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The Long Intergenic Noncoding RNA 00707 Sponges MicroRNA-613 (miR-613) to Promote Proliferation and Invasion of Gliomas

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Abstract

Background: Glioma is one of the most deadly malignant tumors in humans. Long non-coding RNA (lncRNA) plays a key role in the occurrence, development and invasion of tumors by regulating oncogenic and tumor suppressor pathways. However, the role and action mechanism of long intergenic non-coding RNA 00707 (LINC00707) in gliomas have not been elucidated. This study aimed to investigate the interaction between LINC00707 and miR-613 as well as its role in gliomas. **Materials and Methods:** The expression levels of LINC00707 and miR-613 were detected by qRT-PCR. The chi-square test was used to analyze the correlation between LINC00707 expression and clinicopathological parameters. CCK-8 and colony formation assays were used to detect glioma cell proliferation; and wound healing and transwell assays were used to detect glioma cell migration and invasion. The relationship between LINC00707 and miR-613 was predicted by Starbase, and verified by qRT-PCR and dual luciferase reporter gene assay. **Results:** LINC00707 was up-regulated in gliomas. Up-regulated LINC00707 increased the proliferation, migration and invasion of glioma cells, and silenced LINC00707 reduced these abilities. The increase of the expression level of LINC00707 down-regulated miR-613 in glioma cells, while the inhibition of the expression level of LINC00707 up-regulated miR-613 in glioma cells. The high expression of LINC00707 was related to the Karnofsky performance status (KPS) score and WHO staging. LINC00707 could offset the ability of miR-613 to inhibit glioma proliferation and invasion. **Conclusion:** LINC00707 promotes proliferation and invasion of glioma cells by sponging miR-613. The regulatory axis of LINC00707/miR-613 provides new insights into the mechanism and treatment of gliomas.

Keywords
LINC00707, miR-613, glioma, proliferation, invasion

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Introduction

Glioma is among the most deadly tumors, which causes more than 400,000 deaths per year; its prognosis is extremely adverse, with a 5-year survival rate less than 15%.[1,2] It is characterized with high relapse rate after surgery, low cure rate and high mortality, due to its anatomical location and invasive growth.[3] For those patients with high-grade gliomas (mainly grade III and IV, based on WHO classification), the prognosis of the patients is even worse.[4] Accumulating researches indicate that the dysregulation of long non-coding RNAs (lncRNAs) and microRNAs (miRs) is associated with the tumorigenesis and progression of gliomas.[5] Clarifying the biological functions of lncRNAs and miRs in glioma can probably provide novel clues for the diagnosis and treatment of this deadly disease, and improve the clinical outcome of the patients.

LncRNA consists of more than 200 bases in length and lacks protein coding ability. It plays a key role in the occurrence, development and invasion of tumors by regulating oncogenic and tumor suppressor pathways.[6,7] Numerous studies have shown that abnormal regulation of lncRNA in a variety of tumors may affect cell proliferation and apoptosis, and it participates in tumorigenesis and drug resistance.[8-12] As a carcinogenic lncRNA, LINC00707 is up-regulated in lung

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adenocarcinoma, gastric cancer, and liver cancer. For example, LINC00707 promotes proliferation and migration of lung adenocarcinoma cells by modulating cell division cycle 42.[13] LINC00707 promotes the proliferation and invasion of gastric cancer by interacting with mRNA stablizing protein human antigen R.[14] LINC00707 also increases cyclin-dependent kinase 14 by sponging miR-206 to promote progression of liver cancer.[15] However, the expression, mechanism and clinical significance of LINC00707 in gliomas have not been fully investigated.

MicroRNAs (miRs) are small (about 22 bases), single-stranded and non-coding RNAs that bind to the complementary sites of the 3′-untranslated region (3′-UTR) of the target gene to suppress or block translation.[16] There is increasing evidence that dysregulation of miRs contributes to the development of cancer, such as miR-613, which suppresses the progression of laryngeal squamous cell carcinoma by modulating PDK-1.[17] miR-613 suppresses retinoblastoma cell proliferation and invasion by targeting E2F5.[18] miR-613 suppresses the progression of gastric cancer by inhibiting brain-derived neurotrophic factors.[19] Many miRs have been shown to regulate the development of gliomas. For example, miR-29a/b/c acts by targeting CDC42 as a tumor suppressor gene for gliomas and predicts the prognosis of patients. Blocking the MIR155HG/miR-155 axis inhibits mesenchymal transition in gliomas.[20] Serum exosome miR-301a is a potential diagnostic and prognostic biomarker for human glioma.[21] There are also articles showing that miR-613 impedes proliferation and invasion of glioma cells by targeting cyclin-dependent Kinase 14 (CDK-14).[22] However, the roles of miR-613 in glioma are not fully elucidated and deserve further study.

This study investigated the expression, function and clinical significance of LINC00707 in gliomas. The expression of LINC00707 was found to be significantly up-regulated in gliomas and correlated with KPS score and WHO staging. We confirmed that LINC00707 could regulate the proliferation, migration and invasion of glioma cells. With the help of StarBase databse (starbase.sysu.edu.cn), we identified that miR-613 was a potential target miR of LINC00707. We further demonstrated that LINC00707 could promote the development of glioma through sponging miR-613, which might reveal the mechanism of growth and invasion in glioma.

Materials and Methods

Tissue Specimens and Cell Lines

89 pairs of primary glioma and normal tissues that were more than 2 cm adjacent to the lesions were collected from Xiangyang Central Hospital from January 2014 to June 2018. The collection and use of patient tissue samples were approved by the Ethics Committee of Xiangyang Central Hospital. None of the patients received preoperative anti-tumor treatment before specimens collection. Immediately after surgical resection, tissue samples were stored with liquid nitrogen at −80 °C. The clinical data of all subjects were shown in Table 1.

Table 1. Correlation Between Linc00707 Expression and Pathological Parameters in Glioma.

Pathological parameters	Number of patients	LINC00707 Expression		Chi-square	*p*-Value
		Low	High		
Age				0.0104	0.9186
<50	34	1222			
≥50	55	2035			
Gender				1.3515	0.2450
Man	49	1534			
Woman	40	1723			
KPS				4.2053	0.0403
≥70	40	1921			
<70	49	1336			
WHO grade				10.0071	0.0067
I	44	2222			
II	20	713			
III/IV	25	322			

Human glioma cell lines U87, U251, SHG-44, A172 and T98G were purchased from China Center for Type Culture Collection (Wuhan, China). Human normal astrocyte cell lines NHA and human embryonic kidney cell line (HEK-293) were purchased from Sanger Biotechnology Co., Ltd. (Hongkou, Shanghai, China). All cells were cultured in RPMI-1640 medium (YajiBio, Minhang, Shanghai, China), 10% fetal bovine serum (FBS, EXcell, Shanghai, China) and 1% penicillin and 1% streptomycin (Invitrogen, Shanghai, China) in a 5% CO_2, 37 °C incubator. The solution was changed once every 3 days until the bottom of culture bottle was covered with cells for passage. Cells in all of the following experiments were taken in the logarithmic growth phase.

Real-Time Quantitative PCR Analysis (qRT-PCR)

All RNAs were extracted with TRIzol reagent (Invitrogen, Shanghai, China) according to the instructions. miR-613 was amplified using TaqMan miR-613 MicroRNA kits (Applied Biosystems, Foster City, CA), and then converted to cDNA with SuperScript First Strand cDNA System (Invitrogen, Shanghai, China). qRT-PCR analysis was performed on an ABI Step One real-time PCR system (Applied Biosystems) of the SYBR Premix Ex Taq kit (Takara, Dalian, China). Quantitative real-time polymerase chain reaction conditions were: predenaturation at 95 °C for 10 minutes, 95 °C for 15 seconds, 60 °C for 15 seconds, 45 cycles, and the fluorescence signal temperature was 60 °C. GAPDH was used as the internal reference to detect the expression level of LINC00707, and U6 as the internal reference to detect miR-613. The $2^{(-\Delta\Delta Ct)}$ method was used for statistical analysis. Each experiment was repeated and measured 3 times.

LINC00707 Primer sequence: 5′-TCACATCTGTGAAAAGAGTGCT-3′ (forward) and 5′-CTGGACTGTGAGTACCAGGC-3′ (reverse);

miR-613 Primer sequence: 5′-GTGAGTGCGTTTC-CAAGTGT-3′ (forward) and 5′-TGAGTGGCAAAGAAG-GAACAT-3′ (reverse);

GAPDH Primer sequence: 5′-GAGTCAACGGATTTG-GTCGT-3′ (forward) and 5′-TTGATTTTGGAGGGAT-CTCG-3′ (reverse)

U6 Primer sequence: 5′-TTGGTCTGATCTGGCACATA-TAC-3′ (forward) and 5′-AAAAATATGGAGCGCTTCACG-3′ (reverse)

Cell Transfection

U251 cells were washed with PBS buffer (Amresco, America), repeated for 3 times, trypsinized for 2 minutes and transferred to a sterile 15 mL centrifuge tube. They were centrifuged and counted, and seeded at 4×10^5 cells per well in a 6-well plate. The fusion rate was about 70%. The serum-free medium was used to dilute the transfection reagent at 3 μL/L, and incubated at 37 °C for 20 minutes. The serum-free medium was used to dilute the liquid in the si-LINC00707, and the control group at a concentration of 50 μmol/L was incubated at room temperature for 5 minutes, mixed with the same volume of transfection reagent, and continued to culture in a 37 °C incubator. After 12 hours, the state of the transfected cells was observed, and the serum-free medium was changed to the complete medium to continue culture. After another 48 hours, the RNA was extracted to verify the transfection efficiency. In the same manner, the overexpression-LINC00707 and the control group were transfected into the glioma cell line U87. The LINC00707 knockout plasmid and the overexpression plasmid were designed and integrated by GenePharma (Shanghai, China). The blank plasmid was served as a control. U251 and U87 cells were transfected with liposome 3000 (Invitrogen, Foster City, CA, USA) according to protocol. Stably transfected cells were selected using Geneticin (Sigma-Aldrich, St Louis, MO, USA).

CCK-8 Assay

Cell growth curves were determined using CCK-8. Cells were harvested in log phase and digested with trypsin (0.25%). A cell suspension was prepared in 10% FBS and placed in the medium. U251 or U87 cells (3000 cells per well) were placed in 96-well plates and incubated for 1, 2, 3 and 4 days, respectively. Then, 10 μl of Enhanced Cell Counting Kit-8 (Beyotime, Beijing, China) was added and incubated at 37 °C for 1 hour. The absorbance was measured at 450 nm. Each experiment was repeated and measured 3 times.

Plate Cloning Formation Assay

Briefly, si-LINC00707 transfected cells were placed in each well of the 6-well plate for 24 hours after transfection (500 cells per well). Then, they were cultured in the RPMI-1640 medium, and added with 10% FBS and 1% streptomycin for 2 weeks. Cell colonies were fixed with 4% paraformaldehyde for 30 minutes, and visible cell colonies were stained for 10 minutes with 0.1% crystal violet (0.1%, Macklin, Shanghai, China). After washing 3 times with PBS buffer (Amresco, America), the stained colonies were imaged and counted using an optical microscope. Each experiment was repeated and measured 3 times.

Wound Healing Assay

Transfected glioma cells (2×10^5) were added to the 12-well plate and incubated at 37 °C for 24 hours at 5% CO_2. A 200 μl sterilizing tip was used to scribe a direct scratch in the middle of the confluent monolayer and the initial distance (0 time) of the scratch was measured under the microscope. The distance of the scratch was measured after incubation for 24 hours and 48 hours in a constant temperature incubator. Each experiment was repeated and measured 3 times.

Transwell Assay

After being dispersed with 0.25% trypsin, the glioma cells were centrifuged, resuspended, and dispersed in a single well of the 24-well culture plate. Matrigel Chambers (8 μm Pore size; Corning, Beijing, China) were used in invasion experiments, but not in migration experiments. 5×10^4 transfected cells were placed in the upper chamber, and added with Matrigel. 10% FBS medium was placed in the lower chamber, and 400 μL of RPMI-1640 was filled. After incubation at 37 °C for 24 hours, cells that failed to migrate were removed from the upper chamber. The Transwell membrane was fixed with 4% paraformaldehyde for 10 minutes and stained with 0.5% crystal violet. After being rinsed off by the tap water, they were counted under an inverted microscope. All experiments were performed in triplicate and repeated 3 times.

Dual Luciferase Reporter Gene Assay

Dual-luciferase reporter assay system (Promega, Madison, WI, USA) was used to perform a luciferase reporter assay. The target fragment of wild type LINC00707 and mutant LINC00707 was constructed and integrated into pGL3 vector (Promega, Madison, WI, USA) to build pGL3-LINC00707-wild type (LINC00707-wt) and pGL3-LINC00707-mutant (LINC00707-mut) reporter vector. HEK 293 cells were co-transfected with LINC00707-wt or LINC00707-mut with miR-613 mimics or a negative control. After 48 hours of transfection, luciferase activity was determined according to the manufacturer's instructions. All experiments were performed in triplicate and repeated 3 times.

Statistical Methods

SPSS software (version 20.0, Chicago, IL, USA) was used for statistical analysis. All data were expressed as mean $\pm$ SD. Statistical analysis was performed using the student t test. Chi square test was used to analyze the correlation between LINC00707 and pathological parameters. Pearson correlation coefficient was calculated to analyze the association between

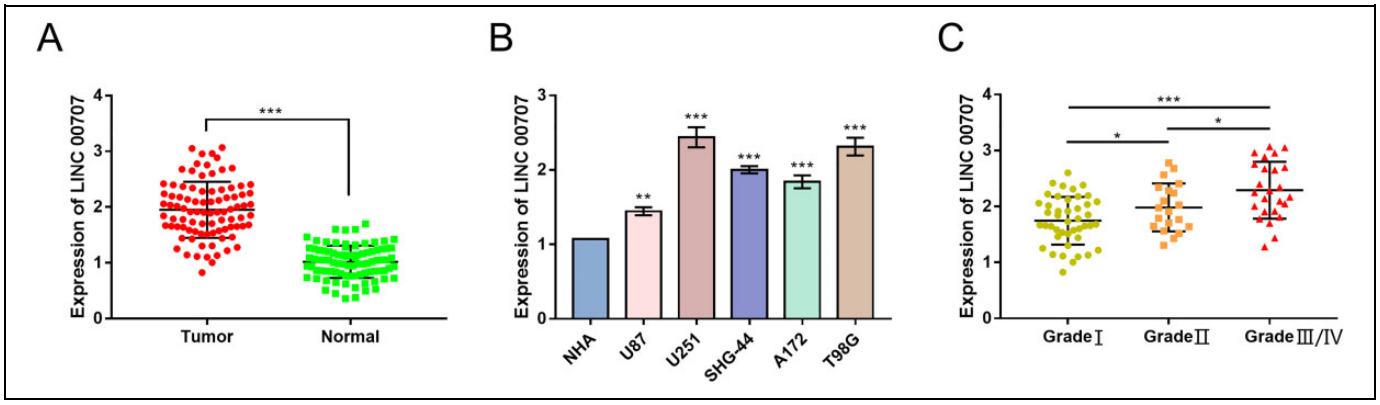

Figure 1. LINC00707 was up-regulated in glioma tissues and cells. A, Expression of LINC00707 in 89 paired primary glioma and normal tissues was determined by qRT-PCR. LINC00707 was found to be up-regulated in glioma tissues. ***P < 0.001. B, LINC00707 expression in glioma cell lines (U87, U251, SHG-44, A172, T98G) and NHA cells were detected by qRT-PCR. C, LINC00707 level was showed to be higher than NHA in all glioma cell lines. **P < 0.05, ***P < 0.01. LINC00707 expression was found to be significantly up-regulated in grade III/IV. *P < 0.05,***P < 0.001.

LINC00707 and miR-613 in clinical samples. $P < 0.05$ was considered statistically significant.

Results

The Expression of LINC00707 in Glioma Was Up-Regulated

To determine whether LINC00707 was abnormally expressed in gliomas, we used qRT-PCR to detect the expression levels of LINC00707 in 89 pairs of glioma and adjacent normal tissues. We found that the expression of LINC00707 was significantly up-regulated in gliomas compared to that in adjacent non-tumor samples ($P < 0.05$, Figure 1A). Then, the expression of LINC00707 in the glioma cell line was detected by qRT-PCR. As shown, the expression of LINC00707 was significantly up-regulated in glioma cell lines compared to that in the normal cell line NHA ($P < 0.05$, Figure 1B). When 89 glioma specimens were stratified by WHO stage, the expression level of LINC00707 was positively correlated with WHO stage ($P < 0.05$, Figure 1C). In addition, according to the expression of LINC00707, we divided the glioma samples into the high and low expression groups of LINC00707, and then analyzed the correlation between the expression of LINC00707 and the clinicopathological features of glioma patients. As shown in Table 1, the high expression of LINC00707 was closely related to KPS score and WHO staging, but not to age and gender. These findings suggested that LINC00707 played an important role in the progression of glioma.

Down-Regulation of LINC00707 Inhibited Proliferation and Migration of Glioma Cells

Since LINC00707 was highly expressed in U251 cells, we chose the U251 cell line for this experiment. To investigate the effect of LINC00707 on proliferation, migration and invasion of glioma cells, we constructed the LINC00707 knockout U251 cells by expressing lentivirus of shRNA (Supplemental Figure 1). Colony formation assay and CCK-8 assay showed that LINC00707 knockout significantly inhibited the proliferation of U251 cells ($P < 0.05$, Figure 2A & B, Supplemental Figure 2). As expected, we observed that LINC00707 knockout significantly reduced U251 cell migration and invasion by transwell migration and invasion assays ($P < 0.05$, Figure 2C & D, Supplemental Figure 3). Wound healing assay didn't get a significant statistic difference, however, we observed that the si-LINC00707 group had a slower speed of healing (Figure 2E, Supplemental Figure 4). The above results indicated that the down-regulation of the LINC00707 had a significant inhibitory effect on the growth and invasion of glioma cells.

Up-Regulation of LINC00707 Promoted Proliferation and Migration of Glioma Cells

Since LINC00707 was lowly expressed in U87 cells, we chose the U87 cell line for gain-of-function experiment. We constructed U87 cells with overexpressed LINC00707 (Supplemental Figure1). Colony formation assay and CCK-8 assay showed that overexpression of LINC00707 significantly promoted the proliferation of U87 cells ($P < 0.05$, Figure 3A & B, Supplemental Figure 2). As expected, we observed that overexpression of LINC00707 significantly increased U87 cell migration and invasion by Transwell assays ($P < 0.05$, Figure 3C & D, Supplemental Figure 3). Although no significant statistical difference was observed in wound-healing assay, we can observe the upregulated trend of U251 cell migration promoted by LINC00707 (Figure 3E, Supplemental Figure 4). The above results indicated that the up-regulation of LINC00707 gene had a significant effect on the growth and invasion of glioma cells.

LINC00707 Acted on miR-613 Directly

Since the cytoplasmic lincRNAs were thought to be a small RNA sponge and could induce inhibition of target miRNA

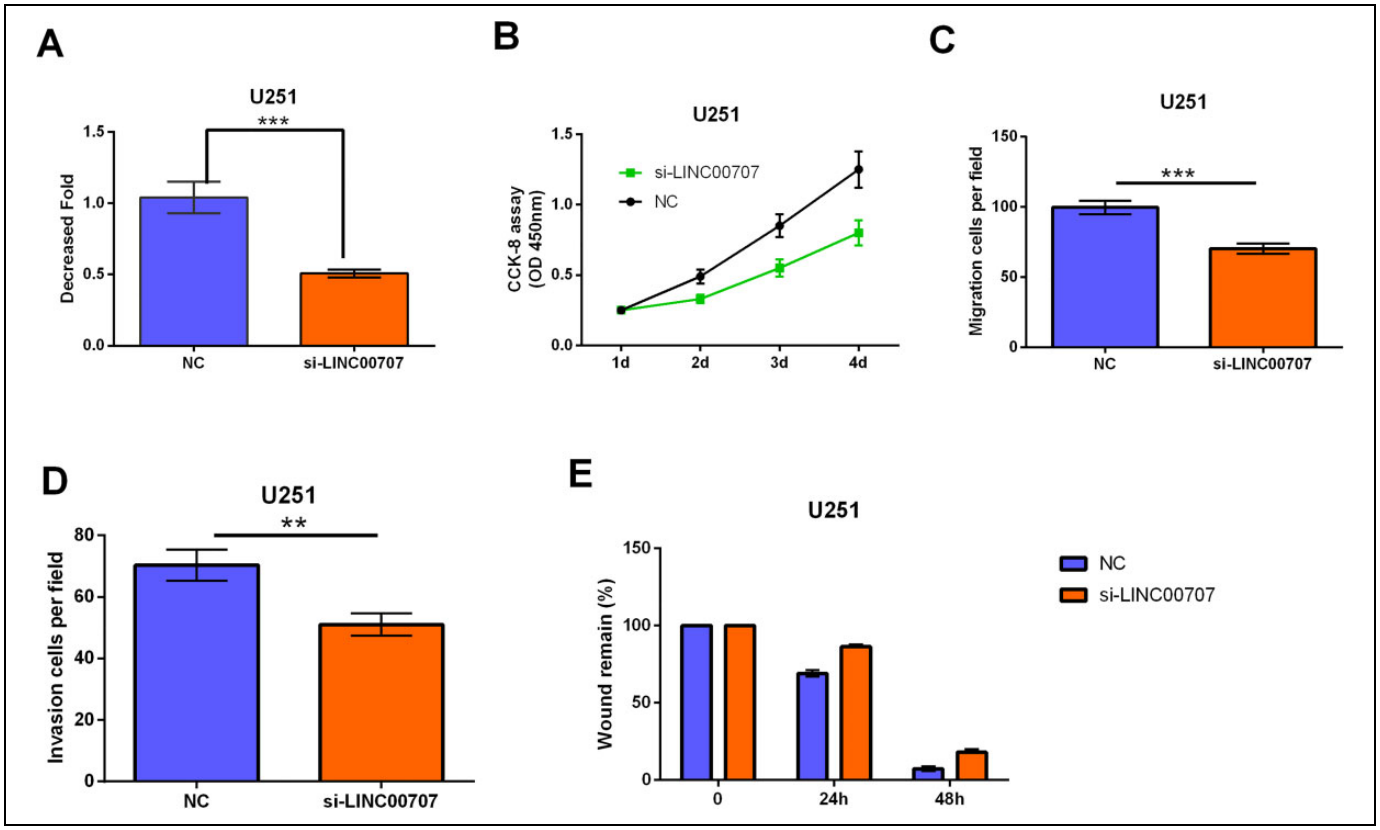

Figure 2. Down-regulation of LINC00707 Inhibited Proliferation and Migration of Glioma Cells. A, Colony formation assay showed that the proliferative ability of U251 cells were inhibited in LINC00707 knockdown (si-LINC00707) group compared to normal control (NC) group. ***P < 0.001. B, CCK-8 assay confirmed that the proliferation of U251 cells were inhibited in si-LINC00707 group compared to NC group. C, Transwell migration assay showed that the migration of U251 cells were inhibited in si-LINC00707 group compared to NC group. ***P < 0.001. D, Transwell invasion assay showed that the invasion of U251 cells were inhibited in si-LINC00707 group compared to NC group. **P < 0.01. E, Wound healing assay showed that the migration of U251 cells were inhibited in si-LINC00707 group compared to NC group.

activity, suggesting that LINC00707 might play its role in this way.[23] We searched the StarBase database for potential target genes for LINC00707. As shown, LINC00707 contained a conserved target site for miR-613 (Figure 4A). We used qRT-PCR to determine the expression of LINC00707 and miR-613 in glioma tissues (Figure 4B). 30 cases were randomly selected from 89 glioma tissues for analysis, and the correlation coefficient r between LINC00707 and miR-613 was calculated to be -0.4256 ($P < 0.05$, Figure 4B). It was initially confirmed that there was a negative regulatory relationship between LINC00707 and miR-613.

To further verify the direct binding of LINC00707 to miR-613, a dual luciferase reporter assay was employed. It showed that overexpression of miR-613 significantly reduced luciferase activity of the luciferase of wild type LINC00707 compared to the negative control, whereas overexpression of miR-613 did not show any impact on luciferase activity of LINC00707-mut (Figure 4C). This demonstrated that miR-613 was a target of LINC00707. After down-regulating LINC00707 of glioma cells U251 and U87, the expression of miR-613 was significantly increased (Figure 4D), further confirming the regulatory relationship between LINC00707 and miR-613.

LINC00707 Regulated Proliferation and Migration of Glioma Cells by Targeting miR-613

To further investigate the role of LINC00707/miR-613 axis in glioma progression, U251 cells with LINC00707 knocked down were co-transfected with miR-613 mimics (Supplemental Figure 1). CCK-8 and colony formation assays demonstrated that transfection of miR-613 mimics reduced the proliferation and colony formation ability of U251 cells, whereas overexpression of LINC00707 inhibited the effects of miR-613 ($P < 0.05$, Figure 5A & B, Supplemental Figure 5). Transwell confirmed that transfection of miR-613 mimics reduced the migration capacity of U87 cells, which could be offset by overexpression of LINC00707 ($P < 0.05$, Figure 5C & D, Supplemental Figure 6). Although no significant statistical difference was observed, the tendency of U251 cell migration to be inhibited by miR-613 was observed, and the effect of miR-613 was counteracted by LINC00707 (Figure 5E,

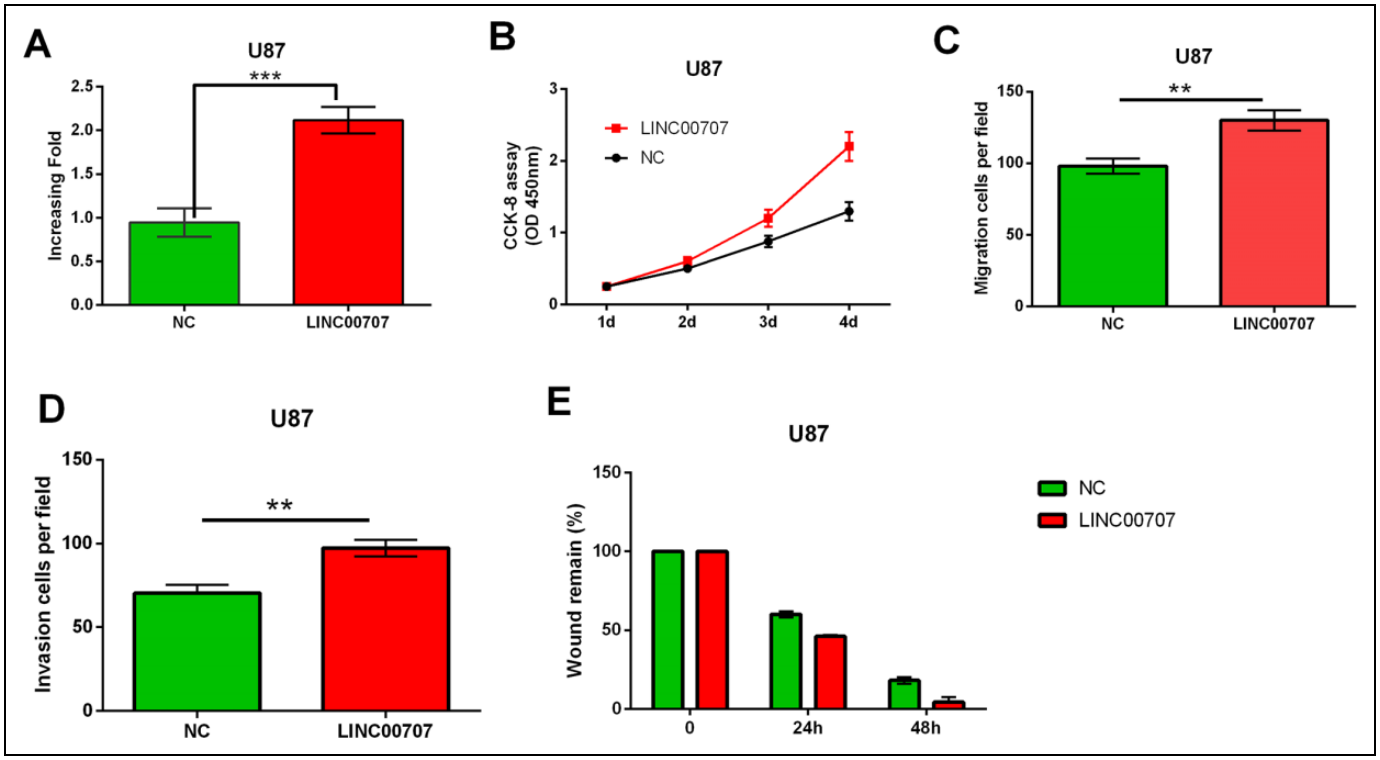

Figure 3. Up-regulation of LINC00707 Promoted Proliferation and Migration of Glioma Cells. A, Colony formation assay showed that the proliferative ability of U87 cells were promoted in LINC00707 mimics (LINC00707) group compared to normal control (NC) group. ***P < 0.001. B, CCK-8 assay confirmed that the proliferation of U87 cells were promoted in LINC00707 group compared to NC group. C, Transwell migration assay showed that the migration of U87 cells were promoted in LINC00707 group compared to NC group. **P < 0.01. D, Transwell invasion assay showed that the invasion of U87 cells were promoted in LINC00707 group compared to NC group. **P < 0.01. E, Wound healing assay showed that the migration of U87 cells were promoted in LINC00707-in group compared to NC group.

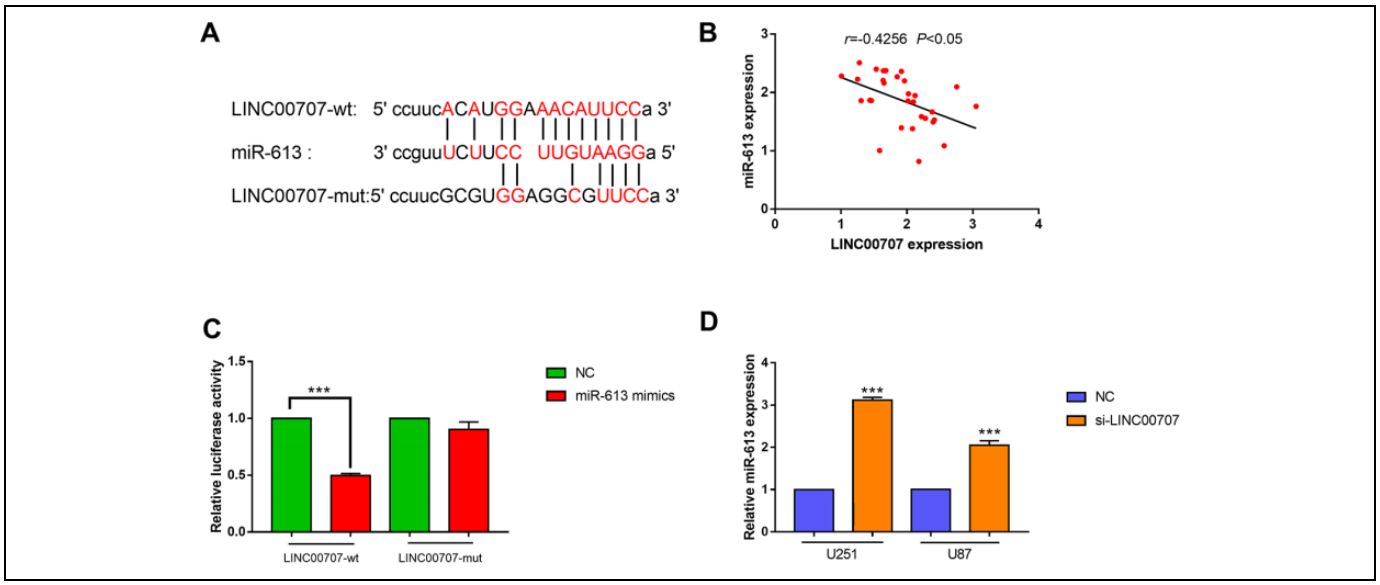

Figure 4. LINC00707 acted on miR-613 directly. A, Schematic diagram of miR-613 putative binding sites in wild type and mutant LINC00707. B, The expression level of LINC00707 and expression of miR-613 in glioma tissues of 30 patients were determined by qRT-PCR respectively. Correlation analysis confirmed the negative correlation between LINC00707 and miR-613. C, Luciferase assay of LINC00707-wt vectors or LINC00707-mut vectors cotransfected with miR-613 mimics and U6 in U251 cells. *** P < 0.001. D, qRT-PCR showed that in U251 and U87 cells miR-613 expression were both inhibited after LINC00707 had been up-regulated. *** P < 0.001.

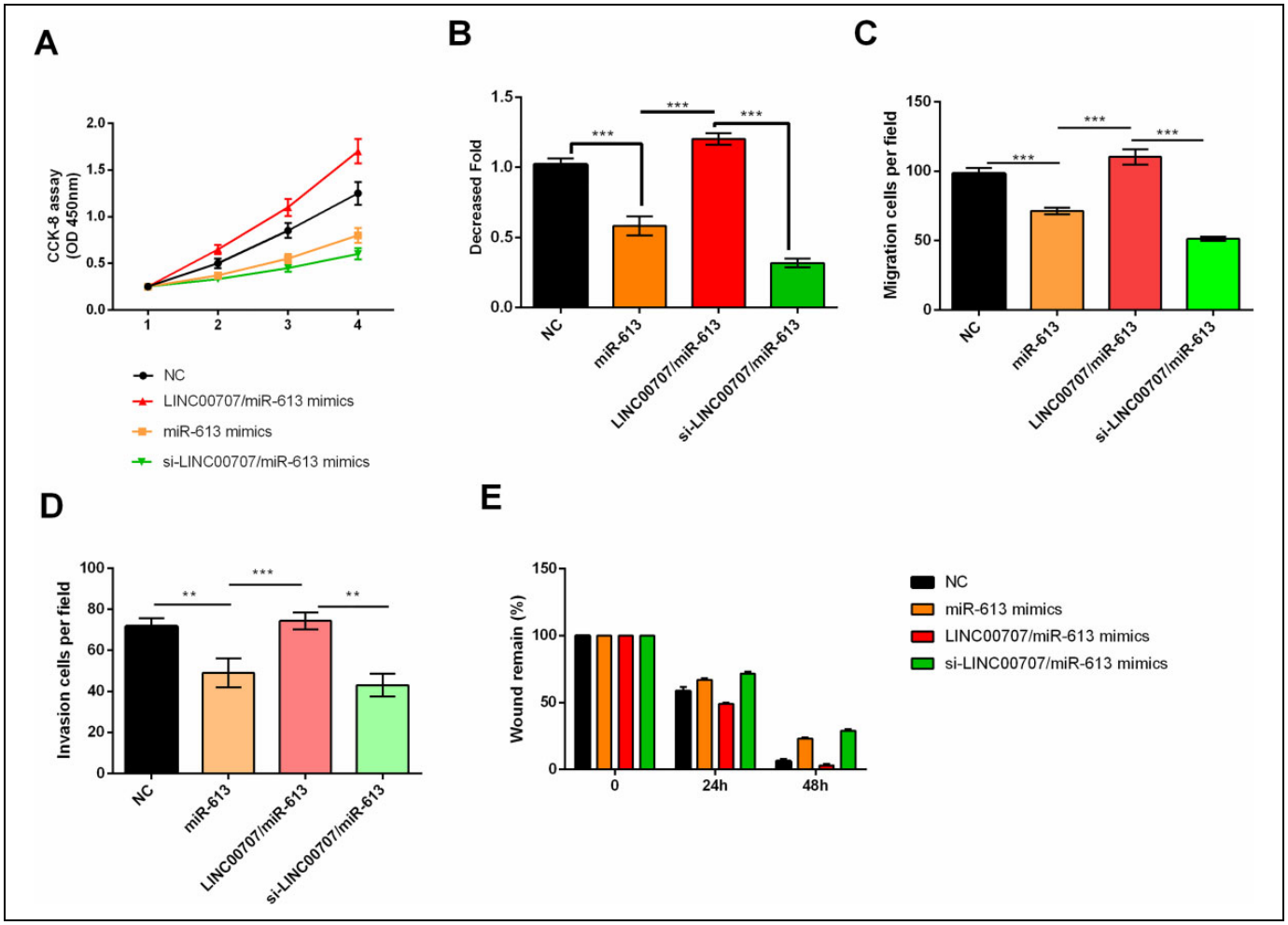

Figure 5. LINC00707 regulated proliferation and migration of glioma cells by targeting miR-613. A, CCK-8 assay showed that U251 cells with miR-613 mimics multiplied slower than NC group. And LINC00707 can offset the effect of miR-613 mimics while si-LINC00707 can promote this kind of effect. B, Colony formation assay showed that miR-613 mimics inhibited the proliferation of U251 cells. LINC00707 can offset this effect while si-LINC00707 can promote this effect. *** $P < 0.001$. C and D, Transwell assay showed that miR-613 mimics inhibited the migration and invasion of U251 cells. LINC00707 can offset this effect while si-LINC00707 can promote this effect. ** $P < 0.01$, *** $P < 0.001$. E, Wound healing assay confirmed that miR-613 mimics inhibited the migration of U251 cells. LINC00707 can offset this effect while si-LINC00707 can promote this effect.

Supplemental Figure 7). Therefore, we concluded that LINC00707 could affect the progression of glioma through sponging miR-613.

Discussions

LncRNAs can function through epigenetic, transcriptional, and post-transcriptional regulations. For example, lncRNA CASC 2 regulates the development of liver cancer by miR-362-5p and NF-κB.[24] LncRNA SNHG15 promotes human breast cancer proliferation, migration and invasion by sponging miR-211-3p.[25] LINC00675 suppresses progression of gastric cancer by enhancing the phosphorylation of Ser83 vimentin.[26] LncRNA plays an important role in the development and invasion of various cancers, so it is important to identify lncRNAs that are diagnostic for glioma. Recent studies have shown that lncRNA colon cancer-associated transcript 1 promotes glioma tumorigenesis by sponging miR-181b.[27] Highly expressed lncRNA CCND2-AS1 promotes glioma cell proliferation via Wnt/β-catenin signaling.[8] Silencing of lncRNA CCDC26 retains the growth and migration of glioma cells in vitro and in vivo by targeting miR-203.[28] lncRNA UCA1 interacts with miR-182 to regulate glioma proliferation and migration by targeting inhibitor of apoptosis-stimulating protein of p53.[29]

As a key regulator in the development and progression of cancer, LINC00707 may become a potential therapeutic target for cancers.[13-15,30] For instance, LINC00707 promotes the progression of hepatocellular carcinoma by activating the ERK/JNK/Akt signaling pathway.[30] However, no studies have fully confirmed the relationship between LINC00707 and glioma. In the current study, we focused on the function and potential mechanism of LINC00707 in glioma. We observed that LINC00707 expression was significantly higher in glioma tissues than that in adjacent normal tissues, and its upregulation

was positively correlated with unfavorable pathological indexes. Gain-of-function and loss-of function experiments were used to explore the effects of LINC00707 on the biological behaviors of glioma cells. The data showed that the LINC00707 mimics significantly promoted the proliferation, migration and invasion of glioma cells. In addition, the inhibition of LINC00707 significantly suppressed these malignant phenotypes of glioma cells.

MiRs are one of the important factors in tumor formation and progression, and their up- or down-regulation has a certain influence on the biological behaviors of tumor cells. For example, dysregulated miR-27a-3p promotes proliferation and migration of nasopharyngeal carcinoma cells by targeting mitogen-activated Protein kinase 10.[31] miR-221 mediates the epithelial-mesenchymal transition of hepatocellular carcinoma by targeting adiponectin receptor 1.[32] miR-202 promotes apoptosis in esophageal squamous cell carcinoma cells by targeting heat shock Transcription factor 2.[33] miR-613 is a proven tumor suppressor that plays a role in suppressing cancer in many cancers including osteosarcoma, renal cell carcinoma and papillary thyroid carcinoma, and its target genes includes C-X-C chemokine receptor type 4, frizzled protein 7, sphingosine kinase 2 and so on.[34-36] In addition, there have been studies confirming that miR-613 exerts a tumor-suppressive effect in gliomas by targeting SRY-box 9 and vascular endothelial growth factor A.[37,38] In this work, we predicted miR-613 as a target for LINC00707 by bioinformatics analysis, confirming the correlation between the 2 in glioma cells. Our data have shown that up-regulation of LINC00707 can inhibit the level of miR-613 in glioma, and down-regulation of LINC00707 has the opposite effect. In addition, transfection of the LINC00707 overexpression vector counteracts the inhibition of proliferation and invasion of glioma cells by miR-613. To our best knowledge, this is the first time the link between LINC00707 and miR-613 is confirmed experimentally. Our work partly explained the mechanism of miR-613 dysregulation in glioma.

This work has certain limitations. Firstly, i*n vivo* experiments are the key to further verify the oncogenic role of LINC00707 in gliomas. What's more, other downstream miRNAs of LINC00707 in glioma needs to be screened and identified; furthermore, to explore the potential of LINC00707 as a biomarker, more samples from multiple centers should be included, and the survival analysis of glioma patients based on the expression of LINC00707 is desirable.

Conclusion

In summary, LINC00707 is highly expressed in gliomas and associated with KPS score, tumor size and TNM stage. LINC00707 promotes proliferation, migration and invasion of glioma cells by sponging miR-613. Therefore, LINC00707 may be a potential biomarker for gliomas, and its inhibitors may be used to treat this deadly disease.

Authors' Note

Conceived and designed the experiments: LHD, HKQ; Performed the experiments: LHD; Statistic analysis: LHD; Wrote the paper: LHD. The data used to support the findings of this study are available from the corresponding author upon request. Our study was approved by the ethics review board of the Affiliated Hospital of Hubei University of Arts and Science (approval no. 2013-125). All patients provided written informed consent prior to enrollment in the study.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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