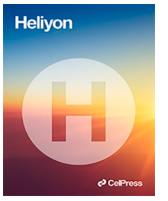
Heliyon 10 (2024) e29549

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Comprehensive analysis of CYBB as a prognostic marker and therapeutic target in glioma: A bioinformatics approach

Yu Wang, Yuhao Wang, Shuai Wang, Chengcheng Wang, Yuhang Tang, Chao Zhang, Dong Yu, Shiqiang Hou, Ning Lin *

Department of Neurosurgery, The Affliated Chuzhou Hospital of Anhui Medical University, The First People's Hospital of Chuzhou, Chuzhou, 239000, China

ARTICLE INFO

Keywords:
Glioma
CYBB
Gene expression
Mitochondria
Prognostic model

ABSTRACT

Background: In the central nervous system, glioma is the most common malignant tumor, and patients have a poor prognosis. Identification of novel marker genes and establishment of prognostic models are important for early diagnosis and prognosis determination.
Methods: Download glioma data from the CGGA and TCG databases. Application of bioinformatics to analyze the impact of CYBB on the clinicopathological characteristics, immunological features and prognosis of gliomas. Using single-cell sequencing data from 7 glioblastoma patients in the CGGA database, the role of CYBB in the tumor microenvironment was analyzed. In addition, a prognostic model was constructed based on CYBB high and low differentially expressed genes and mitochondrial genes.
Results: The expression of CYBB is closely related to various clinical features, immune cell infiltration level, immune checkpoint and survival time of patients. A 10-gene prediction model was constructed based on the differentially expressed genes of low and high CYBB and mitochondria-related genes. Glioma patients with higher risk scores had significantly lower survival probabilities. Receiver operating characteristic curves and nomograms were plotted over time to show the predictive accuracy and predictive value of the 10-gene prognostic model.
Conclusions: Our study shows that CYBB is strongly correlated with clinical characteristics features and prognosis of glioma patients, and can be used as a potential therapeutic target. Prognostic models based on CYBB and mitochondrial genes have good performance in predicting prognosis of glioma patients.

1. Introduction

It is estimated that between 50 % and 60 % of all intracranial malignant tumors are gliomas, which originate from glial and neuronal cells, with significantly higher invasiveness and recurrence rates than other intracranial tumors [1]. In accordance with the World Health Organization (WHO), gliomas are classified as grades II-IV, with the higher the grade, the more serious the cancer [2].

Abbreviations: CGGA, Chinese Glioma Genome Atlas; DEG, Differentially Expressed Gene; GSVA, Gene set variation analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; LASSO, Least absolute shrinkage and selection operator; ROC, Receiver Operating Characteristic; TCGA, The Cancer Genome Atlas; TME, Tumor Microenvironment; WHO, World Health Organization.

* Corresponding author.
E-mail addresses: 2145012095@stu.ahmu.edu.cn (Y. Wang), lin2007512@vip.163.com (N. Lin).

https://doi.org/10.1016/j.heliyon.2024.e29549
Received 19 July 2023; Received in revised form 7 April 2024; Accepted 9 April 2024
Available online 14 April 2024

The treatment and prognosis of patients with different grades of glioma are also different [3,4]. In the present, patients with glioma receive surgical resection with maximum safe range, combined with postoperative radiotherapy and chemotherapy, but prognoses are poor and survival is low [5]. In recent years, more and more molecular markers related to the prognosis of glioma have been discovered. We can better understand glioma's molecular mechanisms by discovering these molecular markers, which can then help with clinical diagnosis and treatment [6,7]. Because single index cannot accurately predict the prognosis of tumor, the combined analysis of multiple indexes is more and more important to improve the accuracy of prognosis prediction [8].

Cytochrome B-245 β-chain (CYBB) is the major catalytic subunit of NADPH oxidase, encoding a protein called NOX2 that plays a central role in phagocytes and leukocytes [9]. NOX2 is considered to be a key enzyme for antibacterial host defense and regulation of inflammation. Knockout of NOX2 can reduce tumor metastasis, and the mechanism may involve improved immune-mediated clearance of metastatic tumor cells [10,11]. NOX2 has been shown to be involved in the development of many cancers. For example: Myeloid leukemia cells express high levels of NOX2, inducing apoptosis of adjacent anti-leukemia lymphocytes to destroy malignant cells [12–14]. EBV-infected gastric cancer cells highly express NOX2 to promote tumor progression [15], and further expressed by non-small cell lung cancer cell lines to mediate tumor cell apoptosis [16]. These studies demonstrate the great potential of CYBB as a novel molecular biomarker and therapeutic target for patients with glioma.

Mitochondria are important organelles in eukaryotic cells. Cells use it to generate energy for survival, to maintain calcium homeostasis, and to initiate apoptosis [17]. A number of diseases, such as diabetes, cancer and neurodegenerative diseases, are associated with mitochondrial dysfunction [18]. Upon mitochondrial dysfunction, mitochondrial reactive oxygen species (mtROS) and metabolites can affect gene expression and subsequently cell activation, proliferation and survival [19,20]. Further damage to the functional activities of other organelles, inducing autophagy, leading to cell injury and death. Importantly, intracellular reactive oxygen species (ROS) are produced in mitochondria and play an important role in maintaining normal cell function [21]. After mitochondrial dysfunction, the increased ROS, as an endogenous DNA damage factor, Inhibits the activity of key energy metabolism enzymes, mediates mitochondrial DNA (mtDNA) damage and mutation, and finally leads to homeostasis and pathological disorders [22]. NOX2 regulates mitochondrial function, structure and bioenergetic capacity by regulating the expression of NOX4 [23]. As mitochondria are involved in tumorigenesis and CYBB regulates mitochondria, analyzing CYBB and mitochondria-related genes and constructing prognostic models based on them may provide new ideas for the treatment of glioma.

In this study, we comprehensively analyzed the relationship between CYBB and various clinical features, immune cell infiltration levels, immune checkpoints and survival time in glioma patients. In addition, we construct a 10-gene prognostic model based on low and high CYBB differentially expressed genes and mitochondria-related genes, this prognostic model has a good predictive effect.

2. Materials and methods

2.1. Data collection

The RNA-seq gene expression data, molecular pathology information and survival data of 693 glioma patients in the training set were downloaded from CGGA (http://www.cgga.org.cn). After excluding the five paraneoplastic data samples in the validation group, the corresponding data for the validation group were obtained from 697 glioma patients in the TCGA (http://cancergenome.nih.gov/), and 325 glioma patients in the CGGA (http://www.cgga.org.cn). Mitochondria related gene set was downloaded from Human. MitoCarta3.0 Data center (*https://www.broadinstitute.org/*), and 1136 genes were obtained (Supplementary Table 1). Information about all patients in this study is publicly available.

2.2. Functional enrichment analysis and gene set variation analysis (GSVA)

The list of CYBB-related genes (Supplementary Table 2, Cor>0.5, $p < 0.05$) was obtained by Pearson correlation analysis and uploaded to the Database for Annotation, Visualization, and Integrated Discovery (DAVID: https://david.ncifcrf.gov/) to obtain gene ontology (GO) and Kyoto Encyclopedia Genome (KEGG) function enrichment analysis results, results in ascending order of P value. In this study, the immune response-related gene set was downloaded from AmiGO2 (http://amigo.geneontology.org/amigo), the correlation between CYBB and immune response-related gene was analyzed by Pearson correlation and visualized in a heat map [P = -log10 (p-value)].

2.3. Single-cell sequencing

The GBM single cell RNA-seq was downloaded from the China Glioma Genome Atlas CGGA and used to analyze the relationship between CYBB and Tumor Microenvironment (TME). The "Seurat" package of R software was used to process the expression matrix of single-cell sequencing data, the gene expression data were normalized, principal component analysis (PCA) was performed using "RunPCA," the "UMAP" package was used for visual analysis, and the "SingleR" package was used for cell annotation.

2.4. Similarity analysis of cell clusters

The characteristic genes in a cell cluster define the similarity of the cell cluster, and the more the number of identical characteristic genes, the higher the similarity of the cell cluster.

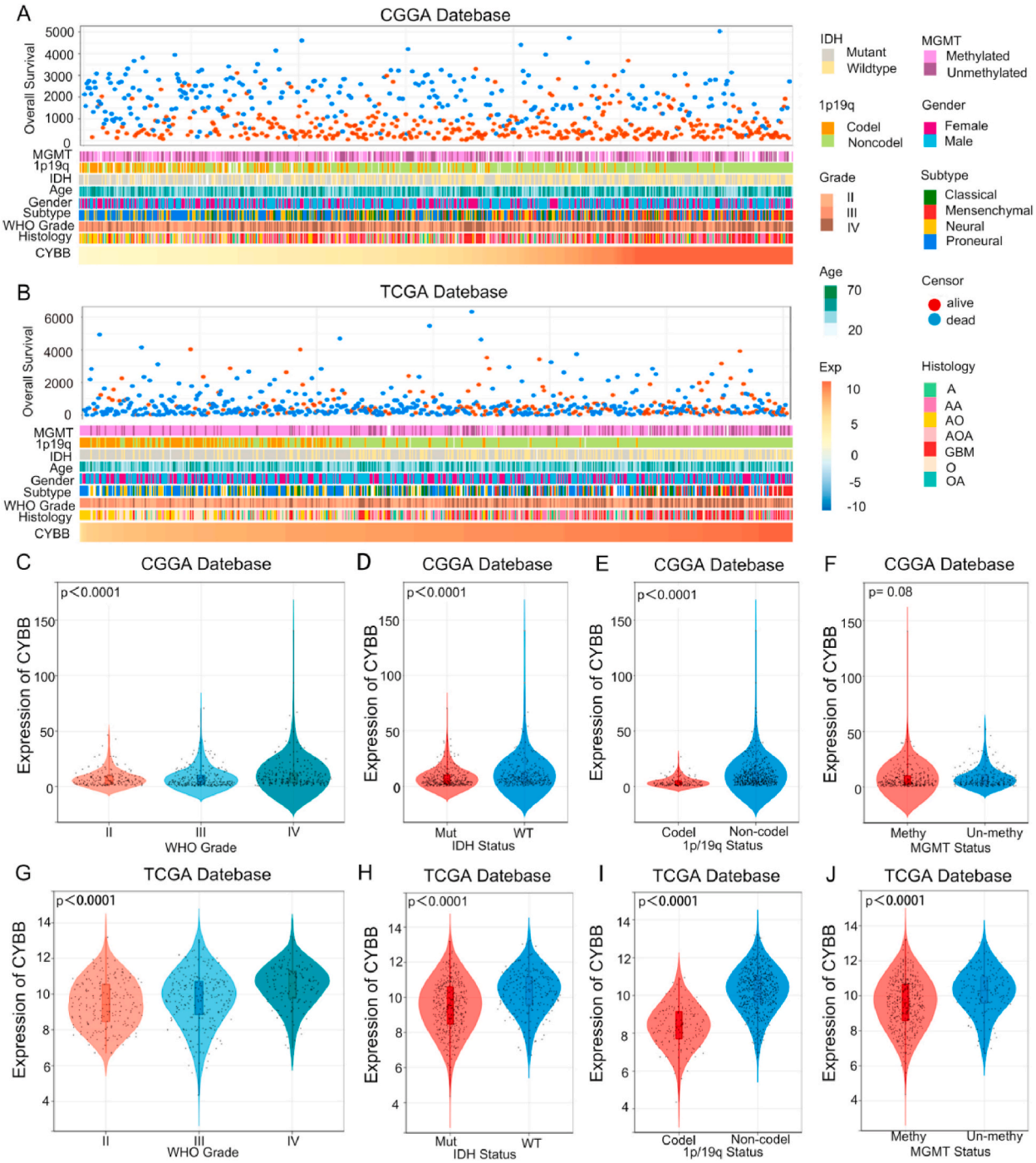


Fig. 1. Relationship between CYBB and clinical and molecular pathological features of glioma patients. A. Overview of clinicopathological features associated with CYBB in gliomas in CGGA (693). B. Overview of clinicopathological features associated with CYBB in gliomas in TCGA. C, G. In CGGA (693) and TCGA, CYBB expression was significantly increased in high-grade gliomas. D, H. In CGGA (693) and TCGA, increased CYBB expression was also seen in wild-type IDH mutation status. E, I. In CGGA (693) and TCGA, increased CYBB expression was seen in gliomas without a 1p/19q codeletion. F, J. In the TCGA database, CYBB expression is greater in gliomas with unmethylated O6-methylguanine-DNA methyltransferase promoters. A statistically significant difference was found in the TCGA database, but not the CGGA (693) database.

2.5. Differentially expressed gene (DEG) analysis

Glioma patients were grouped based on low and high CYBB expression. 619 genes were highly expressed ($p < 0.001$, FC > 2.5) and 733 genes were low expressed ($p < 0.001$, FC < 0.5). (Supplementary Table 3). Visualization of differentially expressed genes in glioma patients using the ggplot2 software package.

2.6. Prognostic features were established and analyzed for their relationship to survival and clinical outcomes

The data from CGGA were utilized to construct the prognostic signature, and the data from the TCGA cohorts were used for validation. Firstly, differentially expressed genes identified between glioma patients with low and high CYBB expression were pooled with a mitochondrial gene pool. Subsequently, the least absolute shrinkage and selection operator (LASSO) regression analysis was used to obtain an optimal predictive model by minimizing the risk of overfitting using "glmnet" software package, and the regression coefficients were obtained. Risk scores were calculated from the corresponding gene expression levels and their lasso regression coefficients. The stratified survival analyses for gliomas were performed using clinical characteristics, including age, gender, grade, IDH, histology, 1p19q, and MGMT. The survival outcomes in two groups were described for all patients stratified in groups through the Kaplan–Meier curves.

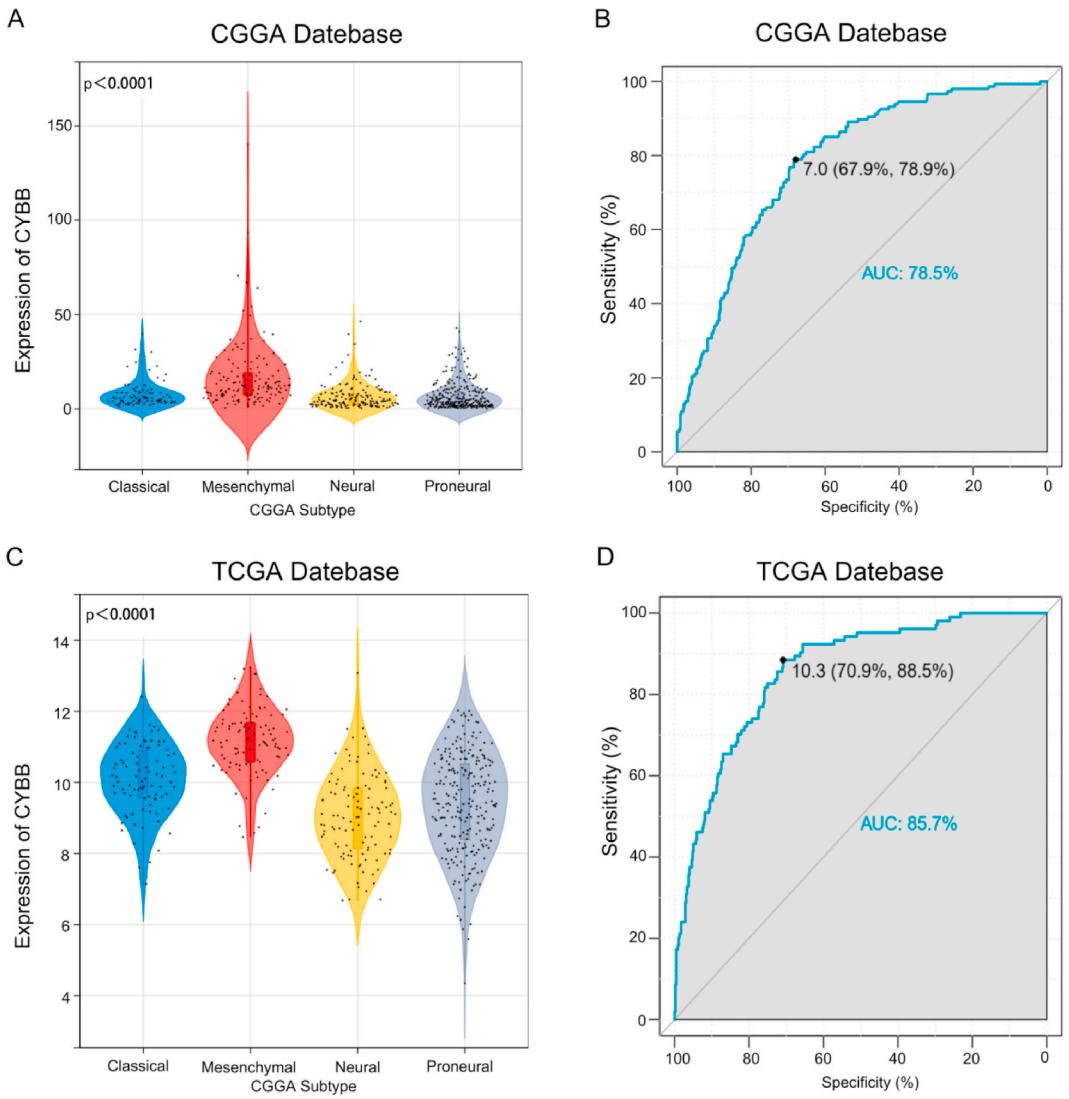


Fig. 2. Differential expression of CYBB in histological subtypes of glioma. A, C. CYBB is enriched in glioma mesenchymal subtypes in CGGA (693) and TCGA. B, D ROC curves show the specificity of CYBB overexpression in glioma mesenchymal subtypes in CGGA (693) and TCGA.

2.7. Nomogram construction

A nomogram was constructed using the "rms" package in R to estimate survival probabilities based on independent prognostic indicators from the TCGA database, utilizing Cox proportional hazard regression models. The performance of the nomogram was evaluated through calibration curve plotting, while discriminative performance was assessed through ROC curve analysis.

2.8. Statistical analysis

IBM SPSS Statistics 25 and R software (4.0.1) were used for all statistical analyses in this study. Rstudio is used with packages such as "survival,""ggplot 2,""pROC,""pheatmap,""corrgram," etc. Pearson was used for correlation analysis. $p < 0.05$ was considered statistically significant.

3. Results

3.1. CYBB is enriched in glioma patients with different clinical characteristics and molecular differences

We found that patients with different levels of CYBB expression exhibited different clinical and molecular pathological features. For example, WHO grade, 1p/19q co-deletion status, MGMT promoter methylation status, IDH mutation status and different histological diagnoses showed differences, and CGGA and TCGA databases showed asymmetric distribution (Fig. 1A and B). In the CGGA database, CYBB expression was proportional to glioma grade (Fig. 1C), was also elevated in IDH wild-type patients (Fig. 1D), and was highly expressed in patients without the 1p/19q co-deletion status (Fig. 1E). TCGA also obtained the same results (Fig. 1G–I). In the TCGA database (Fig. 1J), CYBB was highly expressed in patients who had no MGMT promoter methylation, but this difference wasn't statistically significant in the CGGA database (Fig. 1F). These results demonstrate that CYBB is highly expressed in gliomas. The high expression of CYBB was significantly correlated with WHO grade, 1p/19q co-deletion status and IDH mutation status ($p < 0.05$).

3.2. CYBB can be used as a biomarker of mesenchymal subtypes of glioma

Because histological subtype is a globally recognized molecular diagnostic method for glioma. We further investigated the expression of CYBB in each histological subtype. In both CGGA and TCGA databases, CYBB was highly expressed in mesenchymal isoforms with statistical significance ($P < 0.0001$) (Fig. 2A–C). ROC curve was used to evaluate the expression specificity of CYBB in mesenchymal subtypes of glioma. The results showed that the AUC was 78.9 % ($P < 0.0001$) in the CGGA database (Fig. 2B) and as high as 88.5 % ($P < 0.0001$) in the TCGA database (Fig. 2D). The above results suggest that CYBB is highly expressed in mesenchymal tumors with the worst clinical prognosis, and it may become one of the mesenchymal biomarkers of glioma. Because mesenchymal subtypes of glioblastoma are more aggressive than other types, these results also reconfirm the association of CYBB with the degree of malignancy of gliomas.

3.3. CYBB is involved in biological processes related to immune and inflammatory responses and regulation in glioma

In order to study the biological function related to CYBB, Pearson correlation analysis ($R > 0.5$, $P < 0.001$) was performed using

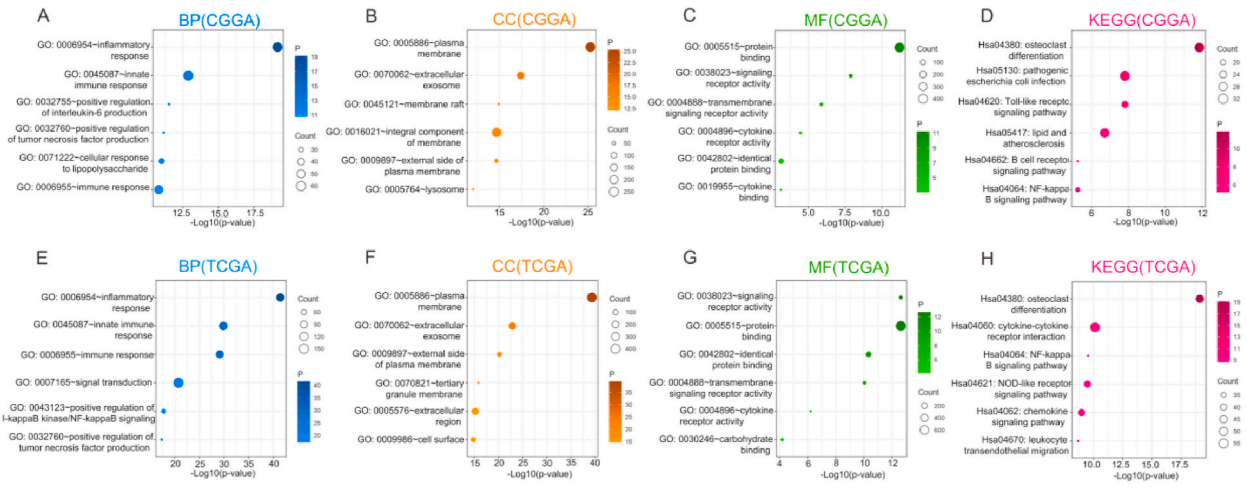


Fig. 3. CYBB is closely related to immune and inflammatory reactions in glioma. A-H. Biological functions, cellular components, molecular functions and signaling pathways related to CYBB in CGGA (693) and TCGA databases.

CGGA and TCGA databases to select the genes most closely related to CYBB. The results of GO and KEGG analysis using the above gene sets are as follows: In the CGGA database, the most relevant biology for CYBB includes inflammation and immune response (Fig. 3A); The cellular components most related to CYBB are the plasma membrane, exosomes, etc. (Fig. 3B); The molecular functions most relevant to CYBB are protein binding and signaling receptor activity, transmembrane signaling receptor activity, etc. (Fig. 3C); The most relevant signaling pathways include osteoclast differentiation, toll-like receptor signaling, B cell receptor signaling, and NF-kappa B signaling (Fig. 3D). Similar results were obtained in the TCGA database (Fig. 3E–H). All these results suggest that CYBB may play an important role in the immune and inflammatory responses of glioma and regulate related biological processes. Therefore, it is expected to become an important immune target of glioma.

3.4. Overexpression of CYBB is involved in glioma-related immune pathways

Apoptosis and necrosis of cancer cells can result from the release of chemokines and cytokines by activated lymphocytes in immunogenic cancer cell death [24]. Therefore, we investigated whether there is a correlation between CYBB activation and immune pathway. Using the CGGA and TCGA databases, Genome Set Variation Analysis (GSVA) was performed to determine the enrichment fraction of the immune

Process. The correlation between enrichment score and CYBB expression was then visualized using a heat map (Fig. 4A), and it can be seen that CYBB expression is positively correlated with most immune functions in vivo. Our results have been re-validated in the TCGA database (Fig. 4B). These results suggest that CYBB is closely related to the immune response of glioma, and may affect the occurrence and progression of glioma through immune pathway.

3.5. CYBB is positively associated with immune checkpoint inhibitors and inflammatory activity

Based on the above analysis, we found that CYBB is not only associated with immune response in glioma patients, but also highly likely to be closely associated with inflammatory activity in TME in glioma patients. Therefore, we further used the CGGA and TCGA databases to analyze whether there was a relationship between CYBB and inhibitory immune checkpoints, randomly selecting common immune checkpoints including TIM3, PDCD1LG2, CD200R1, LILRB4, LILRB2, CD47, ICOS, and HVEM. A significant correlation was found between CYBB and selected inhibitory immune checkpoints (Fig. 5A), resulting in the suppression of the immune response against glioma. In addition, to further understand the inflammatory activity associated with CYBB, we selected seven clusters of

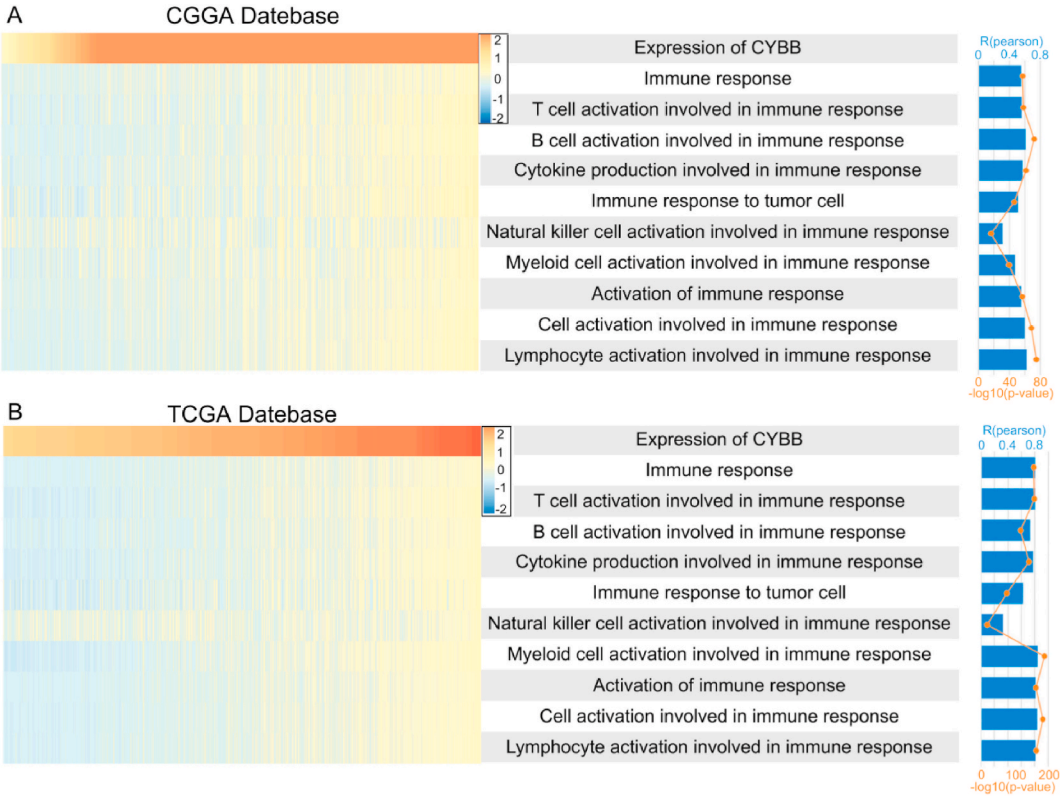


Fig. 4. CYBB and immune function correlation analysis. A, B. heatmaps showing CYBB expression and immune enrichment scores in glioma patients in CGGA (693) and TCGA. The column and line graphs show the R-and *P*-values (P = -log10 (p-value)) of the correlation analysis.

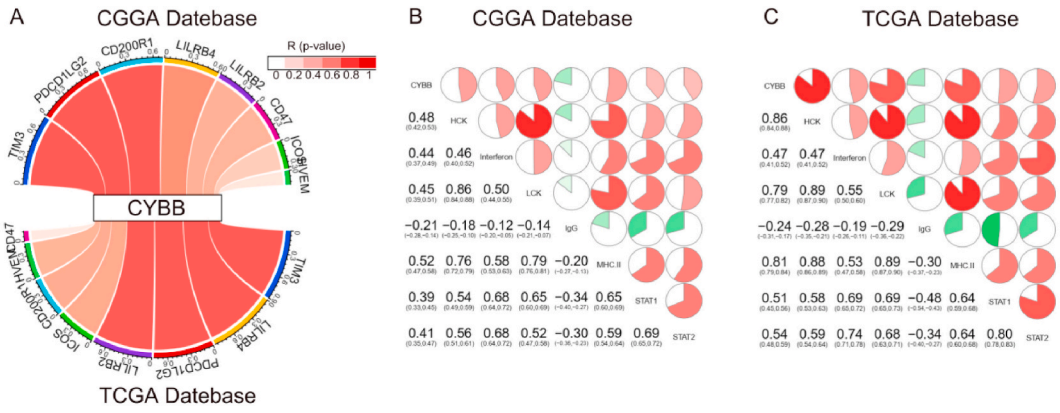


Fig. 5. Correlation analysis of CYBB with immune checkpoint and inflammatory activity. A. Pearson correlation between CYBB and inhibitory immune checkpoint. B, C. CYBB and inflammation-related metagenome clusters. The lower left corner shows the correlation coefficient trend. The two-color gradient and the circle size are proportional to the degree of correlation. The red part represents a positive correlation and the green part represents a negative correlation. Pearson correlation analysis was used.

immune system metabolites as markers of immune status [25,26]. The correlation matrix shows the correlation between CYBB and the 7 metagenome clusters in the CGGA and TCGA databases. Except for the immunoglobulin G (IgG) metagenome, which is negatively correlated with CYBB, the expression of CYBB shows a significant positive correlation with most inflammatory responses (Fig. 5B and C), which may be caused by the recruitment of tumor-mediated immune cells by multiple chemokines in the tumor TME. These results conclude that CYBB is strongly associated with the autoimmune response, possibly by influencing inflammatory activity in the TME, which in turn contributes to a worse prognosis in patients with glioma.

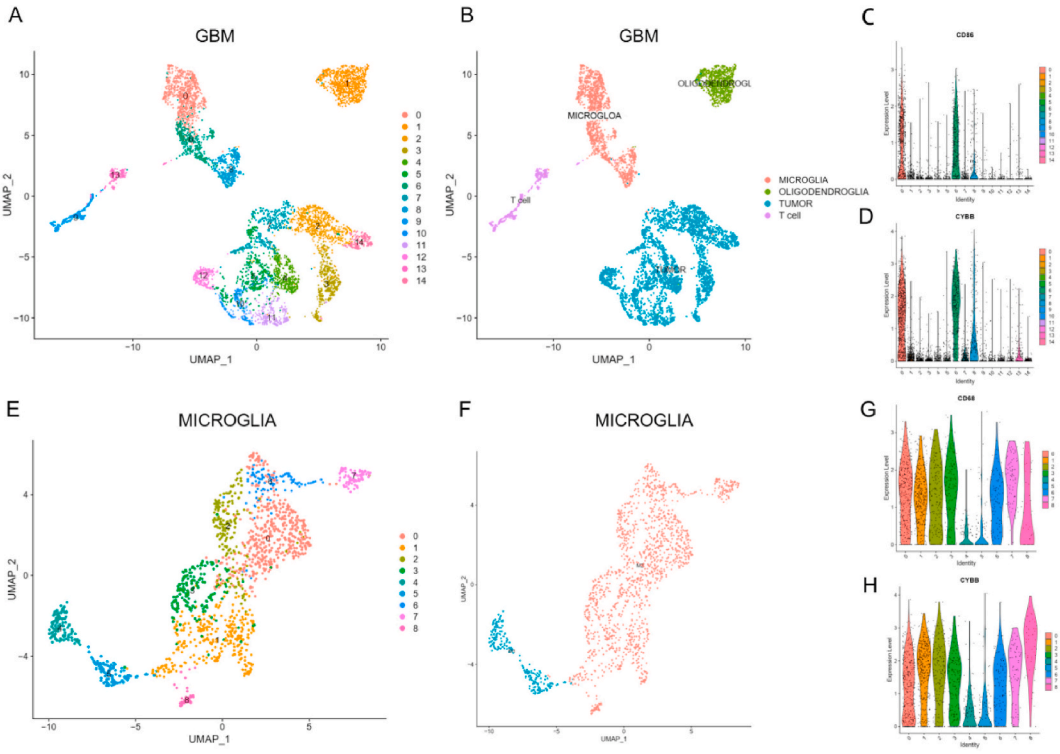


Fig. 6. Expression pattern of CYBB in GBM single cell sequencing. A. Single cell sequencing data cell population from GBM patients. B. GBM single cell sequencing data cell classification. C, D. microglia markers CD86 and CYBB expression in GBM single cell sequencing data. E. single cell sequencing data microglia from GBM patients were extracted. F. GBM single cell sequencing data microglia classification. G, H. M2 microglia markers CD68 and CYBB expression in microglia clusters.

3.6. CYBB is enriched in M2 microglia in GBM

To further assess the relationship between CYBB and TME immunoinfiltrating cell types in gliomas, we analyzed CYBB expression levels in different cell clusters in gliomas using single-cell scRNA-seq. Using GBM single-cell sequencing data, the "UMAP" package of R software divided the single-cell sequencing data of glioblastoma into 15 cell clusters (Fig. 6A), and extracted the differentially expressed genes of each cell cluster. Based on the expression of characteristic markers, cell clusters 0, 6, and 8 were defined as microglia, cell cluster 1 as oligodendrocytes, cell clusters 9 and 13 as T cells, and the remaining cell clusters as tumor cells (Fig. 6B). It was found that CYBB was highly expressed in cell clusters 0 and 6 defined as microglia (Fig. 6C and D), and was specifically enriched in some cell clusters of microglia. Microglia were further divided into 9 clusters (Fig. 6E and F), and CYBB was highly expressed in clusters 0, 1, 2, 3, 6, 7, and 8, consistent with M2 microglia markers (Fig. 6G and H). These results may suggest that CYBB may be mediated by M2 microglia in TME of glioma patients, thereby influencing tumor cell progression and worse prognosis.

3.7. CYBB is an independent prognostic factor affecting the overall survival of glioma patients

To investigate the prognostic value of CYBB in patients with glioma, Kaplan-Meier and Cox proportional hazards analyses were performed using the CGGA and TCGA databases. According to the CGGA database, patients with low CYBB expression had significantly shorter overall survival than those with high CYBB expression ($P < 0.0001$) (Fig. 7A). Similar results were obtained in the TCGA database (Fig. 7B). Thus, patients with high levels of CYBB expression have a poor prognosis. Further analysis using univariate Cox proportional hazards model showed that CYBB was an independent prognostic factor (Tables 1 and 2).

3.8. Screening for genes associated with risk scores

Glioma-associated DEGs were identified based on differential expression of CYBB, and the set of DEGs and mitochondria-associated genes were intersected to obtain 26 common genes (Fig. 8A and B). The results of Lasso regression analysis showed that there were 10 genes: BCL2A1, CASP8, CHCHD10, CKMT1A, CKMT1B, ECSIT, MACROD1, MAOB, NDUFA13, NDUFV1 with regression coefficients of 0.000485976, 0.049873911, −0.000627935, −0.006242697, −0.005087103, −0.001109115, −0.000135429, 0.002896734, 0.000137656, −0.000853945 (Fig. 8C), we constructed a prognostic model based on these 10 genes. Patients with glioma were classified into low and high-risk groups based on risk scores. The links between risk score and 10 genes and patient survival status was further explored (Fig. 8D and E). Next, we further evaluated the relationship between risk score and prognosis of glioma patients, and it was found that the group with high-risk score had worse prognosis ($p < 0.001$, Fig. 8F and G).

3.9. Prognostic risk scores were closely correlated with clinical variables

We have analyzed the association of the risk score with different clinical variables and thus explored its relationship with the development of glioma. The distribution of clinical variables was asymmetric with increasing scores in the CGGA and TCGA (Fig. 9A and B). We then went on to further analyze the correlation between risk scores and clinicopathological factors. It can be concluded that in the CGGA database, WHO class, 1p/19q co-deletion status, IDH mutation status, and age showed a strong correlation ($p < 0.05$) with risk scores (Fig. 9C–F). Similar results were obtained in the TCGA database ($p < 0.01$ Fig. 9G–J). From these results, it is concluded that

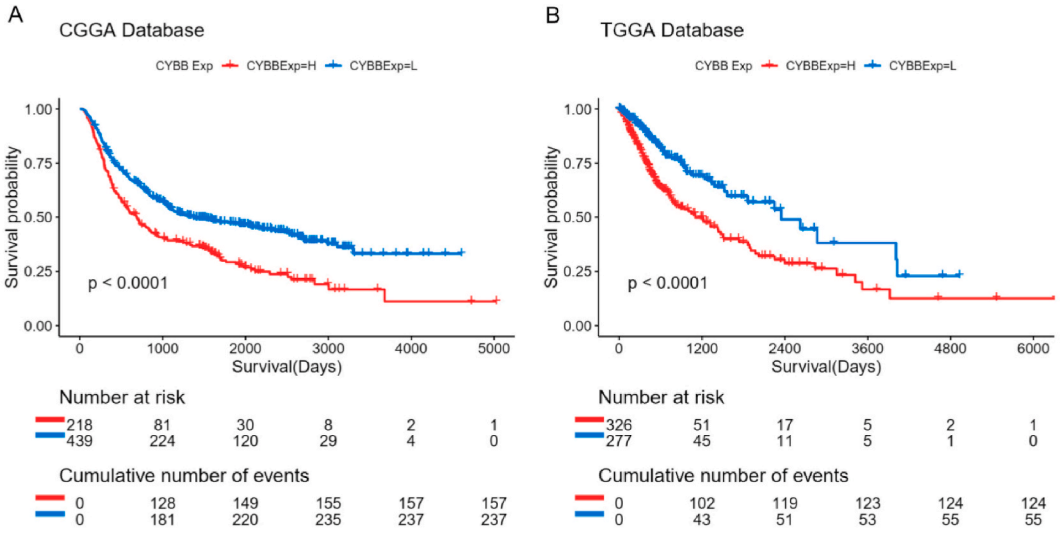


Fig. 7. Kaplan-Meier survival curve of CYBB expression in glioma patients. A, B. Prognostic analysis of CYBB differential expression in the CGGA (693) and TCGA databases.

Table 1
Univariate and multivariate analysis of prognostic parameters in the Chinese Glioma Genome Atlas (CGGA 693) database overall survival (OS).

Variable	Univariate analysis		Multivariate analysis	
	HR (95 % CL)	*P*-value	HR (95 % CL)	*P*-value
CYBB expression	1.011(1.195–1.507)	2.59E-14	0.986(0.976–0.996)	0.0078
PRS type	2.181(1.784–2.666)	2.59E-14	2.527(1.973–3.238)	2.20E-13
WHO grade (II *vs* III)	2.544(1.846–3.508)	1.17E-08	2.386(1.588–3.585)	0.00002
WHO grade (II *vs* IV)	6.967(5.081–9.554)	1.89E-33	4.556(2.960–7.014)	5.56E-12
Age	1.026(1.017–1.034)	1.37E-09	1.009(1.000–1.019)	0.0370
IDH status	0.323(0.262–0.398)	3.37E-26	0.599(0.442–0.811)	0.0009
1p/19q Codel	0.267(0.193–0.371)	3.02E-15	0.368 (0.245–0.555)	1.72E-06
MGMT status	0.795(0.638–0.990)	0.0407	0.908 (0.713–1.155)	0.4329

Abbreviations: CI, confidence interval; HR, hazard ratio; IDH, isocitrate dehydrogenase; WHO, world health organization.

Table 2
Univariate and multivariate analysis of prognostic parameters in the Cancer Genome Atlas (TCGA) database overall survival (OS).

Variable	Univariate analysis		Multivariate analysis	
	HR (95 % CL)	*P*-value	HR (95 % CL)	*P*-value
CYBB expression	1.341 (1.194–1.506)	6.69E-07	1.196 (1.0425–1.373)	0.0106
WHO grade (II *vs* III)	3.256 (1.987–5.335)	2.77E-06	2.202 (1.307–3.710)	0.0030
WHO grade (II *vs* IV)	20.057(12.140–33.136)	1.18E-31	3.850 (2.016–7.354)	0.00004
Age	1.075 (1.062–1.087)	4.28E-34	1.057 (1.040–1.073)	1.38E-12
IDH status	0.090 (0.063–0.128)	2.18E-40	0.364 (0.206–0.645)	0.0005
1p/19q Codel	0.220 (0.129–0.374)	2.30E-08	0.706 (0.356–1.401)	0.3198
MGMT status	0.312 (0.224–0.432)	2.95E-12	0.842 (0.572–1.240)	0.3855

Abbreviations: CI, confidence interval; HR, hazard ratio; IDH, isocitrate dehydrogenase; WHO, world health organization.

the degree of malignancy of gliomas increases with the increase of prognostic risk score.

3.10. Establishment of prediction model based on CYBB and mitochondrial related 10 genes

Next, Cox regression models were used to assess various clinical risk factors. The results of univariate analysis indicates that WHO class, gender, age, IDH mutation status, 1p19q co-deletion status, and risk score (high or low) were all significant (Fig. 10A). Based on the univariate results and considering the importance of chemotherapy for patients with glioma, the factors included in the multivariate analysis were finally determined as follows: WHO grade, age, IDH mutation status, 1p19q co-deletion status, risk score, and whether or not chemotherapy was administered (Fig. 10B). Based on the above independent predictors, an individual prediction model was constructed. Fig. 10C shows that the probability of tumor recurrence (1, 2, 3, 5, and 10 years) in patients with glioma can be estimated using an individualized predictive model. The C index of the nomogram we constructed was 0.752, indicating the accuracy of the model (Fig. 10D). Calibration curves and ROC curves are used to validate the accuracy of the prediction model. The calibration curves showed a high degree of coincidence in both databases, indicating the accuracy of the prediction model (Fig. 10E, F, Fig. S1A). The ROC curves also show the good performance of this predictive model (Fig. 10G, H, Fig. S1B). The above results show that the prediction model has high reliability, good efficiency in external validation, strong prediction accuracy and high clinical application value.

4. Discussion

Patients with glioma have poor prognosis under conventional treatment such as surgery and chemoradiotherapy. As a new treatment strategy, immunotherapy can activate the immune system to attack cancer cells [27,28]. During the immune process, high expression of many immune checkpoints such as PDCD1LG2, HAVCR2 and CTLA4 mediates an inhibitory immune response, leading to tumor immune evasion [29–32]. There are many successful cases of immunotherapy [33], and CART cell therapy targeting inhibition of immune checkpoints is an important progress in cancer treatment [34]. Therefore, the discovery of prognostic biomarkers and the establishment of reliable prognostic models are particularly important in the future treatment of glioma [35,36]. For these reasons, we continue to explore additional biomarkers that may modulate the immune microenvironment and improve the efficacy of brain tumor immunotherapy.

The catalytic core of the oxidase is composed of the membrane-binding subunits CYBB (also known as NOX2 or gp91 phox, where phox refers to phagocytic oxidase) and p22phox (CYBA). NOX is the primary source of superoxide in cells and plays a crucial role in regulating redox signaling and homeostasis. Among the NOX family members, NOX2 was first identified in bone marrow cells and has since been extensively studied in normal and cancer cells. The activation of CYBB results in the accumulation of reactive oxygen species (ROS) and contributes to the development of drug-resistant phenotypes in acute myeloid leukemia (AML) [37]. We investigated the potential value of CYBB in glioma using data from glioma patients in the CGGA and TCGA databases. The results indicates that CYBB

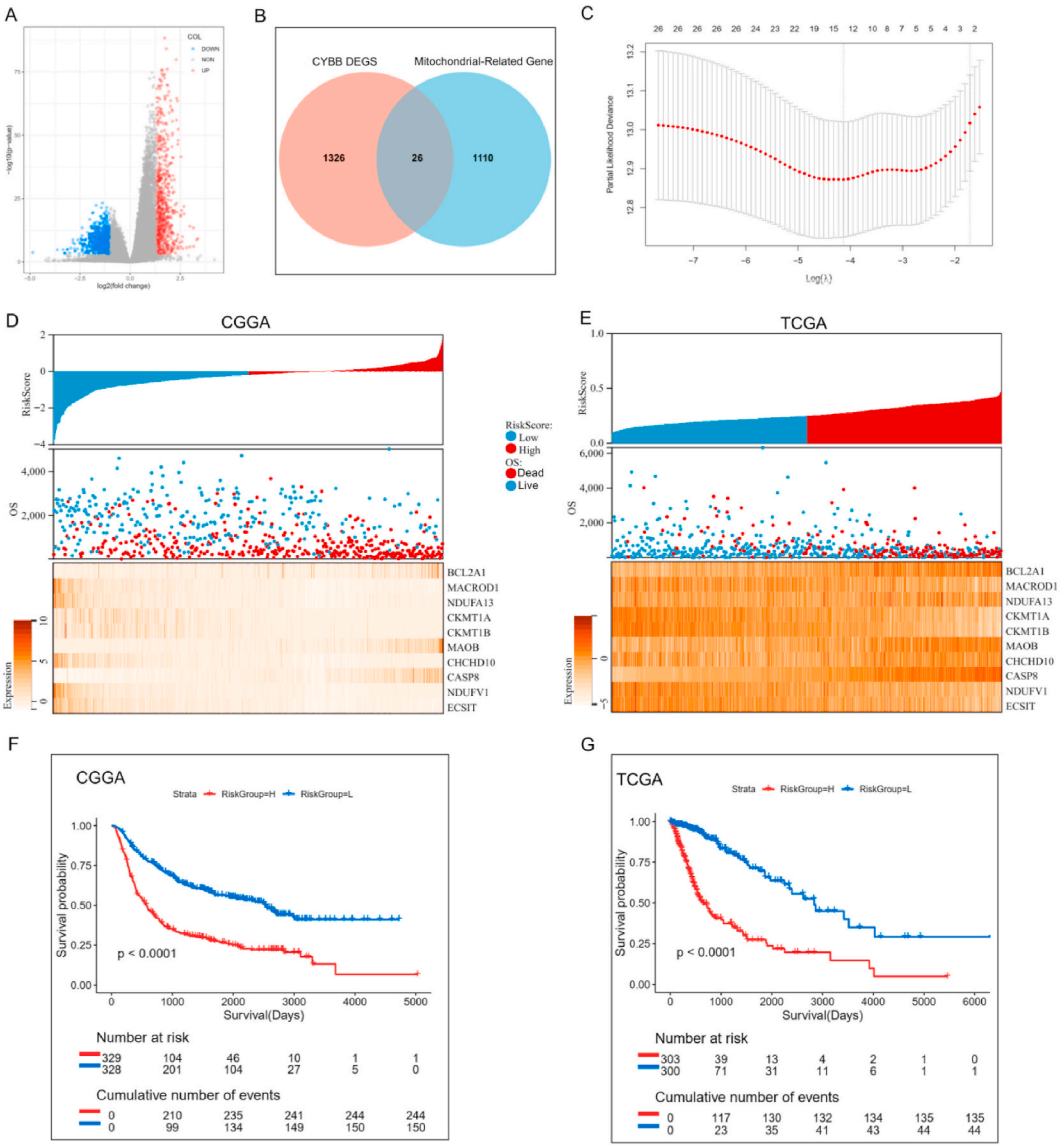


Fig. 8. Screening for genes associated with risk scores. A. Volcano map showing differentially expressed genes in glioma patients grouped by low and high CYBB expression. B. Based on CYBB and mitochondria-related differentially expressed genes. C. An analysis of lasso regression was conducted on 26 candidate genes. D, E. Relationship between risk score and 10 genes and survival status of patients. F. G. Low-risk and high-risk Kaplan-Meier survival curves for glioma patients.

was related to age, WHO grade, 1p/19q codeletion, MGMT promoter methylation and IDH mutation. Through GO and KEGG analysis, CYBB may play a role in the immune and inflammatory response to glioma, and regulate related biological processes. It was also determined that CYBB is not only positively associated with immune checkpoint inhibitors and inflammatory activity, but is also highly expressed in M2 microglia in the tumor microenvironment, potentially affecting tumor cell development. These results suggest that CYBB may be identified as a novel prognostic biomarker and may be an immunologic target for the treatment of glioma.

Mitochondria are responsible for bioenergy metabolism and cellular homeostasis in the body by producing ATP and supplying energy for all life activities. Mitochondrial dysfunction has been linked to various degenerative and metabolic diseases, cancer, and aging [38,39]. Mitochondrial dysfunction is involved in the development of a variety of diseases by altering cellular metabolic pathways, producing disruptions in intracellular redox homeostasis, directly or indirectly causing apoptosis, and generating therapeutic resistance, promoting genetic instability and cancer development [40]. Functional linkage between NOX- and mitochondria-derived ROS (ROS-induced ROS release) has been implicated as a mechanism for amplification and compartmentalization of ROS signaling [41]. Recent evidence suggests that mitochondrial metabolism is essential for tumor growth. Mitochondrial metabolism supports tumor anabolism by providing key metabolites for macromolecular synthesis and producing tumor metabolites. Furthermore, there are several ongoing clinical trials testing the effectiveness of inhibiting mitochondrial metabolism as a novel cancer

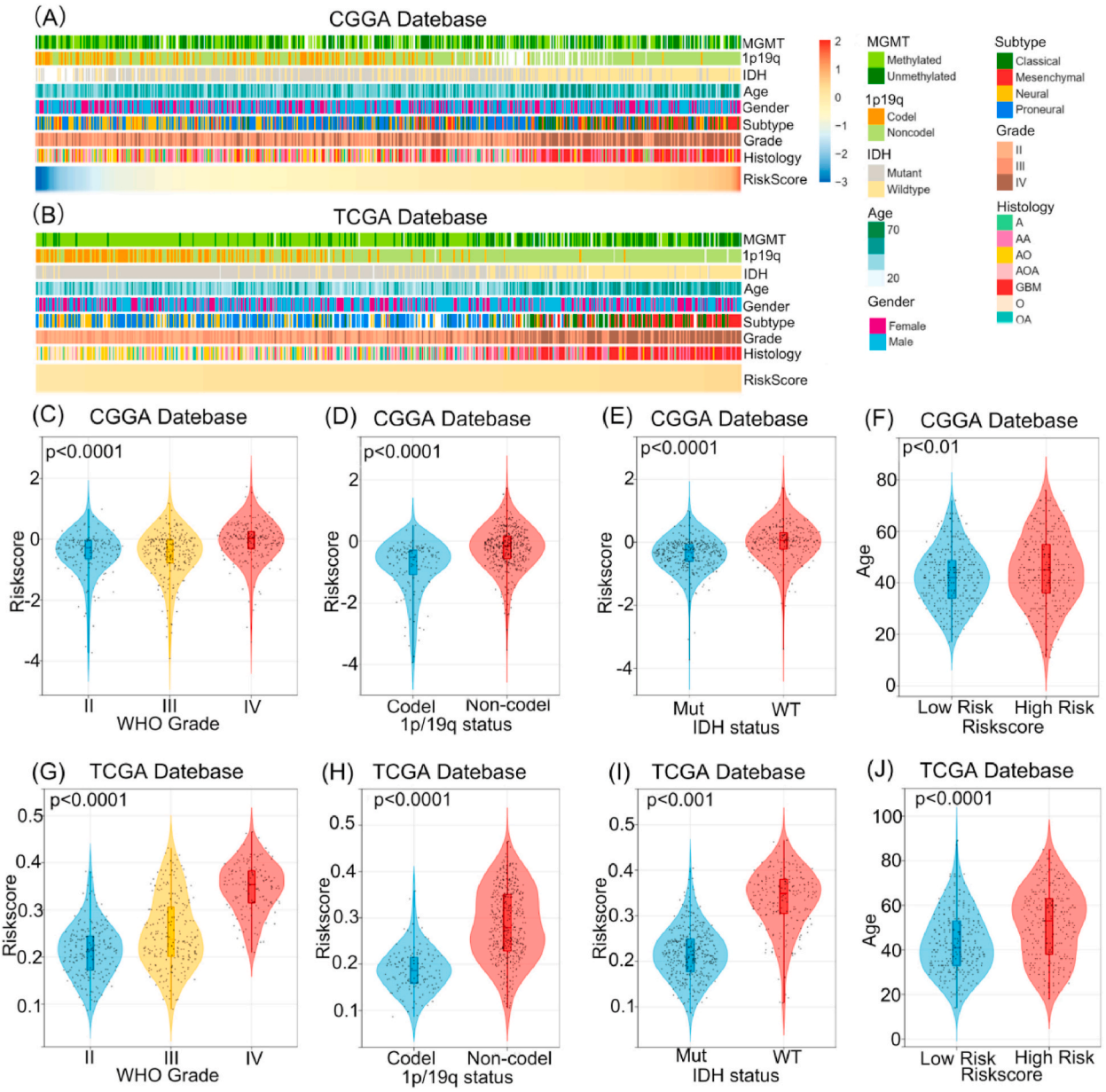


Fig. 9. Relationship of prognostic risk score to clinical variables. A. Overview of prognostic risk score and associated clinicopathological features in CGGA (693). B. Overview of prognostic risk scores and associated clinicopathologic features in TCGA. C-J. Prognostic Risk Score in Relation to WHO Grade, 1p/19q Co-deletion Status, IDH Mutation Status, and Age in Gliomas.

treatment [42].

We pooled differentially expressed genes in glioma patients with low and high CYBB expression with mitochondrial associated genes. The pooled genes were analyzed by lasso regression, and a 10-gene prognostic model based on CYBB and mitochondrial association was constructed. Among the genes included in the model, some have been found to be associated with glioma progression. For example, CKMT1B is a potential prognostic biomarker associated with immune infiltration in low-grade gliomas [43]. OS was significantly shorter in the group of glioma patients with higher expression of CASP8 and was positively correlated with multiple inhibitory immune checkpoints [44]. This supports the effectiveness of our analysis and model building. Glioma patients were divided into low-risk group and high-risk group according to risk score, and Kaplan-Meier curve showed that the prognosis of high-risk group was worse. Finally, to facilitate clinical application, we constructed nomograms based on the above genes, and validated the accuracy of the prediction model using calibration curves and ROC curves. The result shows that the new model has an excellent prediction effect. Since we are bioinformatic and the data are from public databases, we will do more in-depth studies in the future to verify our conclusions and also need to further study the clinical predictive power of our model.

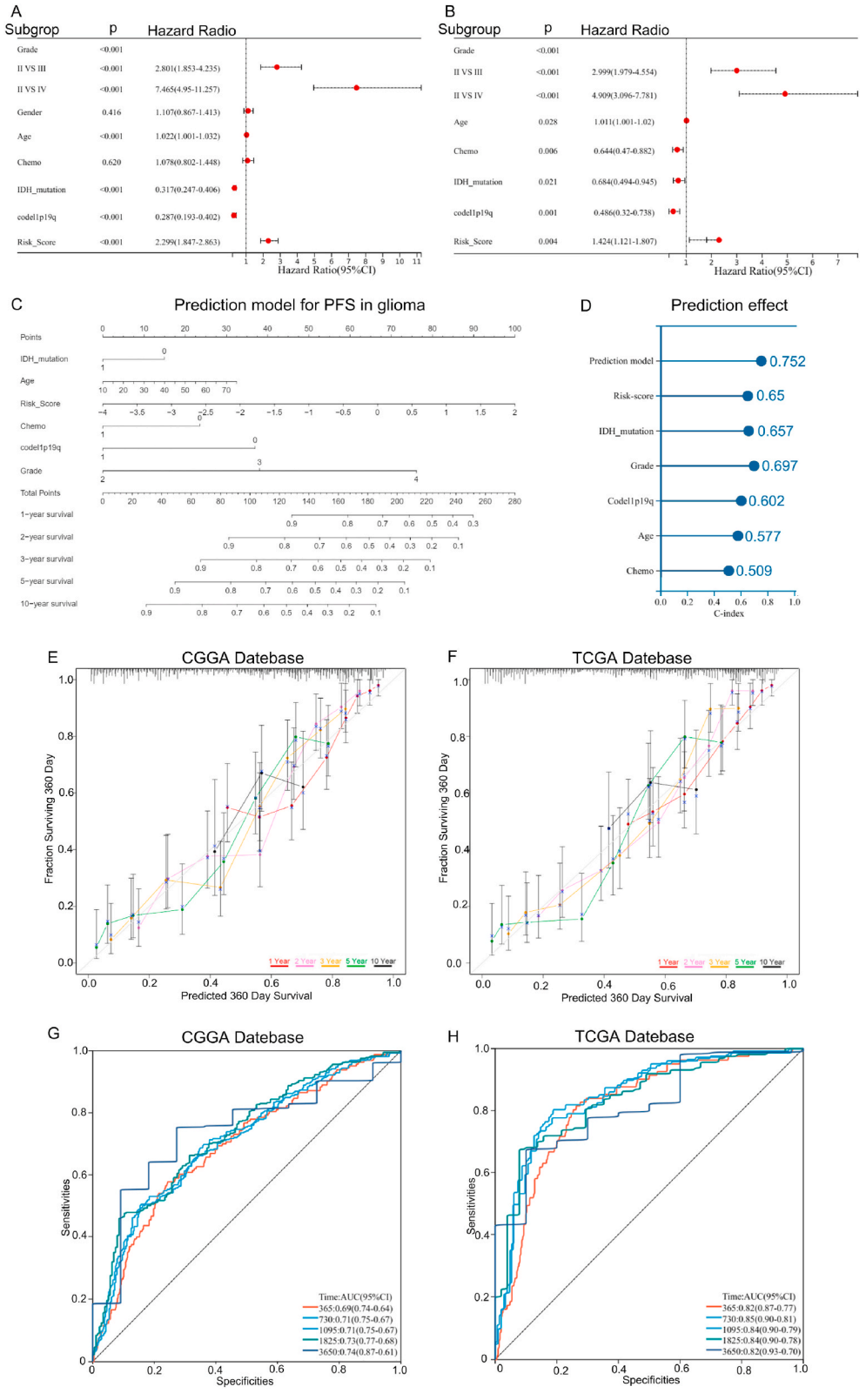


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Fig. 10. Building a prediction model and valuation of prediction model. A. Results of univariate Cox regression analysis based on the CGGA (693). B. Results of multivariate Cox regression analysis based on the CGGA (693). C. Nomogram from CGGA. D. using C index to evaluate the prediction effect of the Nomogram. E. Nomogram calibration curve based on CCGA (693). F. Nomogram calibration curve based on TCGA. G. Establishing a ROC curve of the nomogram according to the CGGA (693). H. ROC curve of nomogram constructed from TCGA.

5. Conclusion

The study indicates that CYBB may be a risk factor for the prognosis of glioma patients and is strongly correlated with disease progression and patient prognosis. Furthermore, a prognostic model based on CYBB and mitochondrial genes was developed, which demonstrated good predictive performance.

Data availability statement

The data presented in the article were all from publicly available databases. Includes the following databases CGGA (http://www.cgga.org.cn); TCGA (*http://cancergenome.nih.gov/*); Human.MitoCarta3.0 Data center (https://www.broadinstitute.org/).

Funding statement

This work was supported by the Major Natural Science Research of Anhui Province University (Grant No. KJ2021ZD0036); Key Natural Science Research of Anhui Province University (Grant No. 2022AH050769); Key research and development plan of Anhui province (Grant No. 2022e07020069).

CRediT authorship contribution statement

Yu Wang: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yuhao Wang:** Methodology, Investigation, Formal analysis, Data curation. **Shuai Wang:** Methodology, Investigation. **Chengcheng Wang:** Formal analysis, Data curation. **Yuhang Tang:** Formal analysis, Conceptualization. **Chao Zhang:** Resources. **Dong Yu:** Resources. **Shiqiang Hou:** Resources, Funding acquisition. **Ning Lin:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29549.

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