IL8 associated with M2 macrophage infiltration as a prognostic biomarker differentiates WHO grade III and grade IV gliomas

Jang-Chun Lin
Shuang Ho Hospital, Taipei Medical University
Cheng-Chin Lee
Taipei Medical University
Yu-Jia Chang
Taipei Medical University
Ai-Wei Lee
Taipei Medical University
Chien-Yu Huang
National Tsing Hua University
Wei-Hsiu Liu (liubear0812bear@yahoo.com.tw)
Tri-Service General Hospital and National Defense Medical Center

Research Article

Keywords: Glioma, IL8, M2 macrophage

Posted Date: September 1st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2004456/v1

Abstract

Malignant glioma can be divided into grade III (Gr. III) and grade IV (Gr. IV). Gr. III glioma patients have significantly better overall survival (OS) than those with Gr. IV glioma, also known as glioblastoma multiforme (GBM). We explored differentially expressed genes (DEGs) from the GSE4290 and GSE109857 datasets between Gr. III and Gr. IV gliomas. Six candidate prognostic genes for GBM were determined from survival analysis of data obtained from The Cancer Genome Atlas (TCGA), and the results were validated via assessments of the OS of Gr. III glioma and GBM patients using data obtained from the Chinese Glioma Genome Atlas (CGGA). Then, the expression levels of CXCL8, also named IL8, had a significant relationship with progression-free survival (PFS) in Gr. IV patients (P = 0.028), and had no effect in Gr. III glioma patients (P = 0.522). Furthermore, the receiver operating characteristic (ROC) curve revealed the critical role of IL8 with an accuracy value of 0.899 for discriminating Gr. IV from Gr. III in TCGA and 0.644 in CGGA. Macrophage (P < 0.001) and neutrophil (P < 0.001) levels were highly related to IL8 levels, especially for M2 macrophage markers. All M2 markers increased the correlative efficiency from primary GBM to the recurrence group. IL8 in GBM has a significant effect on disease prognosis and tumor immunity. IL8-associated M2 macrophage infiltration could be a prognostic biomarker used to classify GBM and Gr. III gliomas.

Introduction

The histological grading of brain glioma is described in the guidelines of the World Health Organization (WHO) tumor classification of the central nervous system (CNS). More precise standards for the subgrouping of glioma could provide more standard guidelines for clinical treatment and more valuable prognostic information. Malignant glioma consists of WHO grade III (Gr. III) glioma and glioblastoma multiforme (GBM), also called grade IV (Gr. IV) glioma. Gr. III glioma includes anaplastic astrocytoma (AA), anaplastic oligoastrocytomas (AOAs) and anaplastic oligodendrogliomas (AOs).(Ahmed et al., 2014) It is generally acknowledged that the prognosis of Gr. III glioma is better than that of GBM. (Noiphithak and Veerasarn, 2017) Nevertheless, it is well known that there are no major prognostic biomarkers that can be used to differentiate WHO gr. II and Gr. IV gliomas.

Patients with malignant glioma, regardless of whether it is Gr. III glioma or Gr. IV glioma, should receive surgical tumor removal(Sanai, 2012) after the establishment of appropriate diagnosis. The most common adjuvant treatment for GBM is concurrent chemoradiotherapy (CCRT) with temozolomide (TMZ) followed by adjuvant chemotherapy.(Stupp et al., 2005; Mirimanoff et al., 2006; Arakawa et al., 2021) According to the molecular analyst report(Kouwenhoven et al., 2009) of European Organisation for Research and Treatment of Cancer (EORTC) study 26951 from a multicenter prospective phase III trial, patients with AOA with mutation of the isocitrate dehydrogenase (IDH) gene and 1p/19p codeletion and those with Gr. III glioma should also receive radiotherapy (RT) and adjuvant chemotherapy with PVC (procarbazine, lomustine, and vincristine).(Kouwenhoven et al., 2009; Wick et al., 2009) Patients with IDH mutant AA should receive postoperative treatments, including standard RT followed by adjuvant TMZ or CCRT with TMZ. However, even after standard adjuvant treatment, WHO Gr. III and Gr. IV glioma patients

exhibit significantly different prognoses. The median survival (MS) of glioma Gr. III patients is approximately 24–60 months(McGirt et al., 2009) and is longer than that of GBM patients, who have a MS of only 11–15 months.(Burnet et al., 2007; Siangprasertkij and Navalitloha, 2008) There was a significantly different prognosis observed between Gr. III and Gr. IV glioma patients. In a series of studies,(Louis et al., 2016) favorable molecular prognostic factors were predicted, including IDH1/2 mutation,(Hegi et al., 2005; Eckel-Passow et al., 2015) 1p/19q codeletion,(Eckel-Passow et al., 2015) loss of alpha-thalassemia mental retardation X-linked syndrome (ATRX),(Abedalthagafi et al., 2013) tumor protein p53 (TP53) wild-type (wt.) expression, telomerase reverse transcriptase (TERT) promoter wt.(Eckel-Passow et al., 2015) expression, and O^6-methylguanine-DNA-methyltransferase (MGMT) promoter hypermethylation,(Hegi et al., 2005) but there are no specific biomarkers that can be used to assess the prognosis of Gr. III and Gr. IV glioma patients. Thus, it is valuable for the medical management of these conditions to further identify the prognostic biomarkers that differentiate WHO Gr. III and Gr. IV gliomas.

Therefore, we explored differentially expressed genes (DEGs) between Gr. III and Gr. IV gliomas. We attempted to base our analysis of Gr. IV Glioma on the results of screens of the prognostic genes from the DEGs. Then, we further validated those high-grade glioma-related prognostic hub genes in an external validation set. Finally, we elucidated the association between prognostic gene signatures and immune function to differentiate Gr. III and Gr. IV gliomas.

Materials And Methods

Microarray data from the Gene Expression Omnibus (GEO)

We downloaded the datasets GSE4290 and GSE109857 from the GEO (https://www.ncbi.nlm.nih.gov/geo) database.(Barrett et al., 2007) The GSE4290 dataset(Sun et al., 2006) was based on the Affymetrix Human Genome U133 Plus 2.0 Array (GPL570) uploaded by Sun et al.,(Sun et al., 2006) containing 31 grade III glioma samples and 71 GBM samples. The GSE109857 dataset,(Zhang et al., 2020) which was based on Agilent-014850 Whole Human Genome Microarray 4x44K G4112F (GPL6480), was uploaded by Zhang et al.,(Zhang et al., 2020) including 34 grade III glioma samples and 89 GBM samples (Table 1).

Table 1
Details of two datasets downloaded from GEO.

Dataset	Grade III	Grade IV	Platform
GSE4290	31	77	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array
GSE109857	34	89	GPL6480 Agilent-014850 Whole Human Genome Microarray 4x44K G4112F
Total	65	166	

Identification of differentially expressed genes (DEGs)

We used the GEO2R-friendly tool(Barrett et al., 2013) to conduct differential gene expression analysis and reanalyzed the microarray data in the GSE4290(Sun et al., 2006) and GSE109857 datasets. In GEO2R, R language was applied for GEO query, and limma packages were used for gene expression analysis. The grade III and grade IV groups were separately selected to identify DEGs. The false discovery rate and *p* values were calculated with a t test and Benjamini–Hochberg method, respectively.(Aubert et al., 2004) The cutoff criteria were adj. P value of < 0.05 and |log[fold change (FC)]|≥1. The volcano plot was generated using the R (3.6.3) package “ggplot2”.

Protein–protein interaction (PPI) network analysis

The PPI network was generated by the STRING website (http://www.string-db.org/).(Szklarczyk et al., 2015) This website tool generates associations between proteins based on genetic context, coexpression, high-throughput experiments, and previous knowledge. The cutoff score was selected as 0.400 (medium confidence). Cytoscape software (version 3.9.1, http://www.cytoscape.org) was used for the analysis of PPI pairs with the CytoNCA app in Cytoscape.(Shannon et al., 2003) A cutoff of ≥ 2 was used to identify hub genes (highly connected genes) by calculating the degree value.

Functional enrichment analysis

The R clusterProfiler package (v3.14.1)(Yu et al., 2012) was employed for functional enrichment analysis of hub genes using the Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) pathway analysis.(Ogata et al., 1999) Statistical significance was defined as a p value of < 0.05 and an FDR value of < 0.25. Visualization of KEGG outputs was conducted by the R (3.6.3) package “ggplot2”.

Survival analysis using the Kaplan–Meier method and validation using the CGGA database

Based on low-grade glioma (LGG) and GBM data from TCGA (https://portal.gdc.cancer.gov/(Ceccarelli et al., 2016; Liu et al., 2018) and the CGGA (https://www.cgga.org.cn/), Kaplan–Meier analysis by the log-rank test was performed using the survival and survminer packages in R. The CGGA mRNAseq_325

dataset, which contained RNA-seq data and clinical information, was used to validate the prognostic value of candidate genes.(Zhao et al., 2021)

Gene expression and receiver operating characteristic curve analysis

The gene expression analysis based on TCGA data was performed by R (3.6.3) and visualized by the ggplot2 package. The Mann–Whitney U test (nonparametric) was used to calculate the difference in gene expression between grade III and grade IV samples in the GBMLGG cohort. Statistical significance was defined as $P < 0.05$. The R pROC package was used to analyze receiver operating characteristic curves, and the ROC plots were generated by ggplot2.

Tumor immune infiltration and immune cell marker correlation analysis

We explored the enrichment level of 23 tumor-infiltrating lymphocytes by using the "GSVA" package based on the ssGSEA (single-sample gene set enrichment analysis) method.(Hänzelmann et al., 2013) Then, we used TIMER (https://cistrome.shinyapps.io/timer/), an interactive web portal, to investigate the relationship between CXCL8 expression and the levels of different gene markers of immune cells.

Results

Identification of DEGs between Gr. III and Gr. IV gliomas from GEO datasets

We present the flowchart of this study in Fig. 1. DEGs were analyzed based on the GSE4290 and GSE109857 gene expression profiles. In the GSE4290 dataset, there were a total of 217 upregulated genes and 177 downregulated genes between Gr. III and Gr. IV, while in the GSE109857 dataset; there were 1022 upregulated genes and 445 downregulated genes. Using the ggplot2 package of R software, we visualized the results of the DEG screening via volcano plots (Fig. 2A). Then, Venn diagrams were used to depict the common up/downregulated DEGs between the two datasets. As a result, 98 genes were identified as differentially expressed, including 62 upregulated genes and 36 downregulated genes (Fig. 2B).

Twenty-three key gene candidates as prognostic factors for Gr. III and Gr. IV gliomas identified via PPI network establishment and KEGG enrichment analysis

The common DEGs were loaded into the STRING database (https://string-db.org/) to obtain PPI pairs, which were then imported into Cytoscape for hub gene identification. A total of 97 nodes and 232 edges were identified from the PPI network. Using the CytoNCA plugin, 48 DEGs were identified as hub genes according to a degree value of ≥ 2 (Fig. 3A).

The 48 hub genes were applied to obtain KEGG enrichment analysis by the DAVID online tool for investigation into the pathways. The significantly enriched KEGG pathways were human papillomavirus infection, PI3K-Akt signaling pathway, ECM-receptor interaction, focal adhesion, AGE-RAGE signaling pathway in diabetic complications, amoebiasis, protein digestion and absorption, proteoglycans in cancer, relaxin signaling pathway, small cell lung cancer, NOD-like receptor signaling pathway, bladder cancer, and malaria (Fig. 3B). To further refine the list of 48 hub genes to the key genes between Gr. III and Gr. In IV gliomas, a total of 21 upregulated and two downregulated hub genes were enriched in KEGG pathways. These 23 genes were identified as “Key genes” (Fig. 3C).

Identification and validation of prognostic genes through Kaplan–Meier analysis and use of the CGGA

To investigate the prognostic value, including the effects on overall survival (OS) and progression-free survival (PFS), of the 23 key genes in GBM and Gr. III glioma, Kaplan–Meier (K-M) analysis was performed using the RNA-seq data and clinical information in TCGA (Table S1). In GBM patients, we found that the expression of THBS1 (HR = 1.40, P = 0.046), CXCL8 (HR = 1.45, P = 0.028), IBSP (HR = 1.52, P = 0.012), COL8A1 (HR = 1.15, P = 0.023) and GBP5 (HR = 1.43, P = 0.035) was associated with a shorter progression-free interval, and the expression of HES5 (P = 0.027) in GBM patients was associated with a poor prognosis in terms of OS. These six genes associated with prognosis in GBM were defined as candidate prognostic genes. After further evaluation of 6 candidate prognostic genes in Gr. III glioma patients, five candidate genes, THBS1 (HR = 1.51, P = 0.035), IBSP (HR = 2.18, P < 0.001), HES5 (HR = 0.47, P < 0.001), COL8A1 (HR = 2.66, P < 0.001) and GBP5 (HR = 2.43, P < 0.001), were found to be related to significantly shorter PFS. Four candidate genes were significantly associated with shorter OS, including IBSP (HR = 1.86, P = 0.005), HES5 (HR = 0.54, P = 0.004), COL8A1 (HR = 2.70, P < 0.001) and GBP5 (HR = 2.09, P = 0.001). Then, we applied the CGGA database, which is based on information from Chinese glioma patients, to validate the prognostic value of six candidate genes. The K–M analysis showed that two candidate genes, THBS1 and CXCL8, were significantly associated with poor overall survival in GBM but not in Gr. III patients (Table 2).

Table 2
A relationship of candidate gene expression with overall survival in Gr. IV or Gr. III glioma

	TCGA				CGGA			
Gene	Grade IV		Grade III		Grade IV		Grade III	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
THBS1	1.19 (0.84–1.67)	0.312	1.15 (0.74–1.78)	0.536	1.76 (1.23–2.52)	0.001*	1.22 (0.72–2.09)	0.456
CXCL8	1.35 (0.96–1.89)	0.077	0.95 (0.61–1.47)	0.801	1.61 (1.12–2.30)	0.006*	1.35 (0.79–2.30)	0.271
IBSP	1.29 (0.92–1.81)	0.131	1.86 (1.20–2.87)	0.005*	1.37 (0.96–1.95)	0.075	1.76 (1.03–3.01)	0.036*
HES5	0.69 (0.49–0.97)	0.027*	0.54 (0.34–0.84)	0.004*	1.05 (0.74–1.50)	0.767	0.85 (0.50–1.46)	0.563
COL8A1	1.15 (0.82–1.61)	0.407	2.70 (1.74–4.20)	< 0.001*	1.40 (0.98–1.99)	0.059	2.60 (1.50–4.50)	0.085
GBP5	1.21 (0.87–1.70)	0.247	2.09 (1.35–3.23)	0.001*	1.32 (0.93–1.88)	0.113	1.82 (1.06–3.12)	0.025*

After consolidating the results of the K-M plots of data regarding TCGA, THBS1, IBSP, COL8A1, GBP5 and HES5 were found to be related to prognosis in both Gr. III and Gr. IV glioma patients, while CXCL8 was only associated with prognosis in Gr. IV glioma patients. Validation of the CGGA K-M plots revealed that the list of candidate prognostic genes could be simplified to THBS1 and CXCL8. This finding might suggest that THBS1 and CXCL8 play critical roles in the disease progression of both gliomas.

CXCL8 is a unique prognostic gene in GBM

To demonstrate the mRNA expression levels of THBS1 and CXCL8 in Gr. III and Gr. IV glioma patients, we visualized the expression levels in TCGA (Fig. 4A, left and B, left). The expression levels of THBS1 and CXCL8 were both significantly higher in GBM patients than in Gr. III glioma patients. According to the K-M curves based on TCGA, THBS1 was a prognostic gene not only in GBM patients (HR = 1.40, P = 0.046) but also in Gr. III glioma patients (HR = 1.51, P = 0.035; Fig. 4A, right). However, CXCL8 only showed an association with PFS in Gr. IV (HR = 1.45, P = 0.028) but not in Gr. III glioma patients (HR = 0.88, P = 0.522;

Fig. 4B, right). As a result, CXCL8 could have an important impact on the prognosis in Gr. III and Gr. IV glioma patients.

Next, we performed ROC curve analysis using CXCL8 levels to further determine the unique role of CXCL8 in discriminating Gr. IV glioma from Gr. III glioma. The area under the curve value was 0.899 for discriminating Gr. IV from Gr. III in the TCGA cohort and 0.644 in the CGGA dataset (Fig. 4C). This finding indicates that when CXCL8 expression in gliomas was elevated in more than 89% of patients in the TCGA dataset and in 64% in the CGGA cohort, the presence of GBM was confirmed.

CXCL8 infiltrates immune cells with emphasis on M2 macrophages in recurrent GBM patients

CACL8 can be secreted by many immune cells, including macrophages, neutrophils, T lymphocytes, eosinophils, fibroblasts and epithelial cells. Using the ssGSEA algorithm, we explored the infiltration levels of 24 immune cell subtypes in Gr. IV gliomas to observe whether CXCL8 expression was associated with immune infiltration. The main immune cells affected by CXCL8 expression were CD8 T cells, cytotoxic cells, DCs, eosinophils, iDCs (immature DCs), macrophages, mast cells, neutrophils, NK CD56dim cells, T cells, T effector memory (Tem), T gamma delta (Tgd), Th1 cells and Th17 cells. Among them, the levels of macrophages ($r = 0.660$, $P < 0.001$) and neutrophils ($r = 0.637$, $P < 0.001$) were highly correlated with CXCL8 expression and levels of immune cell infiltration (Fig. 5A). Furthermore, we evaluated the relationship between the expression of CXCL8 and immune cell markers, including markers of M1 macrophages (iNOS, IRF5, COX2), M2 macrophages (CD163, VSIG4, MS4A4A) and neutrophils (CD66b, CD11b, CCR7), using the TIMER database.(Wang et al., 2022) After adjusting for tumor purity, CXCL8 expression was significantly associated with macrophage markers in GBM, especially M2 markers (Fig. 5B). To reassess the correlation between CXCL8 expression and the levels of M2 macrophage markers in high-grade glioma patients, we conducted a correlation analysis using the CGGA website tool among primary and recurrent high-grade glioma patients. Although we found that CXCL8 expression has a significant effect on both the primary Gr. III and Gr. IV gliomas (Fig. 5C), it was specific to only decreasing significance for recurrent Gr. III glioma of all M2 markers (R value primary→ recurrence of CD163:0.622→0.475; VSIG4: 0.433→0.285, MS4A4A:0.434→0.177). In contrast, all M2 macrophage markers had increasing correlative efficiency from primary GBM to the recurrence group (R value primary→ recurrence of CD163:0.617→0.677; VSIG4: 0.437→0.496, MS4A4A:0.458→0.522).

Taken together, these immune analyses provide meaningful information that CXCL8 might be more involved in M2 macrophage infiltration, with an especially higher correlation coefficient in recurrent GBM than in recurrent Gr. III glioma.

Discussion

Since the 2016 WHO grading system of CNS tumors(Wesseling and Capper, 2018) was established, glioma grading has been reclassified not only using traditional tissue morphology but also by focusing on the status of IDH1, IDH2 mutants and 1p/19p codeletion to identify gliomas according to genomics.

Then, the newest-edition WHO classification of gliomas in 2021(Louis et al., 2021) further included ATRX loss, TP53 wt., TERT promoter mutation, and CDKN2A/B homozygous deletion as pathological diagnostic markers that can be used to distinguish different grades of malignancy of gliomas. Finally, diffuse gliomas were divided into 5 subgroups as follows: 1.) anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO Gr. III; 2.) anaplastic astrocytoma, IDH-mutant, WHO Gr. III; 3.) glioblastoma, IDH-mutant, WHO Gr. IV; 4.) anaplastic astrocytoma, IDH-wildtype, WHO Gr. III; 5.)glioblastoma, IDH-wildtype, WHO Gr. IV.

Compelling evidence indicates that aggressive tumor resection provides disease survival benefits for newly diagnosed GBM patients. The volume of residual GBM tumors as determined from postoperative images is a highly important factor affecting the disease-free time and median survival for GBM patients. (Keles et al., 1999) However, maximal resection of newly diagnosed Gr. III gliomas is recommended even if there is no clear evidence of a benefit on survival, but most expert consensus agrees with this aim for the tumor operation.(Keles et al., 2006) Adjuvant treatments of both Gr. III and Gr. IV gliomas include CCRT with different chemo-regimens, either TMZ or PVC. TMZ plays a more specific therapeutic role in GBM. However, there is a predominant disparity in disease survival between Gr. III and Gr. IV glioma patients. To our knowledge, we present a unique approach for differentiating Gr. III and Gr. IV glioma patients.

CXCL8 (C-X-C motif chemokine ligand 8) is also named interleukin 8 (IL8). Previous studies correlating IL8 and the prognosis of cancers include studies of colorectal cancer (CRC),(Cui et al., 2022; Ichikawa et al., 2022; Schimek et al., 2022) breast cancer,(Abou Shousha et al., 2022; Al-Kharashi et al., 2022) pancreatic cancer,(Li et al., 2022a) gastric cancer(Lin et al., 2022) and glioblastoma.(Chen et al., 2022) Based on our findings, IL8 could have an important impact on the prognosis of Gr. III and Gr. IV glioma patients. In addition, IL8 levels could be used to further differentiate GBM from high-grade glioma.

Immunotherapy has been considered one indication for curing cancers. IL8 can be produced by several immune cells and shows a close relationship with the tumor immune microenvironment. IL8-associated neutrophil recruitment modulates the immune microenvironment of CRC cells.(Schimek et al., 2022) Elevation of IL8 levels induces upregulation of PD-1 presentation in CD8-positive T cells to cause immunosuppression in stomach cancer.(Li et al., 2022b) From those studies, IL8 has been associated with immune cells, including in malignant tumors of the colorectum and stomach and GBM.

Recently, a series of studies related to GBM and IL8 have been conducted that have investigated IL8 expression in tumors, plasma IL8 levels of GBM patients, and treatment-induced IL8 expression. Data regarding GBM has indicated that Annexin-1, as an oncogene, promotes GBM cell escape from immune attack via IL8, which indirectly upregulates the expression of markers associated with the migration of dendritic cells.(Chen et al., 2022) In other clinical studies of plasma IL8 levels and GBM, the plasma levels in 158 patients with Gr. II-IV gliomas whose samples were collected during surgery and the levels of 20 proteins as candidate markers for GBM were significantly different from those with GBM. Finally, a higher level of IL8 in plasma was associated with a lower OS in GBM patients.(Holst et al., 2021) IL8 levels can

discriminate GBM from Gr. III glioma and is a prognostic biomarker associated with poor PFS of GBM patients in TCGA dataset, and an indicator of poor prognosis regarding OS in GBM patients exists in the CGGA cohort.

As a common alkylating agent of adjuvant chemotherapy in GBM, TMZ is indicated to upregulate the activity of the IL8-dependent CXCL2/CXCR2 axis to promote angiogenesis and the production of tumor-associated macrophages. At the same time, combination therapy with TMZ and an antagonist of CXCR2 overcomes therapeutic resistance induced by TMZ.(Urbantat et al., 2021) In our study, IL8 expression was associated with higher levels of immune cells, including macrophages and neutrophils. Increased IL8 expression was also highly correlated with the levels of the immune infiltration of M2 macrophages in primary and recurrent GBM.

In conclusion, even though our study provides a well-known biomarker, IL8, to classify high-grade glioma, IL8 has multiple effects on disease prognosis, tumor immunity and recurrent gliomas. IL8-associated M2 macrophage infiltration could provide both prognostic and recurrent biomarkers to differentiate Gr. III and GBM.

In the future, immunohistochemical staining of high-grade glioma and survival analysis based on IL8 expression in clinical patients might provide further proof that IL8 is a unique factor and therapeutic target of GBM.

Declarations

Funding: This research was funded by the Tri-Service General Hospital (TSGH-E111226 to W.-H.L.), the Shuang Ho Hospital (111TMU-SHH-28 to J.-C.L.) and the Ministry of Science and Technology (MOST 111-2314-B-038 -085 to J.-C.L.).

Disclosure of interest: The authors declare that they have no conflict of interest.

Data availability statement: The datasets used and/or analyzed during the current study are available from the corresponding author on request.

References

1. Abedalthagafi, M., Phillips, J.J., Kim, G.E., Mueller, S., Haas-Kogen, D.A., Marshall, R.E., Croul, S.E., Santi, M.R., Cheng, J., Zhou, S., Sullivan, L.M., Martinez-Lage, M., Judkins, A.R. and Perry, A., 2013. The alternative lengthening of telomere phenotype is significantly associated with loss of ATRX expression in high-grade pediatric and adult astrocytomas: a multi-institutional study of 214 astrocytomas. Mod Pathol 26, 1425–1432.
2. Abou Shousha, S.A., Hussein, B., Shahine, Y., Fadali, G., Zohir, M., Hamed, Y., Hemedah, M., Baheeg, S.A., Ibrahim, A., El Shannawy, M. and Osman, E.M., 2022. Angiogenic activities of interleukin-8,

vascular endothelial growth factor and matrix metalloproteinase-9 in breast cancer. Egypt J Immunol 29, 54–63.

3. Ahmed, R., Oborski, M.J., Hwang, M., Lieberman, F.S. and Mountz, J.M., 2014. Malignant gliomas: current perspectives in diagnosis, treatment, and early response assessment using advanced quantitative imaging methods. Cancer Manag Res 6, 149–170.
4. Al-Kharashi, L.A., Tulbah, A., Arafah, M., Eldali, A.M., Al-Tweigeri, T. and Aboussekhra, A., 2022. High DNMT1 expression in stromal fibroblasts promotes angiogenesis and unfavorable outcome in locally advanced breast cancer patients. Front Oncol 12, 877219.
5. Arakawa, Y., Sasaki, K., Mineharu, Y., Uto, M., Mizowaki, T., Mizusawa, J., Sekino, Y., Ono, T., Aoyama, H., Satomi, K., Ichimura, K., Kinoshita, M., Ohno, M., Ito, Y., Nishikawa, R., Fukuda, H., Nishimura, Y. and Narita, Y., 2021. A randomized phase III study of short-course radiotherapy combined with temozolomide in elderly patients with newly diagnosed glioblastoma; Japan clinical oncology group study JCOG1910 (AgedGlio-PIII). BMC Cancer 21, 1105.
6. Aubert, J., Bar-Hen, A., Daudin, J.J. and Robin, S., 2004. Determination of the differentially expressed genes in microarray experiments using local FDR. BMC Bioinformatics 5, 125.
7. Barrett, T., Troup, D.B., Wilhite, S.E., Ledoux, P., Rudnev, D., Evangelista, C., Kim, I.F., Soboleva, A., Tomashevsky, M. and Edgar, R., 2007. NCBI GEO: mining tens of millions of expression profiles–database and tools update. Nucleic Acids Res 35, D760–D765.
8. Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I.F., Tomashevsky, M., Marshall, K.A., Phillippy, K.H., Sherman, P.M., Holko, M., Yefanov, A., Lee, H., Zhang, N., Robertson, C.L., Serova, N., Davis, S. and Soboleva, A., 2013. NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res 41, D991–D995.
9. Burnet, N.G., Lynch, A.G., Jefferies, S.J., Price, S.J., Jones, P.H., Antoun, N.M., Xuereb, J.H. and Pohl, U., 2007. High grade glioma: imaging combined with pathological grade defines management and predicts prognosis. Radiother Oncol 85, 371–378.
10. Ceccarelli, M., Barthel, F.P., Malta, T.M., Sabedot, T.S., Salama, S.R., Murray, B.A., Morozova, O., Newton, Y., Radenbaugh, A., Pagnotta, S.M., Anjum, S., Wang, J., Manyam, G., Zoppoli, P., Ling, S., Rao, A.A., Grifford, M., Cherniack, A.D., Zhang, H., Poisson, L., Carlotti, C.G., Tirapelli, D.P., Rao, A., Mikkelsen, T., Lau, C.C., Yung, W.K., Rabadan, R., Huse, J., Brat, D.J., Lehman, N.L., Barnholtz-Sloan, J.S., Zheng, S., Hess, K., Rao, G., Meyerson, M., Beroukhim, R., Cooper, L., Akbani, R., Wrensch, M., Haussler, D., Aldape, K.D., Laird, P.W., Gutmann, D.H., TCGA Research Network, Noushmehr, H., Iavarone, A. and Verhaak, R.G., 2016. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. Cell 164, 550–563.
11. Chen, R., Chen, C., Han, N., Guo, W., Deng, H., Wang, Y., Ding, Y. and Zhang, M., 2022. Annexin-1 is an oncogene in glioblastoma and causes tumour immune escape through the indirect upregulation of interleukin-8. J Cell Mol Med.
12. Cui, G., Li, G., Pang, Z., Florholmen, J. and Goll, R., 2022. The presentation and regulation of the IL-8 network in the epithelial cancer stem-like cell niche in patients with colorectal cancer. Biomed

Pharmacother 152, 113252.

13. Eckel-Passow, J.E., Lachance, D.H., Molinaro, A.M., Walsh, K.M., Decker, P.A., Sicotte, H., Pekmezci, M., Rice, T., Kosel, M.L., Smirnov, I.V., Sarkar, G., Caron, A.A., Kollmeyer, T.M., Praska, C.E., Chada, A.R., Halder, C., Hansen, H.M., McCoy, L.S., Bracci, P.M., Marshall, R., Zheng, S., Reis, G.F., Pico, A.R., O'Neill, B.P., Buckner, J.C., Giannini, C., Huse, J.T., Perry, A., Tihan, T., Berger, M.S., Chang, S.M., Prados, M.D., Wiemels, J., Wiencke, J.K., Wrensch, M.R. and Jenkins, R.B., 2015. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. N Engl J Med 372, 2499–2508.
14. Hegi, M.E., Diserens, A.C., Gorlia, T., Hamou, M.F., De Tribolet, N., Weller, M., Kros, J.M., Hainfellner, J.A., Mason, W., Mariani, L., Bromberg, J.E., Hau, P., Mirimanoff, R.O., Cairncross, J.G., Janzer, R.C. and Stupp, R., 2005. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352, 997–1003.
15. Holst, C.B., Christensen, I.J., Vitting-Seerup, K., Skjøth-Rasmussen, J., Hamerlik, P., Poulsen, H.S. and Johansen, J.S., 2021. Plasma IL-8 and ICOSLG as prognostic biomarkers in glioblastoma. Neurooncol Adv 3, vdab072.
16. Hänzelmann, S., Castelo, R. and Guinney, J., 2013. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics 14, 7.
17. Ichikawa, Y., Takahashi, H., Chinen, Y., Arita, A., Sekido, Y., Hata, T., Ogino, T., Miyoshi, N., Uemura, M., Yamamoto, H., Mizushima, T., Doki, Y. and Eguchi, H., 2022. Low G9a expression is a tumor progression factor of colorectal cancer via IL-8 promotion. Carcinogenesis, bgac050.
18. Keles, G.E., Anderson, B. and Berger, M.S., 1999. The effect of extent of resection on time to tumor progression and survival in patients with glioblastoma multiforme of the cerebral hemisphere. Surg Neurol 52, 371–379.
19. Keles, G.E., Chang, E.F., Lamborn, K.R., Tihan, T., Chang, C.J., Chang, S.M. and Berger, M.S., 2006. Volumetric extent of resection and residual contrast enhancement on initial surgery as predictors of outcome in adult patients with hemispheric anaplastic astrocytoma. J Neurosurg 105, 34–40.
20. Kouwenhoven, M.C., Gorlia, T., Kros, J.M., Ibdaih, A., Brandes, A.A., Bromberg, J.E., Mokhtari, K., Van Duinen, S.G., Teepen, J.L., Wesseling, P., Vandenbos, F., Grisold, W., Sipos, L., Mirimanoff, R., Vecht, C.J., Allgeier, A., Lacombe, D. and Van Den Bent, M.J., 2009. Molecular analysis of anaplastic oligodendroglial tumors in a prospective randomized study: a report from EORTC study 26951. Neuro Oncol 11, 737–746.
21. Li, P., Rozich, N., Wang, J., Wang, J., Xu, Y., Herbst, B., Yu, R., Muth, S., Niu, N., Li, K., Funes, V., Gai, J., Osipov, A., Edil, B.H., Wolfgang, C.L., Lei, M., Liang, T. and Zheng, L., 2022a. Anti-IL-8 antibody activates myeloid cells and potentiates the anti-tumor activity of anti-PD-1 antibody in the humanized pancreatic cancer murine model. Cancer Lett 539, 215722.
22. Li, X., Zhai, J., Shen, Y., Zhang, T., Wang, Y., He, Y., You, Q. and Shen, L., 2022b. Tumor-derived IL-8 facilitates lymph node metastasis of gastric cancer via PD-1 up-regulation in $CD8^+$ T cells. Cancer Immunol Immunother.

23. Lin, L., Li, L., Ma, G., Kang, Y., Wang, X. and He, J., 2022. Overexpression of IL-8 and Wnt2 is associated with prognosis of gastric cancer. Folia Histochem Cytobiol 60, 66–73.
24. Liu, J., Lichtenberg, T., Hoadley, K.A., Poisson, L.M., Lazar, A.J., Cherniack, A.D., Kovatich, A.J., Benz, C.C., Levine, D.A., Lee, A.V., Omberg, L., Wolf, D.M., Shriver, C.D., Thorsson, V., Cancer Genome Atlas Research Network and Hu, H., 2018. An integrated TCGA pan-cancer clinical data resource to drive high-quality survival outcome analytics. Cell 173, 400–416.e11.
25. Louis, D.N., Perry, A., Reifenberger, G., Von Deimling, A., Figarella-Branger, D., Cavenee, W.K., Ohgaki, H., Wiestler, O.D., Kleihues, P. and Ellison, D.W., 2016. The 2016 world health organization classification of tumors of the central nervous system: a summary. Acta Neuropathol 131, 803–820.
26. Louis, D.N., Perry, A., Wesseling, P., Brat, D.J., Cree, I.A., Figarella-Branger, D., Hawkins, C., Ng, H.K., Pfister, S.M., Reifenberger, G., Soffietti, R., Von Deimling, A. and Ellison, D.W., 2021. The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro Oncol 23, 1231–1251.
27. McGirt, M.J., Chaichana, K.L., Gathinji, M., Attenello, F.J., Than, K., Olivi, A., Weingart, J.D., Brem, H. and Quiñones-Hinojosa, A.R., 2009. Independent association of extent of resection with survival in patients with malignant brain astrocytoma. J Neurosurg 110, 156–162.
28. Mirimanoff, R.O., Gorlia, T., Mason, W., Van Den Bent, M.J., Kortmann, R.D., Fisher, B., Reni, M., Brandes, A.A., Curschmann, J., Villa, S., Cairncross, G., Allgeier, A., Lacombe, D. and Stupp, R., 2006. Radiotherapy and temozolomide for newly diagnosed glioblastoma: recursive partitioning analysis of the EORTC 26981/22981-NCIC CE3 phase III randomized trial. J Clin Oncol 24, 2563–2569.
29. Noiphithak, R. and Veerasarn, K., 2017. Clinical predictors for survival and treatment outcome of high-grade glioma in Prasat Neurological Institute. Asian J Neurosurg 12, 28–33.
30. Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H. and Kanehisa, M., 1999. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 27, 29–34.
31. Sanai, N., 2012. Emerging operative strategies in neurosurgical oncology. Curr Opin Neurol 25, 756–766.
32. Schimek, V., Strasser, K., Beer, A., Göber, S., Walterskirchen, N., Brostjan, C., Müller, C., Bachleitner-Hofmann, T., Bergmann, M., Dolznig, H. and Oehler, R., 2022. Tumour cell apoptosis modulates the colorectal cancer immune microenvironment via interleukin-8-dependent neutrophil recruitment. Cell Death Dis 13, 113.
33. Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B. and Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13, 2498–2504.
34. Siangprasertkij, C. and Navalitloha, Y., 2008. A multivariate analysis of patients with glioma: a treatment outcome and prognostic factor for survival. J Med Assoc Thai 91, 491–496.
35. Stupp, R., Mason, W.P., Van Den Bent, M.J., Weller, M., Fisher, B., Taphoorn, M.J., Belanger, K., Brandes, A.A., Marosi, C., Bogdahn, U., Curschmann, J., Janzer, R.C., Ludwin, S.K., Gorlia, T., Allgeier, A., Lacombe, D., Cairncross, J.G., Eisenhauer, E. and Mirimanoff, R.O., 2005. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352, 987–996.

36. Sun, L., Hui, A.M., Su, Q., Vortmeyer, A., Kotliarov, Y., Pastorino, S., Passaniti, A., Menon, J., Walling, J., Bailey, R., Rosenblum, M., Mikkelsen, T. and Fine, H.A., 2006. Neuronal and glioma-derived stem cell factor induces angiogenesis within the brain. Cancer Cell 9, 287–300.
37. Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K.P., Kuhn, M., Bork, P., Jensen, L.J. and Von Mering, C., 2015. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 43, D447–D452.
38. Urbantat, R.M., Jelgersma, C., Brandenburg, S., Nieminen-Kelhä, M., Kremenetskaia, I., Zollfrank, J., Mueller, S., Rubarth, K., Koch, A., Vajkoczy, P. and Acker, G., 2021. Tumor-associated microglia/macrophages as a predictor for survival in glioblastoma and temozolomide-induced changes in CXCR2 signaling with new resistance overcoming strategy by combination therapy. Int J Mol Sci 22, 11180.
39. Wang, X.F., Lei, W., Liu, C.M., Yang, J. and Zhu, Y.H., 2022. BOLA3 is a prognostic-related biomarker and correlated with immune infiltrates in lung adenocarcinoma. Int Immunopharmacol 107, 108652.
40. Wesseling, P. and Capper, D., 2018. WHO 2016 classification of gliomas. Neuropathol Appl Neurobiol 44, 139–150.
41. Wick, W., Hartmann, C., Engel, C., Stoffels, M., Felsberg, J., Stockhammer, F., Sabel, M.C., Koeppen, S., Ketter, R., Meyermann, R., Rapp, M., Meisner, C., Kortmann, R.D., Pietsch, T., Wiestler, O.D., Ernemann, U., Bamberg, M., Reifenberger, G., Von Deimling, A. and Weller, M., 2009. NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. J Clin Oncol 27, 5874–5880.
42. Yu, G., Wang, L.G., Han, Y. and He, Q.Y., 2012. clusterProfiler: an R package for comparing biological themes among gene clusters. Omics 16, 284–287.
43. Zhang, B., Wang, Y., Li, H., Feng, L., Li, W. and Cheng, S., 2020. Identification of prognostic biomarkers for multiple solid tumors using a human villi development model. Front Cell Dev Biol 8, 492.
44. Zhao, Z., Zhang, K.N., Wang, Q., Li, G., Zeng, F., Zhang, Y., Wu, F., Chai, R., Wang, Z., Zhang, C., Zhang, W., Bao, Z. and Jiang, T., 2021. Chinese glioma genome Atlas (CGGA): a comprehensive resource with functional genomic data from Chinese glioma patients. Genom Proteom Bioinform 19, 1–12.

Figures

Figure 1

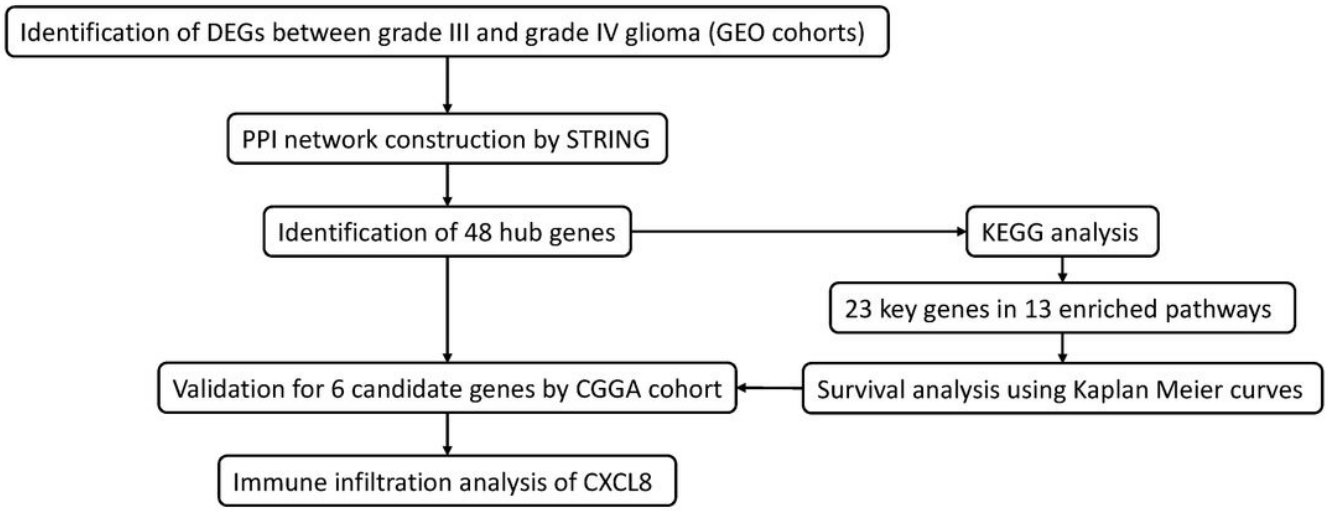

Figure 1

Flow chart of the study design.

Figure 2

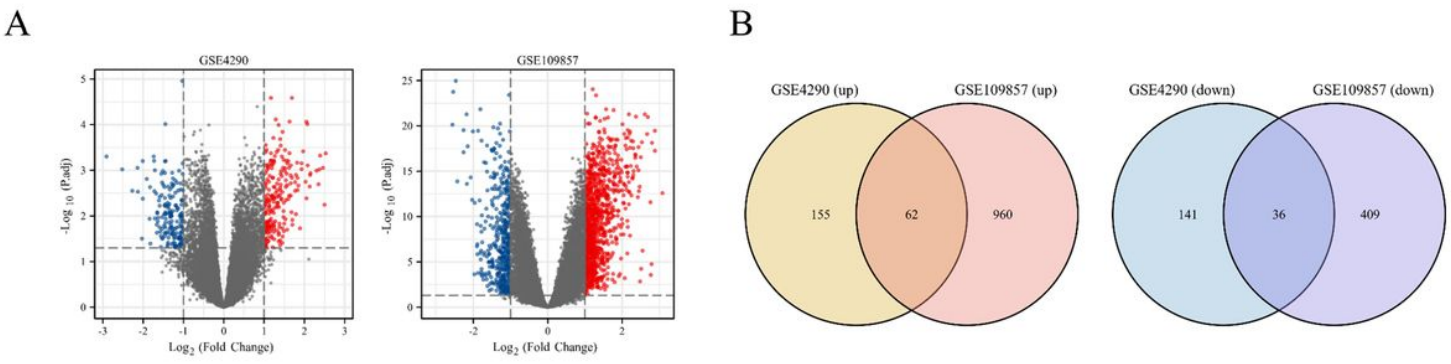

Figure 2

Identification of DEGs between Gr. III and Gr. IV glioma in 2 GEO datasets (GSE4290; GSE109857) (A) Volcano plot displaying the significant DEGs. (B) Venn diagram showing the intersection of upregulated DEGs (left) and downregulated DEGs (right).

Figure 3

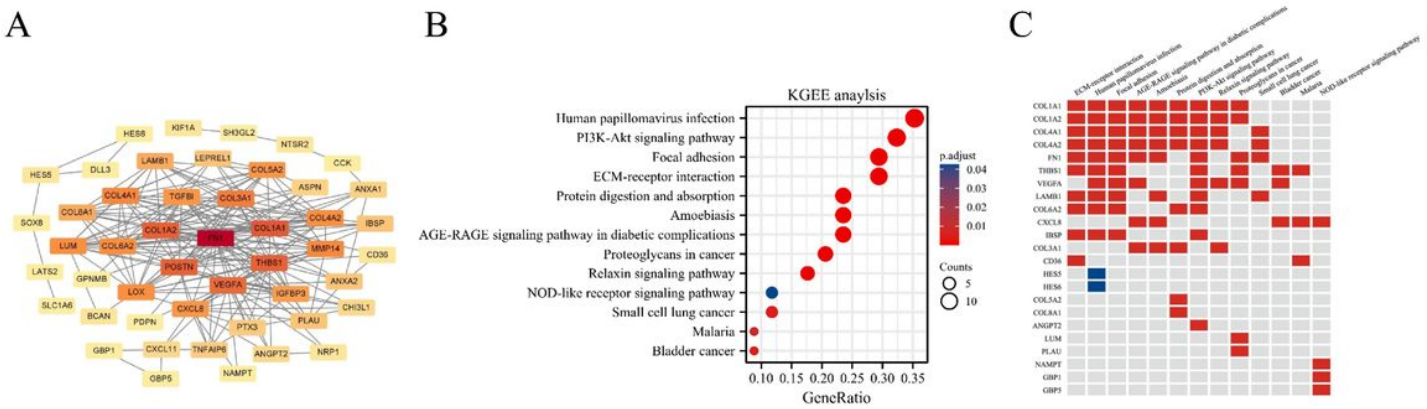

Figure 3

(A) Forty-eight hub genes with a degree ≥ 2 were extracted from the PPI network of common DEGs in the two datasets. (B) KEGG pathway enrichment analysis of 48 hub genes of bubble plot of 13 enriched pathways (p value < 0.05). (C) Twenty-three genes involved in the pathways were considered key genes.

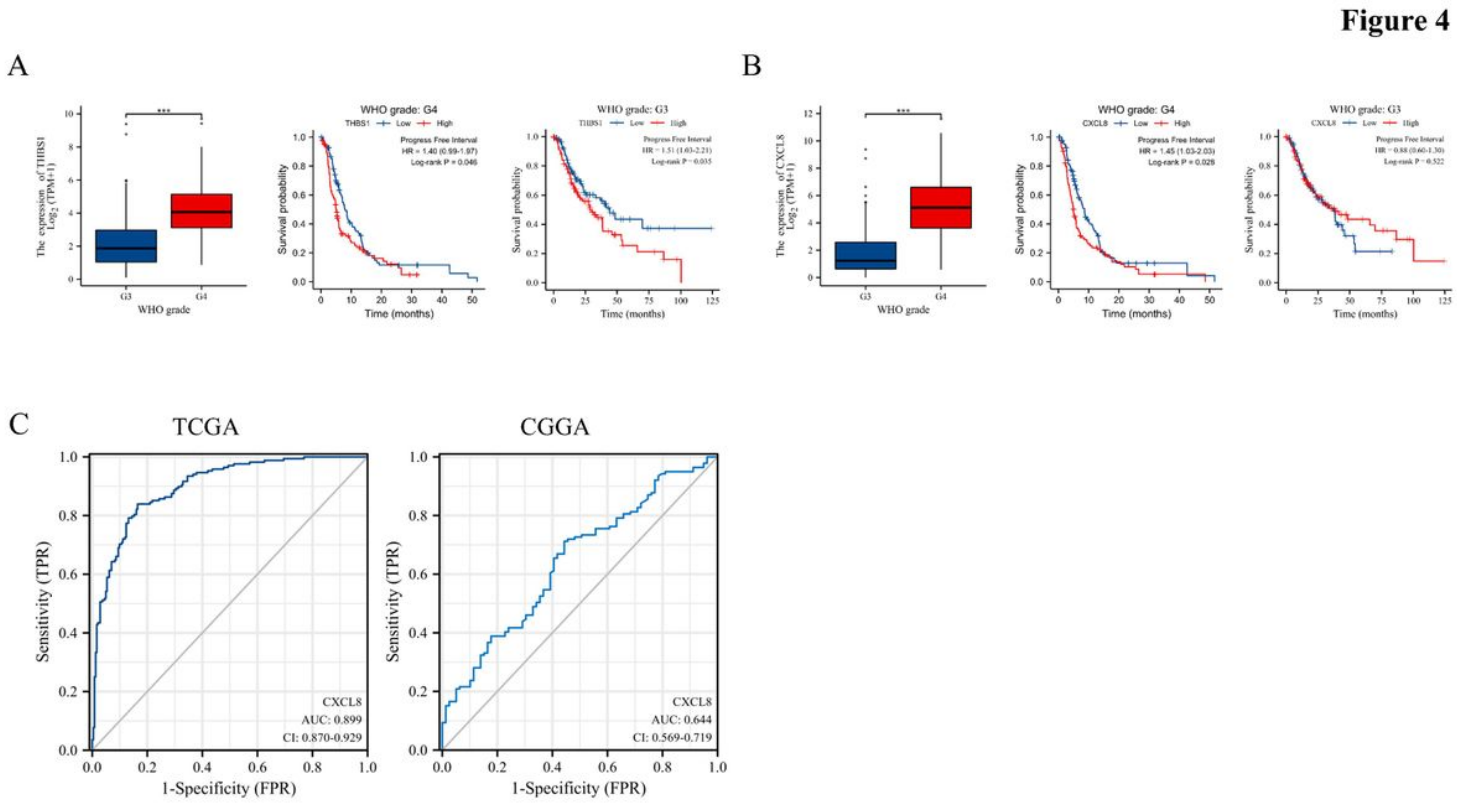

Figure 4

Identification of prognostic genes through Kaplan–Meier survival curves and the mRNA expression levels in Gr. III and Gr. IV glioma samples in TCGA. (A) THBS1; (B) CXCL8. (C) Receiver operating characteristic curve analysis for discriminating grade IV from grade III glioma based on CXCL8 expression.

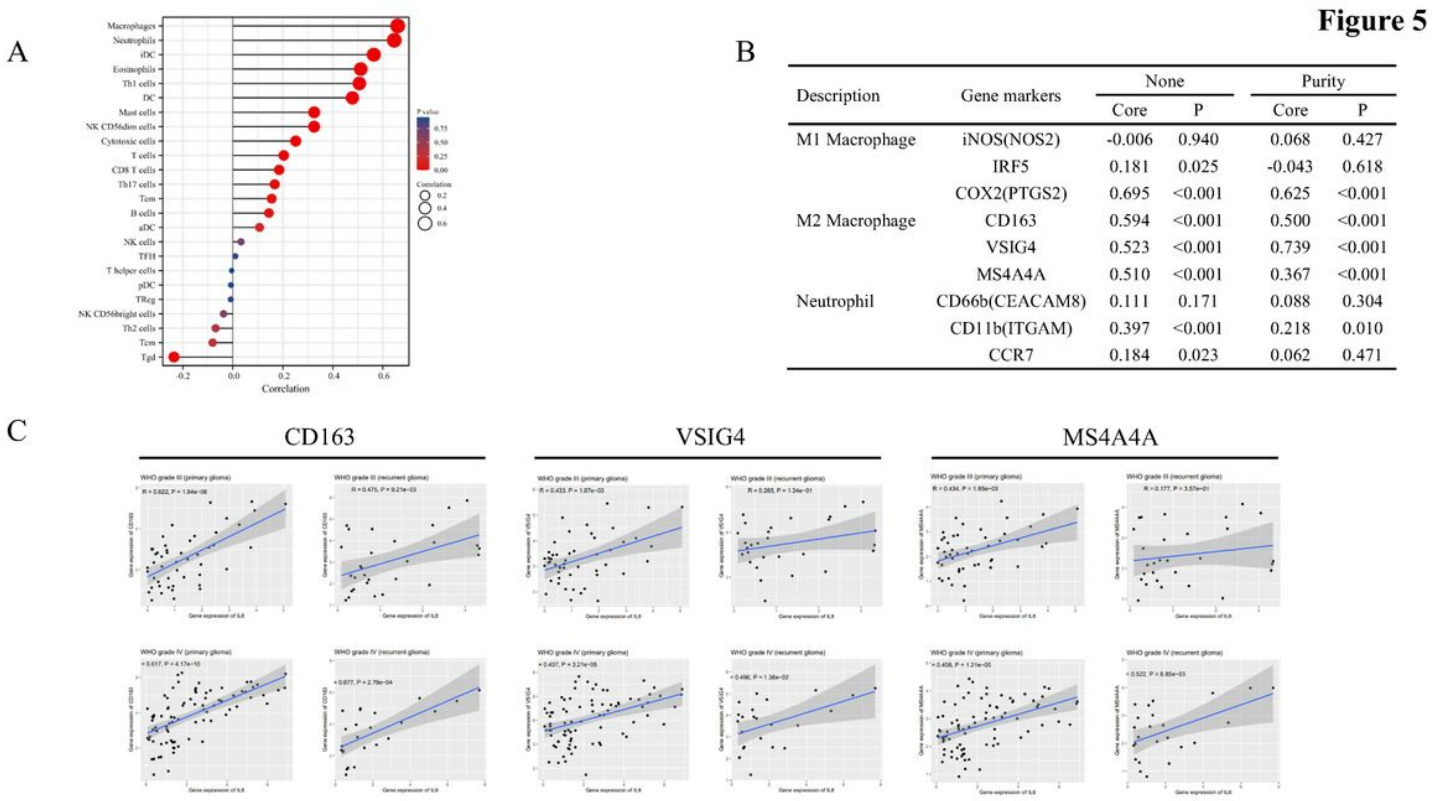

Figure 5

Description	Gene markers	None		Purity	
		Core	P	Core	P
M1 Macrophage	iNOS(NOS2)	-0.006	0.940	0.068	0.427
	IRF5	0.181	0.025	-0.043	0.618
	COX2(PTGS2)	0.695	<0.001	0.625	<0.001
M2 Macrophage	CD163	0.594	<0.001	0.500	<0.001
	VSIG4	0.523	<0.001	0.739	<0.001
	MS4A4A	0.510	<0.001	0.367	<0.001
Neutrophil	CD66b(CEACAM8)	0.111	0.171	0.088	0.304
	CD11b(ITGAM)	0.397	<0.001	0.218	0.010
	CCR7	0.184	0.023	0.062	0.471

Figure 5

Associations between CXCL8 expression and the tumor microenvironment. (A) Analysis of the correlation of CXCL8 expression with infiltrating immune cells in grade IV glioma using the ssGSEA algorithm from the TCGA database. (B) Correlation analysis of CXCL8 expression and the expression of marker genes of different immune cells using the TIMER database. (C) The scatter plots show the relationship between CXCL8 expression and the expression of M2 macrophage marker genes in the CGGA database (CD163, VSIG4 and MS4A4).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplememnttable.docx