

Identification of IFN- β Associated Genes Signature Predicting Overall Survival for Glioblastoma

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Abstract

Background: Brain glioblastoma (GBM) is the most common primary malignant tumor of intracranial tumors. The prognosis of this disease is extremely poor. While the introduction of IFN- β regimen in the treatment of gliomas has significantly improved the outcome of patients, the underlying mechanism remains to be elucidated.

Materials and methods: mRNA expression profiles and clinicopathological data were downloaded from TCGA-GBM and GSE83300 data set from the GEO. Univariate Cox regression analysis and lasso Cox regression model established a novel four-gene IFN- β signature (including PRDX1, SEC61B, XRCC5, and BCL2L2) for GBM prognosis prediction. Further, GBM samples (n=50) and normal brain tissues (n=50) were then used for real-time polymerase chain reaction (PCR) experiments. Gene Set Enrichment Analyses (GSEA) was performed to further understand the underlying molecular mechanisms. Pearson correlation was applied to calculate the correlation between the lncRNAs and IFN- β associated genes. A lncRNA with a correlation coefficient $|R^2| > 0.3$ and $P < 0.05$ was considered to be an IFN- β associated lncRNA.

Results: Patients in the high-risk group shown significantly poorer survival than patients in the low-risk group. The signature was found to be an independent prognostic factor for GBM survival. Furthermore, GSEA revealed several significantly enriched pathways, which might help explain the underlying mechanisms. Our study identified a novel robust four-gene IFN- β signature for GBM prognosis prediction. The signature might contain potential biomarkers for metabolic therapy and treatment response prediction in GBM.

Conclusions: Our study established a novel IFN- β associated genes signature to predict overall survival of GBM, which may help in clinical decision making for individual treatment.

Introduction

Brain glioblastoma (GBM) is the most common primary malignant tumor of intracranial tumors, accounting for ~50% of intracranial tumors with high morbidity and mortality in adults and children. Currently, surgery, radiation, and chemotherapy are the mainstays of GBM management. Chemotherapy is a critical process in the postsurgical treatment of GBM¹⁻³. Alkylating agents, such as temozolomide, remain the standard-of-care in GBM chemotherapy, but responses remain very poor⁴.

The DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) plays an essential role in cellular resistance to alkylating agents⁵. IFN- β can act as a drug sensitizer, enhancing toxicity against various neoplasias, and is widely used in combination with other antitumor agents such as nitrosoureas⁶⁻⁸. IFN- β sensitizes glioma cells that harbor the unmethylated MGMT promoter and are resistant to temozolomide^{6,9,10}. Nevertheless, the specific mechanisms and molecules associated with this phenomenon have not yet been completely elucidated, and further research is needed.

In this study, we explored and analyzed differentially expressed IFN- β associated genes (GSEA-M2567) by systematic bioinformatics analysis. Five hundred and ninety-six were included in TCGA-GBM to train the prognostic model. Univariate Cox regression model found 5 survival-related genes, then Lasso-penalized Cox analysis identified 5 genes to construct the prognostic model. Using the methodology previously described, the proposed approach is validated on the GEO datasets (GSE83300). We found that 4 IFN- β associated genes (PRDX1, SEC61B, XRCC5, and BCL2L2) signature was a robust marker of seizure prognosis in patients with GBM. With CGGA data, we validated that 4 IFN- β associated genes were an independent biomarker of prognosis. Pathway enrichment analysis results show that several modules are enriched in pathways related to GBM.

LncRNA has been demonstrated that played an important role in human disease¹¹, especially in the GBM. LncRNA AC003092.1 regulates TFPI2 expression through ceRNA mechanism, and LncRNA SOX2OT interacts with RNA binding proteins to promote the expression level of SOX2 is involved in the resistance of glioma chemotherapy^{12, 13}. However, the relevance between IFN- β and LncRNA has not been fully elucidated in GBM. Pearson correlation was applied to calculate the correlation between the LncRNAs and IFN- β associated genes. A LncRNA with a correlation coefficient $|R^2| > 0.3$ and $P < 0.05$ was considered to be an IFN- β associated LncRNA. Univariate and multivariate Cox regression were used for survival analysis indicated that AC093278.2, AC004067.1, LINC01116, and AC017104.1 was an independent prognostic factor for GBM patients.

In a word, systems-based biomarker discovery approaches may more accurately reflect the underlying biology, and this laid the foundation for our research on GBM. The present study may lay the foundations for future studies aimed to investigate GBM.

Methods And Materials

Clinical specimens and Data collection

All the 50 human glioma tissue samples and 50 normal brain tissues were obtained from surgery at Jiangxi Cancer Hospital of Nanchang University, China. Samples were then frozen in liquid nitrogen after surgical resection. The study was approved by the Ethics Committee of the Jiangxi Cancer Hospital of Nanchang University with informed consent from the patients or guardians.

Messenger RNA (mRNA) expression profiles and clinical data were obtained from The Cancer Genome Atlas (TCGA, <https://cancergenome.nih.gov/>): glioblastoma multiforme (GBM); the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>): GSE83300 database; and the Chinese Glioma Genome Atlas (CGGA, <http://www.cgga.org.cn/>). IFN- β associated gene set (M2567) was obtained by GSEA using the gene set database (GSEA, <http://www.gsea-msigdb.org/>). All data were analyzed using R software (version 4.0.2).

Identification of differentially expressed gene in TCGA-GBM

Limma package was used to screen the interestingly differentially expressed genes with R 4.0.2. The expression pattern of the 120 IFN- β associated genes mentioned above was then investigated in TCGA. Genes were selected as consistently altered IFN- β associated genes for subsequent prognostic analysis if: (a) they showed consistent expression pattern in TCGA cohort; and (b) they were listed in the GSE83300 data set.

Construction of the prognostic IFN- β associated gene signature

Univariate Cox regression analysis and lasso-penalized Cox regression analysis were performed to identify the prognosis-related IFN- β associated genes and construct the prognostic gene signature. $P < 0.05$ in univariate Cox regression analysis were considered statistically significant. The prognostic gene signature was shown as risk score = (Coefficient $_{mRNA_1}$ \times expression of mRNA $_1$) + (Coefficient $_{mRNA_2}$ \times expression of mRNA $_2$) + \dots + (Coefficient $_{mRNA_n}$ \times expression mRNA $_n$). The R package “survival” and “survminer” was used to explore the optimal cut-off of risk score and drawn the Kaplan–Meier survival curve. In particular, the “surv_cutpoint” function of the “survminer” R package was used to determine the optimal cut-off value to divide patients into high- and low-risk group. The R package “survivalROC” was used to investigate the time-dependent prognostic value of the gene signature. A two-sided log-rank $p < 0.05$ were considered significant for survival analysis.

IFN- β associated lncRNA screening

The profiles of lncRNAs and IFN- β associated genes were obtained from the TCGA RNAseq dataset. Pearson correlation was applied to calculate the correlation between the lncRNAs and differential genes. A lncRNA with a correlation coefficient $|R^2| > 0.3$ and $P < 0.05$ was considered to be IFN- β associated lncRNA.

Construction of the prognostic IFN- β associated lncRNA signature

Construction of the prognostic IFN- β associated lncRNA signature was performed as previously described.

Gene set enrichment analysis (GSEA)

GSEA was applied to investigate potential mechanisms underlying the influence of differential gene expression on GBM prognosis. GSEA was also applied to detect whether a priori defined set of genes showed statistically significant differential expression between the high and low-risk groups. Gene sets with a standard $P < 0.05$ were considered to be significantly enriched.

Immunohistochemistry staining

The paraffin-embedded glioma tissues were cut into thin slices and then installed on glass slides for immunohistochemistry experiments. The specimens were bred with rabbit anti-BCL2L2, anti-PRDX1, anti-XRCC5, and SEC61B antibody (1:200 dilutions, Abcam, USA) at 4 °C overnight, followed by 1 h incubation

of biocatalyst secondary antibody (1:200 dilutions, Santa Cruz Biotechnology, USA) under room condition. Then, the avidin biocatalyst peroxidase complex methods were adopted to determine the target protein's location and relative expression to visualize the bound antibodies.

Result

Construction and validation of the prognostic IFN- β associated genes signature

Five hundred and ninety-six and 120 IFN- β associated genes (70 upregulated and 51 downregulated) were included in TCGA-GBM to train the prognostic model. Differential gene expression analysis identified 14 downregulated and 53 upregulated IFN- β associated genes, respectively (Supplementary Table 1). In this part, Firstly, we displayed the heatmap by differential genes and analyzed these significant genes further (Figure.1A). Univariate Cox regression model found 5 survival-related genes (Supplementary Table 2), then Lasso-penalized Cox analysis identified 5 genes to construct the prognostic model (Supplementary Table 3). Using the methodology previously described, the proposed approach is validated on the GEO datasets (GSE83300) (Supplementary Table 4). Patients were divided into a high- and low-risk group for risk score. GBM patients with high-risk scores indicated poor prognosis (Figure.1B-C). Moreover, increased expression of the 4 different signature genes (PRDX1, SEC61B, XRCC5, and TXN) and reduced expression of the 1 signature genes (BCL2L2) was observed as the risk value increased (Figure.1D-E). Taking all of our results together, four genes were found to be correlated with unfavorable clinical outcomes.

Prognostic significance of the four signature genes expression in GBM

To further validate the expression of the prognostic genes constructing the gene signature, Kaplan-Meier survival analysis was used. Results are presented that the high expression of the SEC61B and XRCC5 is associated with poor prognosis in the GEO dataset (Figure.2A-B). Moreover, the high expression of the SEC61B, XRCC5, and PRDX1 is associated with poor prognosis, and the low expression of the BCL2L2 is associated with poor prognosis in the TCGA dataset (Figure.2C-F). To further verify whether the expression of SEC61B, XRCC5, BCL2L2 and PRDX1 was associated with prognosis in GBM, the GEPIA database (<https://gepia.cancer-pku.cn/>) was used. The expression of SEC61B, XRCC5, and PRDX1 have significantly high expression in tumor samples compared to normal samples, and the expression of BCL2L2 has significantly low expression in tumor samples compared to normal samples (Figure.2G). Further, GBM samples (n=50) and normal brain tissues (n=50) were then used for real-time polymerase chain reaction (PCR) experiments. The results were united with the GEPIA database (Figure.2H). Taken together, four signature-genes expressions are considered to be of clinical significance in GBM.

Validation of the four signature genes in the CGGA database

To further validate these results, we used the Chinese Glioma Genome Atlas (CGGA) database (<http://www.cgga.org.cn/>). Kaplan-Meier survival analysis of CGGA data set shows that high expression of SEC61B, XRCC5 and PRDX1, and low expression of BCL2L2 informs poor patient prognosis (Figure.3A-

D). The expression level of BCL2L2 was significantly decreased with higher grade glioma (Figure.3E). Moreover, the expression of SEC61B, XRCC5 and PRDX1 was significantly increased with higher grade glioma (Figure.3F-H). To further validate these results, we performed immunohistochemical experiments. The immunohistochemistry results obtained in the present study were consistent with the results of the CGGA database (Figure.3I). In all, four signature-genes expressions are further considered to be of clinical significance in GBM.

GSEA analysis of the four signature genes

To further clarify the impact of the four signature genes on GBM, gene ontology and pathway enrichment analyses were performed using GSEA. The results revealed that these genes were mainly enriched in 14 pathways based on the TCGA-GBM database, involved in the calcium signaling pathway, cell cycle, ERBB signaling pathway, GAP junction, Glioma, inositol phosphate metabolism, MAPK signaling pathway, oxidative phosphorylation, phosphatidylinositol signaling system, purine metabolism, ribosome, RNA degradation, spliceosome, and VEGF signaling pathway (Figure.4A). Moreover, In the GEO dataset, these genes were mainly enriched in 9 pathways, involved in the calcium signaling pathway, cell cycle, ECM receptor interaction, ERBB signaling pathway, inositol phosphate metabolism, oxidative phosphorylation, P53 signaling pathway, phosphatidylinositol signaling system, and pyrimidine metabolism (Figure.4B). These genes may be involved in the proliferation of GBM.

The prognostic impact of the IFN- β associated lncRNAs signature for GBM

Considering the critical role of lncRNA in GBM, the identification of important lncRNAs in cancer and developing lncRNA-based therapeutic strategies would be meaningful in the future. Pearson correlation was applied to calculate the correlation between the lncRNAs and IFN- β associated genes. A lncRNA with a correlation coefficient $|R^2| > 0.3$ and $P < 0.05$ was considered to be an IFN- β associated lncRNA. Univariate and multivariate Cox regression were used for survival analysis indicated that AC093278.2, AC004067.1, LINC01116, and AC017104.1 was an independent prognostic factor for GBM patients (Figure.5A-C). Moreover, the high expression of the AC004067.1, AC017104.1, and LINC01116 is associated with poor prognosis, and the low expression of the AC093278.2 is associated with poor prognosis in the TCGA dataset (Figure.5D-G). Further, GBM samples (n=50) and normal brain tissues (n=50) were then used for real-time polymerase chain reaction (PCR) experiments to validate the expression of the IFN- β associated lncRNAs in GBM. qRT-PCR showed that compared with the normal brain tissues, AC004067.1, AC017104.1, and LINC01116 was highly expressed, AC093278.2 was low expressed in GBM (Figure.5H). Taken together, IFN- β associated lncRNAs have a high diagnostic value for GBM.

Discussion

The understanding of cancer treatment has gradually changed powerful with high-throughput sequencing technologies became more pervasive and user-friendly. As the scope of analyzed genes and diseases

expands, bioinformatic analysis is increasingly critical. In the present study, we analyzed the biological functions of the prognostic IFN- β associated genes signature using bioinformatics analysis. This approach may offer clues for therapeutic targets.

Univariate Cox regression model found 5 survival-related genes, then Lasso-penalized Cox analysis identified 5 genes to construct the prognostic model. Using the methodology previously described, the proposed approach is validated on the GEO datasets (GSE83300). We found that 4 IFN- β associated genes (PRDX1, SEC61B, XRCC5, and BCL2L2) signature was a robust marker of seizure prognosis in patients with GBM. With CGGA data, we validated that 4 IFN- β associated genes was an independent biomarker of prognosis and played important roles in many biological processes. For example, the PRDX1 (Peroxiredoxin 1) this gene encodes a member of the peroxiredoxin family of antioxidant enzymes, which reduce hydrogen peroxide and alkyl hydroperoxides¹⁴. PRDX1 forms a heterodimer with p38 α mitogen-activated protein kinase 14 (MAPK14), stabilizing phosphate-p38 α in glioma cells¹⁵ and epigenetic silencing of PRDX1 is frequent in 1p/19q-deleted oligodendroglial tumors and likely contributes to radio- and chemosensitivity of these tumours¹⁶; XRCC5 (X-Ray Repair Cross Complementing 5) is the 80-kilodalton subunit of the Ku heterodimer protein, which is also known as ATP-dependant DNA helicase II or DNA repair protein XRCC5. The Polymorphisms of XRCC5 play an important role in astrocytoma prognosis in the Chinese Han population, which could be used in the determination of astrocytoma prognosis in clinical researches¹⁷; BCL2L2 (BCL2 Like 2) is a member of the BCL-2 protein family. The proteins of this family form hetero- or homodimers and act as anti- and pro-apoptotic regulators. The expression of BCL2L2 mRNA or protein in various cancer cell types. Interestingly, BCL2L2 mRNA is highly expressed in the mesenchymal type of GBM¹⁸. SEC61B (SEC61 Translocon Subunit Beta) is the central component of the protein translocation apparatus of the endoplasmic reticulum (ER) membrane¹⁹. However, to the best of our knowledge, the expression pattern and function of SEC61B in GBM have not been previously reported. Further study is warranted.

Considering the critical role of lncRNA in GBM, the identification of important lncRNAs in cancer and developing lncRNA-based therapeutic strategies would be meaningful in the future. Pearson correlation was applied to calculate the correlation between the lncRNAs and IFN- β associated genes. Univariate and multivariate Cox regression were used for survival analysis indicated that AC093278.2, AC004067.1, LINC01116, and AC017104.1 was an independent prognostic factor for GBM patients. lncRNA genes play important roles in many biological processes. For example, LINC01116 promotes tumor proliferation migration and invasion in glioma cell^{20,21}. However, the role of AC093278.2, AC004067.1, and AC017104.1 in GBM has not been reported, is urgent to be elucidated.

In conclusion, we explored and analyzed differentially expressed IFN- β associated genes by systematic bioinformatics analysis.

Conclusions

Our study established a novel IFN- β associated genes signature to predict overall survival of GBM, which may help in clinical decision making for individual treatment.

Abbreviations

BCL2 Like 2, BCL2L2; Chinese Glioma Genome Atlas, CGGA; Gene Expression Omnibus, GEO; glioblastoma, GBM; O6-methylguanine-DNA methyltransferase, MGMT; Interferon Beta 1, IFN- β ; SEC61 Translocon Subunit Beta, SEC61B; The Cancer Genome Atlas, TCGA; Peroxiredoxin 1, PRDX1; X-Ray Repair Cross Complementing 5, XRCC5;

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Jiangxi Cancer Hospital of Nanchang University with informed consent from the patients or guardians.

Consent for publication

All contributing authors agree to the publication of this article.

Availability of data and materials

All raw data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there are no conflict of interests.

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Author contributions

LC and MY conceived of the study and participated in experiment design and coordination, drafted and revised the manuscript. JZ performed gene differential analysis and survival analysis using TCGA, GEO

and CGGA data. JC collected tissue samples and performed experiment. SL and SZ performed statistical analysis and revised manuscript.

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Figures

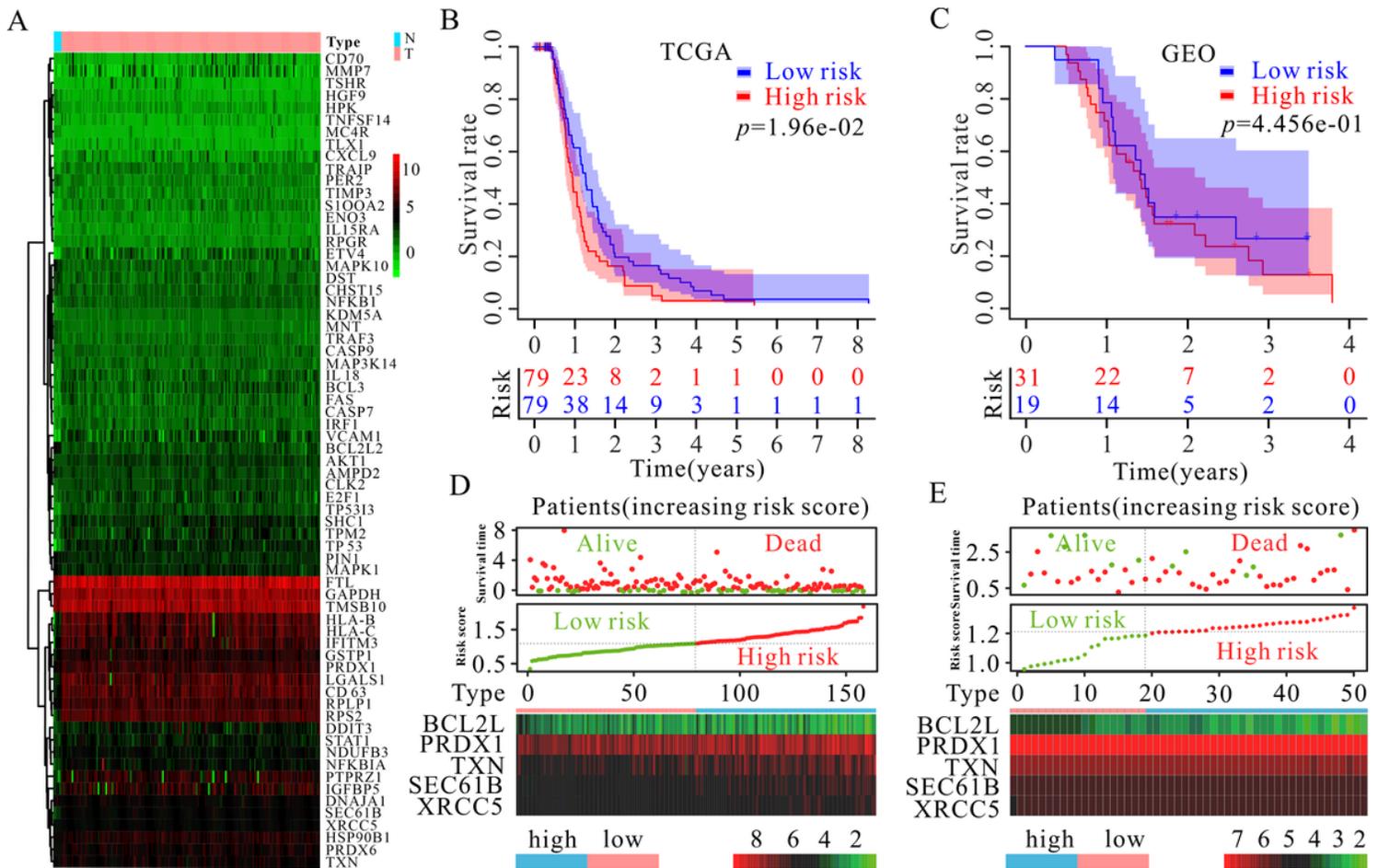


Figure 1

Construction and validation of the prognostic IFN- β associated genes signature. (A) Heatmap showing differential expression of 67 genes between normal brain tissues and GBM tissues that differed significantly ($p < 0.05$). (B) Survival curve of GBM patients based on risk score model in TCGA. (C) Survival curve of GBM patients based on risk score model in GEO. (D-E) The survival duration and status of GBM cases. Interferon-beta associated gene risk score analysis of glioma patients in TCGA and GEO. Heatmap of the 5 key genes expressed in GBM.

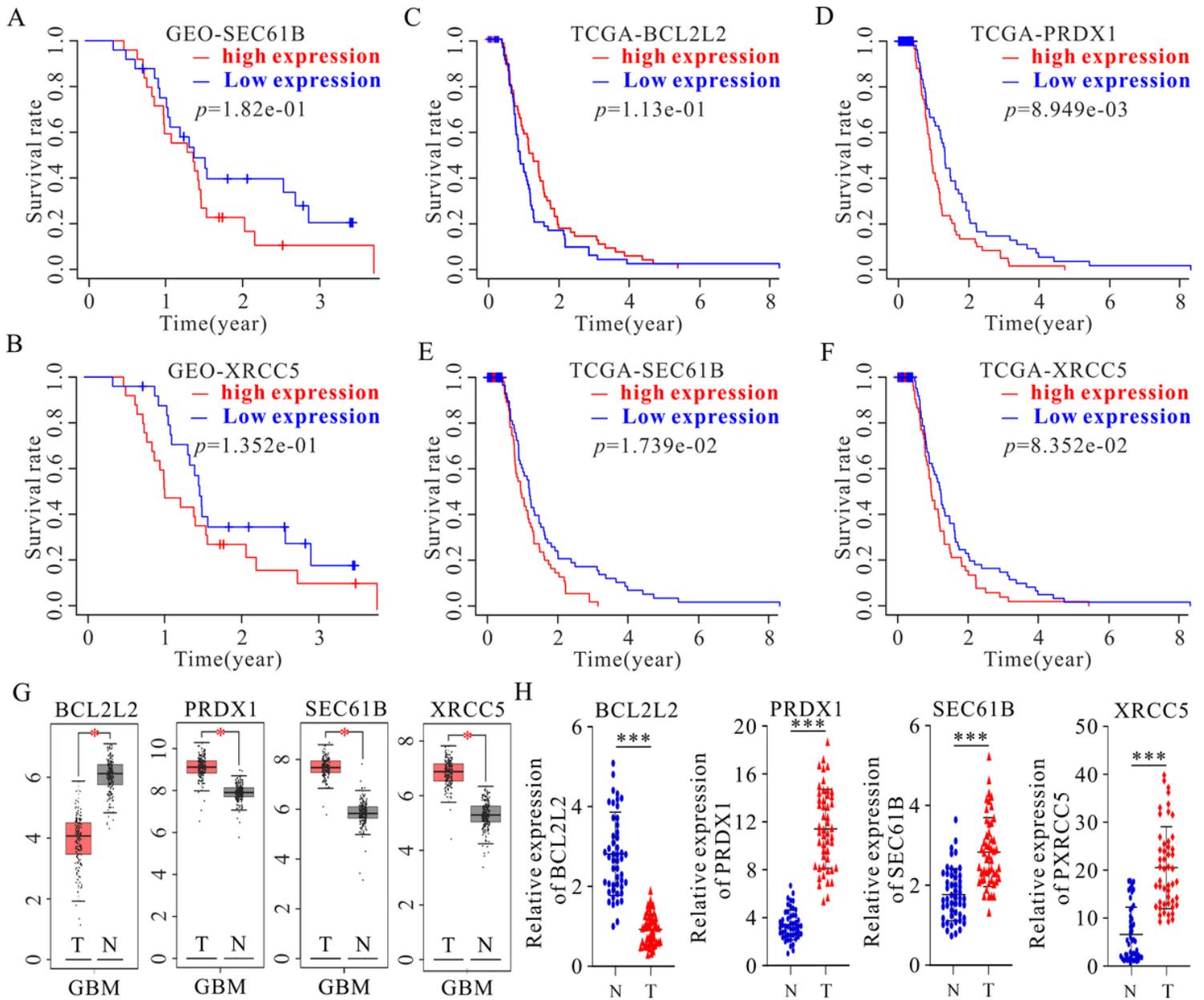


Figure 2

Prognostic significance of four signature-genes expression in GBM. (A-B) Survival analysis of the 2 prognostic interferon-beta associated genes based on the GEO database. (C-F) Survival analysis of the 4 prognostic interferon-beta associated genes based on the TCGA database. (G) The expression analysis of 4 prognostic interferon-beta associated genes according to GEPIA database. (H) Real-time PCR expression analysis of 4 prognostic interferon-beta associated genes in normal brain tissues and GBM tissues (N=50), *** $p<0.001$.

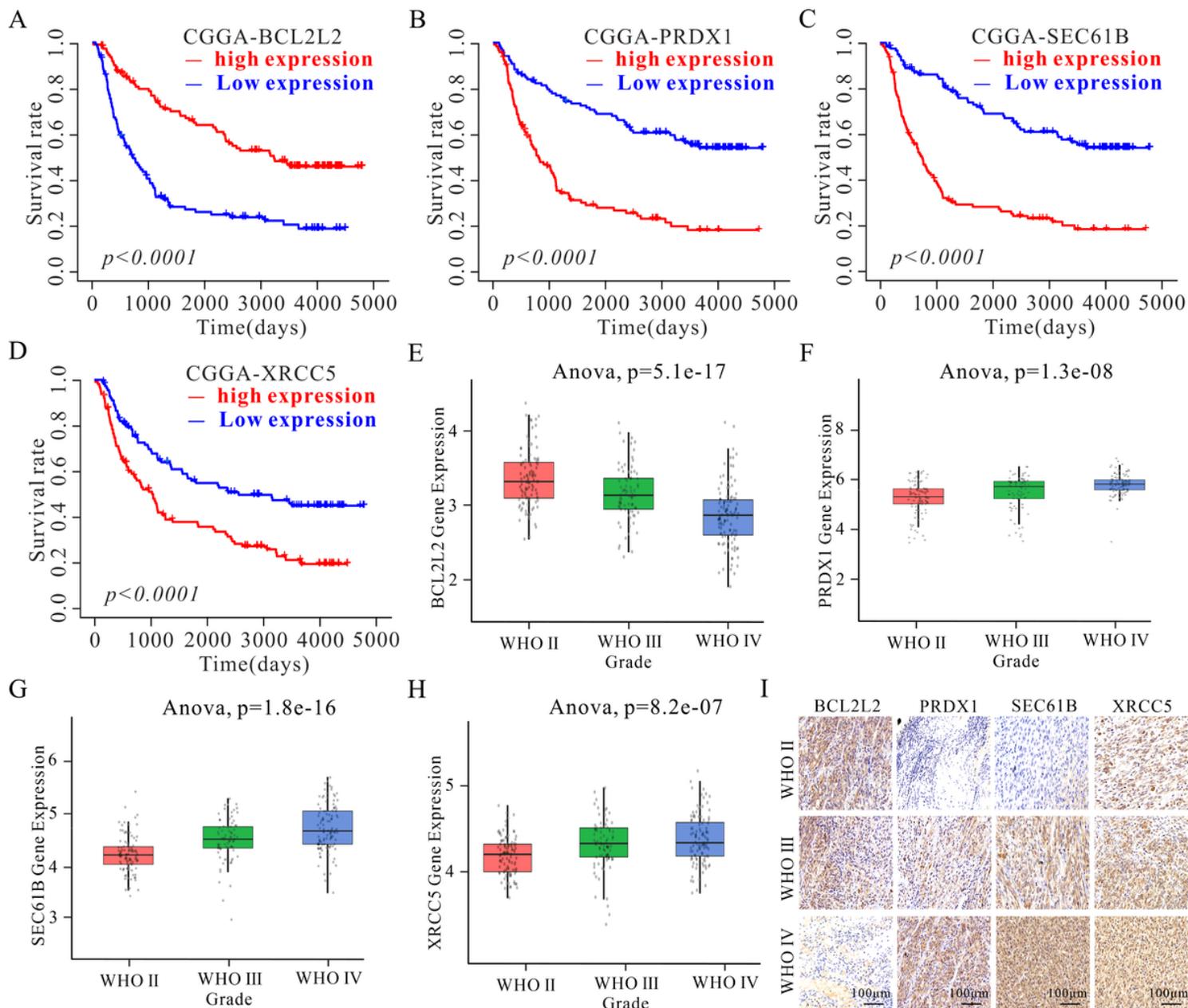


Figure 3

Validation of four signature-genes in CGGA database. (A-D) Survival analysis of the 4 prognostic interferon-beta associated genes based on the CGGA database. (E-H) The expression analysis of 4 prognostic interferon-beta associated genes according to CGGA database. (I) Immunohistochemistry of the 4 prognostic interferon-beta associated genes.

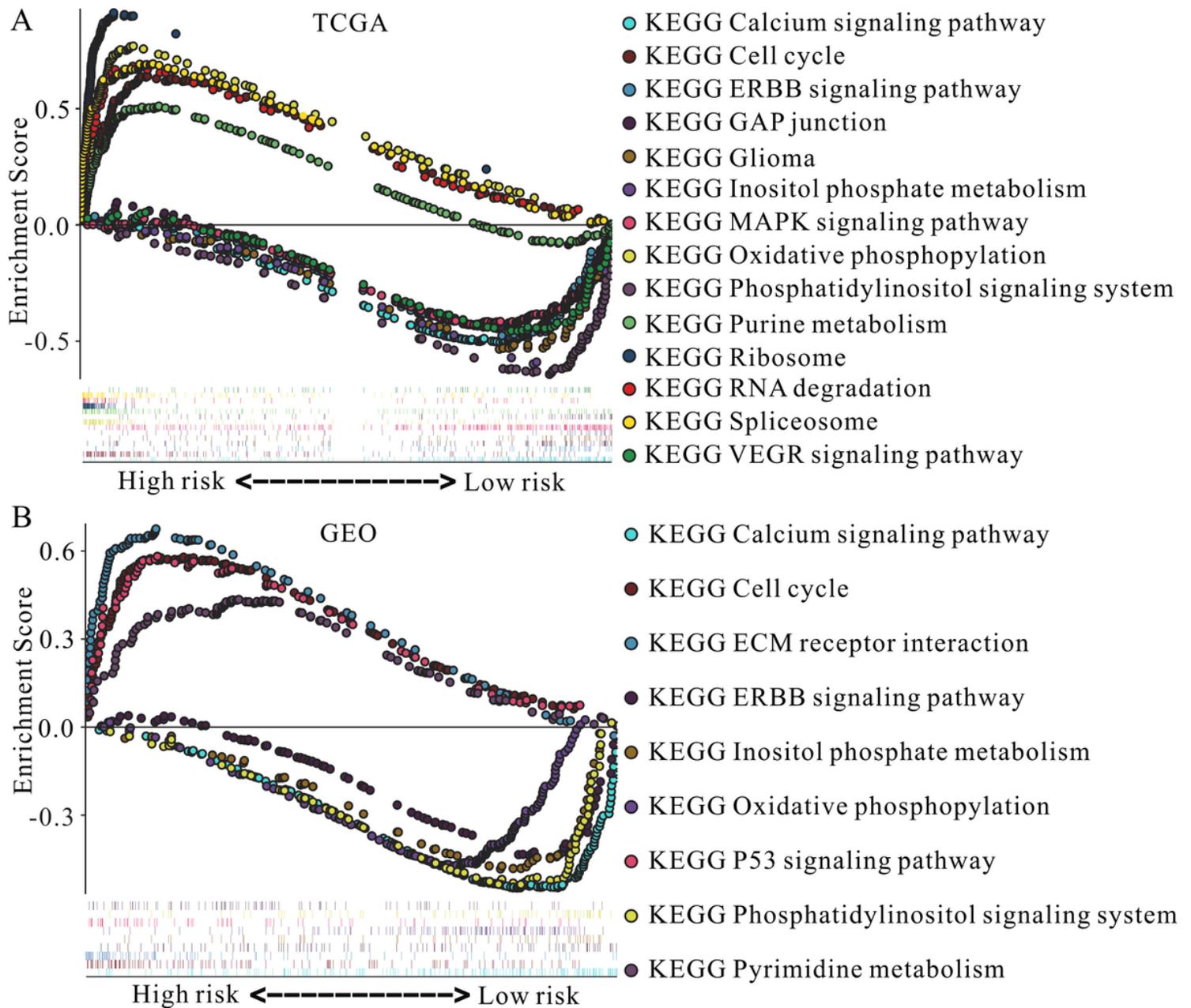


Figure 4

GSEA analysis of the four signature genes. (A-B) GSEA analysis of the 4 prognostic interferon-beta associated genes based on TCGA and GEO database.

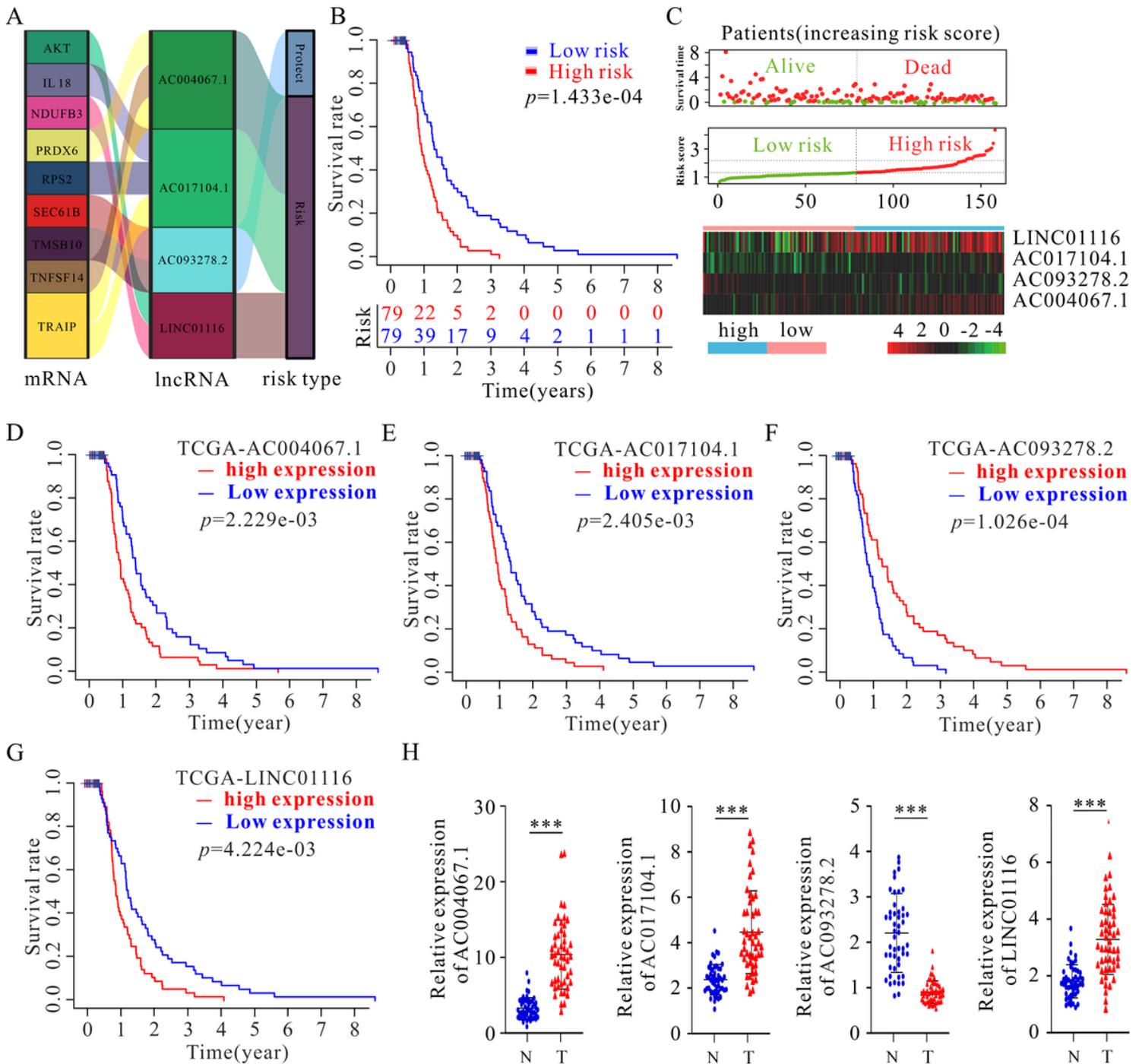


Figure 5

The prognostic impact of the IFN- β associated lncRNAs signature for GBM. (A) Network of prognostic lncRNAs with co-expressed interferon-beta associated lncRNAs in GBM. (B) Survival curve of GBM patients based on risk score model in TCGA. (C) The survival duration and status of GBM cases. Interferon-beta associated lncRNAs risk score analysis of GBM patients in TCGA. Heatmap of the 4 key lncRNAs expressed in GBM. (D-G) Survival analysis of the 4 prognostic interferon-beta associated lncRNAs based on the TCGA database. (H) Real-time PCR expression analysis of 4 prognostic interferon-beta associated lncRNAs in normal brain tissues and GBM tissues (N=50), *** $p<0.001$.