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A multi-omics prognostic model of cuproptosis affects the prognosis of stomach adenocarcinoma

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Abstract

Background Cuproptosis, a form of cell death associated with copper ions, has been linked to the pathogenesis of various cancers, including gastric cancer. Investigating the role of cuproptosis-related genes through multi-omics analysis can enhance our understanding of disease mechanisms and improve prognosis prediction.

Objective This study aims to elucidate the role of cuproptosis-related genes in gastric cancer from a multi-omics perspective.

Materials and Methods We utilized multi-omics sequencing data from TCGA and GEO databases to explore the relationships between cuproptosis genes and gastric carcinogenesis, clinical phenotypes, and prognosis. This analysis encompassed mutation, copy number variation, methylation, mRNA expression, alternative splicing, and APA alterations. Additionally, we examined the regulatory roles of cuproptosis genes in gastric cancer through ceRNA interactions, gene mutations, and DNA methylation. A multi-omics prognostic model for gastric cancer was subsequently constructed.

Results Our findings revealed that CDKN2A was the most frequently mutated gene in gastric cancer. Overall mutations in cuproptosis genes and copy number alterations of PDHB significantly impacted gastric cancer prognosis. Methylation, alternative splicing, and APA alterations of CDKN2A also influenced patient outcomes. Notably, MTF1, a key gene in cuproptosis, was found to affect apoptosis and invasion in gastric cancer cell lines.

Conclusion We successfully developed a multi-omics prognostic model for gastric cancer that offers significant predictive value for patient outcomes.

Keywords Cuproptosis, Stomach adenocarcinoma, Multi-omics prognostic model, Stomach adenocarcinoma

Introduction

Stomach adenocarcinoma(STAD) is one of the most prevalent malignancies globally, ranking as the sixth highest in incidence and the third leading cause of cancer-related deaths in 2020. In China, STAD accounted for an estimated 43.5% of newly diagnosed cases[1, 2]. Despite advancements in treatment strategies over the past decade, 30–66% of patients remain untreated due to early asymptomatic stages and delayed diagnosis[3, 4]. Furthermore, the 5-year recurrence rate after surgical resection is alarmingly high, reaching up to 70%[3, 4]. Consequently, there is an urgent need for the development of more effective prognostic models to address the high incidence and recurrence rate of STAD.

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The uncontrolled proliferation of cancer cells is closely associated with the disruption of cellular death mechanisms. Copper death, a novel form of programmed cell death, significantly influences tumor outcomes. As an essential nutrient, copper serves as a co-factor for various enzymes and plays a crucial role in several physiological processes[5]. Dysregulation of copper homeostasis has been implicated in cancer development and progression[6–9]. Following absorption from the small intestine, copper is delivered to the stomach, with excess levels stored in hepatocytes. Elevated serum copper levels have been reported in STAD patients and animal models, suggesting that maintaining copper homeostasis may serve as an adjunctive therapy for STAD. However, the mechanisms by which copper influences the initiation, progression, and outcomes of STAD remain largely unclear.

Recently, Tsvetkov et al. identified a new form of cell death termed cuproptosis, wherein copper binds directly to the lipoylated components of the tricarboxylic acid (TCA) cycle. This binding leads to the aggregation of copper-bound lipoylated mitochondrial proteins and destabilizes iron-sulfur cluster-containing proteins, resulting in proteotoxic stress and subsequent cell death. Key genes associated with cuproptosis have also been identified[10]. Previous studies have indicated that the metabolic reprogramming of STAD is characterized by the inhibition of the TCA cycle, which is essential for meeting the energy and biomass requirements of tumor initiation and progression[11–13]. Therefore, the genes involved in cuproptosis may offer new insights into prognostic strategies for STAD patients.

Leping Li et al.[14] conducted a study on copper death in STAD using public data from TCGA to examine the expression patterns of copper death-associated genes in STAD. They developed a molecular classification based on these expression patterns to predict prognosis of gastric cancer. However, their analysis focused exclusively on mRNA expression levels, overlooking the potential impact of genes on disease across various omics levels, as outlined by the central dogma. Given the significance of cuproptosis, our objective was to gain a comprehensive understanding of its specific effects in gastric cancer.

To achieve this, we employed a multi-omics approach, analyzing gene mutations, copy number variations, DNA methylation, mRNA expression, and alternative splicing to investigate the relationship between cuproptosis and tumors. Additionally, we examined the possible regulatory roles of cuproptosis genes from multiple perspectives. Finally, we constructed a multi-omics model centered on cuproptosis characteristics in gastric cancer. Our study provides a holistic analysis of the effects of cuproptosis in gastric cancer and opens new avenues for future cuproptosis-based treatment strategies (Additional file 1).

Material and methods

Data collection

Based on previously published literature, we summarized ten copper death-related genes: FDX1(Ferredoxin 1 Gene), LIAS(Lipoic Acid Synthetase), LIPT1(lipoyltransferase 1), DLD(Dihydrolipoamide dehydrogenase), DLAT(Dihydrolipoamide S-acetyltransferase), PDHA1(Pyruvate Dehydrogenase E1 Subunit Alpha 1), PDHB(Pyruvate Dehydrogenase E1 subunit Beta), MTF1(Metal Regulatory Transcription Factor 1), GLS(Glutaminase), and CDKN2A(Cyclin Dependent Kinase Inhibitor 2A), which are key genes in the occurrence of copper death. Based on these ten genes, we conducted subsequent multi-omics analysis[15].

RNA-seq, WGS (whole genome sequence), methylation microarray data and clinical parameter data from TCGA-STAD were downloaded using UCSC XENA to comprehensively analyze the effect of cuproptosis-related genes on STAD. The TCGA dataset consisted of 375 tumor samples and 33 normal samples GSE13911 is an expression profile containing 38 cancers versus 31 normal microarrays. Using GSE13911, we analyzed and verified whether Cu-death-related genes are differentially expressed. GSE62254 was designed to investigate whether genes associated with gastric cancer predicted survival in patients with gastric cancer (300 samples). All prognostic analyses are shown in Additional file 4: Table S1.

Analysis of altered cuproptosis DNA levels

Because mutations do not necessarily affect gene function, we selected loss-of-function (LOF) mutations for follow-up analysis to illustrate the importance of mutations. LOF-associated mutation types included intragenic mutations, nonsense mutations, cut-site mutations, and translation initiation site mutations. First, we analyzed the association between overall cuproptosis gene mutations and gastric cancer by grouping patients according to whether they had mutations or not. Exact testing identified prognosis-associated mutated genes with mutually exclusive and reciprocal mutations.

We examined the relationship between changes in copy number of the cuproptosis gene and tumor prognosis and clinical parameters, depending on whether the copy number was increasing or decreasing.

The CHAMP package was used for differential expression analysis of gastric cancer methylation data. Since the methylation of genes is primarily occurred in the promoter region, we calculated the average methylation level of each promoter region of the cuproptosis gene.

Next, prognostic analysis was performed to observe the relationship between the methylation level of the gene promoter region and the prognosis of gastric cancer. Subsequently, we analyzed that the relationship of these genes with the clinical phenotype of gastric cancer was analyzed by selecting the methylation genes that affect expression and prognosis.

Analysis of cuproptosis RNA levels

We first investigated differentially expressed genes affecting gastric cancer using RNA-seq data from TCGA-STAD and expression data from GSE13911. Then, we searched for copper death genes that affect the prognosis of STAD using prognostic data from TCGA-STAD and GSE62254. Further analysis was conducted on gastric cancer signature genes in relation to clinical parameters and immune cells.

Based on the RNA-seq data of TCGA- STAD, we were able to obtain information on the variable splicing events of genes as well as alternative polyadenylation (APA) alterations. The relationships between these two features and gastric cancer prognosis and conventional clinical parameters were investigated.

Prognostic model construction and clinical characterization

MOVICS is an R package for multi-omic genetic analysis[16]. A prognostically significant multi-omic signature for cuproptosis was utilized to conduct multi-omic clustering analysis using MOVICS. To identify suitable subtype groupings, we assessed the cluster prediction index (CPI) and gap statistic analysis based on multi-omics data. Then, ten clustering algorithms were used to classify patients into different subtypes; finally, a combined classification was obtained by consensus clustering and subtypes were identified with high robustness. A total of ten clustering algorithms, including iClusterBayes, moCluster, CIMLR, IntNMF, ConsensusClustering, COCA, NEMO, PINSPlus, SNF, and LRA, were used in the clustering analysis. MOVICS is capable of segmenting the sample into several groups using multi-omic data by integrating various algorithms (Cluster1, Cluster2, etc.). Additionally, the Kaplan–Meier algorithm was utilized to examine the relationship between the copper mortality model and overall survival, progression-free survival, and disease-specific survival in STAD. The relationship between the copper mortality prediction model and STAD clinical parameters was further investigated. We analyzed the relationship between the clinical parameters such as age, sex, radiotherapy status, TNM stage, recurrence status, and copper mortality model using the clinical information from TCGA-STAD.

Prognostic model functional analysis

Differential expression analysis was performed on the RNA-seq data using the "limma" package based on the grouping of the cuproptosis prognosis model. | Log2 fold change > 1.0 and adjusted $p < 0.05$ were selected as criteria for the screening of differentially expressed genes. Possible functional effects were further investigated in prognostic models. A multi-species gene response pathway database, the Kyoto encyclopedia of genes and genomes (KEGG), was used. KEGG enrichment analysis of the differential analysis results using the GSEA algorithm was carried out using the clusterProfiler package[17]. The GSVA algorithm was also used to evaluate the immune infiltration scores and tumor characteristic pathway scores of STAD patients and to analyze the effect of cuproptosis model on immune cells and tumor characteristic pathways.

Cell function experiments

MTF1 overexpression: To investigate the effects of MTF-1 overexpression in MKN-45 gastric cancer cells, the following protocol is employed. MKN-45 cells are cultured in RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum (FBS) at 37 °C with 5% CO_2. For transfection, cells are seeded at a density of 1–2 × 106 cells per 80 cm flask and allowed to reach semi-confluence. A recombinant vector encoding MTF-1 is then transfected into the cells using Lipofectamine 2000 according to the manufacturer's instructions. Post-transfection, cells are incubated for 48–72 h to allow for MTF-1 overexpression. Transfection efficiency is assessed via flow cytometry or Western blot analysis. Cell invasion and apoptosis experiments were performed using overexpressed cell lines, all of which were repeated three times (Additional file 4).

Cell-Transwell Assay: 72 h after seeding, the Transwell inserts were removed from the culture wells. The medium inside the inserts was discarded, and cells remaining inside the inserts were gently wiped off using cotton swabs moistened with PBS. To stain the migrated cells, 1 mL of crystal violet staining solution was added to each well of a clean 24 well plate, allowing the cells to stain for 3 to 10 min. After staining, the inserts were washed 2 to 3 times with PBS to remove excess stain. The washed inserts were then photographed to document the results of the cell migration assay.

Apoptosis Assay: Apoptosis was assessed 72 h post-transfection. Cells were detached using trypsin without EDTA (Ethylene diamine tetra acetic acid). The cells were then centrifuged at 300 g for 5 min at 4 °C to facilitate collection. Following centrifugation, the cell pellet was washed twice with PBS. After discarding the supernatant,

cells were resuspended in 100uL of'1×Binding Buffer. To stain the cells, Sul of Annexin V-FITC and 10uL of PI Staining Solution were added to the cell suspension, followed by gentle mixing. The cell suspension was then incubated for 10–15 min at room temperature in the dark. After incubation, 400uL of diluted 1×Binding Buffer was added to the samples, which were then mixed well prior to flow cytometric analysis.

Statistical analysis

The data were statistically analyzed and visualized using R software v4.1.2. Measurements are expressed as means (standard deviations). Wilcoxon rank-sum test was used to estimate the difference between the MTF1 overexpression group and the control group. Kaplan–Meier log-rank sum analysis was used to assess survival differences between subgroups of patients. COX regression was used to assess the prognostic impact of cuproptosis on STAD. $P < 0.05$ was considered statistically significant.

Results

Genetic mutation analysis

All ten cuproptosis genes were found to be mutated to varying extents in the mutation analysis. The mutation frequency of CDKN2A was the highest, with a total of 18 patients having mutations (Fig. 1A). Notably, CDKN2A mutations were predominantly located in the structural domain of the gene (Fig. 1B). Coexpression/reciprocal analysis of cuproptosis-related genes was performed, which revealed some co-occurrence of multiple mutations, including LIAS and DLAT (Fig. 1C). We analyzed the relationship between mutations in each cuproptosis gene and the prognosis of gastric cancer. After the analysis, we found that there was no relationship between the mutation or non-mutation of each gene on its own and the prognosis of gastric cancer. Furthermore, by classifying all genes according to whether they were mutated or not, the relationship between overall cuproptosis gene mutations and gastric cancer prognosis was observed. After analysis, it was found that gene mutation prognosis affected the overall survival of gastric cancer (Fig. 1D and Fig. 1E). In the analysis of the relationship between overall mutation with and without cuproptosis and conventional clinical parameters, our analysis showed that overall mutations were associated with age, MSI and TCGA subtype.

Copy number analysis

Our results showed that the copy number of the cuproptosis gene was differentially altered in gastric cancer. DLD was primarily associated with increased copy number, whereas LIAS was predominantly linked to decreased copy number (Fig. 2A). Only PDHB copy number alterations were associated with prognosis, with increasing copy number associated with better tumor prognosis when analyzing copy number variations and prognosis in gastric cancer (Fig. 2B). Our study aimed to investigate the potential impact of PDHB copy number variations on the prognosis of gastric cancer. To achieve this, we examined the correlation between PDHB changes and gastric cancer MSI scores, which were obtained from cBioPortalData. Our findings indicate a positive association between PDHB levels and MSI scores in gastric cancer (Additional file 2: Fig. S1). Most of the cuproptosis gene copy number alterations were associated with MSI (Fig. 2C) when we analyzed the relationship between cuproptosis gene copy number alterations and clinical phenotype. Furthermore, MTF1 was also associated with tumor genotyping (Fig. 2D).

Methylation analysis

Among the methylation analysis, four methylation sites were found to be highly expressed in gastric cancer through differential methylation analysis, and interestingly, all these methylation sites were located in CDKN2A (Fig. 3A). After screening for methylation sites in the promoter region, nine genes were that exhibited methylation in this region. Only PDHA1 had no methylation sites. According to the prognostic analysis, a total of five methylated genes impacted the prognosis of gastric cancer. These five genes were all hypermethylated with poor prognosis. Five bases within LIAS were part of the LA complex, while the other four genes (CDKN2A, DLAT, GLS, and MTF1) were part of PDX complex. Among these, CDKN2A methylation is associated with tumorigenesis and prognosis in gastric cancer (Fig. 3B through F). In our analysis of clinical parameters, CDKN2A was associated with several clinical parameters including age, MSI, and TCGA genotyping (Fig. 3G–I).

Variable splicing analysis

Exon skipping is the only type of variable splicing that occurs in all genes and is also the most common type of variable splicing among all variable splicing events in all cuproptosis (Fig. 4A). All variable splicing events in LIAD are exon skipping. Conversely, CDKN2A has multiple variable splicing events. Prognostic analysis of variable splicing events revealed that there were five genes which had an impact on prognosis and were associated with variable splicing events. Among these, increased ES in CDKN2A is associated with increased patient survival (Fig. 4B), and AT in GLS is associated with improved survival (Fig. 4C). Furthermore, multiple exon skipping in LIPT1 gene resulted in different prognostic effects (Fig. 4D). Clinical parameters were correlated with prognostic variable splicing events. It

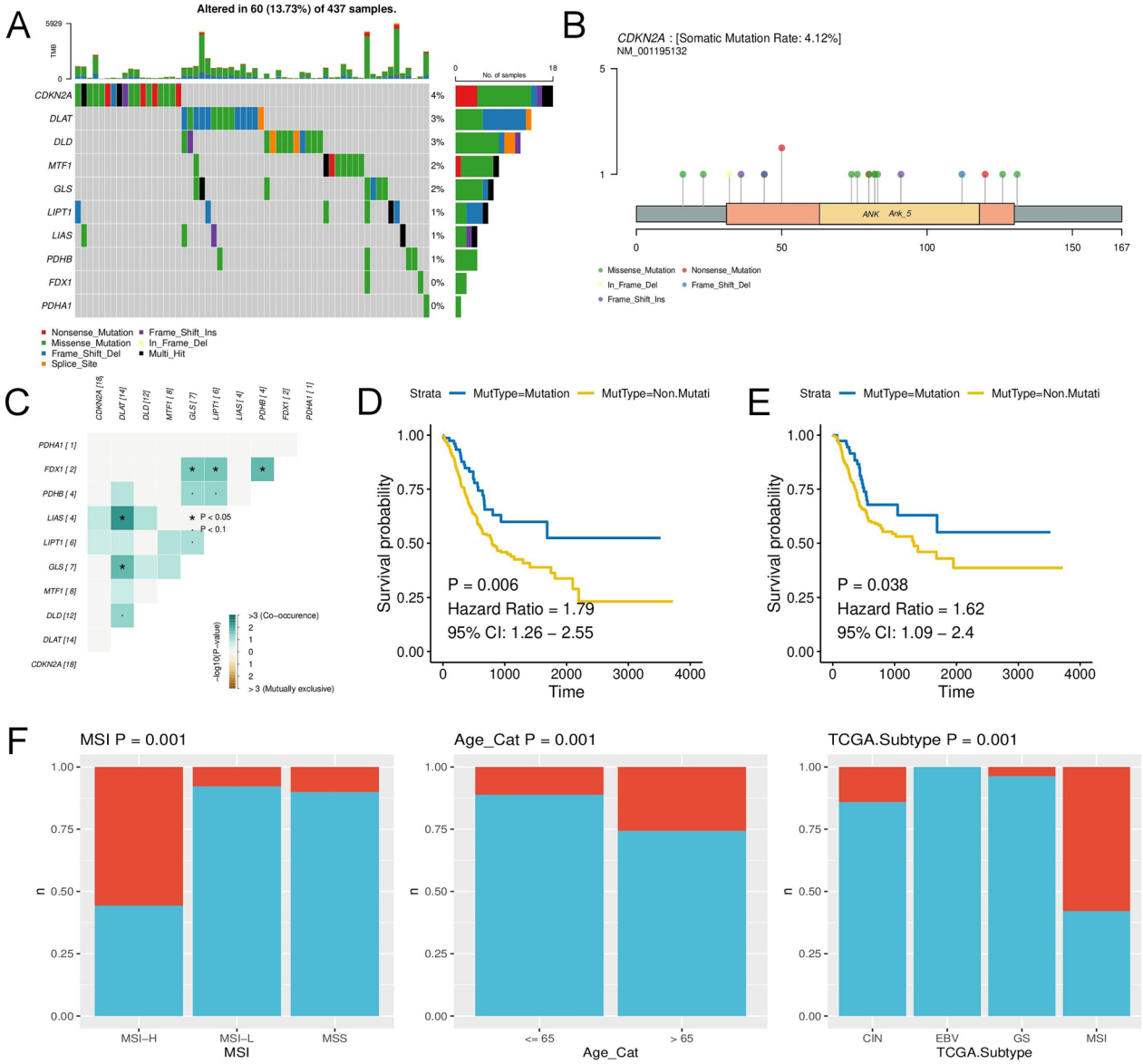

Fig. 1 Genomic changes of Copper Death-related genes in gastric cancer. **A** Genomic Changes of Copper Death-Related Genes in Gastric Cancer. CDKN2A has the most mutation sites in gastric cancer, followed by the DLAT gene. **B** Specific Gene Mutation Sites in CDKN2A. In the linear structure of the gene, each lollipop graph is a specific mutation site. The higher the graph, the more people there are. **C** There is a potential for co-occurrence of mutations in multiple genes associated with copper-induced cell death. **D** Mutations in the copper death gene can lead to better tumor prognosis. **E** Relationship between Gene Mutation Status and Prognosis of Tumor Progression-Free Survival (PFS). **F** The presence of Copper Death Gene Mutations varied across various tumor phenotypes, with a higher incidence in MSI-H tumors and in older individuals, while no mutations were observed in the EBV-infected tumor subtype

was found that both LIPT1 and GIS gene variegation was nearly universally associated with clinical parameters of gastric cancer. Of these, GIS had different splicinging, with the more AT splicing events occurring correlating with a more severe the T phase (Fig. 4E). In the MSI staging system, the exon skipping of LIPT1 was more highly expressed in the MSS staging system and the mixed staging system (Fig. 4F).

A total of nine out of 10 cuproptosis genes showed APA alterations (except MTF1) in the APA gene assay for gastric cancer. In addition, long APA was associated with poorer prognosis in gastric cancer (Fig. 4G). We further analyzed the relationship between APA and clinical involvement and found that the prognosis-related DLAT was associated with the T stage, the stage classification, and the MSI stage (Fig. 4H). We also found that G

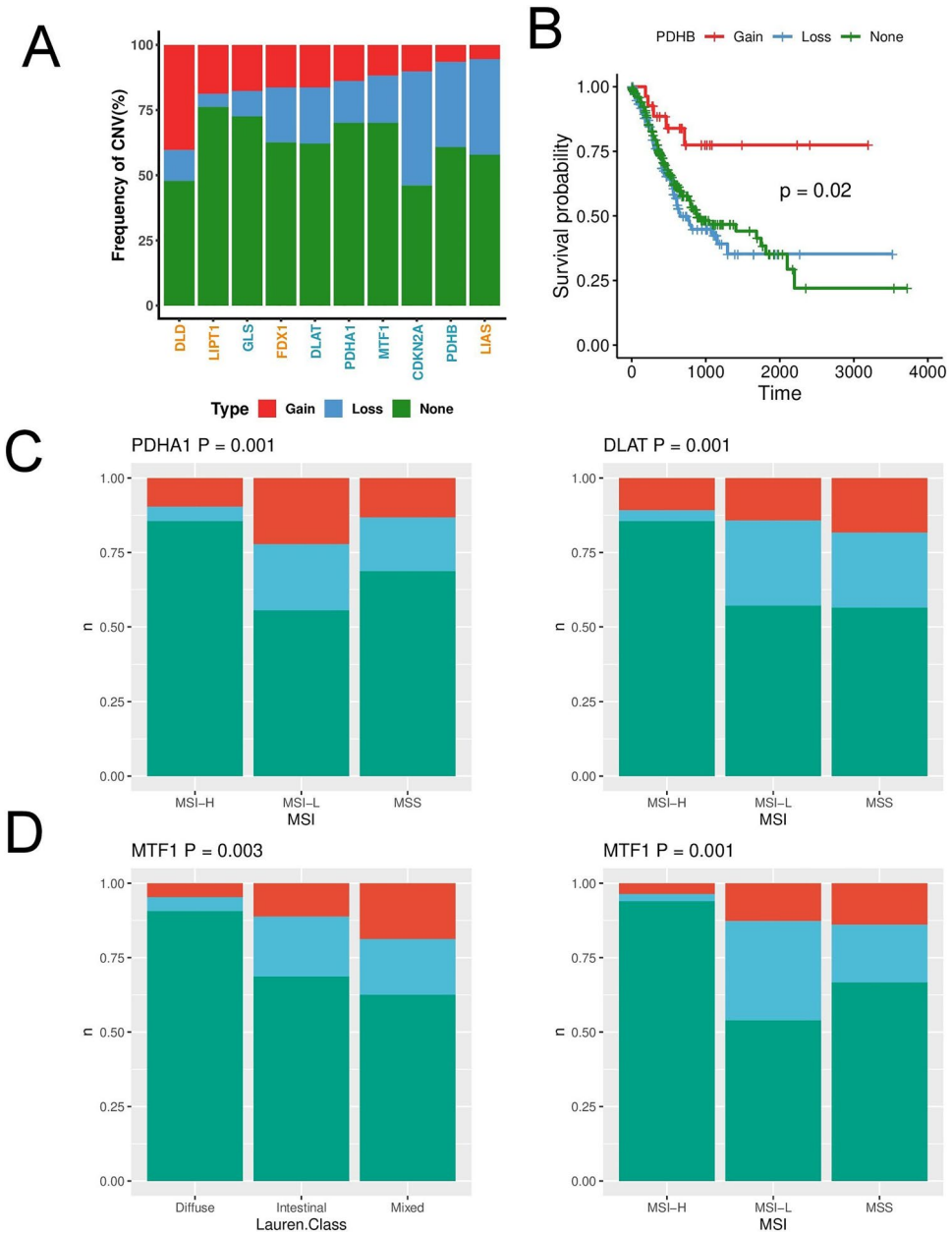

Fig. 2 Alterations in the copy number of genes related to copper-induced cell death impact alterations in the characteristics of tumors. **A** Copy number alteration frequency of cuproptosis, where red represents copy number gain, blue represents copy number loss, and green represents no change in copy number. **B** PDHB gene copy number alterations affect the prognosis of gastric cancer. **C** The relationship between copy number alterations of PDHA1 and DLAT genes and gastric cancer MSI. **D** MTF1 gene copy number alterations affect the classification of gastric cancer and MSI subtypes

LS, FDX1, and DLD were associated with staging in the previous TCGA genotyping. All three were higher in the CIN subgroup (Fig. 4I).

Gene expression analysis

The TCGA database was utilized to conduct a differential expression analysis of 10 genes. The results revealed that three genes (CDKN2A, DLAT, and GLS) exhibited differential expression in gastric cancer (Fig. 5A). GSE13911 is an expression profile microarray that includes 38 cancer vs 31 normal genes. Differential expression analysis using GSE13911 revealed that only GLS was highly expressed in gastric cancer (Fig. 5B). Prognostic analysis of cuproptosis genes using TCGA

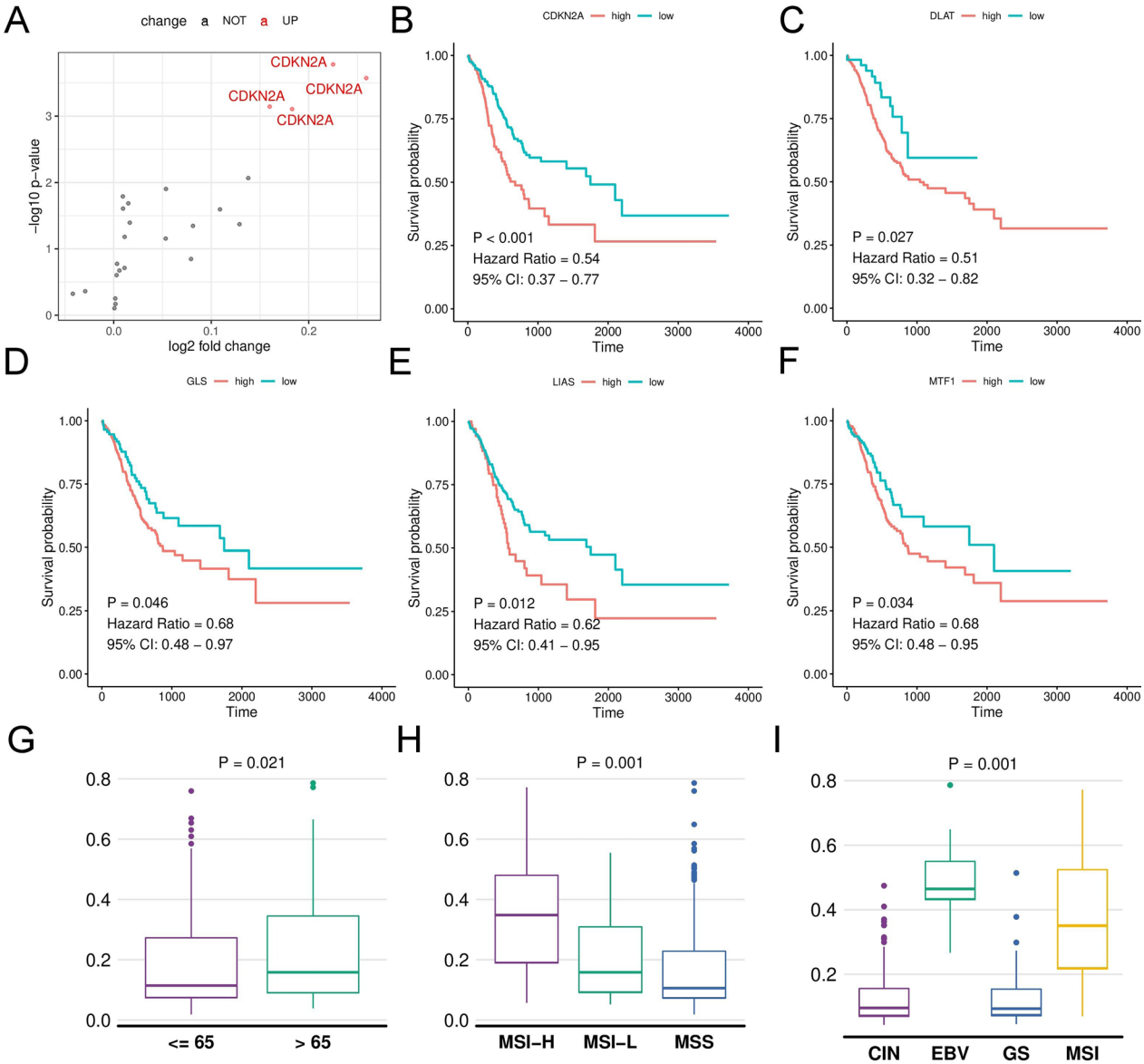

Fig. 3 Alterations in DNA methylation of genes related to copper-induced cell death impact alterations in the characteristics of tumors. **A** DNA methylation of the CDNK2A gene increases in gastric cancer. **B**–**F** Survival curves showing the effect of cuproptosis gene on the prognosis of gastric cancer. **G**–**I** The impact of cuproptosis gene on clinical features of gastric cancer (age, MSI, and pathological classification)

revealed that a total of five out of ten cuproptosis genes were associated with prognosis (LIPT1, DLD, PDHA1, PDHB, and MTF1). After analysis, a total of five genes were found to affect the prognosis of gastric cancer (LIPT1, DLD, PDHA1, PDHB, and MTF1). Cross-tabulation analysis revealed that a total of three genes affected gastric cancer prognosis (PDHA1, PDHB, and MTF1) in both data sets (Fig. 5C). Among them, the expression of PDHB and MTF1 had a protective effect against gastric cancer, while PDHA1 had a risk. To gain a deeper understanding of the correlation between copper death genes and clinical phenotypes of gastric cancer, including TNM staging, MSI staging, and gene expression staging, we conducted an analysis of the gene expression differences among various phenotypes. Our findings revealed that the CIN subtype exhibits high expression levels of CDKN2A and GLS genes(Fig. 5D), while the intestinal subtype shows high expression levels of PDHB and PDHA1 genes(Fig. 5E). Furthermore, our analysis of MSI subtypes also

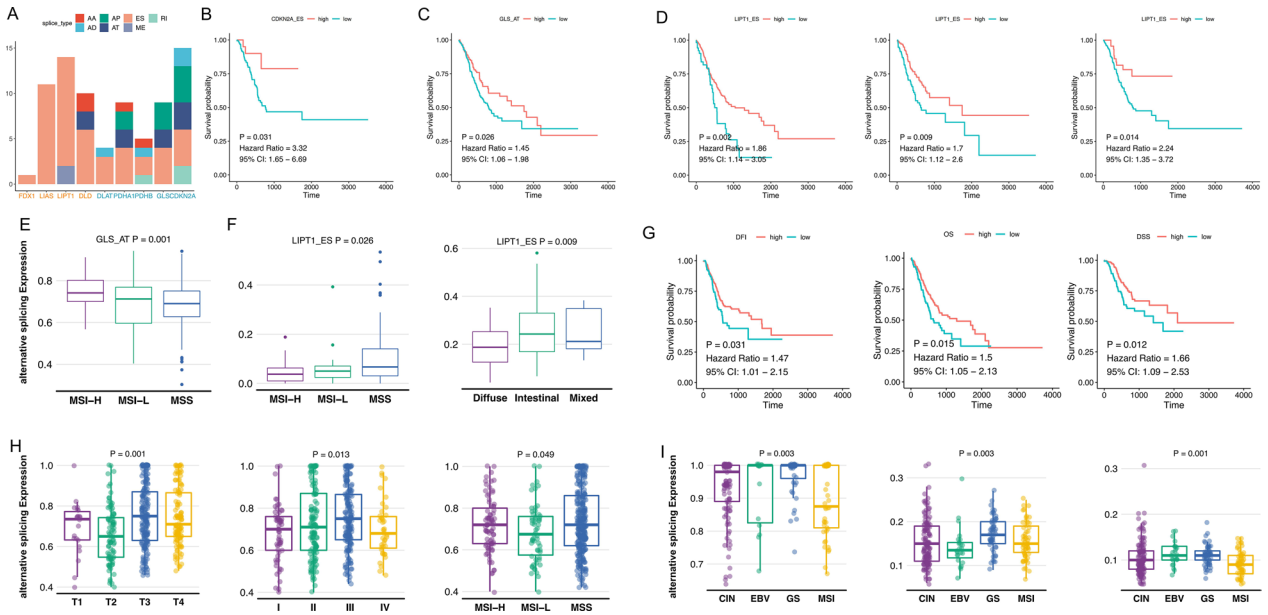

Fig. 4 Features of alternative splicing changes in the cuproptosis gene. **A** Different types of alternative splicing in cuproptosis gene. Different colors in the figure represent different variable shear types. **B**–**D** Copper death genes can undergo alternative splicing, resulting in improved patient survival. **C**–**F** The influence of cuproptosis alternative splicing on clinical characteristics of tumors (MSI and pathological classification). **G** Alternative splicing changes can lead to better tumor prognosis in gastric cancer patients. H-I. The relationship between APA in cuproptosis and clinical features

revealed a connection between these two genes and MSI subtypes (Fig. 5F).

Cuproptosis gene expression regulation and functional analysis

In the analysis of the regulation of cuproptosis by DNA mutations, we identified eight genes with mutations. We further analyzed the effect of these eight genes on cuproptosis gene expression. We found that only CDKN2A mutation affected gene expression, and the mutated gene exhibited high levels of expression (Fig. 6A). In the methylation analysis, we identified a total of two genes that received methylation regulation (FDX1 and CDKN2A) (Fig. 6B-6E), among which CDKN2A had three methylation sites associated with expression. One of the methylation sites in the genomic region was positively correlated with expression, while two methylation sites in the first exon region were negatively correlated with expression. Among the transcriptional regulation predictions, we found that a total of 46 transcription factors were involved in the regulation of cuproptosis genes. Among them, MTF1 was associated with 21 transcription factors (Fig. 6F). Regarding transcription factors, CTCF(CCCTC binding factor) was associated with eight cuproptosis genes and YY1 was associated with six genes. Within the ceRNA regulatory network, six genes were regulated by miRNAs, which were mainly associated with 13 miRNAs (Fig. 6G). Further analysis of the LncRNAs interacting with these miRNAs revealed that hsa-miR-5047 was associated with 35 lncRNAs.

Based on the previous analysis, we found that a total of four genes were associated with gastric cancer (one with the development of gastric cancer and three with the prognosis of gastric cancer). Therefore, these four genes were selected for functional analysis. First, we predicted the interacting proteins of the four genes using the BIOGRID database. Furthermore, we observed the possible functions of the cuproptosis genes associated with gastric cancer through GO analysis and KEGG pathway analysis, finding that the interacting proteins in GO-BP analysis were primarily related to "mitochondrial gene expression/mitochondrial translation" (Fig. 7A). In the KEGG pathway enrichment, the interacting proteins were found to be mainly related to the cell cycle (Fig. 7B). Based on the single gene GSEA analysis, we examined the relationship between the four genes and tumor-related pathways. After the analysis, we found that the four genes were associated with 22–48 pathways respectively. Among them, there were a total of 21 pathways that were common to all four genes. These include pathways related to glycolysis and fatty acid metabolism (Fig. 7C). Finally, we analyzed the relationship between cuproptosis genes and immune cells and immune checkpoints. The analysis revealed that the four genes were not related to most of the immune cells, but only to T helper cells. Among the immune checkpoint analysis, MTF1 was associated with

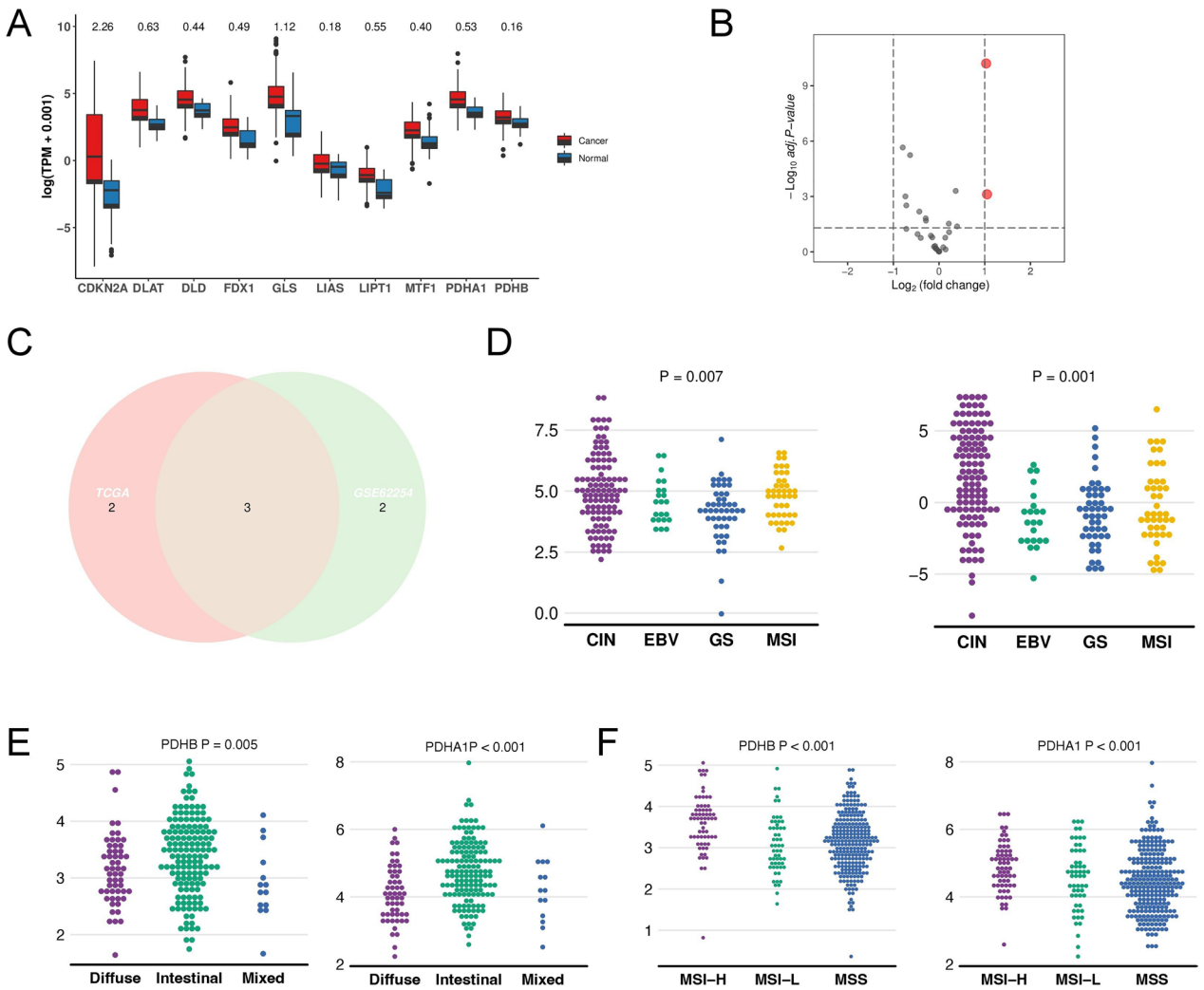

Fig. 5 The mRNA expression features of cuproptosis in gastric cancer. **A** Multiple copper death-related genes are differentially expressed in gastric cancer and are elevated in cancer. **B** Differential expression of cuproptosis in GSE13911. **C** Venn diagram showing the impact of cuproptosis on prognostic genes in both TCGA and GSE62254 datasets. **D**–**F** The influence of cuproptosis gene on clinical characteristics of gastric cancer

multiple immune checkpoints, and the other three genes did not correlate well with immune checkpoints.

Multi-omics prognostic model and functional analysis of copper-dead gastric cancer

In light of the above multi-omics analysis, we found that cuproptosis-related genes impact the prognosis of gastric cancer in different histologies. Based on this analysis, we used multi-omics data that affecting the prognosis of gastric cancer (copy number changes of the PDHB gene, methylation changes of CDKN2A, DLAT, GLS, MTF1, and LIAS, alternative splicing data of five genes, DLAT APA change data, gene expression data of LIPT1, DLD, PDHA1, PDHB, and MTF1) to construct a multi-omics prognostic model. In this study, we developed a prognostic model for gastric cancer using unsupervised clustering. We found that the best results were obtained when the highest mean statistic of the number of subtypes was 2 (Fig. 8A), based on the results of the CPI analysis and the disparity statistics. Then, to make the classification more robust, we classified the patients into cluster 1 (CS1) and cluster 2 (CS2) using ten clustering techniques for validation through consensus clustering (Fig. 8B). The similarities of the samples in the consensus clusters were also assessed by contour analysis, where the contour score of CS1 was 0.51 and that of CS2 was 0.31. Therefore, the two clustering results were clearly different from each other (Fig. 8C). When prognostic analysis was performed on the constructed multi-omics model, we found that the model was associated with all

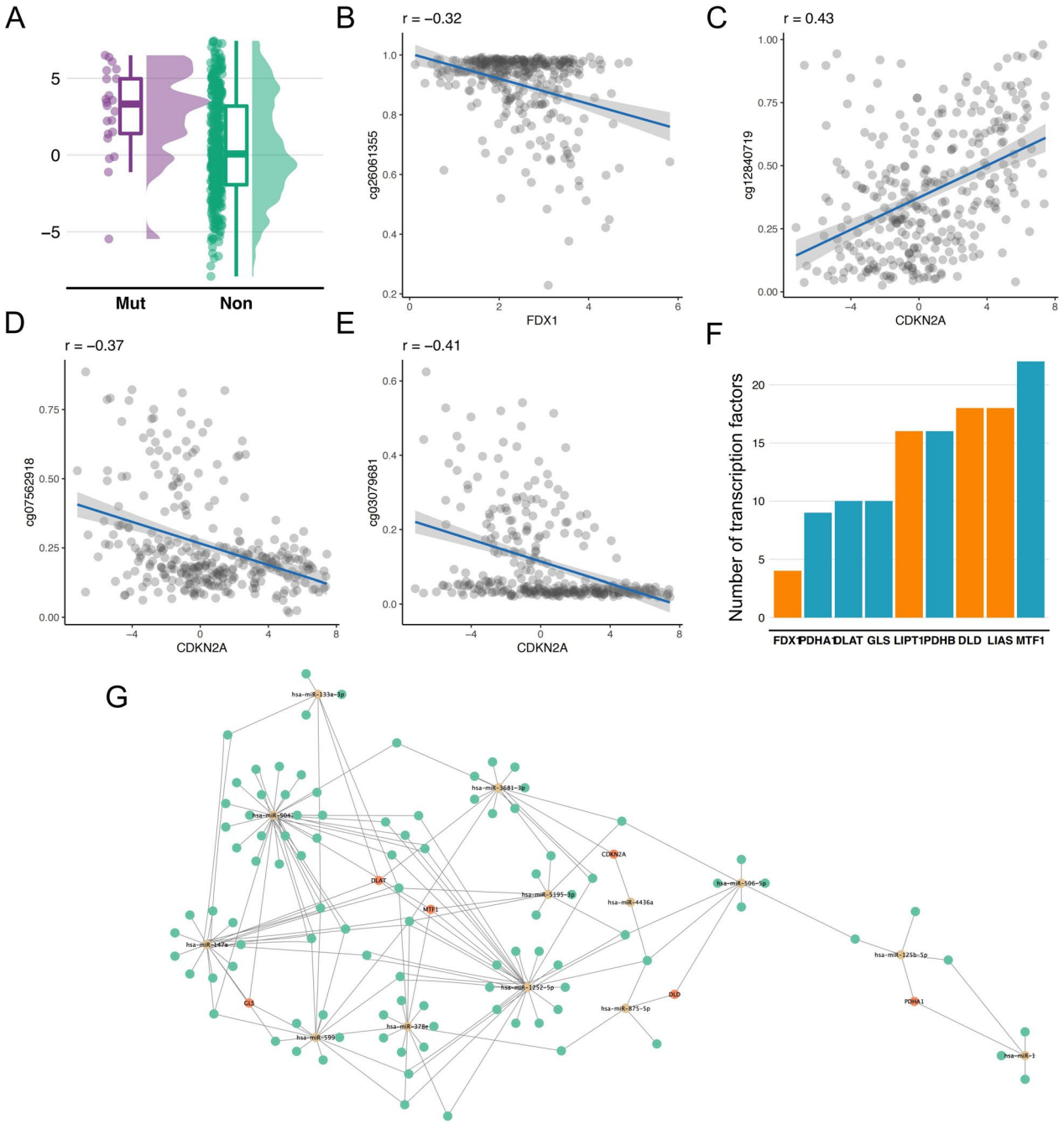

Fig. 6 Prediction of the expression regulation of core cuproptosis genes. **A** CDKN2A gene mutation affects gene expression. **B**–**E** Gene methylation impacts the expression of the genes themselves. **F** Prediction of transcription factors' regulation of cuproptosis expression. **G** 15 miRNAs play a key role in the entire ceRNA regulatory network

four prognostic indicators of the tumor (Fig. 8D-F). The multi-omics model was mainly associated with age and microsatellite instability (MSI) (Fig. 8G), and further analysis of the relationship between the model and clinical parameters revealed that the multi-omics model was primarily associated with age and MSI.

Finally, we recently analyzed the relationship between the model tumor-related pathways to understand the specific function of the prognostic model. The analysis showed that the prognostic model was associated with the TP53 signaling pathway, the IL2 signaling pathway, and the IL6 signaling pathway (Fig. 9A). We analyzed the

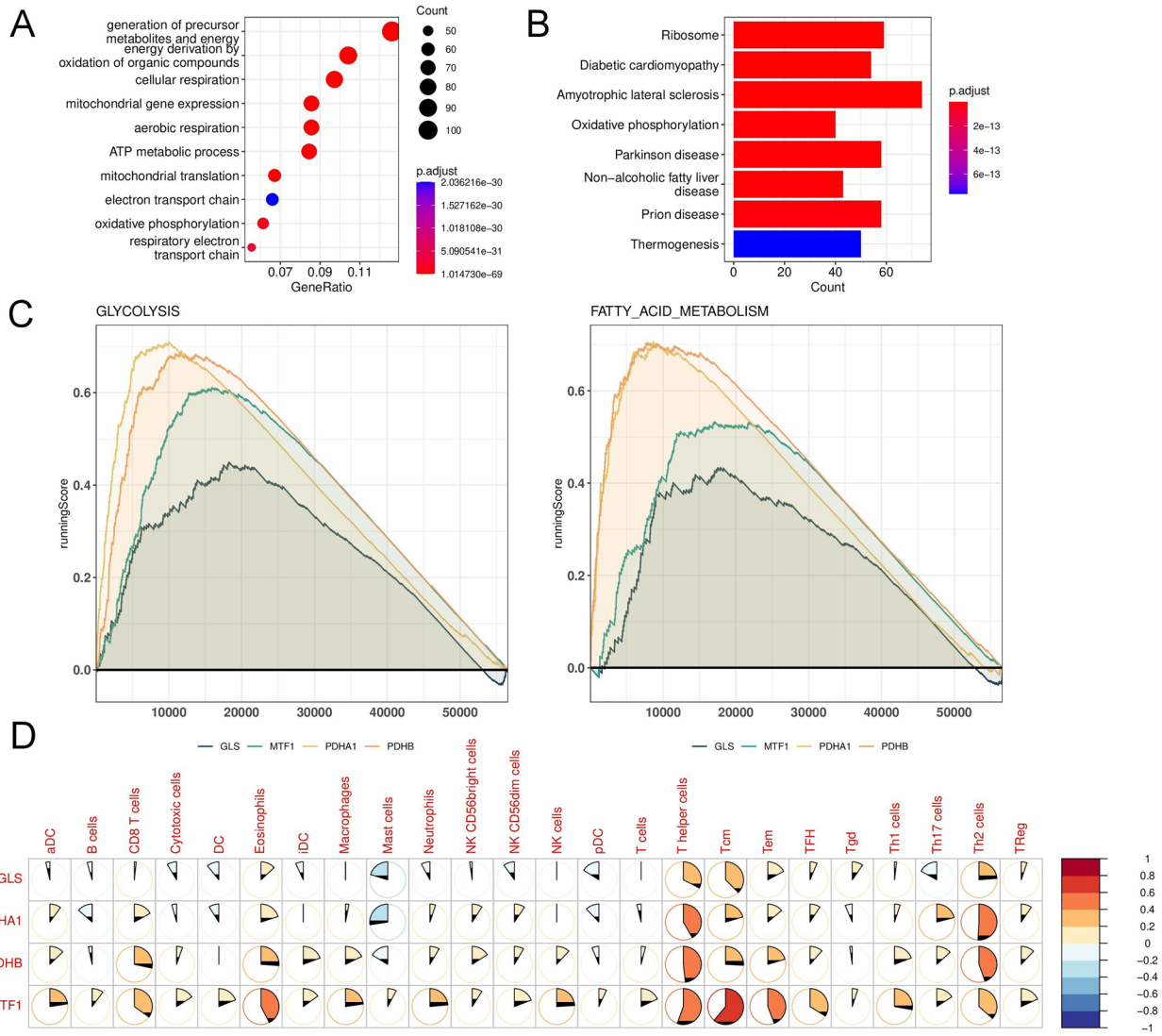

Fig. 7 Functional prediction of cuproptosis core genes. **A** Bubble chart of GO analysis of core genes involved in cuproptosis. **B** Bar graph of KEGG pathway analysis of core genes involved in cuproptosis. **C** Four genes are key to affecting metabolic signaling pathways such as glycolysis and fatty acid metabolism. **D** Relationship between cuproptosis and immune cells. The redder color in the figure represents a positive correlation between genes and immune cells, and the bluer color represents a negative correlation between genes and immune cells

relationship between the prognostic model and immune cells and immune examination. We found that prognostic model was associated with Th17, Th2, and Th1 cells (Fig. 9B), and immune checkpoints including CD86, CD80, and CD40 (Fig. 9C).

The core gene MTF1 affects the biological function of gastric cancer

In the construction of our multi-omics model, MTF1 was found to affect the prognosis of STAD across multiple omics. Additionally, MTF1 ranked first in the importance score of each variable in the model. To gain a deeper understanding of the impact of the MTF1 gene on this disease, we conducted experiments involving the overexpression of MTF1 in gastric cancer cell lines (MGC803) to assess its effects on the biological properties of gastric cancer (Fig. 10A). Our initial investigation focused on the influence of MTF1 on cell apoptosis, and the results revealed that overexpression of MTF1 effectively reduced apoptosis in gastric cancer cells (Fig. 10B). Furthermore, we also observed the effects of MTF1 on tumor cell invasion and migration. As depicted in Fig. 10C, MTF1 enhanced tumor cell invasion. To ensure the reliability of our findings, we conducted experiments using a different gastric cancer cell line (HGC-27) and found that the results were

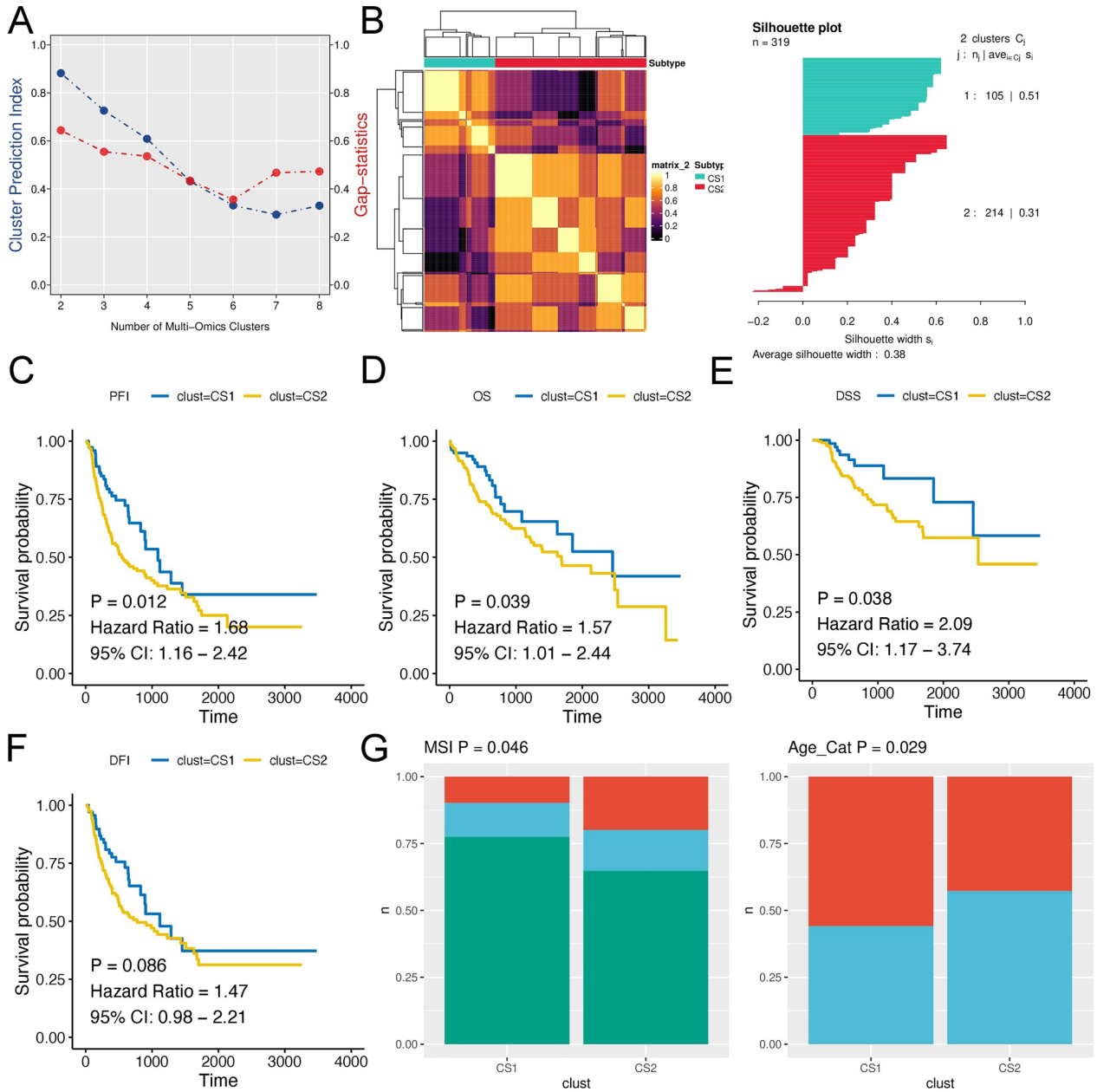

Fig. 8 Construction of cuproptosis multi-omics model. **A** Selection of classification for cuproptosis multi-omics model. The model clusters best when it starts to fall, so 2 groups are selected. **B** Visualized correlation heatmap of cuproptosis multi-omics model. **C**–**F** Impact of cuproptosis multi-omics model on gastric cancer prognosis. **G** Relationship between cuproptosis multi-omics prognosis model and clinical parameters

consistent with those obtained from MGC803 (see Additional file 3: Fig. S2).

Discussion

Cuproptosis is a newly identified and copper-induced form of cell death. However, the correlations between cuproptosis-related genes and the development and prognosis of STAD have not yet been explored. In the present study, we conducted a comprehensive multi-omics analysis of ten key genes. Through this analysis, we found that whether at the mutation level, copy number alteration, or even gene mRNA expression, cuproptosis-related genes affect gastric cancer development and prognosis at multiple levels.

Proteins are the primary executors of life activities; thus, a further differential expression analysis on data

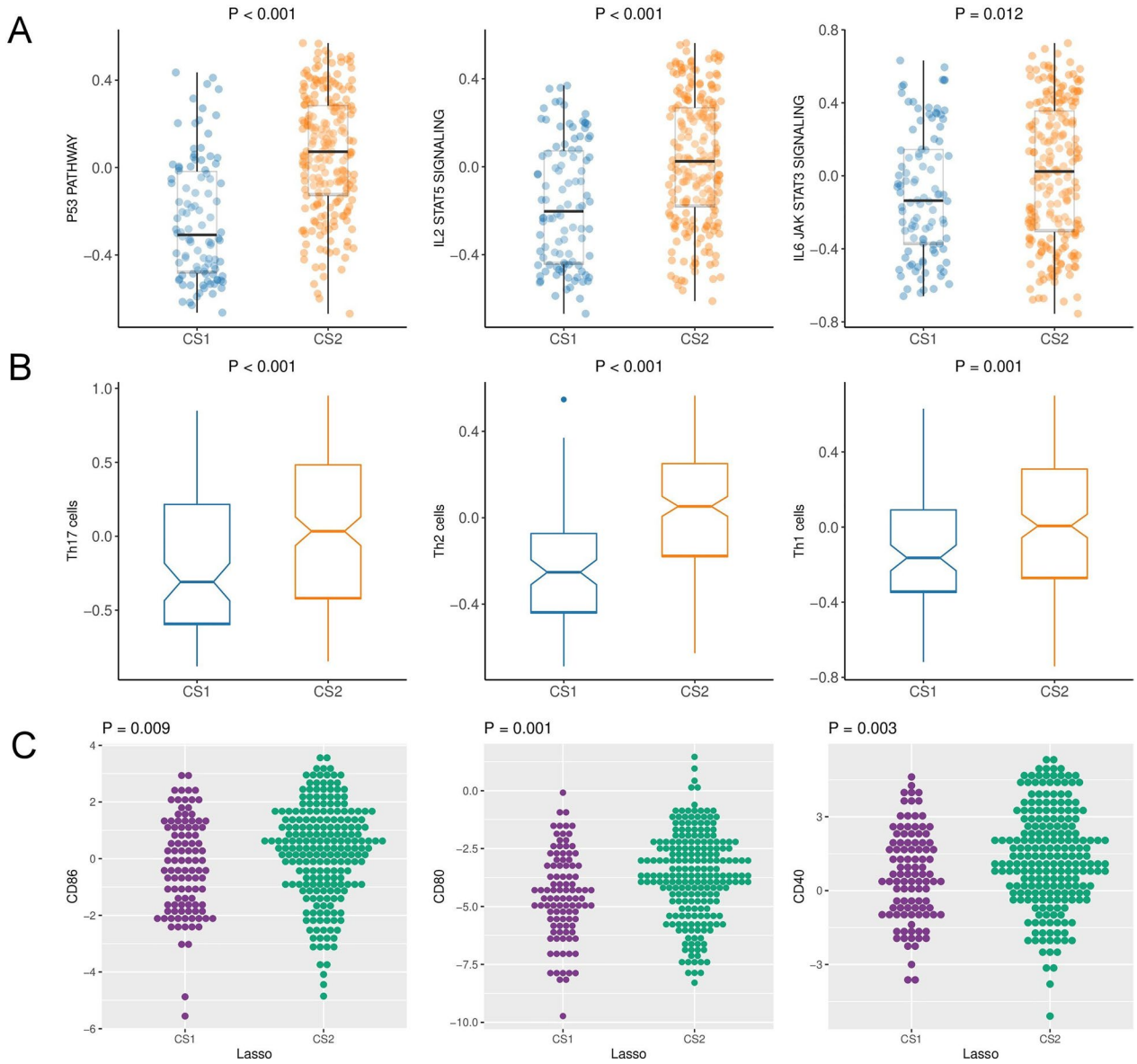

Fig. 9 Functional prediction of cuproptosis multi-omics prognosis model. **A** Tumor-related pathways are all elevated in the CS2 model. **B** CS2 typing triggers the activation of immune cells. **C** Relationship between cuproptosis multi-omics prognosis model and immune checkpoints

from three independent datasets was conducted. We identified 16 cuproptosis-related genes that were differentially expressed between tumor and normal tissues in STAD patients, GO analysis demonstrated that pathways related to the cell cycle were enriched. In addition, we found that a total of three cuproptosis-related genes that were highly correlated with prognosis of STAD and pathway related to TCA cycle were revealed in further KEGG pathway enrichment analysis. Other types of cell death, such as apoptosis, necrosis, necroptosis, pyroptosis, and ferroptosis, have been shown to play important role in neoplasia and have also long been associated with prognosis and therapy of STAD. The role of cuproptosis in STAD has not been examined, and our study provided a comprehensive and up-to-date status of cuproptosis in STAD, with the hope of revealing potential and attractive target for STAD pharmacological treatment.

The CDKN2A was highly expressed in the stomach tumor tissue and was associated with advanced cancer stage and poorer outcomes [18–20]. DARS2, encoded mitochondrial aspartyl tRNA synthase, was strongly upregulated by Hypro in STAD tumor tissues and promoted hepatocarcinogenesis through accelerating cell cycle progression and attenuating cell apoptosis [21].

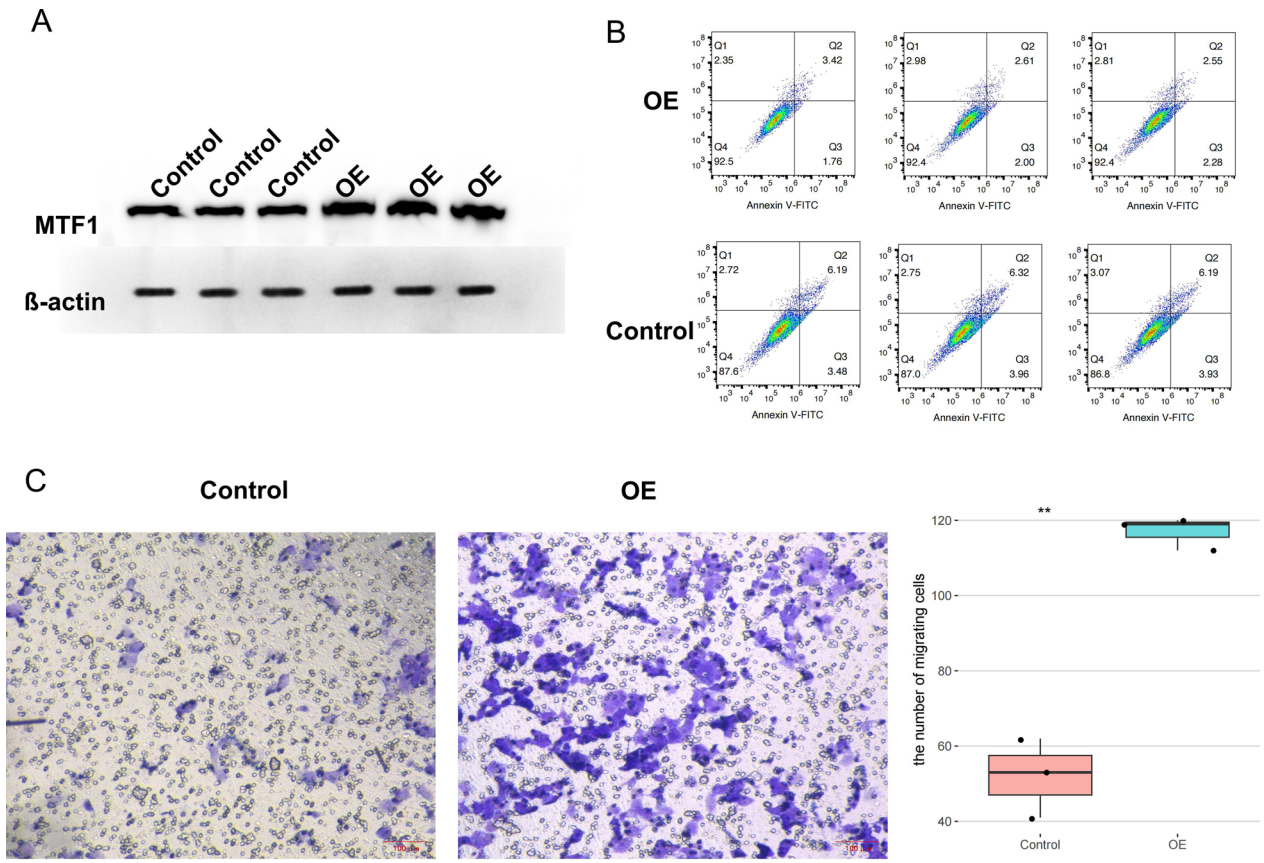

Fig. 10 MTF1 affects apoptosis, invasion and migration of gastric cancer. **A** WB plot of MTF1 gene overexpression. **B** MTF1 gene overexpression leads to reduced apoptosis in tumor cell lines. C MTF1 gene overexpression leads to increased tumor cell invasion

The CANX (calnexin) is an ER-resident lectin chaperone and was involved in the HLA molecules folding and assembly [22–24], which has a vital role in anti-tumor immunity [25–27]. Taken together, all of the three genes were involved in the development and progression of STAD. And the potential regulatory mechanisms of the three characteristic cuproptosis-related genes were also explored, which might provide novel strategy for the treatment of STAD.

A novel multi-omics cuproptosis prognostic model was then constructed for the first time in our study. Gastric cancer prognostic models have traditionally relied on a single type of omic data. However, due to the complex nature of this tumor, predicting its prognosis based on a single omic data can be challenging. By utilizing multi-omic sequencing data, we can select features from a comprehensive perspective to better elucidate the prognosis of gastric cancer, resulting in increased stability. Copper could directly bind and induce the oligomerization of lipoylated DLAT thus resulting in copper toxicity [15]. Increased glutamine metabolism is a hallmark of cancer. Glutaminase (GLS) has been shown upregulated in STAD and was associated with poorer clinical outcome [28, 29]. Various GLS inhibitors aimed to selectively halt the tumor cells growth had been examined in preclinical studies [28]. Cyclin dependent kinase inhibitor 2A (CDKN2A), also named P16, mainly functioned in cell cycle regulating. CDKN2A was proved highly expressed in the stomach tumor tissue than in normal tissues [19] and has been shown correlated with advanced cancer stage and poor prognosis [18–20], which was consistent with our findings. Both GLS and CDKN2A were previously identified as anti-cuproptosis genes [15]. Prohibitin (PHB) is a mitochondrial chaperone that regulates cell growth. The deficiency or knockout of PHB resulted in stomachinjury and STAD [30, 31]. NDUFS2 is a core subunit of mitochondrial complex I and was essential for acute oxygen-sensing, ROS generation, mitochondrial energetics, and cell growth [32, 33]. NDUFA9, another subunit of mitochondrial complex I was involved in stabilizing the junction between membrane and complex I assemble [34]. Hippocalcin-like 1 (HPCAL1) was associated with hepatocarcinogenesis with reduced expression in the STAD cell lines and tissues, and was considered as

tumor suppressor in STAD, lost expression of which has been correlated with poorer clinical outcome [35].

And based on the identified prognostic risk score, we classified the patients into two groups. STAD patients in CS1 group were shown to have significantly shorter OS, DFI, DSS, and PFI. A total of 2090 genes were differentially expressed between these two groups, and immune response-related pathways, including the B cell receptor signaling pathway, TNF signaling pathway and IL-17 signaling pathway, were enriched. In addition, CS1 group was associated with higher immune infiltration level of aDC, iDC, $CD8^+$ T cells, eosinophils, macrophages, NK cells, T helper cells, Tcm cells, Tem cells, Tfh cells, Th1 cells, and Th2 cells, while with lower level of cytotoxic cells, DC cells and mast cells. Although cytotoxic $CD8^+$ T cells are main anti-tumor immune cells, increased expression of PD1 could lead to $CD8^+$ T cell dysfunction, increased infiltration of exhausted $PD1^{Hi}CD8^+$ T cells in tumor tissue was associated with poor survival of STAD patients [36]. And ICIs blocked the interaction of checkpoint molecules with their ligands, and reversal of $CD8^+$ T cells dysfunction and exhaustion was considered as major mechanism for ICIs anti-tumor therapy [37]. Moreover, our results also found that human leukocyte antigen (HLA) class I (including HLA-A, B, C, E, F, and G) and HLA class II (including HLA-DP, DQ, DR, DO, and DM) were markedly evaluated in the CS1 group. HLA class I was the key component in antigen presentation, acted anti-tumor function by presenting intracellular tumoral antigen to the cell surface, which then recognized by cytotoxic $CD8^+$ T cells [26, 38]. And several members of HLA family had been shown association with different clinicopathological features of STAD [39–41]. Loss of HLA class I was associated with tumor immune escape and poor response to ICIs [26, 27], while HLA expression predicted better response to pembrolizumab, a second-line therapy for advanced STAD [42]. The identified prognostic risk score thus may predict a better response to ICIs for patients in CS1 group [43]. While the functional status of immune cells and the under mechanism of cuproptosis affect the immune cells remain large unclear, which needed further explored.

Conclusions

In summary, the present study comprehensive analyzed the signature of cuproptosis-related genes and molecular alteration in STAD. The prognostic risk score based on nine of cuproptosis-related genes was constructed for the first time, which classified patients into two groups. Patients in the CS1 group shown obviously shorter OS, DFI, DSS, and PFI, while significantly higher of immune infiltration level and immune checkpoints expression, predicting better response to ICIs. Our results would also provide insights in novel therapeutic strategies based on cuproptosis and cuproptosis-related genes for STAD.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-025-01894-0.

Additional file 1.

Additional file 2.

Additional file 3.

Additional file 4.

Author contributions

Yinying Wu, Yangwei Fan, and Xuyuan Dong designed this experiment. Danfeng Dong, Yu Shi, and Meichen Wang analyzed the data. Jia Wang and Yuqian Yang write article. Nan Yang, Fengyun Ou, and Enxiao Li reviewed the article.

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All data are from TCGA and GEO public sequencing data.

Declarations

Declarations

Ethical Approval

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Competing interests

The authors declare no competing interests.

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References

1. Gao H, Li L, Geng K, Teng C, Chen Y, Chu F, Zhao Y. Use of proton pump inhibitors for the risk of gastric cancer. Medicine (Baltimore). 2022;101(49):e32228.
2. Hormati A, Hajrezaei Z, Jazi K, AslaniKolur Z, Rezvan S, Ahmadpour S. Gastrointestinal and pancratohepatobiliary cancers: a comprehensive review on epidemiology and risk factors worldwide. Middle East J Dig Dis. 2022;14(1):5–23.
3. Hui Y, Tu C, Liu D, Zhang H, Gong X. Risk factors for gastric cancer: a comprehensive analysis of observational studies. Front Public Health. 2022;10:892468.
4. Singh S, Surendran S, Yacob M, Paul N, Samarasam I. Impact of Covid-19 pandemic on upper gastrointestinal cancer services: experience from an oncosurgical unit. Natl Med J India. 2022;35(4):206–9.
5. Mohammed Asiri S, Levina A, New EJ, Lay PA. Investigations of cellular copper metabolism in ovarian cancer cells using a ratiometric fluorescent copper dye. J Biol Inorg Chem. 2022;5:14.
6. Lv H, Zhu Y, Xue J, Jia X, Chen J. Targeted drug delivery system based on copper sulfide for synergistic near-infrared photothermal therapy/photodynamic therapy/chemotherapy of triple negative breast cancer. Langmuir. 2022;38(50):15766–75.
7. Li S, Yu W, Xie F, Luo H, Liu Z, Lv W, Shi D, Yu D, Gao P, Chen C, et al. Neoadjuvant therapy with immune checkpoint blockade, antiangiogenesis, and chemotherapy for locally advanced gastric cancer. Nat Commun. 2023;14(1):8.

8. Cascinu S, Di Bartolomeo M, Lonardi S, Beretta G, Fornaro L, De Vita F. The evolving strategies for the management of patients with metastatic gastric cancer: a narrative review and expert opinion. Front Med (Lausanne). 2022;9:1002435.
9. Capriotti G, Piccardo A, Giovannelli E, Signore A. Targeting copper in cancer imaging and therapy: a new theragnostic agent. J Clin Med. 2022;12(1):652.
10. Cai J, Xie H, Yan Y, Huang Z, Tang P, Cao X, Wang Z, Yang C, Wen J, Tan M, et al. A novel cuproptosis-related lncRNA signature predicts prognosis and therapeutic response in bladder cancer. Front Genet. 2022;13:1082691.
11. Abu El Maaty MA. 1,25(OH)(2)D(3) disrupts glucose metabolism in prostate cancer cells leading to a truncation of the TCA cycle and inhibition of TXNIP expression. Biochim Biophys Acta Mol Cell Res. 2017;1864(10):1618–30.
12. Anderson NM, Mucka P, Kern JG, Feng H. The emerging role and targetability of the TCA cycle in cancer metabolism. Protein Cell. 2018;9(2):216–37.
13. Marquez J, Flores J, Kim AH, Nyamaa B, Nguyen ATT, Park N, Han J. Rescue of TCA cycle dysfunction for cancer therapy. J Clin Med. 2019;8(12):963.
14. Chong W, Ren H, Chen H, Xu K, Zhu X, Liu Y, Sang Y, Li H, Liu J, Ye C, et al. Clinical features and molecular landscape of cuproptosis signature-related molecular subtype in gastric cancer. Imeta. 2024;3(3):e190.
15. Tsvetkov P, Coy S, Petrova B, Dreishpoon M, Verma A, Abdusamad M, Rossen J, Joesch-Cohen L, Humeidi R, Spangler RD, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. Science. 2022;375(6586):1254–61.
16. Lu X, Meng J, Zhou Y, Jiang L, Yan F. MOVICS: an R package for multi-omics integration and visualization in cancer subtyping. Bioinformatics. 2020;6:96.
17. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation (Camb). 2021;2(3):100141.
18. Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Rebouissou S, Couchy G, Meiller C, Shinde J, Soysouvanh F, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet. 2015;47(5):505–11.
19. Luo JP, Wang J, Huang JH. CDKN2A is a prognostic biomarker and correlated with immune infiltrates in hepatocellular carcinoma. Biosci Rep. 2021;41(10):48.
20. Zhang C, Feng S, Tu Z, Sun J, Rui T, Zhang X, Huang H, Ling Q, Zheng S. Sarcomatoid hepatocellular carcinoma: From clinical features to cancer genome. Cancer Med. 2021;10(18):6227–38.
21. Qin X, Li C, Guo T, Chen J, Wang HT, Wang YT, Xiao YS, Li J, Liu P, Liu ZS, et al. Upregulation of DARS2 by HBV promotes hepatocarcinogenesis through the miR-30e-5p/MAPK/NFAT5 pathway. J Exp Clin Cancer Res. 2017;36(1):148.
22. Jackson MR, Cohen-Doyle MF, Peterson PA, Williams DB. Regulation of MHC class I transport by the molecular chaperone, calnexin (p88, IP90). Science. 1994;263(5145):384–7.
23. Diedrich G, Bangia N, Pan M, Cresswell P. A role for calnexin in the assembly of the MHC class I loading complex in the endoplasmic reticulum. J Immunol. 2001;166(3):1703–9.
24. Anderson KS, Cresswell P. A role for calnexin (IP90) in the assembly of class II MHC molecules. EMBO J. 1994;13(3):675–82.
25. Alam A, Taye N, Patel S, Thube M, Mullick J, Shah VK, Pant R, Roychowdhury T, Banerjee N, Chatterjee S, et al. SMAR1 favors immunosurveillance of cancer cells by modulating calnexin and MHC I expression. Neoplasia. 2019;21(10):945–62.
26. Dersh D, Holly J, Yewdell JW. A few good peptides: MHC class I-based cancer immunosurveillance and immunoevasion. Nat Rev Immunol. 2021;21(2):116–28.
27. Gu SS, Zhang W, Wang X, Jiang P, Traugh N, Li Z, Meyer C, Stewig B, Xie Y, Bu X, et al. Therapeutically increasing MHC-I expression potentiates immune checkpoint blockade. Cancer Discov. 2021;11(6):1524–41.
28. Du D, Liu C, Qin M, Zhang X, Xi T, Yuan S, Hao H, Xiong J. Metabolic dysregulation and emerging therapeutical targets for hepatocellular carcinoma. Acta Pharm Sin B. 2022;12(2):558–80.
29. Masisi BK, El Ansari R, Alfarsi L, Rakha EA, Green AR, Craze ML. The role of glutaminase in cancer. Histopathology. 2020;76(4):498–508.
30. Barbier-Torres L, Lu SC. Prohibitin 1 in liver injury and cancer. Exp Biol Med (Maywood). 2020;245(5):385–94.
31. Fan W, Yang H, Liu T, Wang J, Li TW, Mavila N, Tang Y, Yang J, Peng H, Tu J, et al. Prohibitin 1 suppresses liver cancer tumorigenesis in mice and human hepatocellular and cholangiocarcinoma cells. Hepatology. 2017;65(4):1249–66.
32. Bandara AB, Drake JC, James CC, Smyth JW, Brown DA. Complex I protein NDUFS2 is vital for growth, ROS generation, membrane integrity, apoptosis, and mitochondrial energetics. Mitochondrion. 2021;58:160–8.
33. Dunham-Snary KJ, Wu D, Potus F, Sykes EA, Mewburn JD, Charles RL, Eaton P, Sultanian RA, Archer SL. Ndufs2, a core subunit of mitochondrial complex I, is essential for acute oxygen-sensing and hypoxic pulmonary vasoconstriction. Circ Res. 2019;124(12):1727–46.
34. Stroud DA, Formosa LE, Wijeyeratne XW, Nguyen TN, Ryan MT. Gene knockout using transcription activator-like effector nucleases (TALENs) reveals that human NDUFA9 protein is essential for stabilizing the junction between membrane and matrix arms of complex I. J Biol Chem. 2013;288(3):1685–90.
35. Zhang Y, Liu Y, Duan J, Yan H, Zhang J, Zhang H, Fan Q, Luo F, Yan G, Qiao K, et al. Hippocalcin-like 1 suppresses hepatocellular carcinoma progression by promoting p21(Waf/Cip1) stabilization by activating the ERK1/2-MAPK pathway. Hepatology. 2016;63(3):880–97.
36. Ma J, Zheng B, Goswami S, Meng L, Zhang D, Cao C, Li T, Zhu F, Ma L, Zhang Z, et al. PD1(Hi) CD8(+) T cells correlate with exhausted signature and poor clinical outcome in hepatocellular carcinoma. J Immunother Cancer. 2019;7(1):331.
37. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, Xu W, Harmon S, Giles JR, Wenz B, et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature. 2017;545(7652):60–5.
38. Rock KL, Reits E, Neefjes J. Present yourself! by MHC Class I and MHC Class II molecules. Trends Immunol. 2016;37(11):724–37.
39. Tariq F, Khan W, Ahmad W, Riaz SK, Khan M, Sherwani S, Haque S, Malik MFA, Iftikhar MJ, Khan S, et al. Effect of MHC linked 7-gene signature on delayed hepatocellular carcinoma recurrence. J Pers Med. 2021;11(11):96.
40. Mai H, Chen J, Chen H, Liu Z, Huang G, Wang J, Xiao Q, Ren W, Zhou B, Hou J, et al. Fine mapping of the MHC region identifies novel variants associated with HBV-related hepatocellular carcinoma in Han Chinese. J Hepatocell Carcinoma. 2021;8:951–61.
41. Chen W, Cai MY, Wei DP, Wang X. Pivotal molecules of MHC I pathway in human primary hepatocellular carcinoma. World J Gastroenterol. 2005;11(21):3297–9.
42. Hong JY, Cho HJ, Sa JK, Liu X, Ha SY, Lee T, Kim H, Kang W, Sinn DH, Gwak GY, et al. Hepatocellular carcinoma patients with high circulating cytotoxic T cells and intra-tumoral immune signature benefit from pembrolizumab: results from a single-arm phase 2 trial. Genome Med. 2022;14(1):1.
43. Hsu CL, Ou DL, Bai LY, Chen CW, Lin L, Huang SF, Cheng AL, Jeng YM, Hsu C. Exploring markers of exhausted CD8 T cells to predict response to immune checkpoint inhibitor therapy for hepatocellular carcinoma. Liver Cancer. 2021;10(4):346–59.

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