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Original article

LncRNATCF7 promotes the growth and self-renewal of glioma cells via suppressing the miR-200c-EpCAM axis

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ABSTRACT

Accumulating evidence has demonstrated that long non-coding RNAs (lncRNAs) are involved in tumor initiation and development. Recent studies illustrated that lncRNATCF7 was highly expressed in lung cancer and liver cancer, however, the expression pattern and function of lncRNATCF7 in glioma remains to be elucidated. Here, we found that lncTCF7 was highly expressed in glioma tissues and cell lines. Overexpression of lncTCF7 promoted the proliferation and migration of glioma cells. Down-regulation of lncTCF7 significantly suppressed the tumorigenesis of glioma. Mechanistically, lncTCF7 enhanced the self-renewal of glioma cells via up-regulating the expression of epithelial cell adhesion molecule (EpCAM). The detailed molecular mechanism uncovered that lncTCF7 bound to miR-200c and decreased the abundance of miR-200c, which consequently attenuated the negative regulation of miR-200c on EpCAM. Collectively, these data provide evidence to demonstrate the critical role of lncTCF7 in the tumorigeneis of glioma, which suggested that lncTCF7 might be a promising target in the treatment of glioma.

1. Introduction

Glioma, the predominant type of brain malignancy, is among the most common and deadliest human cancers [1,2]. Although tumor resection and the combination of radio-and chemotherapies have improved the outcome of glioma patients, the five-year survival rate of patients with high invasive glioma still remains very poor [2–5]. The unknown physiological processes that contribute to the initiation and development of glioma have been a big challenge to explore efficient treatment strategies targeting glioma. Therefore, understanding the molecular mechanism and identifying novel biomarkers for the diagnosis and treatment of glioma is quite necessary.

Long non-coding RNAs are characterized as transcripts longer than 200 nucleotides without protein-coding capacity [6–10]. Increasing evidence has demonstrated that lncRNAs function as gene regulatory elements that involved in the occurrence and development of human cancers [11–20]. LncRNAs regulate cell growth and differentiation via a variety of molecular mechanisms including chromatin remodeling, lncRNA-protein as well as lncRNA-miRNA interaction [21]. Aberrant expression of multiple lncRNAs has been illustrated to suggest the tumor suppressive or oncogenic potential of lncRNAs in human cancers. Recent studies demonstrated that lncRNATCF7 was highly expressed in liver and lung cancers and promoted the self-renewal of cancer stem cells [22,23]. During the tumorigenesis, the epithelial-to-mesenchymal transition (EMT) has been considered as the critical step in cancer invasion, metastasis and therapeutic resistance [24,25]. The oncogenic function of lncTCF7 has been found in lung cancer and liver cancer [23], however, the function of lncTCF7 in glioma still remains unknown.

In this study, we investigated the expression and function of lncTCF7 in glioma. Our results provide evidence to verify that lncTCF7 was significantly upregulated in glioma tissues and cell lines. Overexpression of lncTCF7 promoted the growth of glioma cells. Mechanistically, lncTCF7 enhanced the expression of EpCAM by down-regulating miR-200c, the negative regulator of EpCAM. These results suggested that lncTCF7 is a novel target in the therapeutic of glioma.

2. Materials and methods

2.1. Tissue collection and cell lines

Glioma tissues and paired para-carcinoma tissues were collected from the glioma patients who were diagnosed and underwent surgical surgery at the Cangzhou people's hospital. The clinical information of these patients was available and the informed consents have been obtained prior to the investigation. The tissue samples were snap-frozen by liquid nitrogen and stored at −80 °C.

Glioma cell lines U251 and A172 were obtained from the Cell Bank

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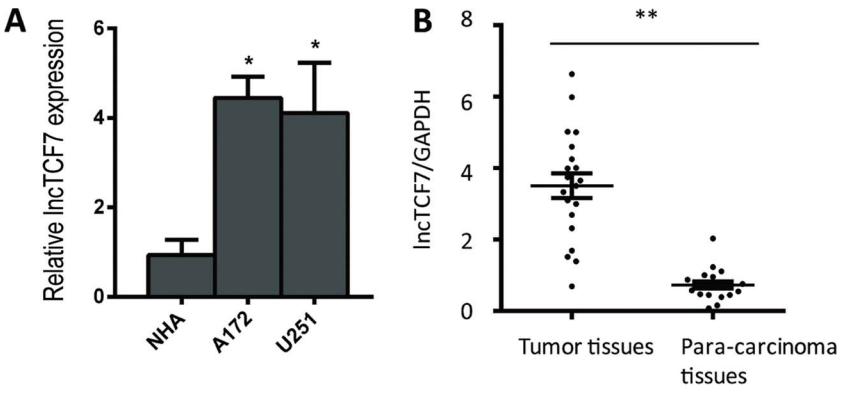

Fig. 1. LncTCF7 was highly expressed in glioma tissues and cell lines. (A) The expression abundance of lncTCF7 in normal NHA cells and glioma cell lines A172 and U251 was examined. HOX6as was used as the control. ***$P < 0.001$. (B) The expression level of lncTCF7 in both glioma tissues and para-carcinoma tissues was detected by qRT-PCR analysis. HOX6as was used as the control. ***$P < 0.001$.

of the Chinese Academy of Sciences (Shanghai, China). The cells were culture in DMEM culture supplemented with 10% FBS (Invitrogen) and maintained in the atmosphere at 37 °C with 5% CO_2.

2.2. qRT-PCR

The total RNA was extracted with the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer' protocol. The first strand cDNA was synthesized with the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, Rockford, IL, USA). The relative expression of lncTCF7 was normalized to the abundance of lncRNA HOX6as, which has been identified as a suitable normalizer in glioma and normal brain [26]. qRT-PCR was performed using the TaqMan Multiplex Master Mix (Invitrogen, Grand Island, NY, USA) with the Roche LightCycler® 96 (LC96) real-time PCR platform.

2.3. Western blot

Glioma cells transfected with overexpressed or depleted lncTCF7 were harvested and lysed with the lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China). The protein concentration was determined using the BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA). Equal amount of protein was loaded and separated by the SDS-PAGE and then transferred onto the polyvinylidene fluoride (PVDF) membrane. The following antibodies were used in the immunoblot analysis: anti-EpCAM (4A7, ab180624), anti-Nanog (ab109250), anti-SOX2 (ab137385), and anti-GAPDH (ab9484) were purchased from Abcam. The membrane was incubated with the primary antibodies for 2 h at room temperature and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz). The protein signal was detected with the enhanced chemifluorescence system (GE Healthcare, Piscataway, NJ, USA).

2.4. Cell transfection

To generate lncTCF7 stably expressed cells, glioma cell was seeded into the 6-well plate overnight. The expressing vector pcDNA-3.1-lncTCF7 and empty vector was transfected into the cells using the Lipofectamine 3000 Transfection Reagent (Life Technology, New York, NY, USA) respectively. To obtain the stable cell clones, cells transfected with the indicated plasmids were incubated with the G418 (Sigma Aldrich) for 7 days. To down-regulate the expression of lncTCF7, glioma cells were transfected with shRNA1-lncTCF7 or shRNA2-lncTCF7. The knockdown efficiency of lncTCF7 was determined by qRT-PCR analysis. The interfering sequences corresponding to the distinct regions of lncTCF7 were listed: shRNA1-lncTCF7: CACCTAGGTGCTCACTGAA, shRNA2-lncTCF7: AGCCAACATTGTTGGTTAT.

2.5. Cell viability assay

Glioma cells transfected with the indicated expression vector were seeded at the 96-well plate with a density of 2×10^3 cells per well. The cell viability was determined with the Cell Counting Kit-8 (Sigma) according to the manufacturer's instructions. The absorbance of each well was detected at 450 nm at the indicated time.

2.6. Wound healing assay

Glioma cells were cultured in the 6-well plate and a vertical wound was generated with a 200 μl Tip. Cells were washed twice with phosphate-buffered saline (PBS) and the cultured for the indicated time. The wound region was detected by the microscopy.

2.7. Statistical analysis

The results were presented as mean ± s.d. from three independent experiments. Statistical analysis was performed with the SPSS statistics V22.0 software. The significance was determined by the Student's *t*-test or one-way analysis of variance (ANOVA). $P < 0.05$ was considered as statistically significant.

3. Results

3.1. LncTCF7 was highly expressed in glioma tissues and cell lines

To detect the specific expression trend of lncTCF7 in glioma, the relative expression level of lncTCF7 in glioma tissues and cell lines was analyzed with HOXA6as as the internal control, which has been identified as a suitable normalizer in glioma and normal brain by Kraus et al. [26]. The data showed that using HOX6as as the control, the expression of lncTCF7 was significantly increased in glioma cell lines U251 and A172 (Fig. 1A). To confirm this data, the relative expression abundance of lncTCF7 was detected with 20 paired glioma tissues and para-carcinoma tissues with HOX6as as the control. The data of qRT-PCR analysis showed that lncTCF7 was significantly overexpressed in glioma tissues (Fig. 1B). These results suggested that lncTCF7 was highly expressed in glioma tissues and cell lines.

3.2. LncTCF7 regulated the growth of glioma cells

To detect whether the aberrant expression of lncTCF7 was involved in the tumorigenesis of glioma, lncTCF7 overexpressed A172 and U251 cells were generated by transfecting with pcDNA-3.1-lncTCF7 (Fig. 2A). The cell viability of A172 cells harboring overexpressed lncTCF7 was determined by the CCK-8 assay. The data showed that highly expressed lncTCF7 significantly promoted the cell viability of A172 cells compared with that of cells expressing control vector (Fig. 2B). Consistent with this result, increased cell viability was also observed in U251 cells with overexpressed lncTCF7 (Fig. 2C). The influence of lncTCF7 on the tumorigenesis of glioma cells was also evaluated by the cell migration assay. As shown in Fig. 2D, overexpression of lncTCF7 significantly accelerated the motility of both