High expression of TXNDC11 indicated unfavorable prognosis of glioma

Peng Peng¹, Fangling Cheng², Yuting Dong³,⁴,⁵, Zirong Chen¹, Xiaolin Zhang¹, Dongsheng Guo¹, Xingjiang Yu⁶, Yiyang Lu⁷, Yuyong Ke⁸, Bin Zhang³,⁵,⁹, Ximiao He³,⁴,⁵, Feng Wan¹

¹Department of Neurosurgery, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; ²Department of Surgery, Hepatic Surgery Center, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; ³Department of Physiology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; ⁴Center for Genomics and Proteomics Research, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; ⁵Hubei Key Laboratory of Drug Target Research and Pharmacodynamic Evaluation, Huazhong University of Science and Technology, Wuhan, China; ⁶Department of Histology and Embryology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; ⁷School of Data Science, Chinese University of Hong Kong, Shenzhen, China; ⁸Department of Neurosurgery, Renmin Hospital of Yangxin County, Huangshi, China; ⁹The Institute for Brain Research, Collaborative Innovation Center for Brain Science, Huazhong University of Science and Technology, Wuhan, China

Contributions: (I) Conception and design: B Zhang, X He, F Wan; (II) Administrative support: Y Ke; (III) Provision of study materials or patients: P Peng, F Cheng, Y Dong; (IV) Collection and assembly of data: P Peng, F Cheng, Y Dong, Z Chen, X Zhang, D Guo; (V) Data analysis and interpretation: X Yu, Y Lu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Bin Zhang; Ximiao He. Department of Physiology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Hangkong Road 13, Wuhan 430030, China. Email: binzhang@hust.edu.cn; XimiaoHe@hust.edu.cn; Feng Wan. Department of Neurosurgery, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Jiefang Avenue 1095, Wuhan 430030, China. Email: wanruiyan@hotmail.com.

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
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 °C 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 hematoxylin (Vector Laboratories,
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**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 CIBERSORT 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 TCGA 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.

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<0.001] (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.
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

| Table 1 Baseline patient characteristics |
|------------------------------------------|
| Clinical characteristics | TCGA (n=1,114), n (%) | CGGA (n=325), n (%) |
| Age | 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 (36.3) |
| O | 191 (17.2) | 64 (19.6) |
| OA | 130 (11.7) | NA |
| GBM | 596 (53.5) | 139 (42.7) |
| Missing | 3 (0.27) | 4 (1.2) |
| IDH1 mutation | | |
| Mutation | 373 (33.4) | 175 (53.8) |
| Wildtype | 224 (20.1) | 149 (45.8) |
| Missing | 517 (46.1) | 1 (0.3) |
| 1p19q-codeletion-status | | |
| Codeletion | 169 (15.1) | 67 (20.6) |
| Non-codeletion | 495 (44.4) | 250 (76.9) |
| Missing | 450 (40.4) | 8 (2.4) |

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

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