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LINC01207 Predicts Poor Prognosis and Suppresses Cell Growth and Metastasis via Regulating GSK-3β/β-Catenin Signaling Pathway in Malignant Glioma

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Statistical Analysis C
Data Interpretation D
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Background: Recent literature has revealed that LINC01207 plays a vital part in tumorigenesis and malignancy progression. However, the potential mechanisms of LINC01207 in malignant glioma are still unknown.

Material/Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was applied to analyze LINC01207 mRNA levels in malignant glioma cell lines and tissue samples. The correlation between LINC01207 mRNA levels and clinical characteristics was explored, and the relative survival rate was observed using the Kaplan-Meier method. To examine the function of LINC01207, we performed cell viability, EdU assay, cell cycle assay, Transwell assay, and wound-healing assay to analyze relative cell proliferation, migration/invasion ability. Finally, qRT-PCR and western blot were used to investigate the potential mechanisms.

Results: LINC01207 mRNA was lowly expressed in malignant glioma cells and cancer tissue samples. Low expression of LINC01207 was associated with Karnofsky performance score (KPS), invasion condition, and tumor grade. Moreover, multivariate analysis confirmed LINC01207 expression and tumor grade were significant independent predictors of poor survival in malignant glioma. LINC01207 markedly inhibited cellar proliferation and viability via inducing G0/G1 phase cell cycle arrested and repressed cell metastasis through restraining epithelial-to-mesenchymal procession *in vivo*. In addition, we detected a reduction in the protein levels of β-catenin and p-GSK-3β, while GSK-3β expression was upregulated.

Conclusions: In summary, LINC01207 served as a tumor-related tumor suppress gene for malignant glioma through inhibiting of GSK-3β/β-catenin signaling pathway.

MeSH Keywords: **Cell Growth Processes • Glioma • Prognosis**

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Background

Malignant glioma (glioblastoma, GBM) has been reported as one of the most common invasive malignancies in the human brain with high recurrence rates, high mortality, and low cure rates [1]. GBM has a high rate of recurrence and poor prognosis due to the invasiveness of the tumor. According to 2020 Cancer Statistics, the incidence and mortality of brain and other nervous system tumors was the number one reason for malignant tumor death among men younger than 40 years old and women younger than 20 years old, which seriously threatens the life and health of younger adults [2]. In addition, the morbidity and mortality of GBM cases has also increased year by year [1,2]. In spite of huge advances in diagnostic techniques and surgical and chemotherapeutic approaches, the prognosis of GBM is still dismal [3,4]. Therefore, specific prognosis and reliable biomarkers in GBM are still needed, indicating the need to find novel and available tumor markers for the early prediction, progression, and prognosis of GBM.

Previous studies have shown that dysregulation of long non-coding RNA (lncRNA) expression is associated with tumorigenicity and poor prognosis in many cancer types, including GBM [5,6]. For example, lncRNA-MALAT-1 mRNA expression was upregulated in bladder transitional cell carcinoma (BTCC) cell lines and tissues samples, and was reported to participate in BTCC carcinogenesis and metastasis. Also, the upregulation of lncRNA-MALAT-1 was statistically correlated with a high clinicopathologic period and also poor BTCC patient survival [7]. LncRNA-SNHG20 was highly expressed in gastric cancer and could obviously regulate epithelial-mesenchymal transition (EMT) progression in gastric cancer by antagonizing GSK-3β/β-catenin signaling pathway [8]. LncRNA-SNHG3 in GBM was significantly upregulated and its decreased expression halted the progression of cancer cells in G0/G1 phase, and the growth and migration of tumor cells are inhibited by the negative regulation of the expression of β-catenin [9].

LINC01207, which is located at 4q32, contained 3 exons and 2 introns and has an important effect on the regulation of gene transcription and protein translation including in pancreatic cancer, lung adenocarcinoma, and colorectal adenocarcinoma [10–12]. However, the LINC01207 expression and its related mechanism in GBM have not yet been reported. In this study, we identified LINC01207 as a novel GBM specific lncRNA, which was significantly downregulated in GBM. The aim of this study was to characterize the tumor-activity and related molecular mechanisms of LINC01207 in GBM.

Material and Methods

Cell culture and reagents

The human malignant glioma cell lines BT142, LN-229, U87-MG, U251, U138-MG, and Hs 683, and normal astrocyte (NHAs) were obtained in American Type Culture Collection (ATCC, Manassas, USA). These cell lines were maintained in RPMI-1640 (Gibco-BRL,

Table 1. Association between the LncRNA LINC01207 expression and clinicopathological characteristics in glioma patients.

Parameters	Characteristics	LINC01207 expression		P value
		High	Low	
Gender	Male	16	25	0.418
	Female	20	19	
Age	<50	12	17	0.396
	≥50	24	27	
KPS	<80	9	11	0.019
	≥80	27	33	
Histopathology	Conventional	13	21	0.863
	Chondroid	23	23	
Invasion condition	Yes	14	20	0.025
	No	22	24	
Tumor grade	I–II	12	19	0.039
	III–IV	24	25	

LncRNA – long non-coding RNA; KPS – Karnofsky performance score.

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Table 2. Multivariate analyses for overall survival by Cox regression test.

Factors	HR	95% CI		*P* value
		High	Low	
Gender	0.982	0.342	3.512	0.567
Age	0.876	0.461	3.471	0.342
KPS	0.877	0.412	3.461	0.456
Histopathology	0.786	0.181	1.089	0.182
Invasion condition	0.722	0.271	1.291	0.319
Tumor grade	8.221	1.351	11.831	0.037
LINC01207 expression	9.586	1.231	91.681	0.021

KPS – Karnofsky performance score.

Table 3. List of RT-PCR primers used in this study.

Primer	Sequence (5'-3')	Product size (bp)
STAT3-F	CCAATGGAATCAGCTACAGC	236
STAT3-R	GCTGATAGAGAACATTCGACTC	
ALDH1-F	GAAATGTCATCCTCAGGCAC	171
ALDH1-R	ATCTCCTTCTTCTACCTGGC	
OCT-4-F	GCCTACCGTGGTATTAGATGTC	484
OCT-4-R	CCTTATGCAGTTGCTCTCC	
KLF4-F	TCCCATCTTTCTCCACGTTC	262
KLF4-R	TCCAGGAGATCGTTGAACTC	
SOX2-F	AGCAACGGCAGCTACAGCA	281
SOX2-R	TGGGAGGAAGAGGTAACCACAG	
CD44-F	ACAACTGGTGATGGAGACTCATCC	546
CD44-R	CAGAGTGGCTTATCATCTTGG	
Ecad-F	TACACTGCCCAGGAGCCAGA	103
Ecad-R	TGGCACCAGTGTCCGGATTA	
Vimentin-F	GACCAGCTAACCAACGACAA	150
Vimentin-R	GTCAACATCCTGTCTGAAAGAT	
N-cad-F	CGAATGGATGAAAGACCCATCC	174
N-cad- R	GGAGCCACTGCCTTCATAGTCAA	
LINC01207-R	CAGACACAGGCCATTCAGTC	269
LINC01207-F	CTTCTTCACCAGAAGCATTCC	
β-actin-F	TCCTGTGGCATCCACGAAACT	315
β-actin-R	GAAGCATTTGCGGTGGACGAT	

F – forward; R – reverse; RT-PCR – real-time polymerase chain reaction.

Karlsruhe, Germany) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 100 IU/mL penicillin, and 100 µg/mL streptomycin (Baishitong, Chongqing, China). All cells were adherently grown in 5% CO_2 at 37°C and in saturated humidity.

GBM patients and tissue specimens

A total of 80 GBM tissue specimens (including cancers and the paired non-cancer tissue specimens) and normal brain clinical samples obtained from patients who received surgery in the Department of Neurosurgery, The Second Affiliated Hospital of Harbin Medical University between 2008 and 2010. We did not consider patients who had any neurosurgery, chemotherapy, or radiation therapy, or long-term use of non-steroidal anti-inflammatory drugs or corticosteroids before surgery. All of these tissue samples were collected and immediately restored in a liquid nitrogen tank, then kept at -80°C until use. All cancer cases were diagnosed histologically by 2 experienced pathologists according to the World Health Organization (WHO) standard, based on the use of both histology and molecular genetic features and previous studies [13,14]. This study was approved by the Institutional Ethics of the Second Affiliated Hospital of Harbin Medical University. The clinical characteristics of all the clinical samples are shown in Tables 1 and 2. All data included in this study are available upon request by contacting the corresponding author.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from GBM, paracancerous tissues and cell lines using TRIzol Kit (Invitrogen Inc, Carlsbad, CA, USA) according to the manufacturer's protocol. Then 5 µg of total RNA was taken for reverse transcription to cDNA according to the qRT-PCR kit instructions (TaKaRa, Otsu, Shiga, Japan). The qRT-PCR was carried out as described previously [15], and was used to examine the relative expression of target genes mRNA

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using SYBR® Green Real-time glioma Master Mix (TaKaRa) on an ABI 7500 fast real-time PCR system (ABI, USA). The $2^{-\Delta\Delta Ct}$ method was performed to calculate the relative mRNA expression of these genes [10,11]. Primers (Table 3) were synthesized by HuaDa Gene Company (Shenzhen, China).

Cell transfection

LN-229 and Hs 683 cells were pre-seeded into the 6-well plates with 5×10^5 cells/mL RPMI-1640 for transfection. Until cultured confluence of 70% to 80%, cells were transfected transiently with pcDNA3.1-LINC01207 or pcDNA3.1(+)- vectors using Lipofectamine™ 3000 reagent (Invitrogen) and incubated in serum-free RPMI-1640 medium followed the product manual. After 6 to 8 hours transfection, the culture medium was replaced with fresh 1640 containing 10% FBS containing 400 µg/mL G418 (Invitrogen) for 2 weeks. The stable-expressed LN-229 and Hs 683 cells were collected for functional assays.

Cell proliferation assay

The cell viability was detected by Cell Counting Kit-8 (CCK-8) assay as previous described [15]. Each well of 96-well plates had added to it 3000 of the aforementioned cells. After adherent cells growth, the cells were incubated with CCK-8 solution (Dojindo Molecular Technologies, Kumamoto, Japan) for 2 hours at 37°C in the dark, and quantified spectrophotometrically at a wavelength of 490 nm using a Microplate Reader (Bio-rad, USA) every 24 hours until 72 hours. The assays were done in triplicate. independently.

EdU assay

The EdU assay was carried out using a commercial EdU Staining Proliferation Kit as described previously (Beyotime, China) [15] In brief, vector and experimental group cells were exposed to EdU for 4 hours in the dark and then fixed with 4% ice methanol for 1 hour. EdU-positive and the total cells were counted under fluorescence microscopy in 5 random non-overlapping fields per coverslip. Experiments were done in triplicate.

Cell cycle assays

After being transfected for 48 hours, vector and experimental group cells were digested, collected, and seeded into 6-well plates, in triplicate. Experimental and vector groups were harvested and immobilized with 75% ice-cold ethanol at 4°C for at least 12 hours. Then, the treated cells were washed with phosphate-buffered saline (PBS) twice and subsequent stained with 50 mg/mL propidium iodide (PI, Beyotime) supplied with 50 mg/mL RNase A (DNase-free, Beyotime) at 37°C for 30 minutes, and sorted using a FACSCalibur instrument (BD Biosciences, San Jose, CA, USA). The percentage of cells in each phase was determined with flow cytometry (BD Biosciences). Data were analyzed using ModFit version 4.0 software (BD Biosciences, Franklin Lakes, NJ, USA).

Cell migration assay

The cell migration abilities were evaluated by using Transwell assay. For the Transwell assay, vector and experimental cells were digested, collected, and replanted in upper Transwell chambers through a membrane of 8-µm pore size (Corning, USA) at a density of 5000 cells/well while RPMI-1640 medium containing 20% of FBS was added to the lower chamber. After being cultured for another 48 hours, invaded cells in the lower chambers were immobilized in 70% ice ethanol and stained with 0.1% crystal violet, then cells were counted and calculated under a microscope in 5 random fields. As in wound-healing assay, vector and experimental cells were maintained in serum-free medium and seeded in a 6-well plate to a confluence of about 95%. After discarding the supernatant, a P-20 pipette tip was used to create culture wound and washed with PBS 3 times to remove the separated cells, cultures were then photographed and assessed under an inverted microscope at every 12 hours to analyze the wound healing process by phase-contrast microscopy.

Protein extraction and western blot

Total proteins were extracted from clinical tissue samples and cancer cells by radio-immunoprecipitation assay (RIPA) lysis buffer (Thermo Fisher Scientific, USA) containing protease inhibitor (Biyuntian, Shanghai) for 30 minutes at 4°C as described previously [16,17]. Vector and experimental group cells were collected, washed 3 times with ice-cold PBS and then protein was extracted using RIPA lysate buffer containing protease inhibitor (Biyuntian, Shanghai). Denatured proteins (50 ug) were separated using 12% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and blotted onto nitrocellulose membranes (polyvinylidene difluoride). The membranes transferred by cell lysates were subsequently incubated with primary antibodies including: β-catenin (1: 1000; ab16051; Abcam, USA), GSK-3β(1: 1000; ab141295; Abcam), p-GSK-3β (1: 1000; sc-373800; Santa Cruz, USA), E-cadherin (1: 1000; ab197751; Abcam), N-cadherin (1: 1000; ab202030; Abcam), vimentin (1: 1000; ab92547; Abcam), OCT4n (1: 1000; ab181557; Abcam), and SOX2 (1: 1000; ab97959; Abcam). After 24 hours, the membranes were washed with PBS twice and then incubated with horseradish peroxidase-conjugated goat anti-rabbit for 2 hours at 37°C, immunoreactive bands were analyzed with by applying Imaging System (Thermo Fisher Scientific, USA). All assays were repeated thrice.

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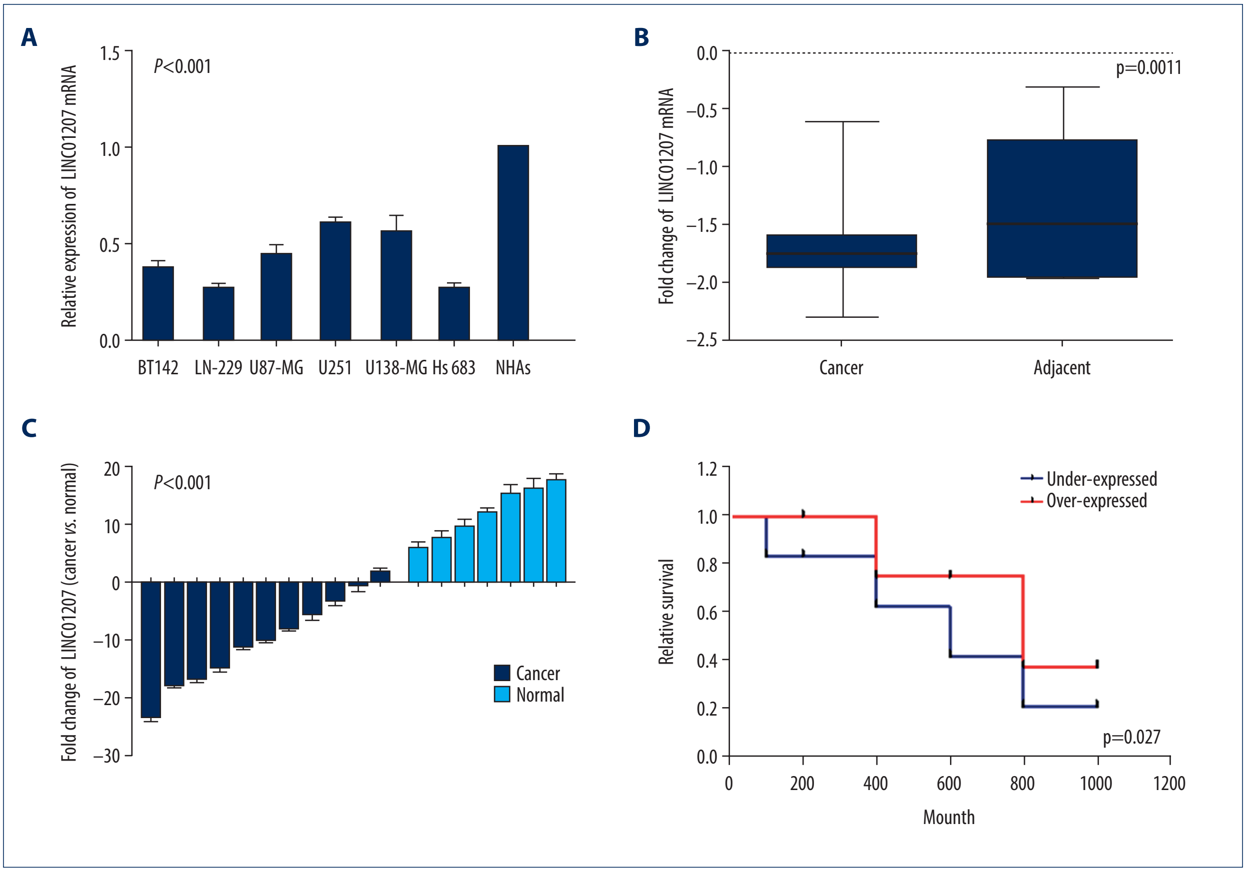

Figure 1. Expression of LINC01207 mRNA in GBM cell lines and samples. (**A**) qRT-PCR detect LINC01207 mRNA levels in cancer cell lines (BT142, LN-229, U87-MG, U251, U138-MG and Hs 683) and normal NHAs, *P*<0.001. (**B**) qRT-PCR detect LINC01207 mRNA in GBM samples and matched adjacent healthy samples *P*=0.0011. (**C**) qRT-PCR detect LINC01207 mRNA in GBM and brain samples, *P*<0.001. (**D**) Relationship between LINC01207 expression and survival time in GBM, *P*=0.027. GBM – glioblastoma (malignant glioma); qRT-PCR – quantitative real-time polymerase chain reaction.

Statistical analysis

All data were analyzed using IBM SPSS Statistics software version 20.0 (Chicago, IL, USA). All data were expressed as means±standard deviation (SD). Differences between 2 samples were assessed by 2-tailed Student's *t*-test and the significance of the differences was analyzed by one-way analysis of variance (ANOVA) using Bonferroni post hoc test in the case of multiple comparisons. Differences were considered to be statistically significant when *P*<0.05.

Results

Decreased expression of LINC01207 in GBM samples and cancer cells

Firstly, we examined the expression level of LINC01207 mRNA in 7 cell lines, and our finding indicated that LINC01207 mRNA was downregulated in GBM cell lines compared to normal NHAs cells (Figure 1A, *P*<0.001). Furthermore, we detected the LINC01207 mRNA expression in GBM tissues, matched adjacent healthy tissues, and normal brain tissues, and the results indicated that LINC01207 mRNA was statistically downregulated in GBM tissues compared with matched adjacent healthy tissues (Figure 1B, *P*=0.0011). Meanwhile, the mRNA levels of LINC01207 was also decreased in GBM samples compared with normal brain samples (Figure 1C, *P*<0.001). Kaplan-Meier analysis indicated that the downregulation of LINC01207 was statistically related to poor overall survival in GBM patients (Figure 1D, *P*=0.027).

LINC01207 expression and clinicopathologic characteristics of GBM patient

We further analyzed the relationship between LINC01207 expression and its clinicopathologic features of GBM. The clinical cases were separated into 2 groups base on the median LINC01207 expression: LINC01207 low expression group and LINC01207 high expression group. The statistical analysis

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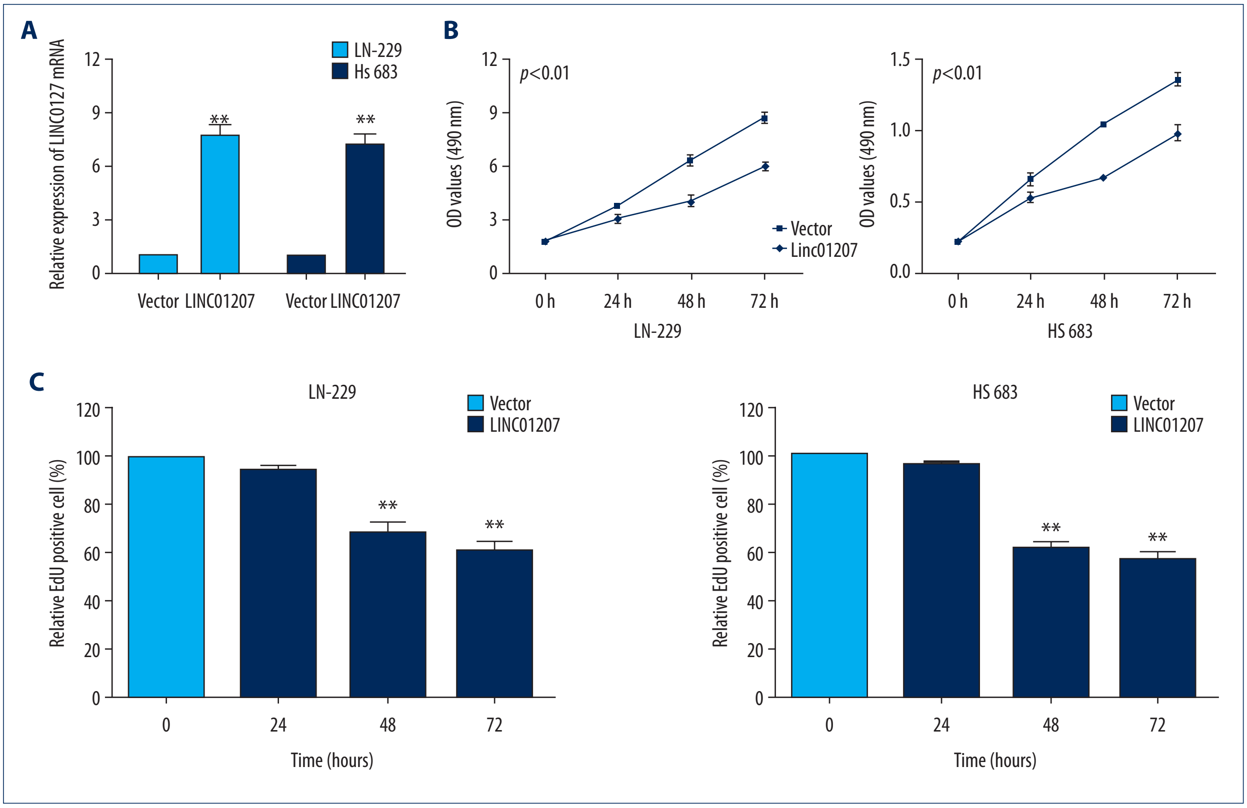

Figure 2. LINC01207 inhibited proliferation of GBM cells. (**A**) qRT-PCR analysis of the extopic-expression of LINC01207 in GBM cell lines, ** *P*<0.01. (**B**) CCK-8 assay for cellular proliferation, ** *P*<0.01. (**C**) EdU assay for cellular viability, ** *P*<0.01. GBM – glioblastoma (malignant glioma); qRT-PCR – quantitative real-time polymerase chain reaction; CCK-8 – Cell Counting Kit-8.

suggested that the lower group was related to Karnofsky performance score (KPS) (*P*=0.019), invasion condition (*P*=0.025), and tumor grade (*P*=0.039). However, no statistics association was found among other factors including gender (*P*=0.418), age (*P*=0.396), histopathology (*P*=0.863) in GBM (Table 1). We further explored the prognostic role of LINC01207 expression in GBM patients; multivariate analyses were applied to analyze these risk factors. As shown in Table 2, multivariate analysis indicated that LINC01207 expression (*P*=0.021) and tumor grade (*P*=0.037) were independent predictors for the overall survival of GBM patients, while age, gender, KPS, histopathology, and invasion condition were not.

The re-expression of LINC01207 inhibited the cell proliferation of GBM cells

To explore the potential roles of LINC01207 in GBM cell lines, we chose the lowest LINC01207 mRNA expression of LN-229 and Hs 683 as experimental groups. The transfection efficiencies were detected according to the relative expression levels of LINC01207 by using qRT-PCR (Figure 2A, *P*<0.01). As shown in Figure 2B, over-expression of LINC01207 caused an obvious suppression of cell proliferation than vector ones (Figure 2B, *P*<0.01). Meanwhile, the EdU assay also showed that LINC01207 statistically inhibited cell proliferation (Figure 2C, *P*<0.01). These results implied that LINC01207 suppressed the GBM cell proliferation *in vivo*.

LINC01207 caused the cell cycle arrested in GBM cells

Flow cytometry was applied to detect whether over-expression of LINC01207 suppressed LN-229 and Hs 683 cells viability and proliferation via regulating the cell cycle. Results showed that LINC01207 over-expressed LN-229 and Hs 683 obviously increase the relative rate of cells in the G0-G1 phase by about 20% to 35% (Figure 3, *P*<0.001), implying that the inhibition of cell viability by LINC01207 was probably through delaying cell cycle arrested in G0/G1 phase.

Over-expression of LINC01207 suppressed cell migration

Cell proliferation and cell metastasis were 2 main manifestations for malignancies; thus, we next explored the effects of LINC01207 on cell metastasis. Wound-healing assays also showed that LN-229 and Hs 683 over-expressed LINC01207 cells migrated into the wounded areas slower than the vector

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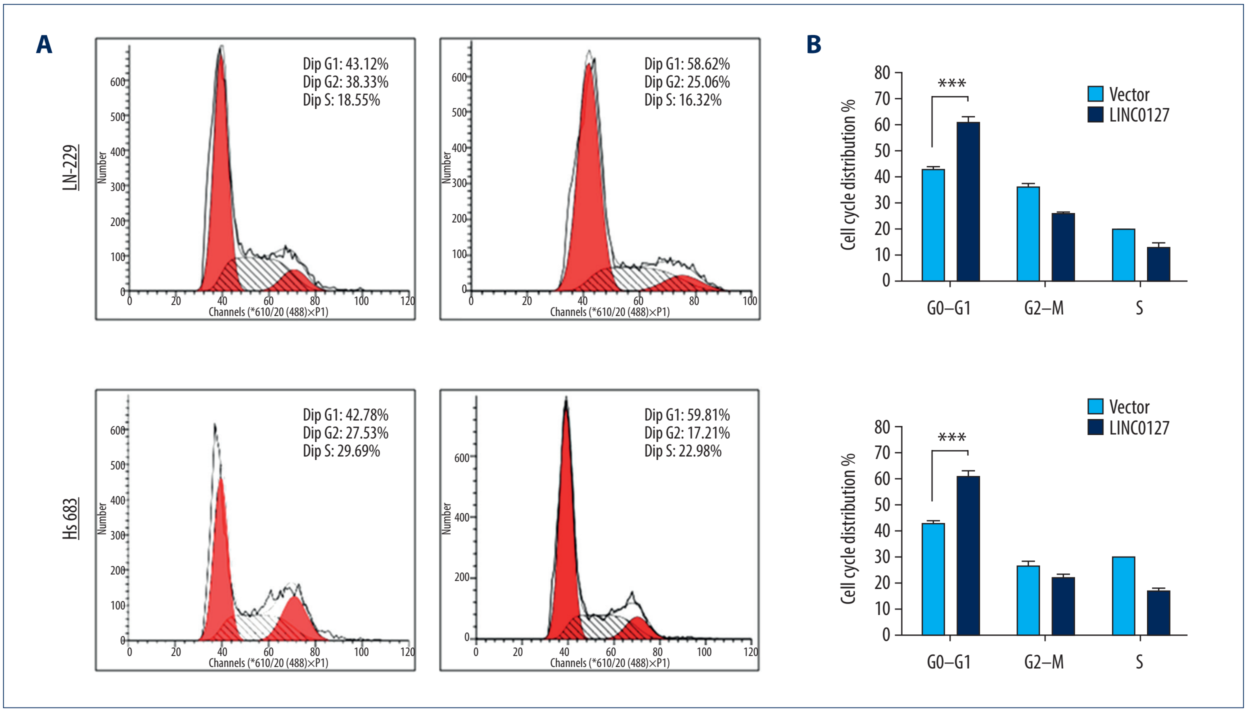

Figure 3. LINC01207 induced cell G0/G1 phase arrest. (**A**) Representative distribution of cell cycles in LN-229 and Hs 683 cell lines. (**B**) Summary of flow cytometry data, *** *P*<0.001.

cells at 48 hours, respectively (Figure 4A, *P*<0.01). EMT processing regulated multiple malignant phenotypes concerning tumor migration/invasion, containing the circulating cancer cells, cancer angiogenesis and resistance to radiotherapy and chemotherapy [14]. We next examined the EMT related markers in LN-229 and Hs 683 over-expressed LINC01207 cells. RT-PCR showed that LINC01207 decreased part of stem cell markers in LN-229 and Hs 683 cell lines in mRNA levels (Figure 4B). Meanwhile, qRT-PCR indicated that E-cadherin mRNA was increased in over-expressed LINC01207 cells, as vimentin, and N-cadherin mRNA and protein downregulated, also with the same results in OCT4 and SOX2 expression levels (Figure 4C, *P*<0.001). Moreover, we examined some downstream expression of stem cell markers in protein levels. Results indicated that LINC01207 declined some stem cell markers in LN-229 and Hs 683 cell lines (Figure 4D), and also parts of stem cell markers such as OCT4 and SOX2 had the same change with mRNA expression (Figure 4D).

Re-expression of LINC01207 inhibited GBM cell invasion

Previous studies have reported that lncRNAs could inhibit/promote cancer cell invasion [18,19]. However, the effect of LINC01207 in the invasion of GBM cells remains unknown. Transwell assays were implied to investigate the invasion ability of GBM cells, and results showed that over-expression of LINC01207 in LN-229 and Hs 683 cells resulted in an obvious decrease compare to normal vector groups (Figure 5A, 5B, *P*<0.001). These data showed that over-expression of LINC01207 suppressed cell metastasis in GBM cells.

LINC01207 regulated GSK-3β/β-catenin signaling pathway in the GBM cells

A number of studies had reported that lncRNAs could influence β-catenin activity [20,21]. To investigate the underlying effects of LINC01207 on GSK-3β/β-catenin pathway, western-blot was applied to analyze the relative protein expression of GSK-3β, p-GSK-3β, and β-catenin in LINC01207 over-expression LN-229 and Hs 683 cell lines. As was shown, the expression level of GSK-3β in LINC01207 over-expression GBM cell lines was obviously increased, while the β-catenin and p-GSK-3β were decreased (Figure 6A, 6B, *P*<0.001). In conclusion, these results suggested that LINC01207 could influence GBM progression by activating of GSK-3β/β-catenin signaling pathway.

Discussion

It has been reported that mammalian genomes encode millions of lncRNAs, which are recognized as "transcription noise" or "junk" genes. Meanwhile, accumulated research has well-documented that a variety of lncRNAs were abnormally expressed in GBM and their abnormal expression played vital roles in malignancy development and OS [22–24]. However, to find new and specific target genes and also to reveal the responsible

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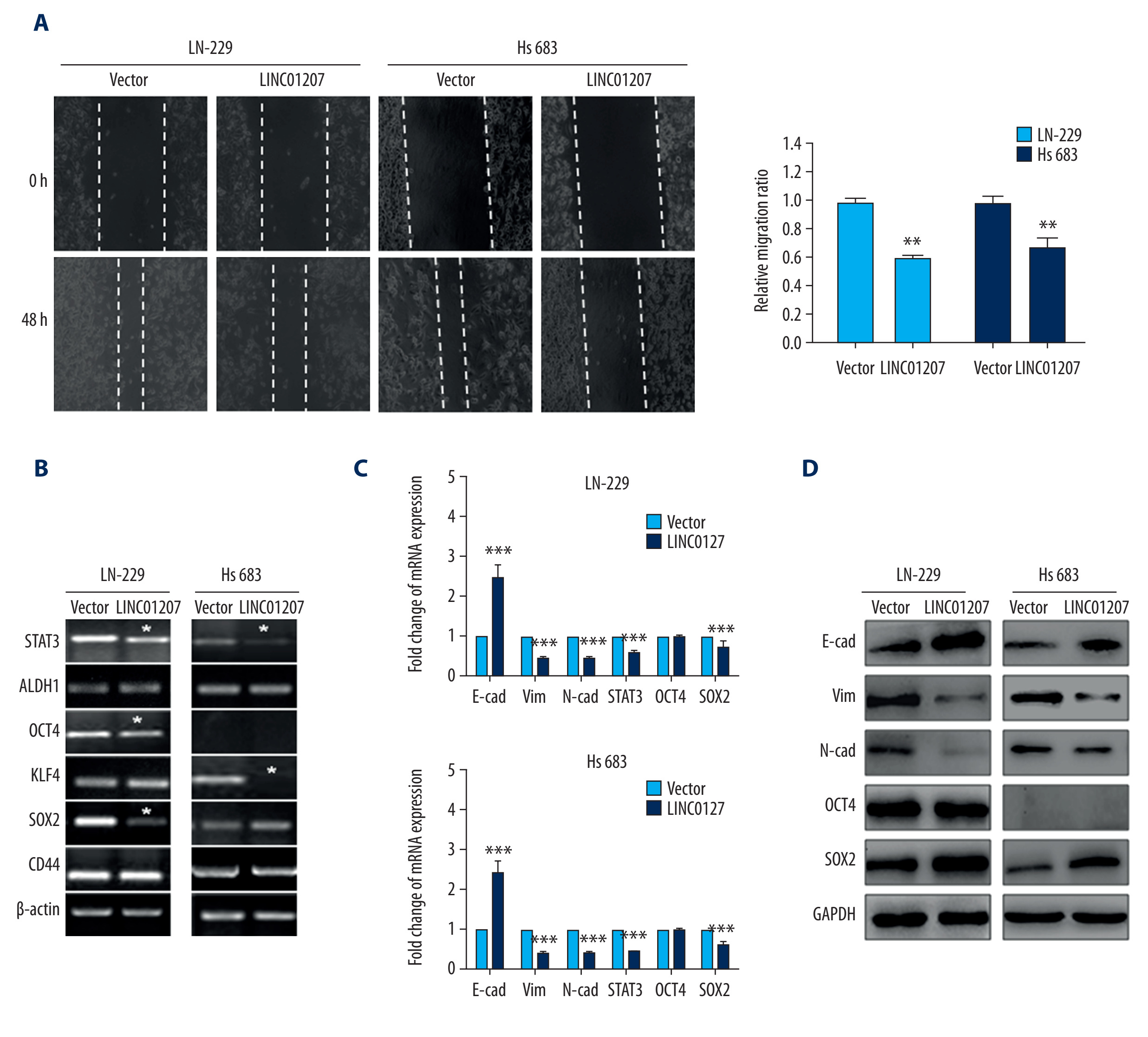

Figure 4. LINC01207 inhibited cell motility in LN-229 and Hs 683 cells. (**A**) Left is representative images of wound-healing assays; right is quantification of wound-healing assay; ** *P*<0.01. (**B**) qRT-PCR indicating downstream stem cell markers expression; * indicates significantly downregulated bands. (**C**) Relative mRNA expression by qRT-PCR; *** *P*<0.001. (**D**) Relative protein expression by western blot. qRT-PCR – quantitative real-time polymerase chain reaction.

mechanisms for the GBM tumorigenesis and progression are critical for the development of effective therapeutic strategies for patients with GBM. Thus, identification and characterization of the functional roles of dysregulated lncRNAs in GBM and their underlying mechanisms might be significant for cancer research, and helpful for finding novel therapeutic targets.

Published studies have indicated that LINC01207 expressed differentially in various carcinomas might be partly explained by its various functions. Wang et al. reported that LINC01207 expression levels was specifically increased in lung adenocarcinoma, which could promote lung adenocarcinoma cellar viability *in vivo* and *in vitro* [10]. Zeng et al. also found that LINC01207 abnormal expression was statistically connected with colon adenocarcinoma patients overall survival, the related genes including LINC01207 and LINC01555 were engaged in cAMP signaling and mucin-type O-glycan biosynthesis pathways and could function as an effective molecular target in diagnosis and treatment of colorectal adenocarcinoma [25]. However, the expression and basic cancer-related functions of LINC01207 in GBM have not been clearly studied.

In order to uncover the function of LINC01207 in GBM, we first detected the relative mRNA levels of LINC01207 in cancer cell lines and samples. The results showed that LINC01207 mRNA was downregulated in cancer cell lines and samples compared

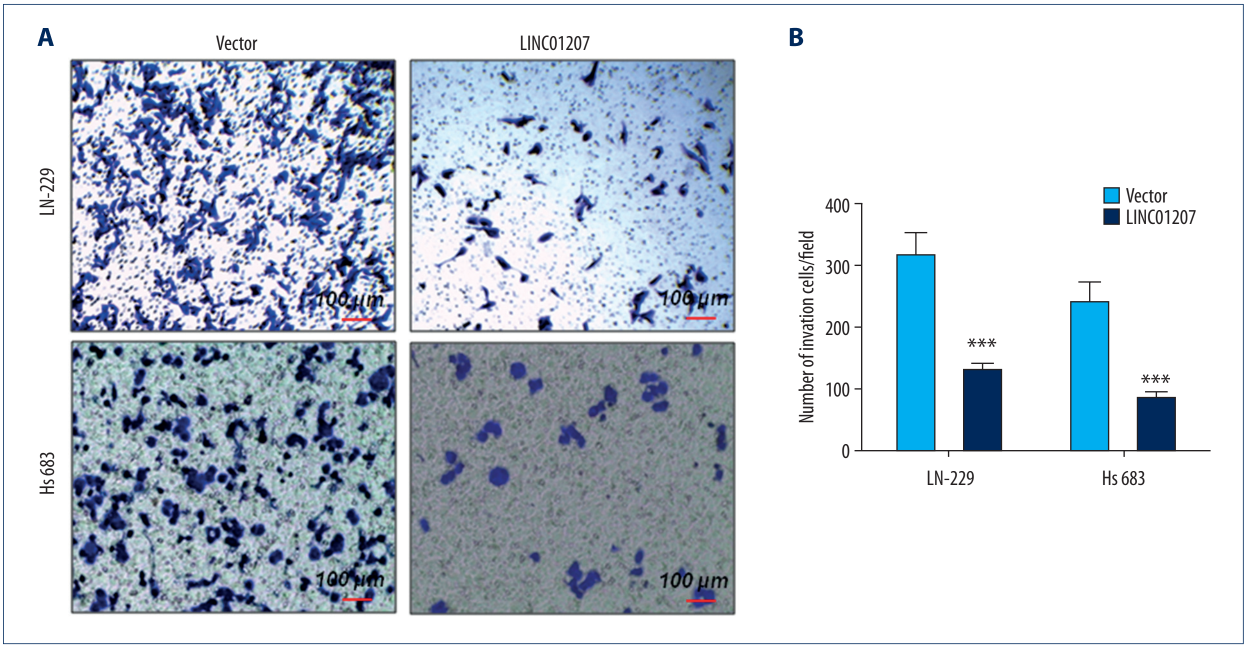

Figure 5. LINC01207 suppressed invasion of GBM cells. (**A**) The invasion of LN-229 and Hs 683 cells. (**B**) Quantitative analysis of invasion cell numbers; the number of control vector samples was set to 100%, data represented the mean values of 3 independent experiments. *** *P*<0.001. GBM – glioblastoma (malignant glioma).

with normal NHAs, paired non-cancer tissues and brain tissues. Downregulated expression of LINC01207 was associated with KPS, invasion condition and tumor grade. Meanwhile, Kaplan-Meier analysis indicated that GBM patients with lower LINC01207 expression had relatively poor overall survival. Next the multivariate Cox regression test analysis suggested that low expression of LINC01207 mRNA was an independent prognostic factor for poor prognosis. These results suggested that LINC01207 abnormal expression in GBM was importantly correlated with tumorigenesis. To detect whether LINC01207 acted as a functional gene *in vitro*, we transfected LINC01207 plasmids into LN-229 and Hs 683 cells. CCK-8 and EdU assay demonstrated that over-expression of LINC01207 decreased cellular proliferative and viability of GBM cells through causing cell cycle arrested. It had been reported that lncRNAs could influence cell migration/invasion in multiple carcinomas [18,19]. Whether or not LINC01207 could influence cell migration/invasion in GBM cells was unknown. In this study, we found LINC01207 downregulation obviously inhibited LN-229 and Hs 683 cells migration/invasion, which was consistent with previous reports. Other studies had proven that induction of EMT in breast cancer cells was not related to enhancing tumor-initiating capacity, but instead, conferred a CD44+/CD24- phenotype and the malignant properties, including cell proliferation, metastasis, chemotherapy and radiation resistance [26]. In our research, over-expression of LINC01207 upregulated E-cadherin mRNA and protein levels, also downregulate vimentin and N-cadherin expression levels, thus LINC01207 was a regulator of EMT progression. Meanwhile, LINC01207 influenced the expression of EMT downstream markers, which was consistent with the previous reports.

Aberrant regulation of the Wnts extensively participated in occurrence and progression of many diseases, including neurodegenerative diseases, multiple cancers, and autoimmune diseases [27,28]. EMT grants cells with stem-like characteristics, including metastasis with acquired with mesenchymal as well a loss of epithelial properties, and some studies have revealed that EMT could be induced by multiple pathways, such as the Wnt, MAPK, and PI3K pathways [29–31]. Also, some lncRNAs have been shown to participate in the main components of the Wnt/β-catenin pathway and influence the onset of tumorigenesis. For instance, lncRNA-OTUD6B-AS1 expression was decreased in human clear cell renal cell carcinoma and suppressed cell viability by regulating the Wnt signaling pathway [32].

Lin-TUG1 was abnormally expressed in colorectal cancer and could improve its therapeutic efficacy via targeting TUG1-Wnt/β-catenin signaling pathway [33]. Our study showed that LINC01207 re-expressed in LN-229 and Hs 683 cells restrained the expression of EMT related markers; our experiments also suggested that SOX2 mRNA and proteins were downregulated in this procession since SOX2 was a receptor for hyaluronic acid and influenced differences in cancer features and phenotypes containing stemness [34]. These findings revealed that LINC01207 may function as an effective regulator of GBM stemness. Meanwhile, the expression level of GSK-3β was obviously increased, while the β-catenin and p-GSK-3β were

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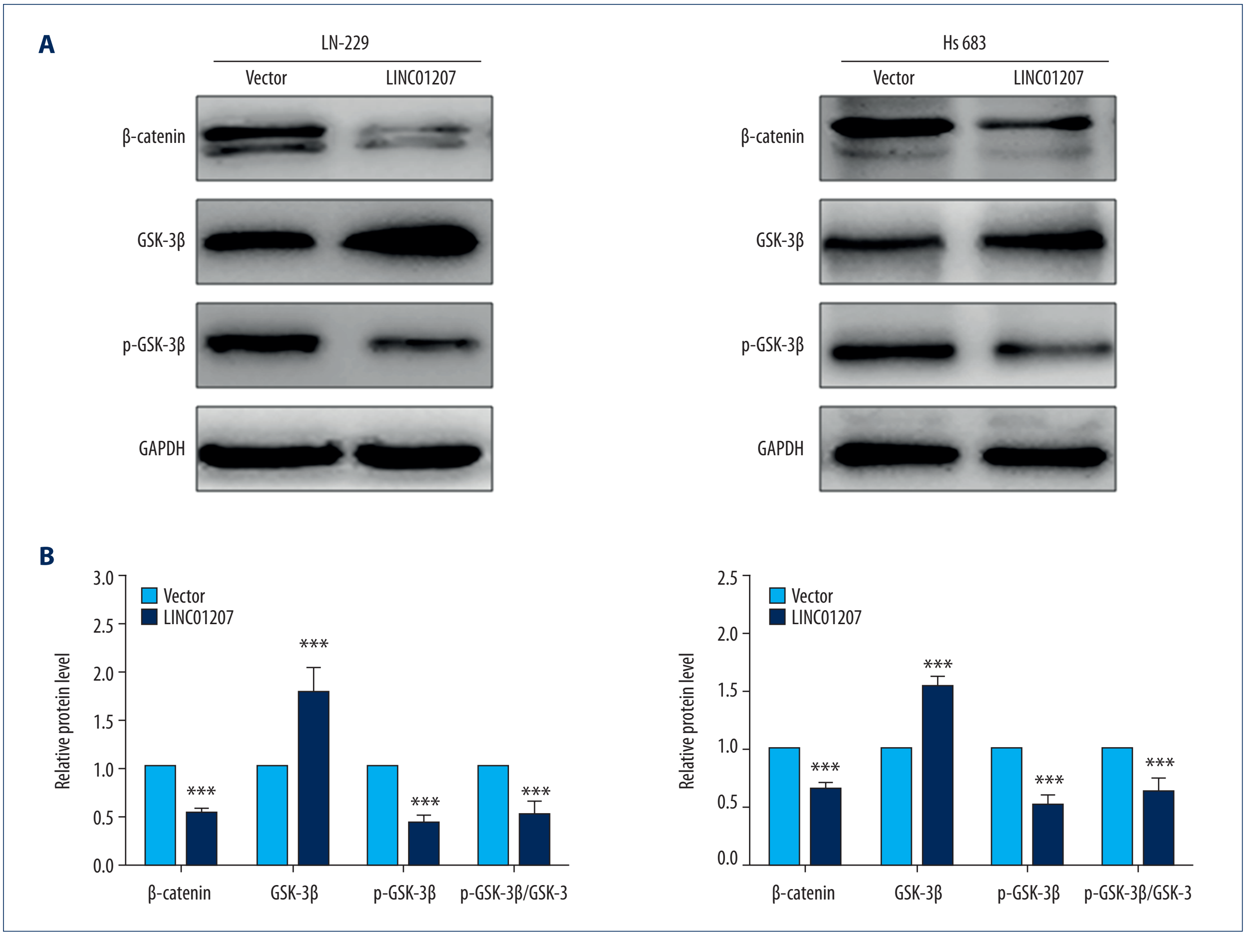

Figure 6. LINC01207 antagonist GSK-3β/β-catenin signaling pathway. (**A**) Western blotting detected the expression of GSK-3β/β-catenin signaling pathway related proteins after LINC01207 over-expression. (**B**) Quantitative analysis of GSK-3β/β-catenin signaling pathway related proteins. *** *P*<0.001.

decreased in experimental group cells, which was consistent with previous studies. Cumulatively, our research suggested that LINC01207 significantly suppressed the GSK-3β/β-catenin signaling pathway, a crucial role mechanism in occurrence and development of GBM.

Conclusions

In summary, our study results showed that LINC01207 was downregulated in GBM. We further founded that LINC01207 re-expressed in LN-229 and Hs 683 cells suppressed cellar proliferation, viability and metastasis by modulating GSK-3β/β-catenin signaling pathway. Taken together, our studies present potential new therapy for the diagnosis and treatment of GBM.

Availability of data and materials

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

Ethics approval and consent to participate

The present study was approved by institutional Ethics Renmin Hospital, Hubei University of Medicine. Patients provided written informed consent.

Conflict of interest

None.

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