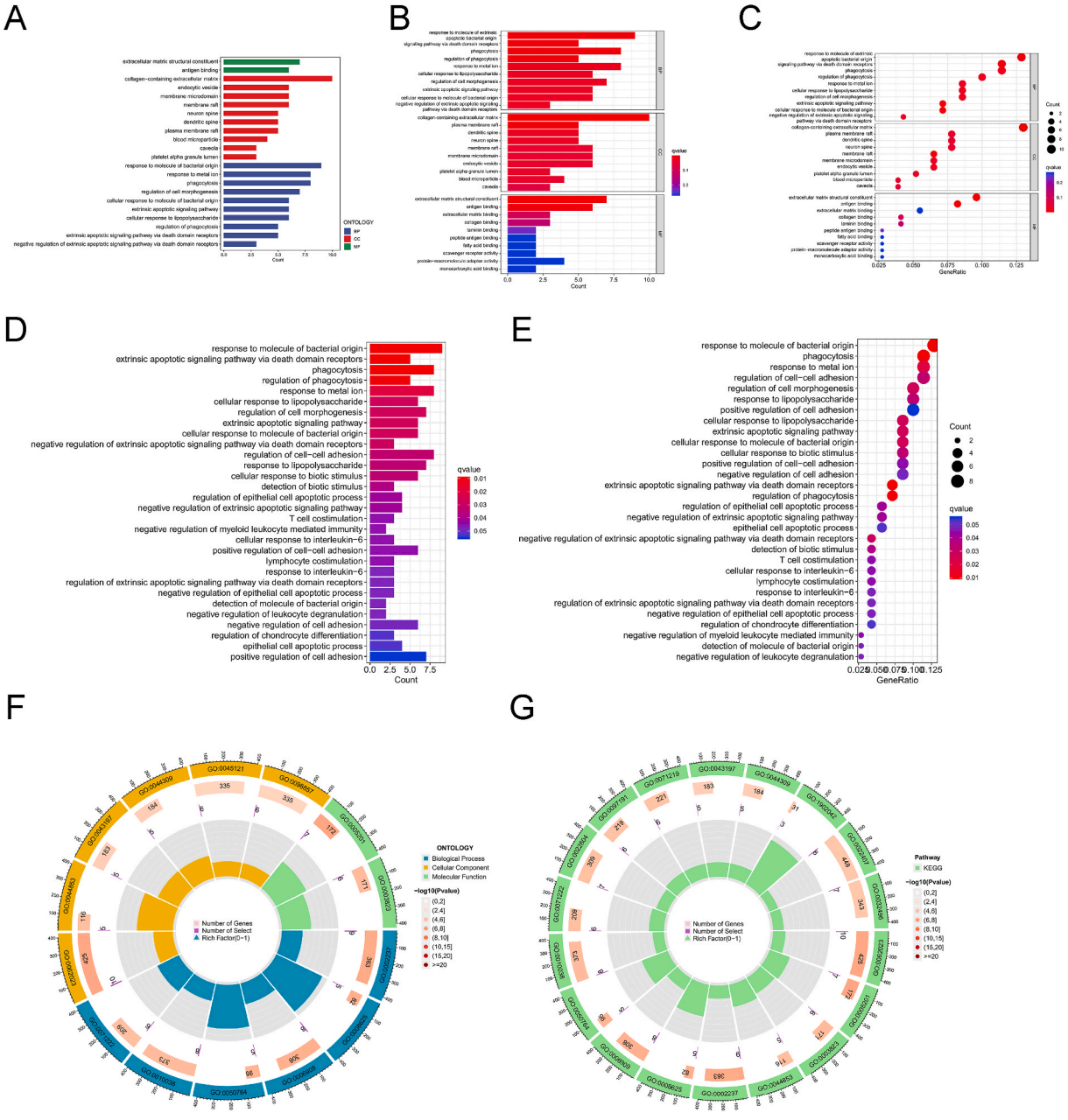

Fig. 6. Pathway enrichment analysis of CRGs in gastric cancer patients of TCGA: (A,B,C,F) the enriched item in the gene ontology analysis (D,E,G) the enriched item in the Kyoto Encyclopedia of Genes and Genomes analysis.

3.3. Principal component analysis

By performing principal component analysis, we were able to identify the lncRNAs to be incorporated in the model construction for the high and low risk groups. Principal component analysis was performed for cuproptosis-related genes, cuproptosis-related lncRNAs,

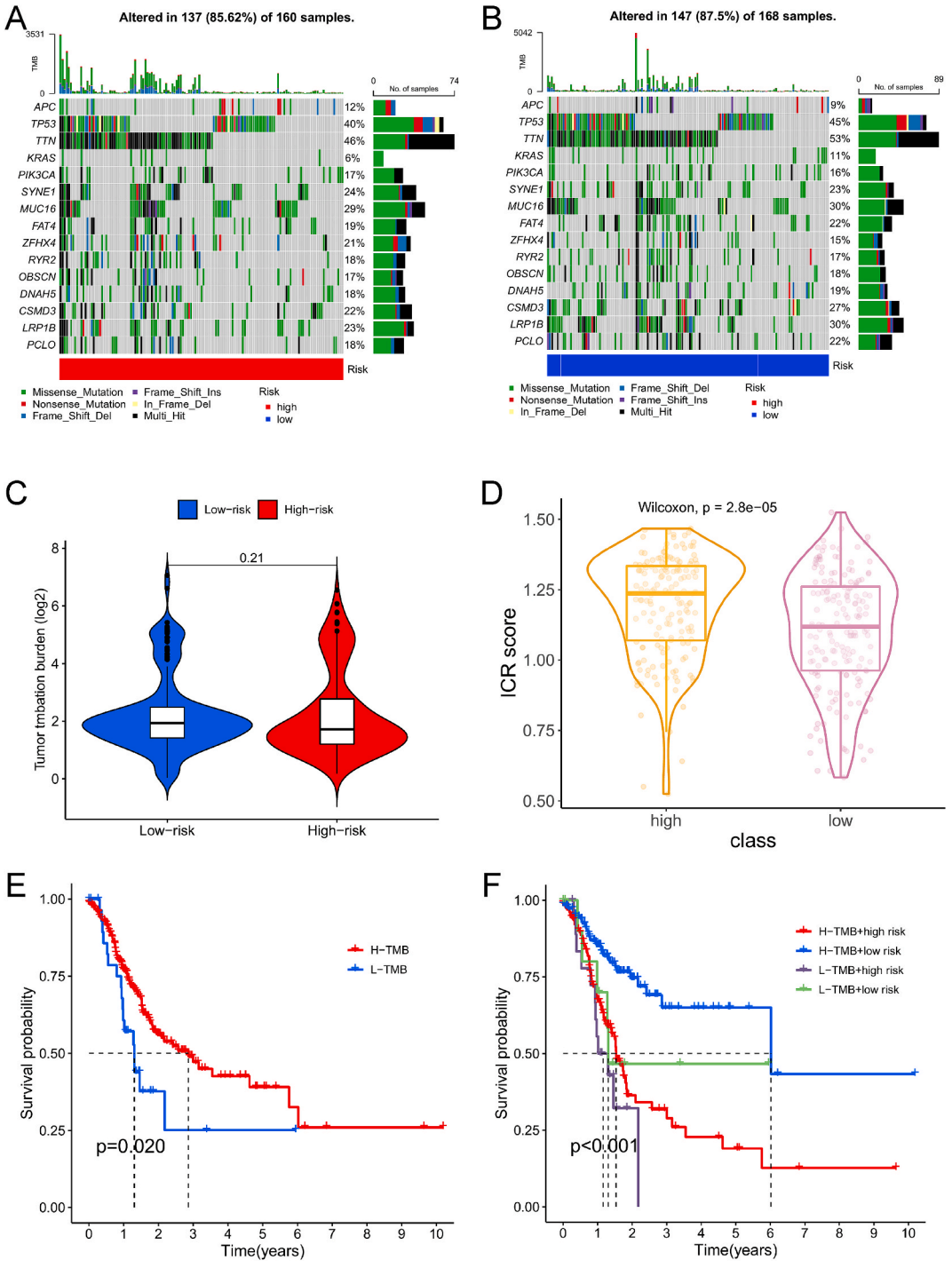

Fig. 7. Genetic characteristics of CRG_score and tumor somatic mutation of gastric cancer: (A, B) The waterfall plot of tumor somatic mutation established by those with low- and high-risk group. (C) Tumor mutation burden in high - and low-risk groups (D) Tumor Immune Dysfunction and Exclusion (TIDE) score in gastric cancer (E,F) The overall survival of the patients stratified by both the CRG-score signature and TMB using Kaplan–Meier curves.

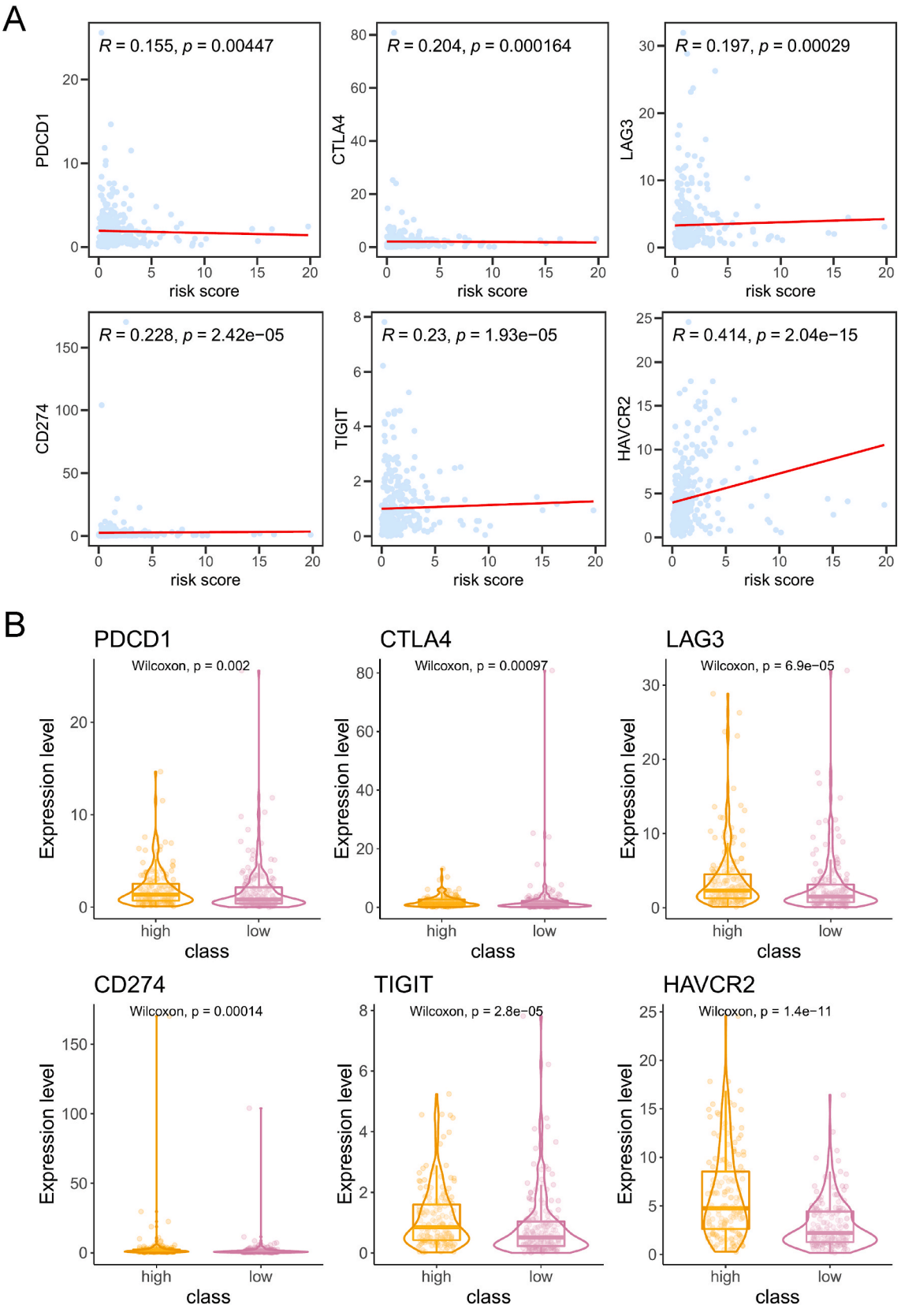

Fig. 8. The difference and correlation between risk score and expression of immune checkpoint in gastric cancer in the TIMER database.

all genomes and model lncRNAs, and the results were visualized in Fig. 3 (A – D).

3.4. Construction and validation of risk predictive model

Multivariate Cox regression analysis was performed on 255 lncRNAs associated with cuproptosis, and seven lncRNAs were found to be significantly correlated with OS. We divided the samples into a training set and a test set (featuring equal numbers) according to a random cycle; then, we constructed a risk prognosis model. The seven prognostic lncRNAs in the two groups were AC008915.2, AC011005.4, AC023511.1, AC139792.1, AL355312.2, LINC01094 and LINC02476. We found that the Kaplan-Meier survival curves of the training and validation sets showed that the survival outcomes of the high-risk and low-risk groups were significantly different, and the OS of the high-risk group was worse than that of the low-risk group. In addition, the overall area under the ROC curve (AUC) over 1, 3 and 5 years also confirmed that the model had good predictive performance; the AUC for 1, 3 and 5 years were 0.648, 0.677 and

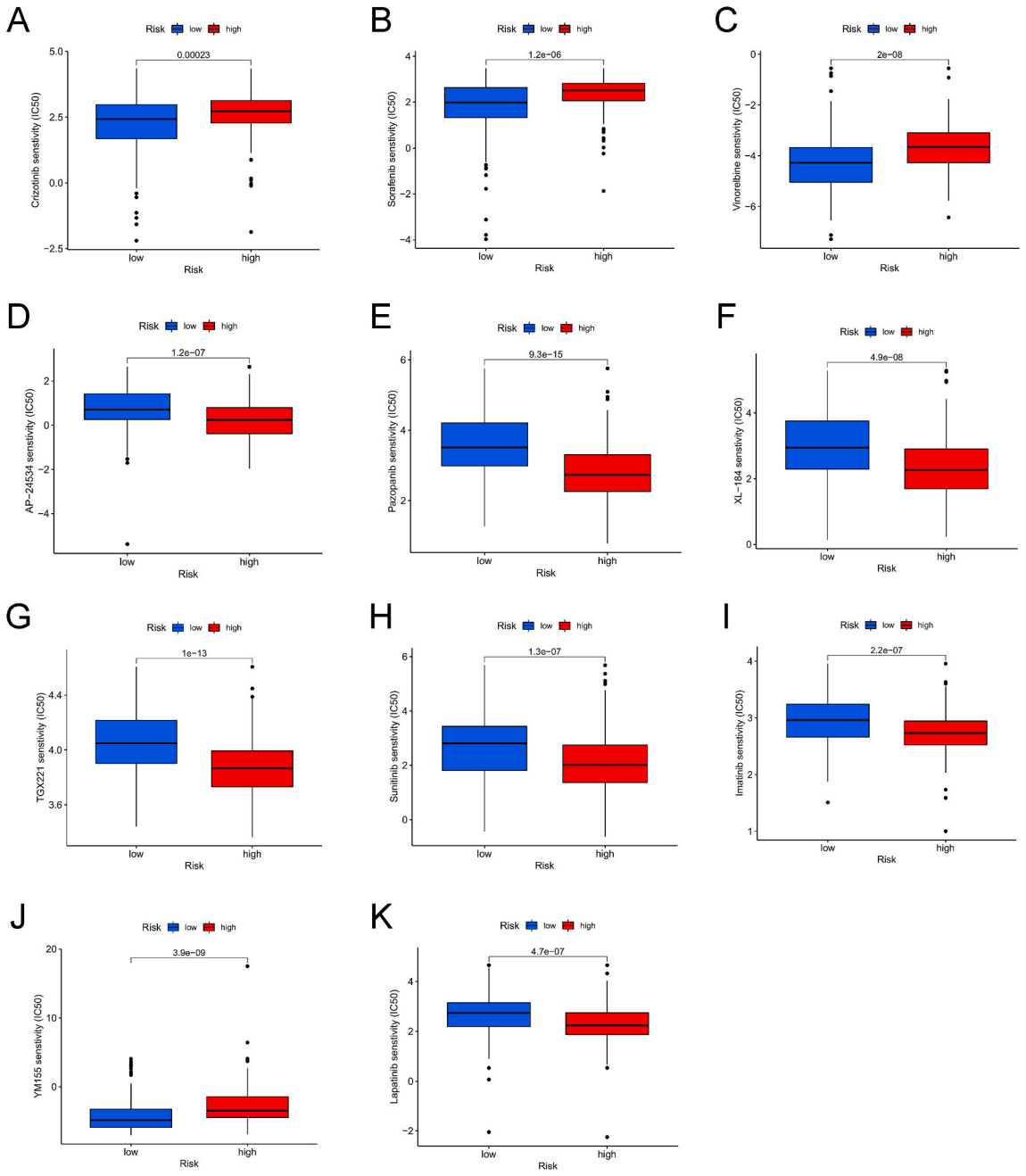

Fig. 9. Drug sensitivity (A–K) estimated drug sensitivity in patients with high and low Cuproptosis-Related lncRNAs risk.

0.652, respectively. An ROC curve was drawn by combining the model we constructed with age, gender and tumor stage. The AUC of risk score was 0.648 while that of tumor stage was 0.603, thus indicating that risk score and tumor stage were better than other clinical parameters for the prediction of survival in patients with gastric cancer. The progression-free survival (PFS) differed significantly between the high-risk group and the low-risk group. When risk score, age, tumor grade and tumor stage were included in multivariate Cox regression analysis, we found that risk score, age and tumor stage were independent prognostic factors, and the results were visualized in Fig. 4 (G-H). Therefore, the new model exhibits good accuracy for predicting prognosis, and the results were visualized in Fig. 4 (A – F). Finally, the qRT-PCR results of 5 pairs of gastric cancer samples indicated that there were significant differences in the expression of AC008915.2, AC023511.1 and LINC02476 (Fig. 4I).

3.5. Development and validation of the nomogram for gastric cancer

Generation of a C index curve proved that the new prediction model had high accuracy for predicting the survival, tumor stage and risk score of patients with gastric cancer. To better predict the survival time of gastric cancer patients, we constructed a nomogram. We found that the 1-year survival probability of patients with a comprehensive score of 180 for age, gender, risk score and tumor stage was 0.947. The 3-year survival probability was 0.847 and the probability of survival over 5 years was 0.814. Next, we validated the model for clinical grouping. The OS of the high-risk group and the low-risk group of patients with stage I-II and III-IV were compared; we confirmed that the OS of the high-risk group was significantly shorter than that of the low-risk group. These data indicate that this risk model can accurately identify and predict the survival of patients with early or advanced gastric cancer, and the results were visualized in Fig. 5 (A – E).

3.6. GO and KEGG enrichment analysis between risk groups

Next, we performed GO enrichment analysis and noted significant enrichment of the dead region receptor, apoptotic gene set. KEGG analysis identified several enriched pathways, including death receptor structure domain of exogenous apoptosis signaling pathways, molecular reaction on the origin of bacteria, phagocytosis, response to the metal ions, and exogenous apoptosis signaling pathways. In addition, some of the DEGs were involved in gastric cancer, apoptosis metal ions, and many signaling pathways (Fig. 6). Studies have shown that naringenin can induce internal and external apoptosis signaling pathways in cancer cells and has a potential therapeutic effect with regards to inhibiting cancer. Lithium can promote apoptosis signaling induced by activation of the Fas death domain receptor [17,18]. A recent study developed an electrochemical sensor based on carbon fiber electrodes and showed that this could accurately achieve targeted tracking and effective inhibition of tumors through an ultra-precision and multiple monitoring of metal ion-mediated miRNA delivery system, thus proving that the metal ion pathway plays an important role in the transmission of biological information in tumors. In terms of tumor regulation, these data indicate that copper ions may mediate the transmission of key information [19], and the results were visualized in Fig. 6(A–G).

3.7. Comparison of the immune microenvironment and drug sensitivity between risk groups

Next, we performed mutation burden correlation analysis; 137 of the 160 cases in the low-risk gastric cancer group and 147 of the 168 cases in the high-risk gastric cancer group possessed mutations which were mainly related to *TTN*, *TP53*, *MUC16* and *LRP1B* genes. The mutation rates for the low-risk group and the high-risk group were 85.62% and 87.5%, respectively.

Next, we investigated differences in the IC50 of immunotherapy and various therapeutic drugs in patients with gastric cancer by immune score, checkpoint analysis and drug sensitivity analysis. Analysis showed that *PDCD1*, *CTLA4*, *LAG3*, *CD274*, *TIGIT*, the expression of various immune HAVCR2 checkpoint in high-risk group were significantly higher than in the low-risk group. There was a significant positive correlation between immune scores ($R > 0$, $P < 0.05$). In particular, HAVCR2 ($R = 0.414$; $P < 0.05$) exhibited a significantly positive correlation; thus, treatment with this immune checkpoint inhibitor was more likely to be of benefit to patients with gastric cancer. According to these findings, long noncoding RNAs associated with cuproptosis may exert a crucial influence on the immune microenvironment of gastric cancer.

To this end, we performed drug sensitivity analysis. The IC50 of Crizotinib, Sorafenib, and Vinorelbine was lower than that of the high-risk group, and the sensitivity of other targeted drugs was higher than that of the high-risk group, including Imatinib, Sunitinib, Pazopanib, Lapatinib, XL184, YM155, TGX221, AP-24534. In our analysis of cancer drugs, we found a statistical difference between the high-risk and low-risk groups: the lower the IC50 value of the low-risk group, the more sensitivity to the drug, and the results were visualized in Figs. 7(A–F) 8(A, B), 9(A – K).

4. Discussion

Apoptosis is the process that controls programmed cell death in the human body. In the adjuvant treatment of malignant tumors, traditional chemotherapy drugs mainly play a role by promoting the apoptosis of tumor cells. However, the resistance of tumors to drugs is also caused by apoptotic mechanisms [20]. Many researchers have investigated the apoptosis pathway in tumor cells. New modes of programmed cell death, such as pyroptosis, ferroptosis and cuproptosis, have been gradually proven to be associated with the apoptosis of tumor cells. Related studies have shown that copper is a key factor affecting cell metabolism; however, the rate of copper secretion is greater than the rate of copper production; an imbalance in the cellular levels of copper can lead to direct binding to fat and protein in the tricarboxylic acid (TCA) cycle, thus inducing mitochondrial dysfunction and increasing mitochondria-dependent

cytotoxicity mediated by the induction of apoptosis [21]. Copper is also directly involved in cell proliferation, angiogenesis, tumor metastasis, epithelial-mesenchymal transition, and is closely related to the formation of the tumor microenvironment [22,23]. Studies have reported that the ATP7A-LOx pathway promotes tumor growth and metastasis in breast cancer, lung cancer cell lines and mouse models by regulating the intracellular transmission of copper [24]. The anti-angiogenesis mechanism induced by copper in tumor cells includes the accumulation of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and hydrogen peroxide, and the inhibition of the proteasome. Studies have shown that copper is widely used in chemotherapy, phototherapy, biological imaging and other fields [25]. In the present study, functional analysis showed that TCA circulation-related pathways were abundant and that CRGs were associated with the prognosis of gastric cancer. There is increasing evidence that cuproptosis plays an integral role in tumor immunity, inflammation, and the tumor immune microenvironment. Nowadays, precision therapy has become an important trend, and the accurate prediction of individual patient biology is the premise of individualized therapy; predictions based on tumor cuproptosis-related lncRNAs provides an effective method. To this end, we performed an unsupervised consensus analysis of 13 prognostic cuproptosis-related lncRNAs and examined their association with OS and PFS in gastric cancer.

Next, we constructed and validated an applicable clinical prognostic model based on the risk score of cuproptosis genes and the clinical data from patients. Next, we explored the tumor immune microenvironment between high and low risk populations based on risk scores. The differences in TMB, immune score, and immune checkpoint expression levels were evaluated between high-risk and low-risk groups. Importantly, our targeted drug sensitivity analysis can guide clinical practice and provide evidence for the selection of effective drug therapy in patients with gastric cancer. We constructed a prognostic nomogram based on cuproptosis-related genes and clinical factors, and then verified that the model had good predictive ability. The models were divided into high-risk and low-risk groups according to lncRNAs related to cuproptosis genes. The Kaplan-Meier survival analysis revealed a significantly higher overall survival (OS) in the low-risk group compared to the high-risk group, with a statistically significant difference observed in their respective survival rates. Surprisingly, most of the lncRNAs associated with cuproptosis were differentially expressed in tumors and normal tissues. These genes were significantly associated with OS and PFS, thus indicating their potential role. The predictive value of this score is of great significance for predicting the prognosis of gastric cancer. Further studies on immune properties, immune checkpoint distribution, and sensitivity to gastric cancer therapy are now needed to determine appropriate treatment options.

Univariate Cox models and Lasso regression analysis were used to filter cuproptosis-related lncRNAs. Then, we calculated regularized regression coefficients using the elastic net regression Cox model, which combined Ridge and LASSO regression algorithms to improve the predictive performance of the prognostic index on independent data. Finally, we identified seven lncRNAs for modeling. It has been previously reported that the seven cuproptosis-related lncRNAs are related to tumor development or prognosis. Studies have shown that LINC02476 plays an important role in the growth and metastasis of HCC cells through the Mir-497/HMGA2 axis; this may represent a new molecular target for the treatment of HCC [26]. Studies have also shown that high expression levels of LINC01094 in gastric cancer predict a poor prognosis and is related to the epithelial-mesenchymal transition pathway and macrophage infiltration [27]. We performed Kaplan-Meier survival analysis and found that the risk score was a prognostic factor. Univariate and multivariate Cox regression analyses included risk score, age, and tumor stage; we found that risk score and tumor stage were independent predictors of a poor prognosis. ROC curve analysis showed that the model had good predictive performance over 1–5 years. There were differences in survival rates between the high-risk and low-risk groups with different stratifications, thus indicating that this model is generally applicable for patients with different stages of gastric cancer progression. This confirms that the prediction performance of the model is robust.

To clarify the correlation between Cuproptosis-associated lncRNAs and four clinical features of gastric cancer, we created a prognostic nomogram. Analysis showed that high-risk population, age, and tumor stage were significant risk factors affecting the prognosis of patients with gastric cancer. By univariate analysis and multivariate analysis, we identified tumor stage and cuproptosis-related lncRNAs as independent risk factors for survival and prognosis in gastric cancer. In addition, overall survival (OS) at 1, 3, and 5 years was predicted more efficiently in the entire cohort. According to the prognostic value of gastric cancer and the correlation with clinical features, four prognostic indicators (age, gender, tumor stage, risk score) were established and analyzed by LASSO Cox regression. The risk coefficients of age, tumor stage and risk score were 1.043 (1.022–1.064),1.72 (1.337–2.212) and 1.018 (1.007–1.028), respectively.

The current treatment methods for gastric cancer mainly include surgical resection, chemotherapy, targeted therapy, radiotherapy, and immunotherapy. Although the treatment of gastric cancer undergone significant development, the strategy and efficacy of treatment for gastric cancer are still unsatisfactory. Immunotherapy is an accurate and effective treatment for gastric cancer [28,29]. Although immunotherapy for gastric cancer has brought new hope to many patients, the efficacy in some patients remains unsatisfactory due to tumor heterogeneity and differences in immune mechanisms; consequently, the immune microenvironment is still a key focus of current research [30]. Immunotherapy has become a very important component of treatment for gastric cancer. Edoku describes the key aspects of immunotherapy, including anti-PD1 and anti-CTLA4. Through effective immunotherapy, the survival of patients with gastric cancer has been continuously improved, and the status of clinical practice has progressed significantly. Many researchers have focused on the selection of effective immunotherapy biomarkers. Previous studies have reported that clinical trials of keynote-061 and keynote-062 regimens in the treatment of patients with gastric cancer with MSI-h reported a higher OS, and that the progression-free survival (PFS) and objective response rate (ORR) of anti-pd1 therapy were higher than those of chemotherapy [31,32]. Our present study showed that the high-risk group was significantly higher than the low-risk group with regards to a range of immune checkpoints, and that risk scores were all significantly and positively correlated. In particular, HAVCA, an immune checkpoint, may exert a better effect on immunotherapy. Our findings will provide important guidelines for the development of novel immunotherapy strategies of target sites.

Our study confirmed that the cuproptosis gene risk score can be used to comprehensively evaluate CRG expression patterns and

corresponding survival prognostic characteristics and immune cell infiltration characteristics. These data will contribute to our understanding of the specific mechanisms of cuproptosis-related genes in gastric cancer and help to determine the immunophenotype of gastric cancer and guide more accurate and effective clinical treatments. In addition, the risk associated with cuproptosis genes can also be used as an independent prognostic biomarker. Our results provide new ideas and directions for clinical research.

This study also has some limitations that need to be considered. For example, all studies were carried out with TCGA-STAD for cohort studies; The limitation of the results in this study is that only gastric cancer transcriptome data were selected, and proteomics was not used to verify the association between lncRNAs- related copper death genes. Additional data from *in vivo*, *in vitro*, and clinical studies need to be acquired for specific validation. In future studies, we will explore the specific molecular mechanisms underlying the effects of cuproptosis-related genes in the immune microenvironment of gastric cancer, so as to provide a research basis for the clinical practice of gastric cancer immunotherapy.

5. Conclusion

We identified a practical prognostic model based on genes associated with gastric cancer and cuproptosis. We comprehensively analyzed the relationship between cuproptosis and gastric cancer prognosis, the tumor immune microenvironment, and factors related to immunotherapy and targeted drug sensitivity. Our results provide a foundation for exploring new targets and immunotherapies.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable•

Availability of data and material

The datasets used or analyzed in the current study are available from web links to public databases.
TCGA(https://genome-cancer.ucsc.edu/)

Funding

Project supported by Youth Foundation of Guangxi Zhuang Autonomous Region Health Commission (No.Z20190422) and Guangxi Medical and Health Key Cultivation Discipline Construction Project (2021063).

Authors' contributions

Bo Zhao, Wei Wu contributed equally to this work. Bo Zhao conceived and designed the experiments , wrote the paper. Wei Wu performed the experiments. Liang Liang, Yongjun Chen and Xiaoyong Cai contributed reagents, materials, analysis data in the manuscript. Weizhong Tang analyzed and interpreted the data for the revised manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to express their gratitude to EditSprings (https://www.editsprings.cn) for the expert linguistic services provided. And thanks to TCGA AND GENEMANI databases for providing the data, and numerous bioinformatic software R packages.

Abbreviations

CRGs	Cuproptosis-related genes
TME	Tumor microenvironment
TIME	Tumor immune microenvironment
TCA	tricarboxylic acid
OS	Overall survival
ROC:	Receiver operating characteristic
TMB	Tumor mutation burden

MF Molecular function
BP Biological pathways
CC Cellular components

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