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Long non-coding RNA LINC01503 predicts worse prognosis in glioma and promotes tumorigenesis and progression through activation of Wnt/β-catenin signaling

H. WANG[1], Z.-G. SHENG[2], L.-Z. DAI[1]

[1]Department of Neurosurgery, Shenzhen People's Hospital, The Second Clinical Medical College of Jinan University, Shenzhen, China
[2]Department of Neurosurgery, Tianjin Medical University First Center Clinical College, The First Center Hospital of Tianjin, Tianjin, China

Hao Wang and Zhi-Guo Sheng equally contributed

Abstract. – OBJECTIVE: Long-noncoding RNAs (lncRNAs) have been recently shown to be involved in the regulation of numerous biological processes including tumor progression. In this study, we aimed to explore the role of lncRNA LINC01503 (LINC01503) in the development and progression of glioma.

PATIENTS AND METHODS: Relative levels of LINC01503 were evaluated in tumor tissues from 133 patients with glioma as well as from cultured glioma cell lines. The correlation among LINC01503 levels, pathological types, and survivals of glioma patients were also determined using the Kaplan-Meier method and multivariate analysis. Next, we investigated the effect of LINC01503 on the proliferation, colony formation, apoptosis, migration and invasion in the U251 and LN299 cells. Relative protein expression was analyzed by Western blot assays.

RESULTS: We found that LINC01503 expression level was significantly up-regulated in glioma tissue and cells, and that its overexpression was significantly correlated with KPS, tumor size and WHO grade in glioma patients. Kaplan-Meier analysis showed that patients with higher levels of LINC01503 had significantly poorer overall survival and disease-free survival than those with lower expression of this lncRNA in glioma patients. Multivariate analysis further confirmed that LINC01503 is an independent prognostic factor in patients with glioma. Functional assays with *in vitro* showed that knockdown of LINC01503 in the U251 and LN299 cell lines suppressed cells growth, colony formation, invasion and migration, and promoted apoptosis. Mechanistic investigation showed that LINC01503 can modulate Wnt/β-catenin signaling, as determined by that knockdown of LINC01503 decreased the TOP-FLASH activity and β-catenin, cyclin D1 and c-myc.

CONCLUSIONS: Our findings suggested that LINC01503 conferred oncogenic function in glioma and may be a new prognostic biomarker and novel therapeutic strategy for this malignancy.

Key Words:
LncRNA, LINC01503, Glioma, Wnt/β-catenin signaling, Function, Prognosis.

Introduction

Glioma represents one of the most lethal solid tumors with one of the highest mortality rates characterized by vascular proliferation, aggressive migration, and pseudopalisades and an adverse clinical outcome[1,2]. Up to date, the classification and clinical treatment of this tumor have been largely based on the histological features of glioma[3]. Glioblastomas (GBM) are high-grade gliomas, accounting for 70% of gliomas[4]. Although a variety of therapies and drugs have been used for the treatment of glioma, the prognosis o GBM still remains poor due to the less apoptosis, distant metastasis, and limited knowledge of its potential molecular mechanisms[5-7]. Like other types of tumors, the reasons of GBM are varied, and include inhibition of tumor suppressor and activation of oncogenes[8]. Further studies are necessary to better understand the pathogenesis of GBM.

Long non-coding RNAs (lncRNAs), sized from 200 nt to 100 kb, are a class of newly identified non-coding RNA with limited or lack of an open reading frame of significant length[9].

Corresponding Author: Li Meng Dai, MD; e-mail: laopwqq0771@163.com

Initially, non-coding RNAs were regarded as 'junk'; however, evidence showed that lncRNAs are emerging as new regulators in the cellular processes such as growth, differentiation, cell cycle, apoptosis, chromosome division, and disease pathogenesis[10,11]. Interestingly, several lncRNAs have been proved to be abnormally expressed in tumors and played important roles in tumor progression[12,13]. For instance, lncRNA RGMB-AS1 was reported to be overexpressed in lung adenocarcinoma and its knockdown suppressed tumors cell proliferation, migration and invasion regulating RGMB expression[14]. Higher expression of lncRNA TP73-AS1 was found to be associated with poorer prognosis of osteosarcoma patients and promote proliferation and metastasis via modulating miR-142[15]. In addition, it was reported that lncRNA MALAT1 was lowly expressed in glioma and its overexpression could cells proliferation by suppressing miR-155 expression[16]. These findings revealed that lncRNAs may be a potential prognostic biomarker and therapeutic target for tumors, including glioma.

Recent studies have reported that LINC01503 expression was significantly up-regulated in colorectal cancer[17] and squamous cell carcinoma[18], and may contribute the progression of above two tumors. However, the expression pattern and biological function of LINC01503 in glioma are still not known, which need to be explored. Our present study aimed to explore the expression and prognostic value of LINC01503 in patients. Furthermore, the potential function and the detailed mechanism of LINC01503 involved as well as the target signaling were also investigated.

Patients and Methods

Human Tissue Samples

Glioma tissues and pair-adjacent normal tissues were obtained from 133 patients with the primary glioma between April 2009 and June 2013 at Shenzhen People's Hospital of The Second Clinical Medical College of Jinan University and The First Center Hospital of Tianjin of Tianjin Medical University First Center Clinical College. The collection and use of patient tissue samples were approved by the Ethics Committee of Shenzhen People's Hospital of The Second Clinical Medical College of Jinan University and The First Center Hospital of Tianjin of Tianjin Medical University First Center Clinical College, and written informed consent was obtained from all the patients. None of the patients had received preoperative treatment before collecting specimens. The tissue specimens were immediately preserved at -80°C using liquid nitrogen after surgical resection. The clinical information of all the subjects was shown in Table I.

Cell Culture and Cell Transfection

Human glioma cell lines A172, LN229, LN18, U251 and T98G cells, and normal human astrocytes NHA cells were purchased from Sanger Biotechnology Co., Ltd. (Hongkou, Shanghai, China). These cells were all maintained using

Table I. Clinicopathological features associated with LINC01503 expression in 133 glioma patients.

Parameters	No. of cases	LINC01503 expression		p
		High	Low	
Age				0.345
< 50	69	31	38	
≥ 50	64	34	30	
Gender				0.876
Man	83	41	42	
Woman	50	24	26	
KPS				0.046
≥ 70	83	35	48	
< 70	50	30	20	
Size				0.007
< 5 cm	86	34	52	
≥ 5 cm	45	29	16	
WHO grade				0.004
Low	95	39	56	
High	38	26	12	

Roswell Park Memorial Institute-1640 (RPMI-1640) medium (YajiBio, Minhang, Shanghai, China) with fetal bovine serum (FBS) (10%) and antibiotics (1%) at 37°C in a humidified incubator containing 5% CO_2. For cell transfection, we applied a SunBioTM Trans-EZ transfection reagent (SunBio, Pudong, Shanghai, China). Briefly, the cells were placed in 6-well plates and grown to about 70% confluence. Next, 10 μl transfection reagent was mixed with siRNAs (10 pmol) for about 20 min. Subsequently, the mixture was added into the plates and the cells were cultured for experiments. The small interfering RNAs (siRNAs) targeting LINC01503 (siRNA#1, siRNA#2, siRNA#3) and negative control siRNAs (si-control) were purchased from Saier Biotechnology Co., Ltd. (Jinnan, Tianjin, China).

RNA Purification and Real-Time Quantitative RT-PCR (qRT-PCR)

Total RNA from tissue specimens as well as cells was extracted and purified using a total RNA isolation kit (DongSheng, Guangzhou, Guangdong, China). To quantify the expression of LINC01503, β-catenin, cyclin D1 and c-myc, the isolated total RNA (2 μg) was firstly reverse transcribed into cDNA using a Roche Transcriptor First Strand cDNA Synthesis kit (HengFei, Minhang, Shanghai, China). Then, the qRT-PCR analysis was carried out using a SYBR Green qPCR Master Mix (MedChemExpress, Pudong, Shanghai, China) on a QuantStudio 12K Flex Real-time PCR apparatus (ORST, Chengdu, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as endogenous controls for the measurement of LINC01503, β-catenin, cyclin D1 and c-myc. Expression levels were quantified using the methods of $2^{-\Delta\Delta Ct}$. The primer sequences were listed in Table II.

Western Blot Analysis

Total proteins from indicated siRNAs-transfected cells were extracted using a total protein extraction kit (NiuPu, Haidian, Beijing, China). The protein concentration was then detected using a BCA assay kit (RongBio, Minhang, Shanghai, China). Subsequently, SDS polyacrylamide gels (10%) were utilized to separate the proteins (20 μg per lane), and the proteins were sequentially transferred onto Millipore polyvinylidene difluoride (PVDF) membranes (JissKang, Qingdao, Shandong, China). Prior to incubate with primary antibodies, the membranes were blocked using 5% BSA. After washing with TBST, the anti-β-catenin antibody (1:800; Abcam, Cambridge, MA, USA), anti-cyclin D1 antibody (1:1000; CST, Beverly, MA, USA), anti-c-myc antibody (1:900; Boster, Wuhan, Hubei, China), anti-GAPDH antibody (1:2000; Epitomics, Hangzhou, Zhejiang, China), were immunoblotted with the membranes for 12 h at 4°C. Thereafter, the membranes were washed three times and matched secondary antibodies were applied to incubate the membranes. Next, the proteins were detected with a Quantum CX5 infrared scanner (VILBER, Fengtai, Beijing, China) using an ECL Western Blotting Substrate kit (YRBio, Changsha, Hunan, China).

Table II. Primer sets used in the present study.

Names	Sequences (5′-3′)
LINC01503: F	GGGACGGAGACAAATGACGG
LINC01503: R	GCAGGCTCCCTGACACGTA
GAPDH: F	GGAGCGAGATCCCTCCAAAAT
GAPDH: R	GGCTGTTGTCATACTTCTCATGG

Cell Counting Kit-8 (CCK-8) Assays

CCK-8 assays were carried out to determine the cellular growth curves. In short, the treated U251 or LN299 cells (3000 cells per well) were placed in 96-well plates and subsequently incubated for 1, 2, 3 and 4 days. After incubation, a CCK-8 assay kit (MeiLun, Dalian, Liaoning, China) was applied and 10 μl CCK-8 solution was added into each well. The optical density (OD) was determined at a wavelength of 450 nm using an iBIO-RADMark microplate reader (Bio-Rad, Pudong, Shanghai, China).

5-Ethynyl-20-Deoxyuridine (EdU) Assays

To evaluate cell proliferation, U251 or LN299 cells were separately planted into 48-well plates, and indicated siRNAs transfection was conducted as described above. After 48 h, a Cell-Light Cell Proliferation kit (Ribobio, Guangzhou, Guangdong, China) was applied, and EdU reagent (50 μM) was incubated with the cells for 2-3 h. Subsequently, after the nuclei were stained with DAPI (1 μg/ml) for 10 min, the cells were observed using an inverted fluorescence microscope (RX50; HGO, Suzhou, Jiangsu, China).

Colony Formation Assays

Briefly, the indicated siRNAs-transfected cells were placed into each well of the 6-well plates (500 cells per well), and cultured in RPMI-1640 medium (with 10% fetal bovine serum (FBS) and

1% antibiotics) for about 2-3 weeks. Thereafter, the visible cell colonies were stained using crystal violet (0.1%; Macklin, Pudong, Shanghai, China) for 15-20 min after being fixed with methanol. After rinsing with phosphate-buffered saline (PBS) for three times, a RX50 microscope (HGO, Suzhou, Jiangsu, China) was employed to photograph pictures.

Flow Cytometry Analysis

We used a BD Biosciences Annexin V-FITC assay kit (SiXinBio, Songjiang, Shanghai, China) to assess the influence of LINC01503 on apoptosis. The indicated siRNAs-transfected cells were harvested, and resuspended in 1 × binding buffer (500 μl) supplemented with Annexin V-FITC (5 μl) and PI (5 μl). After incubation in the dark for 20 min, the cells were washed using ice-cold PBS. Then, the apoptosis was evaluated by a Thermo Fisher Attune NxT flow cytometer (Biopike, Xicheng, Beijing, China).

Wound-Healing Assays

U251 or LN299 cells transfected with corresponding siRNAs were planted in 6-well plates and cultured to form single confluent cell layers. Then, a 100 μl pipette tip was used to scratch the cell layers, and a wound was generated. A RX50 microscope (HGO, Suzhou, Jiangsu, China) was then applied to take pictures (0 h). After 48 h, the wounded areas were also photographed (48 h).

Transwell Assays

Transwell assays were carried out using Coring transwell chambers containing 8 μm pore size inserts (Sproutstrong, Chaoyang, Beijing, China). Next, 1×10^5 treated cells suspended in 200 μl medium (without serum) were placed into the upper sides of transwell chambers. In the lower chamber, we added 600 μl medium (with 15% FBS). After incubation for 24 h at 37°C with 5% CO_2, the invaded cells were stained using crystal violet (0.1%; Macklin, Pudong, Shanghai, China). After being washed with PBS for three times, the cells invaded through the membranes were photographed using A RX50 microscope (HGO, Suzhou, Jiangsu, China).

TOPFlash Luciferase Assays

We evaluated the activity of Wnt/β-catenin signaling by TOPFlash luciferase assays. In short, the indicated siRNAs were first separately transfected into U251 or LN299 cells as described above. Subsequently, we applied lipofectamine 2000 to co-transfect the following plasmids: TOPFlash reporter, FOPFlash reporter and Renilla TK-luciferase vector, into the treated U251 or LN299 cells. Finally, a Promega dual luciferase reporter detection kit (Cobioer, Nanjing, Jiangsu, China) was used to assess the luciferase activity of these cells. All the plasmids were purchased from Addgene (Cambridge, MA, USA).

Statistical Analysis

Statistical analysis in this study was performed using SPSS (vision, 19.0; SPSS, Inc., Chicago, IL, USA). The methods of one-way ANOVA and Student's *t*-test were used to analyze these data, and multiple comparisons between groups were performed by S-N-K method. The expression of LINC01503 was assessed for associations with clinicopathological characteristics using Chi-square test. Survival curve was assessed by the Kaplan-Meier method, and differences were analyzed by the log-rank test. A Cox proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values. The results were considered statistically significant at $p<0.05$.

Results

LINC01503 was Upregulated in Patients with Glioma

Our first goal was to investigate whether LINC01503 expression could be detected and whether its expression was abnormally expressed in glioma. As shown in Figure 1A, the results of RT-PCR showed that the expression of LINC01503 was remarkably increased in glioma tissues compared with match normal brain tissues (Student's *t*-test, $p < 0.01$). Addition, the glioma tissues with advanced stages displayed a higher level of LINC01503 (Figure 1B). Moreover, the expression level of LINC01503 in five human glioma cell lines was also detected by qRT-PCR and the result indicated that LINC01503 was significantly up-regulated in five glioma cell lines compared with that in NHA (Figure 1C). U251 and LN299 cells had the highest expression of LINC01503, thus these two cell lines were used for loss-of-function experiments. Overall, our results revealed that LINC01503 was increased both in human glioma tissues and cells lines.

LINC01503 Indicated the Poor Prognosis of Glioma Patients

In order to clarify the clinical value of LINC01503 in glioma, 153 patients were divided

Table III. Multivariate analysis of the associations of prognosis with various clinicopathologic parameters and LINC01503 expression in glioma patients.

	Overall survival			Disease-free survival		
Parameter	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age	1.665	0.671-2.442	0.231	1.428	0.842-2.331	0.156
Gender	1.742	0.744-2.449	0.157	1.455	0.832-2.311	0.188
KPS	1.853	0.884-2.863	0.087	1.542	0.749-2.664	0.113
Size	2.986	1.349-4.458	0.011	3.051	1.486-4.774	0.025
WHO grade	3.247	1.482-5.449	0.003	3.451	1.582-6.149	0.001
LINC01503 expression	2.875	1.437-5.025	0.007	2.985	1.573-5.327	0.004

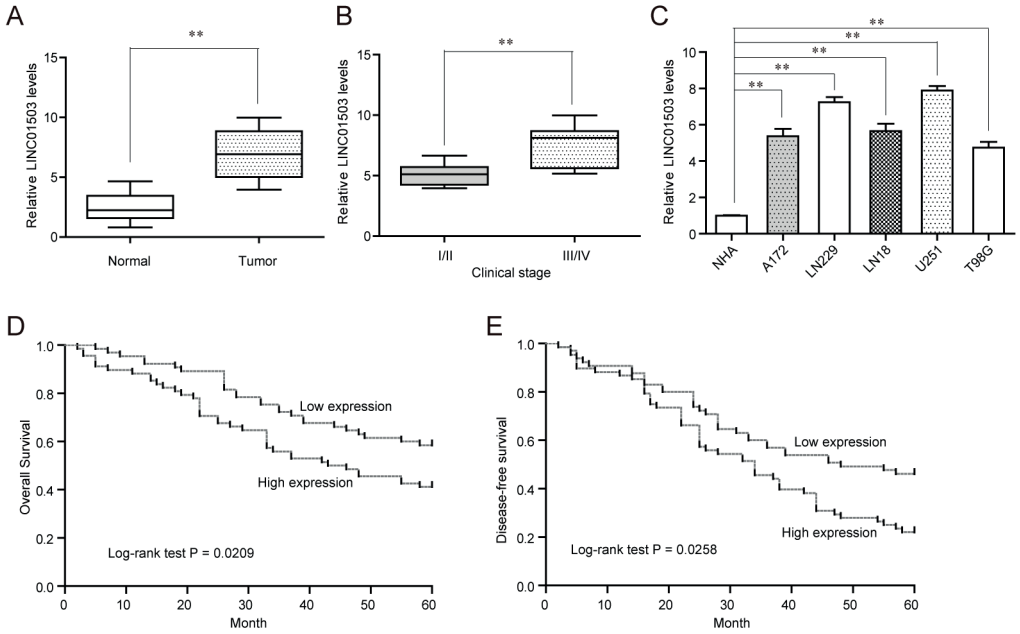

Figure 1.* Highly expressing levels of LINC01503 in glioma were associated with poor prognosis.** ***(A) qRT-PCR analysis of LINC01503 expression in glioma tissues and normal brain tissues. ***(B)*** LINC01503 expression was significantly higher in GBM patients with advanced stages. ***(C)*** The relative expression of LINC01503 was up-regulated in human glioblastoma cell lines (A172, LN229, LN18, U251 and T98G) compared with normal human glial cells (NHA). ***(D)*** The patients with high LINC01503 expression had a significantly shorter overall survival (p= 0.0209) than those with low LINC01503 expression. ***(E)*** The patients with high LINC01503 expression had a significantly shorter overall survival (p = 0.0258) than those with low LINC01503 expression. * $p < 0.05$, ** $p < 0.01$.

into low and high group according to the median expression of LINC01503. Chi-square test was used to reveal the correlation between LINC01503 levels and clinicopathological parameters of CRC. As shown in Table III, we found that high expression of LINC01503 was associated with KPS (p = 0.046), size (p = 0.007) and WHO grade (p = 0.004). However, LINC01503 expression level was not associated with other parameters such as gender (p = 0.876) and age (p = 0.345) in glioma patients (Table I). To further demonstrate the potential clinical utility of LINC01503, we explored the prognostic influence of LINC01503 on overall survival and disease-free survival in 133 glioma patients. As shown in Figure 1D, the five-year survival rate was 38% in the high LINC01503 group and 54% in the low LINC01503 group. The result of Kaplan-Meier analysis revealed that the overall survival of patients with high LINC01503 expression was significantly shorter than those with low LINC01503 expression (p = 0.0209). On the other hand, the positive roles of LINC01503 were also shown in the disease-free survival (Figure 1E). More importantly, multivariate analysis showed that LINC01503 expression was an independent prognostic factor for both overall sur-

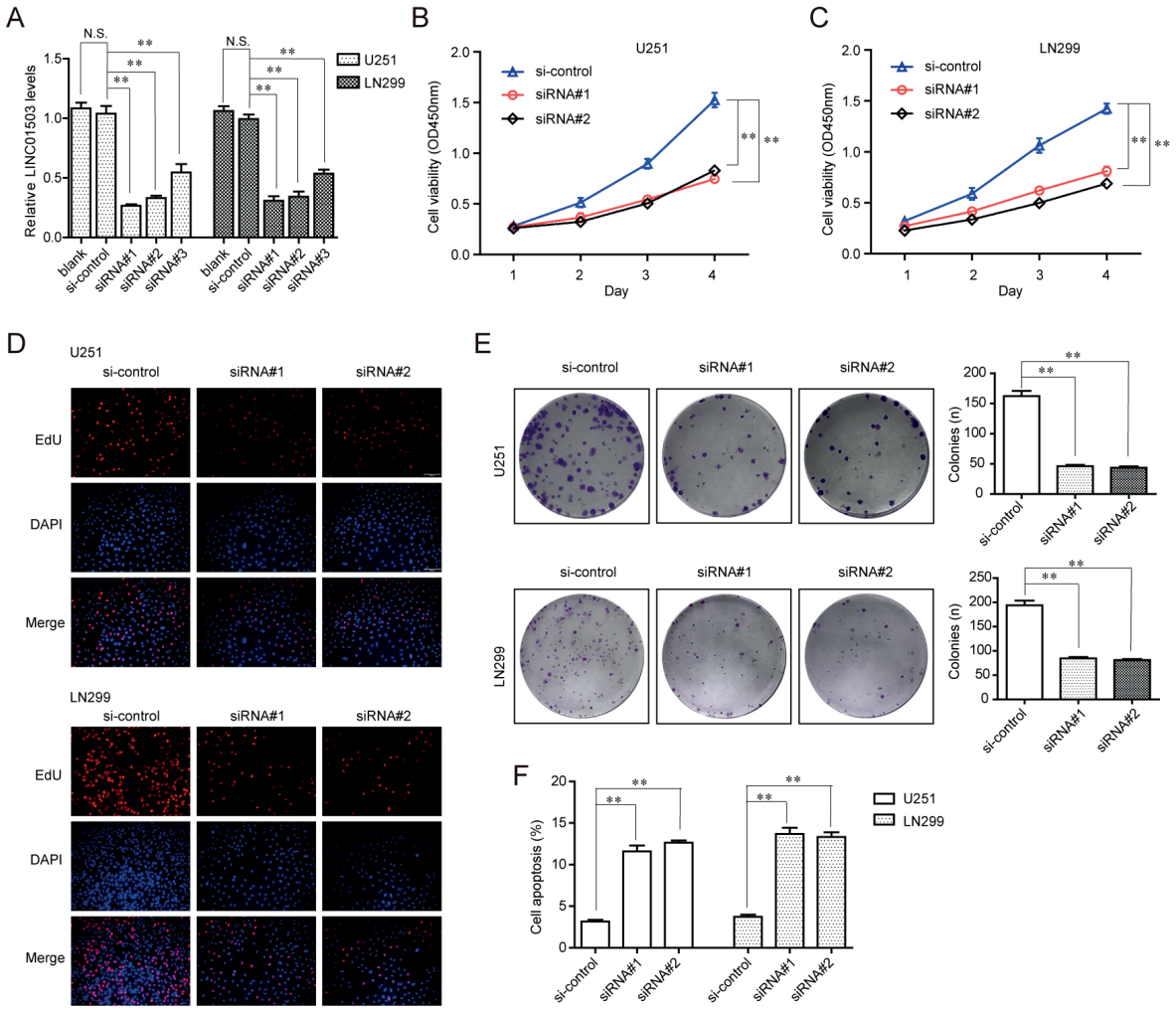

Figure 2. The effects of LINC01503 on cellular growth and apoptosis of U251 and LN299 cells. ***(A)*** Relative expression levels of LINC01503 in U251 and LN299 cells after transfection of LINC01503 siRNAs (siRNA#1, siRNA#2 and siRNA#3) and control siRNAs (si-control). ***(B*** and ***C)*** The cellular growth curves were determined by CCK-8 assays. ***(D)*** EdU assays detected the cell proliferation. The proliferative cells were labeled red and the nuclei were stained with DAPI (blue). ***(E)*** Colony formation assays evaluated the colony formation capacity of U251 and LN299 cells after transfection of indicated siRNAs. ***(F)*** Flow cytometry analysis examined the cell apoptotic rates. * $p < 0.05$, ** $p < 0.01$.

vival (HR= 2.875, 95% CI: 1.437-5.025, p =0.007) and disease-free survival (HR= 2.985, 95% CI: 1.573-5.327, p =0.004) of glioma patients. Taken together, these results suggested that LINC01503 may be involved in regulation of clinical progression of glioma patients.

LINC01503 Depletion Suppressed Proliferation of GBM Cells and Promoted Cell Apoptosis

To investigate the cellular function of LINC01503 in GBM cells, we next performed loss-of-function studies using siRNA transfection. Firstly, we synthesized three siRNAs targeting LINC01503 (siRNA#1, siRNA#2 and siRNA#3) and separately transfected into U251 or LN299 cells. Then, qRT-PCR analysis was utilized to determine the knockdown efficiency of these siRNAs and the data suggested that the knockdown efficiency of both siRNA#1 and siRNA#2 was more than 60% (Figure 2A). Therefore, in the following studies, we used siRNA#1 and siRNA#2 to conduct the experiments. We next evaluated the cellular proliferation of GBM cells after LINC01503 deficiency. The growth curves generated by CCK-8 assays indicated that down-regulation of LINC01503 resulted in greater inhibition of cellular proliferation in U251 or

LN299 cells (Figure 2B and C). Similarly, EdU assays also demonstrated that transfection of LINC01503 siRNAs led to significant suppression of U251 and LN299 cell proliferation (Figure 2D). In addition, the cell colony formation assays confirmed that knockdown of LINC01503 remarkably reduced the number of cell colonies (Figure 2E). We next carried out flow cytometry to detect the cell apoptosis and the results revealed that the cell apoptotic rates of LINC01503 U251 and LN299 cells were notably decreased when they were transfected with LINC01503 siRNAs (Figure 2F). Therefore, these studies suggested that LINC01503 acted as a key player in the tumor development of GBM.

LINC01503 Deficiency Impaired the Metastatic Potentials of GBM Cells

To gain insight into the potential role of LINC01503 in the metastasis of GBM, we next performed transwell and wound healing assays to evaluate the changes of metastatic potentials in GBM cells when their LINC01503 was knocked down by indicated siRNAs. The results from transwell assays indicated that, after transfection of LINC01503 siRNAs, the invaded cell number of U251 and LN299 cells was markedly decreased when compared with that of the control cells transfected with si-control (Figure 3A). We next wondered whether the migratory capacity of GBM cells was depressed after the cells were transfected with LINC01503 siRNAs. Therefore, the wound healing assays were conducted using U251 and LN299 cells. As expected, the wounded closure of LINC01503 siRNAs-transfected U251 and LN299 cells was remarkably abrogated, which implied that silence of LINC01503 resulted in obvious suppression of migratory ability of GBM cells (Figure 3B and C). Taken together, these results validated that LINC01503 was capable to affect the metastasis of GBM.

Knockdown of LINC01503 Suppressed the Activity of Wnt/β-Catenin Signaling in GBM Cells

The molecular mechanisms underlying the potential effects of LINC01503 on GBM cell growth and metastasis were then investigated. A plethora of studies had revealed that lncRNA was capable to affect the expression of crucial signaling pathways involved in tumor growth and metastasis. Therefore, we next evaluated the express-

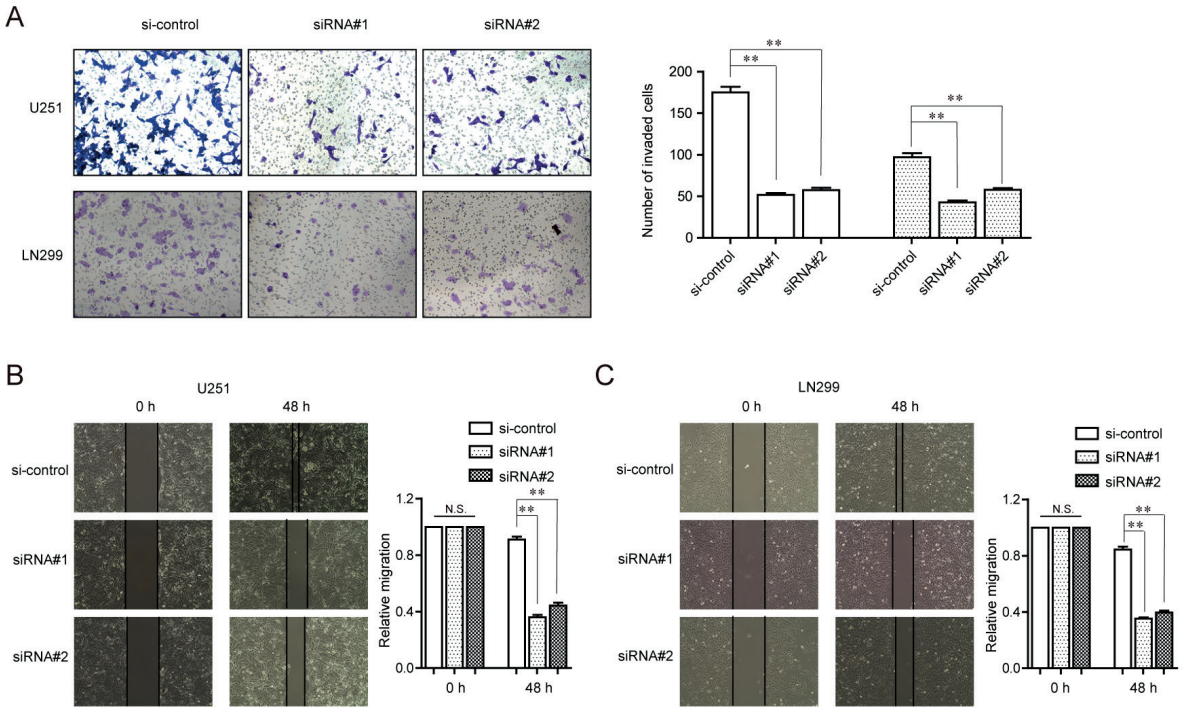

Figure 3. The effects of LINC01503 on the metastasis of U251 and LN299 cells. ***(A)*** Representative images of the transwell invasion assays. The data showed that the invaded cells in the LINC01503 knockdown groups were significantly lower than the controls. ***(B*** and ***C)*** Transfection of LINC01503 siRNAs inhibited the migration of U251 and LN299 cells. * $p < 0.05$, ** $p < 0.01$.

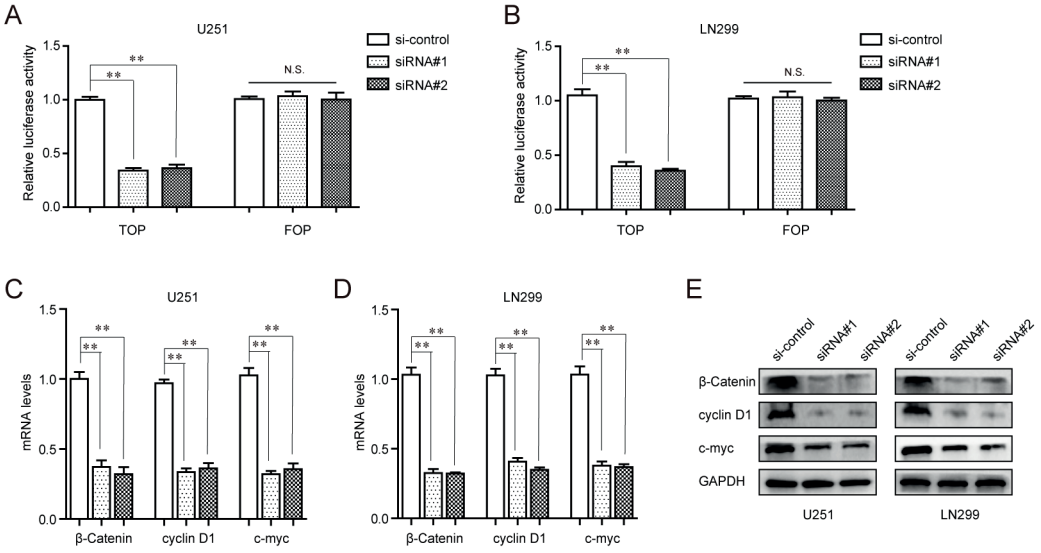

Figure 4. The effects of LINC01503 on the acitivity of Wnt/β-catenin signaling pathway in U251 and LN299 cells. ***(A*** and ***B)*** TOPFlash luciferase assays were applied to determine the activity of Wnt/β-catenin signaling in U251 and LN299 cells. ***(C*** and ***D)*** The qRT-PCR assays were performed to detect the mRNA expression of β-catenin, cyclin D1 and c-myc in U251 and LN299 cells when they were transfected with LINC01503 siRNAs. ***(E)*** The protein expression of β-catenin, cyclin D1 and c-myc was evaluated using Western blot analysis. $*p < 0.05$, $**p < 0.01$.

ing changes of several important components involved in a key signaling pathway relevant with tumor development and progression: Wnt/β-catenin signaling, in GBM cells after transfection of LINC01503 siRNAs. Firstly, the TOP/FOP flash reporter assays were conducted and the results confirmed that repressing the expression of LINC01503 caused a significant decline of the relative luciferase activities in U251 and LN299 cells, which suggested that LINC01503 depletion served as critical roles in regulating Wnt/β-catenin signaling (Figure 4A and B). Furthermore, we performed qRT-PCR analysis to assess the mRNA expression of several crucial molecules involved in Wnt/β-catenin signaling. The results confirmed that the LINC01503 siRNAs-transfected U251 and LN299 cells expressed markedly lower mRNA levels of β-catenin, cyclin D1 and c-myc, compared with cells transfected with si-control (Figure 4C and D). Western blot assays were also carried out and the results showed that silence of LINC01503 remarkably reduced the protein expression of β-catenin, cyclin D1 and c-myc in U251 and LN299 cells, which was consistent with the results of qRT-PCR assays (Figure 4E). To sum up, these data certified that depletion of LINC01503 inhibited the activity of Wnt/β-catenin signaling in GBM cells.

Discussion

Evidence revealed that protein-coding genes and some differentially expressed ncRNAs are important regulatory molecules in tumorigenesis and development[19-21]. Recently, the function of lncRNAs in tumor progression attracted growing attention. More and more lncRNAs were identified to appear to promote or inhibit the development and progression of cancer and to play a potential functional role in the diagnosis, prognosis and treatment of malignancies[22-24]. Unfortunately, few lncRNAs have been functionally elucidated in detail and several important questions needed to be addressed. According to current studies reporting on the associations between lncRNAs and cancers, lncRNAs can be divided into two subgroups (tumor promoter and tumor suppressor). Recently, Xie et al[18] firstly reported that LINC01503 was highly expressed in squamous cell carcinoma and promoted the tumor cells proliferation and metastasis, suggesting it as a tumor promoter in this disease. Following that, Lu et al[17] showed that LINC01503 expression was increased in colorectal cancer and its knockdown can tumor cells proliferation and invasion via modulating miR-4492/FOXK1 signaling, further suggesting its oncogenic roles in tumors. Thus, we wondered

whether LINC01503 had a similar role in glioma. In this study, for the first time, we detected the expression levels of LINC01503 in glioma tissues and cell lines, finding that LINC01503 was significantly highly expressed in glioma tissues and cell lines. Then, we further explored its clinical significance in patients using Chi-square test, finding that high expression of LINC01503 was associated with KPS, size and WHO grade. Further survival assays revealed that overall rates and disease-free survivals of patients with high LINC01503 expression were significantly worse than those of patients with low LINC01503 expression. Multivariate analysis confirmed that high LINC01503 expression was an independent indicator of unfavorable prognosis for glioma patients. To our best knowledge, this is the first time to report the prognostic value of LINC01503 in glioma patients.

Evidence confirmed that dysregulation of lncRNAs was involved in the regulation of tumor cells proliferation, migration and invasion[25]. Previous studies also detected that LINC01503 promoted cells proliferation and metastasis in colorectal cancer[17] and squamous cell carcinoma[18]. Thus, we further explored the potential function of LINC01503 in U251 and LN299 cells. RT-PCR confirmed that LINC01503 expression was down-regulated in U251 and LN299 cells transfected with si-LINC01503. *In vitro* assays revealed that knockdown of LINC01503 significantly suppressed the proliferation, migration and invasion, and promoted apoptosis, indicating that LINC01503 may act as a therapeutic target for glioma.

Wnt signaling pathway is important in developmental processes, cell growth and differentiation[26]. Wnt/β-catenin pathway, highly conserved during evolution, is a well-studied signaling pathway which ranks as the best understood Wnt signaling pathway[27,28]. The Wnt/β-catenin pathway involves different proteins that are needed for cell proliferation and differentiation in different tissues[29,30]. It has been demonstrated dysregulation of this signaling pathway cause uncontrolled cell growth and cell malignant transformation. Subsequently, more and more efforts have made to develop therapeutic strategies through specifically targeting the Wnt/β-catenin pathway[31,32]. Considering that several lncRNAs had been reported to display their functional effects by modulating Wnt/β-catenin pathway, we also focused on the possible association between LINC01503 and this signaling pathway[33,34]. As expected, down-regulation of LINC01503 in U251 and LN299 cells remarkably decreased TOP/FOP transcriptional activity. Moreover, the results of Western blot showed that β-catenin, cyclin D1 and c-myc were dramatically reduced in U251 and LN299 LINC01503 depletion cell lines. These results demonstrated that LINC01503 could promote the activation of Wnt/β-catenin pathway, and thus was involved in the regulation of glioma cells proliferation, metastasis and apoptosis. Such research could uncover significant information for glioma therapy.

Conclusions

We showed that LINC01503 expression in glioma was significantly up-regulation and was closely correlated with tumor size, WHO grade and poor prognosis of glioma patients. Furthermore, LINC01503, as an oncogenic lncRNA, promoted proliferation, inhibited apoptosis and promoted the migration and invasion of glioma cells by modulating Wnt/β-catenin pathway. On the basis of these results, LINC01503 may be used as a prognostic factor and could be considered as a potential target for glioma patients in the future.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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