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Original Article

A high expression of MTERF3 correlates with tumor progression and predicts poor outcomes in patients with brain glioma

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Abstract: Mitochondrial transcription termination factor 3 (MTERF3) is a negative regulator of mitochondrial transcription. MTERF3 is overexpressed in liver cancer, pancreatic cancer, lung cancer, and breast cancer. However, whether MTERF3 is up-regulated in brain glioma is still unclear. The aim of this study was to investigate the expression and clinicopathological significance of MTERF3 in brain glioma and to analyze its potential prognostic value in brain glioma. Immunohistochemistry, Western blot, and a semi-quantitative RT-PCR were performed to analyze the protein and mRNA expression levels of MTERF3 in 28 human brain glioma tissues and 10 noncancerous brain tissues. The expression data of MTERF3 and its clinical information in brain glioma were downloaded from the TCGA dataset using R 2.15.3 software. The relationship between the expression of MTERF3 and its clinicopathological characteristics and its prognostic value was analyzed. A Cox regression model was used for a multivariate analysis of the factors affecting the prognosis of brain glioma. The immunohistochemistry results showed that the MTERF3 protein is located in the cytoplasm, and the positive expression rate of the MTERF3 protein in brain glioma tissues is 64.29%. We found that the positive expression rate of the MTERF3 protein in high-grade glioma tissues (81.25%) is higher than it is in low-grade glioma tissues (41.67%). The expression levels of the MTERF3 mRNA and protein in brain glioma tissues are significantly higher than they are in the noncancerous brain tissues. Moreover, the expression of MTERF3 is significantly correlated with age, tumor type, and pathological classification ($P<0.05$). A Kaplan-Meier analysis showed that a high expression level of MTERF3 mRNA indicated a poor prognosis (log rank $P<0.01$). Furthermore, a multivariate Cox regression analysis showed that age and tumor type were independent prognostic factors for brain glioma patients. A GEPIA analysis suggested that the expression levels of MTERF3 are positively correlated with the TFAM, TFB1M, TFB2M, MTERF1, MTERF2, TEFM, and MFN1 genes, but negatively correlated with the PINK1 gene. The expression level of MTERF3 had no correlation with the MTERF4 gene. In conclusion, these data indicate that the expression of MTERF3 in glioma tissue samples can be used as a prognostic factor for patients with glioma and that a high MTERF3 expression correlates with a poor prognosis in glioma patients.

Keywords: MTERF3, brain glioma, immunohistochemistry, TCGA dataset, prognosis

Introduction

Brain glioma is a primary malignant tumor originating in the neuroectoderm, with the highest incidence in the central nervous system [1]. Brain gliomas account for 49.7% of primary intracranial tumors in adults, including glioblastoma, diffuse astrocytoma, anaplastic oligodendrocytic astrocytoma, etc. [2, 3]. Most gliomas are highly malignant and aggressive, and surgical treatment is the primary therapeutic approach at present. The invasive growth pattern of the tumor may result in an incomplete surgical resection that could lead to a high postoperative recurrence rate and a poor prognosis. Therefore, there is a need for comprehensive treatment combined with postoperative radiotherapy and chemotherapy [4]. In recent decades, with the in-depth study of gliomas, it has been demonstrated that the growth and invasion of brain gliomas are closely related to a variety of genes [5-7]. The survival rate

of patients can be improved by combing updated gene therapy and molecular targeted therapy. However, no specific and effective key target gene has been found in gliomas, and the discovery of new therapeutic targets has become a frontier topic for the comprehensive treatment of glioma. The human mitochondrial transcription termination factor 3 (MTERF3) protein, is also called mitochondrial transcription termination domain containing 1 (MTERFD1) [8]. MTERF3 is the most conserved member of the human MTERF protein family, which consists of 417 amino acid residues and five conserved MTERF motifs [9]. Previous studies have shown that mammalian MTERF3 is a negative regulator of mitochondrial DNA (mtDNA) transcription [10]. Through binding to the promoter region of mtDNA, MTERF3 can inhibit the duplication of the mtDNA double-strand and its transcription and translation levels, thereby slowing down cell energy production [10]. Further, MTERF3 is a necessary gene in mammalian embryo development. MTERF3 gene knockout causes a delay in embryo development and embryo lethality [11]. The inactivation of MTERF3 in the myocardium and in skeletal muscle tissue may lead to abnormal mtDNA transcription, resulting in severe respiratory chain defects and decreased oxidative phosphorylation [12, 13]. It has been shown that MTERF3 gene amplification and overexpression are common in various types of solid tumors. In addition, an abnormal protein expression of MTERF3 may be involved in the occurrence, development, and metastasis of malignant tumors [14]. So far, there is still no relevant report regarding the expression of MTERF3 in human gliomas and its relationship with the prognosis of gliomas. In this study, semi-quantitative RT-PCR, immunohistochemistry, and Western blot were performed to detect the expression and distribution of MTERF3 mRNA and protein in human glioma and noncancerous brain tissues. The purpose of this study was to elucidate the relationship between MTERF3 and the clinicopathological features of human gliomas and that relationship's prognostic significance by using the TCGA dataset, so as to shed light on the role of the MTERF3 gene in the occurrence and development of brain glioma.

Materials and methods

Clinical specimens

Brain glioma specimens (n = 28) were collected from the Department of Neurosurgery, the First People's Hospital of Dali Bai Autonomous Prefecture from June 2013 to December 2015. All the specimens were confirmed by pathology and were kept at -80°C and placed in an ultra-low temperature freezer within 30 min after isolation for cryopreservation. Of the 28 cases, there were 15 males and 13 females, ranging in age from 14-62 years, with an average age of 49.25 ± 12.67 years. All the patients were diagnosed for the first time, i.e., without a treatment history of chemotherapy or radiotherapy before their operations. According to the WHO classification criteria for nervous system tumors (revised version 2016), the collected tumor tissues were classified into two groups: the WHO grade I~II samples were classified as low-grade glioma, and the WHO grade III~IV samples were classified as high-grade glioma. Of these, there were 2 cases of grade I, 10 cases of grade II, 13 cases of grade III, and 3 cases of grade IV. In addition, 10 cases of noncancerous brain tissues with cranial decompressions due to brain trauma during the same period were selected as controls. The study was approved by the Medical Ethics Committee of Dali University (2014-12), and all the subjects signed an informed consent. The resected specimens were divided into two groups: one for the detection of MTERF3 mRNA, and the other for the detection of the MTERF3 protein. All the specimens for immunohistochemical detection were immersed and fixed in a 10% neutral formalin solution, embedded in conventional paraffin, and sliced continuously at 4 μm.

Experimental materials

The primary antibody in the immunohistochemical detection was the rabbit anti-human MTERF3 polyclonal antibody (Cat# ab230232) purchased from Abcam (1:100 dilution; Cambridge, MA, USA). The secondary antibody was horseradish peroxidase labeled goat anti-rabbit IgG (Shanghai Beyotime Biotechnology Co., Ltd.). The SP immunohistochemical kit and the DAB chromogenic kit were provided by Fujian Maixin Biotech Co., Ltd. The TRIzol reagent, the reverse transcription kit, and the *Taq* DNA polymerase were purchased from Promega (USA). RT-PCR primers were synthesized by Shanghai Sangon Biotech Co., Ltd.

MTERF3 protein levels in noncancerous brain tissues and glioma tissues were detected using the immunohistochemical SP method

Immunohistochemistry was performed using a ready-to-use SP kit. The paraffin-embedded tis-

sue slices were baked in an oven at 60°C for 15 min. The slices were deparaffinized with dimethylbenzene and rehydrated in a different concentration of ethanol. The endogenous peroxidase was blocked with 3% hydrogen peroxide for 10 min. Following a full rinsing with PBS, the slices were immersed in a 0.01 M citric acid buffer (pH = 6.0) and received microwave antigen thermal repair for 10 min, followed by cooling at room temperature. To avoid nonspecific staining, the sections were treated with normal goat serum, followed by incubation overnight with an anti-MTERFD1 antibody (1:100 dilution) (Cat# ab230232, Cambridge, MA, USA) at 4°C. Then the slide was washed with PBS three times (3 min each); the secondary antibody was applied and incubated at room temperature for 1 h. After washing with PBS three times (3 min each), the DAB chromogenic agent was added and incubated for 5-10 min, with the development time controlled under a microscope, and the reaction was stopped by rinsing with distilled water. After hematoxylin for re-staining and a full rinsing with distilled water, 1% ethanol-hydrochloric acid was used for differentiation, followed by another washing with distilled water thoroughly. Cell staining was observed under the microscope after routine dehydration, xylene treatment, and mounting with neutral resin.

Analysis of immunohistochemical SP results

Two experienced pathologists independently evaluated the percentage of positive tumor cells and their staining intensity. The expression of the MTERF3 protein was defined as a brownish-yellow staining in the cytoplasm. At a high microscopy magnitude (400 ×), five fields of vision were randomly selected and the proportion of positive staining cells was counted in each visual field. The MTERF3 staining intensity scores were assigned as follows: 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The scores for the percentage of positive tumor cells were determined as follows: 1 (0-25%), 2 (26-50%), 3 (51-75%) and 4 (76-100%). The immunoreactive score (IRS) of each section was calculated by the product of the staining intensity and the percentage of tumor cells. According to the IRS, the staining patterns were divided into three classes: weak (IRS: 0-3), moderate (IRS: 4-6), and strong (IRS: 8-12).

Determination of MTERF3 mRNA expression in noncancerous brain tissues and glioma tissues by semi-quantitative RT-PCR

Primer Premier 5.0 software was used to design the PCR primers according to sequences provided by GenBank, and the primer homology was compared through the NCBI/Primer-BLAST website [15]. Primer sequences of the target gene MTERF3 were: MTERF3-F: 5'-ATATCCTCTGACAATTGCT-3'; MTERF3-R: 5'-GAATGATCCACATAGTCTCG-3', expected band is 320 bp. The primer sequences of the reference gene β-actin were: β-actin-F: 5'-TGGGACGACATGGAGAAAA-3'; β-actin-R: 5'-CAGGAAGGAAGGCTGGAAG-3', with its amplified fragment length of 567 bp. A 100 mg frozen specimen was used for the isolation of total RNA, with a TRIzol solution. Total RNA was extracted using the one-step method according to the instructions of the TRIzol kit, and then the cDNA was synthesized according to the manufacturer's instructions. cDNA was used as template for PCR, and the PCR reaction conditions were as follows: predenaturation at 95°C for 5 min; 95°C for 30 s, 56°C for 30 s, and 72°C for 30 s, for a total of 30 cycles; followed by an extension at 72°C for 5 min. After agarose gel electrophoresis, the PCR products were imaged by the ChemiDoc XRS+ gel imaging system and analyzed by Image Lab™ 5.2.1 software. The MTERF3 mRNA expression was determined by the ratio of the accumulated optical density between the target strip and the internal reference strip. The experiment was repeated three times.

Protein isolation and Western blotting

Protein samples were harvested from both brain glioma tissue and noncancerous brain tissue lysates, and the protein concentrations were determined using a bicinchoninic acid kit. The proteins were separated by 12% SDS-PAGE and transferred to PVDF membrane. The membrane was blocked with 5% nonfat milk in PBS for 2 h at room temperature and then incubated overnight at 4°C with anti-MTERF3 antibody. After being washed with PBS, the membrane was probed with a corresponding HRP secondary antibody for 2 h at room temperature. Finally, the protein signals were detected semi-quantitatively with a Tano™ Hig-sig ECL Western Blotting Substrate.

Table 1. Prognostic factors and their assignment in glioma patients

Influencing factor	Assignment explanation
Age	≤50 years old = 1, >50 years old = 2
Gender	Male = 1, Female = 2
Pathological type	Anaplastic astrocytoma = 1, Anaplastic oligoastrocytoma = 2
	Astrocytoma = 3, Oligoastrocytoma = 4, Oligodendroglioma = 5
WHO classification	I+II = 1, III+IV = 2
History of headache	With = 1, Without = 2, No record = 3
Tumor location	Left = 1, Right = 2, Middle = 3
Sample type	Primary tumor = 1, Recurrent tumor = 2
MTERF3 expression	Low expression of MTERF3 = 1, High expression of MTERF3 = 2
Survival time	Actual survival time (month)
Patient outcome	Survival = 0, Death = 1

Glioma data collection from the TCGA database

By using Bioconductor/TCGA biolinks package in R 2.15.3 software, mRNA expression (SeqV2) data were downloaded from the TCGA database (http://tcga-data.nih.gov/tcga/) and preprocessed to analyze the relative expressions of MTERF3. Meanwhile, the clinical data, the cumulative overall survival (OS), and the disease free survival (DFS) data were downloaded for a correlation analysis of the clinicopathological parameters and the prognosis analysis.

Screening of glioma dataset in TCGA and correlation analysis of clinical pathological parameters

The data from a total of 530 glioma tissues and 10 noncancerous tissues were downloaded using R 2.15.3 software. The clinical data of the glioma patients in the TCGA dataset were filtered to keep cases with clinical parameters and survival data. After the dataset was filtered, 522 cases with complete clinical parameters and survival data were incorporated. According to second-generation mRNA-sequencing data, the expression of MTERF3 mRNA in glioma tissues ranged from 124.62 to 600.14, with a median of 226.73. If the expression of MTERF3 mRNA in a sample of glioma tissue was greater than the median, it was defined as a high expression of MTERF3; otherwise, it was considered a low expression of MTERF3. Accordingly, MTERF3 mRNA was highly expressed in 261 cases and lowly expressed in 261 cases.

The correlations between MTERF3 with other genes in the glioma tissues

GEPIA (Gene Expression Profiling Interactive Analysis) was used to analyze the correlation between MTERF3 and other gene expression levels. We analyzed the glioma tissues dataset downloaded from TCGA. The selection of mitochondrial regulatory genes included: TFAM (mitochondrial transcription factor); TFB1M (mitochondrial transcription factor B1); TFB2M (mitochondrial transcription factor B2); MTERF1 (mitochondrial transcription termination factor 1); MTERF2 (mitochondrial transcription termination factor 2); MTERF4 (mitochondrial transcription termination factor 4), TEFM (mitochondrial transcription elongation factor); MFN1 (mitofusin 1); PIKN1 (PTEN-induced putative kinase 1).

Statistical analysis

The patients' clinical data, such as age, gender, pathological type, pathological classification, history of headache, tumor location, sample type, MTERF3 expression, survival time, and outcome, were quantitatively assigned after the establishment of the patient clinical database. After assignment, all the data were input for the statistical analysis (**Table 1**). SPSS version 22.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis, and Graphpad Prism version 7.01 software were used for mapping. The normal distribution of MTERF3 mRNA expression in the noncancerous brain tissues and the glioma tissues was analyzed using the Shapiro-Wilk method. The results suggested that the expression level of

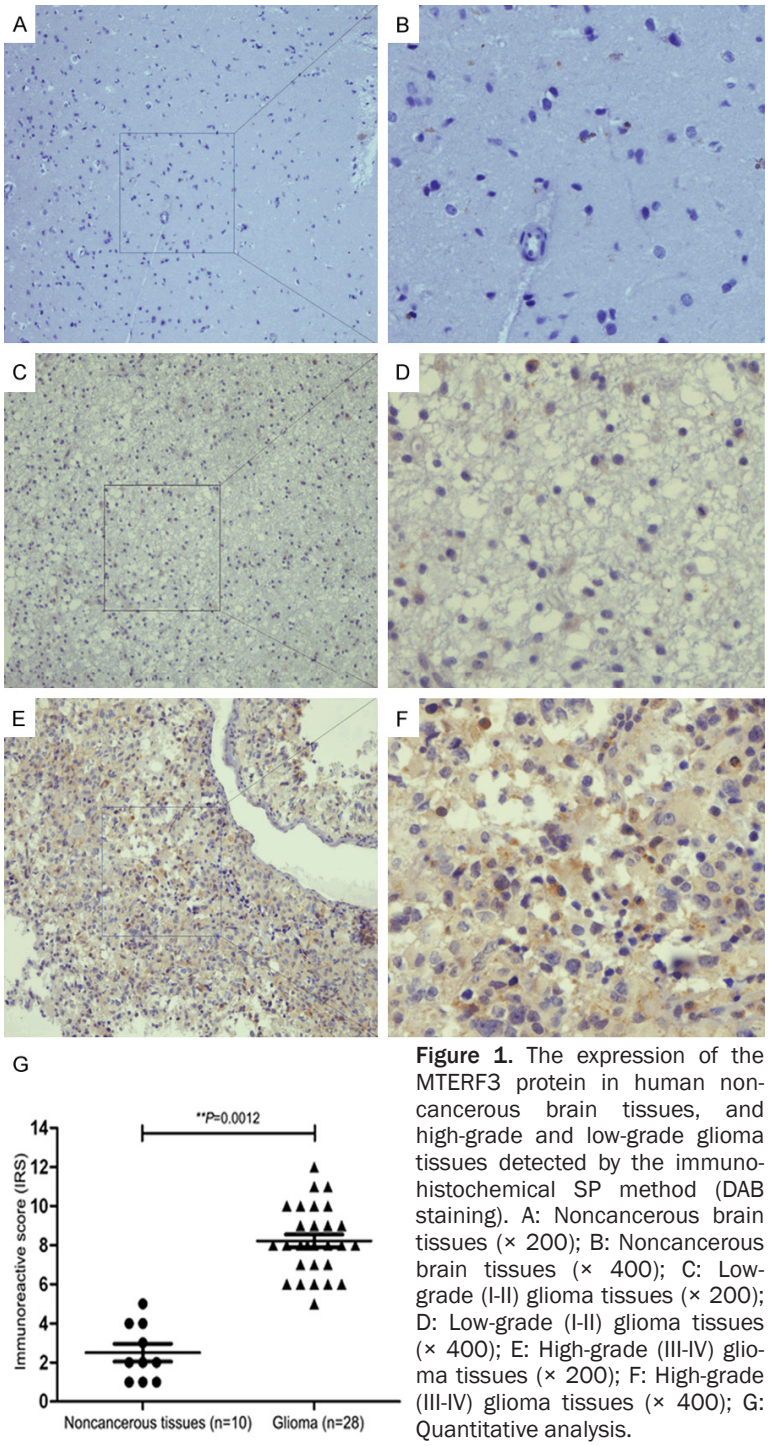

Figure 1. The expression of the MTERF3 protein in human noncancerous brain tissues, and high-grade and low-grade glioma tissues detected by the immunohistochemical SP method (DAB staining). A: Noncancerous brain tissues (× 200); B: Noncancerous brain tissues (× 400); C: Low-grade (I-II) glioma tissues (× 200); D: Low-grade (I-II) glioma tissues (× 400); E: High-grade (III-IV) glioma tissues (× 200); F: High-grade (III-IV) glioma tissues (× 400); G: Quantitative analysis.

MTERF3 mRNA did not conform to a normal distribution. Consequently, the expression level of MTERF3 in the noncancerous brain tissues and glioma tissues was compared using a Mann-Whitney U non-parametric test. The measurement data was expressed as the mean ± standard deviation ($\bar{x} \pm s$), and the categorical data was presented as a frequency (percentage). The correlation between MTERF3 expression and the clinicopathological parameters of the brain glioma patients was calculated using an χ^2 test and Fisher's exact probability test. A Kaplan-Meier analysis detected the effect of MTERF3 on the survival of glioma patients, and a Log-rank test was used to compare the survival rates. Further, a Cox proportional hazards model was applied to analyze the possible risk factors affecting the prognoses of patients with glioma. Pearson's correlation analysis was used to analyze the relationship between the MTERF3 expression level with other genes in the brain glioma patients. The inspection level α was 0.05, and $P \leq 0.05$ and $P \leq 0.01$ referred to significant difference and highly significant difference, respectively.

Results

The expression of the MTERF3 protein in noncancerous brain and glioma tissues

The immunohistochemical staining results showed that the positively stained MTERF3 proteins have fine brown-yellow granules and are localized in the cytoplasms of the gliomas. The expression of MTERF3 was weakly positive in the grade I and II gliomas, but it was moderately and strongly positive in the grade III and IV gliomas, respectively (**Figure 1**). Of the 28 glioma specimens, 18 were positive for MTERF3, and the positive rate was 64.29% (18/28). The positive rate of MTERF3 was 41.67% (5/12) in the low grade gliomas (grade I-II) and 81.25% (13/16) in the high grade gliomas (grade III-IV). In contrast, in the noncancerous brain tissues, the positive expression rate of MTERF3 was only 10% (1/10), and there was a significant difference between the groups ($P<0.01$).

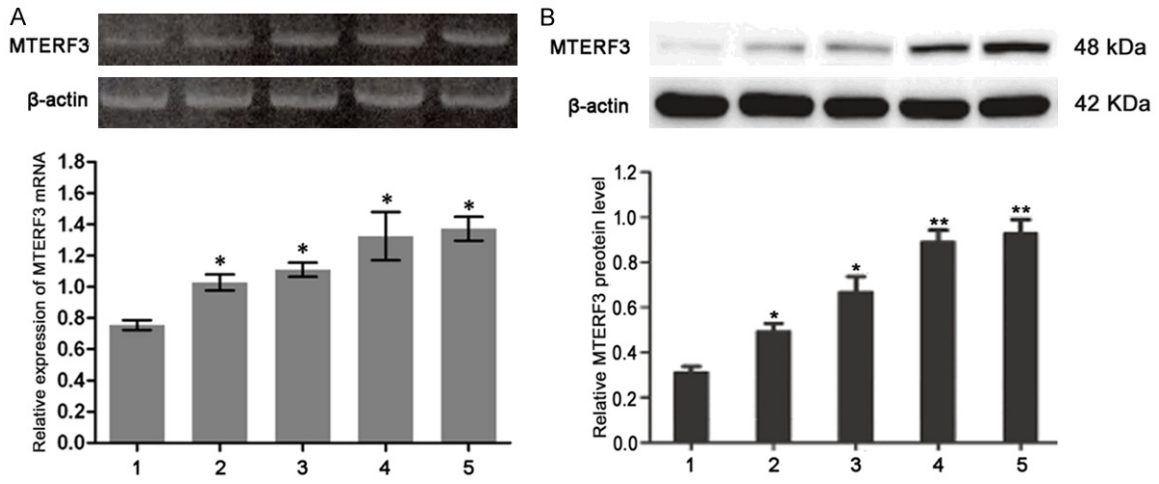

Figure 2. The expressions of the MTERF3 mRNA and protein in human noncancerous brain tissues, and high-grade and low-grade glioma tissues as detected by RT-PCR and Western blot (*P<0.05 *vs.* noncancerous brain tissues). A. The expression of MTERF3 mRNA in human noncancerous brain tissues, and high-grade and low-grade glioma tissues detected by semi-quantitative RT-PCR. 1: noncancerous brain tissues; 2: grade I glioma tissues; 3: grade II glioma tissues; 4: grade III glioma tissues; 5: grade IV glioma tissues. B. The expression of the MTERF3 protein in human noncancerous brain tissues, high-grade and low-grade glioma tissues detected by Western blot. 1: noncancerous brain tissues; 2: grade I glioma tissues; 3: grade II glioma tissues; 4: grade III glioma tissues; 5: grade IV glioma tissues.

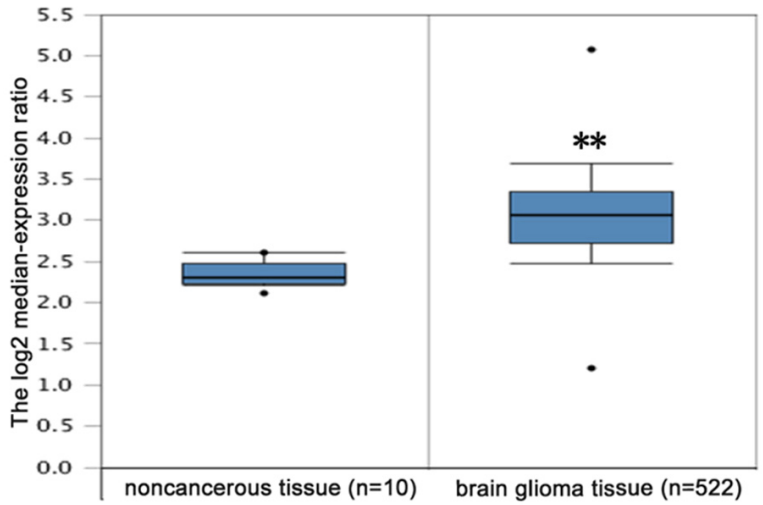

Figure 3. The expression of MTERF3 mRNA in human noncancerous brain tissues and glioma tissues in the TCGA dataset (*P<0.01 *vs.* noncancerous brain tissues).

The expression of MTERF3 mRNA in noncancerous brain tissue and human glioma tissue

A semi-quantitative RT-PCR showed that the expression levels of MTERF3 mRNA in low-grade and high-grade glioma tissues were significantly higher than they were in the noncancerous brain tissues (P<0.05) (**Figure 2A**). The Western blot result also demonstrated that the expression levels of the MTERF3 protein in the low-grade and high-grade glioma tissues were significantly higher than in the noncancerous brain tissues (P<0.05) (**Figure 2B**).

The differences between the expression levels of MTERF3 mRNA in noncancerous brain tissue and in human glioma in the TCGA dataset

In the TCGA database, the MTERF3 mRNA expression levels in different types of gliomas and noncancerous brain tissues were obtained, and the relevant data was compared and analyzed using a box plot, as illustrated in (**Figure 3**). The results indicated that the expression of MTERF3 mRNA in human glioma tissue was significantly higher than it was in noncancerous brain tissue, with a highly significant statistical difference ($P = 1.12 \times 10^{-9}$).

The correlation between MTERF3 mRNA expression and the clinicopathological characteristics of the glioma patients

In the TCGA dataset of glioma, the expression of MTERF3 mRNA was low in 261 cases and high in 261 cases. The expression of MTERF3

Table 2. Correlation analysis of MTERF3 mRNA expression with the clinicopathological parameters of glioma patients n (%)

Clinical indicators	n	MTERF3 expression		*P* value	χ^2
		High expression (%)	Low expression (%)		
Age (year)					
≤50	364	167 (45.88)	197 (54.12)	0.004	8.169
>50	158	94 (59.49)	64 (40.51)		
Gender					
Male	275	129 (46.91)	146 (53.09)	0.136	2.221
Female	247	132 (53.44)	115 (46.56)		
Pathological type					
Anaplastic astrocytoma	129	93 (72.09)	36 (27.91)	<0.0001	37.93
Anaplastic oligoastrocytoma	78	32 (41.03)	46 (58.97)		
Astrocytoma	66	31 (46.97)	35 (53.03)		
Oligoastrocytoma	131	63 (48.09)	68 (51.91)		
Oligodendroglioma	118	42 (35.59)	76 (64.41)		
WHO classification					
I+II	254	101 (39.76)	153 (60.24)	<0.0001	20.74
III+IV	268	160 (59.70)	108 (40.30)		
History of headaches					
With	173	93 (53.76)	80 (46.24)	0.310	2.340
Without	299	147 (49.16)	152 (50.84)		
No record	50	21 (42.00)	29 (58.00)		
Tumor location					
Left	257	132 (51.36)	125 (48.64)	0.463	1.538
Right	258	127 (49.22)	131 (50.78)		
Middle	7	2 (28.57)	5 (71.43)		
Sample type					
Primary tumor	508	253 (49.80)	255 (50.20)	0.588	0.294
Recurrent tumor	14	8 (57.14)	6 (42.86)		

mRNA is significantly correlated with age (P = 0.004), pathological type (P<0.001) and WHO classification (P<0.001), but not with gender, headache history, tumor location or sample type (P>0.05) (**Table 2**).

The correlation between the MTERF3 mRNA level and the prognoses of glioma patients

The cumulative OS and DFS of patients with high and low expressions of MTERF3 mRNA were analyzed using the Kaplan-Meier method in combination with the follow-up data. The Kaplan-Meier analysis revealed that the cumulative OS of patients with a high expression of MTERF3 mRNA was significantly lower than the OS of patients with a low expression of MTERF3 mRNA [(28.79 ± 10.60) months *vs.* (35.98 ± 13.32) months, *Log-rank P* = 0.001, HR = 2.03)]. In addition, the DFS of patients with a high expression of MTERF3 mRNA was lower than the DFS of patients with a low expression of MTERF3 mRNA [(23.80 ± 14.72) months *vs.* (28.95 ± 16.57) months, *Log-rank P* = 0.004, HR = 1.54)], thereby highlighting a significant statistical difference between the groups (*Log-rank P*<0.01). These findings suggest that a high expression of MTERF3 mRNA could be a risk factor for the prognosis of patients with glioma, and a high expression of MTERF3 mRNA is significantly associated with the shortening of survival (**Figure 4**).

The prognostic value of the clinicopathological parameters in glioma patients

Univariate and multivariate analyses were performed to identify the factors influencing the survival of patients with glioma. In the univari-

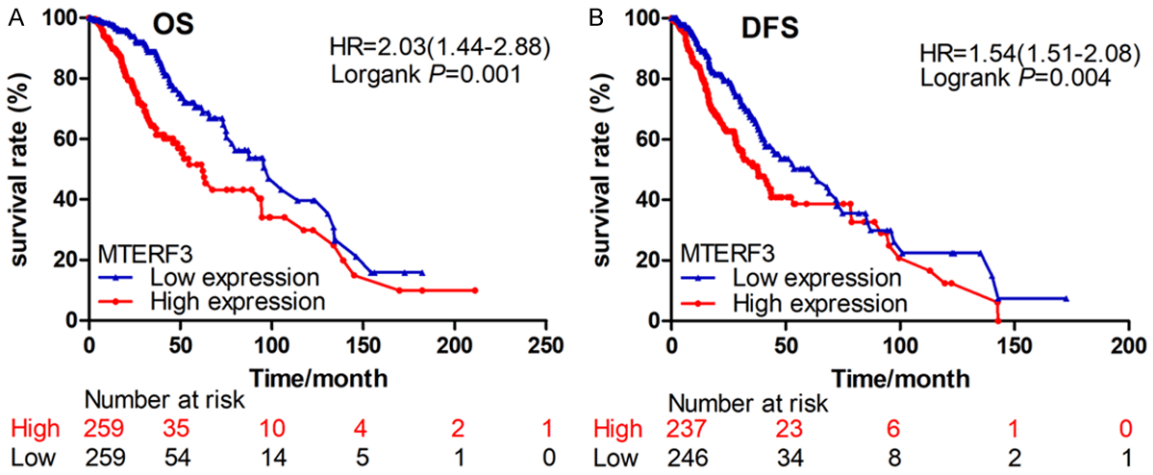

Figure 4. The relationship of the MTERF3 mRNA expression with the survival times of glioma patients. (A) Overall survival and (B) disease free survival were analyzed in 522 patients with brain glioma.

Table 3. Cox proportional hazards regression analysis of clinicopathological factors affecting the prognosis of patients with glioma

Cinicopathological characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (>50 *vs.* ≤50)	3.54	2.47~5.08	<0.001	3.79	2.54~5.66	<0.001
Gender (Male *vs.* Female)	1.11	0.79~1.57	0.539	1.04	0.72~1.48	0.865
Pathological type (*vs.* Anaplastic astrocytoma)						
Anaplastic oligoastrocytoma	0.62	0.38~1.01	0.056	0.41	0.25~0.72	0.001
Astrocytoma	0.23	0.11~0.48	<0.001	0.52	0.19~1.39	0.189
Oligoastrocytoma	0.41	0.25~0.65	<0.001	0.58	0.30~1.00	0.050
Oligodendroglioma	0.26	0.16~0.44	<0.001	0.39	0.17~0.91	0.025
WHO classification (III+IV *vs.* I+II)	3.28	2.25~4.79	<0.001	1.99	1.01~3.91	0.046
History of headaches (*vs.* No record)						
With	0.97	0.50~1.18	0.917	1.34	0.67~2.67	0.404
Without	1.18	0.62~2.21	0.616	1.32	0.68~2.58	0.408
Tumor location (*vs.* Left)						
Right	0.78	0.55~1.11	0.947	1.86	0.56~1.17	0.253
Middle	1.04	0.31~3.45	0.165	0.80	0.53~6.72	0.327
Tumor type (Recurrent tumor *vs.* Primary tumor)	1.12	0.54~2.30	0.762	2.13	0.88~4.92	0.095
MTERF3 expression (High *vs.* Low)	2.03	1.44~2.88	<0.001	1.28	0.89~1.89	0.179

ate analysis, factors such as age, pathological type, and the expression of MTERF3 mRNA influenced the patients' prognosis (P<0.05), but gender, history of headaches, tumor location, and sample type had no influence on the prognosis (P>0.05). Factors with P<0.1 in the above analysis were included in a Cox regression model for a multivariate analysis. The corresponding results suggest that age and patients' pathological type were the independent factors leading to a poor prognosis in patients with glioma (P<0.05) (**Table 3**).

The differences of the expression levels of the MTERF3 gene and the levels of other genes

The results of Pearson's correlation analysis showed that the expression levels of the MTERF3 gene in the glioma samples were positively correlated with the levels of the TFAM (P = 3.3e-06, R = 0.2), TFB1M (P = 0, R = 0.49), TFB2M (P = 0, R = 0.56), MTERF1 (P = 0, R = 0.58), MTERF2 (P = 6.2e-13, R = 0.31), TEFM (P = 0, R = 0.51) and the MFN1 (P = 0, R = 0.48) genes, but negatively correlated with the levels

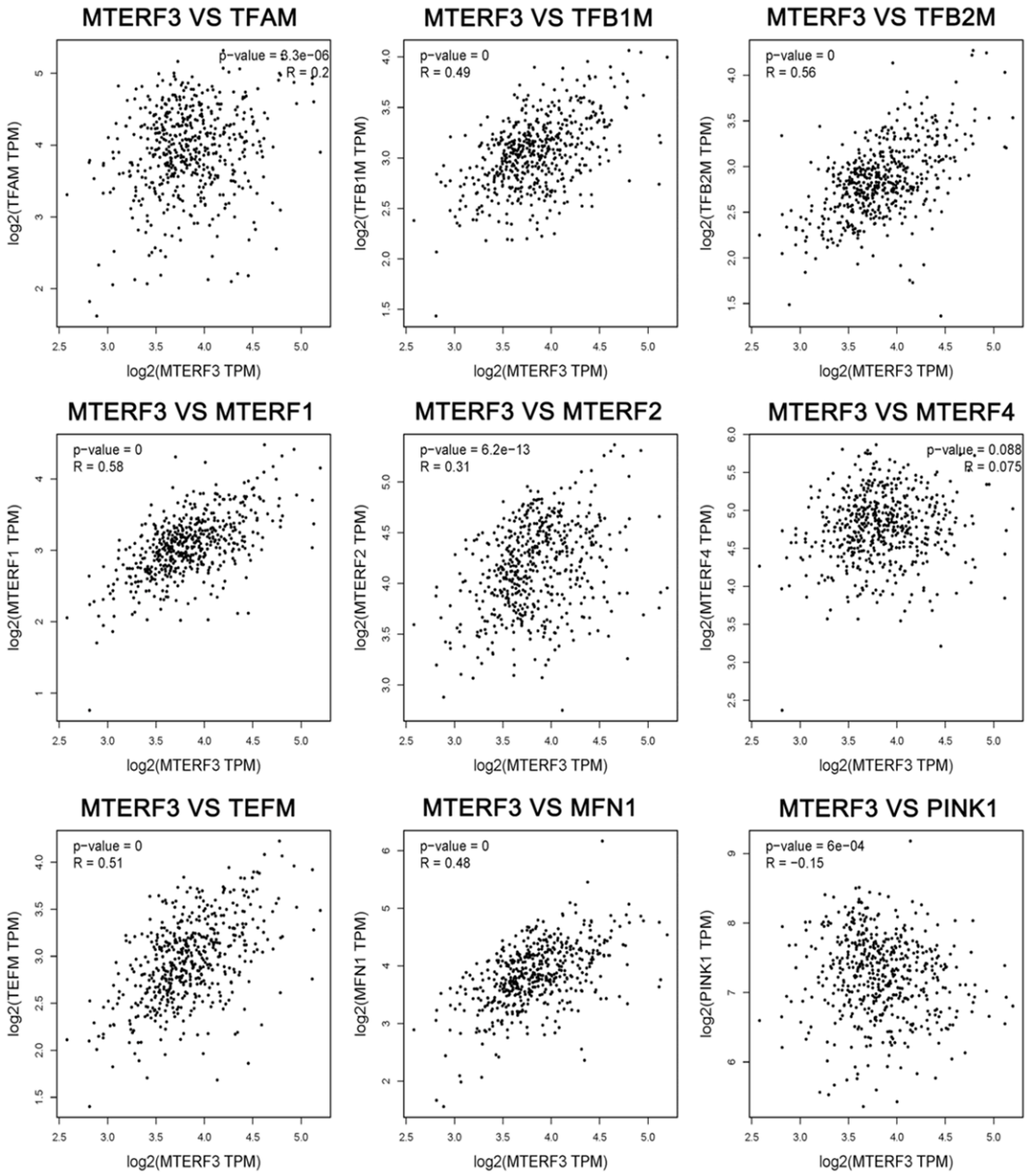

Figure 5. The correlations between the mRNA expression levels of the MTERF3 gene and the mRNA expression level of other mitochondrial regulatory genes in brain glioma.

of the PIKN1 (*P* = 6e-04, R = -0.15) gene. Meanwhile, there was no correlation between the MTERF3 and MTERF4 gene expression levels (*P*>0.05) (**Figure 5**).

Discussion

The MTERF family is a large protein family, which consists of four subfamilies (MTERF1-F4) found widely in metazoans and plants [16]. The MTERF3 protein is the most conserved member and is a relatively primitive MTERF protein in terms of evolution [17, 18]. MTERF3 contains five conserved mitochondrial transcription terminator motifs, including three leucine zippers [19]. It can inhibit transcription by combining with the promoter region of mtDNA, but MTERF3 knockout can increase the activity of the

mtDNA double-strand at the transcription start site in cells. MTERF3 can exert a major inhibitory role in mtDNA expression and can reduce the level of oxidative phosphorylation by decreasing the synthesis of respiratory enzymes, thereby reducing the synthesis of cell energy [20]. As reported in previous studies, MTERF3 not only negatively regulates mtDNA transcription, but it also modulates the assembly of large ribosome subunits, thus affecting ribosome biosynthesis and mitochondrial energy production [21]. With in-depth research, there is a growing realization that mitochondria play an important role in the occurrence and development of malignant tumors. Mitochondria are described as "cellular power plants". Normal cells produce ATP by oxidative phosphorylation, with sufficient oxygen to provide energy for cellular activity. However, a decreased level of oxidative phosphorylation in mitochondria may result in an imbalance between oxidative phosphorylation and glycolysis, which consequently induces the malignant transformation of cells [22, 23]. Mitochondria may also be associated intimately with abnormal energy metabolism, elevated reactive oxygen species (ROS), tissue infiltration and tumor metastasis simultaneously [24-26]. Compared to adjacent normal tissues, MTERF3 has been reported to be highly expressed in liver cancer, pancreatic cancer, lung cancer and breast cancer [14]. In addition, Zhang et al. showed that MTERF3 could promote tumor growth both *in vivo* and *in vitro*, similar to the role of cellular oncogenes [14]. Further, a high expression of MTERF3 is suggested to be positively correlated with tumor metastasis and invasion, estrogen or androgen independence and tumor immune resistance [14]. Previous studies have shown that mitochondrial gene mutations and changes in mitochondrial dynamics are closely associated with the occurrence and development of glioma [27-29]. Nevertheless, as an important factor regulating mitochondrial gene expression, MTERF3 has not been reported to be associated with the prognosis of patients with glioma.

In this study, the expressions of MTERF3 in 28 glioma specimens and 10 noncancerous brain tissues was detected by immunohistochemistry. The results showed that MTERF3 was primarily located in the cytoplasms. The positive rate of MTERF3 expression in gliomas was 64.29%, which was higher compared to noncancerous brain tissues. Meanwhile, the positive rate of MTERF3 expression in high-grade gliomas (81.25%) was significantly higher compared to low-grade gliomas (41.67%). The results of a semi-quantitative RT-PCR showed that the expression of MTERF3 mRNA in different grades of human gliomas was significantly higher compared to noncancerous brain tissues, and the difference was statistically significant ($P<0.05$). The above results indicate that MTERF3 may be involved in the occurrence, development and malignancy of glioma. Owing to the relatively smaller number of glioma samples collected in the study, the R 2.15.3 software was used to download the clinicopathological data of 522 glioma patients and their complete clinical data and the expression levels of MTERF3 mRNA from the TCGA database in order to confirm the credibility of the results. According to our analysis, a high expression of MTERF3 mRNA was correlated with the age, pathological type, and WHO classification of the glioma patients ($P<0.01$), but no obvious correlation was observed with gender, headache history, tumor location or sample type ($P>0.05$). Similarly, the results also suggest that increased an expression of MTERF3 mRNA is closely related to the progression of glioma. The Kaplan-Meier analysis and log-rank test results showed that the overexpression of MTERF3 mRNA significantly correlated with OS and DFS in glioma patients. In other words, the cumulative survival of patients with high expressions of MTERF3 was significantly shorter compared to patients with low expressions of MTERF3 [(28.79 ± 10.60) months vs. (35.98 ± 13.32) months, *Log-rank P* = 0.001, HR = 2.03)]. Thus, a high expression of MTERF3 could be used as a molecular marker for poor prognosis in glioma patients. Our gene correlation analysis showed that the TFAM (P = 3.3e-06, R = 0.2), TFB1M (P = 0, R = 0.49), TFB2M (P = 0, R = 0.56), MTERF1 (P = 0, R = 0.58), MTERF2 (P = 6.2e-13, R = 0.31), TEFM (P = 0, R = 0.51) and MFN1 (P = 0, R = 0.48) gene expression levels and the expression of MTERF3 were positively correlated ($P<0.05$, $R>0$), but were negatively correlated with the expression levels of the PINK1 genes ($P<0.05$, $R<0$). In the results, there was no correlation between the MTERF3 and MTERF4 gene expression levels ($P>0.05$).

Conclusion

In summary, our study revealed the differences between the expressions of MTERF3 in non-cancerous brain tissues and gliomas, suggesting that MTERF3 could be involved in the occurrence and development of human gliomas. Combined with the analysis of the relationship of MTERF3 expression with glioma patients' clinicopathological features and the prognosis of gliomas, MTERF3 could be considered a molecular marker for predicting the prognosis of gliomas and also as a potential target for gene therapy. As a newly discovered oncogene, the MTERF3 gene needs to be further studied to validate to its function and mechanism in human brain gliomas.

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Disclosure of conflict of interest

None.

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