

RESEARCH ARTICLE

Long noncoding RNA FTX is associated with prognosis of glioma patients

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Abstract

Background: Long noncoding RNAs play influential roles in the progression of many types of human malignancies. The present study aimed to explore the prognostic value of long noncoding RNA FTX (FTX) on patients with glioma.

Methods: FTX expression in glioma specimens and matched adjacent non-neoplasm specimens was examined by a quantitative real-time polymerase chain reaction assay. Furthermore, assays of the relationships between FTX expression and clinicopathologic characteristics of patients with glioma were also performed. Kaplan–Meier methods were applied for the assays of the overall survival (OS) and progression-free survival (PFS) of patients and Cox regression assays were used to analyze the clinical value of FTX used as a possible biomarker.

Results: FTX levels were significantly up-regulated in glioma specimens compared to the paired non-neoplasm specimens ($p < 0.01$). Furthermore, high FTX expression in neoplasm tissues was dramatically associated with World Health Organization grade ($p = 0.001$) and Karnofsky Performance Score ($p = 0.009$). Kaplan–Meier assays with 187 patients revealed that patients with high level of FTX expression displayed poorer OS ($p = 0.002$) and PFS ($p = 0.000$). Subsequently, multivariable Cox regression analysis identified FTX expression as an independent prognostic factor of unfavorable survivals in glioma (OS: $p = 0.001$; PFS: $p = 0.002$).

Conclusions: These findings indicated that FTX may be a novel predictor for prognostic assessment of glioma patients. However, studies conducted with larger numbers of patients are essential to confirm our findings.

KEYWORDS

glioma tumor, molecular genetics, neuroscience, oncology

1 | INTRODUCTION

Gliomas are the most common and deadly brain neoplasms of the central nervous system, accounting for 2.3% of all original tumor cases

and 2.8% of cancer-associated deaths worldwide.^{1,2} According to the World Health Organization (WHO) criteria, glioma can be classified into four grades: diffuse astrocytoma, pilocytic astrocytoma, anaplastic astrocytoma and glioblastoma multiform (GBM).³ Despite new improvement to the standard of care therapy for glioma, the 5-year survival rate of GBM patients is less than 3%.⁴ The

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invasiveness and the chemoresistance of GBM are the main barriers to an effect therapy. Therefore, it is imperative to exploit novel prognostic biomarkers, which can guide new treatment modalities.

Long non-coding RNAs (lncRNAs) are a large class of non-coding transcripts that are >200 bases in length.⁵ It has been confirmed that lncRNAs displayed critical roles in cellular processes, such as cellular growth, survival, migration, invasion and differentiation.^{6–8} Recently, more and more evidence has indicated that lncRNAs are capable of performing as oncogenes or tumor suppressors, or as a predictor of prognosis in various tumors, including glioma.^{9,10} For example, Liu *et al.*¹¹ reported that overexpression of LOC730101 promoted the proliferation of lung cancer cell by regulation of Wnt/ β -catenin signaling. Wang *et al.*¹² demonstrated that the deregulated expression of lncRNA HULC may influence the clinical outcome of cervical cancer. A recent study by Chao *et al.*¹³ reported that low lncRNA TUSC7 expression predicted a significantly shorter survival time in glioma patients, and its overexpression suppressed glioma migration and invasion by sponging miR-23b. However, to date, only a few lncRNAs have been functionally annotated in glioma.

lncRNA FTX (FTX), located within the X-inactivation center, is a newly identified lncRNA.¹⁴ Previous studies reported that FTX was evidently highly expressed in glioma¹⁵ and renal cell carcinoma,¹⁶ whereas down-regulation of FTX was observed in hepatocellular carcinoma.¹⁷ These results suggest FTX may exerted different function in different types of tumors. To the best of our knowledge, whether FTX had a clinical influence on the long-term survivals of glioma patients had not been reported. The present study aimed to explore the expression pattern and clinical involvement of FTX in glioma patients.

2 | MATERIALS AND METHODS

2.1 | Patients and specimens

In total, 187 paired primary glioma specimens and matched non-neoplasm tissues were acquired from patients with glioma undergoing surgery in the First Hospital of Shandong First Medical University. Informed consent was obtained from all patients. The diagnosis of all specimens was based on pathological evidence. All samples were immediately frozen and stored at -80°C for further experiments. Based on the standard of the WHO classification and grading system, staging and grading was pathologically determined. Before surgery, all of the patients had not received chemotherapy, radiotherapy or immunotherapy. The study was approved by the Research Ethics Committee of the first affiliated hospital of Shandong First Medical University.

2.2 | Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from glioma samples and matched adjacent normal samples using TRIzol1 reagent (Invitrogen, Jiangning, Nanjing,

TABLE 1 Primers used in the present study

Gene	Primer sequences
FTX	Forward primer: 5'-TATGCCACCTAGCCTTTCTACA-3'
	Reverse primer: 5'-ATCTCTTCA AAAGCGGCATAAT-3'
GAPDH	Forward primer: 5'-GCACCGTCAAGGCTGAGAAC-3'
	Reverse primer: 5'-TGGTGAAGACGCCAGTG GA-3'

China). Total RNA was quantified using a Nanodrop ND-1110 spectrophotometer (NanoDrop, Hangzhou, Zhejiang, China). We purchased a Reverse Transcription Kit (Takara, Dalian, Nialing, China), which was used to reverse transcribe the isolated RNA into cDNA. A qRT-PCR was conducted with SYBR Premix Ex[®] Taq (TaKaRa, Otsu, Shiga, Japan) on a CFX96 Real-Time PCR Examination System. GAPDH was used as an endogenous control. Table 1 shows the primers designed by Gema Technology (Pudong, Shanghai, China). The relative expression of FTX was calculated by the use of the $2^{-\Delta\Delta\text{Ct}}$ methods. The assays were performed in triplicate.

2.3 | Statistical analysis

All data quantification and statistical analysis were performed using SPSS, version 19.0 software (IBM Corp., Armonk, NY, USA). The differences between groups were analyzed using Student's *t*-test. The correlations of FTX with the clinical factors of patients was determined by a chi-squared test. Overall survival (OS) and progression-free survival (PFS) were evaluated by Kaplan–Meier analyses with a log-rank test. The prognostic value of FTX was examined by a Cox regression assay. $p < 0.05$ was considered a statistically significant.

3 | RESULTS

3.1 | FTX up-regulation in glioma specimens

To explore the possible role of FTX in glioma development, the expression of FTX was determined in 187 pairs of glioma tissues and

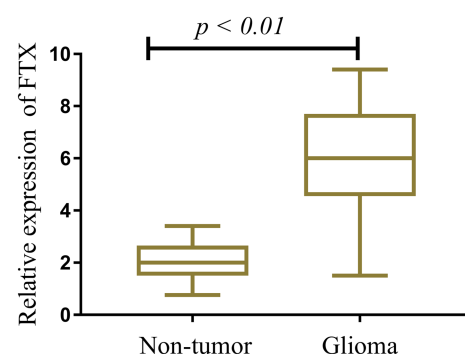


FIGURE 1 FTX expression in 187 glioma tissues samples and corresponding normal tissues

adjacent non-neoplasm tissues. This revealed that FTX expression was increased in specimen tissues compared to matched adjacent non-neoplasm tissues ($p < 0.01$) (Figure 1). Our results indicated that FTX up-regulation may contribute to glioma development.

3.2 | Relationship between FTX and clinicopathologic features of glioma patients

Given the distinct overexpression of FTX in glioma, we further considered whether FTX had possible clinical relevance in glioma patients. We categorized the patients into low and high expression groups according to the mean value of FTX expression. Subsequently, data assays with a chi-squared test revealed that the expression of FTX was inversely correlated with progressive clinicopathologic features, including WHO grade ($p = 0.001$) and Karnofsky Performance Score (KPS) ($p = 0.009$) (Table 2). However, correlations of FTX expression with other parameters, including age, gender and extent of resection, were not observed (Table 2).

3.3 | FTX overexpression was a clinical regulatory factor in glioma patients

The prognostic value of FTX expression with respect to glioma patient survival was evaluated via Kaplan–Meier survival assays. Our results suggested that patients with high FTX expression showed significantly reduced 5-year OS and PFS ($p = 0.002$ and $p = 0.000$, respectively) (Figures 2 and 3). Then, Cox regression assays were applied to evaluate the prognostic value of FTX

TABLE 2 Correlations between FTX expression and clinicopathologic features in glioma patients

Variable	n	FTX expression		p value
		Low	High	
Age (years)				0.533
≥ 45	116	55	61	
< 45	71	37	34	
Gender				0.127
Male	89	49	40	
Female	98	43	55	
Extent of resection				0.534
Partial	57	30	27	
Total	130	62	68	
WHO grade				0.001
I/II	111	66	45	
III/IV	76	26	50	
KPS score				0.009
≥ 80	123	69	54	
< 80	64	23	41	

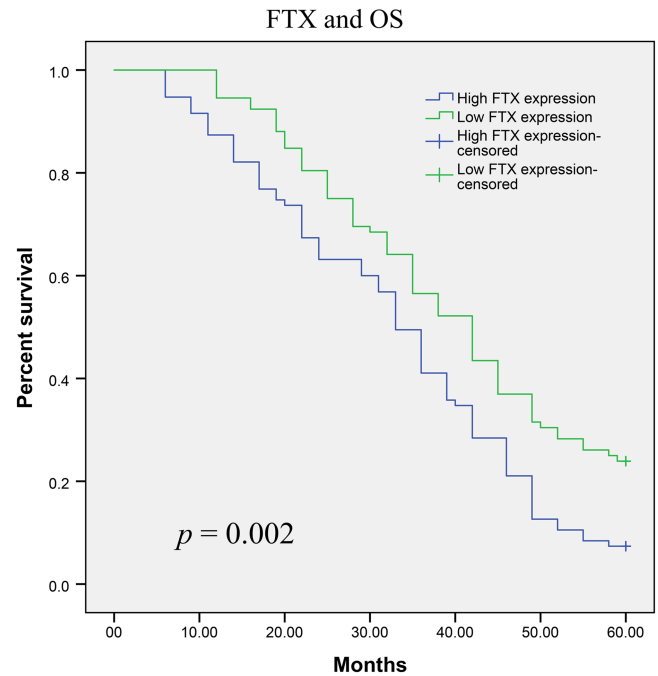


FIGURE 2 Glioma patients with high FTX expression had a significantly shorter OS than those with low FTX expression ($p = 0.002$, log-rank test)

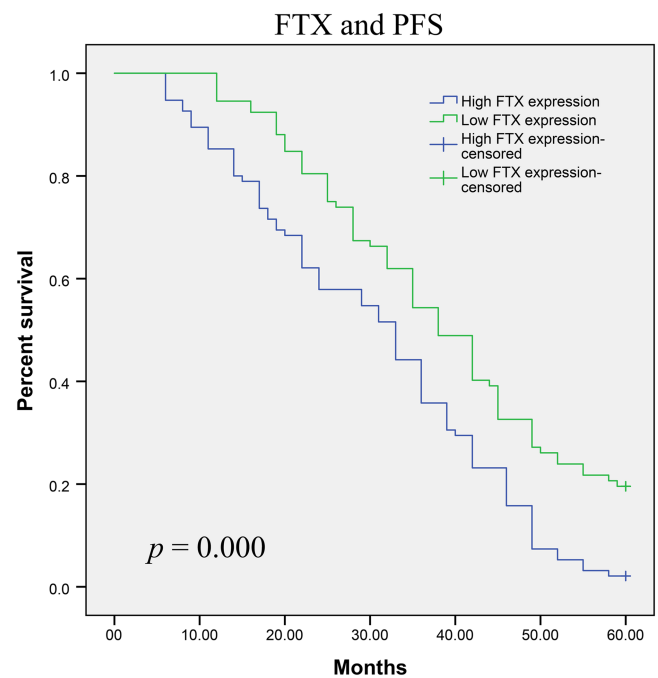


FIGURE 3 Glioma patients with high FTX expression had a significantly shorter PFS than those with low FTX expression ($p = 0.000$, log-rank test)

expression in glioma. As shown in Table 3, FTX was identified as an independent prognostic factors for both OS ($p = 0.001$) and PFS ($p = 0.001$).

TABLE 3 Cox regression model for multivariate analysis of glioma prognostic factors

Variable	Overall survival			Progression-free survival		
	HR	95% CI	p	HR	95% CI	p
Age	1.423	0.832–1.849	0.317	1.631	0.944–2.213	0.186
Gender	1.679	0.927–2.432	0.144	1.832	0.774–2.632	0.084
Extent of resection	1.321	0.755–1.934	0.138	1.583	0.893–1.737	0.155
WHO grade	3.674	1.663–6.343	0.001	4.362	1.993–7.822	0.001
KPS score	3.228	1.342–5.489	0.004	3.893	1.542–6.138	0.007
FTX expression	5.358	1.775–8.569	0.001	6.323	2.213–9.934	0.001

CI, confidence interval; HR, hazard ratio.

4 | DISCUSSION

Considering that the survival rate of glioma is still low, novel and more effective prognostic biomarkers are immediately essential for guiding the individual treatment of glioma patients.¹⁸ To date, traditional staging systems may not be effective for predicting outcome in individual patients.^{19,20} Recently, because of advances in the investigation of glioma carcinogenesis, more and more key molecular events are being identified.²¹ Many scientists try to find effective biomarkers such as tumor-related genes, microRNAs and long noncoding RNAs.^{22–24} Compared with other biomarkers,²⁵ lncRNAs are considered to be ideal biomarkers because of their important regulation effect in glioma progression.

Metastasis and recurrence are the main cause of mortality in tumor patients. Because tumor progression is very complex, the underlying mechanism for tumor metastasis and recurrence is also very unclear. Previous studies have reported that FTX was involved in the regulation of metastasis and clinical prognosis in various types of human malignancies. For example, Liu *et al.*¹⁷ found that FTX was decreased in liver cancer and indicated a poor prognosis. Furthermore, forced FTX expression inhibited the proliferation and metastasis of tumor cells by modulating miR-374a/MCM2. He *et al.*¹⁶ reported that FTX may function as a neoplasm promoter in renal cell carcinoma and its knockdown could suppress proliferation, migration and invasion. More importantly, recent findings by Zhang *et al.*¹⁵ highlighted the promoting role of FTX in glioma through negatively regulating miR-342-3p. Although the tumor-promotive role of FTX in glioma was confirmed by an *in vitro* assay, its prognostic significance in glioma remains unknown.

In the present study, to explore the biological implication of FTX in glioma patients, our group examined the levels of FTX in glioma tissues and matched normal tissues. Unsurprisingly, in accordance with previous reports, the results of the present study showed that significant up-regulation of FTX was observed in glioma tissues. In addition, we found a similar phenomenon in which high expression of FTX in glioma was significantly associated with various clinicopathologic characteristics, such as WHO grade and KPS score. These results indicated that FTX is a biomarker for a poor prognosis in glioma patients. The subsequent survival assay

confirmed our hypothesis. Finally, multivariate assays confirmed that FTX expression was an independent prognostic marker for PFS and OS in glioma patients.

5 | CONCLUSIONS

In conclusion, for the first time, the current data offer convincing evidence for FTX being frequently up-regulated in glioma and it comprise a useful poor prognostic biomarker for glioma patients. In the future, the molecular mechanisms of FTX that are involved in glioma must be investigated further.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

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