European Review for Medical and Pharmacological Sciences 2017; 21: 3605-3610

Long non-coding RNA TUSC7 expression is independently predictive of outcome in glioma

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Abstract. – OBJECTIVE: Down-regulation of long non-coding RNA tumor suppressor candidate 7(TUSC7) contributes to tumorigenesis in several human cancers including glioma. However, the prognostic value of TUSC7 in glioma remains unclear. The present study aimed to investigate the clinicopathological and prognostic value of TUSC7.

PATIENTS AND METHODS: The expression level of TUSC7 in glioma tissues and matched normal tissues were detected by qRT-PCR. Then, the association of serum TUSC7 expression level with various important clinicopathological parameters and survival rates was evaluated. The Cox regression analysis was used to evaluate the effect of independent prognostic factors on survival outcome.

RESULTS: The relative level of TUSC7 was significantly lower in glioma tissues compared to the adjacent normal brain tissues ($p < 0.01$). In addition, a lower expression of TUSC7 was observed in high-grade glioma tissues than in low-grade glioma tissues ($p < 0.01$). Furthermore, the low expression of TUSC7 was associated with poor clinicopathological characteristics of glioma, including WHO grade ($p = 0.002$) and KPS ($p = 0.026$). Then, the low TUSC7 level was correlated with shorter disease free survival (DFS) and overall survival (OS) than low level (both $p = 0.05$). Finally, univariate and multivariate Cox analysis showed that TUSC7 was an independent prognostic indicator for OS and DFS.

CONCLUSIONS: These results provided evidence that TUSC7 may be a potential biomarker in the prognosis of glioma.

Key Words:
Long non-coding RNA, TUSC7, Glioma, Prognosis.

Introduction

Gliomas is known as the most common and lethal type of intracranial tumor in adult with a histological grade that ranges from low (WHO I-II) to high grade (WHO III-IV)[1-3]. Based on the histological appearance, gliomas can be subdivided into astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas[4]. Although recent advances in diagnosis and treatment have improved patient prognosis, the outcome of glioma remains poor[5]. One important reason behind this, it is that early detection and effective treatment strategies for glioma patients were limited[6]. Biomarkers to predict the prognosis of glioma patients could be extremely useful to develop effective treatment strategies. Thus, identification of molecular biomarkers with clinicopathologic and prognostic significance are very important.

It is clear that up to 70% of our genome is transcribed into RNA that does not serve as protein coding genes[7]. Long non-coding RNAs (lncRNAs) is one of those RNAs. As a newly discovered class of non-coding genes, growing studies suggest that lncRNAs may function as master gene regulators capable of controlling protein-coding and noncoding genes[8,9]. It was reported that lncRNAs participated in different biological processes, including modulation of proliferation, migration, invasion and apoptosis[10]. In addition, various lncRNAs play a crucial role in carcinogenesis. For instance, lncRNA ANRIL expression up-regulated in ovarian cancer and its over-expression promoted EOC cell proliferation both *in vitro* and *in vivo*[11]. LncRNA H19 was observed to promote colorectal tumor growth by recruiting and binding to eIF4A3[12]. However, the effect of most lncRNAs in progression of tumor remains largely unknown.

TUSC7 is a lncRNA consisting of four exons, which are more than 2 kb in length, and it is located at 3q13.31[13]. Recently, Shang et al[14] reported that TUSC7 functioned as a tumor suppressor in glioma. Also, they found that low TUSC7 expression was associated to poor prognosis in glioma

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patients. However, the sample size was very small, which may lead to the accuracy was low. In the present study, we collected 206 patients to further identify the prognostic value of TUSC7 in glioma patients.

Patients and Methods

Patients and Clinical Specimens

Paired glioma tissues and adjacent nontumor tissues were obtained from 206 pathological diagnosed glioma patients at the Beijing Tongzhou District Chinese Medicine Hospital between October 2010 and October 2012. All patients recruited in this study were not subjected to preoperative radiotherapy and/or chemotherapy. All human glioma tissues were categorized according to the WHO classification. The tissue specimens included 133 grade I-II tumors and 73 grade III-IV tumors. Specimens were immediately snap-frozen in liquid nitrogen and stored at -80°C until processing. This study was approved by the Hospital Ethics Committee, and all patients signed an informed consent form before surgery.

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from the tumor tissues and matched normal tissues of 206 glioma patients using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instruction. For qPCR, RNA reverse transcribed to cDNA from 1 μg of total RNA was reverse transcribed in a final volume of 2 μl using random primers. qPCR was performed to detect the TUSC7 expression level using a SYBR Premix Ex Taq™ kit (TaKaRa Bio, Dalian, China) according to the manufacturer's protocol. The TUSC7 primers were: 5'-TTTATGCTTGAGCCTTGA-3' (sense) and 5'-CTTGCCTGAAATACTTGC-3' (antisense). GAPDH was used as an internal control for mRNA. GAPDH primers were: 5'-AGAGGCAGGGATGATGTTCTG-3' (sense) and 5'-GACTCATGACCACAGTCCATGC-3'. All results were expressed as the means ± SD of at least three independent experiments. The relative expression of TUSC7 was calculated and normalized using the $2^{-\Delta\Delta Ct}$ method relative to GAPDH.

Statistical Analysis

Analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). The X^2 test was used to show differences in categorical variables. Kaplan-Meier survival curves were constructed to estimate the association between TUSC7 expression and the patients' OS and DFS. Survival times of patients who died from other causes were not taken into consideration. A prognostic analysis was carried out using univariate and multivariate Cox regressions models. p-values < 0.05 were considered to be significant.

Results

TUSC7 is Down-Regulate in Glioma

To validate the expression pattern of TUSC7 in human glioma, TUSC7 expression levels in 206 pairs of human glioma tissues and adjacent normal tissues were quantified by RT-PCR. As shown in Figure 1A, we found that the relative level of TUSC7 was significantly lower in glioma tissues compared to the adjacent normal brain tissues ($p < 0.01$). Next, we further compared the expression levels of TUSC7 in different grade glioma tissues. As shown in Figure 1B, it was observed that the expression of TUSC7 was lower in high-grade glioma tissues than in low-grade glioma ($p < 0.001$). These results suggested that TUSC7 may be involved in the development of glioma.

Downregulation of TUSC7 Associates with Advanced Clinicopathological Features of Glioma

TUSC7 expression is reduced in glioma, which implies that it functions as a tumor suppressor in glioma. About the relationship between TUSC7 expression levels and clinicopathological characteristics, we divided glioma patients into two groups. The median expression level of serum TUSC7 (2.65-fold) was used as the cut-off points to define the high expression level or low expression level. The results were shown in Table I, and we found that low TUSC7 expression levels were positively correlated with high WHO grade ($p = 0.002$) and KPS ($p = 0.0026$). However, there were no changes between TUSC7 expression levels and other factors including age, sex, tumor size, tumor location and tumor recurrence (all $p > 0.05$).

Correlation Between TUSC7 Expression and Prognosis in Glioma Patients

Then, we explore the prognostic value of TUSC7 in glioma patients. Overall survival curves in high PVT1 group and low PVT1 group were shown in

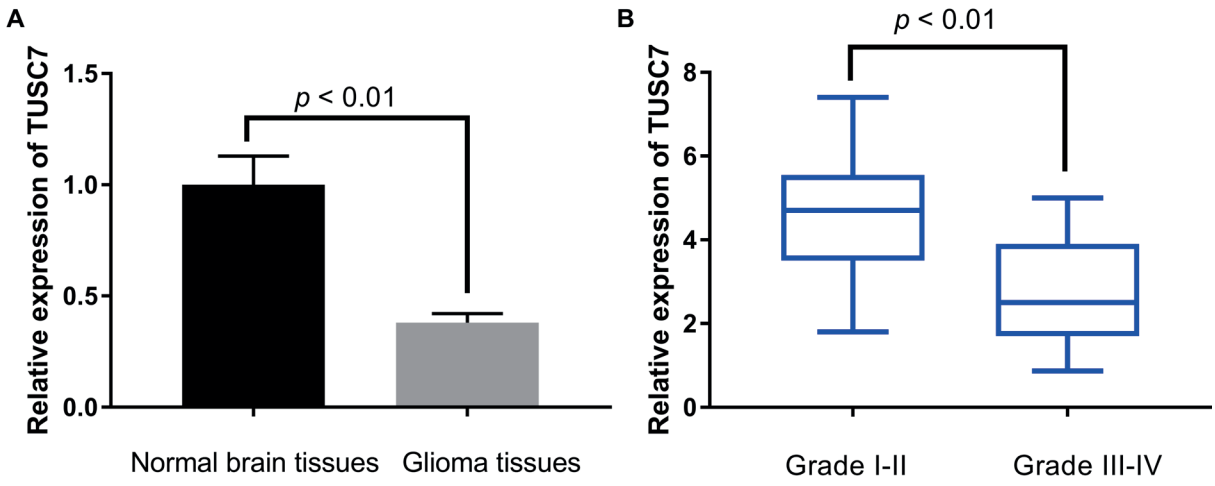

Figure 1. The expression of TUSC7 is downregulated in human glioma tissues. ***A,*** The TUSC7 relative expression levels were determined by qRT-PCR in glioma tissues and the adjacent non-neoplastic tissues. ***B,*** Expression of TUSC7 in high-grade glioma tissues and low-grade glioma.

Figure 2A. As was expected, OS of glioma patients with low TUSC7 expression were significantly poorer than glioma patients with high TUSC7 expression ($p = 0.008$). Furthermore, we used similar methods to compare the DFS in glioma patients. As shown in Figure 2B, we found that patients with a higher expression of TUSC7 had a longer survival time. To determine the prognostic value of the TUSC7 expression level in glioma, univariate and multivariate analyses were performed. The results of univariate and multivariate analysis for DFS and OS are summarized in Table II. We found that TUSC7 expression was associated with both OS ($p = 0.005$) and DFS ($p = 0.007$) in univariate Cox proportional hazards regression analysis. At last, a further multivariate Cox analysis confirmed

Table I. Association between TUSC7 expression and different clinicopathological features of human glioma.

Characteristics	Total, n (%)	TUSC7 expression, n (%)		p
		Low	High	
Age (y)				NS
< 50	91 (44.2)	41 (45.1)	50 (54.9)	
≥ 50	115 (55.8)	63 (54)	52 (56)	
Sex				NS
Male	144 (70)	70 (48.6)	74 (51.4)	
Female	62 (30)	34 (55)	28 (45)	
Tumor size (cm)				
< 5	138 (67)	67 (48.6)	71 (51.4)	
≥ 5	68 (33)	37 (54.4)	31 (45.6)	
Tumor location				NS
Supratentorial	162 (78.6)	82 (50.6)	80 (49.4)	
Subtentorial	44 (21.4)	22 (50)	22(50)	
WHO grade				0.002*
I-II	133 (65)	78 (58.6)	55 (41.4)	
III-IV	73 (35)	26 (35.6)	47 (64.4)	
KPS				0.026*
< 80	115 (56)	66 (57.4)	49 (42.6)	
≥ 80	91 (44)	38 (41.8)	53 (58.2)	
Tumor recurrence				NS
Absent	152 (74)	79 (52)	73 (48)	
Present	54 (26)	25 (46.3)	29 (53.7)	

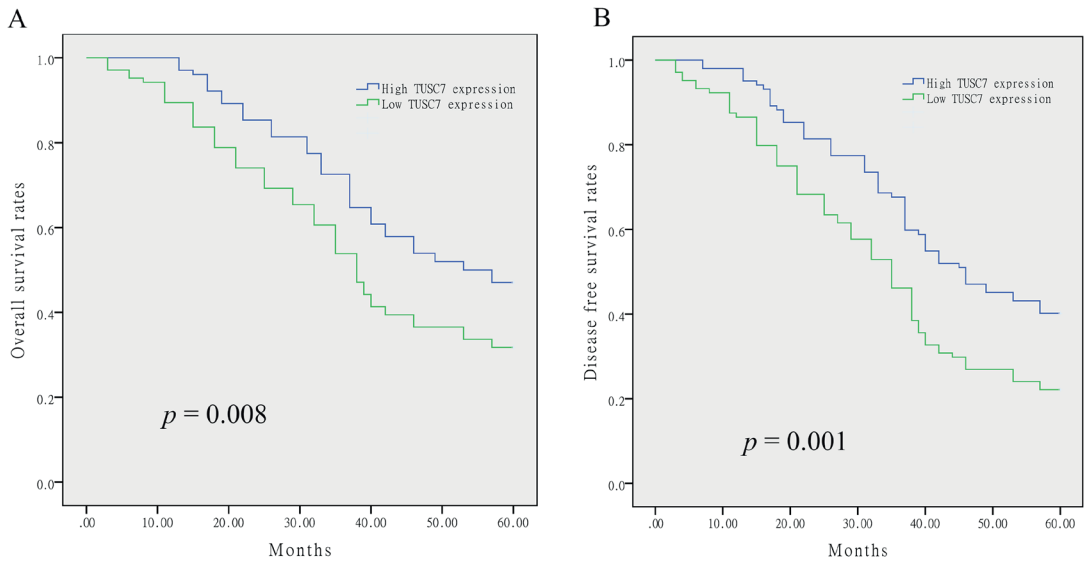

Figure 2. Kaplan-Meier survival curves according to TUSC7 level. ***A,*** Overall survival ($p = 0.008$, log-rank). ***B,*** Disease-free survival ($p = 0.001$, log-rank).

that TUSC7 expression was the independent risk factor for both OS ($p = 0.012$) and DFS ($p = 0.009$) in glioma.

Discussion

Glioma accounts for a majority of malignances of the central nervous system. Up to date, the treatment of glioma remains challengeable[15,16]. Developing new diagnosis and prognosis strategies were useful for the treatment of this malignancy. In the past decades, several clinical predictors of survival have been identified, such as age, preoperative functional status, and tumor extent[17]. However, these factors have a relatively low specificity. Therefore, the exploration of promising novel predictive factors is urgently needed to improve the prognosis of glioma.

Recently, some evidence indicated that TUSC7 played a tumor-suppressive role in several tumors. For example, Wang et al[18] found that forced expression of TUSC7 inhibited hepatocellular

Table II. Univariate and multivariate analyses of prognostic variables of DFS and OS in glioma patients.

Parameters	Univariate analysis		Multivariate analysis	
Disease free survival			–	–
Age (y)	1.251 (0.784-1.893)	NS	–	–
Sex	0.913 (0.652-1.773)	NS		
Tumor size (cm)	1.317 (0.568-1.734)	NS	–	–
Tumor location	1.688 (0.893-2.123)	NS	–	–
Tumor recurrence	1.452 (0.811-1.933)	NS	–	–
KPS	2.897 (1.588-4.332)	**0.001***	2.342 (1.421-3.892)	**0.004***
WHO grade	3.231 (1.778-5.672)	**0.001***	2.993 (1.622-5.213)	**0.001***
TUSC7 expression	1.773 (1.321-3.993)	**0.005***	1.529 (1.231-3.562)	**0.009***
Overall survival				
Age (y)	1.232 (0.823-1.773)	NS	-	–
Sex	0.833 (0.532-1.536)	NS	–	–
Tumor size (cm)	1.299 (0.632-1.462)	NS	–	–
Tumor location	1.472 (0.723-1.938)	NS	–	–
Tumor recurrence	1.323 (0.872-1.932)	NS	–	–
KPS	2.562 (1.329-3.982)	**0.003***	2.213 (1.139-3.263)	**0.009***
WHO grade	2.879 (1.673-4.672)	**0.001***	2.652 (1.337-4.213)	**0.002***
TUSC7 expression	1.572 (1.133-3.472)	**0.007***	1.321 (1.023-2.983)	**0.012***

*$p < 0.05$

carcinoma cell metastasis, invasion, and epithelial-to-mesenchymal transformation (EMT) through competitively binding miR-10a. Also, they use strong evidence to show that TUSC7 may be potential biomarker for hepatocellular carcinoma prognosis. Cong et al[19] found that TUSC7 functions as a tumor suppressor in osteosarcoma by both *in vitro* and *in vivo* experiments. A recent study by Wang et al[20] indicated that upregulation of TUSC7 expression could inhibit proliferation of lung cancer cell *in vitro* and patients with lower TUSC7 expression had worse overall survival compared with the high expression cases. These findings revealed the significant tumor-suppressive role in these tumors. More importantly, Shang et al[14] found that TUSC7 was poorly expressed in tissues and cell lines of glioma. Furthermore, by various cell experiments, they found that up-regulation of TUSC7 inhibited the proliferation, migration and invasion of glioma cells and promoted cellular apoptosis largely bypassing miR-23b. Their findings highlighted the vital role of TUSC7 in the progression of glioma. However, in their work, they performed the survival analysis with a very small sample size. Obviously, the reliability of the results was weak. Our present report aimed to solve this problem.

In the present, we identified TUSC7 as a promising biomarker for predicting prognosis of glioma patients. Firstly, we found that TUSC7 expression was significantly down-regulate in glioma tissues and lower TUSC7 expression was associated with advanced tumor-grade. Secondly, the low expression of TUSC7 was associated with poor clinicopathological characteristics of glioma, including WHO grade and KPS. Thirdly, Kaplan-Meier survival curves demonstrated that OS and DFS were significantly lower in patients with low TUSC7 level than in those with high levels. Furthermore, univariate and multivariate Cox analysis confirmed that TUSC7 was an independent prognostic indicator for OS and DFS. Our results were constant with previous study[14]. Our present paper provided strong evidence that TUSC7 could be used for a novel prognostic marker for glioma.

Conclusions

We indicated that reduced TUSC7 is associated with poor survival in glioma patients, suggesting TUSC7 protein is a potential prognostic biomarker for glioma.

Funding Supports

The study was supported by the Fund for the Science and Technology Activities of Excellent Overseas Students in 2015 (Ministry of Human Resources and Social Security; grant no.008-0064) and The Basic and Clinical Research Project Foundation of Capital Medical University (grant No. 303-01-007-0132).

Conflict of Interest

The Authors declare that they have no conflict of interests.

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