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Original Article

Knocking down LINC01116 can inhibit the regulation of TGF-β through miR-774-5p axis and inhibit the occurrence and development of glioma

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Abstract: Background: Many studies have shown that non-coding RNAs (ncRNAs), including long non-coding RNA (LncRNA) and micro RNA (miRNA), play a crucial regulatory role in glioma. LINC01116 is a newly discovered LncRNA, and the relationship between LncRNA and glioma is still under exploration. Method: LncRNAs with potential differences were screened through GEO database, and the expressions of LINC01116 and miR-744-5p/TGF-β1 in glioma tissues were tested using qRT-PCR. Changes in proliferation and migration/invasion of glioma were tested using CCK-8 and transwell assay. The expression changes of TGF-β1 were tested using qRT-PCR and Western blot. Targeted binding among LINC01116, miR-744-5p and TGF-β1 was verified using double luciferase reporter, RNA Immunoprecipitation (PIR) and RNA pull-down experiments. The effect of LINC01116 on tumor growth was determined by tumor allografting test. Results: GEO database and clinical research revealed that the expression level of LINC01116 in glioma increased, and the elevation of LINC01116 was closely related to the adverse prognosis of clinical patients. Functional experiments showed that the inhibition of LINC01116 could up-regulate miR-744-5p-mediated proliferation and metastasis of glioma cells. Western blot analysis and qRT-PCR analysis showed that LINC01116 regulated TGF-β1 by mediating miR-744-5p. Further cell behavior experiments showed that LINC01116 acted as miR-744-5p sponge to inhibit proliferation and metastasis caused by TGF-β1. Finally, the analysis of animal models in vivo showed that LINC01116 could regulate the tumor growth of glioma. Conclusion: LncRNA LINC01116 acts as an oncogene and promotes TGF-β1 mediated proliferation and metastasis by acting as competitive endogenous RNA (ceRNA) in glioma.

Keywords: LINC01116, miR-744-5p, TGF-β1, glioma, ceRNA

Introduction

Gliomas are common primary malignancies in brain, and it accounted for 70% of primary malignancies in brain [1]. There are data showing that 4-5 out of 100,000 people suffer from the disease every year [2]. At present, the clinical therapy of glioma mainly includes surgery, radiotherapy and chemotherapy [3]. Among them, surgical resection combined with radiotherapy and temozolomide adjuvant chemotherapy is the first choice [4]. Although a diagnosis and treatment plan for glioma has been formulated in recent ten years, the prognosis of glioma patients is still poor [5]. The data have shown that the median survival time of glioma patients is usually within 12 months after diagnosis, and even under the most favorable conditions, the estimated survival time will not exceed 2 years [6]. Therefore, an increasing number of researches are devoted to exploring the potential mechanism of gene regulation of glioma and developing more effective therapeutic schemes.

Long-chain non-coding RNA (LncRNA) is one of the members of non-coding RNA, with the length exceeds 200nt [7]. Studies have found

that LncRNA can regulate the expression level of transcribed and post-transcribed genes, resulting in protein inactivation or instability [8]. Many studies have proved that LncRNA can regulate the growth and metastasis of glioma. For example, [9] LncRNA UCA1 interacts with miR-182 to regulate glioma proliferation and migration by targeting iASPP. Other studies have found that [10] LncRNA HSP90AA1-IT1 promotes glioma through targeted miR-885-5p-CDK2 pathway. LINC01116 is a newly discovered LncRNA and is found to be highly expressed in mammary carcinoma [11], ovarian carcinoma [12] and osteogenic sarcoma [13]. Moreover, some studies found that [14] LINC01116 can regulate the tumorigenesis of glioma by targeting VEGFA, indicating that LINC01116 plays an important role in the occurrence of glioma, but we are still unclear about its in-depth mechanism.

Competing endogenous RNA (ceRNA) is a newly proposed theory, and it combines with microRNA (miR) through response elements to affect miR-induced gene silencing [15]. Studies revealed that [11] LncRNA can restrain the growth of tumor by binding to miR with specific binding sites. In this research, we found that miR-744-5p and LINC01116 have targeted binding sites through online prediction website for LINC01116 to bind miR. Previous reports indicated that miR-744-5p can target downstream target genes to inhibit the development of glioma.

Therefore, this study aimed to explore whether LINC01116 can inhibit the growth of glioma by regulating miR-744-5p and can provide potential targets for clinical treatment.

Methods and data

GEO database analysis

We logged into https://www.ncbi.nlm.nih.gov/gds/to get GSE103227 chip and obtain matrix file Series Matrix File(s). LINC01116 in matrix file was extracted and the expression level of LINC01116 in glioma was further analyzed. In addition, GSE103229 chip was selected to extract miR-744-5p in matrix file and further analyze its expression level in glioma.

Collection of clinical samples

A total of 50 pairs of glioma samples and PTBE tissues of patients treated in Kaifeng Central Hospital from May 2012 to May 2014 were collected during surgery. The samples were then transported in liquid nitrogen at -80°C for later use. The informed consent forms were obtained from the patients and their families. This study conformed to the ethics committee of Kaifeng Central Hospital and was in accordance with Helsinki Declaration.

Cell culture

NHA (normal human astrocytes), U251, H4, SW1783 and LN229 (glioma cells) were obtained from ATCC (USA), and the collected cells were cultured in DMEM (FBS; Sigma-Aldrich, St.Louis, MO, USA) including 10% fetal bovine serum. The cells were cultured in an incubator at 37°C with 5% CO_2.

Cell transfection

Cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Glioma cell lines were transfected with siRNA. siRNA (si-NC, si-LINC01116, si-TGF-β1), miRNA mimetic inhibitors (miR-NC, miR-744-5p-mimics, miR-744-5p-inhibit) and over-expression plasmids (pcDNA, pcDNA-LINC01116) were synthesized by Shanghai Gene Pharmaceutical Co., Ltd. In order to establish a nude mouse model, we also constructed a human recombinant lentivirus vector (SH-LINC01116) stably expressed by LINC 01116, packaged the lentivirus carrying sh-LINC01116 in human embryonic kidney cell line 293T, and collected the virus. U87, U251 and LN229 cells were infected with lentivirus, then puromycin was selected to establish a stable cell line, and the blank vector was used as the control (sh-NC).

qRT-PCR

The obtained samples (tissues, cells) were extracted with TRIzol reagent to obtain total RNA. The purity, concentration and integrity were detected using UV spectrophotometer and agarose gel electrophoresis. cDNA was synthesized from 1 μg RNA of random primers using a high capacity cDNA reverse transcription kit (Applied Biosystems, USA). One-step SYBR PrimeScript RT-PCR and ABI 7500 PCR were used for amplification. The amplification system and amplification conditions were configured and operated according to the kit instructions. PCR amplification was carried out for a total of 40 cycles, with 3 repeat wells for

each sample, and the experiment was carried out for 3 times. In this study, GAPDH was applied as internal reference for LncRNA and mRNA, U6 for miR. $2^{-\Delta\Delta ct}$ was used to analyze the data [16].

Western blotting (WB)

The collected cells were extracted with RIPA buffer solution to obtain total protein, the protein concentration was adjusted to 4 μg/μL using BCA kit, and 12% SDS-PAGE was used for electrophoresis separation. The protein was transferred to PVDF membrane after ionization, stained with Ponceau S working solution, rinsed in PBST for 5 min, washed, and sealed with 5% defatted milk powder for 2 h. anti-TGF-β1 and anti-GAPDH (overnight at 4°C) were incubated with HRP coupled secondary antibody (1:5000) for 2 h to test the blot. WB was observed by enhanced chemiluminescence system. Protein bands were quantified by optical density analysis using Quantity One software.

Cell proliferation detection

Cell proliferation was detected by CCK-8. Transfected cells were adjusted to 1×10^3 cells/well and inoculated into 96-well plates. Then 10 μL/well CCK-8 solution (Beyotime, Shanghai, China) was put in each well at 0 h, 24 h, 48 h and 72 h respectively. The absorbance (OD) was detected by microplate reader at 450 nm.

Cell invasion and migration detection

The migration and invasion of glioma were determined by Transwell assay. The cell invasion experiment was as follows: after 48-hour transfection with plasmid, the cells were starved for 6 hours and inoculated into 24-well cell culture inserts (BD Biosciences) with 8μm pore membranes. The membrane coating was applied for migration and invasion detection in the absence of Matrigel. Migrating and invading cells were stained with crystal violet, and five fields were counted using an optical microscope. Each experiment was repeated three times.

Cell apoptosis detection

Apoptosis was measured by flow cytometry. The specific steps were as follows: the transfected cells were collected, adjusted to $1 * 10^3$ cells/well, and inoculated in a 6-well plate. After incubation for 24 hours, cells were harvested and rinsed twice with phosphate buffered saline. Then the cells were resuspended in Annexin V-fluorescein isothiocyanate (Annexin V-FITC; 5 μL) and propidium iodide (5 μL) by Annexin V-FITC cell apoptosis detection kit. The apoptosis rate was measured by FACS Calibur.

Double fluorescein report

LINC01116 and TGF-β1 fragments containing miR-744-5p binding sites and wild-type or mutant sequences of binding sites were cloned into pmirGLO bifluorescase vectors to obtain report vector wild-type (LINC01116-WT, TGF-β1-WT) and mutant (LINC01116-MUT, TGF-β1-MUT). LINC01116/TGF-β1 wild-type and mutant vectors and miR-744-5p-mimics or miR-NC were co-transfected into glioma using Lipofectamine 2000. After 48 h, the relative luciferase activity (Promega, USA) was measured by double luciferase reporter gene assay.

RNA immunoprecipitation (RIP)

According to the manufacturer's instructions, the EZ-Magna RIP RNA binding protein immunoprecipitation kit was used for RIP analysis. Glioma cells were lysed using RIP lysis buffer, and the cell lysis solution was cultured with RIP buffer. Magnetic beads coupled with human anti-Ago2 antibody and mouse immunoglobulin G were used as the control. Co-precipitated RNA was isolated using TRIzol reagent and measured using quantitative RT-PCR (qRT-PCR).

Subcutaneous tumor formation in nude mice

Ten male BALB/c thymus-free nude mice (aged 4-6 weeks) were purchased from Charles River (Beijing, China) and randomly divided into sh-LINC01116 group and sh-NC group. The mice in sh-LINC01116 group were inoculated with 1×10^6 glioma cells, which were transfected with sh-LINC01116 in the right abdomen of each mouse. The mice in sh-NC group was inoculated with the same amount of sh-NC cells. The tumor volume of nude mice was calculated every 7 days [(length × width^2)/2]. The mice were killed 28 days later, tumor tissues of nude mice were collected and detected. All animal experiments were conducted referring

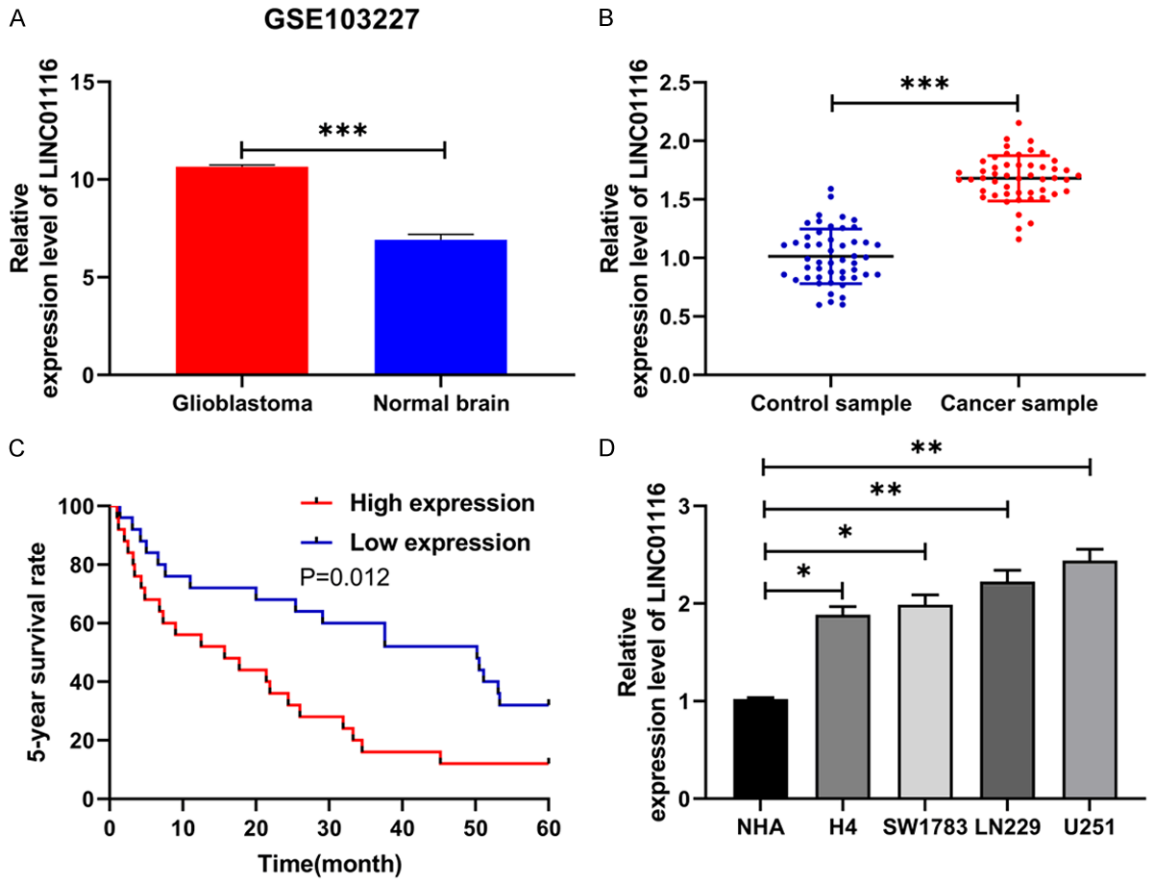


Figure 1. Relationship between the expression level of LINC01116 in glioma and the survival of patients. A. Relative expression of LINC01116 in GSE103227 chip. B. qRT-PCR was used to detect the relative expression level of LINC01116 in tumor tissue of glioma patients. C. K-M survival analysis of the relationship between LINC01116 and 5-year survival of patients with glioma. D. qRT-PCR was used to detect the relative expression level of LINC01116 in glioma cell line. * indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001.

to the Animal Welfare [17] and conformed to the Ethics Committee of Kaifeng Central Hospital.

Statistical methods

GraphPad 7 was applied to visualize the required pictures and analyze the data. K-S test analysis was used to detect the data, which was expressed as mean ± standard deviation (Means ± SD), and independent sample t test was applied for inter-group comparison. The counting data were represented as percentage (%) and compared by Chi-square test, which was expressed as χ^2. One-way ANOVA was applied for comparison among multiple groups, which was expressed as F. LSD-t test was used for post-event comparison, repeated measurement ANOVA was used for comparison at multiple time points, which was expressed as F. Bonferroni was used for back testing, Pearson test was used for analysis of correlation of genes, K-M survival curve was used for visualizing the total survival condition of patients, Log-rank test was used for analysis. P<0.05 was regarded as statistical differences.

Results

LINC01116 was high in glioma and had a poor prognosis

We first explored LINC01116 in GSE103227 chip in order to verify the expression level of LINC01116 in glioma. We found that LINC01116 in glioma samples was higher than that in control samples (**Figure 1A**). qRT-PCR detection also found that LINC01116 in cancer sample was higher than that in control sample (**Figure 1B**). In addition, we divided

Table 1. Relationship between LINC01116 and pathological data of gliomas

Factor	LINC01116 relative expression level		*P* value
	Low expression (n=25)	High expression (n=25)	
Gender			0.569
Male (n=28)	13	15	
Female (n=22)	12	10	
Age			0.354
≥60 (n=35)	19	16	
<60 (n=15)	6	9	
Tumor size			0.024
≥5 cm (n=13)	3	10	
<5 cm (n=37)	22	15	
WHO grade			0.008
I-II (n=38)	23	15	
III-IV (n=12)	2	10	
PTBE			0.157
≥1 cm (n=25)	15	10	
<1 cm (n=25)	10	15	

Note: PTBE, periperitoneal brain edema; WHO, World Health Organization.

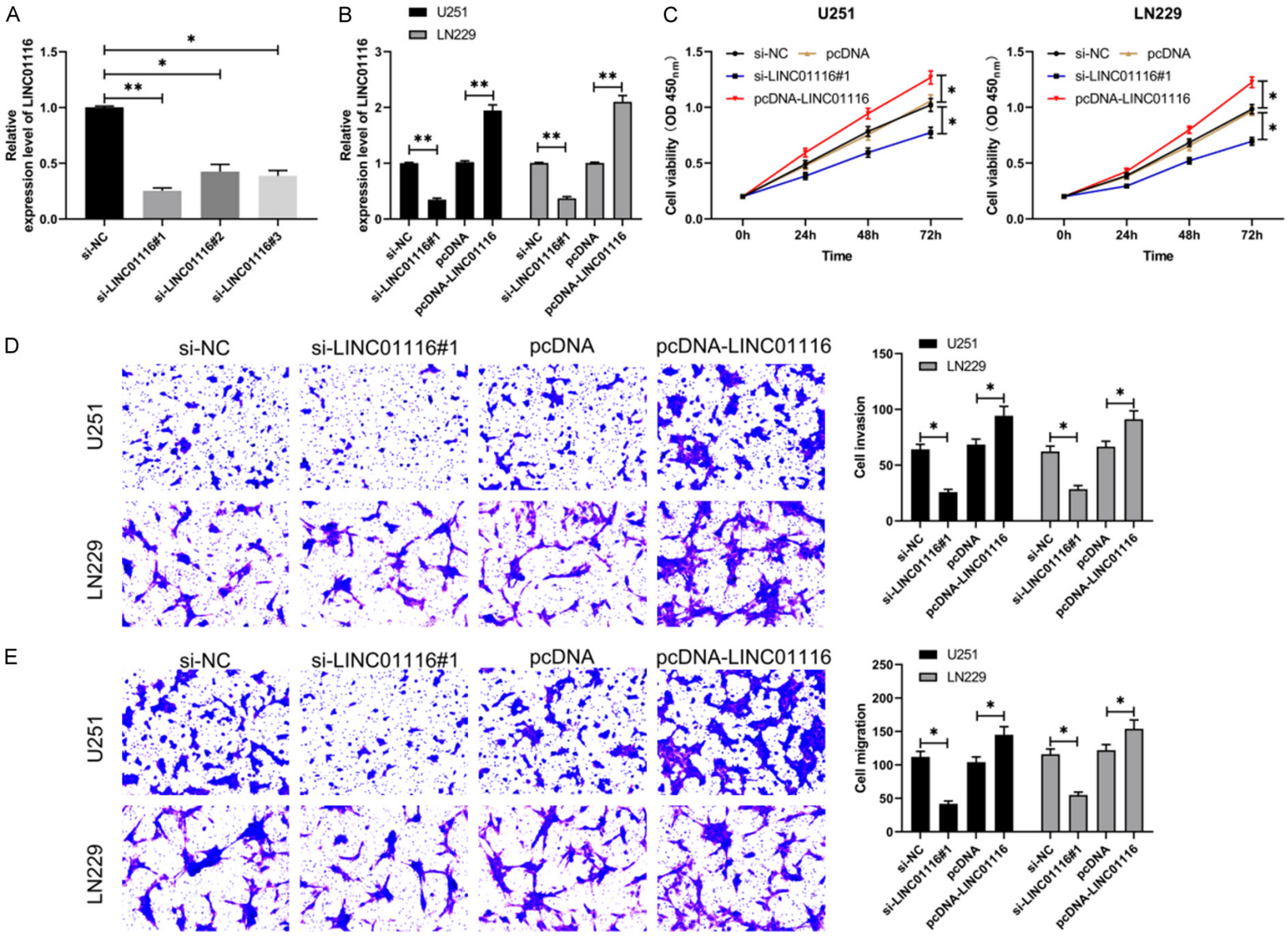
patients into high and low expression groups according to the median value of LINC01116, and further observed the relationship between LINC01116 and pathological data of patients. The results showed that LINC01116 expression was closely related to WHO staging and tumor size (**Table 1**). Survival analysis revealed that the 5-year survival of patients with high expression of LINC01116 was lower than that with low expression (**Figure 1C**), indicating that LINC01116 in glioma was increased (**Figure 1D**).

LINC01116 could regulate the growth and metastasis

We first screened the si-LINAC01116 vector (si-LINAC01116#1) (**Figure 2A**), and transfected si-LINAC01116#1 with glioma cells (U251, LN229) (**Figure 2B**) to determine the effect of LINAC01116. The effect of si-LINAC01116#1 transfected with glioma cells on the growth was determined through experiments. CCK-8 experiment found that the proliferation of cells transfected with si-LINC01116#1 was significantly inhibited compared with si-NC (**Figure 2C**), while Transwell experiment found that the invasion and migration of cells transfected with si-LINC01116#1 were evidently reduced compared with si-NC (**Figure 2D**, **2E**). The cell flow experiment showed that the transfection of si-LINC01116#1 induced apoptosis of glioma cells (**Figure 2F**). However, after transfection of pcDNA-LINC01116 into glioma cells, the proliferation, invasion and migration were evidently enhanced, and the apoptosis of cells was inhibited. This suggested that LINC01116 may be a potential target for glioma treatment.

LINC0116 can be used as MIR-744-5p sponge, and knocking down LINC0116 can reverse the promotion of miR-744-5p-inhibit on the growth and metastasis of glioma cells

ceRNA is an important mechanism for LncRNA to participate in tumorigenesis and development. In order to further explore the mechanism of LINC01116, we predicted the potential binding of LINC01116 to miR, and found that miR-744-5p and LINC01116 had targeted binding sites (**Figure 3A**), and GSE103229 chip analysis revealed that miR-744-5p was low expressed in glioma (**Figure 3B**). We detected miR-744-5p in glioma tissue by qRT-PCR, and the results were consistent with the results in the chip (**Figure 3C**). It was also revealed that miR-744-5p was negatively correlated with LINC01116 in glioma patient tissues through correlation analysis (**Figure 3D**). This suggested that there may be a targeting relationship between LINC01116 and miR-744-5p. In order to verify the relationship, we conducted RIP and dual luciferase reporting experiments respectively. The results showed that both LINC01116 and miR-744-5p could be enriched by Ago2 antibody, and miR-744-5p-mimics could inhibit the fluorescence activity of LINC01116-WT (**Figure 3E**). In addition, we also detected miR-744-5p in glioma cells transfected with si-LINC01116#1 and pcDNA-LINC01116. The results showed that miR-744-5p in cells transfected with si-LINC01116#1 increased significantly, while the expression level of miR-744-5p in glioma cells transfected with pcDNA-LINC01116 decreased (**Figure 3G**). The above research confirmed that LINC01116 specifically bound to miR-744-5p. Finally, cell experiments were applied to verify that LINC01116 regulates the growth and metastasis through miR-744-5p. It was found that the proliferation, invasion and migration were evidently enhanced after transfection of miR-744-5p-5p-inhibit, and the apoptosis of


A
Relative expression level of LINC01116
si-NC
si-LINC01116#1
si-LINC01116#2
si-LINC01116#3
B
U251
LN229
Relative expression level of LINC01116
si-NC
si-LINC01116#1
pcDNA
pcDNA-LINC01116
C
U251
LN229
si-NC
si-LINC01116#1
pcDNA
pcDNA-LINC01116
Cell viability (OD 450nm)
0h
24h
48h
72h
Time
D
si-NC
si-LINC01116#1
pcDNA
pcDNA-LINC01116
U251
LN229
Cell invasion
E
Cell migration

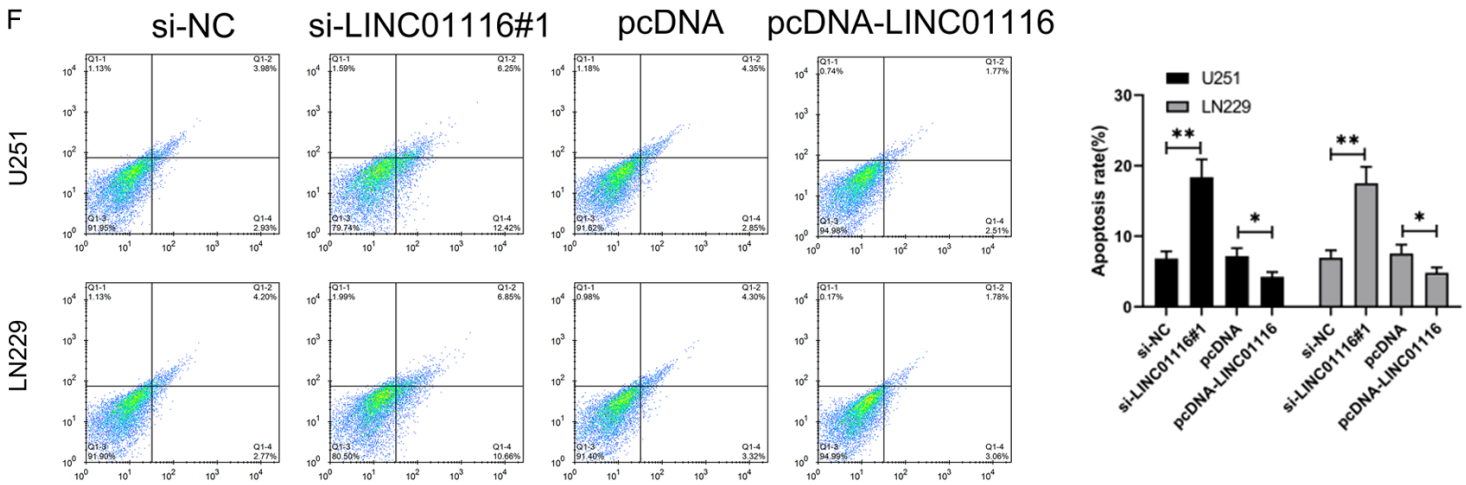


Figure 2. Regulation of the effect of LINC01116 on the growth and metastasis of glioma cells. A. qRT-PCR detection of relative expression level of LINC01116 in si-RNA interference vector. B. qRT-PCR detection of transfection efficiency after constructing pcDNA-LINC01116 vector. C. CCK-8 test was used to detect the changes of glioma cell proliferation after transfection of si-LINC01116#1. D, E. Transwell test was used to detect the changes of invasion and migration of glioma cells after transfection of si-LINC01116#1. F. Flow cytometry was used to detect the induction of apoptosis of glioma cells after transfection of si-LINC01116#1. * indicates $P<0.05$, ** indicates $P<0.01$.

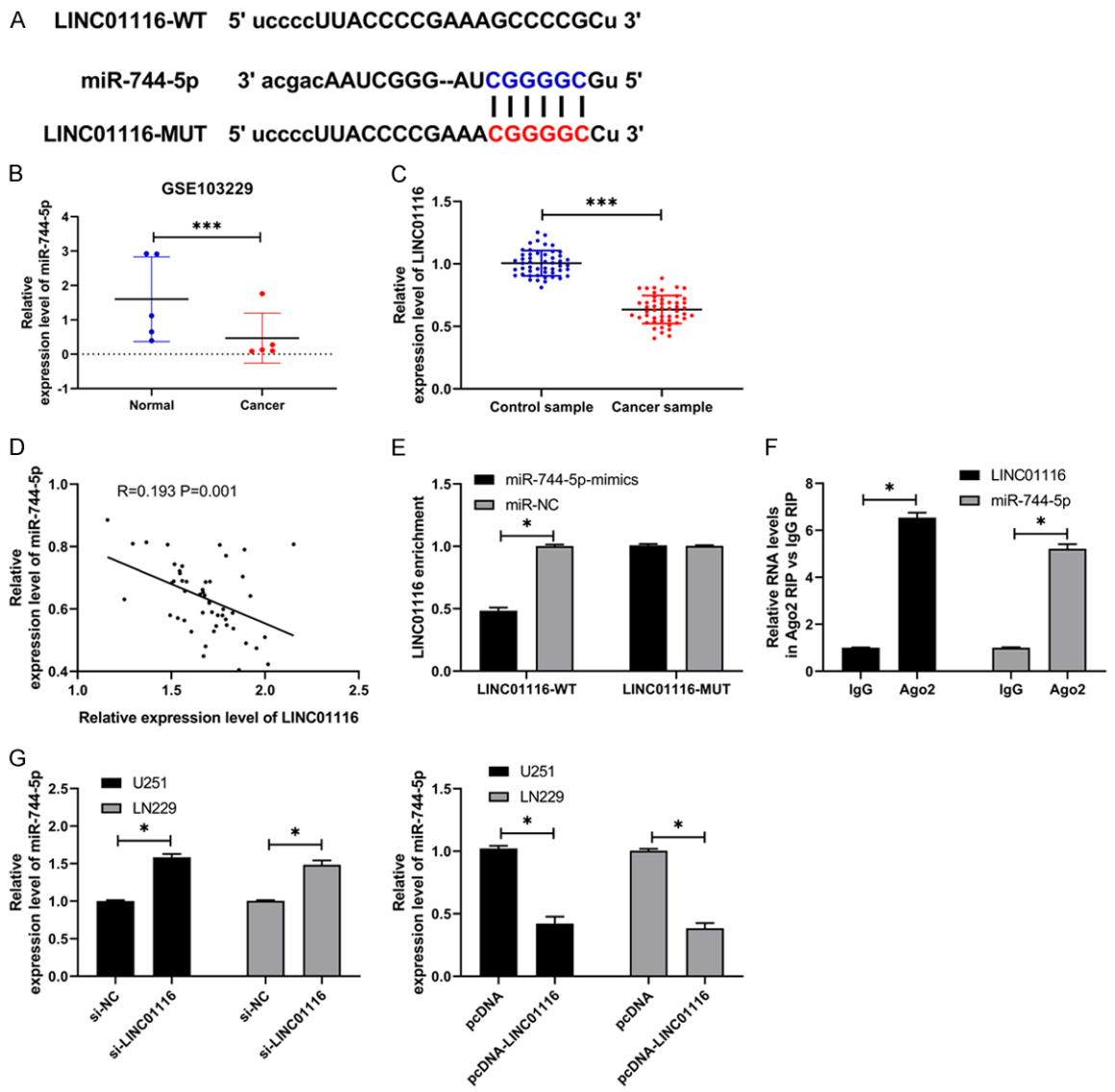
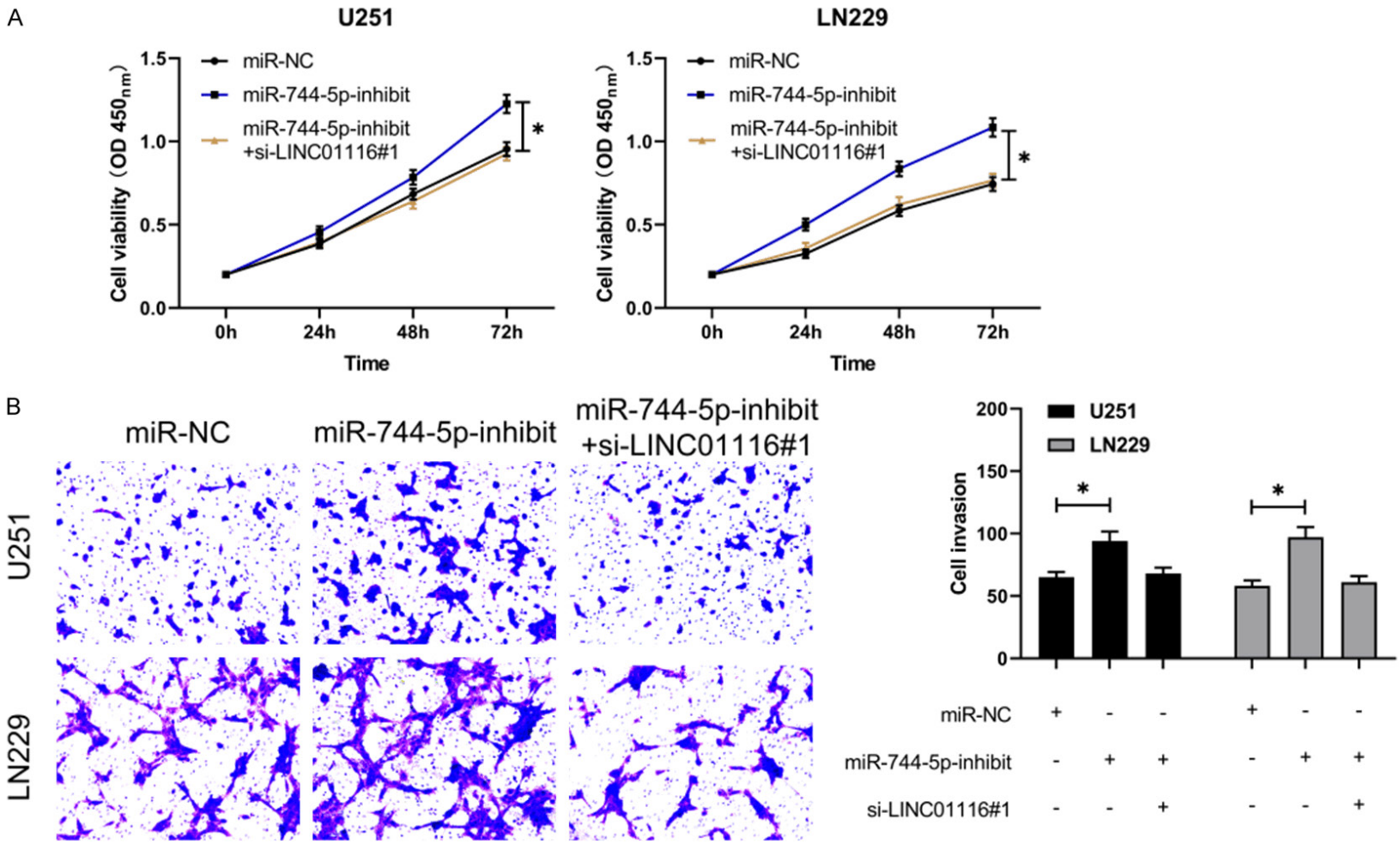


Figure 3. LINC01116 could be used as miR-744-5p sponge. A. Specific binding site of LINC01116 and miR-744-5p. B. Expression of miR-744-5p in GSE103229 chip. C. qRT-PCR was used to detect miR-744-5p relative expression level in tumor tissues of glioma patients. D. Pearson test was used to analyze the correlation between LINC01116 and miR-744-5p in tumor tissue of glioma patients. E. Double luciferase report confirmed that LINC01116 specifically binds to miR-744-5p. F. RIP experiment confirmed that LINC01116 specifically binds to miR-744-5p. G. qRT-PCR was used to detect miR-744-5p relative expression level in glioma cells transfected with si-LINC01116 and pcDNA-LINC01116. * indicates P<0.05, *** indicates P<0.001.

cells was inhibited. However, the proliferation, invasion and migration of cells co-transfected with si-LINC01116 and miR-744-5p-inhibit were inhibited compared with those transfected with miR-744-5p-5p-inhibit, and the apoptosis rate was increased (**Figure 4A-D**). However, compared with glioma cells transfected with miR-NC, there was no difference, which indicated that LINC01116 could act as miR-744-5p sponge to inhibit the growth of glioma.

miR-744-5p targeting TGF-β1 inhibited growth and metastasis of glioma

We predicted the downstream target gene of miR-744-5p through online prediction software TargetScan, starBase, miRDB, and miRTarBase to determine the downstream target gene of miR-744-5p (**Figure 5A**). As a result, we found that TGF-β1 and miR-744-5p had targeted binding sites (**Figure 5C**). We found that TGF-β1 was


A
U251
LN229
miR-NC
miR-744-5p-inhibit
miR-744-5p-inhibit
+si-LINC01116#1
Cell viability（OD 450nm）
1.5
1.0
0.5
0.0
0h
24h
48h
72h
Time
*
B
miR-NC
miR-744-5p-inhibit
miR-744-5p-inhibit
+si-LINC01116#1
U251
LN229
Cell invasion
200
150
100
50
0
U251
LN229
*
*
miR-NC + - - + - -
miR-744-5p-inhibit - + + - + +
si-LINC01116#1 - - + - - +

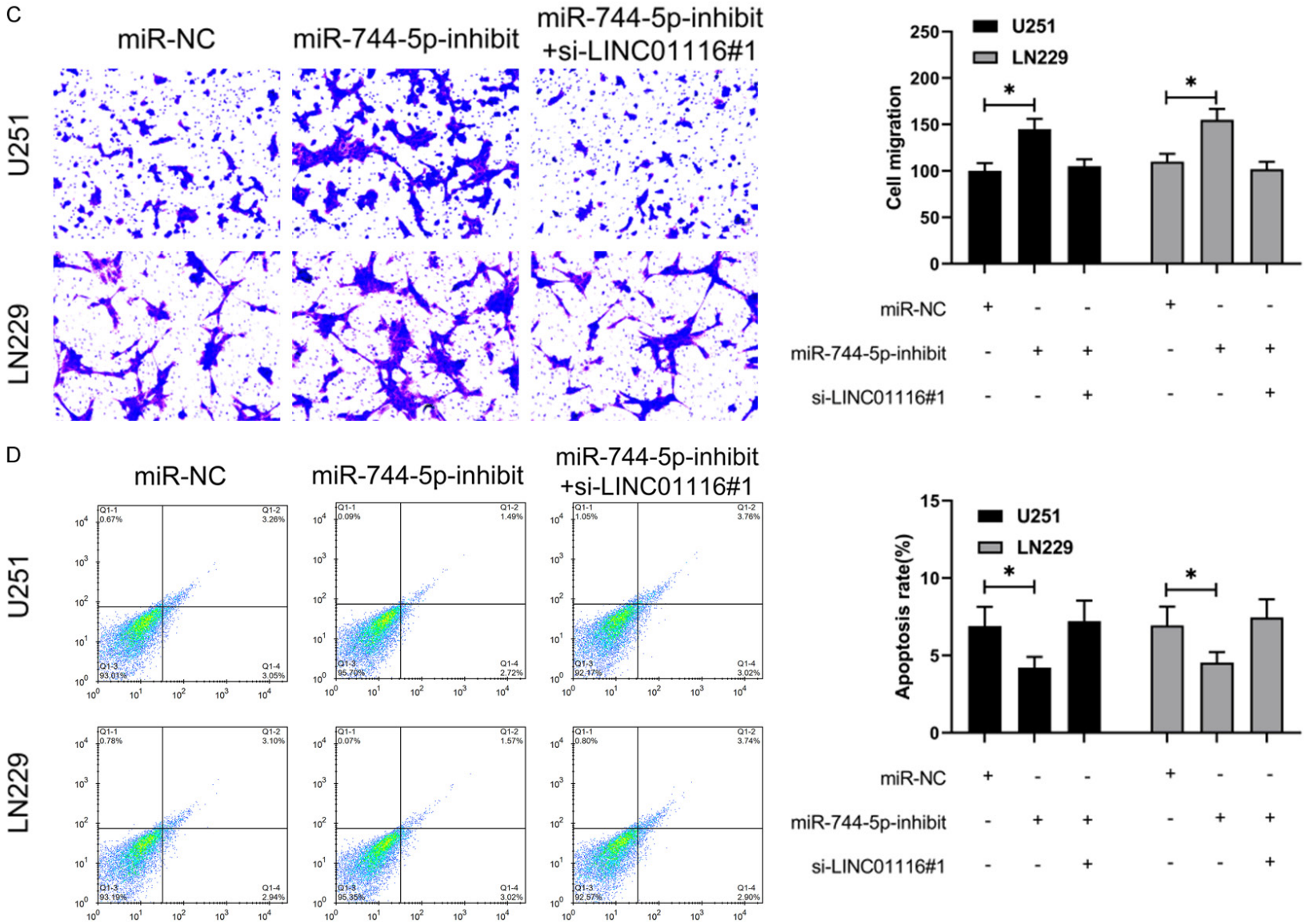


Figure 4. LINC01116 could be used as miR-744-5p sponge to inhibit the growth of glioma. A. CCK-8 experiment was used to detect the changes of glioma cell proliferation after transfection of si-LINC01116#1 and miR-744-5p-inhibit. B, C. Transwell test was used to detect the changes of invasion and migration of glioma cells after transfection of si-LINC01116#1 and miR-744-5p-inhibit. D. Flow cytometry was used to detect the induction of glioma cell apoptosis after transfection of si-LINC01116#1 and miR-744-5p-inhibit. * indicates $P<0.05$.

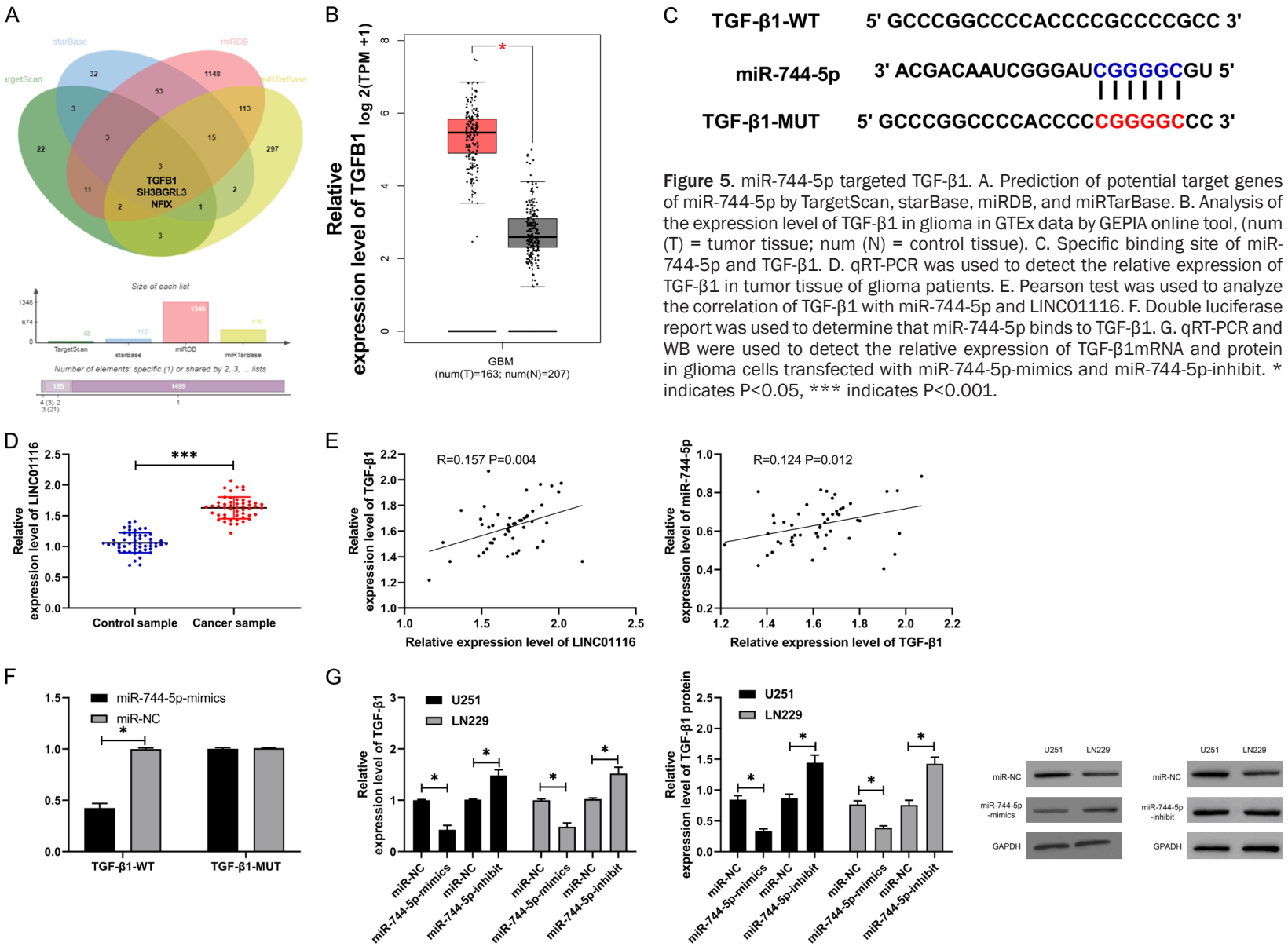


Figure 5. miR-744-5p targeted TGF-β1. A. Prediction of potential target genes of miR-744-5p by TargetScan, starBase, miRDB, and miRTarBase. B. Analysis of the expression level of TGF-β1 in glioma in GTEx data by GEPIA online tool, (num (T) = tumor tissue; num (N) = control tissue). C. Specific binding site of miR-744-5p and TGF-β1. D. qRT-PCR was used to detect the relative expression of TGF-β1 in tumor tissue of glioma patients. E. Pearson test was used to analyze the correlation of TGF-β1 with miR-744-5p and LINC01116. F. Double luciferase report was used to determine that miR-744-5p binds to TGF-β1. G. qRT-PCR and WB were used to detect the relative expression of TGF-β1mRNA and protein in glioma cells transfected with miR-744-5p-mimics and miR-744-5p-inhibit. * indicates P<0.05, *** indicates P<0.001.

highly expressed in glioma through analysis on the online website GEPIA, and the expression level of TGF-β1 in tumor tissue of glioma patients was also increased through qRT-PCR (**Figure 5B**, **5D**). In addition, we found that miR-744-5p and TGF-β1 expression in tumor tissue of glioma patients were negatively correlated through correlation analysis, and TGF-β1 and LINC01116 were positively correlated (**Figure 5E**). Through the verification of the targeted regulatory relationship of miR-744-5p with TGF-β1, we found that miR-744-5p-mimics could inhibit the fluorescent activity of TGF-β1-WT through double luciferase report test, and TGF-β1mRNA and protein expression were inhibited after transfecting miR-744-5p-mimics through qRT-PCR and WB, while TGF-β1mRNA and protein expression levels increased after transfecting miR-744-5p-inhibit. This indicated that miR-744-5p could target TGF-β1 (**Figure 5F**). Finally, in order to verify that miR-744-5p targeted TGF-β1 to regulate the development of glioma, we found through cell experiments that the increase of proliferation, invasion, migration ability and the decrease of apoptosis rate of glioma cells transfected with pcDNA-TGF-β1 were reversed by co-transfection miR-744-5p-mimics (**Figure 6A-D**). This suggested that miR-744-5p targeted TGF-β1 to inhibit the growth and metastasis of glioma (**Figure 5**).

Up-regulation of LINC01116 promoted growth and metastasis by regulating miR-744-5p/TGF-β1 axis

We constructed a co-transfected glioma cell line to determine that LINC01116 can affect the growth of glioma by regulating miR-744-5p/TGF-β1 axis. By observing the growth and metastasis of the co-transfected cells, we found that after miR-744-5p-mimics and si-TGF-β1 were respectively transfected into glioma cells, the proliferation, invasion and migration were evidently inhibited, and the cell apoptosis was induced. However, after we co-transfected pcDNA-LINC01116 with miR-744-5p-mimics and co-transfected pcDNA-LINC01116 with si-TGF-β1 into cells, the inhibition of proliferation, invasion and migration and the induced apoptosis were reversed (**Figure 7A-D**). This showed that LINC01116 could participate in the growth and metastasis of glioma cells through miR-744-5p/TGF-β1 axis.

Knocking down LINC01116 could inhibit the growth of glioma

At the end of the study, we observed the growth of glioma cells after knocking down LINC01116 through in vivo experiments. It was found that the tumor volume and weight after injecting sh-LINC01116 were evidently reduced, and miR-744-5p in nude mice tumor injected sh-LINC01116 was significantly increased compared with sh-NC (**Figure 8A**), while the expression level of TGF-β1 was decreased through WB experiments. At the same time, we also found that the expression level of TGF-β1 protein in nude mice tumor tissue injected sh-LINC01116 was also decreased (**Figure 8B**, **8C**). This suggested that LINC01116 may be a potential target for the treatment of glioma.

Discussion

Glioma is common malignant tumor in brain, and it accounts for about 50%-60% of brain malignancies [18]. Moreover, the prognosis is very poor, and the 5-year survival is not high [19]. Therefore, it is very important to find potential biomarkers to improve the prognosis. In recent years, an increasing number of studies have found that [20] non-coding RNA acts in regulating the occurrence and development of tumors. In this study, we observed that LINC01116 participated in the occurrence of glioma. Moreover, we also found that the 5-year survival of patients with high LINC01116 was reduced, so LINC01116 is expected to become a potential observation indicator of glioma.

LINC01116 is a newly discovered LncRNA located on human chromosome 2q31.1. Previous reports on LINC01116 in tumors were mostly concentrated on osteosarcoma [13] and nasopharyngeal carcinoma [21]. The research on LINC01116 and glioma was relatively few, which was only explored by Zhang [22] and Ye [14] et al. Moreover, the specific mechanism is still unclear. In this study, we confirmed through clinical research that LINC01116 is highly expressed in glioma patients and the prognosis is poor. However, we are not yet clear about its relevant mechanism. Therefore, we conducted in vitro experiments. By constructing different expression vectors of

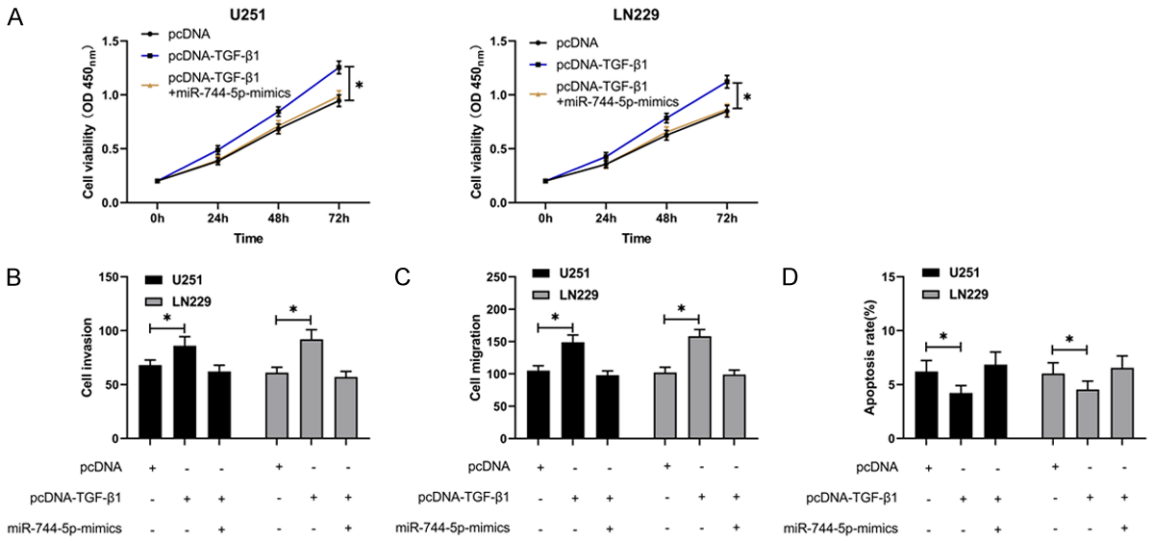
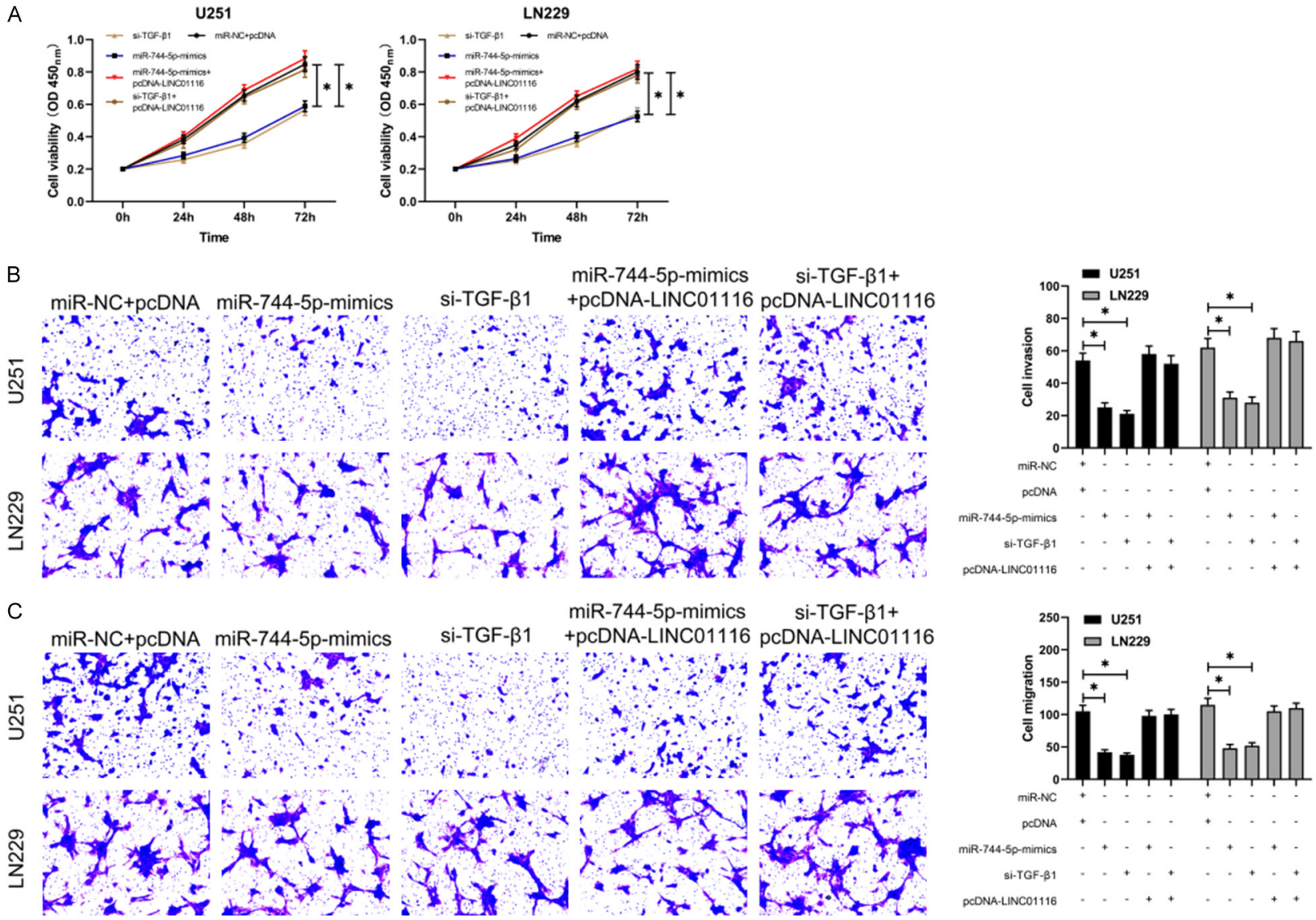


Figure 6. miR-744-5p targeting TGF-β1 inhibited growth and metastasis of glioma. A. CCK-8 test was used to detect the changes of glioma cell proliferation after transfection of miR-744-5p-inhibit and pcDNA-TGF-β1. B, C. Transwell test was used to detect the changes of invasion and migration of glioma cells after transfection of si-LINC01116#1 and miR-744-5p-inhibit. D. Flow cytometry was used to detect the induction of glioma cell apoptosis after transfection of si-LINC01116#1 and miR-744-5p-inhibit. * indicates P<0.05.

LINC01116, we found that the growth and metastasis could be changed by regulating LINC01116, which suggested that LINC01116 is expected to become a potential target for the treatment of glioma. However, we still need further research on the specific mechanism of the occurrence of glioma.

The main function of lncRNA is to hide miR to act as "sponge" and reduce the expression and activity of miRNA [23]. In order to find the potential miR of LINC01116, we found that miR-744-5p and LINC01116 had targeted binding sites through bioinformatics analysis. miR-744-5p is located on the human 17p12 chromosome. Previous research found that miR-744-5p is differentially expressed in multiple cancers, such as lung cancer [24], liver cancer [25], and colon cancer [26]. However, there is no relevant research on the relationship between miR-744-5p and glioma. First of all, we found miR-744-5p low expression through GEO chip analysis, and the expression in glioma tissue was also reduced. In addition, Pearson test determined that miR-744-5p and LINC01116 were negatively correlated. This revealed that miR-744-5p and LINC01116 had targeted regulatory relationship. In order to verify the relationship between the two, we confirmed the targeted regulatory relationship between LINC01116 and miR-744-5p through RIP and double luciferase reporting experiments, and found miR-744-5p in glioma cells transfected with pcDNA-LINC01116 and si-LINC01116#1 through qRT-PCR experiments. Finally, in order to confirm that LINC01116 participates in the growth and metastasis of brain gliomas by regulating miR-744-5p, we constructed co-transfected cells. Through observation, it was found that knocking down LINC01116 could reverse the increase of proliferation, invasion, migration and inhibition of apoptosis of brain glioma cells after transfecting miR-744-5p-inhibit.

Studies have found that LncRNA can participate in tumor genesis by regulating miR downstream target genes [27]. For example, [28] LncRNA LINC00319 accelerates the progression of ovarian cancer through the miR-423-5p/NACC1 pathway. In addition, Gao et al. [29] suggested that LncRNA HOXA-AS2 regulated malignant glioma behavior and angiogenesis simulation through the miR-373/EG FR axis. In this study, in order to further explore the further mechanism of LINC01116, we predicted the target gene downstream of miR-774-5p through 4 online prediction websites, and found that TGF-β1 and miR-774-5p had targeted binding sites. TGF-β1 is one of the secretory


A
U251
LN229
si-TGF-β1
miR-NC+pcDNA
miR-744-5p-mimics
miR-744-5p-mimics+ pcDNA-LINC01116
si-TGF-β1+ pcDNA-LINC01116
Cell viability (OD 450nm)
Time
0h
24h
48h
72h
B
C
miR-NC+pcDNA
miR-744-5p-mimics
si-TGF-β1
miR-744-5p-mimics +pcDNA-LINC01116
si-TGF-β1+ pcDNA-LINC01116
U251
LN229
Cell invasion
Cell migration
miR-NC
pcDNA
miR-744-5p-mimics
si-TGF-β1
pcDNA-LINC01116

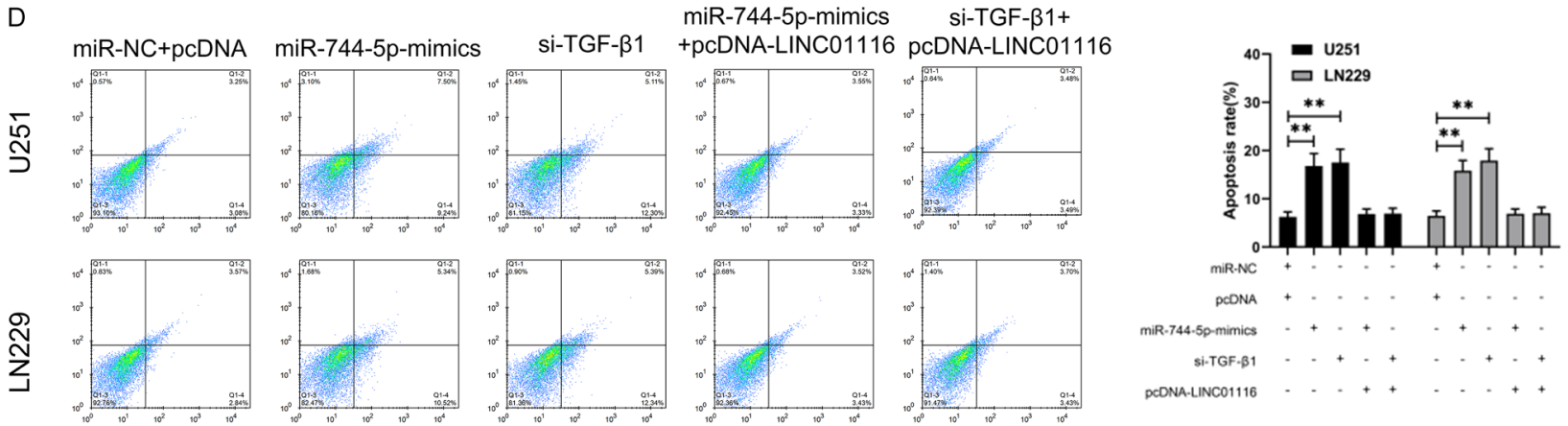


Figure 7. Up-regulation of LINC01116 promoted the growth and metastasis of glioma by regulating miR-744-5p/TGF-β1 axis. A. CCK-8 test was used to detect the changes of glioma cell proliferation after co-transfection. B, C. Transwell test was used to detect the invasion and migration changes of glioma cells after transfection and co-transfection. D. Flow cytometry was used to detect the induction of apoptosis of glioma cells after transfection and co-transfection. * indicates P<0.05, ** indicates P<0.01.

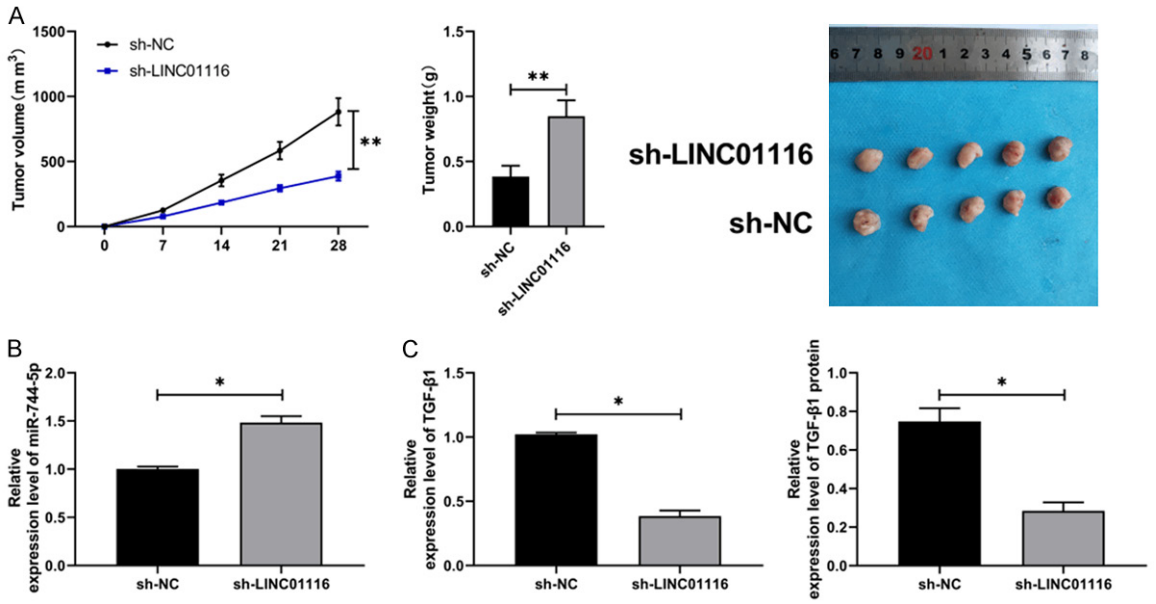


Figure 8. Knocking down LINC01116 could inhibit the growth of glioma. A. Tumor volume and weight in nude mice decreased significantly after injecting sh-LINC01116. B. qRT-PCR was used to detect miR-744-5p relative expression in nude mice tumor tissue. C. qRT-PCR and WB were used to detect the relative expression of TGF-β1 mRNA and protein in tumor tissues of nude mice. * indicates P<0.05, ** indicates P<0.01.

ligand members of TGF-β (transforming growth factor-β) superfamily protein. The encoded protein regulates cell proliferation, differentiation and growth, as well as the expression and activation of other growth factors, including interferon γ and tumor necrosis factor α [30]. Previous studies have found that [31, 32] TGF-β1 is highly expressed in glioma, and we have also confirmed this through clinical tests. TGF-β1 was positively correlated with LINC01116 and negatively correlated with miR-774-5p. This suggested that LINC01116 may participate in the growth of glioma by mediating miR-774-5p/TGF-β1 axis. In order to verify our conjecture, we first verified the relationship of miR-774-5p with TGF-β1. Then, double luciferase report revealed that miR-774-5p-mimics could inhibit the fluorescent activity of TGF-β1-WT. TGF-β1 mRNA and protein in glioma cells transfected with miR-774-5p-mimics and miR-744-5p-inhibit were determined by qRT-PCR and WB experiments to verify this results. In addition, we also found that miR-774-5p-mimics could inhibit pcDNA-TGF-β1 by promoting cell proliferation, invasion, migration and inhibiting cell apoptosis. This showed that miR-774-5p could inhibit the growth of glioma cells by regulating TGF-β1.

At the end of the study, in order to confirm that LINC01116 participates in the occurrence of glioma by mediating miR-744-5p/TGF-β1 axis, we co-transfected pcDNA-LINC01116 with miR-744-5p-mimics and si-TGF-β1 respectively. Through rescue experiments, it was found that pcDNA-LINC01116 could reverse the inhibition of miR-744-5p-mimics and Si-TGF-β1 on cell proliferation, invasion and migration, and increase the apoptosis rate. In addition, we also found through in vivo experiments that the tumor volume and weight were decreased evidently after injection of sh-LINC01116, and the expression level of miR-774-5p in tumor tissue elevated while the expression levels of TGF-β1 mRNA and protein decreased.

This research confirmed that LINC01116 participates in the occurrence of glioma by mediating miR-744-5p/TGF-β1 axis, but there are still some defects. Firstly, the diagnostic value of LINC01116 in glioma is still unclear. Some studies have found that LncRNA has high clinical value in the diagnosis of tumor. We tested a small number of samples this time and we have not detected peripheral blood, exosomes, and peripheral blood mononuclear cells. Secondly, the in-depth mechanism of TGF-β1 has not been proved in this study. Whether

TGF-β1 can participate in the occurrence of glioma by mediating other signal pathways needs further exploration. Therefore, we hope to carry out more research in the future to supplement our research results.

To sum up, LINC01116 is highly expressed in glioma and the prognosis is poor. LINC01116 can participate in the occurrence of glioma by mediating miR-744-5p/TGF-β1 axis and is expected to become a potential therapeutic target for glioma.

Conclusions

To sum up, LINC01116 is highly expressed in glioma and the prognosis is poor. LINC01116 can participate in the occurrence of glioma by mediating miR-744-5p/TGF-β1 axis and is expected to become a potential therapeutic target for glioma.

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Disclosure of conflict of interest

None.

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