CSRP2 is involved in immune regulation and drug sensitivity in glioma cells by the LEF1- AS1/miR-128-3p/CSRP2 axis

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Abstract

Background: Glioma is one of the most common intracranial malignancies. As competing for endogenous RNA (ceRNA), long chain non-coding RNA (lncRNA) can affect the binding of microRNA (miRNA) to mRNA and thus affect the occurrence and development of glioma, which has been suggested as a key role in the development of human cancers. This study was to investigate the effects of TCGA on the development of glioma.

Results: Here, we screened the differential genes by differential analyses on the matrix data for glioma in TCGA and GEO databases and selected the potential prognostic markers among them. Surprisingly, LEF1-AS1 was identified capable of influencing the survival of glioma patients. The results derived from the proportional risk (Cox) regression model showed a significant association between LEF1-AS1 overexpression and poorer prognosis (HR = 1.518, $P < 0.001$). The ROC model validated the feasibility of LEF1-AS1 (AUC = 0.702). MiR-128-3p, the target gene for LEF1-AS1, was screened using the Bioinformatics Online website and found down-regulated with increasing glioma grade in glioma tissues. Then the target gene for miR-128-3p, CSRP2, was obtained through miRTarBase, TargetScan, and miRDB. The survival curve of CSRP2 showed that CSRP2 with low expression corresponded to a better prognosis in glioma patients. The level of CSRP2 was positively correlated with the infiltration ones of CD4+ T and CD8+ T cells in glioma tissues. In addition, the CellMiner database predicted three drugs (Elesclomol, Entinostat, and Staurosporine) with high sensitivity to CSRP2 expression, and their molecular structures were retrieved using the PubChem22 database.

Conclusions: In summary, the LEF1-AS1/miR-128-3p/CSRP2 axis may play a vital role in the development of glioma and provide guiding significance for the early diagnosis and treatment for it.

Introduction

Glioma is one of the most common intracranial malignancies [1], and glioblastoma multiforme (GBM, grade IV) therein acts as the most common and malignant primary brain tumor [2]. Due to a highly aggressive growth pattern, frequent resistance to treatment, as along with tumor heterogeneity and susceptibility, patients with gliomas demonstrate poor prognosis [3]. In recent years, advances in the basics of surgical techniques, chemotherapy, and radiation therapy have contributed little to the survival of patients with glioblastoma [4]. Therefore, studies on the molecular mechanisms underlying glioma invasion and metastasis to find drugs targeting glioma are important for glioma treatment and prognosis.

The competing endogenous RNA (ceRNA) network includes messenger RNA (mRNAs), microRNA (miRNA), and long non-coding RNAs (LncRNAs). LncRNAs, RNA molecules more than 200 bases in length, cannot encode protein [5–8]. LncRNAs have been suggested involved in the development and progression of many tumors, including breast [9], pancreatic [10], and lung cancers [11], in a variety of ways. MiRNAs, RNA molecules not encoding proteins and approximately 22 bases in length, can inhibit

the translation of targeted mRNA transcripts [12]. Among them, the transcripts of lncRNAs possess significant similarities to classical mRNAs. LncRNAs can function as ceRNAs to isolate miRNAs from their target mRNAs by binding to miRNAs or mRNAs, thereby inhibiting miRNAs [13]. Cysteine protein 2 (CSRP2), containing two LIM structural domains as a member of the LIM pure family of cysteine-rich proteins [14], is widely expressed in various tissues [15]. Among them, LIM proteins participate in many cellular functions by regulating protein-protein interactions and play a vital part in various biological processes, such as signaling transduction, cytoskeleton formation, cell proliferation, and differentiation [16]. In addition, the research findings suggest a key role of CSRP2 in gastric [17], colorectal [18], and breast cancers [19]. However, whether it is regulated by lncRNA or miRNA and affects the biological activity of glioma remains unclear.

In the present work, we constructed ceRNA regulatory networks employing bioinformatics tools to identify key genes in glioma and predict derived drugs that may treat glioma. Our findings may contribute to the development of new therapeutic targets for GBM.

Results

Identification of differentially expressed genes and construction of lncRNA-miRNA-mRNA network diagram

LncRNAs, miRNAs, and mRNAs were anomalously expressed in gliomas compared to normal brain tissues. To explore its potential biological functions, a total of 3914 differential genes were identified in the TCGA database, including 407 up-regulated lncRNAs, 577 down-regulated lncRNAs, 1340 up-regulated mRNAs, and 1590 down-regulated mRNAs (Fig. 1A, B). We used the R package "survival" to perform KM survival analyses on differential genes with $P < 0.001$ as the screening condition using TCGA data, and identified three lncRNAs (AC080013.1, LINC01574, and LEF1-AS1) with significant survival effects on glioma patients (Fig. 1C, Supplementary Fig. 1A, B). To increase the accuracy, we used the online site of GEPIA for survival validation of these 3 genes and found that LINC01574 and LEF1-AS1 had more impact on patient survival duration, with lncRNA-AC080013.1 exerting no impact on that, however (Fig. 1D, Supplementary Fig. 1C, D). The expression profile microarray of GSE41032 detecting the expression of miRNAs between glioma stem cells (GSC) and normal neural ones (NSC), was performed using GEO2R ($|\log2FC| \geq 1$, $P \leq 0.05$). The results showed that 31 elevated differential expression miRNAs (DEmiRNAs) and 69 decreased ones in the GSE41032 microarray (Fig. 1E). We predicted 134 miRNAs targeting LINC01574 and LEF1-AS1 using the miRcode online website. Subsequently, the differential miRNAs in GSE41032 were intersected with miRNAs targeting lncRNA. A total of 4 DEmiRNAs were identified, namely miR-27a-3p, miR-670-5p, miR-128-3p, and miR-138-5p (Fig. 1F). Of particular note, LEF1-AS1 has a targeting relationship with miR-27a-3p, miR-670-5p, and miR-128-3p, and LINC01574 with miR-138-5p. Subsequently, we used three online databases, miRTarBase, TargetScan, and miRDB, to predict the target proteins for miR-27a-3p, miR-670-5p, miR-128-3p, and miR-138-5p, and a total of 226 mRNAs targeting the above four miRNAs were obtained. There were only 24 mRNAs after taking intersection with differential mRNAs in TCGA (Fig. 1G). GO analyses were conducted using the Metascape online database

and found these 24 mRNAs focused on the regulation of the G1/S cell cycle, regenerating glial cells, regulation of protein complex assembly, and actin structural organization (Fig. 1H). Then, we constructed a network of lncRNA-miRNA-mRNA with targeting relationships and analyzed it using Cytoscape software (Fig. 1I). Meanwhile, the top 10 degrees of lncRNAs, miRNAs, and mRNAs were identified using Cytoscape's plug-in Cytohubba (Fig. 1J). The aforementioned 10 genes were selected as the key genes for this study.

Expression of key genes in glioma and normal samples

To determine the differences in expression of key differential genes in glioma tissues versus normal ones, we used the GEPIA database to query their expression. The results showed that lncRNA-LEF1-AS1 was up-regulated in GBM tumor tissues (Fig. 2A). Four mRNAs, PDIA5 (Fig. 2B), WEE1 (Fig. 2C), CSRP2 (Fig. 2D), and ABCA1 (Fig. 2E) were up-regulated and one mRNA (RELN) was down-regulated (Fig. 2F). In addition, we used GSE41032 to derive the expression of miR-27a-3p, miR-670-5p, and miR-128-3p which has a targeting relationship with LEF1-AS1 in normal neural stem cells versus glioma ones. Low levels of miR-128-3p (Fig. 2G) and miR-670-5p (Fig. 2H) were found in glioma stem cells (Tumor) ($P < 0.05$), while the expression of miR-27a-3p was not statistically significant (Supplementary Fig. 1E). To establish an important ceRNA with significant prognostic value in GBM, we selected miR-128-3p, possessing the most obvious difference in GSE41032, for the subsequent study. Therefore, according to the ceRNA principle, we selected the LEF1-AS1/miR-128-3p/WEE1, PDIA5, CSRP2, ABCA1 axes. Then, we analyzed the levels of lncRNAs, miRNAs, mRNAs in different glioma grades of GBM. The results showed that LEF1-AS1(Fig. 2I), along with the mRNAs of CSRP2 (Fig. 2K), PDIA5 (Fig. 2L), and WEE1 (Fig. 2M) increases with glioma grade, while miR-128-3p (Fig. 2J) decreases with that. And ABCA1 expression was less correlated with glioma grade (Fig. 2N).

Clinical significance of LEF1-AS1 and CSRP2 in patients with glioma

To determine whether the more correlated RNAs were associated with GBM prognosis, OS analyses on GBM patients were performed using the online database CGGA. As a result, one DEmiRNA (Fig. 3A) and four DEmRNAs (PDIA5, WEE1, CSRP2) were found associated with prognosis ($P < 0.001$) (Fig. 3B-D). These data suggest that LEF1-AS1 probably promotes the expression of WEE1, PDIA5, and CSRP2 by sponging miR-128-3p as a ceRNA. Furthermore, expression correlation analyses showed that the expression of WEE1, PDIA5, and CSRP2 was positively correlated with that of LEF1-AS1. And we selected CSRP2 which has not been studied in glioma for the subsequent study (Fig. 3E-G). Next, the LEF1-AS1/miR-128-3p/CSRP2 axis in the ceRNA network was selected as a potential predictive model. To determine whether the levels of the LEF1-AS1 and CSRP2 are influenced by clinical characteristics, we performed the correlation between levels of LEF1-AS1 as well as CSRP2 and clinical factors. Therefore, univariate and multivariate Cox regression analyses were performed to identify OS-related features. In the univariate Cox regression model for LEF1-AS1, certain prognostic factors (glioma grade, surgical treatment, TMZ treatment, IDH, and 1p19q) were strongly associated with OS in the TCGA cohort of glioma patients, and LEF1-AS1 (hazard ratio [HR] = 1.518, $P < 0.001$) overexpression was significantly

associated with poorer prognosis (Fig. 3H, J). However, in the univariate Cox regression model for CSRP2, certain prognostic factors in the TCGA cohort of glioma patients (glioma grade, surgical treatment, TMZ treatment, IDH, and 1p19q) were strongly associated with OS, and CSRP2 (HR = 1.004, $P < 0.001$) overexpression were significantly associated with poorer prognosis (Fig. 3J). Meanwhile, multifactorial Cox regression analyses on LEF1-AS1 showed significant association between high expression of LEF1-AS1 and poor prognosis (LEF1-AS1 HR = 1.219, $P = 0.006$, CSRP2 HR = 1.002, $P = 0.005$) (Fig. 3I). And multifactorial Cox regression analyses on CSRP2 showed that a high level of CSRP2 was significantly associated with poor prognosis (CSRP2 HR = 1.002, $P = 0.005$) (Fig. 3K). Using the R language, we derived the risk scores of LEF1-AS1 and CSRP2 based on which the patients were divided into high-risk and low-risk groups, respectively. As shown in Figs. 3L and 3M, patients with glioma died earlier in the high-risk group for LEFA1-AS1 and CSRP2. We also analyzed the disease-specific survival (DSS) of both groups, and glioma patients in the high-risk group presented shorter DSS (Fig. 3N, O). In addition, we also validated the ROC prediction model, and the AUC values of LEF1-AS and CSRP2 were 0.702 and 0.747, respectively, demonstrating the feasibility of our model (Fig. 3P, Q). Therefore, LEF1-AS1 and CSRP2 may be independent prognostic factors for patients with glioma.

Prognostic value of CSRP2 low expression in glioma tissues

Based on the aforementioned, we conclude that CSRP2 plays an important role in the development and progression of glioma. Next, we explored its further potential role in glioma. Immunohistochemical (IHC) staining using the Human Protein Atlas (HPA) database confirmed high expression of CSRP2 in glioma tissues and low in normal ones (Fig. 4A). It has been shown that IDH mutations promote gliomas by disrupting chromosomal topology and permitting aberrant modulation of the interplay inducing oncogene expression. Unlike primary glioblastomas and IDH gliomas which are fast-growing and treatment-resistant, gliomas harboring IDH mutations grow slowly and have a relatively good prognosis. And, oligodendrogliomas with 1p/19q codeletion, the prototypical IDH mutant cancer, display both enhanced radio- and chemo-sensitivity. In particular, lower expression of CSRP2 was found in IDH mutant patients (Fig. 4B) who have better overall time than the wild-type ones (Fig. 4C). Codeletion of chromosome arms 1p and 19q (1p/19q codeletion) highly benefit diagnosis and prognosis in gliomas. And CSRP2 low expression was lower in the 1p/19q codeletion phenotype (Fig. 4D) and the combined deletion of 1p/19q would be more favorable to the prognosis of patients (Fig. 4E). The above results suggest that low expression of CSRP2 may be associated with the development of glioma and patient healing.

Relationship between CSRP2 expression and immune cells in glioma tissues

In some tumors, tumor-infiltrating lymphocytes (TIL) are independent predictors for sentinel lymph node (SLN) status and survival. TIMER was employed to investigate the relationship between CSRP2 expression and immune infiltration levels in glioma tissues. Firstly, analysis of the "Gene" module showed that CSRP2 expression was significantly correlated with tumor purity and positively correlated with the infiltration levels of CD4 + and CD8 + T cells in glioma tissues (Fig. 5A, B). Then, we further evaluated the correlation between CSRP2 expression and immune cell marker genes, and CSRP2 expression was shown

positively correlated with immune cell marker genes of CD4 and CD8B (Fig. 5C, D). Meanwhile, the results showed that the expression of CSRP2 was positively correlated with immune checkpoints of EGF and DDX60 (Fig. 5E, F). In conclusion, CSRP2 may be closely related to the development of glioma.

GSEA functional annotation and drug sensitivity

GSEA was applied to investigate the relationship between CSRP2 expression in glioma samples and cancer-related signaling pathways based on the clinically and FDA-validated data from the CellMiner database. The enrichment of glutathione metabolism, oxidative phosphorylation, glycine, serine and threonine metabolism, peroxisome, pyruvate metabolism, and fatty acid metabolism in the group with high expression of CSRP2 compared with the group with low expression of CSRP2 (Fig. 6A). Meanwhile, the CellMiner database was used to predict the potential therapeutic agents for CSRP2. It was found that drugs of Elesclomol (Fig. 6B), Entinostat (Fig. 6C), and Staurosporine (Fig. 6D) have high sensitivity to CSRP2, and it is reasonable to believe that these three drugs could be effective for glioma treatment. In addition, we have identified the molecular structures of Elesclomol (Fig. 6E), Entinostat (Fig. 6F), and Staurosporine (Fig. 6G), contributing to their application in the treatment of glioma.

Conclusions

We successfully predicted the LEF1-AS1/miR-128-3p/CSRP2 ceRNA network with the help of TCGA, GEO, and CGGA online databases, which contributed to our understanding of glioma development, progression, and invasion, and the ROC model predicted the feasibility of our model and explored that CSRP2 expression was positively correlated with CD4 + and CD8 + immune cells. Three targeted agents were then also identified, providing new ideas for the treatment of glioma.

However, there are some limitations to our study. First, our sample size is still too small, and we only made use of TCGA, GEO, and CGGA databases for our study. Secondly, we did not perform correlative clinical trials on key genes in the ceRNA network. Finally, we only identified the LEF1-AS1/miR-128-3p/CSRP2 axis by making predictions on lncRNAs. However, one thing we must note is that the ceRNA mechanism is very complex and remains to be further investigated.

Material And Methods

Data sources construction of lncRNA-miRNA-mRNA network diagram

To identify and compare the gene expression between GBM and normal tissues, we searched and downloaded the gene expression data and clinical information related to glioma patients from the Cancer Genome Atlas (TCGA, https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga), and also used the R language "edgeR" package to select the differential genes, with $P < 0.01$, $|\log2FC| \geq 2$ as the threshold. In addition, we combined the clinical data with $KM < 0.001$ (Kaplan-Meier analysis) conditions to perform overall survival (OS) analyses of glioma patients to identify survival-related differential genes. To improve accuracy, we employed the Gene Expression Profile

Interaction Analysis (GEPIA, http://gepia.cancer-pku.cn/) database for differential genes. The widely used Gene Expression Omnibus database (GEO) was searched and from which the publicly available microarray expression profile dataset of GSE41032 was downloaded. GSE41032, composed of 3 cases of glioma stem cells (GSC) versus 3 normal neural ones (NSC), was identified using GEO2R with $P \leq 0.05$ and $|\log2\ FC| \geq 1$ as the threshold to identify the differential miRNAs among them. Then, the above differential genes were employed to construct the lncRNA-miRNA-mRNA network diagram. First, we used the miRcode online database (http://www.mircode.org/) to predict key lncRNA downstream genes based on the lncRNAs identified above. In addition, to verify the generalizability and credibility of further supporting data, we used miRTarBase (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php), TargetScan (http://www.targetscan.org/vert_80/), and miRDB (http://mirdb.org/) databases to predict the target genes for differential miRNAs (DEmiRNA) and select those common in all of the three databases. The online tool Venny (https://bioinfogp.cnb.csic.es/tools/venny/index.html) was also used to construct a Venn diagram with TCGA differential mRNAs (DEmRNA) and identify the genes shared among them. Then the Metascape online database (https://metascape.org/gp/index.html#/main/step1) was applied to analyze their potential functions with enrichment analyses. Meanwhile, lncRNA-miRNA-mRNA targeting relationship data were imported into Cytoscape 3.6.0 to construct the lncRNA-miRNA-mRNA network graph. And visualization in Cytoscape using cytoHubba plugin to identify the extent of genes in the network and key genes in it was performed. The expression of screened key genes was employed to verify whether the hypothesis of low ceRNA expression and poor prognosis was satisfied through GEPIA.

Survival and correlation analyses as well as the selection of models with glioma specificity

Data from datasets in the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn/) database and clinical information were used to predict the expression of key genes and the effects on the survival of glioma patients. Meanwhile, the ceRNA hypothesis suggests that levels of lncRNAs and miRNAs are negatively and positively correlated with mRNAs expression, respectively. Therefore, the CGGA database was used to predict the correlation among key genes.

Clinical significance of the LEF1-AS1 and CSRP2 in patients with glioma

We analyzed the clinical significance of the LEF1-AS1 and CSRP2 in glioma patients and calculated risk scores to determine the relationship between risk scores and survival status of glioma patients concerning glioma grade, surgical treatment, TMZ treatment, and mutations in isocitrate dehydrogenase (IDH) and deletion of 1p19q. And we also validated the feasibility of the model.

Prognostic value of low CSRP2 expression in glioma tissues

The ROCR and pROC packages in R were used to compute receiver operating characteristic (ROC) curve, ROC AUC, and P-values between ROC curves. We then applied ROC analyses to calculate the expression of LEF1-AS1 and CSRP2 as well as the area under the ROC curve (ROC-AUC). ROC-AUC was to measure ROC performance. For the prognostic value of low expression of CSRP2, we used immunohistochemical

(IHC) staining from the Human Protein Atlas (HPA) database to verify the tissue changes corresponding to the high expression of CSRP2. Using clinical data from patients in the CGGA Chinese glioma database, we analyzed the expression of IDH and 1p/19q combined deletion in glioma patients and discussed the prognostic impact of both on the prognosis of glioma patients.

Relationship between CSRP2 expression and immune cells in glioma tissues

TIMER uses RNA-Seq expression profiling data to detect the infiltration of immune cells in tumor tissues. Among them, TIMER provides the infiltration of 6 types of immune cells (B, CD4 + T, CD8 + T, Neutrophils, Macrophages, and Dendritic cells). Using TIMER, we explored the relationship between CSRP2 expression and immune cells and relevant markers in glioma tissues using R language. In addition to this, we also investigated the correlation between CSRP2 expression and immune checkpoints to explore the possible implications of CSRP2 expression concerning immune cells in the development of glioma.

GSEA functional annotation and drug sensitivity

To explore the effect of gene expression on this pathway, a GSEA enrichment analysis was performed. First, we extracted the CSRP2 expression data of TCGA-GBM. We used the first 50% of CSRP2 expression as the high expression group and the last 50% as the low one. All mRNAs in TCGA were selected and GSEA analysis was performed using the GSEA desktop version (version: 4.0.320, version 4.0.3) with FDR $q < 0.05$ and $P < 0.05$ as screening conditions. In parallel, CSRP2 was used to perform sensitivity prediction of clinically and FDA-validated drugs from the CellMiner database (https://discover.nci.nih.gov/cellminer/home.do). CellMiner is based on 60 cancer cells listed by the National Cancer Institute (NCI) for correlating specific anticancer and other drugs with their targets and gene expression. The PubChem22 database (https://pubchem.ncbi.nlm.nih.gov/) was used to retrieve the molecular structures of the identified drugs.

Discussion

GBM, the highest grade of malignant astrocytoma (WHO grade IV), is the most common and fatal primary central nervous system (CNS) tumor in adults [20–21]. Despite the availability of surgical radiotherapy treatment for glioma, its prognosis remains poor [22]. Therefore, it is particularly important to investigate its pathogenesis. In this study, we identified potential prognostic markers for GBM by comprehensive bioinformatics analyses and searched for targeted drugs with high sensitivity to these markers, which might contribute to new ideas and insights for GBM treatment.

ceRNAs can regulate gene expression by competitively binding microRNAs which can cause gene silencing by binding mRNAs [23]. ceRNA mechanisms have been shown involved in multiple tumors [24–25]. HMGA2 functions as a competing endogenous RNA to promote lung cancer progression [26], STAT3-mediated upregulation of lncRNA HOXD-AS1 as a ceRNA facilitates liver cancer metastasis by regulating SOX4 [27], and LINC01133 as ceRNA inhibits gastric cancer progression by sponging miR-106a-3p to regulate APC expression and the Wnt/beta-catenin pathway [28] and affect tumor development in

different ways [29–30]. Meanwhile, based on the ceRNA hypothesis, we gradually predicted downstream miRNAs and mRNAs from lncRNAs and finally successfully constructed the ceRNA network, in which all genes were associated with a poor prognosis for GBM. Importantly, our model fully conforms to the rules of the ceRNA network.

Through Pubmed search, we found that miR-27a-3p targets the GSK3 vector and promotes proliferation and migration of triple-negative breast cancer through the Wnt/ catenin pathway [31]. Moreover, inhibited the malignant progression of prostate cancer and promoted the proliferation of hepatocellular carcinoma cells by miR-138-5p through targeting FOXC1 [32] and FOXC1 through PROX1 [33], respectively, was also found. However, only miR-128-3p fits the ceRNA rule and has not been studied in glioma. In this experiment, miR-128-3p was found to possess a target binding site with CSRP2 through the database, and it is reasonable to speculate that miR-128-3p is involved in the progression of glioma development.

CSRP2 has been shown to promote the proliferation and cell cycle progression of human B-cell lymphoma Ramos cells through activating the Erk - CREB pathway: Pb1650, while no relevant data on CSRP2 and glioma has been reported [34]. In this paper, the oncogene CSRP2, among the differential genes in glioma, was identified by bioinformatics, and patients with high expression of CSRP2 showed significantly lower survival rates. The present work suggests the involvement of CSRP2 in the development and progression of glioma in several directions.

Here, we verified the relationship between LEF1-AS1 and CSRP2 expression in clinical samples and found that abnormally low expression of CSRP2 and LEF1-AS1 in GBM was strongly associated with age, sex, surgical treatment, TMZ treatment, IDH, and 1p19q. And IDH mutability was shown more dangerous compared to the wild-type [35]. The expression profile of the 1p19q gene is closely related to the malignancy and prognosis of 1p/19q non-coding glioma [36]. And the feasibility of our model was verified by the ROC model.

Previous studies have reported that immune infiltration can affect patient prognosis [37–38]. In this study, CSRP2 expression in glioma tissues was significantly correlated with tumor purity, infiltration levels of CD4 + T and CD8 + T cells, and immune cell markers of CD4 and CD8B in glioma tissues, which opens up the possibility of early diagnosis and treatment of glioma. In addition, GSEA enrichment pathways exhibited that high expression of CSRP2 was significantly enriched in multiple amino acid metabolic pathways, while there is clear literature demonstrating that tumor cells reorganize metabolic pathways to accommodate their increased energy nutrient requirements, reduced equivalents, and cellular biosynthesis [39–40]. In addition, using the CellMiner database Elesclomol, Entinostat, and Staurosporine, three drugs with high sensitivity to CSRP2, which are clinically validated and FDA-validated, and identifying the molecular results of these three drugs, it is expected that studies will discover targeted drugs for glioma.

In conclusion, we established a network of ceRNAs (LEF1-AS1-miR-128-3p-CSRP2) associated with glioma prognosis, which helps to understand the correlation among lncRNA,miRNA, and mRNAs. In

addition, we found that the ceRNA-based LEF11-AS1/CSRP2 axis may be a new important prognostic factor involved in GBM, which contributes to the investigation of pathogenesis and treatment for GBM.

Declarations

DATA AVAILABILITY

All data collected and used in the study are publicly available. All the procedures were performed in accordance with the relevant guidelines and regulations. The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

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AUTHORS'CONTRIBUTIONS

ZW, JLY, CL, CXL, YCG, LW, and LHL designed the study. ZW, JLY, CL, and CXL performed the bioinformatics analysis and interpretation of the data. ZW, JXY, CL, and CXL wrote the manuscript. LW and LHL revised the manuscript and gave final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

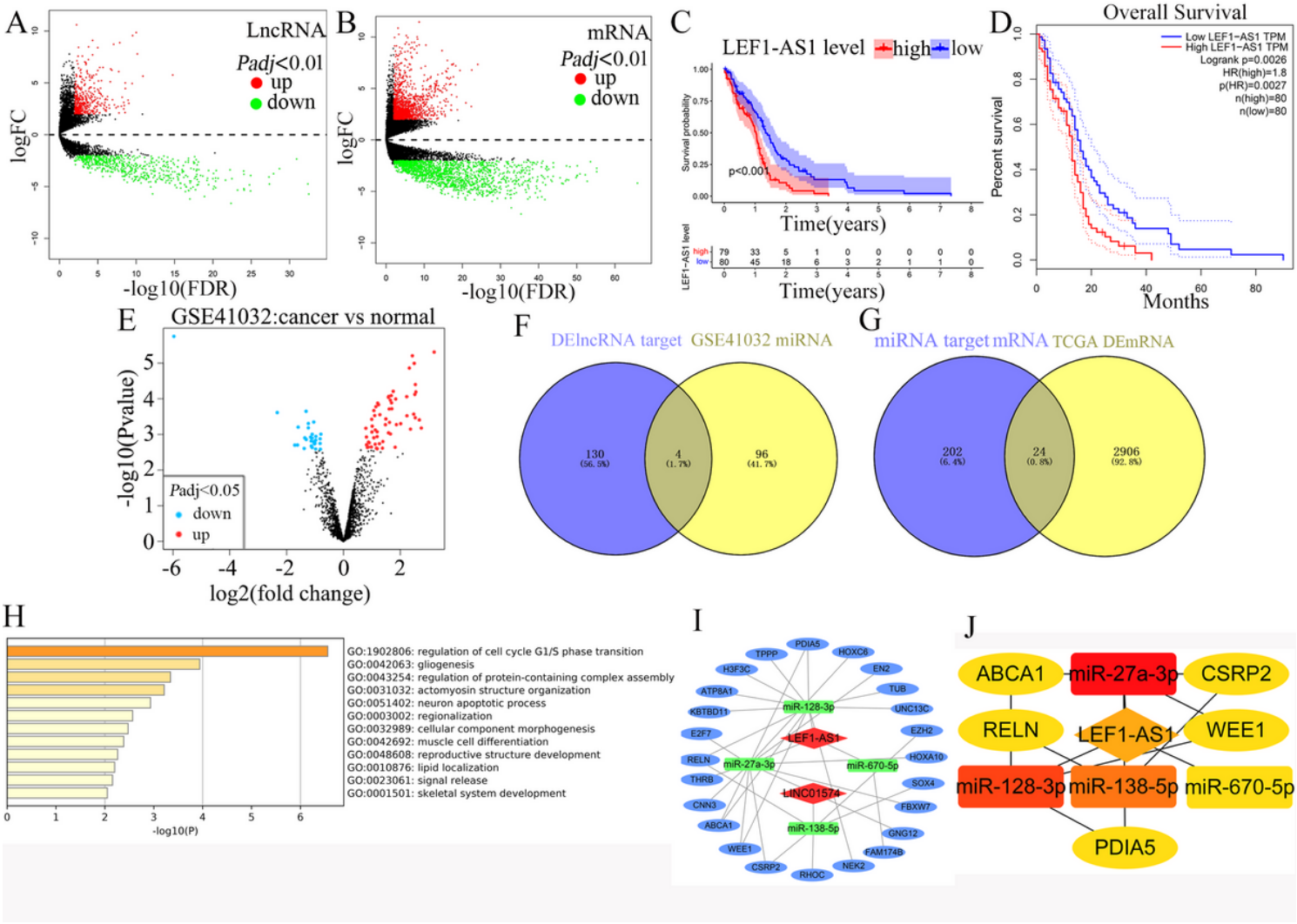

Figure 1

Identification of differentially expressed genes and construction of lncRNA-miRNA-mRNA network diagram. (A) Volcano plot of differential lncRNAs in the TCGA database. The red and green spots represent up-regulated and down-regulated lncRNAs with the cutoff criteria of |log2FC| ≥ 2 and $P < 0.01$. The black spots represent lncRNAs with no prominent expression difference. (B) Volcano plot of differential mRNAs in the TCGA database. The red and green spots represent up-regulated and down-regulated mRNAs with the cutoff criteria of |log2FC| ≥ 2 and adjusted $P < 0.01$. The black spots represent mRNAs with no prominent expression difference. (C) Survival curves of LEF1-AS1 in patients with glioma using TCGA data. (D) Survival curves of LEF1-AS1 in patients with glioma using GEPIA. (E) Volcano plot of differential miRNAs in the GSE41032. The red and green spots represent up-regulated and down-regulated miRNAs with the cutoff criteria of |log2FC| ≥ 1 and $P \leq 0.05$. The black spots represent miRNAs with no prominent expression difference. (F) Venn diagram of differential miRNAs in GSE41032 and lncRNA targeted miRNAs. (G) Venn diagram of target gene mRNAs targeting miRNAs and mRNAs in the TCGA database. (H) GO enrichment analyses on 24 mRNAs. (I) LncRNA-miRNA-mRNA network diagram. The diamond, rectangle, and oval represent lncRNAs, miRNAs, and mRNAs respectively. (J) The

first 10 degrees of ligated mRNAs, miRNAs, and lncRNAs. The diamond, rectangle, and oval represent lncRNAs, miRNAs, and mRNAs, respectively.

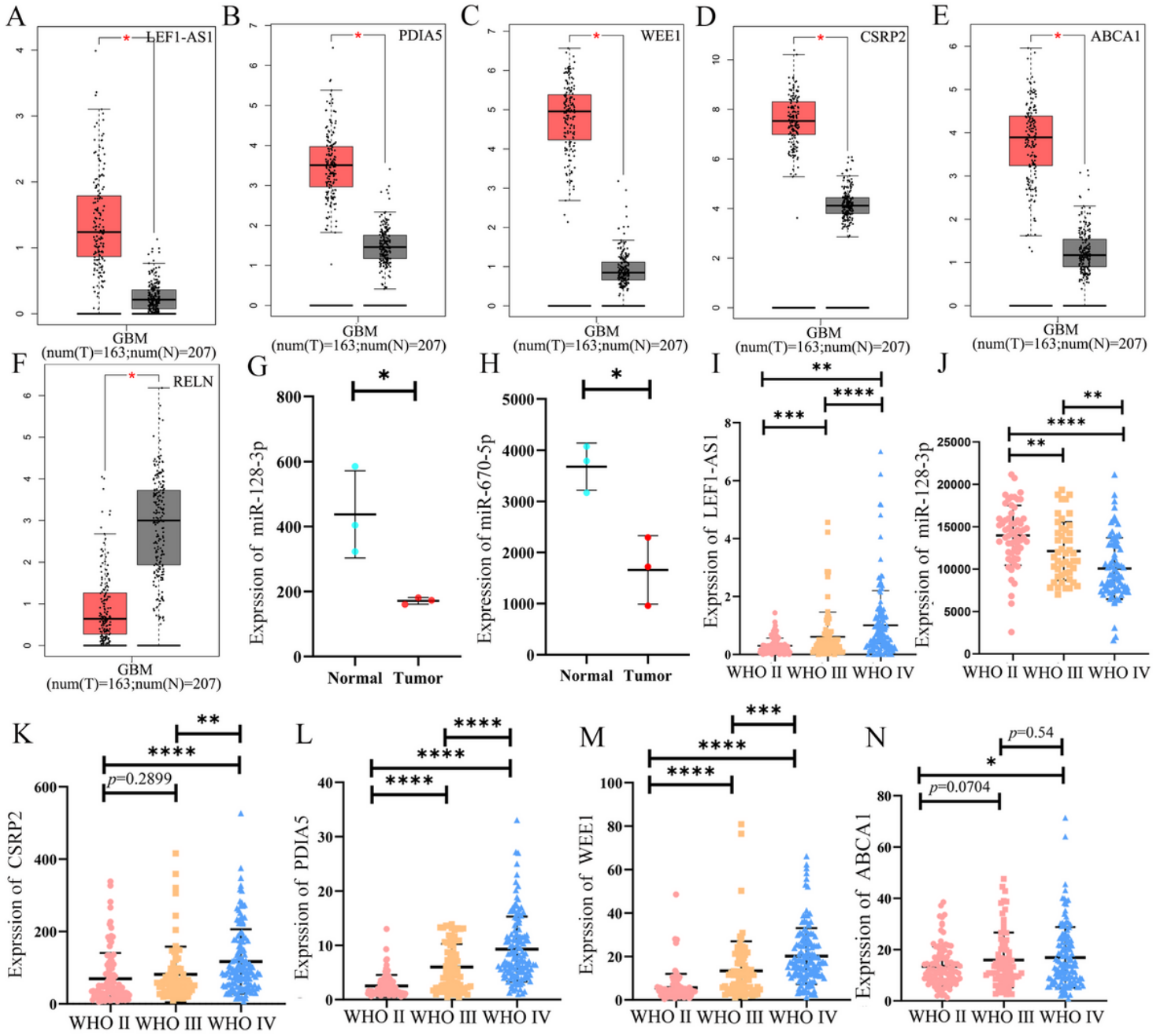

Figure 2

Expression of key genes in glioma and normal samples. (A-F) Expression of LEF1-AS1, PDIA5, WEE1, CSRP2, ABCA1, and RELN in GEPIA. (G-H) Expression of miR-128-3p and miR-670-5p in normal neural stem cells (Normal) and glioma ones (Tumor). (I-N) Expression of LEFA-AS1, miR-128-3p, CSRP2, PDIA5, WEE1, and miR-128-3p in CGGA in patients with different glioma grades. *$P < 0.05$ **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

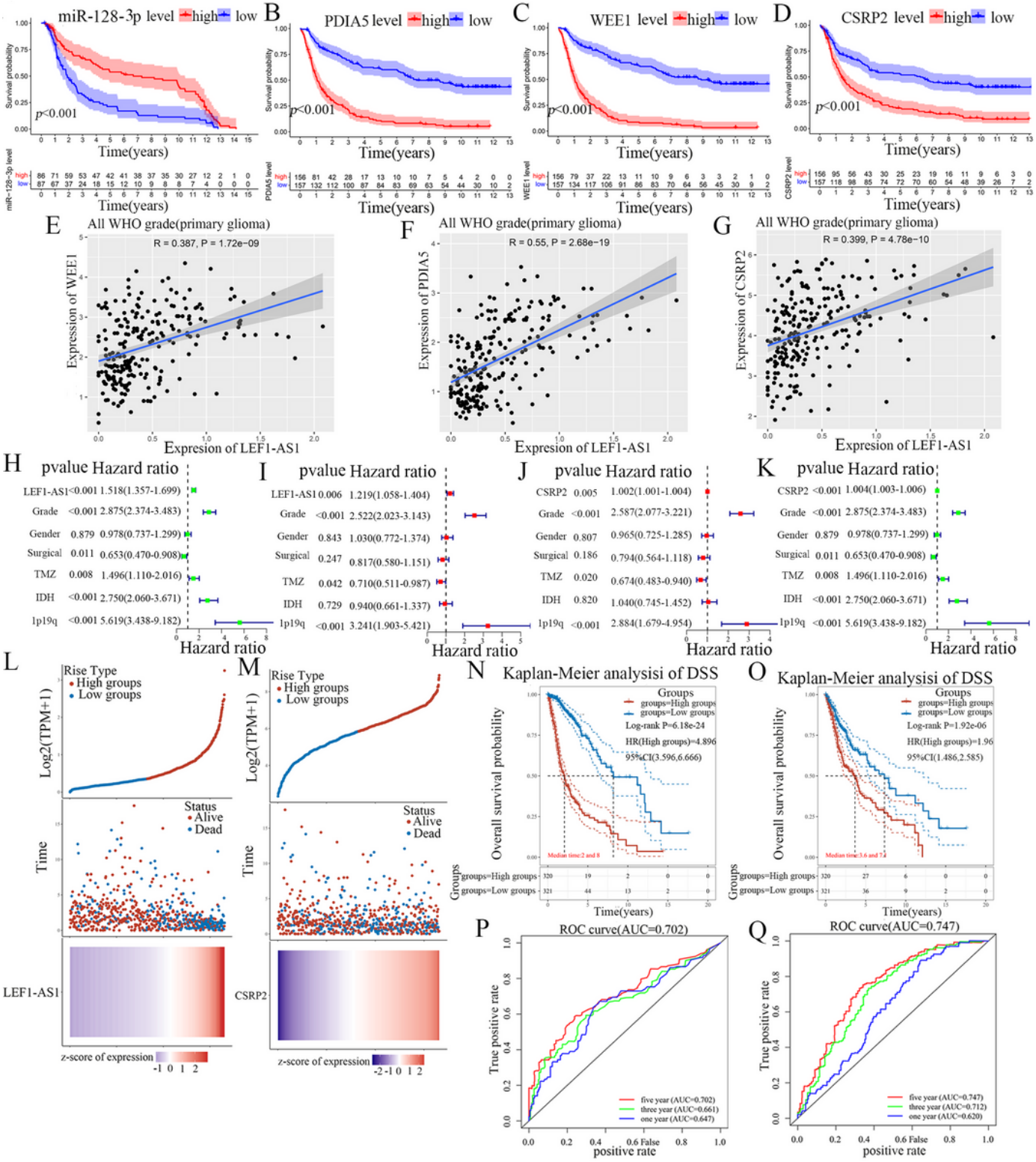

Figure 3

Clinical significance of LEF1-AS1 and CSRP2 in patients with glioma. (A) Relationship between miR-128-3p expression and prognosis for glioma patients. (B) Relationship between PDIA5 expression and prognosis for glioma patients. (C) Relationship between WEE1 expression and prognosis for glioma patients. (D) Relationship between CSRP2 expression and prognosis for glioma patients. (E) Expression of WEE1 is positively correlated with the expression of LEF1-AS1. (F) Expression of PDIA5 is positively

correlated with the expression of LEF1-AS1. (G) Expression of CSRP2 is positively correlated with the expression of LEF1-AS1. (H) Forest plot of univariate Cox regression between LEF1-AS1 expression and glioma prognosis in CGGA cohort. (I) Forest plot of multivariate cox regression of between LEF1-AS1 expression and prognosis of glioma CGGA cohort. (J) Forest plot of univariate Cox regression between CSRP2 expression and glioma prognosis in CGGA cohort. (K) Forest plot of multivariate cox regression of CSRP2 expression and prognosis of glioma CGGA cohort. (L) Classification of glioma patients according to median values of LEF1-AS1 expression and survival status of glioma patients. (M) Glioma patients classification based on the median value of CSRP2 sample expression. (N) Glioma patients from high-risk groups of LEF1-AS1 exhibited shorter DSS than that in the low-risk one. (O) DSS of glioma patients in CSRP2 high-risk groups were shorter than those in the low-risk group. (P) ROC model validation of LEF1-AS1. (Q) ROC model validation of CSRP2.

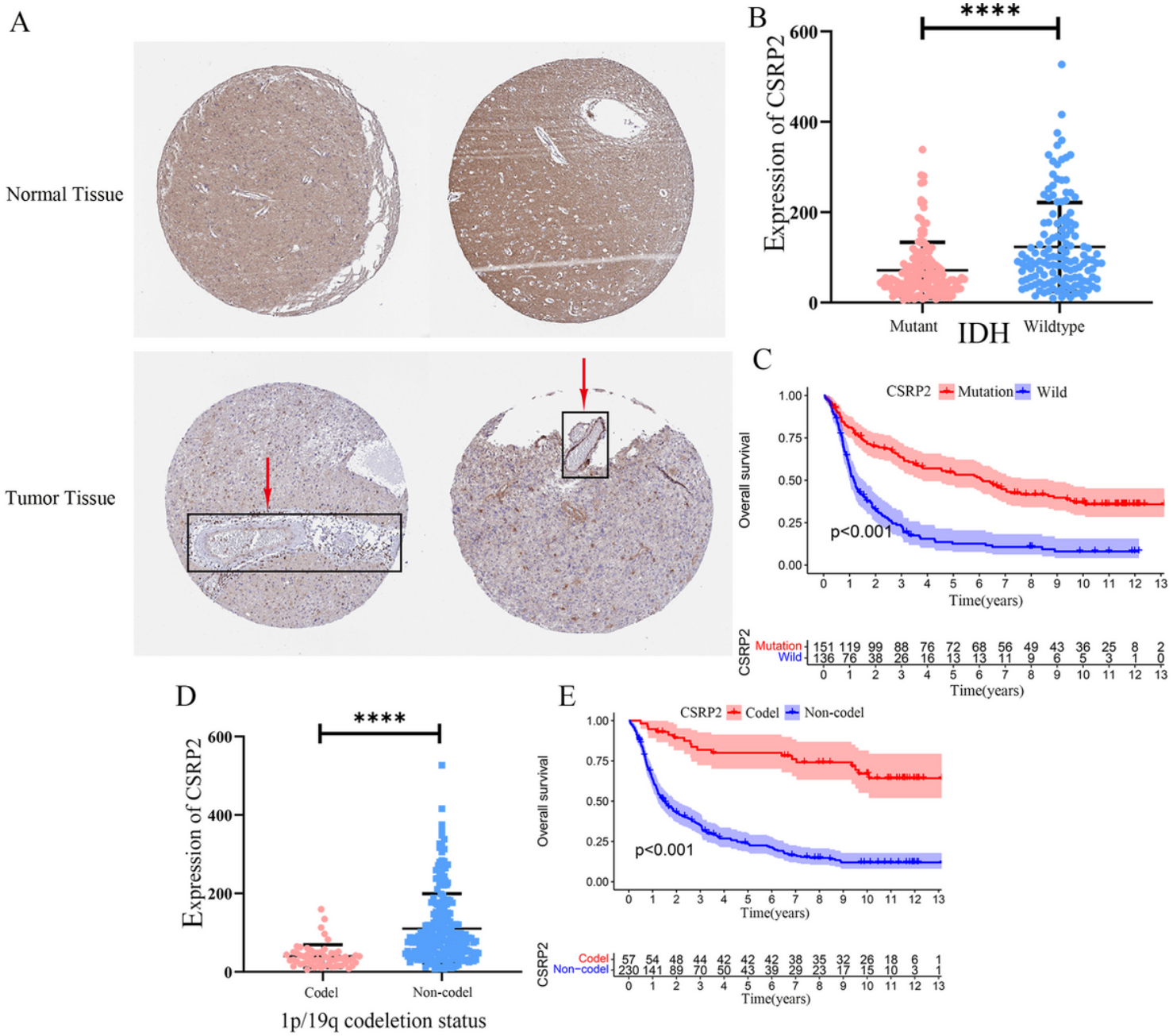

Figure 4

The feasibility of model validation and prognostic value of low CSRP2 expression in glioma tissues. (A) Validation of CSRP2 expression in glioma tissues using immunohistochemical (IHC) staining from the Human Protein Atlas (HPA) database. (B) CSRP2 expression is higher in IDH mutant patients than in IDH wild-type patients. (C) IDH wild-type patients have a better prognosis compared to IDH mutant patients. (D) CSRP2 expression is lower in patients with 1p/19q co-deletion (1p/19q codeletion) than in patients without deletion. (E) Combined deletion of 1p/19q would be more prognostic for patients. The relationship between CSRP2 expression in glioma tissue and immune cells.

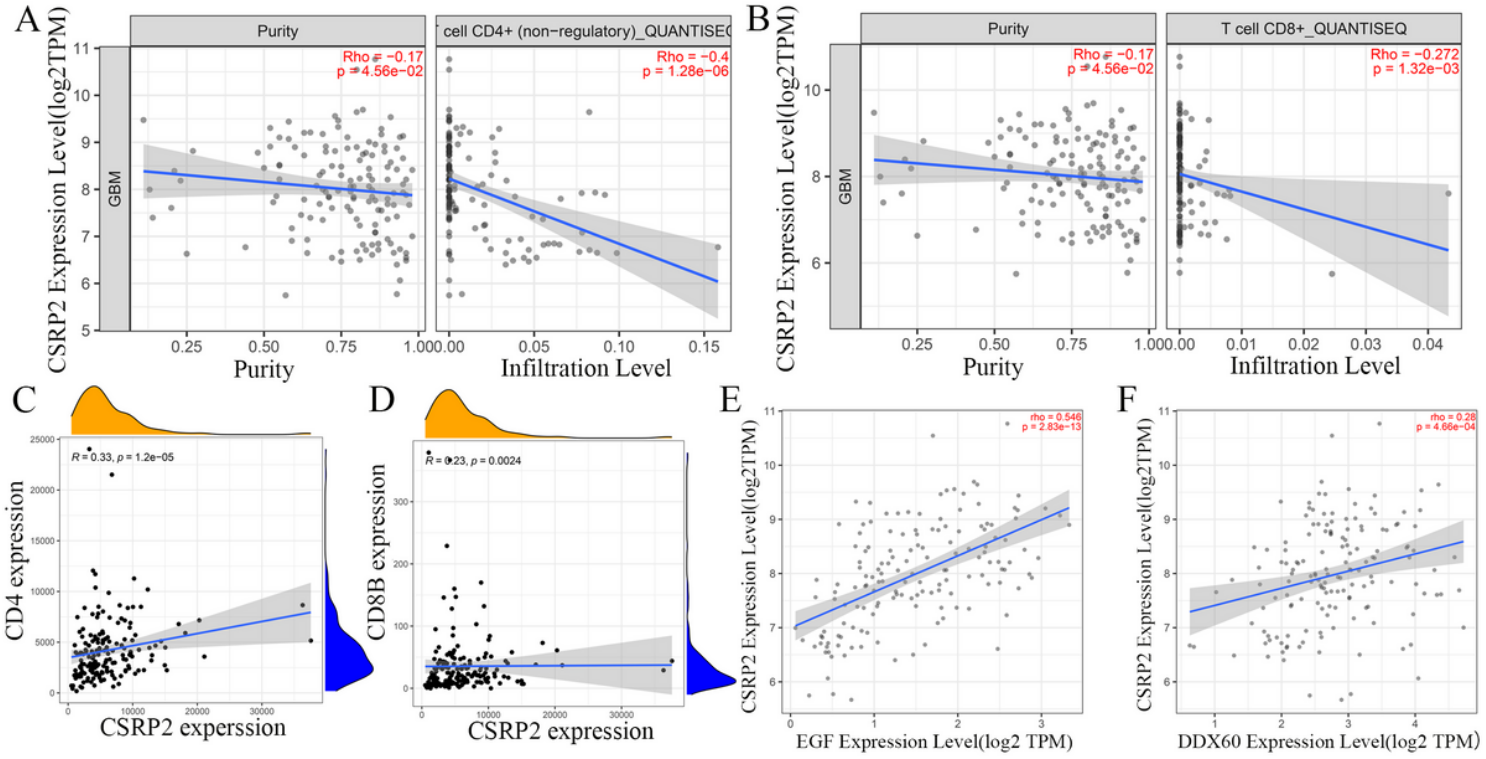

Figure 5

Relationship between CSRP2 expression in glioma tissues and immune cells. (A) Relationship between CSRP2 expression in glioma tissues and immune cells. The infiltration levels of CD4+ T cells in glioma tissues were significantly and positively correlated. (B) The infiltration levels of CD8+ T cells in glioma tissues were significantly and positively correlated. (C) CSRP2 expression was positively correlated with immune cell marker genes CD4. (D) CSRP2 expression was positively correlated with immune cell marker genes CD8B. (E) The expression of CSRP2 was positively correlated with the immune checkpoints EGF and DDX60. (F) The expression of CSRP2 was positively correlated with the immune checkpoints EGF and DDX60.

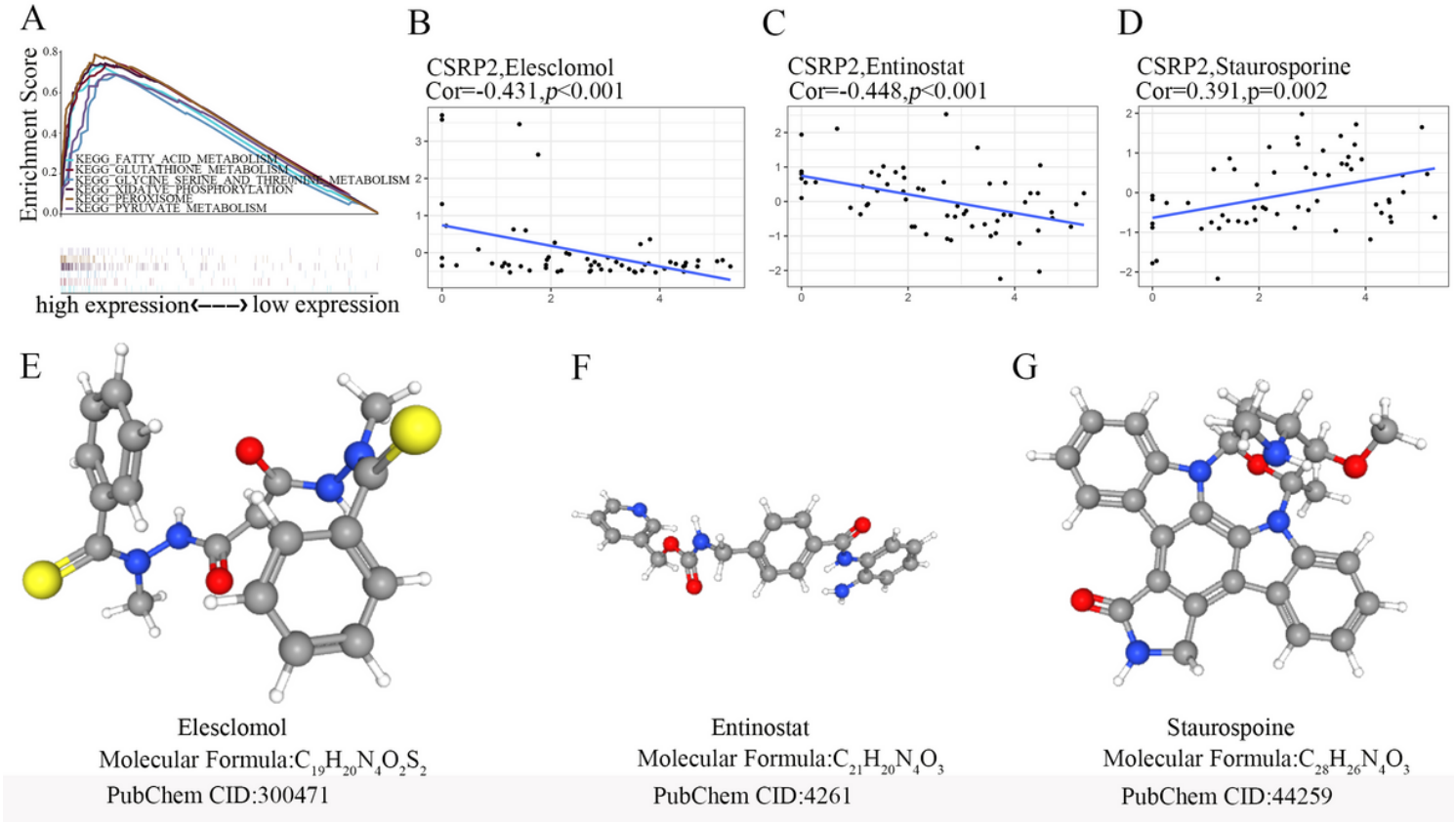

Figure 6

Relationship between CSRP2 expression and GSEA functional annotation and drug sensitivity. (A) The high expression CSRP2 group was significantly enriched in multiple amino acid metabolic pathways compared to the low expression CSRP2 group. (B) Elesclomol was predicted to have high sensitivity with CSRP2 using the CellMiner database. (C) Entinostat was predicted to have high sensitivity with CSRP2 using the CellMiner database. (D) Staurosporine was predicted to have high sensitivity with CSRP2 using the CellMiner database. (E) Molecular structure formulae of Elesclomol sensitive targeted drugs. (F) Molecular structure formulae of Entinostat sensitive targeted drugs. (G) Molecular structure formulae of Staurosporine sensitive targeted drugs.

Supplementary Files

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