Original Article

High expression of *TXNDC11* indicated unfavorable prognosis of glioma

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Background: Thioredoxin domain containing 11 (*TXNDC11*) has been implicated in numerous cancers. Nevertheless, the function of *TXNDC11* in glioma is not well described. This study aimed to assess clinical significance of *TXNDC11* in glioma based on bioinformatics analysis and immunohistochemical (IHC) staining.

Methods: GEPIA2, The Cancer Genome Atlas (TCGA), and Gene Expression Omnibus (GEO) databases were employed to detect the levels of *TXNDC11* transcript in glioma. Gene expression profiles and data from the methylation chip with clinical details from TCGA and Chinese Glioma Genome Atlas (CGGA) of glioma samples were examined. The methylation of *TXNDC11* in glioma was evaluated by 450K methylation chip data analysis. The pathways involved in *TXNDC11* expression were screened by gene set enrichment analysis (GSEA). The correlation between *TXNDC11* and immune cells was analyzed. Protein level of *TXNDC11* was detected by IHC staining in glioma specimens.

Results: *TXNDC11* was highly expressed in glioma, and high *TXNDC11* expression was associated with poor overall survival (OS) and worse clinical prognostic variables. The methylation of cg04399632 was statistically different between glioma samples and normal samples, and was negatively correlated with *TXNDC11* expression in glioma patients. Survival analysis demonstrated a poorer prognosis in glioma patients with cg04399632 hypomethylation. *TXNDC11*-high phenotype was associated with certain immune-related pathways and other signaling pathways in glioma. The expression of *TXNDC11* was correlated positively with M2 macrophage infiltration and negatively with M0 and M1 macrophage infiltration. IHC staining confirmed that *TXNDC11* expression increased in higher-grade glioma.

Conclusions: High expression of *TXNDC11* may predict unfavorable prognosis of glioma patients.

Keywords: Thioredoxin domain containing 11 (*TXNDC11*); prognosis; glioma; gene set enrichment analysis (GSEA); macrophage

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Introduction

Gliomas are the most prevalent primary brain tumors and one of the deadliest solid tumors with a high relapse rate and low chance of recovery (1). Despite the improvements in the treatment modalities in the past two decades, clinical therapeutic effect of glioma remains unsatisfactory. With the rapid advancement of biomedical approaches, many glioma biomarkers have been developed (2-5). However, efficient and accurate biomarkers that can predict the prognosis of glioma are few.

Thioredoxin domain containing 11 (*TXNDC11*), also recognized as EF-Hand Binding Protein 1, acts as a redox regulator involved in protein folding of thyroid oxidase (6). Recently, *TXNDC11* has been described as a hub gene in gynecological cancer progression (7). In patients with endometrial carcinoma of the uterine corpus, higher *TXNDC11* expression was correlated with prolonged overall survival (OS). Elevated expression of *TXNDC11* also indicated a good prognosis in hepatocellular carcinoma patients (8). However, much less is known about the epigenetic and genetic status of *TXNDC11* in gliomas.

The current study aimed to define the correlation between *TXNDC11* expression and glioma progression, and identify potential prognostic value of *TXNDC11* based on The Cancer Genome Atlas (TCGA), the Gene Expression Omnibus (GEO), and the Chinese Glioma Genome Atlas (CGGA). We found that *TXNDC11* was highly expressed in glioma and associated with reduced OS and higher WHO grades. Immunostaining confirmed that higher grade was associated with higher expression of *TXNDC11* in samples collected at our hospital. Further, gene set enrichment analysis (GSEA) indicated that high expression of *TXNDC11* was correlated with immune-related pathways and other signaling pathways such as M2 phenotype macrophage infiltration.

We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/tcr-21-1326).

Methods

Dataset generation

Microarray data on glioma patients were retrieved from the public GEO database (https://www.ncbi.nlm.nih.gov/geo) (9) with access number GSE68848 (10). The dataset GSE68848 was used for *TXNDC11* expression analysis between glioma tissues compared with non-tumor tissues. In addition, data on the gene expression profile comprising clinical data from glioblastoma (GBM) (HTSeq-FPKM) and low-grade glioma (LGG) projects were collected from the TCGA (https://cancergenome.nih.gov/) database (11). Moreover, the dataset of mRNAseq_325 of 325 glioma samples with mRNA data and clinical data was acquired from the CGGA database (http://www.cgga.org.cn/) (12). The TCGA and CGGA data were further examined for the correlations between the expression of *TXNDC11* and clinical features (age, WHO grade, histology, etc.). The methylation chip data was downloaded from TCGA to analyze the methylation of *TXNDC11* in gliomas.

Immunohistochemical (IHC) staining

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology and informed consent was taken from all the patients. Seventy-eight specimens of gliomas were collected at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology from September 2014 to September 2016. Tissue sections (4 μm thick) were stained with primary rabbit anti-TXNDC11 antibody (Sigma-Aldrich, St. Louis, MO, USA; Cat# HPA041174) overnight at 4 ℃ and then incubated with biotinylated goat anti-Rabbit secondary antibodies for 1 hour. Finally, the sections were detected with SignalStain® DAB (Cell Signaling Technology, Danvers, MA, USA) and counterstained with QS haematoxylin (Vector Laboratories,

Burlingame, CA, USA).

GSEA

GSEA (4.0.3) was performed to examine biological pathways with statistically relevant differences between high and low *TXNDC11* expression groups. The gene-set permutations were executed 1,000 times for each sample. Gene sets have been shown to be significantly improved with a typical P value <0.05 and a false discovery rate (FDR) <0.05.

Immune response gauging of TIICs via CIBERSORTT in glioma

CIBERSORT (13) (https://cibersort.stanford.edu/) is an algorithm for deconvolution based on gene expression, and has been applied to analyze cell heterogeneity (14,15). To analyze tumor-infiltrating immune cells (TIIC), TCGA gene expression datasets were configured and uploaded to the CIBERSORT web portal with standard signature matrix. The correlation with *TXNDC11* was then calculated.

Statistical analysis

Statistical analyses were performed using R software v3.6.2. Mann-Whitney U and logistic regression tests were used to evaluate the associations between *TXNDC11* and clinicopathologic characteristics. Cox regression analyses and Kaplan-Meier method were used to analyze the effect of *TXNDC11* on OS and other clinical variables. The correlation coefficient analysis was used to assess the correlation between *TXNDC11* expression and immune cell types. P value <0.05 was regarded to be statistically significant.

Results

TXNDC11 transcript levels in glioma based on databases

TXNDC11 transcript levels in gliomas (LGGs and GBMs) and matched normal samples were analyzed with the online tool GEPIA2. The boxplots indicated that *TXNDC11* expression was upregulated in LGGs and GBMs compared to the matched normal samples (*Figure 1A,1B*).

TXNDC11 expression data were collected from TCGA for 698 glioma samples. In tumor tissues, TXNDC11 was significantly overexpressed compared to normal tissues (*Figure 1C*; P<0.001). Furthermore, for validation we used GSE68848 dataset from the GEO database (*Figure 1D*; P<0.05). These results showed increased *TXNDC11* transcript levels in glioma.

Characteristics of TCGA and CGGA glioma patients

We selected 1,114 gliomas of all grades from TCGA as a training cohort and 325 gliomas of all grades from CGGA for validation (*Table 1*). The median age (years) of patients was 51 (from 10 to 89) in TCGA and 42 (from 8 to 79) in CGGA. Males accounted for 58.4% in TCGA and 62.5% in CGGA. There were 249 grade II, 265 WHO grade III and 596 grade IV gliomas in TCGA (GBMs). CGGA dataset included 103 grade II, 79 grade III, and 139 grade IV gliomas. For isocitrate dehydrogenases (IDH) mutation, 373 (33.48%) tumors were identified in TCGA and 517 (46.41%) cases lack IDH details. In CGGA, all the 325 samples were detected for IDH mutation with 175 (53.85%) tumors mutated. For 1p19q-codeletion status, non-codeletion accounted for 44.43% in TCGA and 76.92% in CGGA.

Association of TXNDC11 expression and clinicopathologic features of glioma

We compared the expression of *TXNDC11* in gliomas of different grades (*Figure 2*), and found that high expression of *TXNDC11* was significantly correlated with age (P<0.05), histologic type (P<0.05) and WHO grade (P<0.05) (*Figure 2A,2C,2F*), while not associated with gender in the TGGA dataset (*Figure 2B*). In TCGA, the expression of *TXNDC11* in IDH1 mutated samples was significantly lower than that of the wildtype (P<0.05), and the expression of *TXNDC11* in 1p19q non-codeletion samples was significantly higher than that of the codeletion samples (P<0.05) (*Figure 2D,2E*). These results were validated in the CGGA (*Figure 3A-3F*).

Univariate logistic regression analysis indicated that *TXNDC11* expression was correlated with poor clinical pathological variables (*Table 2*). High expression of *TXNDC11* in glioma was significantly correlated with age [≥51 *vs.* <51, OR =1.89, 95% CI: (1.38–2.58), P<0.001], gender [male *vs.* female, OR =0.69, 95% CI: (0.51–0.94), P<0.05], WHO grade [III *vs.* II, OR =1.52, 95% CI: (1.07–2.16), P<0.05; IV *vs.* II, OR =3.00, 95% CI: (1.99–4.57), P<0.001], histology type [GBM *vs.* astrocytoma, OR =1.59, 95% CI: (1.03–2.47), P<0.05; GBM *vs.* oligoastrocytoma, OR =2.36, 95% CI: (1.47–3.81), P<0.001; and GBM *vs.*

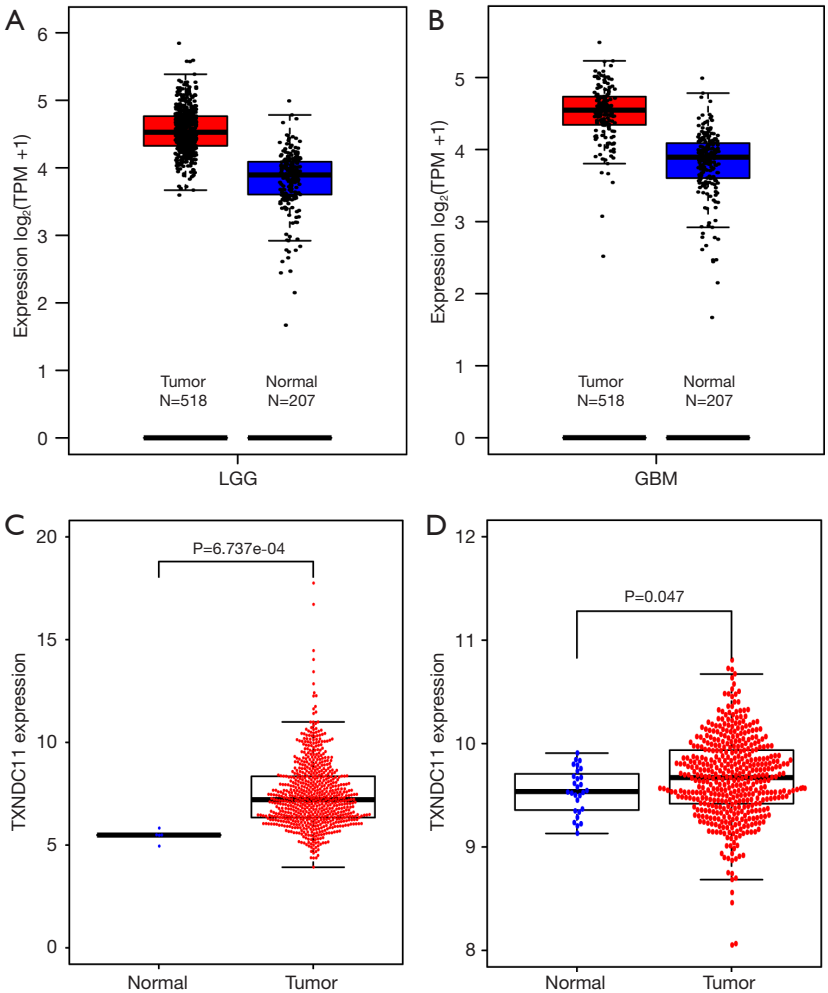

Figure 1 *TXNDC11* expression was upregulated in glioma. (A,B) *TXNDC11* expression was upregulated in LGGs and GBMs compared with the matched normal samples (analyzed with the online tool GEPIA2). (C,D) TCGA cohort, and GSE68848 dataset from GEO confirmed that *TXNDC11* expression was upregulated in glioma. *TXNDC11*, thioredoxin domain containing 11; LGGs, low-grade gliomas; GBMs, glioblastomas; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus.

oligodendroglioma, OR =3.44, 95% CI: (2.22–5.38), P<0.001], IDH1 mutation [wildtype *vs.* mutant, OR =2.14, 95% CI: (1.53–3.0), P<0.001], 1p19q-codeletion-status [non-codeletion *vs.* codeletion, OR =3.8, 95% CI: (2.6–5.63), P<0.001].

Association of TXNDC11 expression and survival outcomes of glioma

We examined OS and *TXNDC11* expression in CGGA and TCGA databases to explore the predictive consequences of *TXNDC11* in glioma prognosis. After excluding patients with missing OS data, Kaplan-Meier analysis showed that the prognosis of glioma patients with high *TXNDC11* expression was poorer than that of patients with low *TXNDC11* expression (*Figure 4A,4B*; P<0.001).

Univariate Cox regression showed that high *TXNDC11* expression was significantly associated with weaker OS [HR =1.14, 95% CI: (1.07–1.22), P<0001] (*Table 3*). Other clinicopathologic parameters associated with poor survival were age [HR =1.07, 95% CI: (1.06–1.08)], WHO grade [HR =4.69, 95% CI: (3.85–5.71)], and histological type [HR =1.95, 95% CI: (1.70–2.23)] (all with P<0.001). Multivariate Cox analysis showed that *TXNDC11* was independently correlated with OS, with HR of 1.09 [95% CI: (1.00–1.18), P=0.04], along with age and WHO grade.

Table 1 Baseline patient characteristics

Clinical characteristics	TCGA (n=1,114), n (%)	CGGA (n=325), n (%)
Age		
Median [range]	51 [10-89]	42 [8-79]
Gender		
Male	651 (58.4)	203 (62.5)
Female	460 (41.3)	122 (37.5)
Missing	3 (0.27)	0
WHO grade		
II	249 (22.4)	103 (31.7)
III	265 (23.8)	79 (24.3)
IV	596 (53.5)	139 (42.8)
Missing	4 (3.6)	4 (1.2)
Histology		
A	194 (17.4)	118 (36.31)
O	191 (17.2)	64 (19.69)
OA	130 (11.7)	NA
GBM	596 (53.5)	139 (42.77)
Missing	3 (0.27)	4 (1.23)
IDH1 mutation		
Mutation	373 (33.48)	175 (53.85)
Wildtype	224 (20.11)	149 (45.85)
Missing	517 (46.41)	1 (0.3)
1p19q-codeletion-status		
Codeletion	169 (15.17)	67 (20.62)
Non-codeletion	495 (44.43)	250 (76.92)
Missing	450 (40.4)	8 (2.46)

TCGA, The Cancer Genome Atlas; CGGA, Chinese Glioma Genome Atlas; IDH, isocitrate dehydrogenases.

The correlation between TXNDC11 methylation and prognosis of glioma

The 450K methylation chip data analysis revealed that the methylation of cg04399632 (the CpG site located in the S shore region of CpG island of *TXNDC11*) was statistically different between glioma and normal samples (*Figure 5A*). In GBM and LGG patients, the methylation of this site was negatively correlated with *TXNDC11* expression (*Figure 5B-5D*). According to the methylation at this site, GBM and LGG patients were divided into hypomethylation group and hypermethylation group. Survival analysis showed that hypermethylation glioma patients had a favorable prognostic value than hypomethylation patients (P<0.05) (*Figure 5E,5F*).

TXNDC11 related signaling pathways based on GSEA

The unfavorable prognosis of glioma patients with high *TXNDC11* expression may be related to signaling pathways commonly involved in cancer initiation and progression. GSEA was used to screen signaling pathways between low and high *TXNDC11* expression samples. In *TXNDC11* high expression cohorts, numerous signaling pathways, particularly inflammation, and immunity-related pathways, were greatly enhanced, including antigen processing and presentation, leukocyte transendothelial migration, B-cell receptor, cytokine-cytokine receptor interaction, Fc gamma R-mediated phagocytosis, cell-mediated cytotoxicity of natural killer (NK), T-cell receptor and pancreatic receptor (*Figure 6A*, *Table 4*).

Correlation analysis showed that *TXNDC11* transcription levels in TCGA were positively correlated with infiltration of M2 macrophages, monocytes, CD8 T cells, NK cells, but were negatively correlated with infiltration of M0 macrophages and M1 macrophages (*Figure 6B*).

Protein expression of TXNDC11 in glioma specimens and HPA database

Finally, we collected clinical samples and performed IHC staining. The analysis of *TXNDC11* expression in 78 glioma specimens of grade II (n=22), grade III (n=13), and grade IV (n=43) showed increased *TXNDC11* expression in high grade tumors (*Figure 7A,7B*). Similar findings were reported by examining IHC data from the HPA database (https://www.proteinatlas.org/), with 66.7% of LGG and 87.5% of high-grade glioma (HGG) being positive for *TXNDC11* staining.

Discussion

Due to therapeutic resistance and high recurrence rate of the infiltrative gliomas after concurrent radio- and chemo- therapies, individualized treatment based on molecular targets has gained more attention. In recent years, important prognosis related molecular aberrations of gliomas have been found, including 1p/19q codeletion, IDH mutation, MGMT promotor methylation, TERT

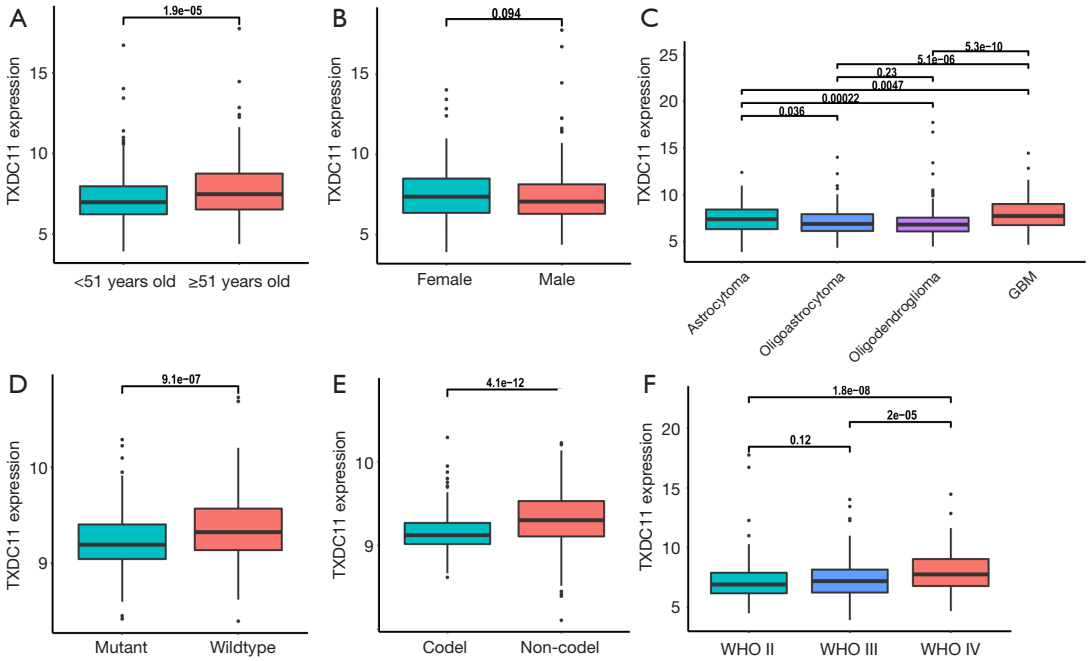

Figure 2 Associations between *TXNDC11* expression and clinicopathologic variables in TCGA cohort. (A) Age, (B) gender, (C) histological type, (D) IDH1 mutation status, (E) 1p19q-codeletion-status, (F) WHO grade. *TXNDC11*, thioredoxin domain containing 11; TCGA, The Cancer Genome Atlas; IDH, isocitrate dehydrogenases.

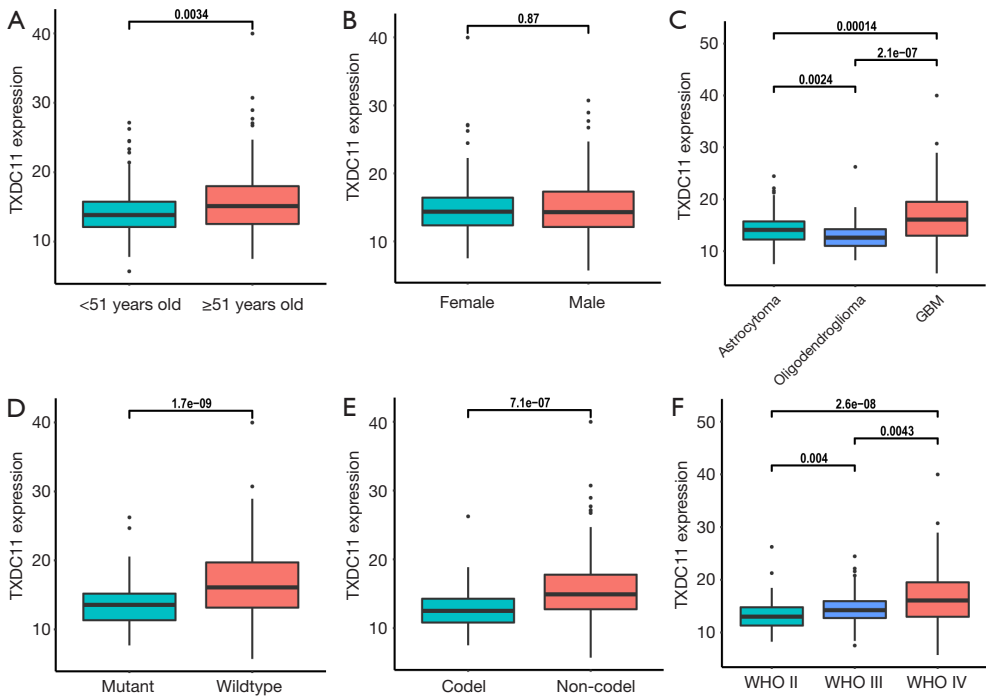

Figure 3 Associations between *TXNDC11* expression and clinicopathologic variables in CGGA-325 cohort. (A) Age, (B) gender, (C) histological type, (D) IDH1 mutation status, (E) 1p19q-codeletion-status, (F) WHO grade. *TXNDC11*, thioredoxin domain containing 11; CGGA, Chinese Glioma Genome Atlas; IDH, isocitrate dehydrogenases.

Table 2 Association of *TXNDC11* expression in TCGA with clinical-pathological characteristics

Clinical characteristics	Total (n)	OR in TXNDC11 expression	P value
Age (≥51 *vs.* <51)	1,111	1.89 (1.38–2.58)	<0.001*
Gender (male *vs.* female)	1,111	0.69 (0.51–0.94)	0.019*
WHO grade			
III *vs.* II	514	1.52 (1.07–2.16)	0.020*
IV *vs.* II	845	3.00 (1.99–4.57)	<0.001*
Histological type			
GBM *vs.* astrocytoma	790	1.59 (1.03–2.47)	0.035*
GBM *vs.* oligoastrocytoma	726	2.36 (1.47–3.81)	<0.001*
GBM *vs.* oligodendroglioma	787	3.44 (2.22–5.38)	<0.001*
IDH1 mutation (wildtype *vs.* mutant)	597	2.14 (1.53–3.00)	<0.001*
1p19q-codeletion-status (non-codeletion *vs.* codeletion)	664	3.80 (2.60–5.63)	<0.001*

*, P<0.05. *TXNDC11*, thioredoxin domain containing 11; TCGA, The Cancer Genome Atlas; GBM, glioblastoma; IDH, isocitrate dehydrogenases.

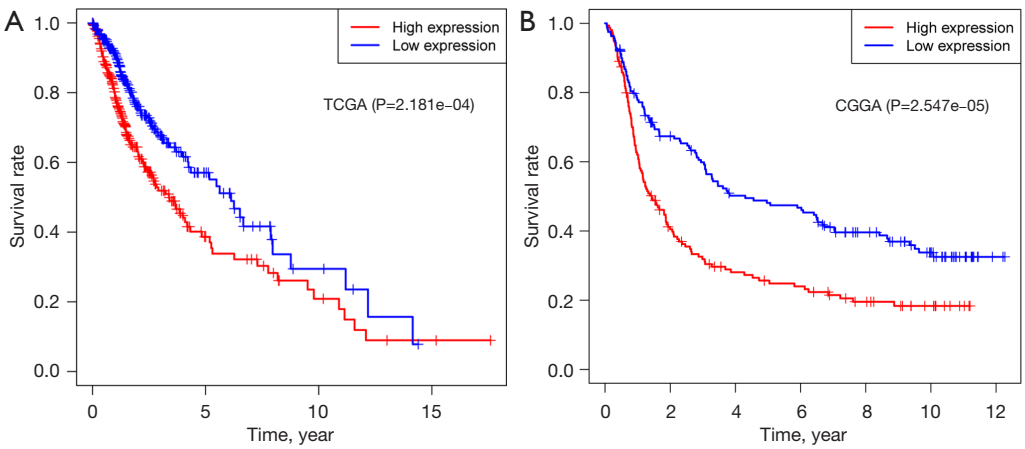

Figure 4 Association of *TXNDC11* expression and OS of glioma patients. (A) TCGA cohort. (B) CGGA cohort. *TXNDC11*, thioredoxin domain containing 11; OS, overall survival; TCGA, The Cancer Genome Atlas; CGGA, Chinese Glioma Genome Atlas.

Table 3 Univariate and multivariate analysis of clinicopathologic characteristics and OS in TCGA cohort

Characteristics	Univariate analysis		Multivariate analysis	
	P value	HR (95% CI)	P value	HR (95% CI)
Age	<0.001*	1.07 (1.06–1.08)	<0.001*	1.04 (1.03–1.05)
Gender	0.1095	1.23 (0.95–1.59)	0.56	1.08 (0.84–1.40)
WHO grade	<0.001*	4.69 (3.85–5.71)	<0.001*	3.75 (2.87–4.91)
Histology	<0.001*	1.95 (1.70–2.23)	0.12	0.89 (0.76–1.03)
TXNDC11 expression	<0.001*	1.14 (1.07–1.22)	0.04*	1.09 (1.00–1.18)

*, P<0.05. OS, overall survival; TCGA, The Cancer Genome Atlas; *TXNDC11*, thioredoxin domain containing 11.

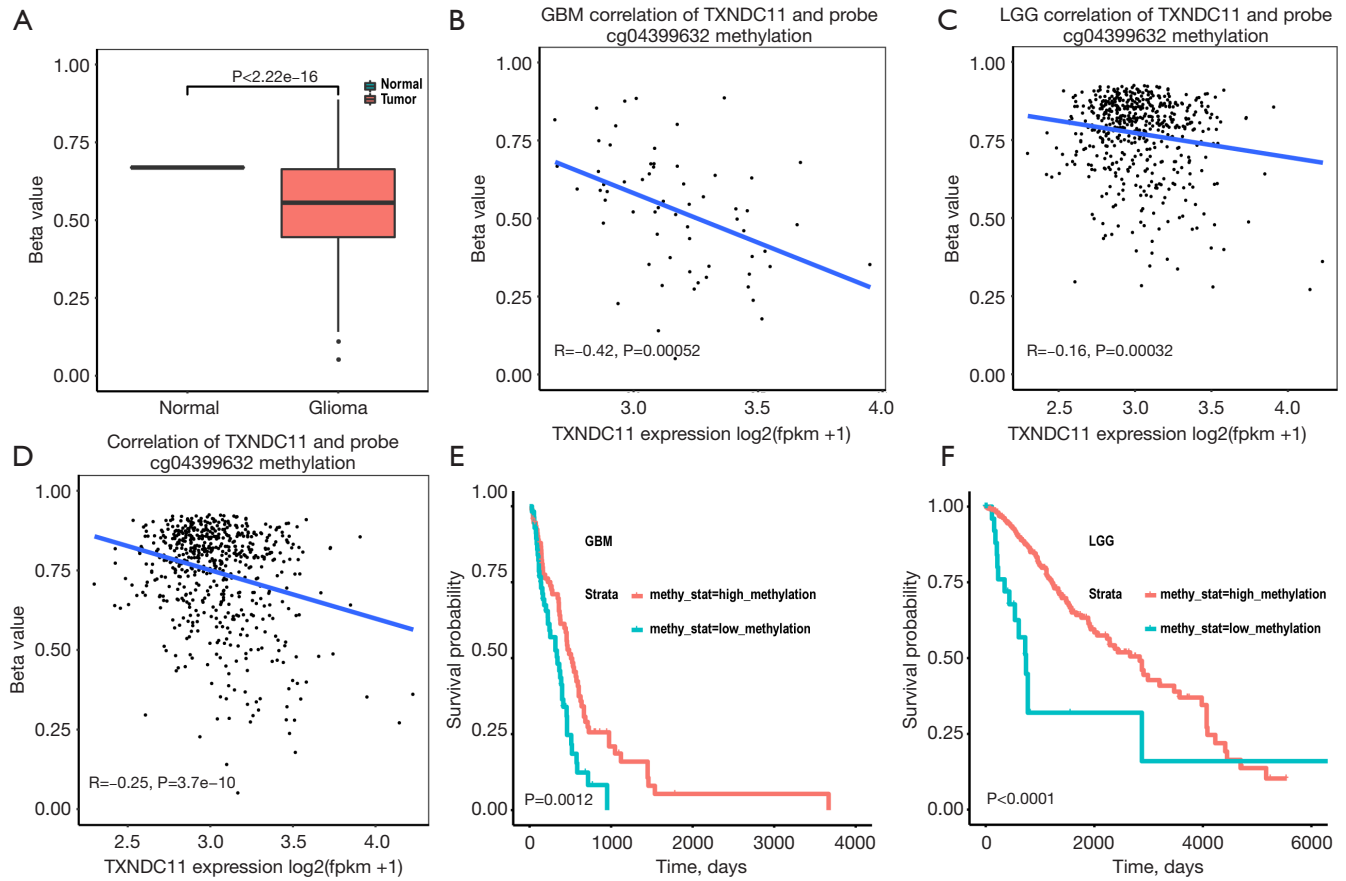

Figure 5 The correlation between *TXNDC11* methylation and OS of glioma patients. (A) The methylation of cg04399632 in glioma and normal tissues. The correlation between *TXNDC11* expression and the methylation of cg04399632 in GBM (B), LGG (C) patients, and all gliomas (D). Influence of the methylation on OS of glioma patients in GBM (E) and LGG (F) patients. *TXNDC11*, thioredoxin domain containing 11; OS, overall survival; LGG, low-grade glioma; GBM, glioblastoma.

mutation, EGFR amplification or EGFR VIII mutation (2).

In this study, we reported that *TXNDC11* had higher expression in glioma than normal tissues and was correlated with clinical features such as older ages, higher WHO grade, 1p19q non-codeletion and IDH wildtype. Survival analyses and Cox regression analyses confirmed that patients with higher *TXNDC11* expression had a shorter OS, and *TXNDC11* was an independent prognostic indicator of OS. Therefore, *TXNDC11* might be a potential oncogene in glioma.

DNA methylation is a crucial epigenetic regulation mechanism for gene expression in cancers (16). The methylation status of MGMT promoter in gliomas can predict patient survival and response to the treatment by temozolomide (17-19). In this study, we found that gliomas had a lower methylation level at cg04399632 and the methylation level of this site was negatively correlated with *TXNDC11* expression. The methylation level at cg04399632 not only affected *TXNDC11* mRNA expression, but also was positively correlated with patient survival.

Accumulating evidence has shown that tumor development and infiltration were related to oxidative stress (20-22). The excessive generation of cellular reactive oxygen species (ROS) and antioxidant system dysfunction cause oxidative stress (23). Free radicals and ROS serve as critical signaling molecules in immune process and inflammation (24,25). *TXNDC11* is a redox regulator that regulates redox status in the plasma membrane, cytosol, endoplasmic reticulum, and the nucleus (6,26,27). Fu *et al.* showed that secretion of *TXNRD1* was elevated in hepatocellular carcinoma patients under oxidative stress and inflammation (28). We assumed that *TXNDC11* may contribute to glioma development via the immune process and inflammation mechanism. We used TCGA data for GSEA,

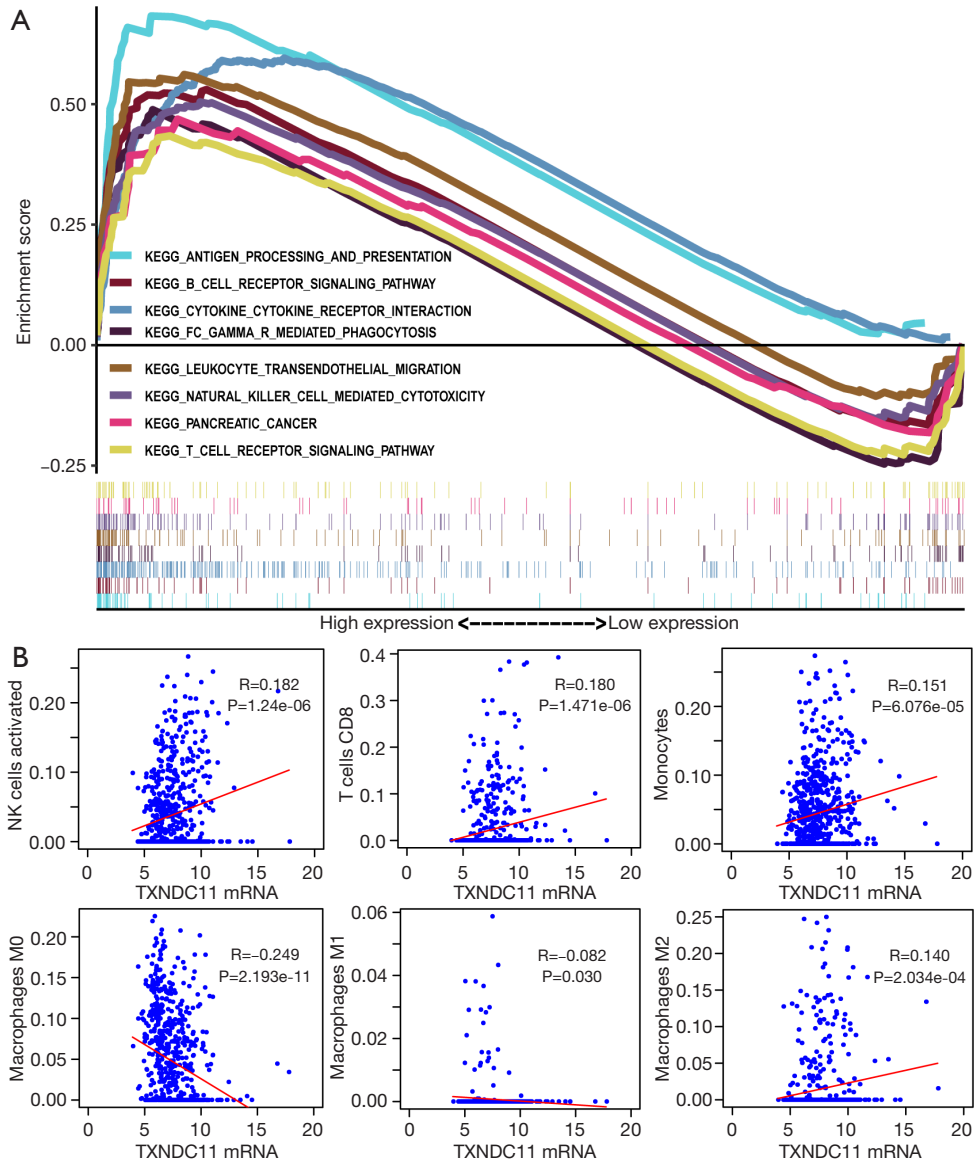

Figure 6 Correlations of *TXNDC11* expression with immune cells in glioma. (A) GSEA analysis of signaling pathways enriched in glioma samples with high expression of *TXNDC11*. (B) *TXNDC11* mRNA levels in TCGA were positively correlated with infiltration of M2 macrophages, monocytes, CD8 T cells, NK cells, but were negatively correlated with infiltration of M0 macrophages and M1 macrophages. *TXNDC11*, thioredoxin domain containing 11; GSEA, gene set enrichment analysis; TCGA, The Cancer Genome Atlas; NK, natural killer.

and found that inflammation and immune-related pathways, along with other pathways commonly involved in cancers, were associated with *TXNDC11* expression.

Previous histopathological and flow cytometry studies revealed that various immune cells such as granulocytes, resident central nervous system (CNS) (microglia) and peripheral macrophages [glioma-associated macrophages (GAMs)], T lymphocytes, and myeloid-derived suppressor cells (MDSCs) were involved in tumor microenvironment (29-32). Heimberger *et al.* found a positive association between the intra-tumoral number of $CD8^+$ cells and the survival of patients with glioma (33). Consistent with their report, we found the correlation between *TXNDC11* transcript level and immune cell infiltration, such as CD8 T cells, NK cells activated, monocytes and macrophages.

GAMs constitute a significant immune cell population

Table 4 Gene sets enriched in high *TXNDC11* expression phenotype

MSigDB collection	Gene set name	NES	NOM P value	FDR q value
Kegg.v6.2.symbols.gmt	KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	2.035	0	0.004
	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	1.986	0.002	0.007
	KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	1.835	0.006	0.018
	KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	1.568	0.042	0.081
	KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	1.792	0.008	0.025
	KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	1.754	0.018	0.028
	KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	1.976	0.002	0.007
	KEGG_PANCREATIC_CANCER	1.728	0.012	0.033

Gene sets with NOM P value <0.05 and FDR q value <0.05 were considered as significantly enriched. *TXNDC11*, thioredoxin domain containing 11; NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate.

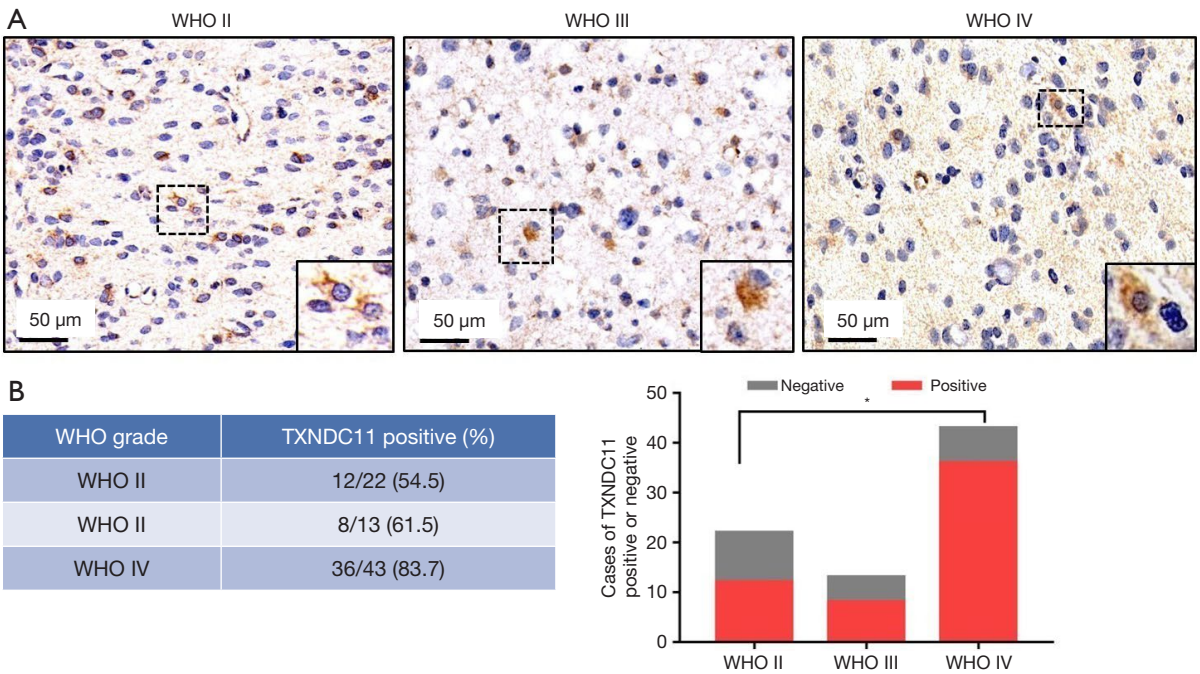

Figure 7 *TXNDC11* expression increased in higher-grade glioma. (A) IHC analysis of *TXNDC11* in glioma. Representative images were shown. Two times magnification of the box in the lower right corner. (B) Tumor type and *TXNDC11* expression in glioma. The fractions and percentages of *TXNDC11*-positive tumors were shown. *, P<0.05. *TXNDC11*, thioredoxin domain containing 11; IHC, immunohistochemical.

in gliomas and contribute to tumor growth and neovascularization (21,34-36). Lu-Emerson *et al.* found that increased GAM number was correlated with poor survival of GBM patients after anti-angiogenic therapy, which suggested that GAMs might participate in the escape of tumor cells from anti-angiogenic therapy, and therefore represent a potential biomarker of therapy resistance and a therapeutic target for GBMs (37). Moreover, M0 macrophages were negatively related to the prognosis of glioma patients, and M2-GAMs could promote the stemness and migration of glioma cells (38,39). In this study, M0 and M1 macrophages were negatively correlated with *TXNDC11* transcript level, while M2 macrophages were positively correlated with *TXNDC11* transcript

level. Therefore, the role of *TXNDC11* in dictating GAM phenotypes and glioma progression need further studies.

In summary, we showed that *TXNDC11* expression was upregulated in glioma and may contribute to unfavorable outcomes. Glioma patients with hypermethylation of *TXNDC11* at cg04399632 site had a better prognosis than those with hypomethylation. *TXNDC11* might be an important regulator of immune microenvironment of gliomas. Taken together, elevated expression of *TXNDC11* may predict unfavorable prognosis of glioma patients.

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Footnote

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Ethical Statement: The authors are 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology and informed consent was taken from all the patients.

References

1. Wen PY, Reardon DA. Neuro-oncology in 2015: Progress in glioma diagnosis, classification and treatment. Nat Rev Neurol 2016;12:69-70.
2. Siegal T. Clinical Relevance of Prognostic and Predictive Molecular Markers in Gliomas. Adv Tech Stand Neurosurg 2016;(43):91-108.
3. Wang Z, Zhang C, Liu X, et al. Molecular and clinical characterization of PD-L1 expression at transcriptional level via 976 samples of brain glioma. Oncoimmunology 2016;5:e1196310.
4. Ozlem OZ. A Novel Mutation in Neurofibromatosis Type 1 with Optic Glioma. Oncologie 2020;22:155-60.
5. Li G, Wang Z, Zhang C, et al. Molecular and clinical characterization of TIM-3 in glioma through 1,024 samples. Oncoimmunology 2017;6:e1328339.
6. Wang D, De Deken X, Milenkovic M, et al. Identification of a novel partner of duox: EFP1, a thioredoxin-related protein. J Biol Chem 2005;280:3096-103.
7. Zhang X, Wang Y. Identification of hub genes and key pathways associated with the progression of gynecological cancer. Oncol Lett 2019;18:6516-24.
8. Cho SY, Kim S, Son MJ, et al. Clinical Significance of the Thioredoxin System and Thioredoxin-Domain-Containing Protein Family in Hepatocellular Carcinoma. Dig Dis Sci 2019;64:123-36.
9. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002;30:207-10.
10. Madhavan S, Zenklusen JC, Kotliarov Y, et al. Rembrandt: helping personalized medicine become a reality through integrative translational research. Mol Cancer Res 2009;7:157-67.
11. Gao C, Zhuang J, Zhou C, et al. Prognostic value of aberrantly expressed methylation gene profiles in lung squamous cell carcinoma: A study based on The Cancer Genome Atlas. J Cell Physiol 2019;234:6519-28.
12. Zhao Z, Meng F, Wang W, et al. Comprehensive RNA-seq transcriptomic profiling in the malignant progression of gliomas. Sci Data 2017;4:170024.
13. Gentles AJ, Newman AM, Liu CL, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med 2015;21:938-45.
14. Ali HR, Chlon L, Pharoah PD, et al. Patterns of

Immune Infiltration in Breast Cancer and Their Clinical Implications: A Gene-Expression-Based Retrospective Study. PLoS Med 2016;13:e1002194.
15. Bense RD, Sotiriou C, Piccart-Gebhart MJ, et al. Relevance of Tumor-Infiltrating Immune Cell Composition and Functionality for Disease Outcome in Breast Cancer. J Natl Cancer Inst 2016;109:djw192.
16. Phillips T. The role of methylation in gene expression. Nature Education 2008;1:116.
17. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352:997-1003.
18. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-96.
19. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009;10:459-66.
20. Wu L, Wang F, Xu J, et al. PTPN2 induced by inflammatory response and oxidative stress contributed to glioma progression. J Cell Biochem 2019;120:19044-51.
21. Goyal S, Chatterjee S, Gupta N. Analysis of Antioxidant Potential of Trigonella foenum-graecum (L.) Extract Against Tumorigenesis. Oncologie 2021;23:89-104.
22. Park HK, Hong JH, Oh YT, et al. Interplay between TRAP1 and Sirtuin-3 Modulates Mitochondrial Respiration and Oxidative Stress to Maintain Stemness of Glioma Stem Cells. Cancer Res 2019;79:1369-82.
23. Liang J, Cao R, Wang X, et al. Mitochondrial PKM2 regulates oxidative stress-induced apoptosis by stabilizing Bcl2. Cell Res 2017;27:329-51.
24. Qiao C, Yang J, Shen Q, et al. Traceable Nanoparticles with Dual Targeting and ROS Response for RNAi-Based Immunochemotherapy of Intracranial Glioblastoma Treatment. Adv Mater 2018;30:e1705054.
25. Coutinho de Souza P, Smith N, Atolagbe O, et al. OKN-007 decreases free radical levels in a preclinical F98 rat glioma model. Free Radic Biol Med 2015;87:157-68.
26. Hanschmann EM, Godoy JR, Berndt C, et al. Thioredoxins, glutaredoxins, and peroxiredoxins--molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. Antioxid Redox Signal 2013;19:1539-605.
27. Timms RT, Menzies SA, Tchasovnikarova IA, et al. Genetic dissection of mammalian ERAD through comparative haploid and CRISPR forward genetic screens. Nat Commun 2016;7:11786.
28. Fu B, Meng W, Zeng X, et al. TXNRD1 Is an Unfavorable Prognostic Factor for Patients with Hepatocellular Carcinoma. Biomed Res Int 2017;2017:4698167.
29. Fecci PE, Mitchell DA, Whitesides JF, et al. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. Cancer Res 2006;66:3294-302.
30. Lohr J, Ratliff T, Huppertz A, et al. Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-β. Clin Cancer Res 2011;17:4296-308.
31. Alexiou GA, Vartholomatos G, Karamoutsios A, et al. Circulating progenitor cells: a comparison of patients with glioblastoma or meningioma. Acta Neurol Belg 2013;113:7-11.
32. Wainwright DA, Dey M, Chang A, et al. Targeting Tregs in Malignant Brain Cancer: Overcoming IDO. Front Immunol 2013;4:116.
33. Heimberger AB, Abou-Ghazal M, Reina-Ortiz C, et al. Incidence and prognostic impact of FoxP3+ regulatory T cells in human gliomas. Clin Cancer Res 2008;14:5166-72.
34. Esiri MM, Morris CS. Immunocytochemical study of macrophages and microglial cells and extracellular matrix components in human CNS disease. 2. Non-neoplastic diseases. J Neurol Sci 1991;101:59-72.
35. Geranmayeh F, Scheithauer BW, Spitzer C, et al. Microglia in gemistocytic astrocytomas. Neurosurgery 2007;60:159-66; discussion 166.
36. Gieryng A, Pszczolkowska D, Walentynowicz KA, et al. Immune microenvironment of gliomas. Lab Invest 2017;97:498-518.
37. Lu-Emerson C, Snuderl M, Kirkpatrick ND, et al. Increase in tumor-associated macrophages after antiangiogenic therapy is associated with poor survival among patients with recurrent glioblastoma. Neuro Oncol 2013;15:1079-87.
38. Zhong QY, Fan EX, Feng GY, et al. A gene expression-based study on immune cell subtypes and glioma prognosis. BMC Cancer 2019;19:1116.
39. Liu Z, Kuang W, Zhou Q, et al. TGF-β1 secreted by M2 phenotype macrophages enhances the stemness and migration of glioma cells via the SMAD2/3 signalling pathway. Int J Mol Med 2018;42:3395-403.

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