Immune heterogeneity and clinicopathologic characterization of IGFBP2 in 2447 glioma samples

Jinquan Cai, a,b,c,† Qun Chen a,b,c,† Yuqiong Cui a,b,c, Jiawei Dong a,b,c, Meng Chen a,b,c, Pengfei Wu a,b,c, and Chuanlu Jiang a,b,c

aDepartment of Neurosurgery, the Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China; bNeuroscience Institute, Harbin Medical University, Harbin, Heilongjiang, China; cChinese Glioma Cooperative Group (CGCG), Beijing, China.

ABSTRACT
Glioblastoma is an immunosuppressive, deadly brain tumor. IGFBP2, a circulating biomarker for cancer diagnosis and a potential immunotherapeutic target, is attracting more and more attention from oncologists and clinicians. Thus, it is urgent to thoroughly investigate the immune biological process of IGFBP2 to understand tumor immune complexity and provide potential evidence for anti-IGFBP2 therapy. Through authoritative public databases, we enrolled a total of 2447 glioma samples with gene expression profiles. Then, the clinical characteristics and immunosuppressive status of IGFBP2 in the glioma samples were analyzed. Immunohistochemical staining detected the expression of immunosuppressive biomarkers. We found that IGFBP2 expression was upregulated in high-grade glioma and GBM and downregulated in IDH mutant glioma. Increased IGFBP2 accompanied PTEN loss and EGFR amplification. Bioinformatic analysis revealed that IGFBP2 is related to immunological processes. We further selected specific immunologic related novel theoretical foundation and found IGFBP2 predominated immunosuppressive activities in GBM. Furthermore, we explored the relationship between IGFBP2 and genes that were well-characterized glioma-mediated immunosuppressive molecules to investigate the potential effect of IGFBP2. We discovered that IGFBP2 was correlated with CHI3L1, TNFRSF1A, LGALS1, TIMP1, VEGFA, ANXA1 and LGALS3, which were classic immunosuppressive biomarkers. Higher IGFBP2 expression predicted unfavorable survival for patients with GBM. Our findings implied that IGFBP2 is involved in immunosuppressive activities and is an independent unfavorable prognostic biomarker for patients with GBM. IGFBP2 is a potential immunotherapeutic target for GBM in future clinical trials.

Introduction
Glioblastoma is one of the most lethal tumors. Neurosurgical removal of the tumor accompanied with radiotherapy and adjuvant temozolomide is the conventional therapy for GBM. However, patients suffering from GBM just have a dismal prognosis with a median survival of less than one year. The discovery of the lymphatic system in the central nervous system gives inspiration to bring a novel theoretical foundation and new prospect for immunotherapy in brain tumors. Plenty of work has demonstrated the mutual effect between GBM and immunity. Multiple related biological processes influencing immune surveillance, such as the PI3K/Akt pathway, some chemokines, FAK, the IGF pathway, HIF-1α, IL-6, TGF-β, CTLA-4 and PD-1/PDL-1, could individually or collectively impact immunosurveillance. IGFBP2 is a member of the secreted IGF family that functions by interacting with circulating IGFs to modulate IGF-mediated signaling. As a secreted protein, IGFBP2 was reported to be a human tumor antigen that elicited T-cell and B-cell immunity in patients with some cancers. The circulating IGFBP2 antibodies may provide a potential approach for diagnosing early cancers in a broad population of patients. IGFBP2 peptide-specific T cells mediated an antitumor effect in a transgenic mouse model of breast cancer. A neutralizing antibody against IGFBP2 could impair downstream IGFBP2-mediated oncogenic signaling pathways and inhibit tumor cell spreading. Heretofore, there have been few reports comprehensively illustrating the immunosuppressive status and genomic alterations in glioma with different IGFBP2 expression. Thus, deeply investigating the immune biological process of IGFBP2 based on current genomic datasets may help to get a good idea of tumor immune complexity and guide potential anti-IGFBP2 therapy.

In the present study, we employed 2447 glioma specimens to further explore the IGFBP2 expression and clinical characteristics in glioma. IGFBP2 was upregulated in GBM and was an unfavorable prognostic biomarker for patients with GBM. Moreover, IGFBP2 was involved in the immunosuppressive response and synergistic with several immunosuppressive members, providing evidence for potential anti-IGFBP2 treatment in glioma immunotherapy.
Results

**IGFBP2 expression was upregulated in high grade glioma, GBM and downregulated in IDH mutant glioma**

IGFBP2 expression was analyzed according to the WHO grade system, histopathology and IDH mutation status (Supplementary Table 1). In the CGGA mRNA microarray dataset, the expression of IGFBP2 was highest in WHO IV glioma (Fig. 1A, \( P = 2.644 \times 10^{-37} \)) and GBM (\( P = 7.691 \times 10^{-39} \)). Furthermore, IDH wild type GBM expressed a higher level of IGFBP2 than IDH mutant GBM (\( P = 1.959 \times 10^{-08} \)). In addition, we also validated that WHO IV glioma and GBM had higher IGFBP2 expression than IDH wild type GBM (\( P = 1.959 \times 10^{-08} \)). In all datasets, GBM with wild type IDH presented a higher expression level of IGFBP2 than IDH mutant GBM (\( P = 4.705 \times 10^{-09} \) and \( P = 6.297 \times 10^{-4} \), respectively). These findings further suggest that higher IGFBP2 expression accompanies higher malignancy in glioma.

**Glioma containing different IGFBP2 expression profiles had distinct genomic and transcriptomic spectrums**

To uncover the molecular characteristics related to the expression pattern of IGFBP2, we collected available mutation and CNV information. The occurrence of the 1p/19q codeletion, a genomic hallmark of oligodendroglioma, decreased along with increasing IGFBP2. While Chr7 amplification accompanied Chr10 loss, a representative event in GBM, was enriched in the high IGFBP2 expression group (Fig. 2A, B). Among patients with high IGFBP2 expression, the most frequently deleted genomic region was 10q23.3, which encompasses the PTEN locus (Fig. 2B, \( P = 4.239 \times 10^{-99} \)). On the other hand, the most commonly amplified region associated with high expression level of IGFBP2 was 7p11.2, which contains EGFR
We conducted PCA to excavate the clinical (age at diagnosis and WHO grade or histological types) and molecular features such as EGFR and PETN alteration. PCA1 and PCA2 represented the top two dimensions showing a good separation between the high-IGFBP2 group and the low-IGFBP2 group in glioma specimens, which account for 45.32%, 36.21% in TCGA, 43.04%, 27.00% in CGGA database and 40.38%, 32.45% in Rembrandt database. Here, we observed a separation between high and low IGFBP2-expressing groups in the CGGA, TCGA and Rembrandt databases (Fig. 2C). PCA1 was correlated with clinical factors – age at diagnosis and WHO grade or histology types. And PCA2 mainly presented the molecular features – EGFR and PTEN alteration (Supplementary Table 3). Frequent mutations in IDH1, ATRX, CIC and NOTCH1 were more enriched in samples with a lower level of IGFBP2 than in samples with a higher level of IGFBP2 (Fig. 2D, $P = 1.157E-16$, $P = 3.274E-11$, $P = 8.622E-63$, $P = 8.267E-06$, respectively).

**Functional enrichment analysis revealed that IGFBP2 positive-related genes were associated with immunologic events**

To explore the biological features of GBM with different IGFBP2 expression, we selected the genes that strongly correlated with IGFBP2 expression (Pearson $r > 0.4$) in the CGGA, TCGA and Rembrandt databases. In the TCGA dataset, IGFBP2-positive-related genes were enriched in innate immune response (Fig. 3A, $P = 3.011E-4$, Benjamini = 0.0419), inflammatory response (Fig. 3A, $P = 7.664E-4$, Benjamini = 0.0458), leukocyte migration (Fig. 3A, $P = 1.420E-3$, Benjamini = 0.0487) and antigen processing and presentation of peptide antigen via MHC class I...
(Fig. 3A, P = 0.0262, Benjamini = 0.0498). We found that genes that were positively correlated with IGFBP2 expression were more involved in innate immune response (Fig. 3B, P = 2.509E-14, Benjamini = 1.704E-11), immune response (Fig. 3B, P = 1.451E-13, Benjamini = 6.568E-11), inflammatory response (Fig. 3B, P = 1.534E-13, Benjamini = 5.209E-11), leukocyte migration (Fig. 3B, P = 1.389E-15, Benjamini = 1.960E-12) and positive regulation of B cell proliferation (Fig. 3B, P = 3.381E-3, Benjamini = 0.0483) in CGGA cohort. IGFBP2-positive-related genes were relevant to antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent (Fig. 3C, P = 6.760E-10, Benjamini = 3.280E-07), leukocyte migration (Fig. 3C, P = 1.906E-3, Benjamini = 0.0421), NIK/NF-kappaB signaling (Fig. 3C, P = 5.660E-6, Benjamini = 8.820E-04) and antigen processing and presentation of peptide antigen via MHC class I (Fig. 3C, P = 1.310E-8, Benjamini = 5.180E-06) in Rembrandt dataset. GSEA confirmed that IGFBP2 was related to immunologic processes in the CGGA (NES = 1.852, FDR = 0.0360), TCGA (NES = 1.917, FDR = 0.0245) and Rembrandt databases (Fig. 3D, NES = 1.936, FDR = 0.0202). We overlapped IGFBP2-positive-related genes of the CGGA, TCGA and Rembrandt datasets and obtained 127 genes (Supplementary Table 4). KEGG Pathway analysis revealed that these genes were mainly involved in immune related pathways, including the interleukin-10 (IL-10) signaling pathway (Fig. 3E, P = 6.321E-3), a recognized immunosuppressive pathway.16,17 These findings imply that IGFBP2 might play a role in immunologic biological processes in GBM.

**Immunosuppression is a predominate feature in GBMs with high IGFBP2**

To get a further comprehensive study of IGFBP2-related immunologic biological processes in malignant brain tumors, we chose classifier gene sets for specific immune cell lineages6 and immunologic effectors19 which were subsequently defined as metagenes (Supplementary Table 5). Based on the single sample GSEA, we observed that IGFBP2 was positively associated with immune cell lineages, such as helper T cells (P = 2.57E-06), cytotoxic T cells (P = 3.01E-06), myeloid cells (P = 9.60E-08), monocytes (P = 1.77E-03), NK cells (P = 6.43E-04), dendritic cells (P = 2.39E-02) and T-cell lineage (P = 1.05E-07) in TCGA dataset (Fig. 4A). A similar pattern of GBM in the CGGA and Rembrandt datasets was observed (Fig. 4B, C, Supplementary Table 6). Next, we parsed these three datasets to show that the proportion of patients with high IGFBP2 had significantly higher mRNA expression of previously well-characterized glioma mediated immunosuppressors compared with immune activators (Fig. 4D-E, TCGA: r = 0.419, r = 0.275; CGGA: r = 0.556, r = 0.549; Rembrandt: r = 0.589, r = 0.459). These results indicate that IGFBP2-related immunologic biological processes might, through a series of immune cells, play an important role in the immunosuppressive response.

**IGFBP2 was synergistic with other immunosuppressive genes in glioblastoma**

As revealed above, IGFBP2 played a key role of immunosuppression in GBM. Therefore, we would like to explore the key immunosuppressive effectors in GBM. We overlapped IGFBP2-positive-related genes of the CGGA, TCGA and Rembrandt datasets with immunosuppressive gene sets and got seven effectors (Fig. 5A). IGFBP2 was found to significantly correlate with CHI3L1 (CGGA, r = 0.642; TCGA, r = 0.552 and Rembrandt, r = 0.555), TNFRSF1A (CGGA, r = 0.590; TCGA, r = 0.463 and Rembrandt, r = 0.579), LGALS1 (CGGA, r = 0.643; TCGA, r = 0.458 and Rembrandt, r = 0.631), TIMP1 (CGGA, r = 0.748; TCGA, r = 0.609 and Rembrandt, r = 0.683), VEGFA (CGGA, r = 0.599; TCGA, r = 0.567 and Rembrandt, r = 0.734), ANXA1 (CGGA, r = 0.590; TCGA, r = 0.409 and Rembrandt, r = 0.641), and LGALS3 (CGGA, r = 0.569; TCGA, r = 0.485 and Rembrandt, r = 0.579) (Fig. 5B-D, Supplementary Table 7). IHC demonstrated that the protein level of IGFBP2 in GBM tissues was positively correlated with immunosuppressive molecules VEGFA (r = 0.6387), ANXA1 (r = 0.5693), LGALS3 (r = 0.5249), CHI3L1 (r = 0.4672), TNFRSF1A (r = 0.5789), LGALS1 (r = 0.5691) and TIMP1 (r = 0.5189) (Fig. 6, Supplementary Fig. 1).

**High expression of IGFBP2 predicted unfavorable survival in GBM**

We employed dichotomization to separate cases for depicting the survival curves according to the median value and the best cutoff point. We evaluated the prognostic value of IGFBP2 in two databases. Patients with GBM containing higher IGFBP2 expression had a significantly shorter survival times than their counterparts (Fig. 7A, B, P = 0.0172, P = 0.0104) in the CGGA RNAseq and mRNA microarray data. We also observed that IGFBP2 was a dismal biomarker for patients with GBM in TCGA mRNA microarray data (P = 0.0012) and RNAseq (Fig. 7C, D, P = 0.0140). Next, we conducted cox regression analysis to evaluate the prognostic value of IGFBP2 expression and other prognostic factors. The results confirmed that the expression of IGFBP2 (P < 0.001) along with karnofsky performance score (KPS) (P = 0.009) and extent surgery (P = 0.017) were prognostic indicators in CGGA RNAseq data (Fig. 7E). In CGGA mRNA microarray data, the expression of IGFBP2 was still associated with survival (P = 0.025) after adjusting for other factors such as TCGA subtype, chemotherapy, radiotherapy and age at diagnosis (Fig. 7F). In TCGA mRNA microarray data, the expression of IGFBP2 (P = 0.032), along with KPS (P < 0.0001), radiotherapy (P = 0.046) and age at diagnosis (P < 0.0001), were prognostic indicators (Fig. 7G). The expression of IGFBP2 (P = 0.008) was still an independent prognostic indicator in TCGA RNAseq data (Fig. 7H). These results indicate that IGFBP2 is an unfavorable predictor for patients with GBM.

**Discussion**

Glioblastoma is the most common malignant primary tumor of the central nervous system (CNS) in adults, contributing to approximately 50% of all gliomas.19 Despite advances in neurosurgery, along with the widely recognized chemotherapy and radiotherapy in newly diagnosed GBM, improvements in patient survival are very limited.20 Therefore, new treatment concepts and therapeutic approaches are urgently needed. Immune therapy is increasingly popular treatment method for...
GBM. Immune checkpoint blockades (e.g., PD-1, CTLA4) and chimeric antigen receptor T-Cell immunotherapy (CAR-T) have shown potential benefits for cancer treatment. Currently, patients suffering from melanoma and lung carcinoma benefit from novel immunotherapeutic treatment. GBM develops in a relatively immune-privileged CNS and thrives in an immunosuppressive microenvironment. In the present study, we would like to explore and describe the hidden relationship between glioma and immunosuppression through bioinformatic analysis.

Figure 3. Functional enrichment analysis reveals that IGFBP2 positive-related genes are associated with immunologic events. (A-C) IGFBP2 associated with biological process by GO analysis in CGGA, TCGA and Rembrandt datasets. (D) GSEA indicated a significantly enhanced immunologic process in cases with high IGFBP2 expression. (E) A total of 127 genes that overlapped IGFBP2-positive-related genes in the CGGA, TCGA and Rembrandt datasets were analyzed by the pathway analysis tool, ClueGO. P < 0.01 indicates P < 0.01.

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Different glioma antigen molecules and immune related genes manifest differential expression patterns among GBM subtypes. The mesenchymal subtype, in which an enrichment of immunologic genes is involved, is sensitive to anti-tumor inflammatory responses, including immunosuppression. First, we enrolled the transcriptome data of more than 2000 glioma patients,
including 447 gliomas from the CGGA, 475 gliomas from the Rembrandt database, and 1241 gliomas from the TCGA database. Through a deep analysis of the biological function of IGFBP2 in GBM, we found that IGFBP2 played an important role in immunologic processes of GBM, especially in immunosuppressive activities (e.g., immunosuppressive checkpoints, tumor-supportive macrophage chemotactic and skewing molecules) and immunosuppressive pathways (IL-10 signaling pathway). IL-10 limits intratumoral dendritic cell activation and production of IL-12, thereby inhibiting cytotoxic T cell responses during chemotherapy. Inflammatory responses are suppressed by IL-10 through inhibiting macrophage activation. The role of IGFBP2 in GBM was strongly synergistic with other immunosuppressive members such as CHI3L1, TNFRSF1A, LGALS1, TIMP1, VEGFA, ANXA1 and LGALS3 in both transcriptome and protein levels.

Tumor-associated macrophages (TAMs) are a lineage of immune cell population present in tumor tissues, which skew towards an M2-altered functional profile and play a crucial role in immune evasion within tumors. M2 macrophage-derived CHI3L1 specifically binds to the interleukin-13 receptor α2 chain (IL-13Rα2) of gastric and breast cancer cells, promoting cancer metastasis. Tumor necrosis factor (TNF) is a potent promoter of carcinogenesis and potentially important target for cancer prevention. Deletion of TNF or TNFRSF1A genes protects mice from 3-methylcholanthrene (MCA)-induced carcinogenesis and prevents MCA induction of cell-mediated suppression of NK-cell/DC crosstalk. LGALS1 is a member of a family of b-galactoside-binding lectins and has been reported that this represents the primary mechanism by which tumor-derived LGALS1 restsains antitumor immunity.

Figure 4. Immunosuppression is a predominate feature in GBMs with high IGFBP2 expression. (A-C) IGFBP2 was related to immune cell lineages in the CGGA, TCGA and Rembrandt datasets. (B-D) IGFBP2 was related to well-recognized glioma-associated immunosuppressive activities.
Figure 5. Correlation of immunosuppressive genes and IGFBP2 in GBM at transcriptome level. (A) A Venn diagram showed the immunosuppressive genes among IGFBP2-positive-related genes of the CGGA, TCGA and Rembrandt datasets and immunosuppressive gene sets. (B-D) Correlation of IGFBP2 and immunosuppressive genes in GBM.

Figure 6. Correlation of immunosuppressive molecules and IGFBP2 in GBM at the protein level. IHC staining showed the correlation of IGFBP2 and immunosuppressive molecules (AXAN1, VEGFA and LGALS3) in GBM tissues.
suppression in glioma could extend patient survival by recruiting NK immune surveillance which could eradicate glioma cells. Many studies demonstrate that elevated levels of TIMP-1 are associated with poor prognosis for a variety of cancers, including breast, colorectal cancer and lung carcinoma, or hematopoietic tumors. There is a linear relationship between TIMP-1 expression and Tregs in pancreatic cancer, suggesting that TIMP1 can promote immunosuppression. VEGFA can contribute to prevent the development of efficient antitumor immune responses by promoting local and systemic immunosuppression. VEGFA/VEGFR-targeting therapies may revert such an immunosuppressive state. Immune responses to glioma could be impaired by the tumor itself through expression of immunosuppressive cytokines such as TGF-β. In breast cancer, up-regulation of ANXA1 enhances TGF-β signaling. LGALS3 has been shown in vitro to possess several immunomodulatory functions, such as weakening the affinity of the T-cell receptor (TCR) for its cognate MHC I–peptide ligand by separating the TCR from its CD8 coreceptor and inducing T-cell apoptosis. Inhibiting LGALS3 in conjunction with CD8+ T-cell–directed immunotherapies could enhance the tumor-specific immune response.

The finding that IGFBP2 is associated with immunosuppressive molecules and implies that IGFBP2 plays a role in the immunosuppressive microenvironment in GBM. IGFBPs seems to increase the activity of IGFs to promote the IGF signaling pathway. These actions might involve in lengthening the half-life of IGF1 and IGF2, and delivering IGF1R. The human mesenchymal stem cells (MSCs) culture supernatant induced CD4+FOXP3+ Tregs that expressed IGF-1R, and IGF-2R, showing antiproliferative activity against CD4+ T cells. The induction of Tregs by human MSC culture supernatant was enhanced by the addition of IGF and suppressed by the inhibition of IGF-1R. CD4+Foxp3+ Tregs could be play an important role in suppressing immune surveillance among tumor environment. IGFBP2 was confirmed to activate integrin β1 and downstream invasion pathways to induce cell motility and activate NF-κB signaling pathway. NF-κB is a major factor to induce immune responses and may be responsible for some cancers, inflammatory and autoimmune diseases. NF-κB is important in determining this balance between the protumour and antitumour properties of macrophages. Activated NF-κB signaling pathways were associated with TIMP-1 overexpression in tumor cells. Such regulation enabled TAMs to sustain the smouldering inflammatory microenvironment present in established metastatic neoplasia. Above all, IGFBP2 might potentially regulate the IGF and NF-κB signaling pathways to impact the immune response in the tumor tissue.

In addition, IGFBP2 expression was further confirmed to be significantly upregulated in highly malignant gliomas and predicted a worse outcome for patients. Next, distinct genomic and transcriptomic spectrums were analyzed. We found that Chr7 (EGFR) amplification accompanied with Chr10 loss (PTEN) occurred more frequent when IGFBP2 expression was elevated. On the other hand, most mutations of IDH1 and 1p/19q codeletion occurred in the relatively lower IGFBP2 group. All these findings also implicated IGFBP2 as a biomarker and potential therapeutic target for glioma immunotherapy.

In conclusion, we explored the clinical roles and immune biological processes of IGFBP2 in more than two thousand diffuse gliomas. IGFBP2 was involved in the immunosuppressive response and synergistic with several immunosuppressive members, acting
as a potential therapeutic target. These findings extend our understanding of anti-cancer immunotherapy in glioma.

**Methods and materials**

**Data collection**

Four kinds of transcriptome data from patients who were diagnosed with glioma (WHO II-IV) were used. The datasets used were The Chinese Glioma Genome Atlas (CGGA) database (RNAseq, n = 137, microarray, n = 310) (http://www.cgga.org.cn), The Cancer Genome Atlas (TCGA) database (RNAseq, n = 702, microarray, n = 539) (http://cancergenome.nih.gov/), the GSE16011 database (n = 284) and the Rembrandt database (n = 475) (https://caintegrator.nci.nih.gov/rembrandt/). The copy number variation (CNV) profile and somatic mutation data were obtained from TCGA data portal (http://cancergenome.nih.gov/).

**Immunohistochemistry**

We obtained 25 paraffin-embedded GBM tissues from patients who provided informed consent under an Institutional Ethics Committee-approved study from the Second Affiliated Hospital of Harbin Medical University. IHC assay was described in our previous research. Brieﬂy, the slices were incubated with primary antibody (IGFBP2, CST, 3922, 1:25; CHI3L1, Invitrogen, PA5-46996, 1:100; TNFRSF1A, Invitrogen, 710368, 1:100; LGALS1, CST, 13888, 1:250; TIMP1, Abcam, ab211926, 1:100; VEGFA, Abcam, ab1316, 1:100; ANXA1, CST, 32934, 1:400 and LGALS3, CST, 46995, 1:100; TNFRSF1A, Invitrogen, 710368, 1:100; LGALS1, Invitrogen, 432934, 1:400 and LGALS3, CST, 87985, 1:600) for 12 h at 4°C, then incubated with secondary antibody (ZSGB, 1:100) at 37°C for 30 min. After washing with a phosphate-buffered solution (PBS) three times for 5 min, the slices were stained with Diaminobenzidine (DAB) for 2 min, rinsed in PBS and counterstained with hematoxylin. Quantitative evaluation was performed by examining each slice using at least three different high-power fields with the most abundant stained cells.

**Statistical analysis**

Differences in variables were assessed by Student’s t-test for two groups or one-way analysis of variance (one-way ANOVA) for at least three groups (WHO grade and histological types as the basal levels, age at diagnosis and gender as other conditions). Comparisons of binary and categorical patient characteristics between subgroups were performed via the Chi-square test. The Kaplan–Meier survival curve and log-rank tests were used to describe survival distributions and assess statistical significance between two groups. The multiple variates cox proportional hazard model and the principal components analysis (PCA) were performed by using SPSS 22.0. GISTIC2.0 was used to assess CNV associated with IGFBP2 expression. GISTIC value less than −1 or more than 1 was defined as a deletion or amplification. Gene ontology (GO) analysis was performed when gene sets (Pearson r > 0.4) were submitted to the DAVID website (http://david.abcc.ncifcrf.gov/home.jsp). ClueGO, a Cytoscape APP, was used to perform pathway analysis. R packages, such as circlize and ComplexHeatmap, were used to produce figures. Biological processes were further analyzed through gene set enrichment analysis (GSEA). Single-sample GSEA (ssGSEA) was used to calculate the enrichment score of every gene set for every sample. Heatmaps were constructed and produced by Gene Cluster 3.0 and Gene Tree View software. P values less than 0.05 were considered statistically significant.

**Abbreviations**

- ATRX: Alpha Thalassemia/Mental Retardation Syndrome X-Linked
- ANXA1: Annexin A1
- CGGA: Chinese Glioma Genome Atlas
- CHI3L1: Chitinase 3 Like 1
- CIC: Capicua Transcriptional Repressor
- CNS: Central Nervous System
- CNV: Copy Number Variation
- EGFR: Epidermal Growth Factor Receptor
- GBM: Glioblastoma
- GO: Gene Ontology
- GSEA: Gene Set Enrichment Analysis
- IDH1: Isocitrate Dehydrogenase (NADP(+))
- IGFBP2: Insulin Like Growth Factor Binding Protein 2
- IHC: Immunohistochemistry
- IL-10: Interleukin 10
- KEGG: Kyoto Encyclopedia of Genes and Genomes
- KPS: Karnofsky Performance Score
- LGALS1: Galectin 1
- LGALS3: Galectin 3
- MSCs: Mesenchymal Stem Cells
- NOCTH1: Translocation-Associated Notch Protein TAN-1
- PBS: Phosphate-buffered Solution
- PCA: Principal Components Analysis
- PTEN: Phosphatase And Tensin Homolog
- ssGSEA: Single-sample GSEA
- TCGA: The Cancer Genome Atlas
- TCR: T-cell Receptor
- TIMP1: Tissue Inhibitor Of Metalloproteinases 1
- TMZ: Temozolomide
- TNFRSF1A: TNF Receptor Superfamily Member 1A
- VEGFA: Vascular Endothelial Growth Factor A
- WHO: World Health Organization

**Disclosure of potential conflicts of interest**

The authors declare no potential conflicts of interest.

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**ORCID**

Jinquan Cai http://orcid.org/0000-0002-6773-3546
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