Comprehensive Analysis of Cuproptosis Regulators With Regard to prognostic and Immune Infiltration in Clear Cell Renal Cell Carcinoma

YaXuan Wang
Nantong University
XiaoLin Wang (cxhwyc2010@163.com)
Nantong Tumor Hospital

Research Article

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Abstract

Background: Renal clear cell carcinoma (KIRC) is one of the most common tumors of the male urinary system. It is highly heterogeneous, and there is still a lack of biomarkers to predict the prognosis of patients with advanced KIRC. Cuproptosis is a new type of cell death recently discovered and plays an important role in tumor. The objective of this study was to investigate the prognostic value of cuproptosis-related genes and immune infiltration in KIRC.

Results: There were significant differences in the expression of copper death-related genes in KIRC. Functional enrichment analysis revealed that the 10 cuproptosis-related genes were mainly related to the Citrate cycle (TCA cycle)、Pyruvate metabolism、Glycolysis / Gluconeogenesis、Carbon metabolism、Central carbon metabolism in cancer、Glucagon signaling pathway、HIF−1 signaling pathway. A cuproptosis-related gene signature stratifying patients into 2 risk score groups was established based on the TCGA cohort, Patients with higher risk scores had worse overall survival. In addition, we also found that cuproptosis-related genes were closely related to immune cell infiltration, TMB, MSI.

Conclusions: In conclusion, we comprehensively analyzed the prognostic and immune infiltration effects of cuproptosis-related genes in KIRC, and identified five genes as prognostic biomarkers of KIRC. our study provides important evidence for the role of cuproptosis in KIRC.

Background

Kidney cancer has been at a high rate in recent years, accounting for about 2.2 percent of all new cancer cases and 1.8 percent of all cancer deaths [1]. Renal cell carcinoma (RCC) is the most common type of kidney cancer. Among them, renal clear cell carcinoma (KIRC) is the common form, accounting for about 75–80%, with an incidence of > 5%. In addition, it has frequent metastases and poor prognosis [2]. Patients with KIRC have a high survival rate with early surgical treatment, whereas patients with advanced clear cell renal cell carcinoma have a consistently poor prognosis. Chemotherapy, immunotherapy, and targeted therapy are the preferred therapies for patients with advanced clear cell carcinoma. However, due to drug resistance, individual differences, and the lack of reliable prognostic biomarkers, the therapeutic outcomes of patients with advanced clear cell carcinoma have been unsatisfactory. Studies have demonstrated that high tumor heterogeneity and delayed detection are major factors contributing to untimely treatment and recurrence [3]. Therefore, it is particularly important to find effective biomarkers that can be used to identify and predict the survival of patients with KIRC and establish prognostic models. By establishing a prognostic model, clinicians can make better individualized survival predictions at the molecular level [4].

Copper ion is an essential cofactor in all living things, but when its concentration exceeds a certain threshold, it will produce toxic effects on the body [5]. Latest study found that the cells in the body depends on the copper ions and by regulating the new way of cell death: “cuproptosis”. Copper-dependent death occurs by means of direct binding of copper to lipoylated components

of the tricarboxylic acid (TCA) cycle, this results in lipoylated protein aggregation and subsequent

iron-sulfur cluster protein loss, which leads to proteotoxic stress and ultimately cell death [6]. Therefore, the killing of cancer cells by copper ion carriers may become a new method for cancer treatment.

The prognostic value of cuproptosis-related genes in KIRC has not been elucidated. Our study analyzed the expression, prognosis, and immune infiltration of cuproptosis-related genes in KIRC, as well as the correlation of cuproptosis-related genes in different data sets. We also constructed a prognostic model of cuproptosis-related genes, hoping to provide a basis for finding prognostic biomarkers and therapeutic targets for KIRC.

Methods And Materials

Data Acquisition

RNA-seq data and clinical information of the ccRCC cohort were downloaded from the Genomic Data Commons (GDC) data portal of The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) in May 2022. Data included 72 normal kidney and 530 ccRCC tissues[7]. GSE53757 dataset from Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) database was used to further analyze the expression of cuproptosis-related genes in KIRC.

Bioinformatics Analysis

Kyoto Encyclopedia of Genes and Genomes(KEGG) analysis and Gene Ontology (GO) (including cellular component (CC),the biological process (BP), and molecular function (MF) categories) analysis were performed using the "GGplot2" package in R software.

Univariate Cox regression analysis was performed on 10 genes associated with cuproptosis, in which candidate genes were selected if they satisfied the screening condition of $P < 0.05$. Based on these prognostic cuproptosis-related genes, LASSO Cox regression analysis was then used to construct the prognostic model. The TCGA KIRC patients were divided into low- and high-risk subgroups according to the median risk score, and the overall survival (OS) time was compared between the two subgroups via Kaplan–Meier analysis. The predictive accuracy of each gene and the risk score were evaluated by performing time receiver-operating characteristic (ROC) analysis. Considering the clinical characteristics, a predicted nomogram was developed to predict the 1-, 3-, and 5-year overall survival. A forest was used to show the P-value, HR and 95% CI of each variable through the “forestplot” R package[8].

Using the Tumour IMmune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/)

to analysed the correlation between prognostic PRG and immune infiltration. In tumour mutation burden (TMB) and microsatellite-instability (MSI) analysis, Spearman’s correlation analysis was performed to calculate the correlation between gene expression and TMB and MSI score.

Statistical Analysis

All the analysis methods and R packages were implemented by R (foundation for statistical computing 2020) version 4.0.3. p value <0.05 was considered statistically significant.

Result

Expression of cuproptosis-related genes in KIRC

It is reported that FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, PDHB, MTF1, GLS, and CDKN2A are recognized as cuproptosis-related genes [9]. We first analyzed the expression of these 10 genes in 530 KIRC and 72 corresponding paracancer samples from TCGA database, and the results showed that the expression of FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, PDHB, MTF1 and GLS in KIRC was significantly lower than that in adjacent tissues. Only CDKN2A expression was significantly higher in KIRC than in adjacent tissues (figure 1A).Spearman correlation analysis showed that CDKN2A was negatively correlated with FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, PDHB, MTF1 and GLS in KIRC in TCGA database, while FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, PDHB, MTF1 and GLS genes were positively correlated (figure 1B). Similarly, we used THE GSE53757 dataset in GEO database to analyze the expression of cuproptosis-related genes in KIRC and adjacent to corresponding cancers. The results showed that the other results were consistent with TCGA database except MTF1 expression in KIRC and adjacent to corresponding cancers(figure 1C). Subsequently, we also analyzed the association between cuproptosis-related genes in the GSE53757 dataset (figure 1D).

Correlation analysis of cuproptosis-related genes in KIRC and clinicopathology

To investigate the association between the expression of cuproptosis-related genes and clinicopathological features of KIRC, we assessed the expression of cuproptosis-related genes in patients with stage I, II, III, and IV KIRC. Results showed that FDX1, LIAS, DLD, DLAT, PDHB, MTF1, CDKN2A expression was significantly different in patients with stage I, II, III and IV KIRC, while LIPT1, PDHA1 and GLS expression were not significantly difference (figure 2A-J). We also demonstrated the high-low expression distribution trend of cuproptosis-related genes on pT stage, pN stage, pM stage and patient survival in KIRC samples (figure 2K-T).

Enrichment analysis of cuproptosis-related genes

To elucidate the function of cuproptosis-related genes in tumors, we analyzed these genes using KEGG and GO databases. KEGG analysis showed that the 10 cuproptosis-related genes were mainly related to the Citrate cycle (TCA cycle)Pyruvate metabolismGlycolysis / GluconeogenesisCarbon metabolism Central carbon metabolism in cancerGlucagon signaling pathwayHIF−1 signaling pathway (figure 3A). GO database analysis showed that copper death related genes participated in sulfur compound biosynthetic process, Coenzyme biosynthetic process, cofactor biosynthetic process, etc (figure 3B-D).

Prognostic analysis of cuproptosis-related genes in KIRC

In order to investigate the correlation between the expression of cuproptosis-related genes and the prognosis of KIRC, seven cuproptosis-related genes with prognostic value were screened out by univariate Cox regression analysis。Kaplan-meier survival curve results showed that high CDKN2A expression was significantly associated with poor prognosis in patients with KIRC，and low DLAT,DLD,FDX1,LIAS,MTF1,and PDHB expression was significantly associated with poor prognosis in patients with KIRC(figure 4A-G). There were no significant differences in the other three genes.

Construction of a cuproptosis-related prognostic gene model

The LASSO Cox regression model was used to select the most predictive genes as prognostic indicators. λ was selected when the median of the sum of squared residuals was the smallest. Five potential predictors (figure 5A-B). MTF1、LIAS、FDX1、DLAT、CDKN2A were identified as prognostic factors for KIRC. The risk score ==(0.1403)*CDKN2A+(-0.1883)*DLAT+(-0.3737)*FDX1+(-0.1023)*LIAS+(-0.1865)*MTF1. Patients with KIRC were divided into two groups based on risk score. The distribution of risk score, survival status and expression of these 5 genes are shown in Figure 5C. Kaplan-meier curves showed that patients with high risk KIRC had a lower overall survival rate than patients with low risk KIRC (median time=5.4 years，p=1.46e-05)(figure 5D)。AUC in the 1-year, 3-year and 5-year ROC curves were 0.702, 0.669 and 0.663, respectively (figure 5E). These results suggest that copper death-related genes can be used as biomarkers for the prognosis of KIRC.

Building a predictive nomogram

Considering the clinicopathologic features and these five prognostic cuproptosis-related genes, we also built a predictive nomogram to predict the survival probability. Univariate and multivariate analyses showed that FDX1 expression, age and pM stage were independent factors affecting the prognosis of KIRC patients (figure 6A-B). The predictive nomogram suggested that the 3-year and 5-year overall survival rates could be predicted relatively well compared with an ideal model in the entire cohor (figure 6C-D).

Correlation analysis of cuproptosis-related genes prognosis model and tumor immune infiltration in KIRC

Cuproptosis plays an important role in the development of tumor immune microenvironment. In this study, we also used TIMER and MCPCOUNTER methods to analyze the correlation between cuproptosis-related genes prognosis model and various immune cells。The results of TIMER method showed that B cell and Macrophage was negatively correlated with cuproptosis-related genes prognosis model(figure 7A) .cuproptosis-related genes prognosis model was positively correlated with T cell CD8+、cytotoxicity score, negatively correlated with Monocyte、Macrophage/Monocyte、Myeloid dendritic cell、Neutrophil、Endothelial cell by MCPCOUNTER method (figure 7B).

Cuproptosis prognosis related genes and immunoassay site related genes expression differences and correlation analysis with TMB,MSI

Immune checkpoint molecules are inhibitory regulatory molecules in the immune system, which are essential for maintaining tolerance, preventing autoimmune reactions, and minimizing tissue damage by controlling the timing and intensity of immune responses[10]. Therefore, we analyzed the expression differences of immunoassay site-related genes in the high and low expression groups of cuproptosis prognosis related genes MTF1, LIAS, FDX1, DLAT and CDKN2A。The results showed that LAG3, PDCD1, CD274 and PDCD1LG2 were significantly different in the high and low expression groups of five cuproptosis prognosis genes(figure 8A-E). TMB and MSI are two emerging biomarkers related to immunotherapy response[11]. We also evaluated the correlations of MTF1, LIAS, FDX1, DLAT, and CDKN2A with TMB and MSI。The results showed that FDX1 and CDKN2A were positively correlated with MSI($p<0.05$)(figure 8F). FDX1 and DLAT were negatively correlated with TMB, while CDKN2A was positively correlated with TMB ($p<0.05$)(figure 8G).

Discussion

Recent studies found that unbalanced copper homeostasis affect tumor growth, causing irreversible damage [12]. Copper is an essential mineral nutrient for all organisms and is essential in mitochondrial respiration, iron absorption, and antioxidant/detoxification processes [13]. Recent studies have also shown that copper plays an important role in cancer, with levels of copper significantly higher in various malignant tumors than in normal tissues, such as breast [14], gallbladder[15], Thyroid [16], lung[17], and Prostate[18]. Changes in cupriin levels have been documented in many types of cancer. These variations result in increased concentrations of intratumoral Cu and alterations in the systemic distribution of copper. Such alterations in Cu homeostasis may promote tumor growth or invasiveness or may even confer resistance to treatments[19]. Therefore, specific changes in copper metabolism appear to have broad clinical promise as prognostic and/or predictive biomarkers.

In this study, we analyzed the expression and correlation of cuproptosis-related genes using TCGA database and GEO database. We found that the expression of FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, PDHB, MTF1 and GLS was lower in KIRC than in normal tissues, while the expression of CDKN2A was higher in KIRC than in normal tissues. we assessed the expression of cuproptosis-related genes in patients with stage I, II, III, and IV KIRC. Results showed that FDX1, LIAS, DLD, DLAT, PDHB, MTF1, CDKN2A expression was significantly different in patients with stage I, II, III and IV KIRC, while LIPT1, PDHA1 and GLS expression were not significantly difference. These results suggest that cuproptosis-related genes are closely related to the pathological staging of KIRC. The proposed prognostic model comprises 5 genes, including MTF1,LIAS,FDX1,DLAT,and CDKN2A. Patients with KIRC were divided into two groups based on risk score, high risk score group had a poor prognosis. Our results also suggest that the prognostic model we constructed is closely associated with immune invasion in KIRC. Therefore, we suggest that cuproptosis-related genes can be used as prognostic biomarkers for KIRC and are closely associated with immune cell infiltration.

Of course, this study has some limitations. The model we built is based on online database, without validation from independent cohorts, so we need more studies to verify our conclusions.

Conclusions

The expression of cuproptosis-related genes is closely related to the clinical characteristics of KIRC. cuproptosis-related genes can be used as a prognostic biomarker of KIRC and are highly correlated with immune infiltration-related cells. In conclusion, our study provides important evidence for the role of cuproptosis in KIRC.

Abbreviations

KIRC Kidney renal clear cell carcinoma

TCGA The cancer genome atlas

GEO The gene expression omnibus

OS Overall survival

HR Hazard ratio

CI Confidence interval

SD Standard deviation

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analysed during the current study are available in the ‘The Cancer Genome Atlas’ (TCGA) and ‘Gene Expression Omnibus’ (GEO) database(https://portal.gdc.cancer.gov/,https://www.ncbi.nlm.nih.gov/geo/).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Wang Yaxuan wrote the full text, and Wang Xiaolin was responsible for reviewing it.

Acknowledgements

Not applicable.

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Figures

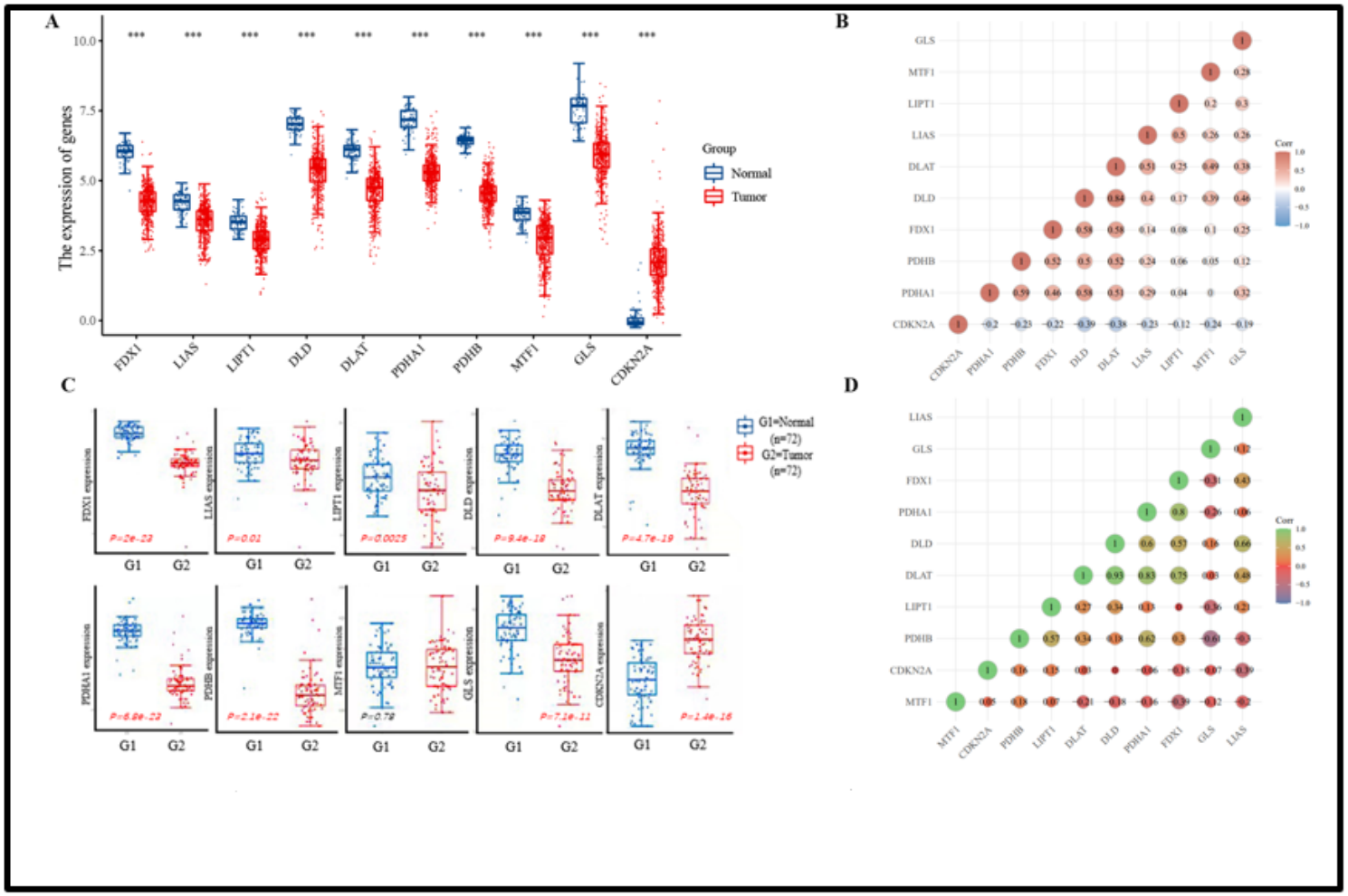

Figure 1

Expression of cuproptosis-related genes in KIRC.(A-B)Expression and correlation of cuproptosis-related genes in TCGA database. (C-D)Expression and correlation of cuproptosis-related genes in GEO database.

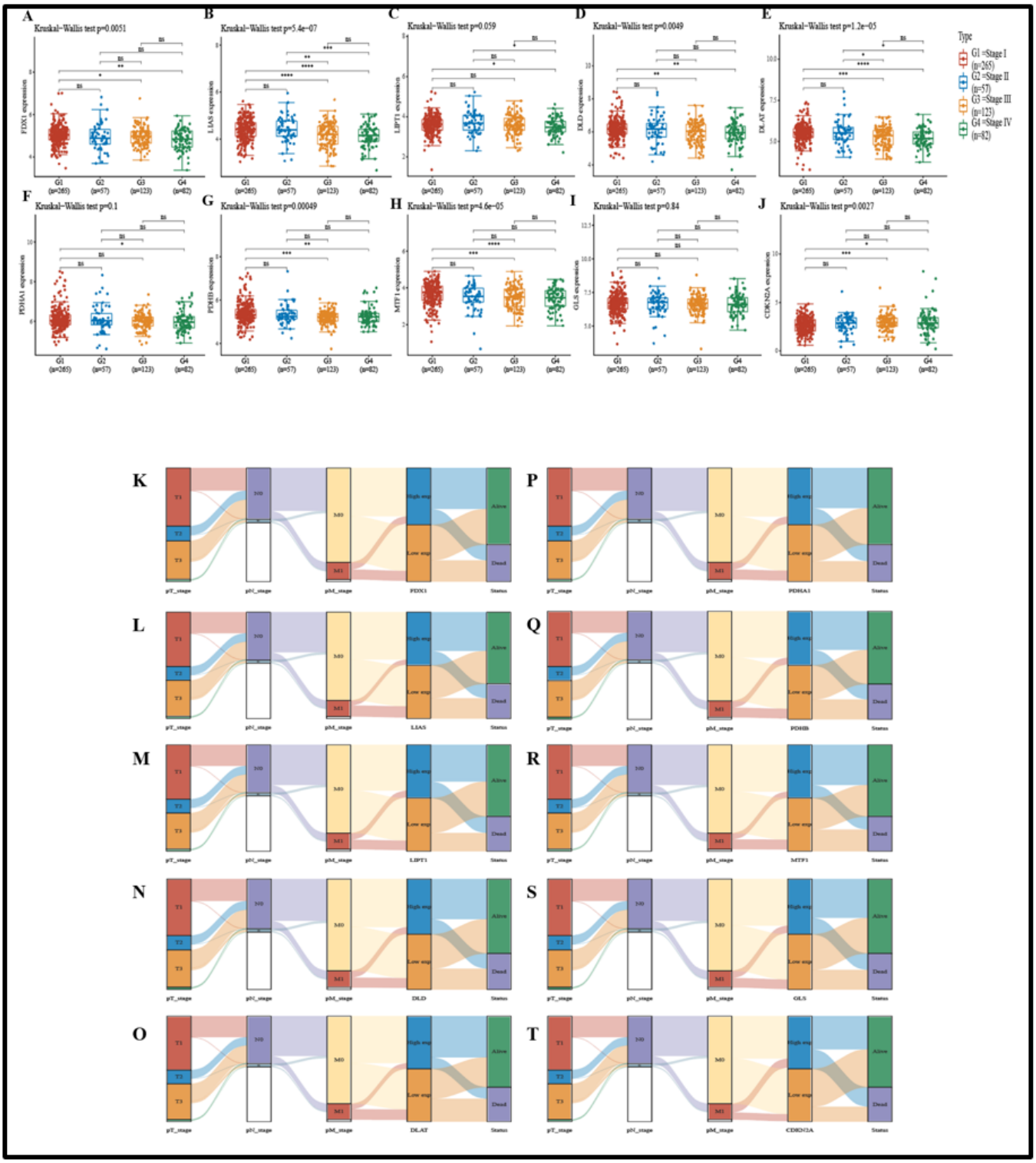

Figure 2

Correlation between cuproptosis-related genes and pathological stages of KIRC. (A-J): Expression of FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, PDHB, MTF1, GLS, and CDKN2A in patients with stage I, II, III, and IV KIRC. (K-T):The width of extended branches in the Sankey diagram corresponds to the size of data flow, which is used to display the high-low expression distribution trend of copper death-related genes on

different stages and patient survival in KIRC tumor samples. Log2 (TPM+1) is used for the logarithmic scale. * p < 0.05，* * p < 0.01，* * * p < 0.001.

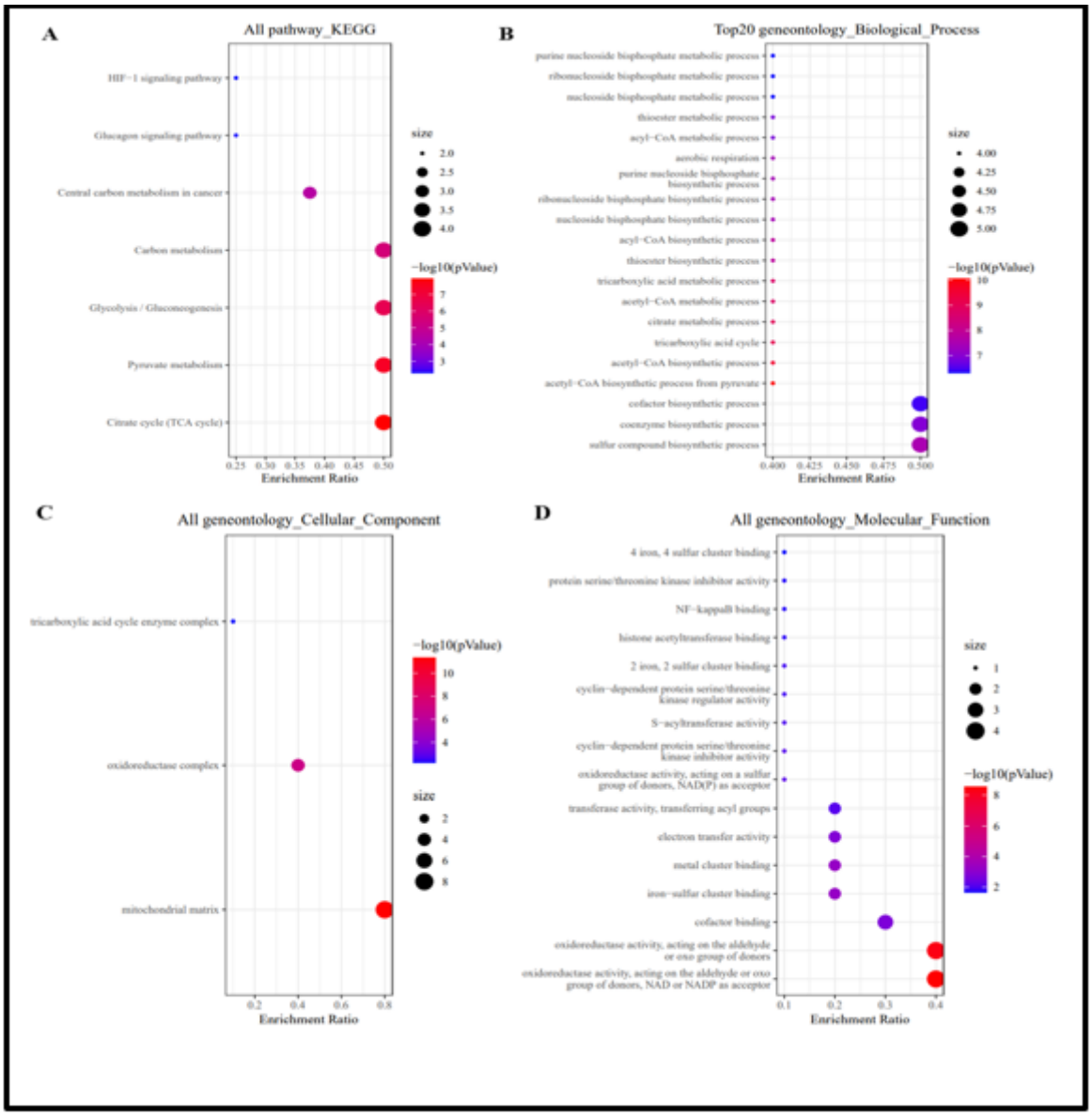

Figure 3

Enrichment analysis of cuproptosis-related genes. (A)KEGG analysis. (B)Biological Process. (C)Cellular Component. (D)Molecular Function

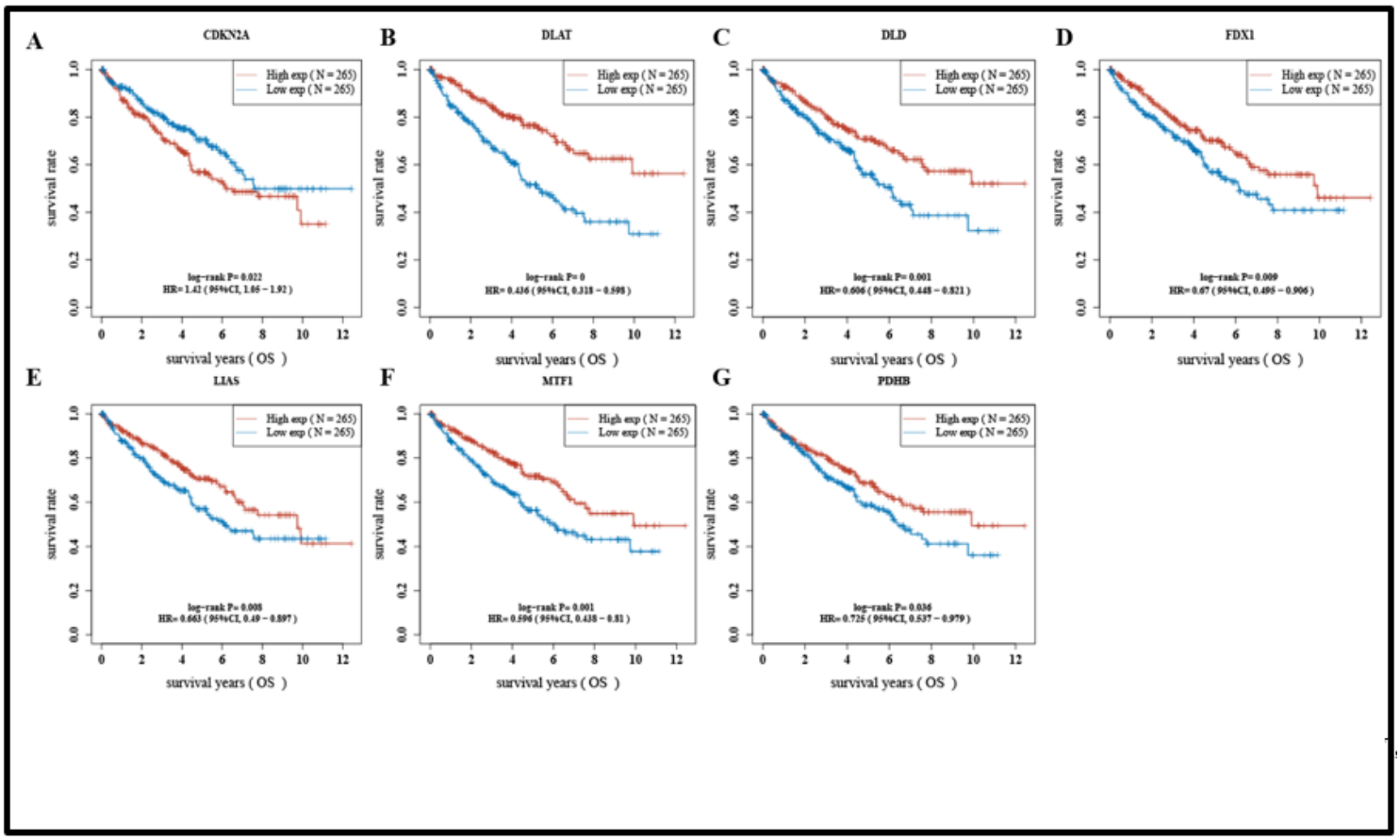

Figure 4

The prognostic value of cuproptosis-related genes in KIRC. (A-G):Overall survival curve of CDKN2A、DLAT、DLD、FDX1、LIAS、MTF1、PDHB.

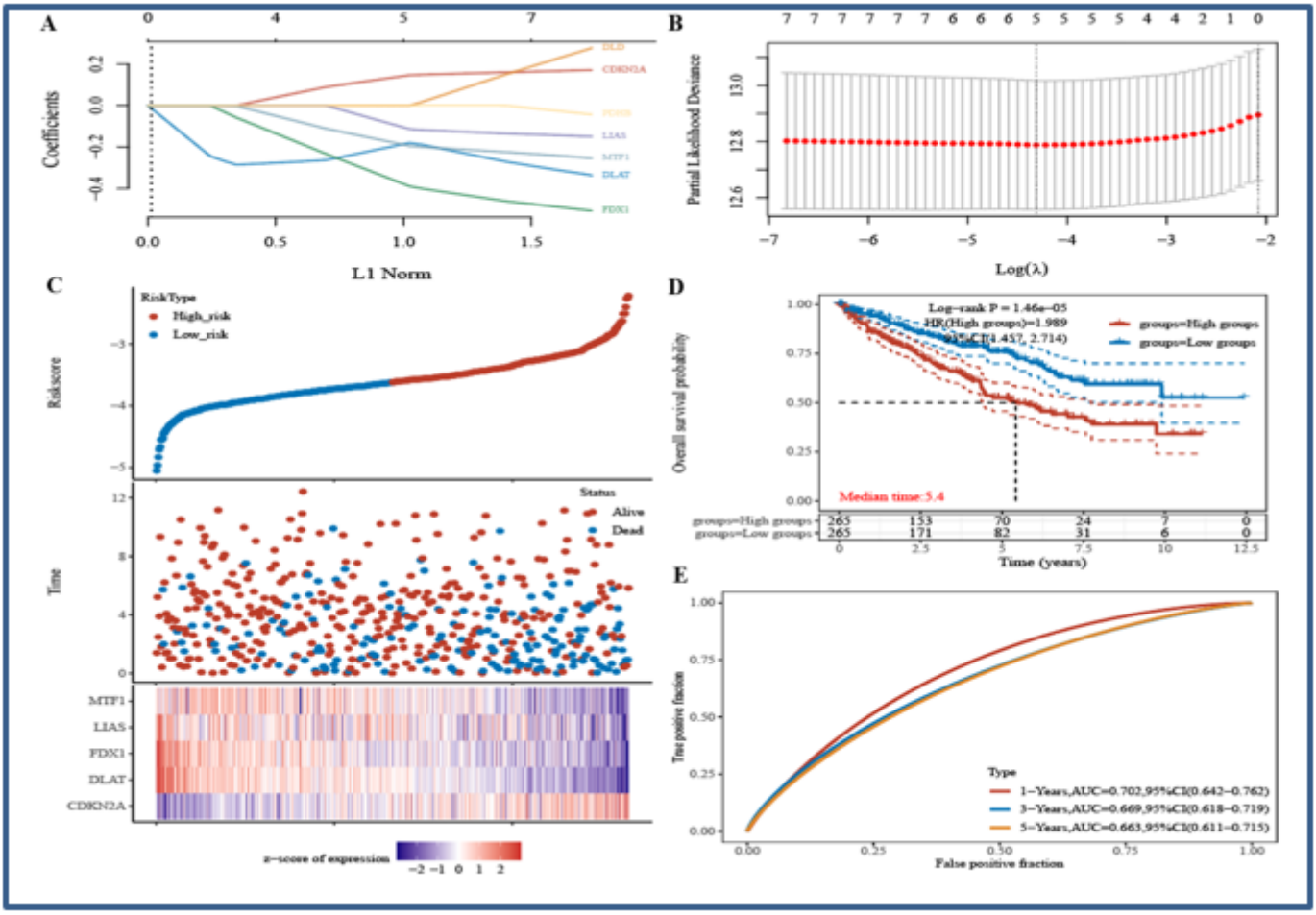

Figure 5

Genetic prognostic model of cuproptosis-related genes. (A): Partial likelihood deviance versus log (λ)was drawn using LASSO Cox regression model. (B): Coefficients of selected features are shown by lambda parameter. (C): Distribution of risk score, survival status, and the expression of five prognostic cuproptosis-related genes in KIRC. (D-E): Overall survival curves for KIRC patients in the high-/low-risk group and the ROC curve of measuring the predictive value.

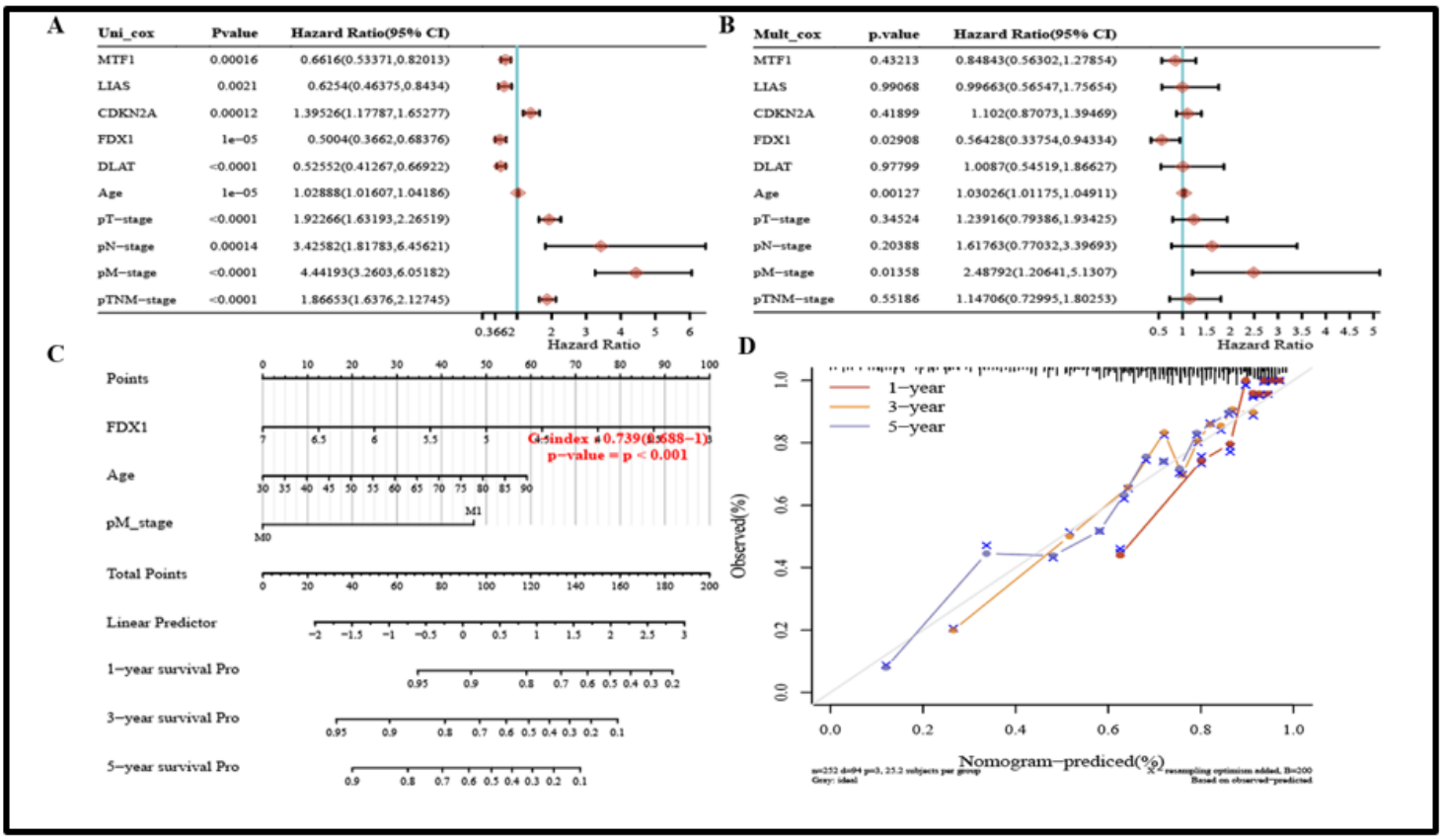

Figure 6

Construction of a predictive nomogram. (A-B): The risk ratios and P values of the components involved in univariate and multivariate Cox regression for clinical parameters of KIRC and five cuproptosis-related genes. (C-D): Nomogram to predict the 1, 3, and 5 year overall survival rate of KIRC patients. Calibration curve for the overall survival nomogram model in the discovery group. A dashed diagonal line represents the ideal nomogram.

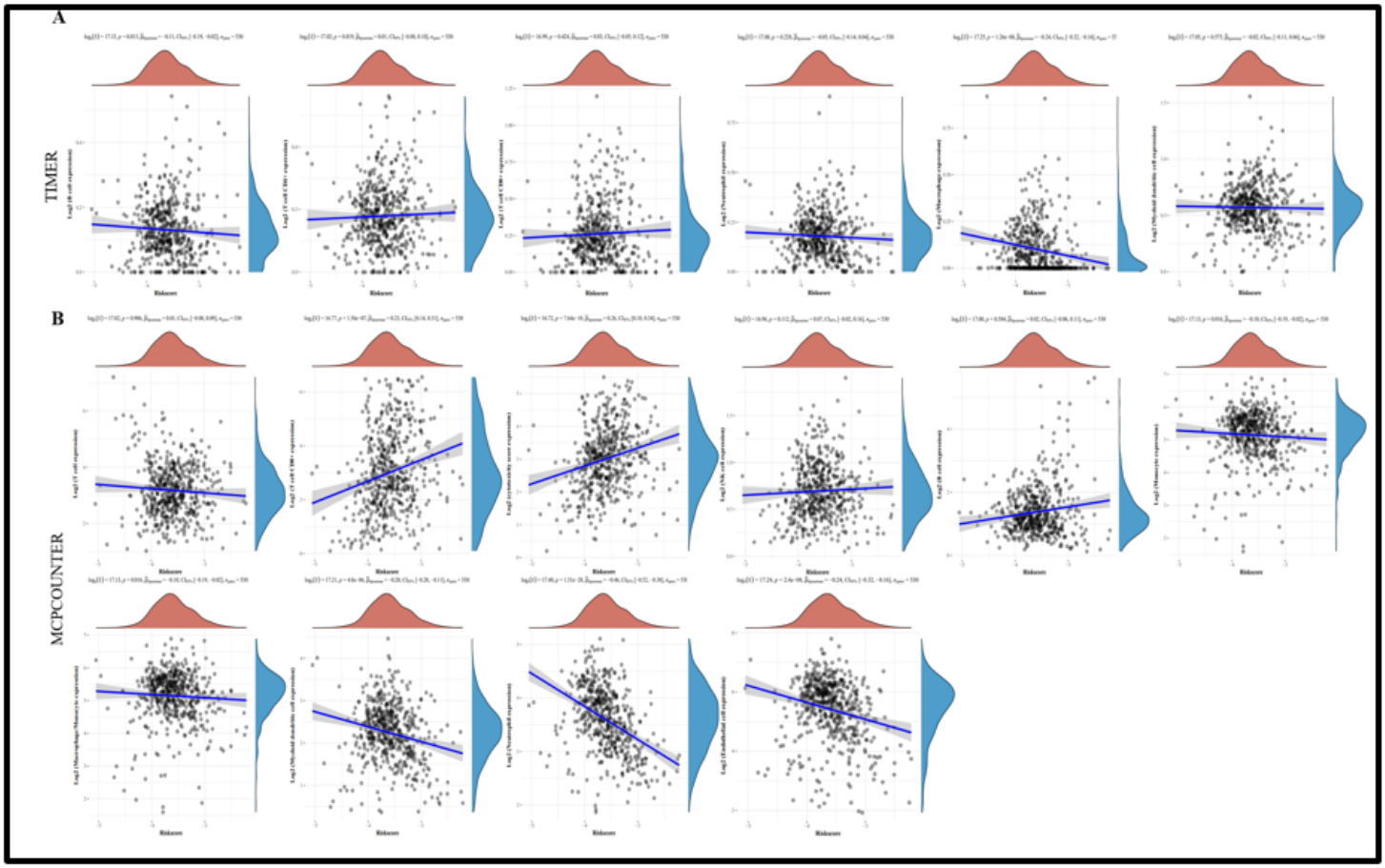

Figure 7

Correlation analysis of cuproptosis-related genes prognosis model and tumor immune infiltration. (A): TIMER method was used to analyze the correlation between cuproptosis-related genes and immune infiltrating cells. (B): MCPCOUNTER method was used to analyze the correlation between cuproptosis-related genes and immune infiltrating cells.

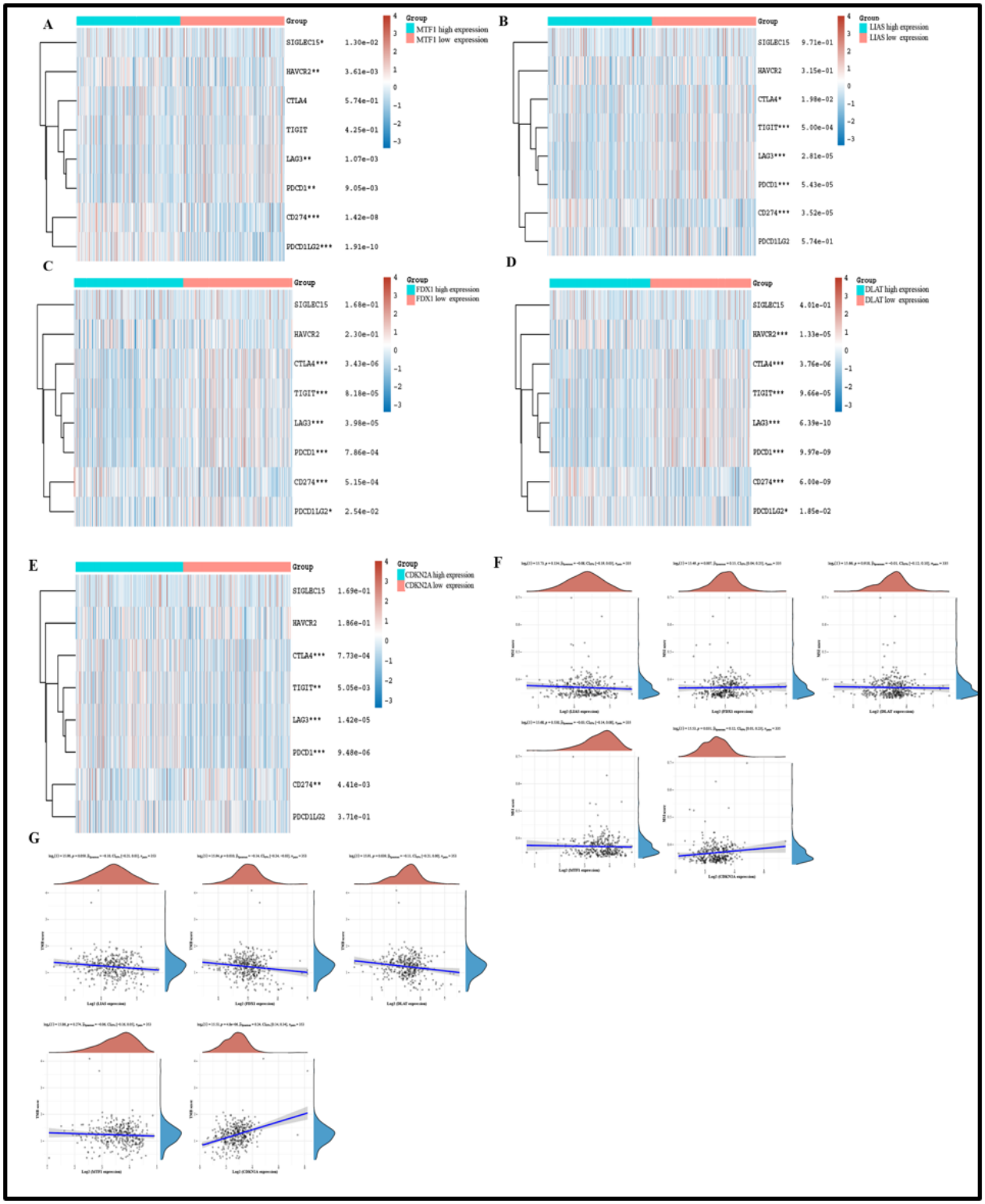

Figure 8

Correlation analysis of cuproptosis prognosis gene with immunoassay sites, MSI, TMB. (A-E): Cuproptosis prognosis related genes and immunoassay site related genes expression differences.(F-G): Correlation analysis of cuproptosis prognosis gene with MSI, TMB.