Independent prognostic value of ISCU and a multi-gene signature of cuproptosis-related genes in glioblastoma multiforme: a TCGA-based study

Author:Jiantong Zheng
ORCID: 0009-0002-4416-9310
Email:xipeier5@outlook.com
Affiliation: Sun Yat-sen University, School of Future Biomedical Sciences, Guangzhou, China

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Abstract
Background
Cuproptosis is a recently identified copper-dependent form of regulated cell death, but its clinical significance in glioblastoma multiforme (GBM) remains poorly understood. This study aimed to systematically characterize the expression and prognostic value of cuproptosis-related genes (CRGs) in GBM using data from The Cancer Genome Atlas (TCGA).
Methods
RNA-seq and corresponding clinical data of 194 GBM patients were downloaded from TCGA. Differentially expressed CRGs were identified using the limma-voom pipeline ($|\log_2 FC|>1$, FDR<0.05). Kaplan-Meier survival analysis and multivariate Cox regression were performed to evaluate the prognostic significance of individual CRGs. A LASSO-Cox regression model with 10-fold cross-validation was constructed to develop a multi-gene risk score, whose predictive accuracy was assessed by time-dependent ROC at 1/3-year, 2/3-year and 1-year. All analyses were computational (dry-lab) and focused on biomarker discovery.
Results
Among 28 CRGs, 26 were differentially expressed in GBM. High expression of ISCU was significantly associated with worse overall survival (log-rank p = 0.0354). After adjusting for age and sex, ISCU remained an independent adverse prognostic factor (HR = 1.61, 95% CI 1.21–2.16, p = 0.0013). A LASSO-Cox risk score incorporating ten other CRGs – including key genes such as SLC31A1, ATP7A and MT2A – achieved time-dependent AUC values of 0.678, 0.673 and 0.678 at 1/3-year, 2/3-year and 1-year, respectively. Notably, ISCU was not selected in this multi-gene signature, indicating that its prognostic information is partially shared with other functionally related CRGs involved in copper transport and metal detoxification. Consequently, the multi-gene score provides complementary prognostic value, whereas ISCU alone remains a practical single-gene biomarker.
Conclusion
ISCU is a novel independent prognostic biomarker in GBM, with high expression predicting poor survival. The multi-gene risk score offers additional predictive information, and both approaches together may facilitate risk stratification in GBM patients. Nevertheless, both approaches are dry-lab based and require further validation in independent cohorts.

Keywords: Glioblastoma multiforme; Cuproptosis; ISCU; Prognostic biomarker; TCGA; Bioinformatics; Iron-sulfur cluster

Introduction

Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor in adults, accounting for nearly half of all malignant gliomas. Despite aggressive multimodal treatment including maximal surgical resection, radiotherapy and concomitant temozolomide chemotherapy, the median overall survival remains dismal at approximately 12–15 months[1,2]. The inevitable tumor recurrence and acquired resistance to standard therapies underscore an urgent need for reliable prognostic biomarkers and novel therapeutic strategies.

In recent years, reprogrammed cell death pathways have gained increasing attention as potential targets for cancer therapy. Among them, cuproptosis was recently identified as a copper-dependent form of regulated cell death distinct from apoptosis, necroptosis, ferroptosis and pyroptosis[3]. Mechanistically, excess copper directly binds to lipoylated components of the tricarboxylic acid (TCA) cycle, particularly lipoylated dihydrolipoamide S-acetyltransferase (DLAT), leading to aggregation of these proteins, subsequent loss of iron-sulfur cluster proteins, and ultimately proteotoxic stress and cell death[3,4]. This unique mode of cell death has been implicated in various tumors, including lung, breast, and colorectal cancers, where cuproptosis-related genes (CRGs) have shown prognostic and predictive value[5–8].

In glioma, a few studies have attempted to construct prognostic signatures based on CRGs, but most of them have focused on low-grade glioma (LGG) or mixed-grade cohorts, leaving the role of cuproptosis in pure GBM largely unexplored[9,10]. Moreover, these studies primarily adopted multi-gene models, while the individual prognostic significance of specific CRGs in GBM has not been systematically evaluated. Given that GBM exhibits distinct molecular and clinical features from LGG, a dedicated analysis restricted to GBM is necessary to identify truly relevant biomarkers.

Among all CRGs, ISCU (iron-sulfur cluster assembly enzyme) plays an indispensable role in maintaining mitochondrial respiratory chain function, as it is required for the assembly of iron-sulfur clusters in complexes I, II and III[11]. Dysregulation of iron-sulfur cluster biogenesis has been linked to impaired oxidative phosphorylation, altered energy metabolism, and increased oxidative stress—processes that are closely associated with tumor progression and therapeutic resistance.Previous studies have linked ISCU to hypoxia adaptation and metabolic reprogramming in cancer cells[12], and its expression has been associated with prognosis in several tumor types. Nevertheless, whether ISCU carries independent prognostic value specifically in GBM has remained unexplored.

In this study, we systematically analyzed the expression patterns and prognostic significance of 28 CRGs using the TCGA-GBM cohort. We identified ISCU as an independent adverse prognostic factor in GBM. Furthermore, we constructed a multi-gene risk score based on other CRGs that showed moderate predictive accuracy, providing complementary information to the single-gene ISCU signature. Our findings establish ISCU as a practical and robust single-gene biomarker and offer new insights into the cuproptosis landscape in GBM.

Methods

RNA-seq data (STAR-Counts workflow) and corresponding clinical information of glioblastoma multiforme (GBM) patients were downloaded from The Cancer Genome Atlas (TCGA) using the TCGAbiolinks R package. Samples with incomplete survival information were excluded, leaving 194 GBM patients with complete overall survival data for analysis[13]. Gene expression levels were log2-transformed after adding a pseudo-count of 1.

A total of 28 cuproptosis-related genes (CRGs) were compiled from previous literature. Differentially expressed CRGs between GBM and normal brain tissues were identified using the limma-voom pipeline[14]. Genes with $|\log_2 \text{fold change}| > 1$ and false discovery rate (FDR) < 0.05 were considered significantly differentially expressed.

For each CRG, patients were divided into high- and low-expression groups according to the median expression level. Overall survival curves were plotted using the Kaplan-Meier method and compared by log-rank tests[15]. To evaluate independent prognostic value, multivariate Cox regression models were constructed including the gene expression group, age and sex as covariates. Hazard ratios (HR) with 95% confidence intervals (CI) were calculated.

To explore a multi-gene risk score, candidate genes were preselected by univariate Cox regression ($p < 0.2$). LASSO-Cox regression with 10-fold cross-validation was then applied to select the optimal gene subset and estimate regression coefficients[16]. The risk score for each patient was calculated as the linear combination of the expression levels of the selected genes weighted by their LASSO coefficients. Time-dependent receiver operating characteristic (ROC) curves were generated to assess the predictive accuracy of the risk score at 1/3-year, 2/3-year and 1-year.

All statistical analyses were performed using R software. The “survival” and “survminer” packages were used for survival analysis, “glmnet” for LASSO-Cox regression, and “timeROC” for time-dependent ROC curves[17]. A two-sided $p < 0.05$ was considered statistically significant unless otherwise specified.

During the preparation of this work, we used DeepSeek to improve the clarity and structure of the text and to assist with revising the content based on feedback. After using this service, we reviewed and edited the content as needed and take full responsibility for the content of the published article.

Results

Differential expression of cuproptosis-related genes in GBM

A total of 28 cuproptosis-related genes (CRGs) were examined in the TCGA-GBM cohort. Compared with normal brain tissues, 26 of the 28 CRGs were significantly differentially expressed in GBM ($|\log_2 FC|>1$, FDR<0.05). Among these, five genes showed the most pronounced changes: GLS (downregulated), SLC31A1 (upregulated), ISCU (downregulated), ATP7B (downregulated) and ATP7A (upregulated). A volcano plot illustrating the expression distribution of all CRGs is shown in **Figure 1**.

ISCU is an independent prognostic factor for overall survival

Based on the median expression level of ISCU, the 194 GBM patients were divided into high- and low-expression groups. Kaplan-Meier analysis revealed that patients with high ISCU expression had significantly shorter overall survival compared with those with low expression (log-rank $p = 0.0354$; **Figure 2A**). The median survival time of the high-expression group was considerably lower than that of the low-expression group, indicating a strong association between ISCU upregulation and poor prognosis.

To determine whether ISCU provides independent prognostic information beyond established clinical factors, we performed multivariate Cox regression including age and sex as covariates. High ISCU expression remained significantly associated with worse overall survival, with a hazard ratio (HR) of 1.61 (95% CI 1.21–2.16, $p = 0.0013$; **Figure 2B**). Age (HR = 1.04, 95% CI 1.02–1.05, $p < 0.001$) and male sex (HR = 1.47, 95% CI 1.09–1.99, $p = 0.012$) were also independently associated with shorter survival (**Table 1**). These results demonstrate that ISCU is an independent adverse prognostic biomarker in GBM.

Figure 1

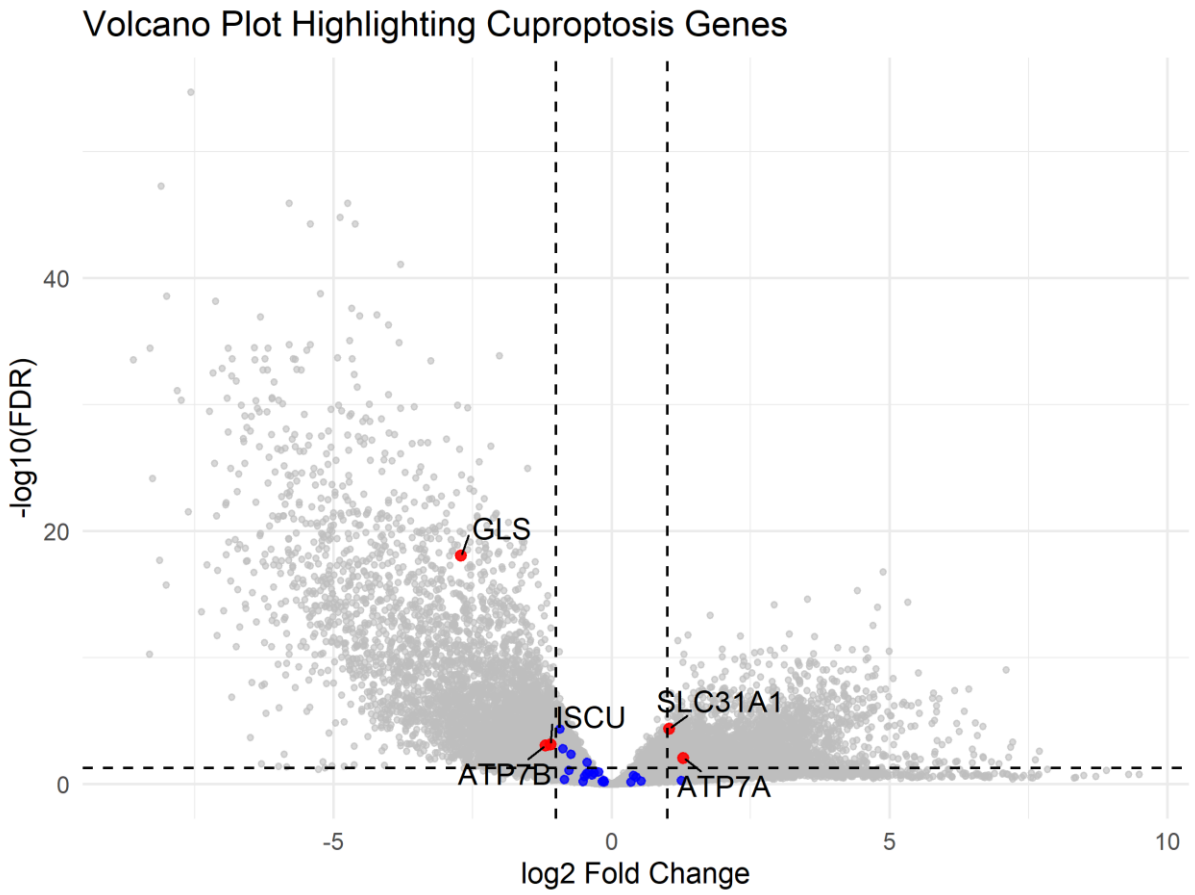


Figure 2A

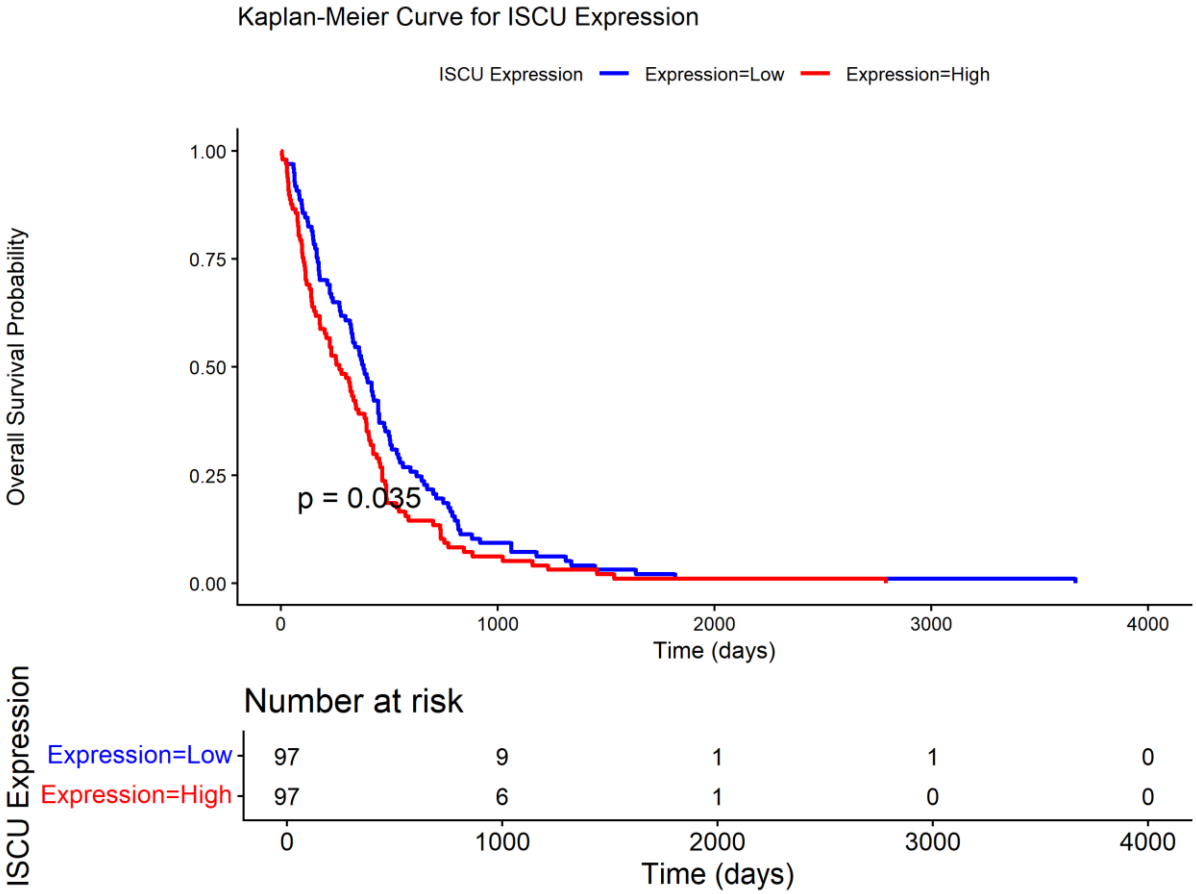


Figure 2B

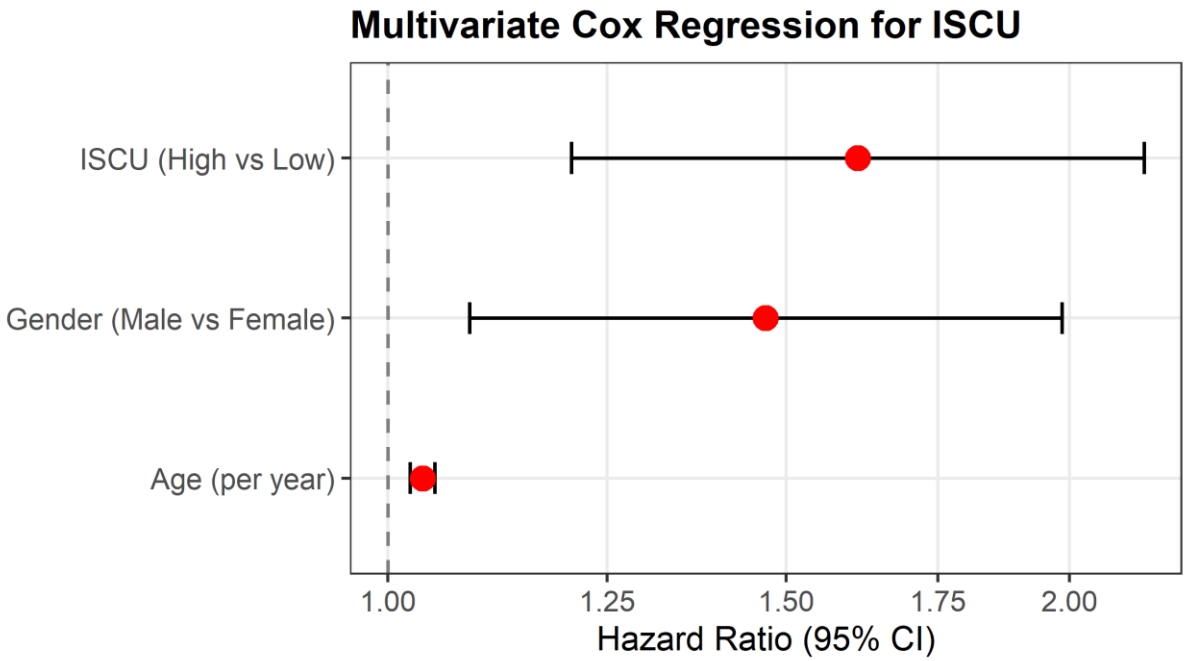


Table 1

Gene	Variable	HR	HR_low	HR_high	p
SLC31A1	gene_groupHigh	1.238256	0.928241	1.651809	0.146077
SLC31A1	age	1.030774	1.018366	1.043333	9.32E-07
SLC31A1	genderMALE	1.408962	1.042419	1.904391	0.025731
ISCU	gene_groupHigh	1.612928	1.205285	2.15844	0.001299
ISCU	age	1.036109	1.023169	1.049213	3.16E-08
ISCU	genderMALE	1.468792	1.086793	1.985063	0.012364

SLC31A1 shows only marginal univariate prognostic value

SLC31A1, which encodes a high-affinity copper importer, was significantly upregulated in GBM tissues ($log_2FC = 1.04$, FDR < 0.05). Univariate survival analysis showed a borderline association between high SLC31A1 expression and worse overall survival (log-rank p = 0.0407; **Supplementary Figure S1A**). However, after adjusting for age and sex in multivariate Cox regression, the association became non-significant (HR = 1.24, 95% CI 0.93–1.65, p = 0.1461; **Supplementary Figure S1B**). Thus, SLC31A1 is not an independent prognostic factor in GBM.

Multi-gene risk score based on other cuproptosis-related genes

To explore whether combining multiple CRGs could improve predictive performance, we performed LASSO-Cox regression on all 28 CRGs. Univariate Cox screening ($p < 0.2$) preselected candidate genes, and the optimal penalty parameter was determined by 10-fold cross-validation. The final LASSO model selected 10 genes: LIAS, PDHB, SLC31A1, ATP7A, SCO1, MT2A, MT3, FDX2, DLST and CDKN2A[18]. A risk score was calculated for each patient as the linear combination of the expression levels of these genes weighted by their LASSO coefficients. Notably, ISCU was not included in this multi-gene signature.

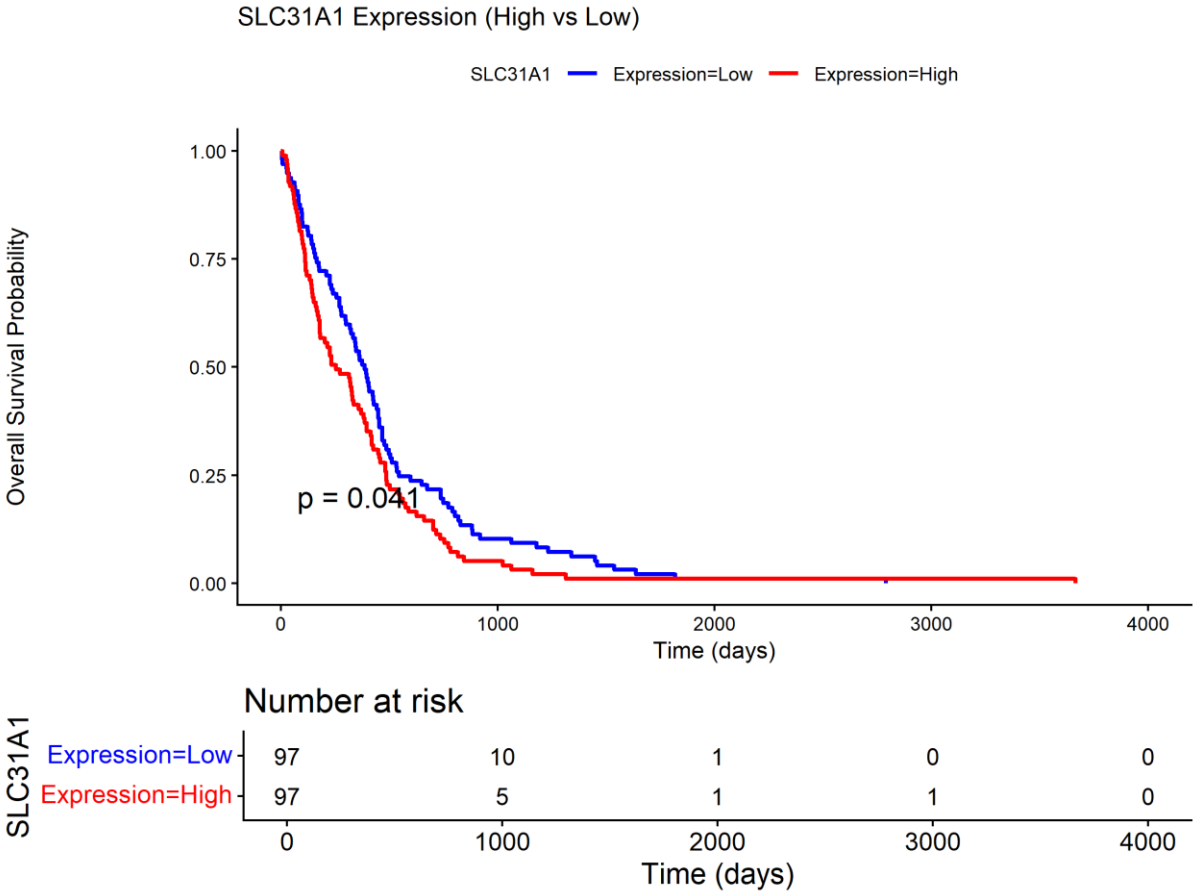
Time-dependent ROC curves were generated to evaluate the predictive accuracy of the risk score at 1/3-year, 2/3-year and 1-year. The AUC values were 0.678, 0.673 and 0.678, respectively (**Figure 3**). Although the multi-gene score exhibited moderate discriminative ability, it did not outperform the simplicity and clinical utility of single-gene ISCU.

To further illustrate the relationship between ISCU expression and the multi-gene risk score, we compared the distribution of risk scores between ISCU high- and low-expression groups. Patients with high ISCU expression showed significantly higher risk scores than those with low ISCU expression (**Figure 4**), but ISCU was not selected in the LASSO model. The absence of ISCU in the LASSO model suggests that the prognostic information carried by ISCU is partially shared with other CRGs that are functionally connected, such as those involved in copper transport (SLC31A1, ATP7A), mitochondrial metabolism (LIAS, PDHB, FDX2) and metal detoxification (MT2A, MT3). Therefore, the multi-gene risk score provides complementary predictive information from a pathway perspective, whereas ISCU alone remains a practical and straightforward single-gene biomarker.

Discussion

In this study, we comprehensively evaluated the expression and prognostic significance of cuproptosis-related genes (CRGs) in glioblastoma multiforme (GBM) using the TCGA-GBM cohort. Our main findings are threefold: (i) 26 of 28 CRGs were differentially expressed in GBM, indicating widespread dysregulation of the cuproptosis pathway; (ii) ISCU was identified as an independent adverse prognostic factor after adjusting for age and sex (HR = 1.61, 95% CI 1.21–2.16, p = 0.0013); and (iii) a multi-gene risk score based on ten other CRGs (excluding ISCU) achieved moderate predictive accuracy (time-dependent AUC ≈ 0.68). These results collectively demonstrate that ISCU is a practical single-gene biomarker, while a multi-gene signature provides complementary information from a pathway perspective.

Supplementary Figure S1A



Supplementary Figure S1B

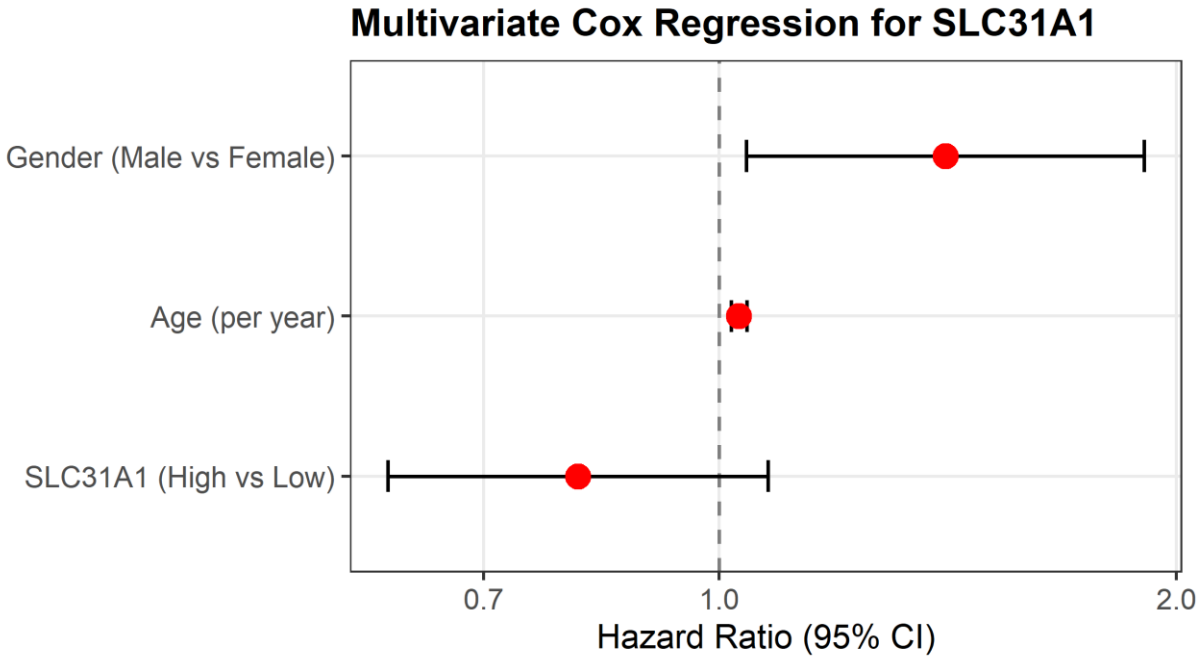


Figure 3

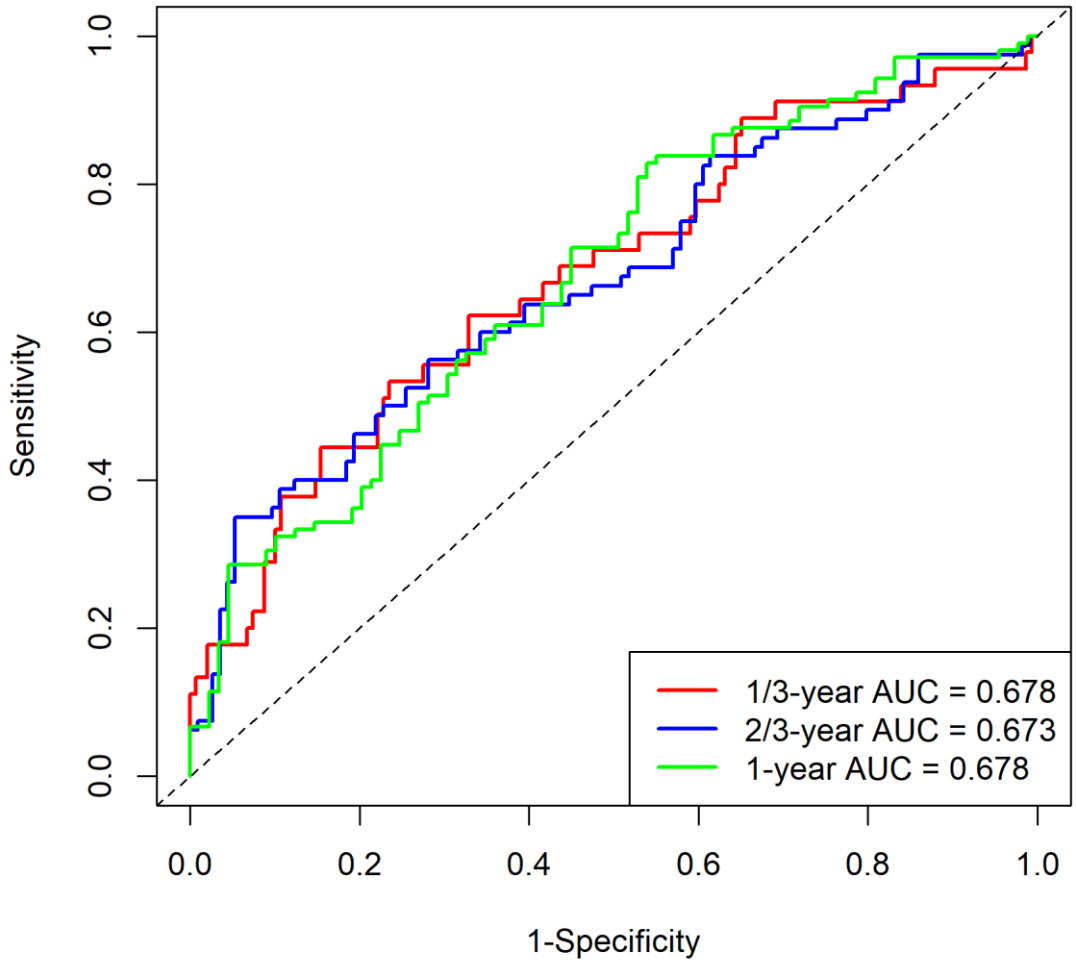
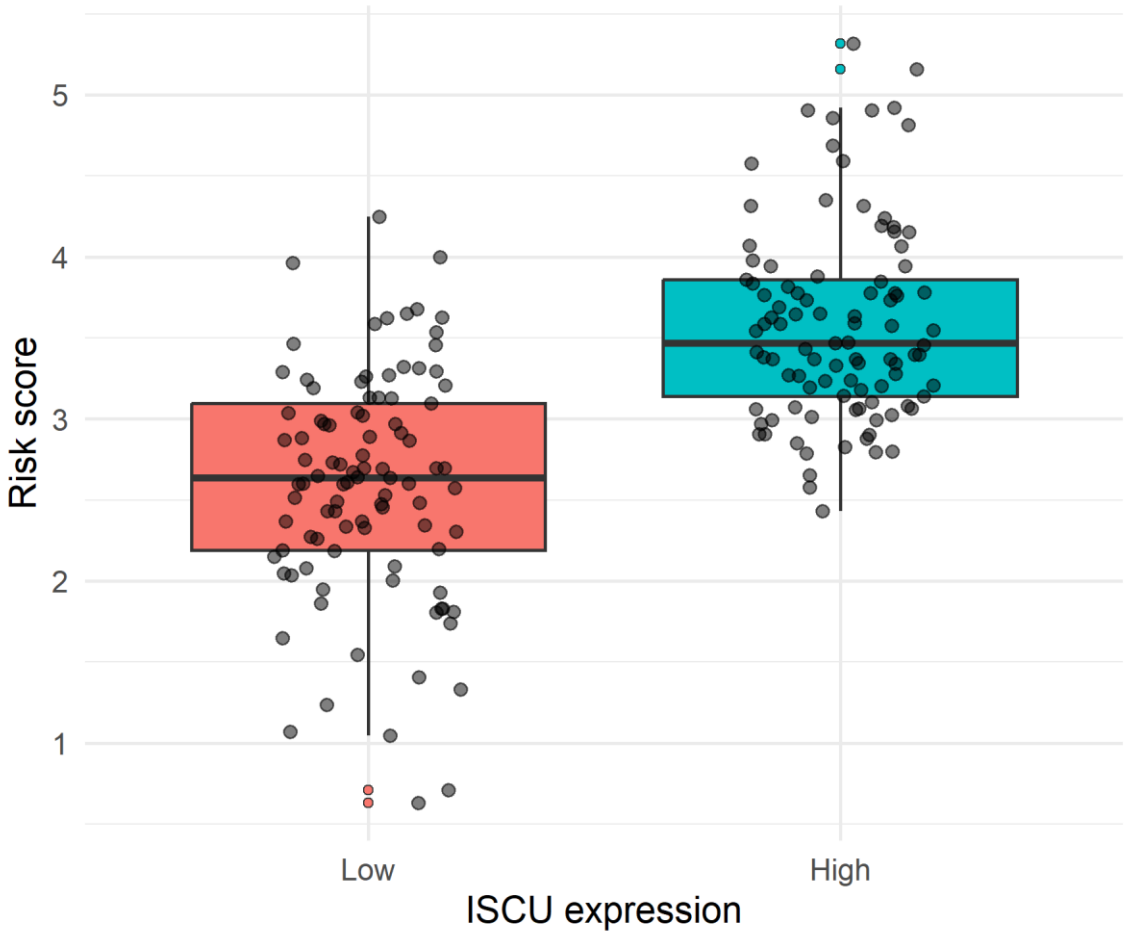


Figure 4



ISCU as an independent prognostic biomarker in GBM

Mechanistically, ISCU-mediated iron-sulfur cluster assembly is critical for maintaining mitochondrial electron transport chain function. Its high expression may thus enhance oxidative phosphorylation and fuel rapid tumor proliferation, while also contributing to resistance against oxidative stress and chemotherapy.

The most important contribution of this study is the identification of ISCU as an independent prognostic factor in GBM. ISCU encodes a key component of the mitochondrial iron-sulfur cluster assembly machinery, which is essential for the activity of respiratory chain complexes I, II and III and for proper cellular metabolism[11,12]. Dysregulation of iron-sulfur cluster biogenesis has been implicated in tumor progression, metabolic reprogramming and resistance to therapy[19,20]. Our finding that high ISCU expression predicts poor survival in GBM is consistent with a recent study showing that ISCU is a core gene in a ferroptosis-related prognostic signature for glioma[21]. However, that study focused on mixed-grade gliomas, whereas our analysis was restricted to pure GBM, providing a more homogeneous and clinically relevant context.

Notably, the prognostic value of ISCU remained significant after adjusting for age and sex, indicating that its effect is not simply a reflection of demographic differences. Moreover, the hazard ratio of 1.61 is comparable to or even stronger than that of age (HR = 1.04) and male sex (HR = 1.47), highlighting its potential clinical relevance. Although the exact mechanism by which ISCU influences GBM prognosis remains to be elucidated, we speculate that high ISCU expression may enhance mitochondrial oxidative phosphorylation, promote tumor cell proliferation, or confer resistance to apoptosis and chemotherapy. Future functional studies are warranted to test these hypotheses.

Why was ISCU not selected in the LASSO-derived multi-gene risk score?

A seemingly paradoxical finding is that although ISCU itself was not selected in the LASSO-Cox model, the risk score was significantly higher in the ISCU high-expression group (data not shown). This suggests that the prognostic information carried by ISCU is largely shared with other CRGs that were selected, such as SLC31A1 (copper importer), ATP7A (copper exporter), LIAS and PDHB (mitochondrial metabolism), and MT2A/MT3 (metal detoxification). Functional redundancy within the cuproptosis pathway likely allows these genes to collectively capture the same biological signal as ISCU, making ISCU's individual contribution non-significant in a multi-gene model that already includes its functional partners. This phenomenon is common in LASSO regression when predictors are highly correlated, and it does not diminish the independent value of ISCU as a single-gene biomarker. In clinical practice, a single-gene test is simpler, cheaper and easier to interpret than a multi-gene signature. Therefore, we propose that ISCU alone can serve as a frontline biomarker for risk stratification in GBM patients, while the multi-gene score may be reserved for research settings or for patients with equivocal ISCU expression.

Comparison with previous cuproptosis-related prognostic models

Several studies have constructed prognostic signatures based on CRGs in gliomas. For example, Xie et al. identified FDX1 as an immune-related biomarker in glioma[22], and Zhao et al. developed a cuproptosis-based model for low-grade glioma[9]. However, most of these studies

either used mixed-grade cohorts or focused on multi-gene signatures without systematically evaluating individual CRGs in pure GBM. Our study fills this gap by rigorously examining the independent prognostic value of each CRG and by directly comparing the single-gene (ISCU) and multi-gene approaches. The AUC values of our multi-gene risk score are comparable to those reported in previous studies using similar methodologies[9,23]. Although a multi-gene score may provide slightly better discrimination than a single gene, the improvement is modest and comes at the cost of increased complexity.

SLC31A1: from borderline significance to non-independence

SLC31A1 (copper importer) was significantly upregulated in GBM and showed a borderline association with survival in univariate analysis. However, after adjusting for age and sex, it became non-significant. This indicates that the apparent prognostic effect of SLC31A1 may be partly confounded by age or gender, or that its effect size is too small to be detected after controlling for other factors. Interestingly, SLC31A1 was selected in the LASSO model, suggesting that it contributes to the multi-gene score through interactions with other genes. This again highlights the difference between single-gene independence and multi-gene complementarity.

Limitations

Several limitations should be acknowledged. First, all analyses were based on a single public cohort (TCGA-GBM). Although the sample size is adequate (n = 194), external validation in an independent GBM cohort is still needed. Unfortunately, most publicly available GBM datasets either lack complete survival data or have very limited normal controls, making direct validation challenging. Second, the retrospective nature of TCGA data may introduce selection bias. Third, the AUC values of the multi-gene risk score (around 0.68) indicate only moderate predictive discrimination, which is expected for a single-pathway signature. Fourth, the biological mechanisms underlying ISCU's prognostic role have not been experimentally verified. Future studies should investigate whether ISCU directly promotes GBM cell proliferation, invasion, or chemoresistance. Fifth, we did not incorporate other clinically relevant variables such as MGMT promoter methylation or IDH mutation status because these data were not available for all samples in our extracted dataset.

Clinical implications and future directions

Despite these limitations, our findings have potential clinical relevance. ISCU is a single gene that can be easily measured by qPCR or RNA-seq, and its high expression robustly identifies patients with poor prognosis. This could help clinicians stratify GBM patients at diagnosis and tailor more aggressive follow-up or treatment for high-risk individuals[24]. The multi-gene risk score, while more complex, may provide additional precision and could be used in research settings or for deeper mechanistic understanding.

Future work should include external validation using independent cohorts (e.g., CGGA, GEO) once sufficient GBM samples with survival data become available. Functional experiments, such as ISCU knockdown or overexpression in GBM cell lines, are needed to confirm its causal role in tumor progression and to explore potential therapeutic targeting[25]. Additionally, investigating the relationship between ISCU expression and standard therapies (e.g., temozolomide) could provide insight into its role in treatment resistance. All these findings are

derived from computational (dry-lab) analyses and warrant further experimental and clinical validation.

Conclusion

In conclusion, this study demonstrates that ISCU is an independent adverse prognostic biomarker in GBM. A complementary multi-gene risk score based on other CRGs provides moderate predictive accuracy. ISCU alone is a practical, reliable and easily measurable marker for risk stratification, and its integration with multi-gene signatures may further improve prognostic assessment in GBM patients. Our findings highlight the importance of cuproptosis in GBM biology and open new avenues for biomarker-driven clinical management.

Data Availability

The data analyzed in this study are publicly available from The Cancer Genome Atlas (TCGA) database. TCGA-GBM RNA-seq and clinical data can be accessed via the TCGA Data Portal (https://portal.gdc.cancer.gov/). The computational code used for analysis is available from the corresponding author upon reasonable request.

Disclosure

The content presented here is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or any other funding agency.

Conflicts of Interest

The authors declare no conflicts of interest.

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Figure 1. Volcano plot of cuproptosis-related genes in GBM.

Differentially expressed genes between GBM and normal brain tissues are shown as gray dots (not significant), blue dots (cuproptosis-related genes with $|\log_2 FC|>1$ but FDR≥0.05), and red dots (significant cuproptosis-related genes with $|\log_2 FC|>1$ and FDR<0.05). Dashed vertical lines indicate $|\log_2 FC| = 1$; dashed horizontal line indicates FDR = 0.05 ($-\log_{10}(0.05) \approx 1.30$). Gene labels are shown for the five significant genes: GLS, SLC31A1, ISCU, ATP7B and ATP7A.

Figure 2. Prognostic significance of ISCU in GBM.

(A) Kaplan-Meier curves for overall survival according to ISCU expression (high vs low, split by median). P value was calculated by log-rank test. Numbers of patients at risk are shown below the x-axis. (B) Forest plot of multivariate Cox regression analysis for ISCU, age and sex. Hazard ratios (HR) with 95% confidence intervals (CI) are plotted. The vertical dashed line indicates HR = 1.

Figure 3. Time-dependent ROC curves of the multi-gene risk score.

The risk score was constructed using LASSO-Cox regression based on ten cuproptosis-related genes (excluding ISCU). ROC curves are shown for 1/3-year, 2/3-year and 1-year survival. Area under the curve (AUC) values are indicated in the legend.

Figure 4. Boxplot of risk scores by ISCU expression groups.

Risk scores derived from the multi-gene signature were compared between patients with high (n = 97) and low (n = 97) ISCU expression (median split). The box represents the interquartile range, the horizontal line the median, and whiskers extend to 1.5× IQR. P value from

Wilcoxon rank-sum test is shown.

Supplementary Figure S1. Survival analysis for SLC31A1.

(A) Kaplan-Meier curves for overall survival according to SLC31A1 expression (high vs low, median split). Log-rank p value is shown. (B) Forest plot of multivariate Cox regression for SLC31A1, age and sex. HR and 95% CI are shown.

Table 1. Multivariable Cox regression analysis for overall survival in GBM patients

Note: HR, hazard ratio; CI, confidence interval. Age was treated as a continuous variable; gender was binary with male as reference.