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Transcription factor ZNF703 activates linc-UBC1 to stimulate the progression of glioma

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Abstract. – OBJECTIVE: To investigate the role of the transcription factor Zinc finger 703 (ZNF703) in influencing the progression of glioma by regulating linc-UBC1 level.

MATERIALS AND METHODS: Linc-UBC1 level in glioma with different staging and tumor sizes was determined. The potential influences of linc-UBC1 on viability, cell cycle progression, and invasiveness of glioma cells were evaluated. Through RNA binding protein immunoprecipitation (RIP) assay and Dual-Luciferase reporter gene assay, the interaction between ZNF703 and linc-UBC1 was assessed. The rescue experiments were conducted to identify the role of ZNF703 in regulating cellular performances of glioma by interacting with linc-UBC1.

RESULTS: Linc-UBC1 was highly expressed in glioma. Its level was higher in glioma with larger tumor size or advanced staging. The knockdown of linc-UBC1 reduced viability, arrested cell cycle in the G0/G1 phase, and attenuated invasiveness of U87 and LN229 cells. The presence of the binding sites was observed in the promoter regions of ZNF703 and linc-UBC1. The overexpression of ZNF703 could alleviate the inhibited proliferative and invasive potentials in U87 and LN229 cells with the linc-UBC1 knockdown.

CONCLUSIONS: The transcription factor ZNF703 promotes the proliferative and invasive potentials in glioma cells by regulating the transcriptional activity of linc-UBC1.

Key Words:
Glioma, Linc-UBC1, ZNF703.

Introduction

Glioma is a type of tumor which originates in the central nervous system, accounting for 40-65% of primary malignant tumors in the brain[1]. In recent years, the incidence of glioma in Chinese adolescents has increased each year[2]. The pathological type of glioma can be divided into 4 grades according to the WHO grading[3]. Higher grade of glioma indicates worse malignancy. Malignant growth of glioma depends on tumor location, structure, and size. In addition, the clinical symptoms of glioma are diverse, including increased intracranial pressure (headache, vomiting, papilledema, and disturbance of consciousness), focal symptoms, and signs (dysmotility, sensory disturbance, and epilepsy)[4]. Currently, the combination treatment of surgery and chemotherapy regimens indeed prolongs the survival of glioma. However, life qualities and clinical outcomes of glioma patients is still unsatisfactory[5]. High rate of recurrence is the major reason for glioma-induced death[6]. Invasive growth of tumors is the underlying cause of poor prognosis[7]. Cellular and molecular biological characteristics suggest that abnormally expressed genes responsible for regulating tumor cell biology are important factors for controlling the progression of glioma.

In 1992, Brockdorff et al[8] discovered non-coding RNAs in 200 nucleotides long, which are distributed in nucleus or cytoplasm, that is, lncRNAs. Many studies have identified the crucial functions of lncRNAs in the biological processes involving almost all processes of gene regulation, including genomic imprinting, chromatin modification, and post-transcriptional activation. LncRNAs are also essential for diverse cellular behaviors[9-12]. Multiple differentially expressed lncRNAs have been identified in tumors, such as HOTAIR, Dreh, lncRNA-ATB, LINC00473, and etc. Abnormal expressions of these lncRNAs may influence the prognosis

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of tumor patients[13-17]. Hence, tumor-related lncRNAs have been well-concerned throughout the world.

Zinc finger (ZNF) proteins are widely found in eukaryotes and participate in many life processes. In recent years, the role of ZNF in tumors has attracted widespread attention. It is reported that ZNF could mediate tumorigenesis by regulating the transcription and expressions of oncogenes[18]. ZNF703 is a newly discovered member of the ZNF family and is an oncogene for breast cancer. It is a positive driver of estrogen receptors[19]. ZNF703 is upregulated in breast cancer and endometrial cancer, which is closely related to tumor invasion and metastasis[20]. The expression change of ZNF703 may affect the occurrence and progression of tumors.

In this paper, we determined the expression pattern of linc-UBC1 in glioma patients with different staging and tumor sizes. Its regulatory effects on viability, cell cycle progression, and invasiveness of glioma cells were evaluated. The interaction between ZNF703 and linc-UBC1 in regulating the progression of glioma was investigated, and it provides novel ideas for clinical treatment of glioma.

Materials and Methods

Cell Culture and Transfection

Normal glial cell line HEB, and glioma cell lines Hs683, U251, U87, and LN229 were provided by Cell Bank (Shanghai, China). They were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640; HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS) (HyClone, South Logan, UT, USA) in an incubator with 5% CO_2 at 37°C. Cell transfection was conducted at 60-70% confluence using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

RNA Extraction and quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The cells or tissues were lysed for extracting the total RNAs using TRIzol method (Invitrogen, Carlsbad, CA, USA) for reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Kumatsu, Japan). RNA concentration was detected using a spectrometer. QRT-PCR was then performed based on the instructions of SYBR Premix Ex Taq™ (TaKaRa, Kumatsu, Japan) for 5 min at 95°C, and 36 cycles for 15 s at 95°C, 30 s at 60°C, and 30 s at 72°C. The relative level was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences were as follows: Linc-UBC1, F: 5'-ATGAAGCCTGACTATGACGT-3', R: 5'-CTAAGCCGAAGGTGACAGCT-3'; ZNF703, F: 5'-ATGCTGACAAGACGGCGGTT-3', R: 5'-CTACTTGCGGCCCTGCAGGA-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), F: 5'-TGCCAGCGGACCGAGTCCGG-3', R: 5'-CTACTTGCGGCCCTGCAGGA-3'.

Cell Counting Kit-8 (CCK-8) Assay

The cells were seeded into 96-well plates with 2×10^3 cells per well. At the appointed time points, 10 μL of CCK-8 solution (Dojindo, Kumamoto, Japan) was added in each well. After 2 h incubation, the absorbance at 450 nm of each sample was measured by a microplate reader (Bio-Rad, Hercules, CA, USA).

Transwell

Cell density was adjusted to 5×10^4 cells/mL. 100 μL of suspension was applied in the upper side, while 500 μL of medium containing 10% FBS and chemokines was applied in the bottom of a transwell chamber (Corning, Corning, NY, USA). After 24 h of incubation, the cells penetrated to the bottom side were fixed in methanol for 15 min, stained with crystal violet for 20 min, and counted using a microscope. The number of invasive cells was counted in 5 randomly selected fields per sample (magnification 200×).

Cell Cycle Determination

The cells were washed with pre-cold phosphate-buffered saline (PBS) twice, digested in 0.25% trypsin for 1 min, and terminated by adding medium. The mixture was centrifuged at 1000 r/min for 5 min. The precipitant was suspended in 500 μL of PBS and incubated in 500 μL of Propidium Iodide (PI) in the dark. At last, cell cycle distribution was analyzed using BD FACSCalibur flow cytometry (Franklin Lakes, NJ, USA).

Chromatin Immunoprecipitation (ChIP)

The cells were subjected to 10-min cross-link with 1% formaldehyde at room temperature. Subsequently, the cells were lysed and sonicated for 30 min. Finally, the sonicated lysate was immuno-precipitated with corresponding antibodies or anti-IgG.

Dual-Luciferase Reporter Gene Assay

Wild-type and mutant-type Luciferase plasmids of linc-UBC1 were constructed. The cells seeded in the 24-well plate with 2×10^5 cells per well were co-transfected with UBC1-WT/UBC1-MUT and pcDNA-ZNF703/NC. 36 hours later, the cells were lysed for determining the relative Luciferase activity (Promega, Madison, WI, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 statistical software (IBM Corp., Armonk, NY, USA) was used for data analysis. Data were expressed as mean ± standard deviation ($\bar{x} \pm s$). The differences between the two groups were analyzed using the Student's *t*-test. The comparison between multiple groups was done using One-way ANOVA test followed by the post-hoc test (Least Significant Difference). $p<0.05$ considered the difference as statistically significant.

Results

Linc-UBC1 Was Upregulated in Glioma

QRT-PCR results showed a higher abundance of linc-UBC1 in glioma tissues relative to normal ones (Figure 1A). Particularly, linc-UBC1 level was higher in glioma tissues larger than 3 cm in tumor size, or those in advanced stage (III+IV) relative to those smaller than 3 cm or in stage I+II (Figures 1B, 1C). Similarly, linc-UBC1 was upregulated in glioma cells compared with that of normal glial cells (Figure 1D). U87 and LN229 cells expressed high abundance of linc-UBC1, which were chosen for the following experiments. The above data suggested that linc-UBC1 was involved in the progression of glioma.

Linc-UBC1 Accelerated Viability, Cell Cycle, and Invasiveness of Glioma

The transfection of si-UBC1 markedly downregulated linc-UBC1 level in U87 and LN229 cells, confirming the pronounced transfection efficacy of si-UBC1 (Figure 2A). Viability in U87 and LN229 cells transfected with si-UBC1 was remarkably reduced at day 1, 2, and 3 (Figure 2B). Besides, the knockdown of linc-UBC1 elevated cell ratio in G0/G1 phase, suggesting the arrested cell cycle (Figure 2C). The transwell assay showed attenuated invasiveness in glioma cells with linc-UBC1 knockdown (Figure 2D). Collectively, linc-UBC1 could promote viability, cell cycle progression, and invasiveness of glioma cells.

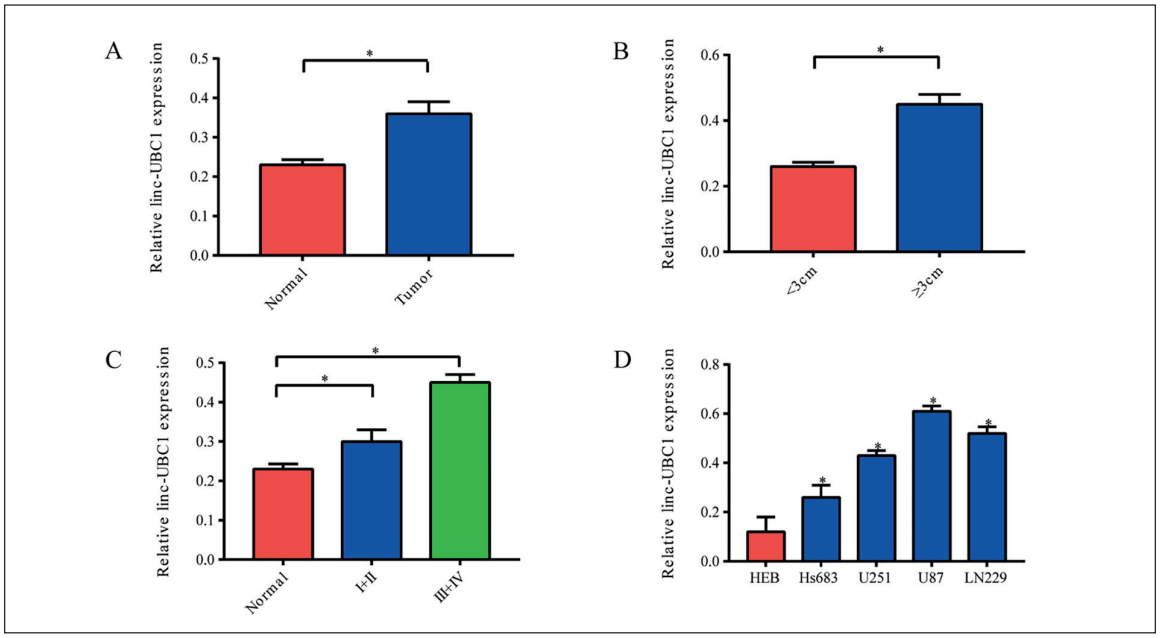

Figure 1. Linc-UBC1 was upregulated in glioma. **A,** Linc-UBC1 level in glioma tissues and normal tissues (n=15). **B,** Linc-UBC1 level in glioma tissues larger or smaller than 3 cm in tumor size. **C,** Linc-UBC1 level in normal tissues, glioma tissues in stage I+II and stage III+IV. **D,** Linc-UBC1 level in normal glial cell line HEB, and glioma cell lines Hs683, U251, U87, and LN229.

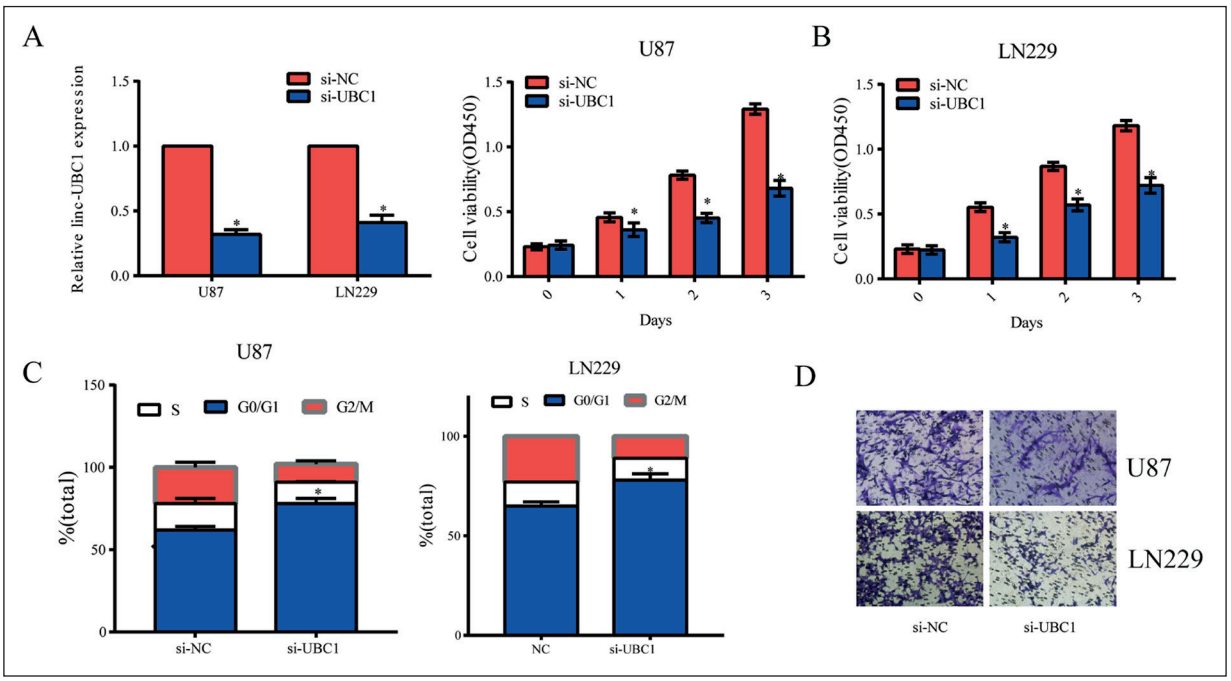

Figure 2. Linc-UBC1 accelerated viability, cell cycle, and invasiveness of glioma. **A,** Transfection efficacy of si-UBC1 in U87 and LN229 cells. **B,** Viability in U87 and LN229 cells transfected with si-NC or si-UBC1 at day 0, 1, 2, and 3. **C,** Cell ratio in S, G0/G1, and G2/M phase in U87 and LN229 cells transfected with si-NC or si-UBC1. **D,** Invasion in U87 and LN229 cells transfected with si-NC or si-UBC1 (magnification: 40×).

ZNF703 Stimulated the Transcription of Linc-UBC1

To further uncover the oncogenic role of linc-UBC1 in glioma, the binding sites in promoter regions of ZNF703 and linc-UBC1 were predicted on Jaspar website. ZNF703 was found to be upregulated in glioma tissues compared to those of the controls (Figure 3A). Next, we constructed pcDNA-ZNF703 and tested its transfection efficacy in U87 and LN229 cells (Figure 3B). In glioma cells transfected with pcDNA-ZNF703, the relative level of linc-UBC1 was remarkably upregulated (Figure 3C). Furthermore, ChIP assay illustrated higher enrichment of linc-UBC1 in anti-ZNF703 relative to anti-IgG, suggesting the interaction between linc-UBC1 and ZNF703 (Figure 3D). Luciferase activity increased in glioma cells co-transfected with pcDNA-ZNF703 and UBC1-MUT (Figure 3E). As a result, ZNF703 positively regulated the expression of linc-UBC1 by activating its transcription.

ZNF703 Regulated Linc-UBC1 to Influence Glioma Cell Behaviors

To further confirm the regulation of ZNF703 on linc-UBC1, the glioma cells were co-transfected with si-UBC1 and pcDNA-ZNF703 to determine the changes in viability and invasiveness. The decreased viability in U87 and LN229 cells with linc-UBC1 knockdown was partially reversed by the co-transfection of si-UBC1 and pcDNA-ZNF703 (Figures 4A, 4B). Besides, the overexpression of ZNF703 reversed the inhibited invasiveness in glioma cells with linc-UBC1 knockdown (Figure 4C).

Discussion

Glioma is the most common primary tumor in the human brain, manifesting as diffuse infiltration and poor prognosis. The recurrent rate of glioma is extremely high even undergoing comprehensive treatment, thus leading to poor clinical outcomes[21]. The pathogenesis of glioma involves multiple genes and pathways. Effective treatment and recurrence prevention of glioma are of significance.

The specific role of linc-UBC1 in the pathogenesis of glioma is unclear. This experiment determined the expression level of linc-UBC1 in glioma tissues and cells. Moreover, the regulatory effects of linc-UBC1 on viability, cell cycle, and invasiveness of U87 and LN229 cells were investigated.

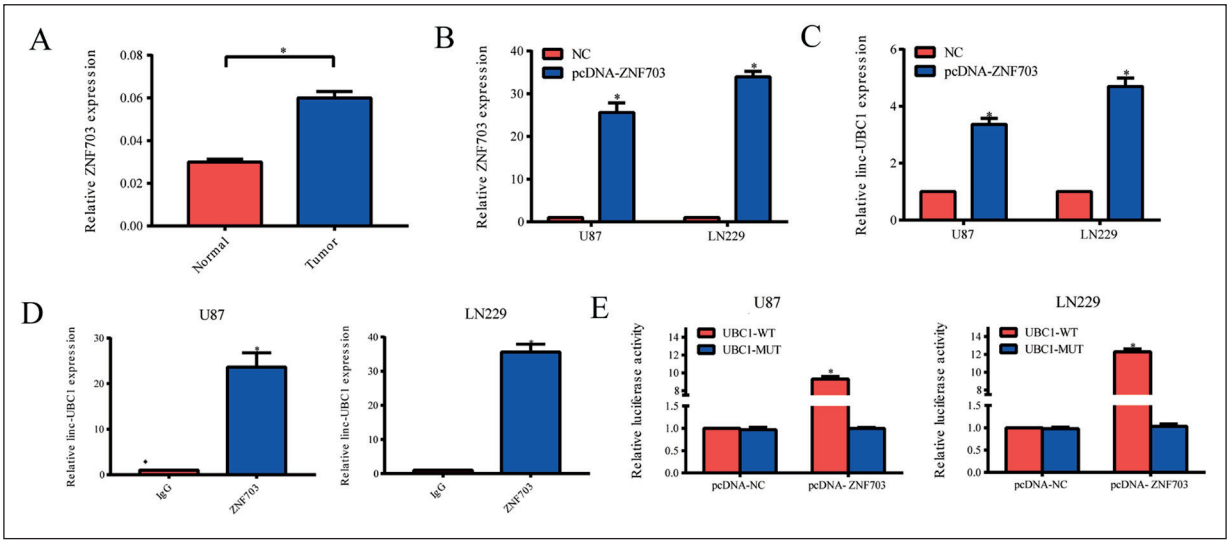

Figure 3. ZNF703 stimulated the transcription of linc-UBC1. **A,** ZNF703 level glioma tissues and normal tissues (n=15). **B,** Transfection efficacy of pcDNA-ZNF703 in U87 and LN229 cells. **C,** Linc-UBC1 level in U87 and LN229 cells transfected with NC or pcDNA-ZNF703. **D,** Immunoprecipitant of linc-UBC1 in anti-IgG and anti-ZNF-703 in U87 and LN229 cells. **E,** Luciferase activity in U87 and LN229 cells co-transfected with UBC1-WT/UBC1-MUT and pcDNA-NC/pcDNA-ZNF703.

ZNF proteins were first discovered by Miller et al[22] in the transcription factor IIIA of Xenopus oocytes. ZNF has a relatively independent finger-like tetrahedral structure formed by the binding of several conserved amino acid residues and zinc ions. ZNF703 regulates the embryonic development by recruiting histone deacetylation complex (HDAC) to regulate gene expressions[23]. Sircoulomb et al[20] analyzed the relationship between ZNF703 level and pathological characteristics in more than 500 breast cancer patients. They demonstrated an increased genetic instabil-

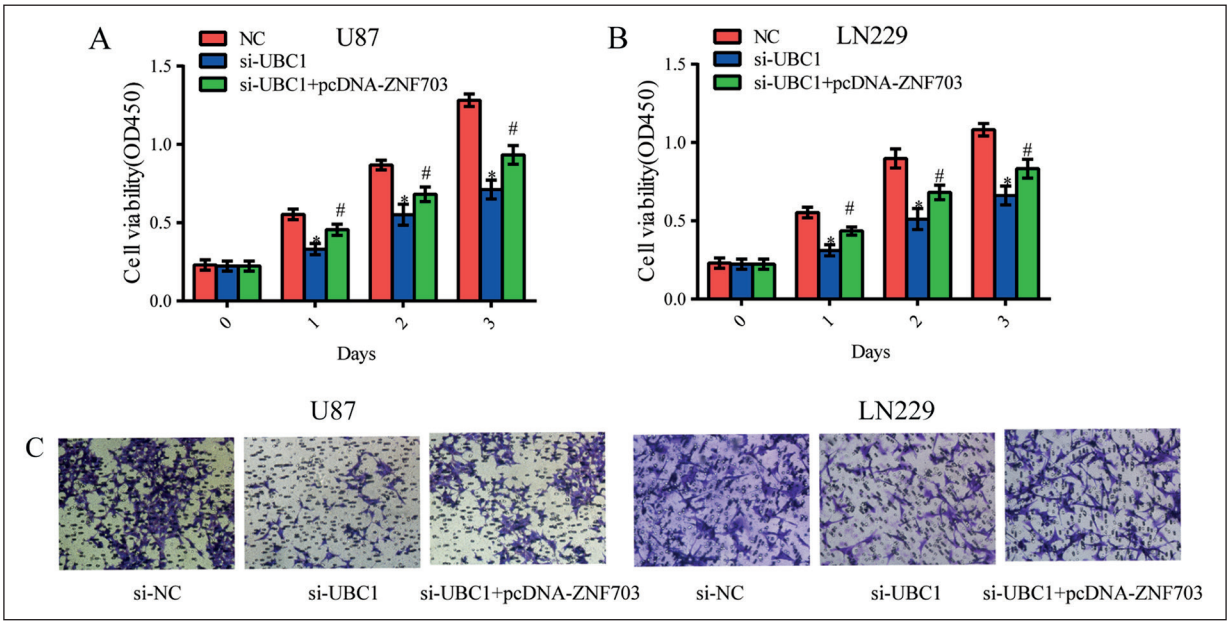

Figure 4. ZNF703 regulated linc-UBC1 to influence glioma cell behaviors. **A,** Viability in U87 cells transfected with si-NC, si-UBC1 or si-UBC1 + pcDNA-ZNF703 at day 0, 1, 2, and 3. **B,** Viability in LN229 cells transfected with si-NC, si-UBC1 or si-UBC1 + pcDNA-ZNF703 at day 0, 1, 2, and 3. **C,** Invasion in U87 and LN229 cells transfected with si-NC, si-UBC1 or si-UBC1 + pcDNA-ZNF703 (magnification: 40×).

ity in breast cancer cells overexpressing ZNF703. High level of ZNF703 predicts worse prognosis in patients with luminal A and luminal B breast cancer. Yang et al[24] pointed out that upregulated ZNF703 in gastric cancer is closely related to infiltration and lymphatic metastasis. It is generally believed that ZNF703 behaves as a negative transcription factor to promote tumor progression.

In this paper, we identified the interaction between ZNF703 and linc-UBC1. Interestingly, the overexpression of ZNF703 could alleviate the inhibited proliferative and invasive potentials in U87 and LN229 cells with the linc-UBC1 knockdown. As a result, ZNF703 stimulated the transcription of linc-UBC1, thus aggravating the progression of glioma.

Conclusions

Linc-UBC1 is upregulated in glioma, which promotes the viability, cell cycle progression, and invasiveness of glioma cells. The transcription factor ZNF703 in glioma cells promotes their proliferative and invasive potentials by regulating the transcriptional activity of linc-UBC1. ZNF703/linc-UBC1 could be utilized as therapeutic targets for glioma.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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