Expression and Function of Tumor Necrosis Factor- α -induced protein 8-like (TIPE) family in glioma

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Abstract

Background: Although novel strategies of glioma are emerging sequentially, the effectiveness of novel treatment remains suboptimal at present. Therefore, profiles of molecular are needed for improving diagnosis, survival prediction and identification of therapeutic targets for glioma. Tumor necrosis factor- α -induced protein 8-like (TNFAIP8/TIPE) family members are involved in tumorigenesis and inflammatory responses but have been poorly studied in glioma. In this study, we aimed to investigate the expression and functions of TIPE family in glioma.

Methods: The expression data of the TIPE family from the Chinese Glioma Genome Atlas (CGGA), the Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) were conducted to analyze TIPEs expression and functional networks in gliomas. Kaplan-Meier analysis and Cox regression were performed to access the overall survival of these genes. The related functional networks were identified using Gene Set Enrichment Analysis and LinkedOmics. The relationship between TNFAIP8 and tumor immune system were analyzed by TISIDB.

Results: The results shown that TIPEs were generally expression in gliomas. Glioma patients with high TNFAIP8 levels tend to be more malignant than those with low expression and are associated with reduced survival. GSEA identified a variety of oncogenic and immune pathway that were tightly linked with TNFAIP8. TISIDB data found that TINAIP8 expression was significantly positively correlated with immune infiltrates cells and immune-related factors.

Conclusions: TNFAIP8 exhibit crucial role in reduced survival in glioma and could serve as a potential prognostic biomarker. The present study revealed the expression patterns and potential functional networks of TNFAIP8 in glioma, providing insights for future research of the role of TNFAIP8 in carcinogenesis.

Background

Gliomas are the most common and lethal type of primary brain tumor with extremely poor prognosis. According to the World Health Organization (WHO) classification of tumors of the central nervous system, gliomas are typically classified as grade II to IV, based on histological features¹. Diffuse lower-grade gliomas (LGG, grades II and III) have highly variable clinical behaviors, which can be predicted based on molecular parameters². Therefore, the 2016 edition of the WHO classification system subdivided gliomas based on molecular subtypes, such as isocitrate dehydrogenase (IDH) mutation and the 1p/19q codeletion (codeletion). The majority of LGG cases without IDH mutation typically and rapidly progress to glioblastoma (GBM, grade IV)³. Even in cases with early management, consisting of surgery, chemotherapy, and radiotherapy, the estimated 2-year overall survival (OS) rate for GBM patients is only 20%⁴. Because of the complicated pathogenesis of glioma, effective clinical treatment remains scarce. Therefore, the continued exploration of the molecular mechanism of glioma remains urgently necessary to improve glioma diagnosis and prognosis and identify more effective therapeutic targets.

TNFAIP8/TIPE, which is regulated by tumor necrosis factor- α (TNF α), has been reported to modulate critical cellular functions, including immune functions, cell migration, and the proliferation/apoptosis axis. The TIPE family consists of four members: TNFAIP8, TNFAIP8-like 1 (TNFAIP8L1/TIPE1), TNFAIP8-like 2 (TNFAIP8L2/TIPE2), and TNFAIP8-like 3 (TNFAIP8L3/TIPE3). The first member of the TIPE family, *TNFAIP8*, was initially discovered in human metastatic head and neck squamous cell carcinoma cells⁵ and was defined as an anti-apoptotic carcinogen. Since then, studies have confirmed that *TNFAIP8* is associated with the development of various cancers, including lung⁶, liver⁷, colon⁸, breast⁹, gastric¹⁰, prostate¹¹, and renal cancer¹². The biological roles played by *TNFAIP8* in malignant tumors include the inhibition of apoptosis and the promotion of cellular proliferation, invasion, metastasis, and drug resistance¹³. *TNFAIP8* also participates in inflammatory and immune responses¹⁴. The second identified member of the TIPE family, *TIPE1* has been found to be widely expressed in diverse cancers. It was reported to be downregulated in colon cancer¹⁵, liver cancer¹⁶, breast cancer¹⁷, osteosarcoma¹⁸, gastric cancer¹⁹, which exerts anti-tumor effects in these cancers by inducing cell arrest and apoptosis. On the contrary, *TIPE1* was also found increased expression in cervical cell lines and tissues, serving as an oncogene in the development of cervical cancer^{20,21}. Another member of the TIPE family, *TIPE2*, has also been widely studied and has been reported to act as a negative regulator of immunity and inflammation. The expression of *TIPE2* has been correlated with many different human diseases, including systemic lupus erythematosus²², myasthenia gravis²³, and cancers²⁴. The decreased expression of *TIPE2* has been observed in small-cell lung cancer²⁵, hepatic cancer²⁴, and gastric cancer tissues²⁶. Similar to *TIPE1*, *TIPE2* acts to inhibiting cancer cell growth by the inducing cell apoptosis²⁵. Compared with the other members of the TIPE family, the biological function of *TIPE3* remains ambiguous. A few reports have reported that the increased expression of *TIPE3* can be detected in colon, cervical, lung, and esophageal cancers^{26,27}, suggesting that *TIPE3* may be involved in cancer cell survival.

Although these four members of the TIPE family have been reported to be closely associated with the development of various tumors, the expression patterns and prognostic characteristics of these TIPE family have not been fully characterized in glioma. Here, we investigated the expression profile of *TIPES* and performed a survival analysis associated with *TIPES* expression. We also examined the functional networks associated with *TNFAIP8* in glioma patients, based on TCGA datasets. The results revealed a significant relationship between *TNFAIP8* expression and glioma outcome. These findings uncover the important oncogenetic role played by *TNFAIP8* in glioma and provide insights for the future study of *TNFAIP8* in carcinogenesis.

Materials And Methods

Cell culture and transfection

The human glioblastoma cell lines U87MG, U251, U118, T98G and normal glial cell HEB were obtained from ATCC. All cells were cultured in Dulbecco's modified eagle medium (DMEM) (Gibco), supplemented

with 10% foetal bovine serum (FBS) (Serana) and maintained in a humidified atmosphere containing 5% CO₂ at 37°C. Cell in logarithmic growth phase or at 80% confluence were used for experiments. TNFAIP8 siRNA and negative control siRNA were synthesized by GenePharma (Shanghai, China). Cell transfection was performed using jetPRIME reagent (Polyplus transfection) according to the manufacturer's instructions.

RNA extraction and quantitative real-time PCR

The expression levels of *TNFAIP8* were analyzed by real-time reverse transcription-polymerase chain reaction (RT-PCR). Total RNA were isolated using TRIzol reagent (Invitrogen). Reverse transcription was performed by PrimerScript RT Master Mix (Perfect Real Time, TAKARA) according to the manufacturer's instructions. All samples were analyzed using ABI 7500 Real-Time PCR system (thermoFisher). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression were used as an internal mRNA quantity control to normalized the expression of target gene. Triplicate quantitative PCR reactions were performed for both target gene and the housekeeping gene using SYBR Green I Premix ExTaq (Tli RnaseH Plus) qPCR reagent (TAKARA) according to the manufacturer's instructions. Each experiment was repeated three independent times. Primers for *TNFAIP8* were forward 5'- GGATTATACCTTTGACCGGAA-3', reverse 5'- AACACATTATTAACCCGTCCA-3', and *GAPDH* were forward 5'- GAAGGTGAAGGTCGGAGTC-3', reverse 5'- GAAGATGGTGGTGGGATTTC-3'.

Cell apoptosis assays

Cell apoptosis was evaluated using an Annexin V-FITC/PI apoptosis kit (Multi Sciences, #AP101, China) on Guava easyCyte 12HT Benchtop Flow Cytometer (Luminex). Cell line T98G were seeded in 6-well plates at 1×10^5 cells per well and transfected with TNFAIP8 siRNA or negative control siRNA. Cells were collected after 48h of siRNA transfection and then washed twice with cold PBS. 1 × Binding Buffer provided in the kit were added to resuspended the washed cell, and stained with annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) for 10 min. The number of positive apoptotic cells is expressed as percentage of total number of cells counted.

Western Blotting

After incubated with siRNA of TNFAIP8 or negative control for 48h. Total protein lysed from T98G cells were extracted and protein expression was analyzed by western blotting. Each experiment was repeated three times. The primary antibody includes and PARP (proteintech, #66520-1-Ig). Mouse monoclonal antibody β-tubulin (CST, #2128) is used as loading control.

Gene expression data analysis

The Gene Expression Omnibus (GEO) profile GSE4290 was used to analyze the expression profiles of TIPE family members in glioma cases²⁸. We also confirmed the gene profiles of TIPE family members in a Chinese Glioma Genome Atlas (CGGA) dataset (DataSet ID: mRNAseq_325). A dataset from The Cancer

Genome Atlas (TCGA) (DataSet ID: TCGA RNAseq), which included 702 samples of different grades of glioma cases, was used to perform the OS analysis for *TIPE* expression in glioma patients and Gene Set Enrichment Analysis (GSEA). The GEO dataset was downloaded from <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4290>. The CGGA dataset and TCGA dataset were downloaded from http://www.cgga.org.cn/download_other.jsp.

GSEA was performed using the GSEA software (version 4.1.0) downloaded from <https://www.gsea-msigdb.org/gsea/downloads.jsp>. GSEA was applied to identify pathway alterations between cohorts expressing high (*TNFAIP8*-high) and low (*TNFAIP8*-low) levels of *TNFAIP8*, according to the median expression level of *TNFAIP8* ($n = 694$). The gene symbols included in the gene set database Hallmark v7.1., which includes 50 gene sets, were used as the gene matrix. Kaplan-Meier analysis was performed between *TIPE*-high and *TIPE*-low cohorts, according to OS ($n = 672$).

LinkedOmics analysis

The LinkedOmics database (<http://www.linkedomics.org/login.php>) was used to study differentially expressed genes correlated with *TNFAIP8* expression in the TCGA_GBMLGG dataset ($n = 669$)²⁹. RNA-sequencing (RNA-seq) data from the HiSeq platform were chosen for this analysis. The results were analyzed statistically using Pearson's correlation coefficient analysis and generated on online analysis directly. All results were graphically presented in volcano plots and heat maps.

Co-expressed gene lists and association results from the LinkFinder analysis were downloaded and ranked according to $P < 0.05$ and $|r| > 0.5$ to perform the functional network and pathway enrichment analysis of *TIPE*s in glioma. GSEA was performed using the Web-based Gene Set Analysis Toolkit (WebGestalt, <http://www.webgestalt.org>)³⁰ to analyze Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Statistical analysis

The log-rank analysis was performed to determine the significance of the Kaplan-Meier survival plot. Spearman's rank correlation analysis was performed to analyze the potential relationships between *TNFAIP8* expression and the clinicopathological features of glioma. Independent factors associated with survival were identified using the Cox proportional hazards model. Statistical analysis was performed using GraphPad Prism 8.0 or SPSS 7.0. $P < 0.05$ was considered significant.

Results

mRNA expression dynamics of *TIPE*s in glioma

To determine the mRNA expression levels of the *TIPE* family members in glioma, the mRNA levels in glioma and normal brain tissues were assessed using the GEO dataset. A total of 180 samples with 23 normal samples, 76 LGG (Grade II & III) and 81 GBM were used for the gene expression analysis. The

results showed that all *TIPE* family genes were generally detected in glioma samples. Compared with the level in normal brain tissues, the *TNFAIP8* expression level was significantly increased in glioma ($p < 0.0001$), with the highest expression levels associated with GBM ($p < 0.0001$). In contrast, *TIPE1* expression was significantly lower in glioma than that in normal brain tissues ($p < 0.0001$, Figure 1). No significant differences were identified for *TIPE2* and *TIPE3* expression levels between glioma and normal cases (Figure 1a). To verify this finding, another dataset was examined, obtained from the CGGA, which contained 345 samples (20 normal brain tissues and 325 glioma cases) and the corresponding clinical information. The results were similar to those obtained from the GEO dataset analysis, showing that *TNFAIP8* expression was elevated in high-grade glioma ($p < 0.0001$), and *TIPE1* expression was decreased in glioma compared with those in normal tissues (Figure 1b).

Seven molecular subtypes have been identified for glioma³¹. Using the TISIDB, we found that *TIPE* expression was distributed in the classic-like, mesenchymal-like, cytosine-phosphate-guanine (CpG) island methylator phenotype (G-CIMP)-low, and LGM6-GBM types in GBM, whereas they were expressed primarily in the G-CIMP-low, G-CIMP-high, codel, classic-like, mesenchymal-like, and pilocytic astrocytoma (PA)-like types in LGG (Figure 1c). These results implied that *TIPE* mRNA was highly expressed in the molecular subtypes of gliomas associated with worse outcomes.

Survival outcome analysis for glioma patients

To determine whether *TIPE* expression was associated with prognosis in glioma patients, we next compared OS rates between the high- and low-*TIPE* expression groups in TCGA dataset and CGGA dataset. Survival analysis between two datasets indicated that *TNFAIP8* and *TIPE1* expression levels were significantly associated with OS ($p < 0.0001$, Figure 2). High levels of *TNFAIP8* (above the median) expression were linked with worse outcomes. Although the expression of *TIPE1* decreased in glioma cases, OS analysis showed that the increased expression of *TIPE1* was also associated with decreased OS ($p < 0.0001$ in TCGA and $p = 0.0007$ in CGGA).

Because a larger survival difference was observed in association with *TNFAIP8* expression than in association with the expression of the other *TIPE*s, we further explored the association between *TNFAIP8* expression levels and clinical signatures in glioma. As shown in Table 1, Spearman's rank correlation analysis between *TNFAIP8* expression levels and clinicopathological features revealed that the expression of *TNFAIP8* was significantly associated with tumor grade ($P < 0.001$), patient age ($P < 0.001$), IDH mutation status ($P < 0.001$), and 1p19q codel status ($P < 0.001$). No significant differences were identified according to gender. Next, we utilized Cox proportional hazard regression models to determine the relative risks indicated by *TNFAIP8* expression levels in glioma prognosis. As shown in Table 2, univariate analyses indicated that *TNFAIP8* expression levels, patient age, tumor grade, IDH mutation status, and 1p19q codel status were associated with prognosis in glioma cases. Multivariate Cox regression analysis found that *TNFAIP8* expression could be used as a factor to predict poor outcomes in glioma patients. Taken together, these results indicated a significant correlation between *TNFAIP8* expression and glioma prognosis.

Table 1 Correlation between *TNFAIP8* expression and clinical characteristics of glioma (Spearman analysis)

Variable	TNFAIP8 expression level	
	Spearman correlation	p-value
Age (>50 vs ≤50)	0.267	<0.0001
WHO gradeⅡⅢ vs Ⅳ	0.503	<0.0001
Gender(Female & Male)	0.09	0.105
IDH status(wild type & mutant)	0.52	<0.0001
1p19q codeletion(non-codel & codel)	0.473	<0.0001

Table 2 Univariate and multivariate cox analysis of *TNFAIP8* in CGGA

Variable	Univariate analysis		Multivariate analysis	
	p-value	HR (95% CI)	p-value	HR (95%CI)
TNFAIP8 expression	<0.0001	1.124(1.090-1.116)	0.037	1.042(1.002-1.084)
Age at diagnosis(>50 vs ≤50)	<0.0001	2.322(1.741-3.098)	0.025	1.014(1.002-1.027)
WHO gradeⅡⅢ, Ⅲ vs Ⅳ	<0.0001	4.773(3.562-6.395)	<0.0001	2.285(1.865-2.800)
IDH status(wild type & mutant)	<0.0001	2.847(2.154-3.763)	0.323	0.8370.588-1.191)
1p19q codeletion(non-codel & codel)	<0.0001	5.977(3.663-9.754)	<0.0001	3.643(2.158-6.15)

Oncogenic role of TNFAIP8 in glioma

To investigate the performance of *TNFAIP8* expression for predicting patient outcomes, we further explored the oncogenic role of *TNFAIP8* in glioma. The enrichment results indicated that 21 gene sets (pathways) were significant enriched (FDR < 0.25, normalized P (N-P) < 0.05, Table S1). The top-ranked pathway, apoptosis, was found to be highly enriched in the *TNFAIP8* high-expression group in glioma (Figure 3). *TNFAIP8*-high was significantly associated with a variety of signaling pathways that have been found to promote tumor progression in glioma, including the mammalian target of rapamycin complex 1 (mTORC1), interleukin-1 (IL1)-signal transduction and activator of transcription 5 (STAT5),

glycolysis, phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT)-mTOR, hypoxia, p53, epithelial-to-mesenchyma transition (EMT), STAT3, INF- γ , KRAS, and angiogenesis pathways.

GSEA was also performed to explore the oncogenic roles of *TIPE1*, *TIPE2*, and *TIPE3* in glioma. The top pathways that were significantly enriched and associated with tumorigenesis were hypoxia [normalized enrichment score (NES) = 1.62, N-P = 0.031] for *TIPE1* (Table S2), apoptosis (NES = 1.76, N-p = 0) for *TIPE2* (Table S3), and MYC-target V2 (NES = 1.94, N-p = 0.012) for *TIPE3* (Table S4).

To assess the oncogenic role of *TNFAIP8* expression in a comprehensive manner, relationship between *TNFAIP8* expression and the tumor immune system of glioma was analyzed using the Tumor and Immune System Interaction Database (TISIDB). The results illustrated that *TNFAIP8* expression was significantly correlated with infiltrating levels of majority tumor-infiltrating lymphocytes (TILs, $r > 0.5$), especially with regulatory T-cells (Tregs), type 1 helper T-cell (Th1), myeloid-derived suppressor cells (MDSCs), and gamma delta T-cells, in both of GBM and LGG cohorts (Figure S1). In contrast, a low correlation was observed between *TNFAIP8* expression and type 2 helper T-cells, eosinophils, and CD56 natural killer cells (Table S5).

The TISIDB data also revealed that *TNFAIP8* expression was significantly correlated with various immunomodulators, including the immune inhibitors IL10 and CD96 and the immunostimulators IL-2 receptor A (IL-2RA) and CD86 (Figure S2). We found that *TNFAIP8* expression had a strong and positive association with major histocompatibility complex class II (MHC II) molecules. In addition, *TNFAIP8* expression was also significantly associated with a series of chemokines and receptors, among which the top two chemokines were CCL2 and CCL5, and the top two receptors were CCR2 and CCR5 (Figure S3).

Enrichment analysis of *TNFAIP8* functional networks in glioma

To further elucidate the molecular mechanism of *TNFAIP8* in glioma, we explored the co-expression genes correlated with *TNFAIP8* expression in glioma. We obtained 20,188 genes associated with *TNFAIP8* expression from the TCGA-GBMLGG dataset. As shown in Figure 4, the volcano plot and heat map illustrated gene sets that were significantly positively and negatively correlated with *TNFAIP8* expression, which demonstrated the widespread impact of *TNFAIP8* expression on the transcriptome. The expression of *TNFAIP8* showed a strong positive correlation with the expression of *CASP4* ($r = 0.873$), *PLBD1* ($r = 0.87$), and *S100A4* ($r = 0.866$), which reflects a regulatory role in cell apoptosis, hydrolase activity, and cell cycle progression and differentiation.

To further examine the *TNFAIP8*-associated genes, we performed GO and KEGG pathway analysis using WebGestalt. As shown in Figure 5, GO analysis demonstrated that genes expressed in correlation with *TNFAIP8* were primarily associated with the primary lysosome, cell-substrate junction, extracellular matrix, and specific granule, where they are involved in neutrophil-mediated immunity, lymphocyte-mediated immunity, cytokine production, and angiogenesis. Their molecular functions involve cytokine binding, endopeptidase activity, extracellular matrix structural constituents, and protease binding. In

addition, the KEGG pathway analysis revealed the enrichment of cytokine-cytokine receptor interactions, complement and coagulation cascades, the TNF signaling pathway, and antigen processing and presentation (Figure 5b). Searching for *TNFAIP8* co-expression genes in the TNF signaling pathway revealed that these associated genes were primarily enriched in pro-apoptosis, Fas-FADD, and necroptosis pathways (Figure 5c).

TNFAIP8 inhibits apoptosis of GBM tumor cells

Based on the *in-silico* analysis that TNFAIP8 may function as tumor promoter in gliomas, we designed experiments to validate this hypothesis. The expression level of *TNFAIP8* in GBM tumor cell lines were significantly higher than normal glial cells (Figure 6a). Among these cell lines, T98G with highest expression of *TNFAIP8* were chose to be knockdown (Figure 6b). Apoptosis assay were demonstrated by flow cytometry analysis, and increased apoptosis rate was found between NC-siRNA and TNFAIP8-siRNA cells (Figure 6c and 6d). WB for PARP1 protein expression in T98G cells treated with NC-siRNA or TNFAIP8-siRNA shown that more cleaved PARP1 were detected in si-TNFAIP8 cells in contrast with that of si-NC cells (Figure 6e).

Discussion

Diffuse gliomas are common and progressive brain tumors characterized by heterogeneity and extensive invasion, rapid recurrence and fatality rates, and highly infiltrative patterns¹. Although novel strategies for glioma treatment continue to emerge, the effectiveness of novel treatments remain suboptimal³². The *TIPE* family members play critical roles in tumorigenesis and inflammatory responses and have been reported to have critical prognostic significance in many cancer types. However, the prognostic roles and functions of *TIPE* expression in glioma has not yet been studied. In this study, we identified and characterized molecular phenotypes, which may aid in our understanding of the molecular mechanisms through which this family of genes affects glioma, paving the way for further studies.

TNFAIP8 is reported to be overexpressed in various human cancer, such as lung cancer, lymphoblastic leukemia, chronic myelogenous leukemia, but lower expression in normal brain compared with other tissues.³³ At present study, our results shown that *TNFAIP8* expression level was higher than normal brain tissues, and increased with increasing glioma grade. Moreover, the expression levels of the various *TIPE* was correlated with malignant molecular glioma phenotypes, such as G-CIMP-low, LGm6-GBM, and mesenchymal-like subtypes, which have been reported as having the worst prognosis outcomes³⁴. The molecular profile of *TIPE*s in gliomas demonstrated that the expression of *TNFAIP8* was correlated with malignant phenotypes and unfavorable prognosis in glioma.

The survival analysis and Tumor and Immune System Interaction Database (TISIDB) database analysis³⁵ further confirmed the prognostic value of *TNFAIP8* expression in glioma (The TISIDB was used to assess the role of *TIPE*s in the tumor-immune system interplay.). First, Kaplan-Meier survival analysis found that the increased expression of *TNFAIP8* was significantly associated with worse survival. And

then multivariate Cox regression analysis indicated that the *TNFAIP8* expression level was an independent prognostic factor for glioma patients. Moreover, analysis of *TNFAIP8* expression in tumor-immune system interactions showed its role in the immune response in gliomas. Previous studies have found that TILs were correlated with either better or worse prognosis in glioma³⁶. In our study, *TNFAIP8* expression was significantly correlated with TIL expression, especially Tregs and MDSCs, which are typically associated with poor prognosis in tumors³⁷. In addition, a number of significant associations were identified during the TISDB analysis between *TNFAIP8* expression and immune-related factors (including IL10, CD96, IL2RA, CD86, HLA-DRA, HLA-DMA, CCL2, CCR2, and CCR5), which play crucial roles in controlling the development and prognosis of glioma. Together, these results robustly demonstrated that *TNFAIP8* might serve as a potential prognostic biomarker for glioma. In past decades, *TNFAIP8* has been confirmed to be associated with the development and prognosis of many malignant tumors but not with brain tumors. To our knowledge, this is the first study to investigate the association between *TNFAIP8* expression and glioma.

To understand the role played by *TNFAIP8* in glioma, GSEA was firstly performed to identify pathway alternations based on the expression of *TNFAIP8*. Interestingly, *TNFAIP8* was found to be highly associated with various critical oncogenic pathways associated with glioma, particularly cell apoptosis and angiogenesis in tumorigenesis. In addition, we also investigated the functional networks associated with *TNFAIP8* in glioma. The enrichment analysis results also demonstrated the oncogenic role of *TNFAIP8*, which was primarily enriched in the pathways associated with the evasion of apoptosis and angiogenesis. These results confirmed the oncogenic roles played by TIPEs in glioma. Since *TNFAIP8* was overexpressed in various human cancers, initial studies have focused on its role in tumorigenesis. Reports shown that *TNFAIP8* could promote cell proliferation, tumor growth, and metastasis through induction of autophagy and inhibiting apoptosis^{14,38}. Recent study suggest *TNFAIP8* also participates angiogenesis processes in colorectal cancer⁸. In this study, we observed a similar role for *TNFAIP8* in the development of glioma. To verify this, we employed lentivirus infection and evaluated the number of cell apoptosis between normal control GBM cells and *TNFAIP8* knockdown GBM cells. The results showed that cells in knockdown of *TNFAIP8* demonstrated significantly higher rate of cell apoptosis. And more cleaved PARP1 was detected in the present of *TNFAIP8* knockdown cells, compared with normal control. PARP is an enzyme involved DNA repair which during apoptosis is cleaved by active caspase-3³⁹. Therefore, the role of anti-apoptotic in GBM is confirmed. But the molecular mechanisms of *TNFAIP8* playing roles in the development of glioma is still unclear, more studies remain necessary.

Conclusion

In conclusion, our research, which deeply mined genomic cancer data sets, revealed that the high expression of *TNFAIP8* correlated with poor glioma prognosis. According to further analyses, we speculate that *TNFAIP8* may play an important role in glioma progression by influencing apoptosis evasion and regulating angiogenesis. The expression level and the anti-apoptosis function of *TNFAIP8* in GBM were validated by experiment assay. Although we have performed a comprehensive and systematic

investigation of the expression and function of TIPE family members in glioma, our findings are mainly based on an *in silico* analysis. More studies using multiple cohorts of glioma cases and *in vivo* and *in vitro* investigations remain necessary to further explore the molecular mechanism of TNFAIP8 in the progress of gliomas.

According to our bioinformatics-based findings, TIPEs family were generally expressed in gliomas, and *TNFAIP8* may play an important role in glioblastoma progression by influencing apoptosis evasion and regulating angiogenesis. The mRNA expression and anti-apoptotic function of TNFAIP8 in GBM cells is confirmed based on experiments. This studies provided evidence that TNFAIP8 is more likely to participate the progress of GBM development in TIPEs family. It may work as an oncogene, which is hopeful to become the molecular target of glioblastoma therapy in the future.

Declarations

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Authors' contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by CQ, TCL and LL. The first draft of the manuscript was written by CQ and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

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The manuscript was edited for correct English language usage, grammar, punctuation and spelling by qualified native English speaking editors at Charlesworth Author Services. Certification was obtained (provided separately).

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Figures

Figure 1

TIPE expression profiles in glioma. (a) Differences in TIPE expression levels between glioma (LGG and GBM) and normal tissues in the GEO database GSE4290, (b) TIPE expression level between glioma (LGG and GBM) and normal tissues in CGGA database (n=325), (c) in different immune glioma subtypes from the TISIDB database. Log counts per million mapped reads (log2CPM).

Figure 2

Overall survival analysis for glioma patients. Kaplan-Meier survival curve for glioma patients according to the expression of TIPEs (a) in the TCGA dataset (n = 673) and (b) in CGGA dataset (n=315). A log-rank test was used.

Figure 3

Gene set enrichment analysis (GSEA) for the oncogenic role of *TNFAIP8* in glioma patients from the TCGA dataset (n = 694). A false discovery rate (FDR) < 25% and normalized-p < 0.05 were used to define significant enrichment. NES: Normalized Enrichment Score

Figure 4

Differentially expressed genes correlated with *TNFAIP8* expression in lower-grade glioma (LGG) and/or glioblastoma (GBM) (n = 669 and n = 158, respectively, LinkedOmics) A Pearson's correlation coefficient analysis was used to analyze the correlations between *TNFAIP8* and differentially expressed genes in glioma. Red color indicates positively correlated genes, and green color indicates negatively correlated genes. Heat maps demonstrating genes that are positively and negatively correlated with *TNFAIP8* expression in glioma (Top 50).

Figure 5

Significantly enriched GO annotations and KEGG pathways for genes co-expressed with *TNFAIP8* in glioma. (a) GO slim summary for the uploaded gene lists. (b) Enrichment results (affinity propagation) from GSEA. The bar width is equal to the normalized enrichment score (NES) in GSEA. Bars with darker shaded colors indicate that the false discovery rate (FDR) for the categories is ≤ 0.05 . (c) KEGG pathway

annotations for the TNF signaling pathway. Red marked nodes are associated with the Leading Edge Gene (Pearson's correlation coefficient $|r| > 0.5$ and $p < 0.05$).

Figure 6

TNFAIP8 inhibits apoptosis of GBM tumor cells. (a) TNFAIP8 mRNA expression in GBM cell lines was significantly higher than that of normal glial cell line HEB. (b) qRT-PCR analysis of TNFAIP8 mRNA expression in T98G cells infected with NC-siRNA or TNFAIP8-siRNA. *** $P < 0.0001$. (c) and (d) Increased apoptosis rate was shown between NC-siRNA and TNFAIP8-siRNA, as demonstrated by flow cytometry analysis. (e) WB for caspase 3 and PARP1 protein expression in T98G cells treated with NC-siRNA or TNFAIP8-siRNA.

Supplementary Files

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