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Expression and prognostic value of ferritinophagy-related NCOA4 gene in low-grade glioma: integration of bioinformatics and experimental validation

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Abstract

Background Low-grade glioma (LGG) is a primary brain tumor with relatively low malignancy. NCOA4 is a key regulator of ferritinophagy-related processes and is involved in the occurrence and development of many cancers. However, the role of NCOA4 in LGG remains poorly understood.

Methods This study comprehensively analyzed several mainstream bioinformatics databases to explore the expression, diagnostic efficacy, clinical pathological features, immune infiltration, prognostic value, and biological functions of NCOA4 in LGG. Immunohistochemistry experiments were conducted using LGG tissue samples collected from our hospital to validate the bioinformatics analysis results.

Results NCOA4 expression was significantly elevated in LGG ($p < 0.05$), with an Area Under the Receiver Operating Characteristic Curve (AUC) of 0.973, suggesting it as a potential diagnostic marker. High NCOA4 expression was associated with younger age (21–40 years), lower malignancy (oligodendroglioma), and better prognosis (IDHmut-non-codel and IDHmut-codel subtypes) (all $p < 0.05$) in LGG. Kaplan-Meier survival curves from three databases showed that high NCOA4-expressing LGG patients had better prognosis (all $p < 0.05$). NCOA4 correlated weakly with B cells, CD8 + T cells, macrophages, and dendritic cells infiltration (all with correlation coefficients $r < 0.3$, and $p < 0.05$) in LGG. Multivariate Cox regression identified NCOA4, age, CD8 T cells, and macrophages as LGG independent prognostic factors (all $p < 0.05$). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses indicated that NCOA4's primary function in LGG is related to autophagy processes (all $p < 0.05$).

Conclusion Our findings suggest that NCOA4 could be a potential prognostic marker and therapeutic target in LGG.

Keywords Low-grade glioma, NCOA4, Ferroptosis, Prognosis, Biomarker

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Introduction

Low-grade gliomas (LGG), classified as WHO grade 2 tumors, and high-grade gliomas (HGG), classified as WHO grade 3–4 tumors, are primary malignant brain tumors [1]. However, LGG exhibits significant intrinsic heterogeneity in their biological behavior compared to HGG [2]. In theory, almost all LGG will eventually progress to HGG [3]. However, the time for LGG to evolve into HGG can be very short or very long. This uncertainty in the timeline of evolution contributes to the uncertainty in LGG prognosis. In addition, due to the poor prognosis of HGG, the research focus has primarily been on HGG, lacking exploration of clinical variables for predicting and evaluating LGG [3]. Therefore, identifying new biomarkers for predicting LGG prognosis and guiding personalized treatment is highly significant.

Nuclear receptor coactivator 4 (NCOA4), an iron-autophagy related protein, is considered a key molecule in promoting ferroptosis in various cancer cells [4]. NCOA4 is a selective cargo transporter for ferritin during autophagy, and its C-terminal domain binds to ferritin and delivers it to autophagosomes, thus promoting ferroptosis [5]. Ferroptosis has a dual role in tumor growth [6]. For example, inducing ferroptosis in pancreatic cancer cells can inhibit cancer metastasis [7]. Conversely, inhibiting ferroptosis in hepatocellular carcinoma accelerates tumor growth and metastasis [8]. Therefore, it is essential to understand the relationship between ferroptosis status and tumor progression in a specific tumor before attempting to use ferroptosis inducers or inhibitors as treatments. Studies have shown that NCOA4 is dysregulated in various types of cancer and is closely related to prognosis. For example, a decrease in NCOA4 expression in ovarian cancer and renal clear cell carcinoma is associated with poor prognosis [9, 10]. However, the expression and prognostic value of NCOA4 in LGG remains unclear.

In this study, we utilized multiple large public databases to explore the expression, diagnostic value, clinical pathological features, immune infiltration, prognostic value, and biological functions of NCOA4 in LGG. We also validated the bioinformatics findings of NCOA4 in LGG by examining the NCOA4 immunohistochemistry results from LGG patients in our hospital. Our data highlights the potential application value and mechanism of NCOA4 in the prognosis and treatment of LGG.

Materials and methods

Study design

Based on the potential of the ferritinophagy-related NCOA4 gene in promoting tumor cell ferroptosis, we proposed a research hypothesis that NCOA4 could serve as a novel diagnostic and prognostic biomarker in LGG. Therefore, in this study, we utilized multiple publicly available databases to mutually validate our research hypothesis and conducted experimental validations as well.

GEPIA2

GEPIA2 (http://gepia2.cancer-pku.cn/#index) is an interactive web server that utilizes standard processing pipelines to analyze RNA sequencing expression data from thousands of cancer tissues and normal tissues from the TCGA and GTEx projects [11]. In this study, we utilized the "Expression DIY" module of GEPIA2 to compare the mRNA levels of NCOA4 between LGG tumor tissues and normal glial tissues. The results were presented using scatter plots and box plots. Besides, we used the "Survival Analysis" module of GEPIA2 to generate Kaplan-Meier curves to observe whether NCOA4 has an impact on the survival of LGG patients. Furthermore, we used the "Similar Genes Detection" module of GEPIA2 to obtain the top 100 genes correlated with NCOA4 in LGG tumor tissues, to explore the biological functions of NCOA4 in LGG.

UCSC Xena

UCSC Xena (https://xena.ucsc.edu/) is a platform for downloading large public datasets, which includes public data from multiple databases such as TCGA and GTEx [12]. To evaluate the diagnostic efficacy of NCOA4 in LGG, we obtained corresponding LGG data from TCGA and normal tissue data from GTEx from UCSC Xena. The RNAseq data in FPKM format from TCGA and GTEx, processed through the Toil pipeline, were successfully extracted [13]. This resulted in RNAseq data from LGG tissues in TCGA ($n = 523$) and corresponding normal tissue RNAseq data in GTEx ($n = 1152$). Next, in R software (version 4.2.1), we used the pROC package (version 1.18.0) to perform Receiver Operating Characteristic (ROC) curve analysis on the extracted RNAseq data. The results were visualized using ggplot2 (version 3.3.6) to assess the diagnostic value of NCOA4 for LGG. The diagnostic efficacy was evaluated using the Area Under the Curve (AUC) of the ROC curve, where AUC values generally range between 0.5 and 1. A higher AUC, closer to 1, indicates better diagnostic performance of NCOA4 in diagnosing LGG.

The human protein atlas

The Human Protein Atlas (https://www.proteinatlas.org/) is an interactive online tool consisting of three sub-atlas, including tissue map, cell map and pathological map [14], which can be used to explore the expression of the target protein in different maps. In addition to collecting immunohistochemical maps of normal tissues, the database also collected immunohistochemical maps of 17 major cancer types, including LGG. In this work,

we used immunohistochemical images downloaded from the database to compare the protein expression levels of NCOA4 in LGG tumor tissues and normal glial tissues.

UALCAN

UALCAN (https://ualcan.path.uab.edu/) is a comprehen sive, user-friendly, and interactive web resource for analyzing cancer OMICS data [15]. It provides easy access to publicly available cancer OMICS data, such as TCGA, and allows users to perform in silico validation of potential genes of interest.In our work, we use UALCAN to understand how the mRNA expression level of NCOA4 changes in different clinicopathological parameter Settings, and the results are represented in a box diagram. In addition, we also used the "Survival" section of the database to generate Kaplan-Meier curve and validate the impact of NCOA4 on the survival of LGG patients.

GlioVis

GlioVis (http://gliovis.bioinfo.cnio.es/) is a user-friendly web application for data visualization and analysis to explore brain tumor expression datasets [16]. In our study, we utilized GlioVis to establish the correlation between NCOA4 mRNA expression levels and factors such as patient's race, patient's gender, patient's age, and TP53 mutation status. Additionally, we also analyzed the prognostic significance of NCOA4 mRNA expression in LGG using this database.

TIMER

TIMER (https://cistrome.shinyapps.io/timer/) is a web server for comprehensive analysis of tumor-Infiltrating Immune Cells [17]. In our work, we obtained the scatterplots using the "Gene" module to analyze the correlation between the expression level of NCOA4 and the infiltration of six immune cells in LGG. The association between the expression of the NCOA4 gene and tumor purity was also investigated. In addition, we explored the relationship between NCOA4 expression and several immune cell markers in the TIMER database. Spearman correlation analysis was employed for all these correlation analyses, and the strength of the correlation was represented by the Spearman correlation coefficient (Cor). Generally, if the absolute value of the Spearman correlation coefficient is less than 0.3, it indicates weak correlation between the two variables. If the absolute value of the Spearman correlation coefficient is between 0.3 and 0.5, it represents moderate correlation, while a correlation coefficient greater than 0.5 indicates strong correlation between the two variables. Furthermore, the "Survival" module in the TIMER database allows users to explore the correlation between one or more immune infiltrating cells and the survival time of patients with the target tumor (LGG), and to flexibly adjust multiple covariates in a multivariate Cox proportional hazards model. The covariates include clinical factors (age, sex, race, tumor stage) and the expression of the target gene (NCOA4). After defining all inputs, TIMER outputs the Cox regression results, including the hazard ratio (HR), 95% confidence interval (CI), and statistical significance.

Patients and clinical samples

To validate the bioinformatic analysis results regarding the expression of NCOA4 in LGG, we conducted experimental validation. Brain tissue wax blocks from LGG patients who underwent surgical resection in the Neurosurgery Department of the Affiliated Hospital of Guizhou Medical University were selected for subsequent immunohistochemical detection of NCOA4 expression. Before selecting the wax blocks, the research team established inclusion criteria: (1) LGG patients underwent extended surgical resection of the tumor; (2) Pathological sections clearly diagnosed as LGG; (3) LGG located in the frontal lobe. Exclusion criteria: (1) Patients who received chemotherapy or radiotherapy before surgery; (2) Patients with malignant tumors in other organs; (3) Patients with incomplete tumor resection. Among the brain tissue wax blocks that met the above inclusion and exclusion criteria, wax blocks from 8 LGG patients were randomly selected to make tissue sections for immunohistochemistry. The pathological sections of each LGG patient were tested for NCOA4 protein expression level by immunohistochemistry three times. This study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (IRB-2022-152) and written informed consent was obtained from all patients.

Immunohistochemistry

The paraffin blocks were made into tissue sections, and the expression of the NCOA4 protein in LGG tumor tissue and adjacent non-tumor tissue was detected by the immunohistochemistry staining. Firstly, the tissue sections were baked in a 65℃ oven for 20 min to dissolve the wax, then the tissue sections were put into xylene for dewaxing, and then they were hydrated in different gradients of ethanol. The hydrated tissue sections were then immersed in a citrate buffer solution for antigen retrieval under high temperature and high pressure conditions. After removing non-specific staining with 3% hydrogen peroxide and blocking with goat serum, the Rabbit Anti-NCOA4 antibody (Bioss, diluted 1:100) was added as the primary antibody and incubated overnight at 4 °C. Then, a goat anti-rabbit IgG (HUABIO, diluted 1:100) was used as the secondary antibody for incubation. After secondary antibody incubation, DAB was used for uniform staining at room temperature. Then, the sections

were subjected to hematoxylin staining, ammonia water bluing, dehydration, and sealing. Finally, the immunohistochemical staining results were observed under a microscope and quantitatively analyzed using the FIJI software. The protein expression level of NCOA4 was represented by the mean optical density (MOD) value. MOD is obtained by dividing the integrated optical density (IOD) by the area of the effective target distribution region.

STRING

STRING (https://cn.string-db.org/) is a protein interaction network database based on public databases and literature information [18]. In our study, we imported NCOA4 into the STRING database to construct a protein-protein interaction (PPI) network consisting of NCOA4 and the 10 most highly related interacting proteins. This PPI network provides information on the interactions between NCOA4 and these 10 proteins. In the PPI network, the confidence score for interactions between proteins is a measure of the reliability of these interactions, and an interaction is considered significant when the confidence score is greater than 0.7.

Biological functions of NCOA4 in LGG

To explore the biological functions of NCOA4 in LGG, we utilized the 10 NCOA4-binding proteins identified from the STRING database and determined 100 genes associated with NCOA4 expression using the LGG Tumor expression datasets from the GEPIA2 database. We constructed a gene list that includes NCOA4 and these associated genes. In R software (version 4.2.1), we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses using the clusterProfiler package (version 4.4.4) [19]. Subsequently, we used the ggplot2 package (version 3.3.6) for data visualization to showcase the primary biological functions of NCOA4 in LGG.

Statistical analyses

The difference in MOD values between LGG tumor tissue and adjacent non-tumor tissue in the immunohistochemistry experiment was determined by a Student's t-test. The ggplot2 R package (version 3.3.6) was used to visualize the data. All bioinformatics analysis results and experimental results are considered statistically significant if $p < 0.05$.

Results

Expression and experimental validation of NCOA4 in LGG

To investigate the differential expression of NCOA4 in LGG, we analyzed the mRNA and protein expression levels using various databases. First, according to the results from the GEPIA2 database, the mRNA expression level of NCOA4 was significantly upregulated in LGG tissues compared to normal gliotic tissues ($p < 0.05$) (Fig. 1A, B). Further, to evaluate the diagnostic value of NCOA4 for LGG, we conducted an ROC curve analysis using the TCGA-LGG RNAseq data and GTEx normal tissue RNAseq data downloaded from the UCSC Xena database. The results showed an AUC value of 0.973, indicating a very high diagnostic efficacy of NCOA4 for LGG (Fig. 1C). Subsequently, in the Human Protein Atlas database, we found that the protein expression level of NCOA4 was significantly upregulated in LGG tissues compared to normal gliotic tissues (Fig. 2). These results suggest that the change trends of NCOA4's mRNA and protein expression levels are consistent in LGG. Finally, to validate the results of the above bioinformatics analysis, we quantitatively analyzed the protein expression of NCOA4 in 8 LGG tissues and their paired adjacent normal tissues using immunohistochemistry staining, with the expression levels represented by MOD values. Experimental results showed that the protein expression level of NCOA4 in LGG tissues was significantly higher than in adjacent normal tissues ($p < 0.001$) (Fig. 3), consistent with the bioinformatics analysis results. These findings suggest that NCOA4 may be a potential diagnostic biomarker for LGG.

Relationship between NCOA4 and clinical pathological features of LGG

To comprehensively understand the relationship between NCOA4 and the clinical pathological features of LGG patients, we conducted statistical analyses using two online databases and visualized the results therein. First, we utilized the UALCAN database to analyze the correlation between NCOA4 levels and the race, gender, age, and TP53 mutation status of LGG patients. The results showed that there was no significant difference in NCOA4 expression among different races, genders, or TP53 mutation statuses. However, it is noteworthy that NCOA4 expression levels were significantly higher in LGG patients aged 21–40 compared to those aged 41–60 ($p < 0.01$) and 61–80 ($p < 0.01$) (Fig. 4C). Subsequently, we used the GlioVis database to investigate the relationship between NCOA4 expression and histology, grade, and subtype. In terms of histology, there was no significant difference in NCOA4 expression between astrocytomas and oligoastrocytomas, nor between oligoastrocytomas and oligodendrogliomas. However, NCOA4 expression was significantly higher in oligodendrogliomas compared to astrocytomas ($p < 0.001$) (Fig. 5A). Regarding grade, NCOA4 expression was significantly higher in grade II patients compared to grade III patients ($p < 0.001$) (Fig. 5B). For subtypes, there was no significant difference in NCOA4 expression between IDHmut-non-codel and IDHmut-codel subtypes. However, NCOA4 expression

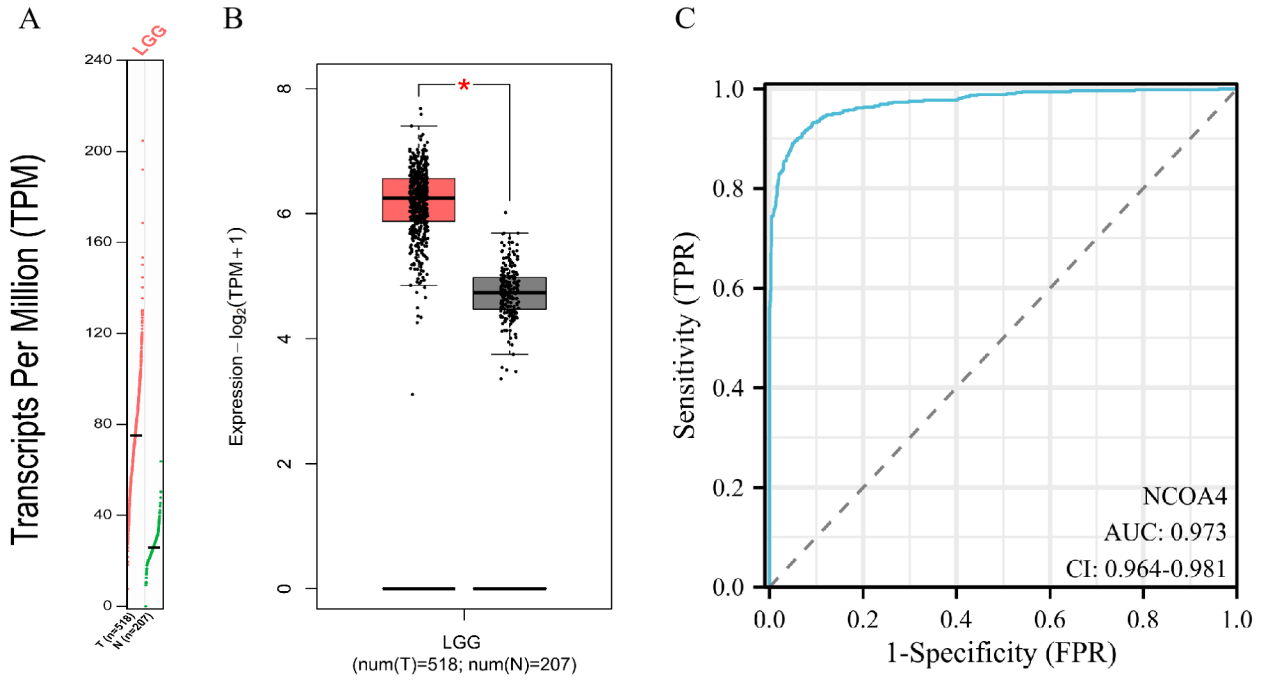


Fig. 1 Expression and diagnostic value of NCOA4 in LGG. (**A**) scatter diagram and (**B**) box plot show that the NCOA4 mRNA expression level is significantly higher in LGG tissues compared to the normal glial tissues from GEPIA2 database, with $*p < 0.05$. (**C**) The ROC curve of NCOA4 in LGG shows an AUC of 0.973, suggesting that NCOA4 can serve as a potential biomarker for LGG

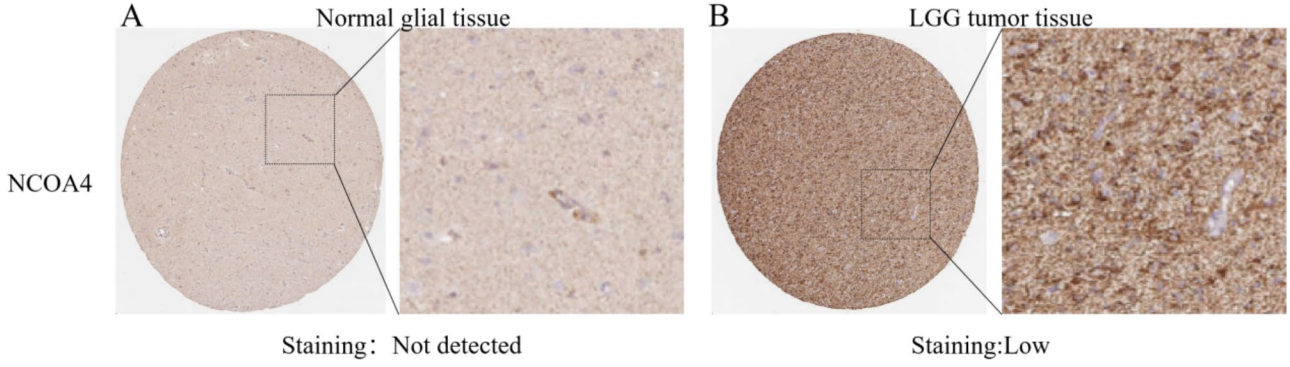


Fig. 2 Representative immunohistochemistry images of NCOA4 from The Human protein Atlas database. (**A**) Normal glial tissue and (**B**) LGG tumor tissue

was significantly higher in LGG patients of the IDHmut-non-codel ($p < 0.001$) and IDHmut-codel ($p < 0.001$) subtypes compared to the IDHwt subtype (Fig. 5C). These findings suggest that NCOA4 expression may be more significant in younger, lower-grade, and better-prognosis LGG patients. Previous studies also support that younger, oligodendroglioma type, grade II, and IDH-mutated LGG patients tend to have a better prognosis [20, 21]. These results provide new insights into understanding the role of NCOA4 in LGG.

Prognostic value of NCOA4 in LGG

The aforementioned analytical results suggest that the expression level of NCOA4 in LGG is elevated, particularly in younger (21–40 years old), lower-grade (oligodendroglioma), and better-prognosis (IDHmut-non-codel and IDHmut-codel subtypes) LGG patients. These observations imply that high expression of NCOA4 could be a marker of favorable prognosis in LGG. To delve deeper into the prognostic value of NCOA4 in LGG patients, the authors conducted independent studies using the GEPIA2 database, the UALCAN database, and the GlioVis database. The results from these three databases corroborate each other, collectively supporting the prognostic value of NCOA4. The study results indicate that Kaplan-Meier survival curves from all three databases show that high expression of NCOA4 is significantly associated with longer survival times in

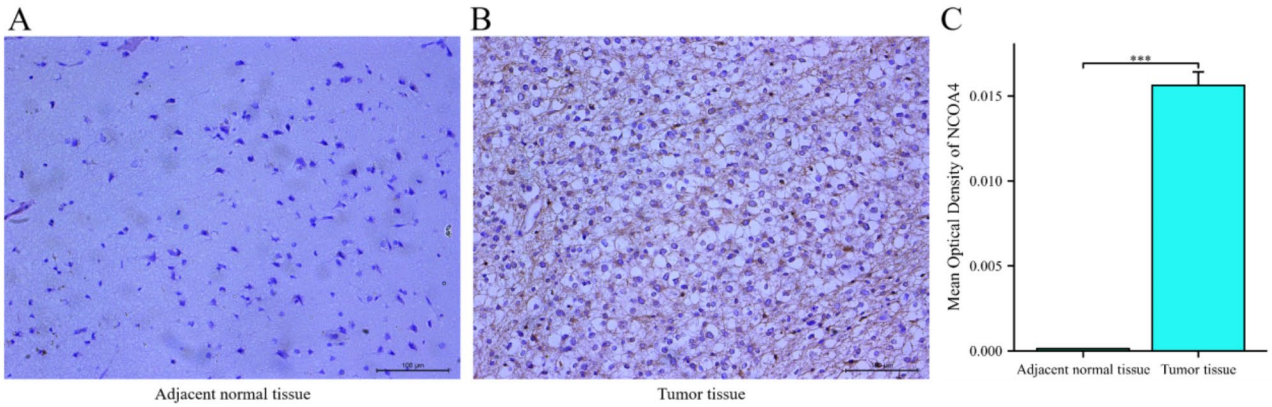


Fig. 3 The Immunohistochemistry experimental results show that the expression level of NCOA4 protein was significantly increased in LGG tumor tissue compared with adjacent normal tissue ($n=8$). (**A**) Representative immunohistochemistry images of NCOA4 in adjacent normal tissue tissue. (**B**) Bar chart of the statistical analysis of NCOA4 protein expression level in LGG tumor tissue. (**C**) Bar chart of the statistical analysis of NCOA4 protein expression level. *** $p<0.001$

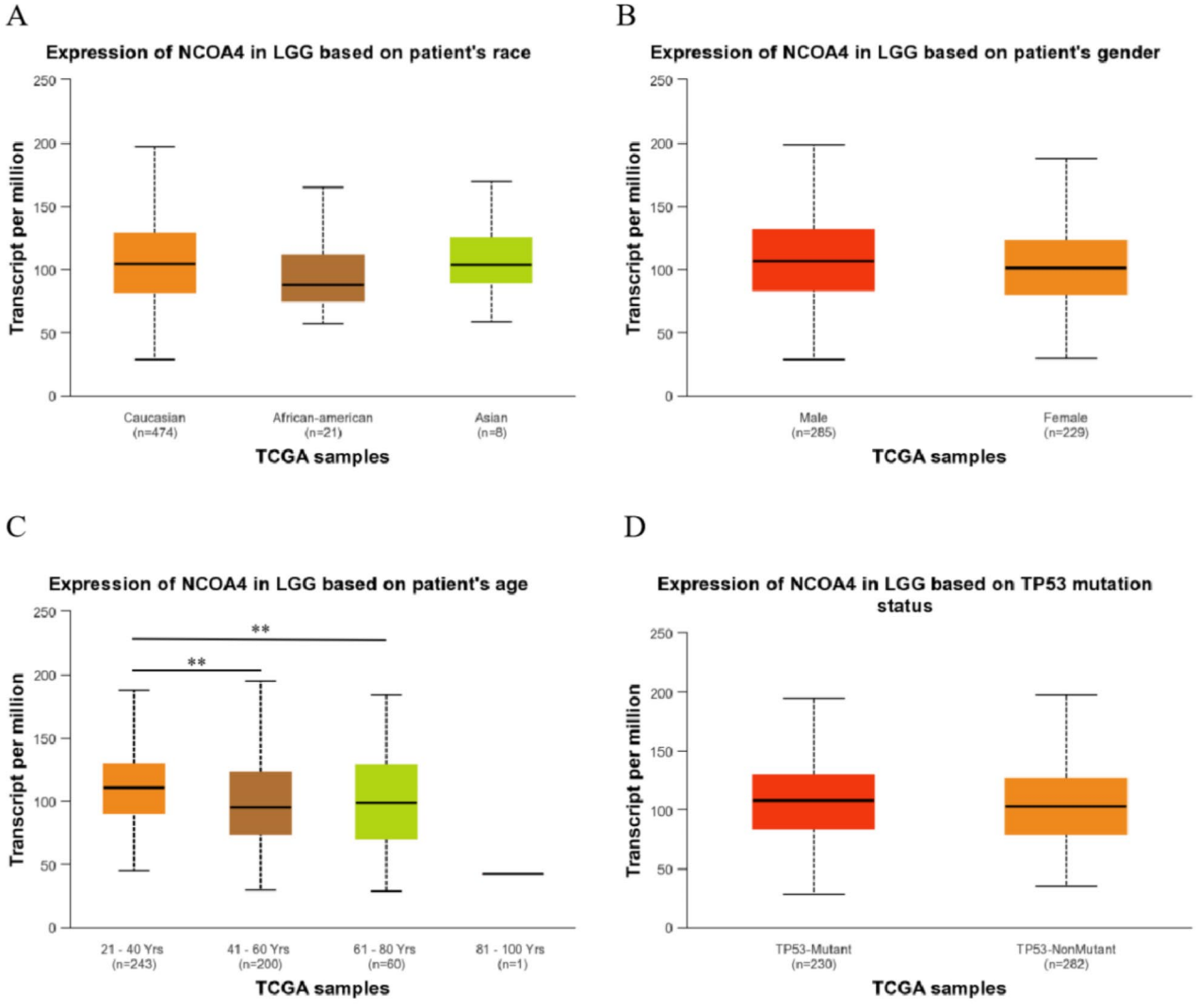


Fig. 4 The relationship between NCOA4 expression level and Patient's race, gender, age, TP53 mutation status from UALCAN database. (**A**) The Patient's race. (**B**) The Patient's gender. (**C**) Patient's age. (**D**) TP53 mutation status. ** $p<0.01$

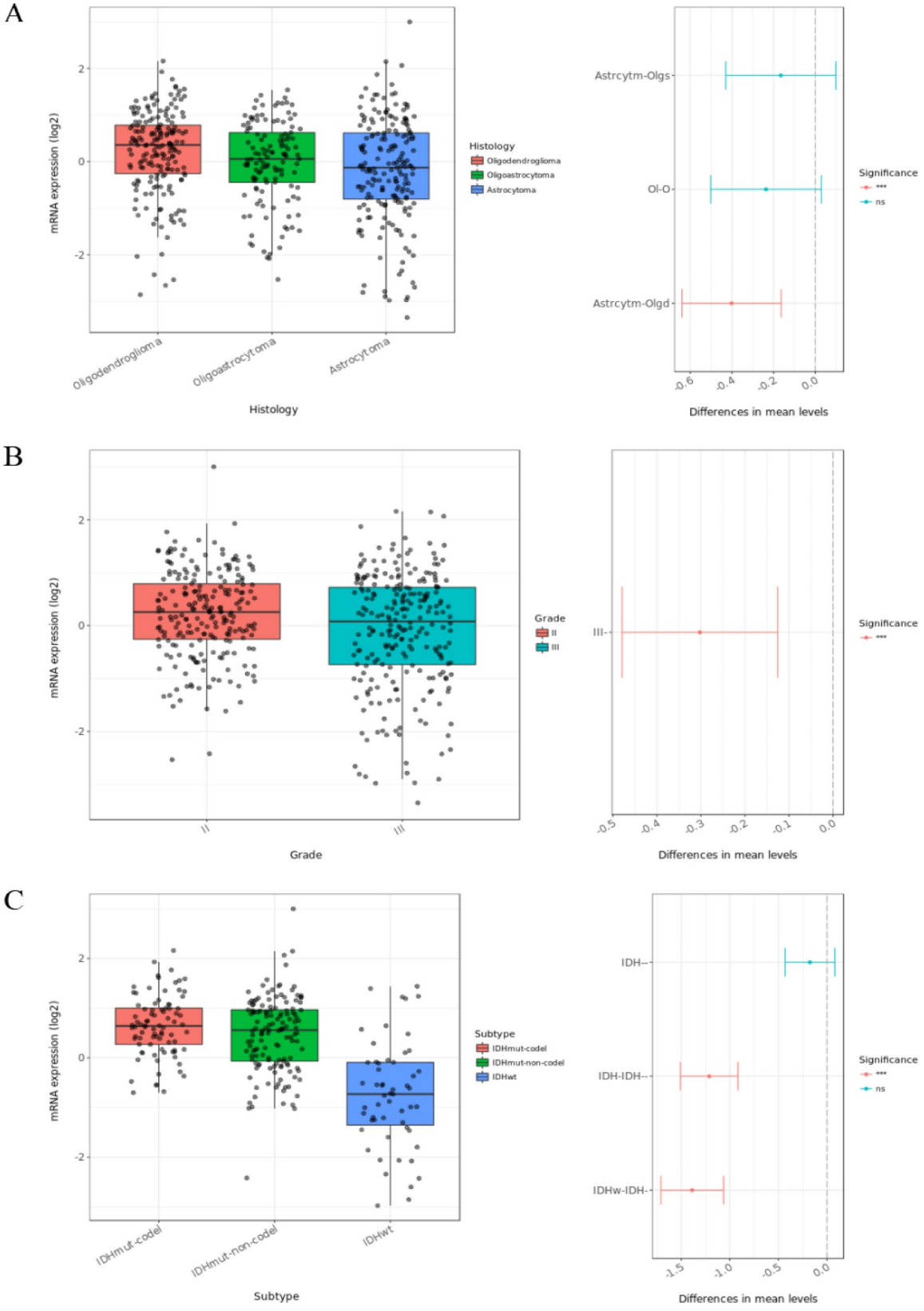


Fig. 5 The relationship between NCOA4 expression level and Histology, Grade and Subtype from GlioVis database. (**A**) Histology. (**B**) Grade. (**C**) Subtype. *** $p < 0.001$

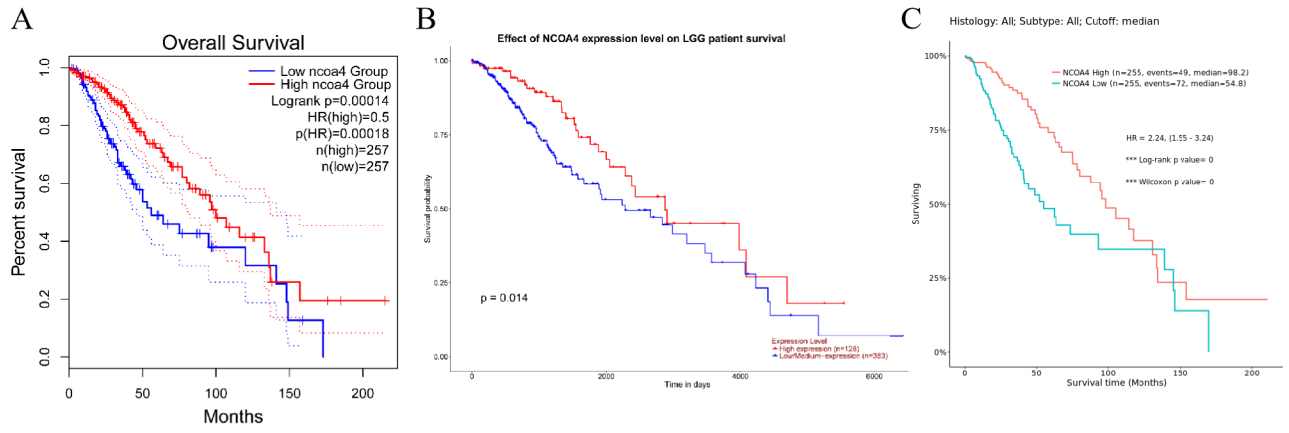


Fig. 6 Kaplan-Meier survival curves of LGG patients with high and low NCOA4 expression in three different databases support the prognostic value of NCOA4 in predicting LGG patient outcomes. (**A**) GEPIA2 database. (**B**) UALCAN database. (**C**) GlioVis database. *** $p < 0.001$

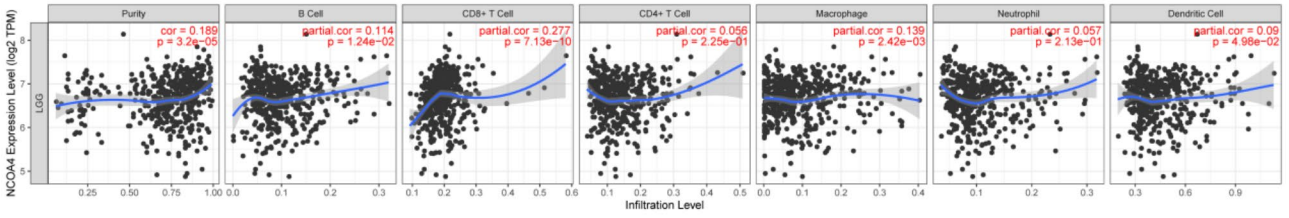


Fig. 7 Correlation between NCOA4 and immune cell infiltration in LGG tissue from TIMER database

LGG patients, with statistical significance (all $p < 0.05$) (Fig. 6A-C). This finding suggests that the expression level of NCOA4 is significantly correlated with the prognosis of LGG and can serve as a valuable predictive biomarker.

Correlation analysis of NCOA4 expression with infiltrating immune cells

Analyzing the correlation between target protein expression and infiltrating immune cells can provide important insights for prognosis assessment and personalized treatment of cancer patients Therefore, we conducted an analysis using the TIMER database to assess the correlation between NCOA4 expression and six types of infiltrating immune cells (B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells), as well as tumor purity. The results showed that in LGG, the expression level of NCOA4 is weakly positively correlated with infiltrating B cells ($r = 0.114$, $p = 1.24e\text{-}02$), CD8 + T cells ($r = 0.277$, $p = 7.13e\text{-}010$), macrophages ($r = 0.139$, $p = 2.42e\text{-}03$), dendritic cells ($r = 0.09$, $p = 4.98e\text{-}02$), and tumor purity ($r = 0.189$, $p = 3.2e\text{-}05$). However, there is no significant correlation between NCOA4 expression and CD4 + T cells or neutrophils (Fig. 7). Further analysis of lymphocyte infiltration markers revealed a weak positive correlation between CD20 and NCOA4 expression in B cells, while CD38 showed a moderate positive correlation with NCOA4 expression. In macrophages, CD68 showed a weak correlation with NCOA4 expression. In dendritic cells, CD1C showed a weak positive correlation with NCOA4 expression, while CD11c (ITGAX) showed a weak negative correlation with NCOA4 expression. It is worth noting that in M1 macrophages, INOS showed a weak negative correlation with NCOA4 expression, while in M2 macrophages, CD163 and MS4A4A showed weak correlations with NCOA4 expression, and MS4A4A showed a moderate positive correlation with NCOA4 expression. This suggests that NCOA4 may play a regulatory role in macrophage polarization processes. Although the correlation analysis showed statistically significant moderate to low positive or negative correlations between NCOA4 expression and certain immune cells and their markers, no strong correlations were identified. Based on these findings, we believe NCOA4 likely has a limited regulatory role in the immune microenvironment of LGG, and further research is needed to understand the biological mechanisms behind these findings.

Cox regression analysis of clinical characteristics, infiltrating immune cells, and NCOA4 on LGG prognosis

We utilized the Cox proportional hazards model from the TIMER database, incorporating various factors including age, gender, race, tumor purity, B cells, CD8 T cells, CD4 T cells, macrophages, neutrophils, dendritic cells, and the expression level of NCOA4, to analyze their impact on the survival time of LGG patients. The results, as shown

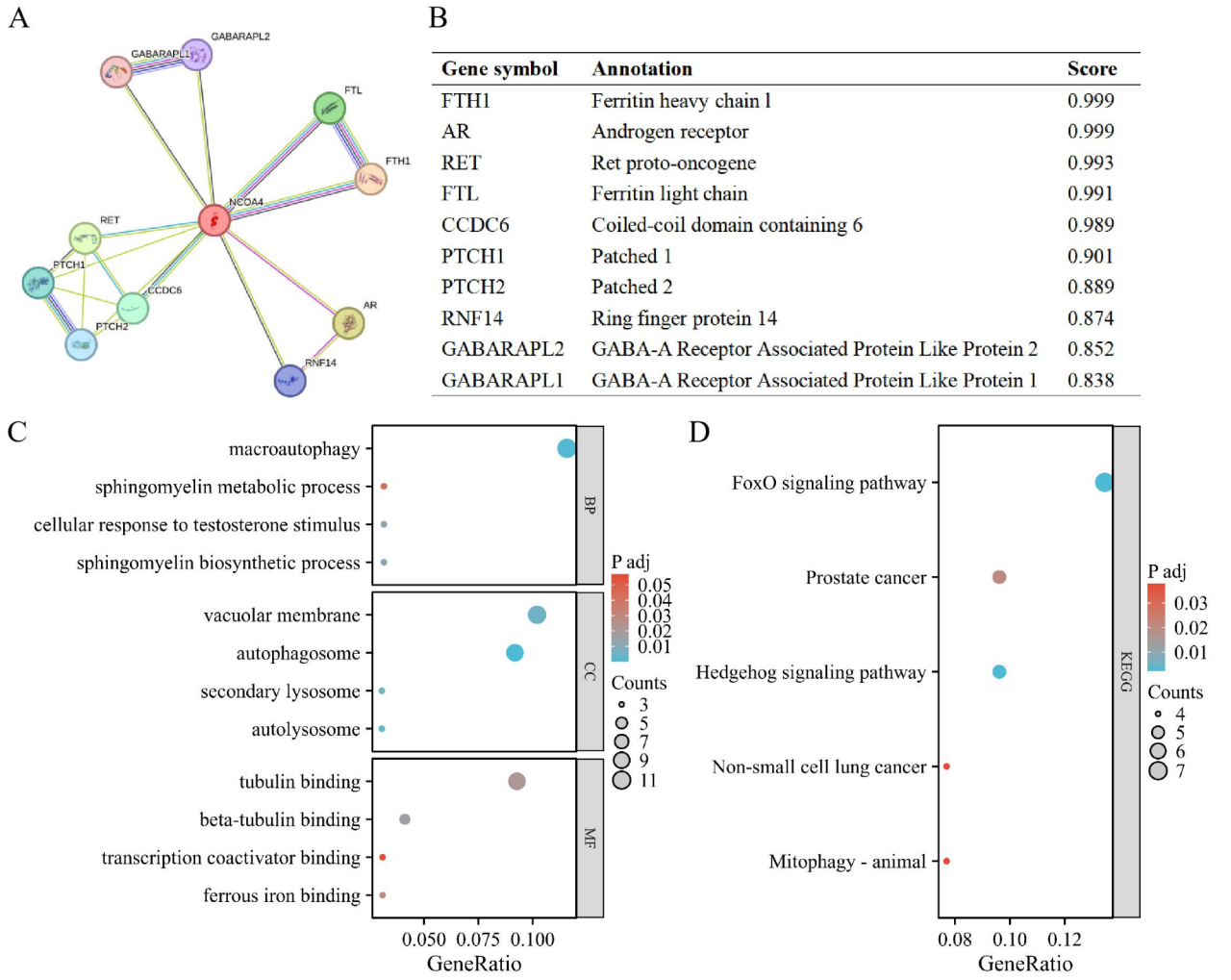
in Table 1, indicate that the regression coefficient for Age is 0.054, with an HR of 1.055, suggesting that for each additional year of age, the risk of death increases by 5.5%, with a p-value < 0.001, indicating statistical significance. The regression coefficient for CD8 Tcell is 7.838, with an HR of 2534.377, indicating that each unit increase in CD8 T cells increases the risk of death by 2534.377 times, with a p-value of 0.022, indicating statistical significance. The regression coefficient for Macrophage is 5.333, with an HR of 207.093, indicating that each unit increase in macrophages increases the risk of death by 207.093 times, with a p-value of 0.015, indicating statistical significance. The regression coefficient for NCOA4 is -1.043, with an HR of 0.352, indicating that each unit increase in NCOA4 reduces the risk of death to 0.352 times, with a p-value < 0.001, indicating statistical significance. In summary, multi-factor Cox regression analysis indicates that age, CD8 T cells, macrophages, and NCOA4 are all independent prognostic indicators for LGG (all $p < 0.05$). Additionally, the results of this section also indicate that after controlling for age, CD8 T cells, macrophages, and other factors, the expression level of NCOA4 remains an independent predictor of prognosis for LGG patients ($p < 0.05$).

Biological function of NCOA4 in LGG

In previous studies [4], NCOA4 has been identified as a crucial regulator of ferroptosis, a form of programmed cell death involving iron-dependent lipid peroxidation. NCOA4 targets ferritin and delivers it to lysosomes for degradation, releasing free iron. Under the action of divalent iron, it catalyzes the lipid peroxidation of highly expressed unsaturated fatty acids on the cell membrane, thereby inducing ferroptosis. Characteristics of ferroptosis include the accumulation of intracellular iron and lipid peroxidation. However, the biological function of NCOA4 in LGG has not been fully validated. To comprehensively understand the biological function of NCOA4 in LGG, we conducted GO and KEGG analyses involving NCOA4's interacting proteins and related genes. First, we used the STRING tool to construct a PPI network of NCOA4, identifying 10 interacting proteins with NCOA4 (Fig. 8A). The confidence scores of these interacting proteins with NCOA4 all exceeded 0.7, indicating significant interactions (Fig. 8B). Next, based on NCOA4 and its 10 interacting proteins, as well as 100 related genes obtained from GEPIA2, we conducted GO and KEGG enrichment analyses to understand the biological functions of NCOA4 in LGG. The GO analysis results (Fig. 8C) revealed multiple biological functions of NCOA4 in LGG (all $p < 0.05$): at the biological process (BP) level, NCOA4 was primarily involved in macroautophagy, sphingomyelin metabolic process, cellular response to testosterone stimulus, and sphingomyelin biosynthetic process. These processes are closely related to cellular autophagy activities, lipid metabolism, and hormone responses. At the cellular component (CC) level, NCOA4 was associated with vacuolar membrane, autophagosome, secondary lysosome, and autolysosome. These organelles play a central role in the autophagy process. At the molecular function (MF) level, NCOA4 exhibited significant roles in tubulin binding, beta-tubulin binding, transcription coactivator binding, and ferrous iron binding. These functions are related to cytoskeletal dynamics, gene expression regulation, and iron metabolism. The GO analysis results all point to lipid metabolism and iron metabolism processes, consistent with previous studies on NCOA4 functions. Additionally, KEGG pathway analysis showed that NCOA4 was involved in FoxO signaling pathway and Hedgehog signaling pathway in LGG (both $p < 0.05$), both of which play crucial roles in cell growth, differentiation, and death (Fig. 8D). In summary, the findings from the GO and KEGG studies highlight the biological functions of

Table 1 Multivariate Cox regression analysis of clinical characteristics, tumor purity, infiltrating immune cells, and NCOA4 in relation to LGG prognosis from TIMER database

	LGG				
	coef	**HR**	**95%CI_l**	**95%CI_u**	***p*.value**
Age	0.054	1.055	1.037	1.073	0.000
Gender (Male)	0.120	1.128	0.750	1.696	0.564
Race (Black)	15.652	6271960.121	0.000	Infinity	0.994
Race (White)	15.939	8357245.098	0.000	Infinity	0.994
Purity	0.294	1.341	0.508	3.541	0.553
B cell	2.662	14.328	0.014	14311.303	0.450
CD8 T cell	7.838	2534.377	3.105	2068921.800	0.022
CD4 T cell	2.151	8.593	0.004	19658.212	0.586
Macrophage	5.333	207.093	2.845	15074.847	0.015
Neutrophil	−8.828	0.000	0.000	1.053	0.051
Dendritic	1.412	4.105	0.058	291.177	0.516
NCOA4	−1.043	0.352	0.236	0.526	0.000



Gene symbol	Annotation	Score
FTH1	Ferritin heavy chain 1	0.999
AR	Androgen receptor	0.999
RET	Ret proto-oncogene	0.993
FTL	Ferritin light chain	0.991
CCDC6	Coiled-coil domain containing 6	0.989
PTCH1	Patched 1	0.901
PTCH2	Patched 2	0.889
RNF14	Ring finger protein 14	0.874
GABARAPL2	GABA-A Receptor Associated Protein Like Protein 2	0.852
GABARAPL1	GABA-A Receptor Associated Protein Like Protein 1	0.838

Fig. 8 The biological functions of NCOA4 in LGG. (**A**) Protein-protein interaction network of NCOA4 from STRING database. (**B**) Annotation of NCOA4-interacting proteins and their co-expression scores. (**C**) GO functional enrichment analysis of NCOA4-related genes in LGG, including biological-process, cellular-components, and molecular-functions. (**D**) KEGG analysis of NCOA4-related gene pathways in LGG

NCOA4 in LGG, particularly its role in the autophagy process. Therefore, we speculate that NCOA4 in LGG may also influence ferroptosis by regulating autophagy and iron metabolism.

Discussion

Our study, for the first time, combined bioinformatics analysis and experimental validation to systematically explore the clinical value of NCOA4 in LGG. Initially, we observed a significant elevation of NCOA4 expression levels in LGG tissues compared to normal controls. The AUC, assessed by the ROC curve, reached 0.973, suggesting that NCOA4 could serve as a potential diagnostic marker for LGG. Secondly, we noted an upregulation of NCOA4 in younger (21–40 years old) LGG patients with lower malignancy (oligodendroglioma) and better prognosis (IDHmut-non-codel and IDHmut-codel subtypes). This finding implies a possible prognostic role of NCOA4. This prognostic value was consistently supported by Kaplan-Meier curve analyses from three different databases: GEPIA2, UALCAN, and GlioVis, all indicating better outcomes for LGG patients with high NCOA4 expression. Thirdly, we discovered a weak positive correlation between NCOA4 expression levels and the infiltration of B cells, CD8 + T cells, macrophages, and dendritic cells in LGG. This association suggests that NCOA4 might influence the tumor's immune microenvironment, but the effects may be limited. Finally, a multivariate Cox regression analysis incorporating the clinical features, infiltrating immune cells, and NCOA4 expression revealed that NCOA4, age, CD8 T cells, and macrophages are independent prognostic indicators for LGG. Additionally, GO analysis indicated that NCOA4's biological function in LGG remains tied to autophagy processes. Similarly, KEGG analysis supported the enrichment of NCOA4 in two cell death signaling

pathways: FoxO signaling pathway and Hedgehog signaling pathway. These discoveries, to some extent, fill gaps in the field of NCOA4-related ferroptosis research in LGG.

To our knowledge, NCOA4 has never been studied in the context of LGG before. Our findings not only offer a new molecular marker for the diagnosis and prognostic evaluation of LGG but also present a potential therapeutic target for future treatment strategies. Specifically, the significantly higher expression of NCOA4 in LGG compared to normal tissues suggests its potential as an effective biomarker to distinguish between LGG and healthy brain tissue. Furthermore, the correlation between NCOA4 expression and specific pathological features of LGG (such as age, grade, and mutational subtype) could enhance the accuracy and specificity of LGG diagnosis. For instance, combining NCOA4 with other known LGG biomarkers like IDH mutation status and 1p/19q deletion might improve diagnostic precision and prognostic assessment. Such a multi-marker approach could provide a more comprehensive disease profile and aid in the development of personalized treatment plans. Moreover, the strong association between NCOA4 expression and LGG patient prognosis indicates its potential utility in predicting treatment responses and survival outcomes. Studies have suggested that modulating NCOA4 expression or its function in ferroptosis could lead to novel therapeutic strategies, particularly in inducing ferroptosis for glioblastomas (GBM) treatment [22, 23]. These modulation methods include, but are not limited to, certain herbal components and chemical agents. Therefore, NCOA4 not only holds promise in LGG diagnosis and prognostic evaluation but also emerges as a potential therapeutic target.

Understanding the role of ferroptosis in cancer is helpful for targeting and intervening in its potential application in systemic therapy, radiotherapy, and immunotherapy [24]. Research has shown that the overexpression of NCOA4 increases the degradation of iron proteins and promotes ferroptosis [25]. Basic experiments have shown that targeting NCOA4 to induce ferroptosis may be a viable strategy for treating GBM. For example, a study in 2021 found that COPZ1 deficiency induced NCOA4-mediated autophagy and ferroptosis in GBM cell lines, and proposed that the COPZ1/NCOA4/FTH1 axis is a new therapeutic target for human GBM [26]. Subsequently, another study in 2022 showed that TTRIM7 regulates NCOA4-mediated iron protein phagocytosis and ferroptosis in GBM cells, and pointed out that TTRIM7 directly binds and ubiquitinates NCOA4 through K48-linked chains to inhibit NCOA4-mediated iron protein phagocytosis and ferroptosis in GBM cells, knocking out TRIM7 makes GBM cells more sensitive to temozolomide treatment [27]. The latest study shows that HECW1 induces NCOA4-regulated U87 cell ferroptosis through ZNF350 ubiquitination and degradation, and proposes the concept of the HECW1/ZNF350/NCOA4 axis [28]. From these studies, we realize that indirectly promoting the upregulation of NCOA4 has a good therapeutic effect on gliomas. NCOA4 seems to be in a very critical position in the ferroptosis pathway of tumor cells.

Through the PPI network in this study, we identified ten proteins interacting with NCOA4, among which FTH1 and FTL were identified as ferritins. Both FTH1 and FTL can degrade through autophagy to increase iron levels, leading to cellular ferroptosis [29, 30]. We paid attention to FTH1 and FTL as they have been studied in LGG contexts [31–34]. A bioinformatics analysis study found that FTH1 is downregulated while FTL is upregulated in LGG, with FTH1 levels showing no correlation with LGG prognosis, whereas increased FTL levels are associated with poor LGG prognosis [35]. The protein with the highest co-expression score with NCOA4 is FTH1, an essential component of transferrin. FTH1 can bind with NCOA4 to degrade ferritins in a ferroptotic manner, releasing substantial iron ions. This process increases the concentration of $Fe2+$ in the cytoplasm, promoting the expression of mitochondrial membrane proteins that transport $Fe2+$ into mitochondria, resulting in mitochondrial lipid peroxidation and ultimately cell death [36]. While NCOA4 is a downstream target of FTH1, studies indicate that FTH1 is unrelated to LGG prognosis [35], whereas our research shows a correlation between NCOA4 and LGG prognosis. This suggests that interfering with NCOA4 affects LGG prognosis, whereas interfering with upstream FTH1 does not. This reinforces the key position of NCOA4 in the ferroptosis pathway promoting LGG tumor cell death. Given its significant roles in LGG diagnosis, prognostic evaluation, and immune infiltration, we believe NCOA4 is a highly promising therapeutic target in LGG and deserves more research investment in the future.

Research has shown that the expression of many iron metabolism-related proteins is altered in GBM cells [37]. In theory, similar changes may also occur in LGG. However, in reality, studying the mechanisms in LGG is more challenging compared to GBM, primarily due to the difficulty in obtaining LGG cell lines compared to GBM cell lines. As a result, many researchers have focused on studying iron death in GBM cells [38–40]. This has led to the current lack of clarity regarding the expression status of many iron metabolism-related proteins in LGG. In this study, we observed an elevation in the expression of NCOA4 in LGG, suggesting the presence of active cell iron death in LGG. Furthermore, we found that the upregulation of NCOA4 is associated with a favorable prognosis in LGG, indicating the presence of

active tumor cell iron death. Based on the critical role of NCOA4 in iron death and our research findings, it seems feasible to target the upregulation of NCOA4 to induce tumor cell iron death as a potential treatment for LGG.

This study has achieved preliminary results in exploring the clinical value of NCOA4 in LGG, but there are some limitations that need to be addressed in future research. For example, although we observed a significant increase in the expression levels of NCOA4 in LGG tissues compared to normal glioma tissues, it is currently unclear which specific cell types express NCOA4 at high levels. This issue needs to be further verified and clarified through subsequent experimental studies.

Conclusion

We have for the first time explored the expression, diagnostic value, clinicopathological features, immune infiltration, prognostic value, and biological function of NCOA4 in LGG. Our current research evidence suggests that NCOA4 is a potential diagnostic and prognostic biomarker for LGG. Although some new findings in this study require more evidence to validate, they undoubtedly provide valuable clues for future research directions. In summary, this study provides a new perspective on the research of NCOA4 in LGG and points out the direction for future research.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-025-04036-4.

Supplementary Material 1: **Table S1.** Correlation analysis of NCOA4 and LGG immune cell markers form TIMER database.

Supplementary Material 2: **Table S2.** Tukey's Honest Significant Difference (HSD) of the relationship between NCOA4 and Histology, Grade and Subtype from GlioVis database

Supplementary Material 3: **Table S3.** NCOA4-related genes in LGG from GEPIA2 database

Supplementary Material 4: **Table S4.** GO and KEGG enrichment analysis

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Author contributions
G.C. and X.Z. conceived the study; X.Z.and J.L. guided the methods; G.C.and X.S. conducted the software; X.S. and R.J. validated the study; X.S. and R.J. analyzed the data; J.Q. and X.D. investigated the research; G.C, J.Q. and X.D. prepared for the resources; G.C and X.S collected the data; G.C. drafted the original manuscript; X.Z.and J.L. reviewed and edited the article; G.C. visualized the study; X.Z.and J.L. supervised the research; X.Z.and J.L. administrated the project. All authors read and approved the final manuscript.

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Data availability
The authors confirm that all data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study involving LGG patients were reviewed and approved by The Ethical Committee of Affiliated Hospital of Guizhou Medical University (No. IRB−2022−152). The LGG patients provided their written informed consent to participate in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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