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Running Title: IGFBP2 in Lower-Grade Gliomas
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Positive Expression of IGFBP2 as a Novel Prognostic Biomarker for Patients with Lower-Grade Gliomas

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Abstract

Background: Previous studies have identified insulin-like growth factor-binding protein 2 (IGFBP2) as a target gene associated with the prognosis of various malignant cancers. This study aimed to explore the role and mechanisms of this prognostic signature in patients with low-grade gliomas (LGGs).

Method: A total of 217 patients with LGGs were retrospectively obtained from the Chinese Glioma Genome Atlas as the training group, whereas an additional 190 cases (GSE107850) were collected from the Gene Expression Omnibus as validation data. The Kaplan–Meier method evaluated the overall survival (OS) between the high IGFBP2 and low IGFBP2 expression groups. Univariate and multivariate Cox analyses were used to identify independent prognostic factors associated with survival. Gene set enrichment analysis (GSEA) was conducted to investigate signaling pathways influencing glioma cell proliferation at the transcriptional level of IGFBP2. Statistical analyses and data visualization were performed using R language (version 3.6.3) and Perl software (version 5.38.1), with significance set at $P < 0.05$.

Results: Kaplan–Meier survival analysis suggested that the group with decreased IGFBP2 expression may have improved OS as compared with the group with high IGFBP2 expression. Increased IGFBP2 expression in gliomas significantly correlated with isocitrate dehydrogenase mutation-wild type. GSEA results revealed that five differential pathways involved in collagen binding, collagen-containing extracellular matrix, collagen metabolic process, collagen trimer, and

extracellular structure organization were significantly enriched in patients with glioma with high IGFBP2 expression.
Conclusion: Our study is the first to show that overexpression IGFBP2 could be an independent glioma biomarker. For patients with LGG overexpressing IGFBP2, radiotherapy may be a preferable choice over chemotherapy.
Keywords: IGFBP2, Glioma, Prognosis, Bioinformatics, Genomics

Introduction

Gliomas represent the most prevalent primary brain tumors, with incidence rates ranging from approximately 4.67/100000 to 5.73/100000 globally.[1] Standardized radiochemotherapy technologies have improved the prognosis of patients with gliomas, including standardized temozolomide (TMZ) chemotherapy and radiotherapy (RT); however, 5-year overall survival (OS) rates remain notably poor.[2] Moreover, high-grade gliomas, such as glioblastomas, exhibit particularly poor outcomes, with only 5% achieving 5-year survival rates.[3] With the development of personalized medicine, following the 2016 World Health Organization (WHO) classification, isocitrate dehydrogenase mutations (IDHs) in gliomas have become increasingly associated with radio-chemo-resistance and patient prognosis.[4] Numerous studies have been conducted to develop clinical and radiological models for glioma prognosis prediction from clinical baseline characteristics (such as sex,[5] tumor size,[6] location,[7] and imaging signatures)[8,9] and multiple genetic biomarkers;[10-12] however, no consensus has been reached. A deeper understanding of the mechanisms involving novel genetic biomarkers in gliomas may elucidate glioma cell invasion development and improve prognostic predictions, which is crucial for establishing individualized treatment strategies.[10]

Emerging evidence suggests that insulin-like growth factor-binding protein 2 (IGFBP2) plays a crucial role in tumorigenesis via various signaling pathways in several malignant tumors, particularly those involved in metabolic pathways in malignant cancers.[13-15] Several studies have highlighted that the IGFBP2 gene was mediated by the L1/ezrin/NF-kB network and contributes to colorectal tumorigenic.[16] Furthermore, the latest study suggested that the IGFBP2 gene signature could facilitate the β-catenin/Wnt pathway to exert pro-tumorigenic function in breast cancer.[14] Recent studies have reported that IGFBP2 may stimulate downstream ILK/NF-κB signaling to drive a complex metabolic adaptation program in glioblastoma subtypes.[17] Additionally, potential findings suggest IGFBP2 via regulation of β-catenin in tumors and the crucial role of the β-catenin pathway in tumor proliferation. However, the mechanism of regulation of glioma by IGFBP2 remains unclear. Therefore, this study aims to investigate the prognostic role and underlying mechanisms of IGFBP2 in patients with gliomas.

Materials and Methods

Materials

Raw mRNA sequencing profiles of 1,018 patients with glioma were retrospectively obtained from the Chinese Glioma Genome Atlas (CGGA), a freely accessible online database containing comprehensive clinical data, such as patient age, sex, IDH mutations, histological grade, and survival information. Additionally, validation data from GSE107850 were acquired from the Gene Expression Omnibus (GEO) database (https://portals.broadinstitute.org/ccle/about) using the GPL6244 platform. Clinical information on OS and gene expression profiles of gliomas were analyzed using the

Perl software (version 5.38.1). We analyzed the correlation between IGFBP2 transcription level and various clinicopathological parameters, including gender, age, tumor grade, IDH mutations, and radiochemotherapy. Raw IGFBP2 gene expression data from the GSE108570 dataset was the validation cohort. Gliomas were classified according to the 2016 WHO classification, including molecular subtypes. To investigate the association between IGFBP2 expression and patient prognosis further, we examined the strong link between IDH mutations in patients with glioma and validated the GSE cohort results.

Additionally, gene set enrichment analysis (GSEA) was performed to identify potential mechanisms underlying the effects of IGFBP2 expression on glioma evolution and development in the CGGA cohort. The gene set several permutations was 1,000 times, with significance defined as $P < 0.05$ for markedly enriched genes. Significantly positive and negative genes correlated with IGFBP2 overexpression were selected to elucidate the potential mechanism underlying glioma invasion. All human participant data were obtained from publicly available sources, namely the CGGA and GEO databases. This study complied with the Helsinki Declaration and passed the review of the Ethics Committee of First Hospital of Qinhuangdao, exempting participants from informed consent (ethics code: 2021Q050).

Statistical analysis

All statistical analyses and data visualization were performed using R language (version 3.6.3) and Perl software (version 5.38.1).[18,19] The Kaplan–Meier method evaluated the OS between the high IGFBP2 and low IGFBP2 expression groups. Univariate and multivariate Cox analyses were used to identify independent prognostic factors associated with survival. A univariate Cox analysis inclusion criterion of $P < 0.05$ was selected for the multivariate tests. Receiver operating characteristic (ROC) curves were generated to assess the accuracy of the models that predicted prognosis using the survival "ROC" package. A significant level of $P < 0.05$ was considered statistically marked.

Ethical Considerations

This study complied with the Helsinki Declaration and passed the review of the Ethics Committee of First Hospital of Qinhuangdao, exempting participants from informed consent (ethics code: 2021Q050).

Results

Survival analysis of IGFBP2 genes in CGGA and GEO databases

The largest sample population comprised 241 patients with low-grade gliomas (LGGs) (grade II = 241), with 217 patients included in the CGGA dataset, excluding those with unobtainable survival information. Table 1 summarizes transcription levels of IGFBP2 and key clinicopathological parameters, including sex, age, tumor grade, IDH mutations, and radio-chemotherapy. A total of 175 tumors exhibited IDH mutation, 42 were wild-type IDH, and 138 eligible patients had 1p19q non-deletion. Among the included patients, 130 were female, and 87 were male. An additional 190 cases were enrolled from GSE107850 GEO (https://www.ncbi.nlm.nih.gov/geo/) as validation data.

Kaplan–Meier analysis revealed that patients in the high-expression group had significantly poorer OS than those in the low-expression group (Figure 1A, $P < 0.001$). Confirming the effect of the signature expression on patient stratification in the external cohort (GSE108570), similar survival outcomes were observed as compared with the CGGA cohort, with the high-risk group exhibiting a poorer prognosis (Figure 1B, $P = 0.003$). We further evaluated the predictive accuracy of the model by computing the area under the curve (AUC),

and the 1-year and 5-year AUC was 0.701 in the CGGA cohort (Figure 2A). The 1-year and 5-year AUC in the GEO cohort were 0.68 and 0.73 (Figure 2B). These results underscored the robust predictive ability of the signature for glioma prognosis.

Patients in the lower IGFBP2 gene expression group trended to benefit from RT (Figure 3A) compared with the high-expression group, with no observed difference in patients assigned to the TMZ groups (Figure 3B). Furthermore, IDH-mutant gliomas with lower IGFBP2 gene expression receiving RT showed a better prognosis (Figure 3C), whereas no difference was observed for patients assigned to the TMZ groups (Figure 3D). PFS assigned for other molecular subtypes according to differential gene expression (median) by Kaplan–Meier analysis is not presented. These findings suggested that IDH-mutant patients with glioma with IGFBP2 lower expression achieve a better prognosis when receiving RT than the high-expression group, with similar results observed in the GEO datasets (Figure 4A-D).

Correlations between overexpression of IGFBP2 and clinical parameters in patients with glioma

To better understand whether the signature expression correlated with clinical parameters in the CGGA cohort, the correlation between IGFBP2 expression and clinicopathological features of the patients was analyzed using R software. The expression level of IGFBP2 was significantly lower in IDH-wild-type gliomas than in IDH-mutant gliomas (Figure 5A), and the relationship between IGFBP2 expression and IDH status was explored in a validation cohort. The expression of IGFBP2 in IDH-wild-type gliomas was higher than that in IDH-mutant gliomas (Figure 5B). Furthermore, the analysis revealed that the overexpression of IGFBP2 was higher in older than younger individuals ($P < 0.001$). Additionally, correlation analysis indicated that IGFBP2 expression increased with the rise in histological grade and tumor type. We found that the expression levels of IGFBP2 did not reach statistical significance in CGGA and GEO cohorts.

Prognostic role of IGFBP2 expression in patients with glioma

To better understand the prognostic role of risk factors in the CGGA cohort, possible prognostic factors from the univariate Cox analysis were included in a multivariate Cox proportional hazards analysis to identify significant risk factors. Figures 6A and B show the results of the univariate and multivariate Cox hazard ratio (HR) analyses from the original CGGA. In comparison with patients in the high-expression group, those in the low-expression group exhibited a significant improvement in OS time (HR: 1. 13[0.58–1.17]). A significant difference in IDH status was observed. Patients with IDH-wildtype had significantly poorer OS (HR and 95% CI: 0.759 [0.589–0.978]), and similar results were found between 1p–19q co-deletion status and OS (HR: 0.419 [0.3–0.586]. To validate the prognostic value of IGFBP2 expression, multivariate analysis confirmed that grade, age, IDH mutation, and IGFBP2 expression are independently associated with OS in the GEO set (Figure 6C, D). These findings strongly indicated that overexpression of IGFBP2 is an independent biomarker for the prognosis of gliomas.

GSEA results (Figure 7A) revealed that five differential pathways involved in collagen binding, collagen-containing extracellular matrix, collagen metabolic process, collagen trimer, and extracellular structure organization were significantly enriched in patients with glioma with high IGFBP2 expression. Five significantly positive and negative genes correlated with IGFBP2 overexpression (Figure 7B). In the validation cohort, consensus biological function analysis of gene ontology (GO) may

contribute to poor prognosis of patients with glioma.

Discussion

Our study identified a novel gene to improve prognostic prediction in glioma, especially positively correlated with IDH-mutant glioma. This signature identified two molecular subgroups significantly associated with OS, a finding validated in the GEO cohort. Notably, IDH-mutant gliomas with lower IGFBP2 gene expression exhibited improved prognosis when treated with RT, whereas no significant difference was observed in patients assigned to the TMZ group. Additionally, our GSEA results highlighted the significant enrichment of five differential pathways—collagen binding, collagen-containing extracellular matrix, collagen metabolic process, collagen trimer, and extracellular structure organization—in patients with glioma with high IGFBP2 expression, as corroborated by GO analysis results.

However, the expression of IGFBP2 in IDH glioma subtypes has not been previously reported. To address this gap, we used two online databases to analyze IGFBP2 expression in patients with LGG. Our findings revealed an association between IGFBP2 expression and IDH tumor type, with IGFBP2 being overexpressed and correlated with poor prognosis in IDH wild-type gliomas, consistent with prior studies.[20-22] Significantly, patients assigned to the IDH-mutated glioma high-expression group and those in the low-expression group demonstrated markedly improved OS times when receiving RT. A mouse model study has also suggested that elevated IGFBP2 expression contributes to glioma progression and an increased incidence of anaplastic oligodendroglioma, further underscoring the potential of IGFBP2 as a therapeutic target in LGGs.[23] Therefore, our results suggest that IGFBP2 could represent a promising target for RT in LGGs.

Molecular subtype analysis has established that IDH mutation status and 1p19q co-deletion status are major prognostic factors for patients with glioma, as previously reported.[4] Our study identified a novel gene that enhances prognostic prediction in gliomas. This signature revealed a significant association between IGFBP2 overexpression and molecular subtype, a finding validated in the GEO cohort. Previous research has indicated that IGFBP2 overexpression is a glioma biomarker with critical functional implications for tumor-grade development and progression in glioma.[24] The tumorigenic process in glioma may involve the activation of biological activity of the C-terminal fragments by IGFBP2.[25] Patients characterized by IDH wild-type status, absence of 1p19q co-deletion, and decreased IGFBP2 expression exhibit a better prognosis, which may be attributed to this mechanism.[26] Our findings regarding glioma molecular subtypes indicate that IGFBP2 protein levels are notably elevated in cases with wild-type IDH. These observations align with earlier reports suggesting that IGFBP2 overexpression in IDH wild-type gliomas is a poor prognostic factor, impacting the CpG island methylator phenotype.[27] Following the publication of the 2016 WHO guidelines,[28] refining glioma management by examining IDH molecular subtypes has significantly improved prognosis, offering a more precise approach than histology alone.

Gene Ontology GO analysis revealed significant enrichment of five differential pathways, including collagen binding, collagen-containing extracellular matrix, collagen trimers, and extracellular structure organization, in patients with glioma with high IGFBP2 expression. These findings are consistent with previous studies that have demonstrated the migration of glioma cells along blood vessels and invasion into the surrounding brain parenchyma.[15] Alterations

in the collagen metabolic process and significant signal pathways, such as the extracellular matrix and extracellular structure organization in the brain, underpin these biological processes.[29] Similar results from the GO analysis were validated in the GEO cohort. The pathway-associated role of IGFBP2 underscores its significance in glioma development, suggesting its potential as a robust biomarker for predicting glioma prognosis.

It has consistently been found that Crosstalk genes (CDC20, CHIL3, KIF20A, HOXA7, and SERPINH1) have a significant positive correlation with IGFBP2 overexpression, as evidenced by previous studies.[10,30,31] For instance, a study by French scientists has indicated that low-expression of CHIL3 could improve OS in patients with glioma, particularly when associated with 1p19q loss and IDH mutational status.[30] Additionally, CDC20 overexpression in high-grade gliomas has been linked to tumor growth in glioma cells and increased Ki67 expression levels.[10,32] Moreover, IGFBP2 overexpression positively regulates the expression of KIF20A, which plays a role in glioma cell invasion and proliferation. These findings are consistent with recent research.[31] The positive correlation between IGFBP2 overexpression and the signatures mentioned above provides robust evidence suggesting that IGFBP2 may significantly contribute to evaluating glioma prognosis. In contrast, five negative genes correlated with IGFBP2 were identified, including TUB, REPS2, NALCN, CDR1, and TNR. However, the impact of these genes on glioma infiltration remains to be reported in the future.

Based on the results presented herein, we propose that IGFBP2 holds promise as a potential novel biomarker for stratifying glioma subgroups. Rather than serving as an update to glioma classification strategies, these findings offer insights into potential targeted treatment approaches. In particular, patients with IDH-mutant-type gliomas exhibiting high IGFBP2 expression may benefit from conventional chemotherapy combined with IGFBP2-targeted inhibitors, leading to improved outcomes. Although our study has been validated in a GEO cohort and shows promise, further research is warranted to validate our findings and to integrate this signature in routine clinical practice as a therapeutic target for gliomas.

The nomograms developed in this study demonstrated favorable predictive performance; however, it is essential to acknowledge some limitations that warrant careful consideration. First, inherent bias may exist in the GEO data obtained from public databases. Therefore, conducting a large-scale multicenter study is imperative to validate our findings. Second, the mechanisms underlying glioma recurrence and progression remain unclear, highlighting the need for further investigations into the role of IGFBP2 in these processes. Finally, the other specific biological functions of IGFBP2 in gliomas remain largely unexplored.

Conclusion

Our findings primarily highlight the potential of IGFBP2 overexpression as a promising biomarker for identifying patients with glioma at risk of poor prognosis. This novel biomarker can facilitate individualized survival risk assessments, and inform treatment decisions in patients with gliomas.

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Authors' Contribution

Wenfei Li: Study design, data gathering, drafting and reviewing the manuscript; Yanguo Li and Jiawei Fan: Data gathering, drafting; Zhuo Shen and Fang Han: Data gathering, drafting; Jun Zhang and Xumei Yin: Data gathering, drafting; All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest

None declared.

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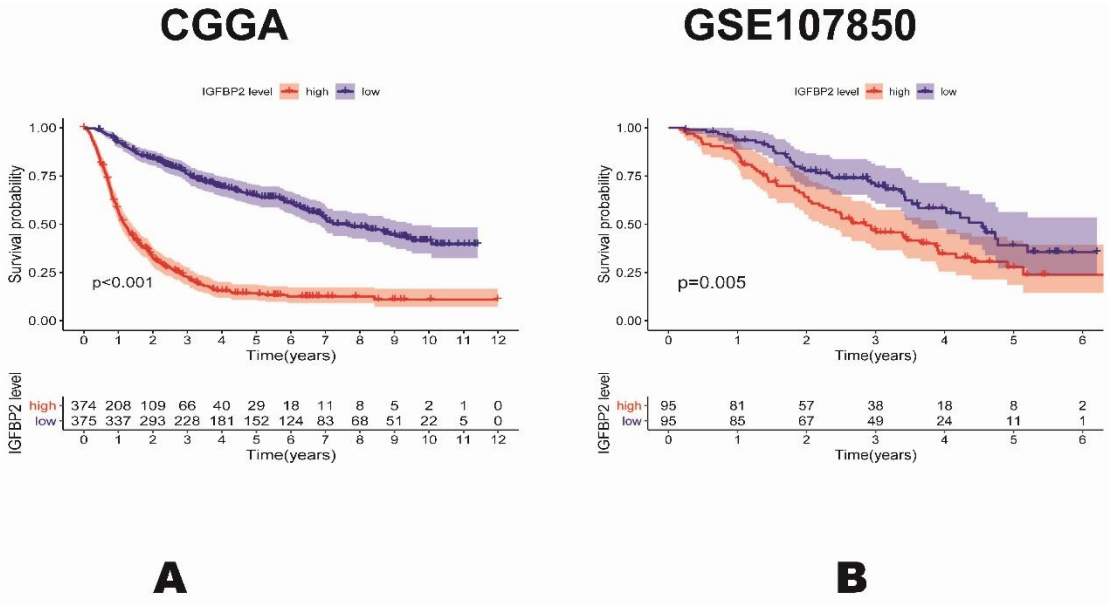

Figure 1. OS according to differential IGFBP2 gene expression (median) by Kaplan–Meier analysis in glioma from (A) CGGA cohort and (B) GEO cohort. PFS according to differential IGFBP2 gene expression (median) by Kaplan–Meier analysis in glioma from (A) CGGA cohort and (B) GEO cohort. Blue line represents low expression; Red line, high expression.

P-values were calculated using log-rank test; OS: Overall survival; IGFBP2: Insulin-like growth factor-binding protein 2; PFS: progression-free survival; CGGA: Chinese Glioma Genome Atlas; GEO: Gene expression omnibus

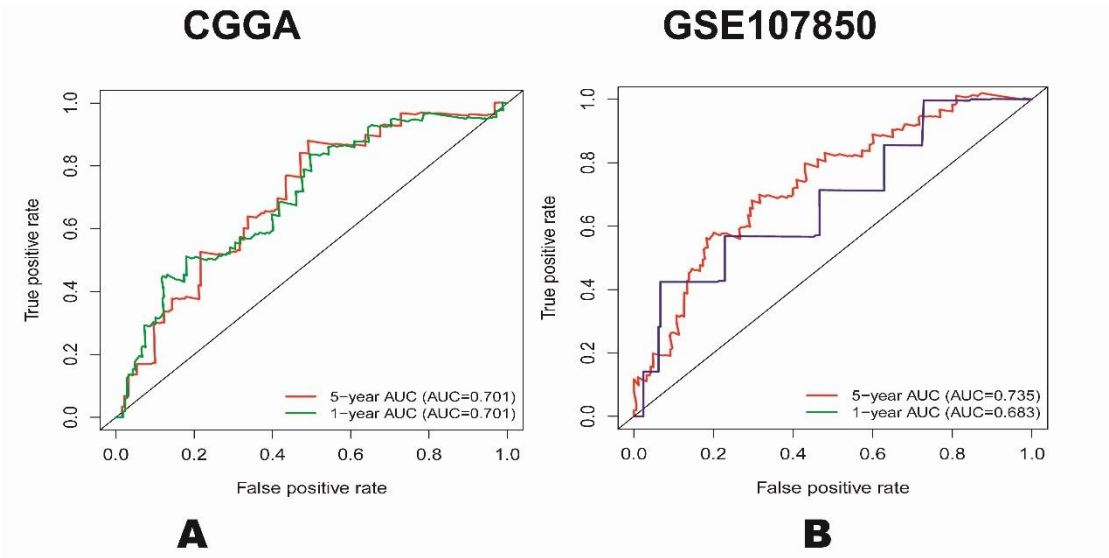

Figure 2. Predictive power of the IGFBP2 by computing AUC in CGGA (A) and GEO (B) cohorts.

CGGA: Chinese Glioma Genome Atlas; IGFBP2: Insulin-like growth factor-binding protein 2; GEO: Gene expression omnibus; AUC: Area under the curve

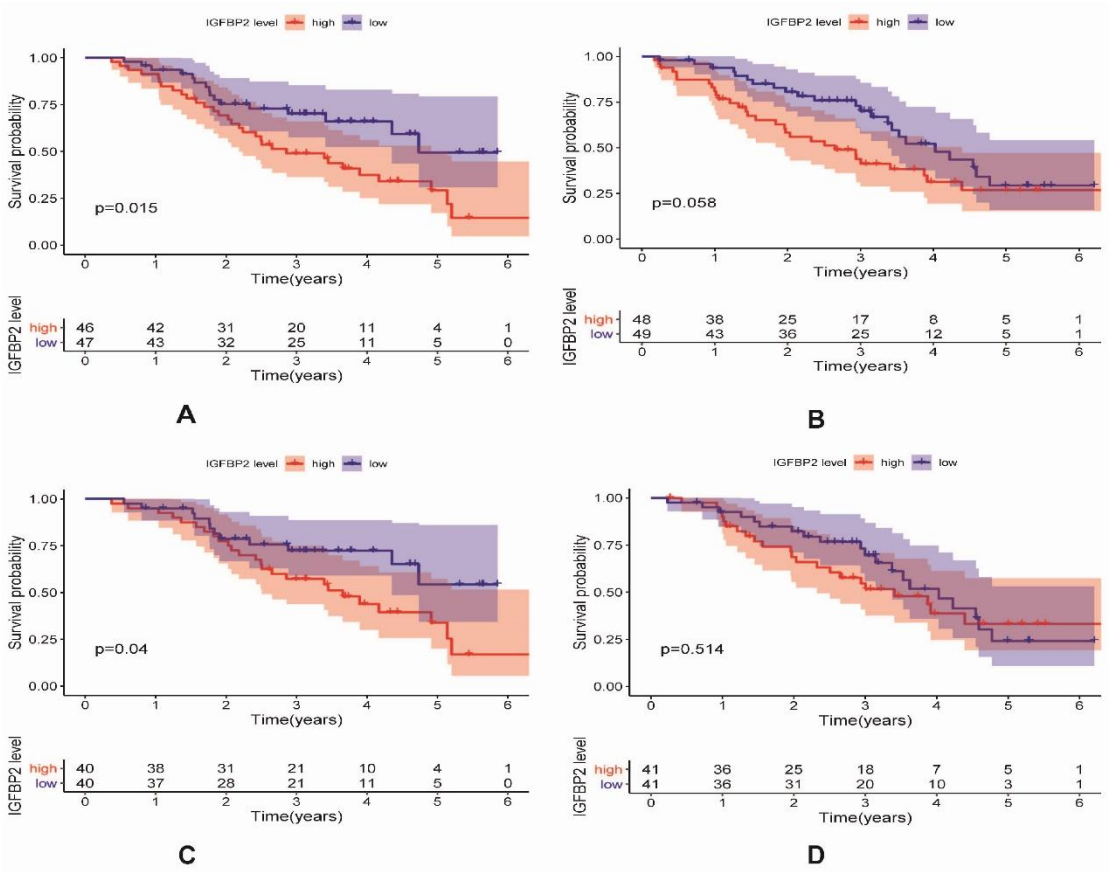

Figure 3. Patients assigned to lower IGFBP2 gene expression show a trend towards benefit from RT(A) compared with high-risk group in CGGA cohort, no difference was observed for patients assigned to TMZ group(B). IDH mutant gliomas with lower IGFBP2 gene expression receiving RT favor with better prognosis(C), while no difference was observed for patients assigned to TMZ group (D).

CGGA: Chinese Glioma Genome Atlas; IGFBP2: Insulin-like growth factor-binding protein 2; RT: Radiotherapy; TMZ: Temozolomide; IDH: Isocitrate dehydrogenase mutations

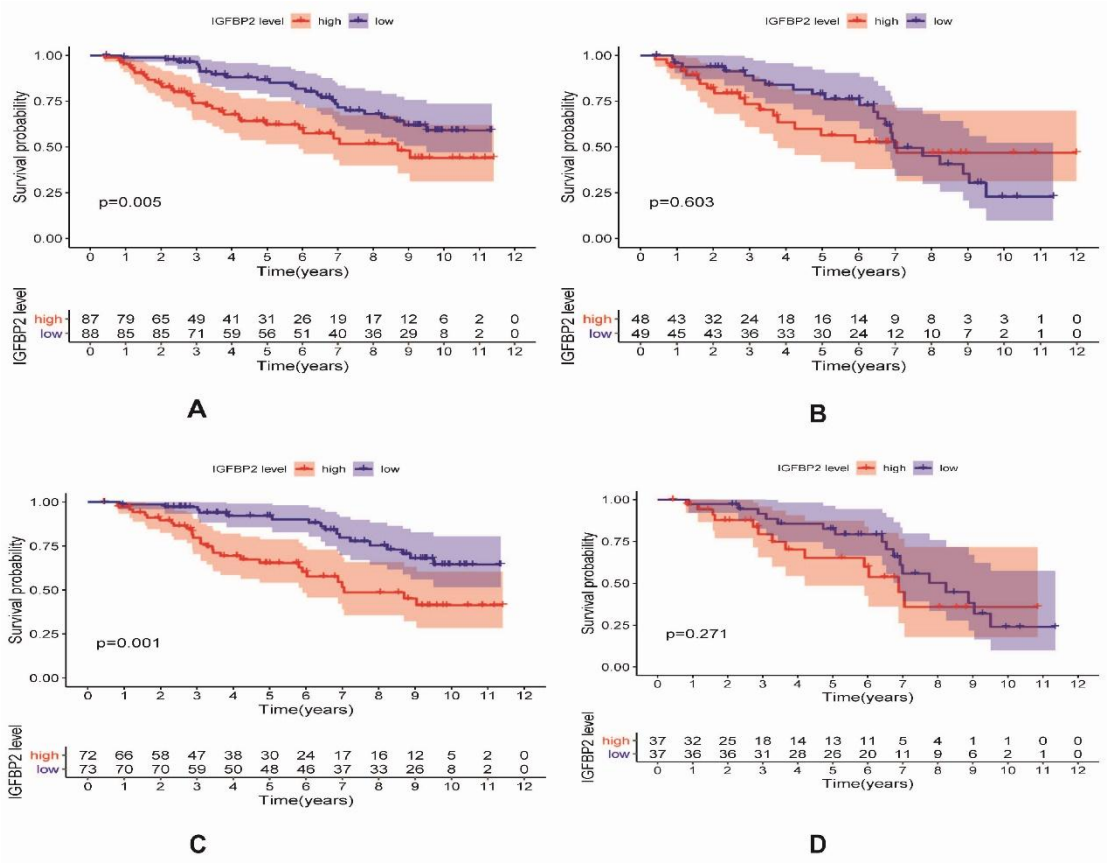

Figure 4. Patients assigned to lower IGFBP2 gene expression show a trend towards benefit from RT (A) compared with high-risk group in GEO cohort, no difference was observed for patients assigned to TMZ group(B). IDH mutant gliomas with lower IGFBP2 gene expression receiving RT favor with better prognosis(C), while no difference was observed for patients assigned to TMZ group (D). PFS assigned to other molecular subtypes according to differential gene expression (median) by Kaplan–Meier analysis not shown.

GEO: Gene expression omnibus; IGFBP2: Insulin-like growth factor-binding protein 2; RT, radiotherapy; TMZ: Temozolomide; PFS: Progression-free survival; IDH: Isocitrate dehydrogenase mutations

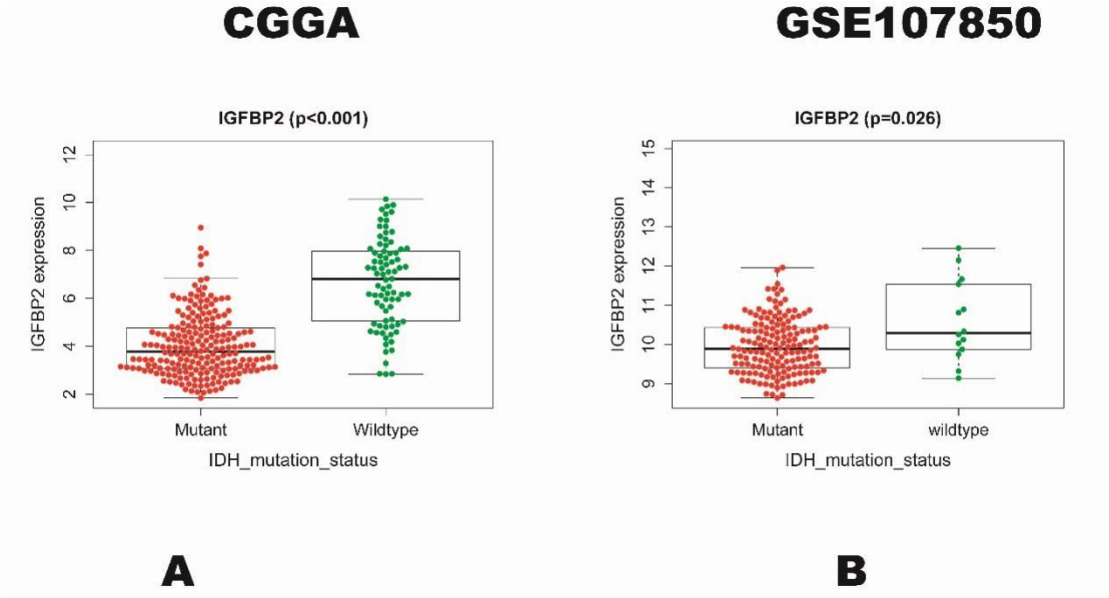

Figure 5. Overexpression level of IGFBP2 was significantly lower in IDH-wildtype than in IDH-mutant gliomas in CGGA (A) and GEO (B) cohorts.

CGGA: Chinese Glioma Genome Atlas; IGFBP2: Insulin-like growth factor-binding protein 2; GEO: Gene expression omnibus; IDH: Isocitrate dehydrogenase mutations

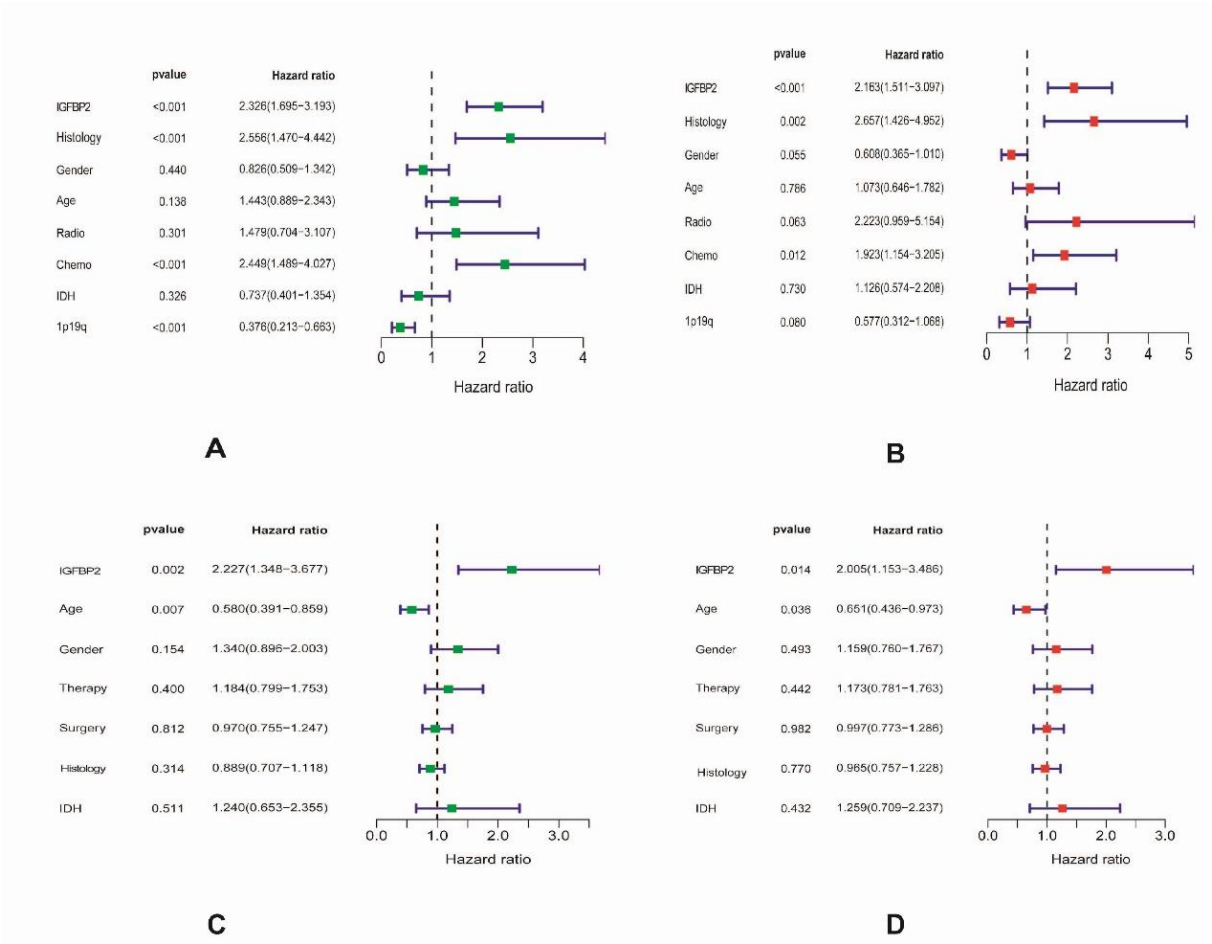

Figure 6. Univariate (A) and multivariate (B) cox regression analysis for OS in glioma patients from CGGA cohort. Univariate (C) and multivariate (D) cox regression analysis for OS in glioma patients from GEO cohort.

CGGA: Chinese Glioma Genome Atlas; IGFBP2: Insulin-like growth factor-binding protein 2; GEO: Gene expression omnibus; OS: Overall survival

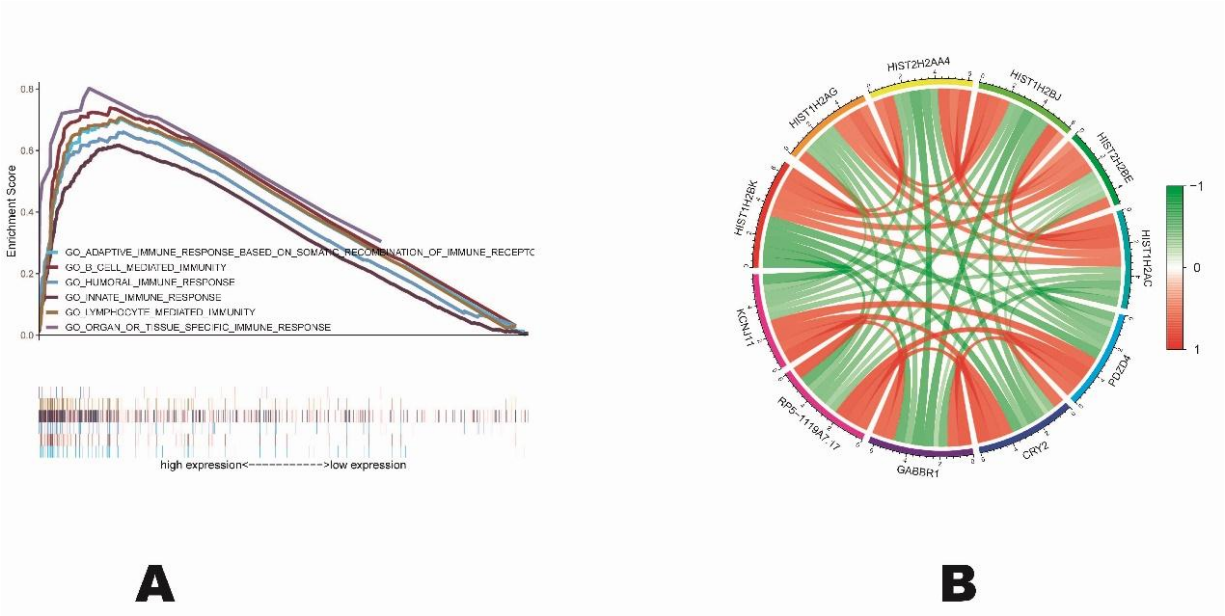

Figure 7. (A) GSEA results showing five differential pathways involved in collagen binding, collagen containing extracellular matrix, collagen metabolic process, collagen trimer and extracellular structure organization were significantly enriched in gliomas patients. (B) Five significantly positive genes (CDC20, CHIL3, KIF20A, HOXA7, and SERPINH1) and negative genes (TUB, REPS2, NALCN, CDR1, and TNR) correlated with overexpression of IGFBP2.

GSEA: Gene set enrichment analysis; IGFBP2: Insulin-like growth factor-binding protein 2

Table1. Demographic and clinical characteristics of LGGs patients

Parameter	CGGA group	GEO group
Age	63.3 ± 8.5	61.7 ± 8.7
Gender		
Male	130	107
Female	87	83
Histology		
Astrocytoma	67	49
Oligoastrocytoma	31	49
Oligodendroglioma	99	92
Unnamed		
IDH status		
Wild type	42	14
Mutant	175	162
Unnamed	NM	14
Surgery		
Partly	NM	113
Total	NM	41
Biopsy	NM	36
Treatment		
RT	175	93
CT	42	97

CGGA: Chinese Glioma Genome Atlas; CT: Chemotherapy, IDH: Isocitrate dehydrogenase mutations; LGGs: Lower-grade gliomas; NM: Not mentioned; RT: Radiotherapy; GEO: Gene expression omnibus