Int J Clin Exp Med 2021;14(2):852-863
www.ijcem.com /ISSN:1940-5901/IJCEM0121891

Original Article

Effect of long intergenic non-coding RNA 00312 on regulating biological behaviors of glioma cells by targeting microRNA-21-3p

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Received September 7, 2020; Accepted October 12, 2020; Epub February 15, 2021; Published February 28, 2021

Abstract: Objective: To clarify the role of long intergenic non-coding RNA 00312 (LINC00312) in epithelial-mesenchymal transition (EMT), invasion, and metastasis of glioma by targeting microRNA-21-3p (miR-21-3p). Methods: The surgical resection samples from glioma patients and normal brain tissues from patients with severe head trauma were obtained, separately, and the expressions of LINC00312 and miR-21-3P in normal brain tissues, glioma tissues, human normal brain glial cells, and human glioma cells were detected, respectively. The localization of LINC00312 was predicted and verified by subcellular localization websites and nucleic-cytoplasmic separation experiment, separately. The dual luciferase reporter assay was used to confirm the interactions between LINC00312 and miR-21-3p. The cell line with the lowest LINC00312 expression among cell lines U251, U87, and A127 was selected for cell experiments. After grouping, the cells were transfected with LINC00312, sh-LINC00312, miR-21-3p mimic, and vector, respectively. At the same time, negative controls were set up. The expressions of EMT-related factors such as N-cadherin, E-cadherin, and Vimentin, as well as invasion and metastasis-associated factors such as MMP-2 and MMP-9, were detected using Western blot analysis. MTT assay, Transwell assay, and flow cytometry were used to assess cell proliferation activity, invasion, and apoptosis, respectively. Results: Compared with the normal brain tissues and glial cells, there was lower expression of LINC00312 but higher expression of miR-21-3p in the glioma brain tissues and glial cells (all $P<0.05$). LncLocator prediction website revealed that the expression of LINC00312 can be observed in both the cell cytoplasm and nucleus, and dual-luciferase reporter assay further confirmed that LINC00312 could play a role in targeting inhibition of miR-21-3p expression. LINC00312 could reduce the expression of N-cadherin and Vimentin but enhance E-cadherin expression. At the same time, LINC00312 could induce cell apoptosis and play a role in inhibiting cell proliferation, cell invasion, and expression of invasion and metastasis-related factors. Conversely, miR-21-3p caused the opposite effect. Moreover, the effect of LINC00312 on biological behaviors in glioma cells could be reversed by miR-21-3p. Conclusion: LINC00312 can serve as a molecular sponge to adsorb miR-21-3p, thus inhibiting the proliferation, invasion, metastasis, and EMT of glioma cells, as well as inducing apoptosis.

Keywords: Long intergenic non-coding RNA 00312, microRNA-21-3p, glioma, epithelial-mesenchymal transition, invasion and metastasis

Introduction

Glioma, as a common primary intracranial tumor, includes oligodendroglioma, astrocytoma, ependymoma, and so on [1, 2]. At present, the etiology of glioma is not clear, which may be related to diet, genetics, ionizing radiation, unhealthy lifestyle habits, and other factors. Early surgical resection, radiotherapy and chemotherapy in the late stage, and immunotherapy are all therapeutic methods for glioma. However, the prognosis for patients with glioma is still very poor, with high rates of recurrence and mortality [3, 4]. Therefore, it is necessary to carry out new researches beneficial to the treatment of glioma.

Long non-coding RNAs (LncRNAs) are considered noncoding RNAs that lack protein-coding functions, playing important roles in transcriptional silencing and activation, chromosome modification, and intranuclear transport [5, 6].

LncRNAs, containing over 200 nucleotides in length, play an important role in many key cellular processes, as well as occurrence and prognosis of cancer [7, 8]. At present, a great deal of research has been carried out to discover the function of LncRNAs and reveal the role of LncRNAs in glioma. Xie et al. (2020) found that long intergenic non-coding RNA 01198 (LINC01198) can activate the PI3K/AKT signaling pathway by targeting PIK3CA and PTEN, thus promoting the occurrence and development of glioma [9]. Huang et al. (2020) found that LncRNA GAS5-AS1 can inhibit the proliferation, migration, and invasion of glioma cells through the miR-106b-5p/TUSC2 signaling pathway [10]. Therefore, the function of different LncRNAs varies in glioma. Long intergenic non-coding RNA 00312 (LINC00312) is mainly located on chromosome 3p25.3-26.3 which is also considered as gene deletion regions related to nasopharyngeal carcinoma, and LINC00312 also plays an important regulatory role in the pathogenesis of nasopharyngeal carcinoma [11]. In addition, it was confirmed that LINC00312 exerts its tumor suppressor function in colorectal cancer and thyroid cancer [12, 13]. However, the role of LINC00312 in glioma is not clear. MicroRNAs (miRNAs) are relatively small noncoding RNAs with the length from 18 to 24 nucleotides, which have certain effects on regulating biological processes such as cell growth, apoptosis, and inflammation. The dysregulation of many miRNAs has been found in glioma. For example, miR-3175 and miR-18a play the role of 'cancer-promoting factor', miR-134 and miR-940 play the role of 'tumor suppressor' in glioma, while miR-21 has been identified to be overexpressed in glioma by Jiang et al. [14-16].

In our study, the expression of LINC00312 in glioma tissues and cells was examined and the role of LINC00312 acting as a ceRNA for miR-21-3p was confirmed. Therefore, this study aims at exploring the role of LINC00312 in glioma.

Materials and methods

General information

The surgically resected tumor brain tissues from 35 patients with glioma treated in The First People's Hospital of Shuangliu District, Chengdu/West China (Airport) Hospital Sichuan University from October 2018 to February 2020, including 19 males and 16 females, were collected for our study. The mean age of patients was 48.6±7.5 years. All these patients had pathologically confirmed primary glioma and none of them underwent chemotherapy, radiotherapy, and other treatment. The malignancy stages were based on the World Health Organization (WHO) classification criteria issued in 2007 including 22 cases in stage I and 13 cases in stage II [17]. Meanwhile, normal brain tissue samples (n=21) were collected from patients with craniocerebral trauma treated in The First People's Hospital of Shuangliu District, Chengdu/West China (Airport) Hospital Sichuan University as a control. All patients and their families signed the informed consent, and the study was approved by the Ethics Committee of The First People's Hospital of Shuangliu District, Chengdu/West China (Airport) Hospital Sichuan University.

Screening and culture of cell lines

Human normal glial cell line (HEB) and glioma cell lines U251, U87, and A127 (purchased from ATCC cell bank in the USA) were cultured in DMEM with 10% fetal calf serum (11039-047, Thermo Fisher, USA) at 37°C in an atmosphere containing 5% CO_2. Cell passage was performed every 2 to 3 days. The cells in the logarithmic growth phase were collected and the expressions of LINC00312 and miR-21-3P in cell lines were detected.

Subcellular localization of LINC00312 and dual-luciferase reporter assay

Prediction of subcellular localization of LINC00312 was conducted using LncLocator subcellular location database (http://www.csbio.sjtu.edu.cn/bioinf/lncLocator/). Nuclear and cytosolic fractions of glioma cells were separated using the AmbionPARIS™ Kit (AM1921, Thermo Fisher, USA). Then the expression of LINC00312 in the cytoplasmic and nuclear fractions was examined by quantitative real-time polymerase chain reaction (qRT-PCR). The miRNAs with binding site that binds to LINC00312 were predicted by the LncRNA SNP database. Later, the 3'UTR fragment of LINC00312 was double-digested and inserted into the pMIR-REPORTTM luciferase vector to construct the LINC00312 wild-type (WT) sequence. The mutation at the binding site

Table 1. Quantitative real-time PCR sequence

Gene	Primer sequences
LINC00312	Forward: 5'-CTCTCCATTGGGTTCACCATTC-3'
	Reverse: 5'-GCGGCAGGTCTTAAGAGATGAG-3'
miR-21-3p	Forward: 5'-GCGGAGAGGGCCCACAGTG-3'
	Reverse: 5'-TGGTGTCGTGGAGTCG-3'
GAPDH	Forward: 5'-TCCACCACCCTGTTGCTGTA-3'
	Reverse: 5'-ACCACAGTCCATGCCATCAC-3'
U6	Forward: 5'-TCCGGGTGATGCTTTTCCTAG-3'
	Reverse: 5'-TTTGCGGTGGAAATGTCCTTTTC-3'

Note: LINC00312: Long intergenic non-coding RNA 00312; miR-21-3p: microRNA-21-3p; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

between LINC00312 and miR-21-3p was performed to construct the mutant-type (MUT) sequence. The WT gene fragment and MUT gene fragment were co-transfected with miR-21-3p and NC into 293T cells, respectively using L Lipofectamine™ 3000 (L3000001, Thermo Fisher, USA) according to the instruction. Luciferase activity was detected with Dual-Luciferase kit (D0010-100T, Beijing Suolai Bao Technology Co., Ltd., China) and automatic chemiluminescence apparatus (DxI800, Beckman Coulter, USA).

Cell grouping and transfection

The glioma cells with the lowest LINC00312 expression and the highest miR-21-3p expression were selected for grouping, as follows: blank group (without transfection), vector group (transfected with empty plasmid), LINC00312 group (transfected with LINC00312 overexpression plasmid), sh-LINC00312 group (transfected with sh-LINC00312 plasmid), miR-21-3p group (cells transfected with miR-21-3p overexpression plasmid), and LINC00312 + miR-21-3p group (transfected with LINC00312 and miR-21-3p overexpression plasmid). All plasmids were purchased from Thermo Fisher Scientific, Inc. The transfection of each group was performed according to the instructions of Lipofectamine™ 3000. When the cell confluence reached 70-90%, 5 μL Opti-MEM medium was mixed with 0.15 μL Lipofectamine™ 3000 reagent as solution A; 0.15 μg DNA and 10 μL Opti-MEM was mixed with 0.4 μL P3000™ reagent as solution B, then solution A and solution B were mixed and incubated at room temperature for 15 min. Finally, the DNA-liposome complexes were added to the cells for routine culture. Subsequent experiments were performed 48 h after transfection.

Quantitative real-time PCR detection

Total RNA was extracted from glioma tumor tissues of glioma patients and normal brain tissues of patients with craniocerebral trauma using the Trizol kit (15596026, Thermo Fisher, USA). After that, cDNA was synthesized using reverse transcription reagent kits (11141ES-10, Shanghai Yeasen Biotechnology Co., Ltd., China). Then the cDNA was inactivated at 90°C for 5 min for subsequent PCR reactions. qRT-PCR reactions were completed using the qRT-PCR kit (125940255, Thermo Fisher, USA). The qRT-PCR reaction system was 10 μL, and the reaction conditions were 95°C for 3 min followed by 40 cycles of 90°C for 30 s, and 60°C for 30 s. The process was conducted according to the kit instructions. This method was also suitable for cell experiments. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference of LINC00312, and U6 was used as the internal reference of miR-21-3p. Primer sequences are presented in **Table 1**.

Western blot analysis

An appropriate amount of protein lysate was added to cells, and then the cells were lysed fully with the lysate on ice for 30 min. After total protein extraction, detection of protein concentration was performed with the BCA kit (PC0020, Beijing Suolai Bao Technology Co., Ltd., China), after which, the protein samples were boiled to denature. After SDS-PAGE gel electrophoresis, the proteins were transferred to nitrocellulose membranes by wet transfer method, then the membranes were blocked with 5% defatted milk powder for 2 h. After that, primary antibodies were added, including anti-Vimentin (1:2000, ab92547, Abcam, UK), E-cadherin (1:1500, ab233611, Abcam, UK), N-cadherin (1:5000, ab76011, Abcam, UK), MMP-2 (1:1500, ab86607, Abcam, UK) and MMP-9 (1:1500, ab76003, Abcam, UK). After incubation at 4°C overnight, secondary antibody anti-IgG was added (ab150077, Abcam, UK). Incubation for 30 min at room temperature was followed. After development in elec-

tro-chemi-luminescence (ECL) developing solution (PE0010, Beijing Suolai Bao Technology Co., Ltd., China), the results were observed under an inverted microscope. GAPDH was selected as an internal reference, and the ratio of the gray value of the target band to that of GAPDH band was calculated to express the results.

MTT assay

The transfected mouse endothelial progenitor cells were inoculated in 96-well plates at 10×10^3 cells per well. Three complex holes were set for each group, and then the cells were cultivated in an incubator at a constant temperature of 37°C and 5% CO_2. Each well was loaded with 20 μL of a 5 mg/mL MTT solution (M1025, Beijing Suolai Bao Technology Co., Ltd., China) prepared in the phosphate buffer saline (PBS) at 24 h, 48 h, and 72 h. After 4 h of culture, the culture was terminated and dimethylsulfoxide (DMSO) (D8371, Beijing Suolai Bao Technology Co., Ltd., China) was added into each well. After shaking and mixing, the optical density (OD) value of each well was measured at 490 nm wavelength using a microplate reader (51119570, Thermo Fisher, USA). The experiment was repeated 3 times.

Transwell detection

Cells from each group were cultured with serum-free medium (N6010, Beijing Suolai Bao Technology Co., Ltd., China) for 24 h. Next, 200 μL of cells were inoculated into the upper compartment of Transwell chamber, and the lower chamber was added with the DMEM medium containing 10% fetal bovine serum. The chambers were cultured at 37°C in an atmosphere containing 5% CO_2 for 24 h. Then, the cells were washed with PBS, fixed with paraformaldehyde, and stained with crystal violet (C8470, Beijing Suolai Bao Technology Co., Ltd., China). Finally, the staining was observed under the microscope.

Flow cytometry

The endothelial progenitor cells after transfection for 48 h were placed into a centrifuge tube. After washing with PBS, an appropriate amount of trypsin cell digestive fluid was added. After digestion, the cells were centrifuged for 5 min and the supernatant was discarded. The Annexin-V-FITC/PI kit was used to stain the cells in the dark. The staining solution was prepared according to the instructions of the apoptosis detection kit (BL1141, Beijing Baiolaibo Technology Co., Ltd., China). Cells were resuspended at a concentration of 1×10^6 cells per 100 μL staining solution. The apoptosis of cells was determined by flow cytometry (CytoFLEX, Beckman Coulter, USA).

Statistical analysis

SPSS 21.0 software was used to analyze the measurement data. Each experiment was repeated at least 3 times to calculate the mean and standard deviation (SD). All data were tested for normality and homogeneity of variance. T-test was used for comparisons between two groups, and one-way ANOVA test was used for multiple group comparison; LSD method was used for further comparison. The rank data were analyzed by the rank-sum test for pairwise comparisons. $P<0.05$ was considered statistically significant.

Results

The expression of LINC00312 was reduced while the expression of miR-21-3p was increased in glioma

The expressions of miR-21-3P and LINC00312 were detected by qRT-PCR. Compared with normal brain tissues, the expression of miR-21-3p in glioma tissues was upregulated, while the expression of LINC00312 was suppressed (all $P<0.05$, **Figure 1A** and **1B**). Relative to HEB, the expression of LINC00312 was suppressed while the expression of miR-21-3p was enhanced in the U251, A127, and U87 glioma cell lines (all $P<0.05$). Furthermore, among these glioma cell lines, U251 cells exhibited the lowest LINC00312 expression but the highest miR-21-3p expression (all $P<0.05$, **Figure 1C** and **1D**). Therefore, the U251 cell line was selected for subsequent experiments. See **Figure 1**.

LINC00312 can inhibit the proliferation, invasion, and epithelial-mesenchymal transition (EMT) of U251 glioma cells and promote cell apoptosis

To explore the effect of LINC00312 in glioma cells, intervention in the expression of LINC-

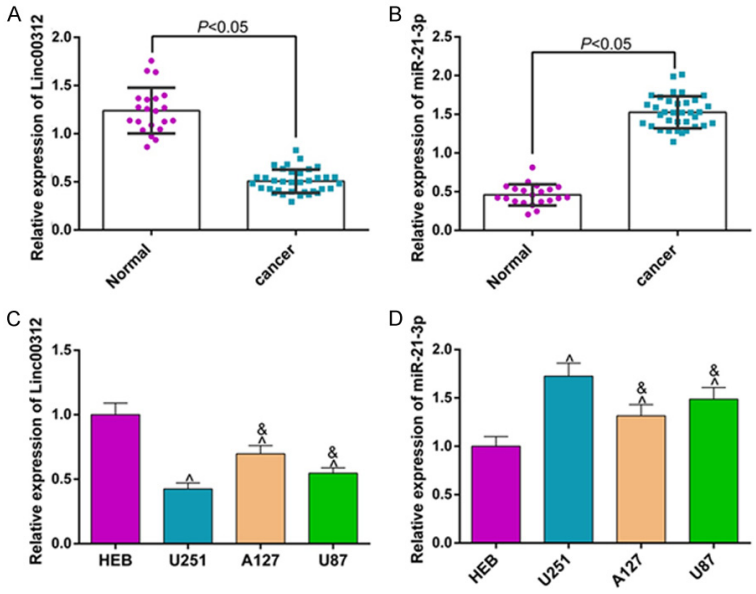

Figure 1. Expression of LINC00312 and miR-21-3P in glioma. A: Expression of LINC00312 in cancer tissues and normal brain tissues; B: Expression of miR-21-3p in cancer tissues and normal brain tissues; C: Expression of LINC00312 in each cell line; D: Expression of miR-21-3p in each cell line. Compared with HEB, ^P<0.05; compared with U251, &P<0.05. LINC00312: Long intergenic non-coding RNA 00312; miR-21-3p: microRNA-21-3p; HBE: Human normal brain glial cell line.

00312 in glioma cells as well as grouping were performed. The results showed that compared with the blank group, cells in the LINC-00312 group exhibited proliferation inhibition (**Figure 2A**), increased apoptosis (**Figure 2B**), decreased cell invasion (**Figure 2C**), reduced N-cadherin, Vimentin, MMP-2, MMP-9 expression and increased E-cadherin (**Figure 2D** and **2E**) (all P<0.05), while the opposite was found for the sh-LINC00312 group (all P<0.05). There was no statistical difference between vector group and blank group (all P>0.05).

LINC00312 can act as a molecular sponge to adsorb miR-21-3p

The LncLocator prediction results revealed a higher expression level of LINC00312 in the cytoplasm. LINC00312 expression was observed in the cell cytoplasm and nucleus after nucleus-cytoplasm separation, indicating that LINC00312 might exert ceRNA function. Therefore, this study focused on the effect of LINC-00312 as a ceRNA to adsorb miR-21-3p in glioma. The prediction results of the LncRNA SNP database suggested the presence of binding sites between LINC00312 and miR-21-3p. A dual luciferase report assay indicated that compared with the co-transfection group of DC and LINC00312 WT fragment, the luciferase activity was significantly inhibited in the co-transfection group of miR-21-3p and LINC00312 WT fragment (P<0.05). There was no statistical difference in luciferase activity between the co-transfection group of DC and LINC00312 WT fragment and the co-transfection group of miR-21-3p and LINC00312 MUT fragment (P>0.05). See **Figure 3**.

miR-21-3p can reverse the effect of LINC00312 on cell proliferation and apoptosis in glioma

MTT assay (**Figure 4A**) and flow cytometry (**Figure 4B**) findings suggested that the cell proliferation activity in the miR-21-3p group was increased and the cell apoptosis rate was inhibited compared with the blank group (all P<0.05), while the opposite was found for the LINC00312 group (all P<0.05). There was no statistical difference between the blank group and LINC00312 + miR-21-3p group as well as between the blank group and vector group (P>0.05). See **Figure 4**.

LINC00312 can rescue the effect of miR-21-3p on the invasion of glioma cells

The results of the invasion of glioma cells (**Figure 5A**) and the expressions of MMP-2 and MMP-9 (**Figure 5B**) showed that compared with the blank group, invasion and the expressions of MMP-2 and MMP-9 were suppressed in the cells of LINC00312 group (all P<0.05), while the opposite was found for the miR-21-3p group (all P<0.05). There was no statistical significance in all indicators between LINC00312 + miR-21-3p group and blank group (all P>0.05), indicating that LINC00312 can rescue the effect of miR-21-3p on cell invasion in glioma. Moreover, there was no statistical difference in all indicators between the vector group and blank group (all P>0.05). See **Figure 5**.

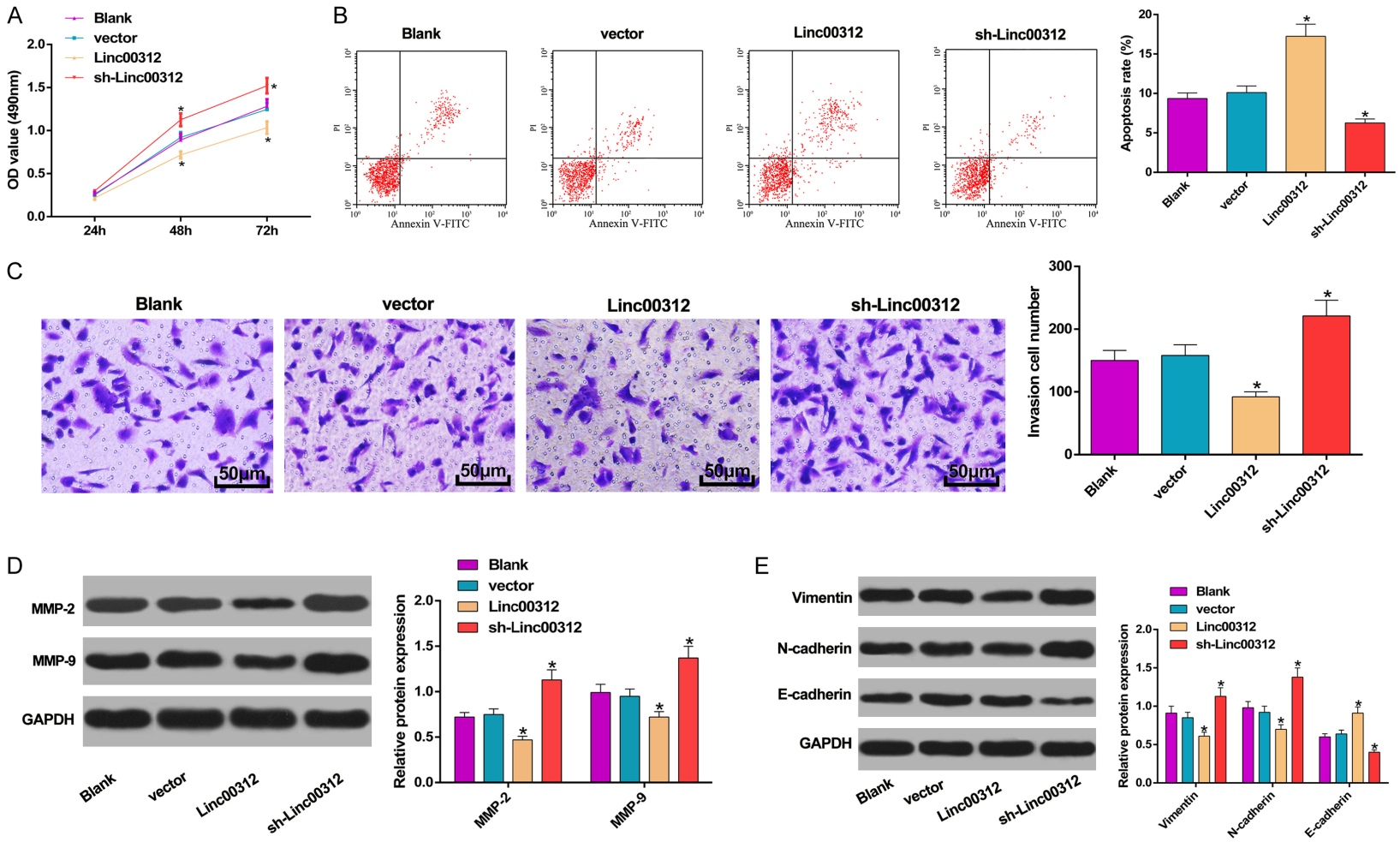

Figure 2. Upregulation of LINC00312 can inhibit cell proliferation, invasion, and EMT and promote cell apoptosis. A: OD value; B: Cell apoptosis; C: Cell invasion (200×); D: Expressions of MMP-2 and MMP-9 proteins in each group; E: Expressions of EMT-related factors in each group. Compared with blank group, *P<0.05. LINC00312: Long intergenic non-coding RNA 00312; miR-21-3p: microRNA-21-3p; EMT: Epithelial-mesenchymal transition; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; OD: Optical density.

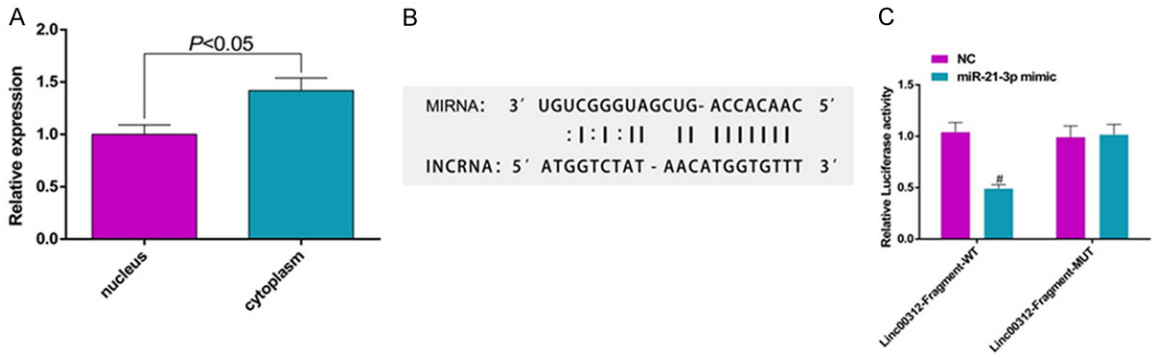

Figure 3. LINC00312 can act as a molecular sponge to adsorb miR-21-3p. A: Expression of LINC00312 in cytoplasm and nucleus; B: Targeting binding sites between LINC00312 and miR-21-3p; C: Dual-luciferase reporter assays result. Compared with the co-transfection group of NC and LINC00312-Fragment-WT, #P<0.05. miR-21-3p: microRNA-21-3p; WT: Wild-type; MUT: Mutant-type.

mi-R21-3p can reverse the effect of LINC00312 on the expressions of EMT-related factors in glioma cells

The results of the Vimentin, N-cadherin, and E-cadherin expression in glioma cells in each group showed that compared with the blank group, there was higher E-cadherin expression but lower Vimentin and N-cadherin expressions in the LINC00312 group (all P<0.05), while the opposite was found for the miR-21-3p group (all P<0.05). There was no significant difference in all indicators among vector group, LINC00312 + miR-21-3p group, and blank group (all P> 0.05). See **Figure 6**.

Discussion

It was found that the 5-year survival rate of patients with glioma is lower than 5% after comprehensive chemoradiotherapy. Therefore, it is imperative to develop new potential targets for the diagnosis and treatment of glioma [18-21]. Studies have found that dysregulation of LncRNAs expression will not only affect the regulation of eukaryotic genomes, but also the growth of malignant cells [22-25]. As a long non-coding RNA, LINC00312 can suppress the progression of breast cancer by regulating miR-9/CDH1 [26].

Previous research found that LINC00312 was upregulated in lung adenocarcinoma and can induce the migration and angiogenesis of cancer by directly binding to YBX1 [27]. However, the effect of LINC00312 in glioma has not been confirmed. In our study, the expressions of LINC00312 in normal and diseased tissues were detected, and the results showed that LINC00312 expression was downregulated in the cancer tissues.

The theory of competitive endogenous RNA (ceRNA) reveals a new biological mechanism about RNA interactions, and ceRNA functions in the cell by competitive binding to miRNA [28-30]. For example, the study by Luo et al. found that LncRNA CASC11 can promote cell proliferation of bladder cancer cells by acting as a ceRNA for miR-150 [31]. Research has also found LncRNA CCAT1 can promote the development of glioma by adsorbing miR-181b [32]. In our study, through nucleic-cytoplasmic separation experiment, we found that LINC00312 was distributed in both cytoplasm and nucleus and exerted function as a ceRNA. Subsequently, through dual luciferase reporter assays, we found that LINC00312 could target miR-21-3p. High expression of miR-21-3p in glioma tissues and cells was observed through tissue and cell experiments.

We also found that LINC00312 could enhance the E-cadherin expression and reduce the N-cadherin, Vimentin, MMP-2, and MMP-9 expressions in glioma cells, whereas the activation of miR-21-3p exerted the opposite effect, which can be reversed by LINC00312 overexpression. E-cadherin, N-cadherin, and Vimentin are common markers associated with EMT, and MMP-2 and MMP-9 are markers related to cell invasion. EMT is characterized by the upregulation of N-cadherin and Vimentin expressions as well as the downregulation of E-cadherin expression. EMT can significantly change the epithelial cell shape and enhance the motility of epithelial cells, thereby enhancing tumor cell metastasis, chemoresistance, and tumor stem-

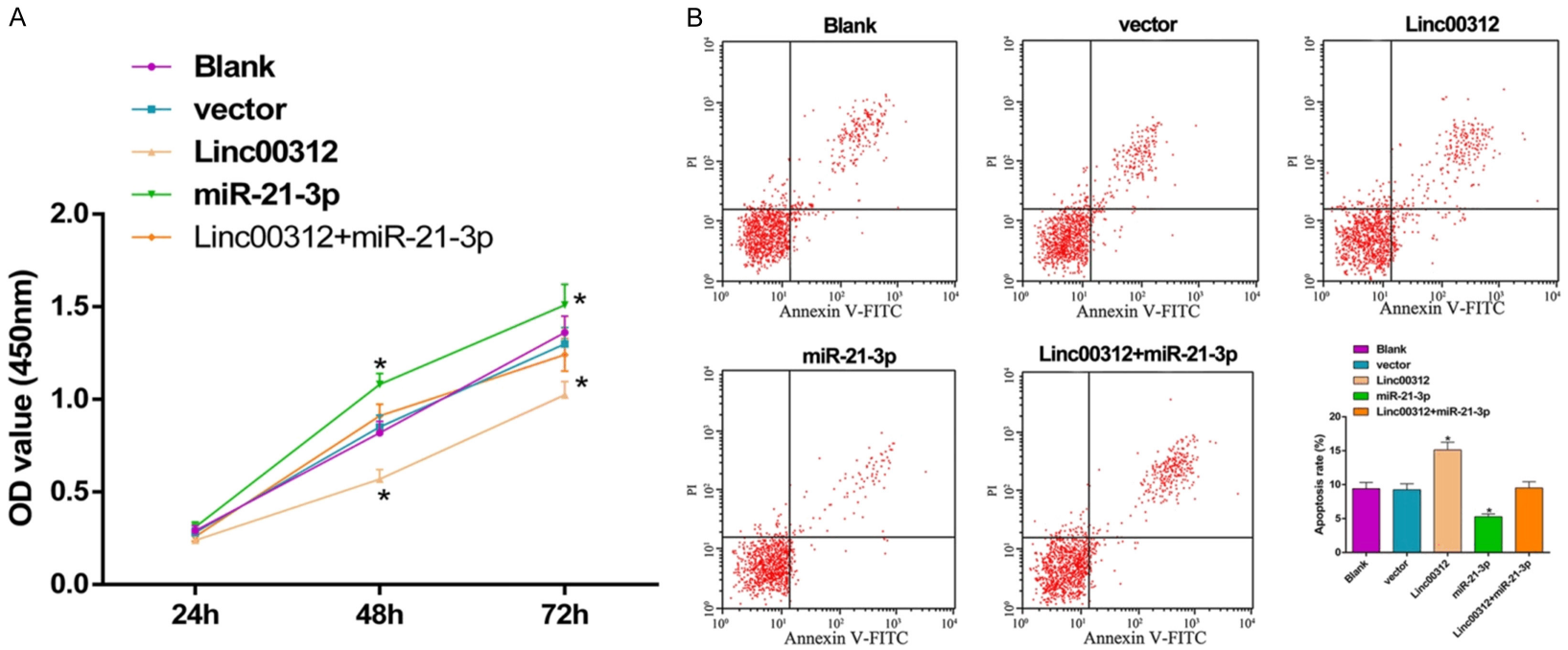

Figure 4. Results of cell proliferation and apoptosis in each group. A: Cell proliferation; B: Cell apoptosis. Compared with blank group, *P<0.05. LINC00312: Long intergenic non-coding RNA 00312; miR-21-3p: microRNA-21-3p; OD: Optical density.

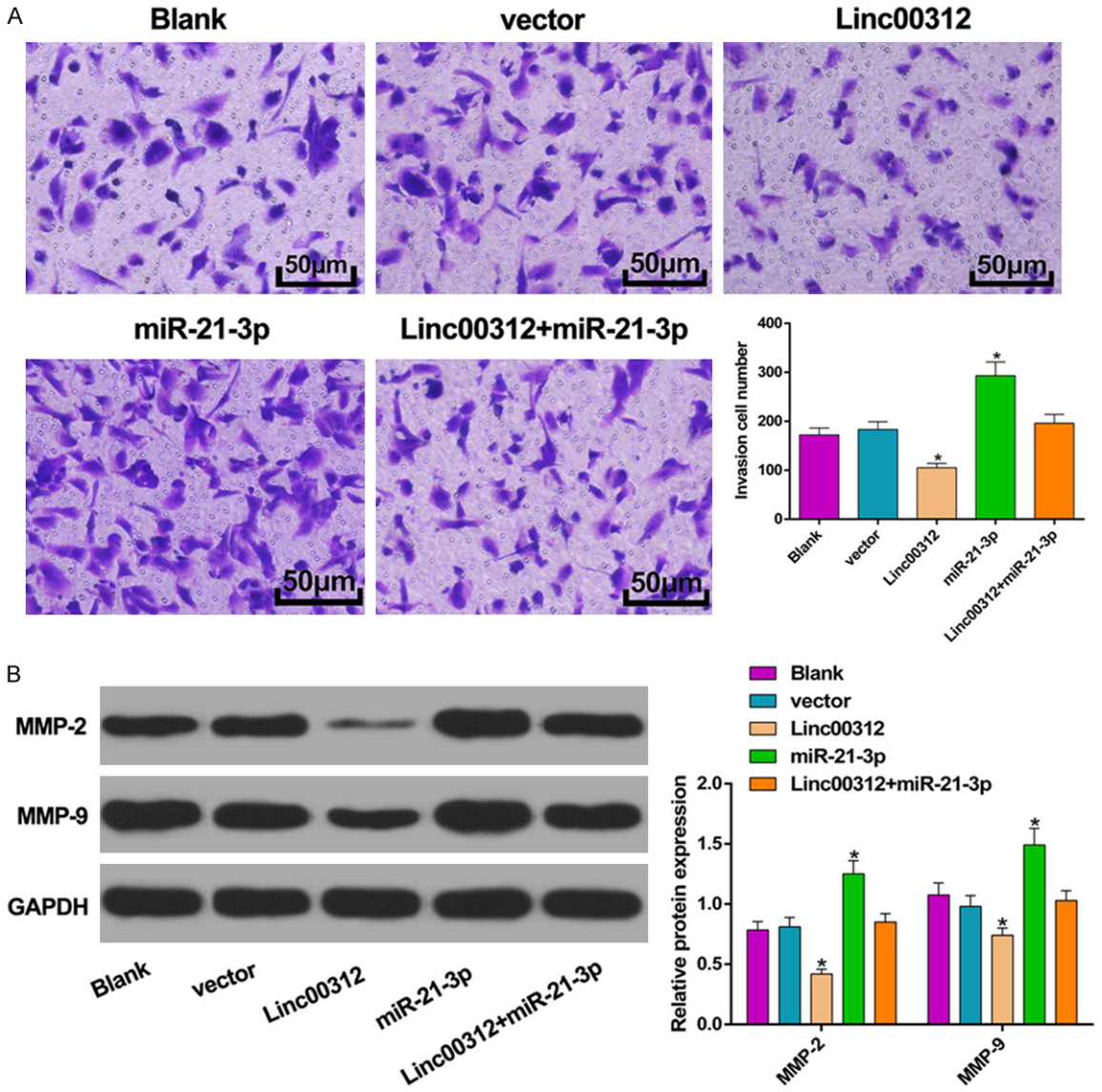

Figure 5. Results of glioma cell invasion. A: Cell invasion in each group (200×); B: Expressions of MMP-2 and MMP-9 in each group. Compared with blank group, *P<0.05. LINC00312: Long intergenic non-coding RNA 00312; miR-21-3p: microRNA-21-3p; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

ness [33]. E-cadherin belongs to the cadherin family together with N-cadherin. E-cadherin is involved in the homotypic intercellular adhesion and the maintenance of cell polarity, which plays an essential role in the maintenance of morphology and structural integrity of epithelial cells, while N-cadherin has the opposite effect, which can promote tumor infiltration and metastasis [34]. Vimentin is a cytoskeleton protein widely distributed in various interstitial cells but is not expressed in normal epithelial cells [35, 36]. MMPs are a class of proteolytic enzymes able to degrade the extracellular matrix and the basal membrane components, which have an influence on de-adhesion of cells and are involved in cancer cell metastasis and invasion [37]. It was found that MMP-2 and MMP-9 can degrade type IV collagen, and the expression levels of MMP-2 and MMP-9 in the cancer tissues are higher than those in the adjacent tissues [38]. Therefore, it is presumed that LINC00312 and miR-21-3p can suppress glioma cell invasion and EMT by influencing the expressions and roles of the above factors.

According to previous studies, miR-21 can inhibit the growth of glioma cells by targeting NF-

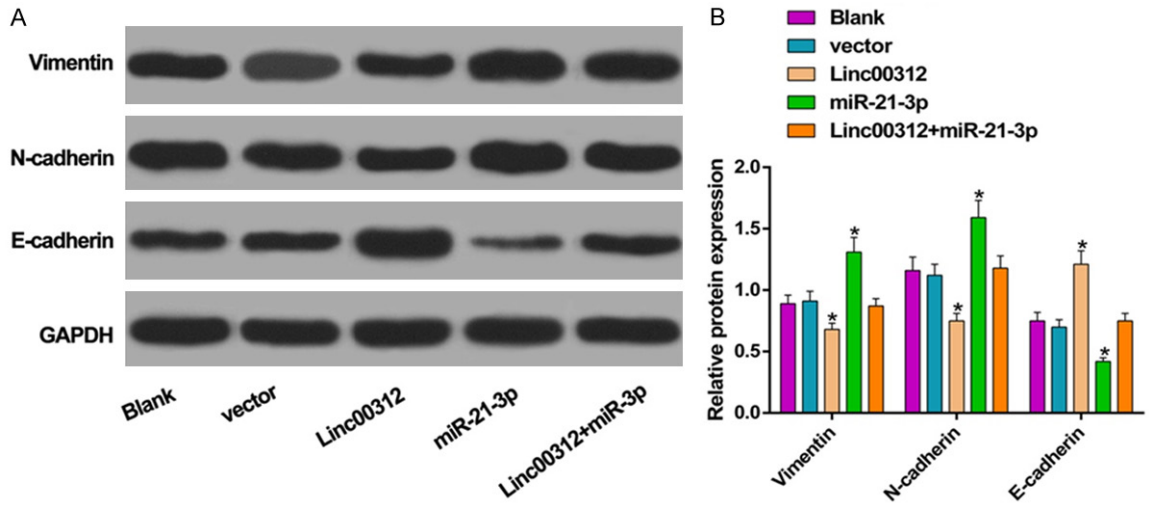

Figure 6. Expressions of EMT-related factors of glioma cells in each group. A: Protein bands of EMT-related factors in each group; B: Quantitative results of protein expressions of EMT-related factors in each group. Compared with blank group, *P<0.05. LINC00312: Long intergenic non-coding RNA 00312; miR-21-3p: microRNA-21-3p; EMT: Epithelial-mesenchymal transition; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

κB or PTEN signaling pathways [39, 40]. LINC00312/miR-21/PTEN signaling pathway has also been reported to play a role in the proliferation and progression of colorectal cancer cells [12]. Although the downstream targets of LINC00312/miR-21-3p signaling pathway in glioma have not yet been clearly identified, we speculated that LINC00312/miR-21-3p signaling pathway may have an effect on glioma by affecting the expression of downstream PTEN. In addition, miR-21 has been found to promote glioma progression by regulating the SPRY1 target and to modulate immune escape of glioma cells by targeting PEG3 [41, 42]. We, therefore, speculated that the effect of LINC00312/miR-21-3p on glioma may be achieved by regulating downstream targets including PTEN, SPRY1, and PEG3, but the specific mechanism remains to be further verified.

To sum up, in this study, we found that LINC00312 could act as a molecular sponge to adsorb miR-21-3p, thus regulating glioma cell proliferation, invasion, and EMT, playing a role in the inhibition of glioma cells. We hope more studies would be performed on glioma to explore more underlying mechanisms and effective potential targets for clinical diagnosis and treatment.

Disclosure of conflict of interest

None.

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References

[1] Huang LF, Li X, Ye H, Liu YJ, Liang XL, Yang C, Hua L, Yan ZX and Zhang X. Long non-coding RNA NCK1-AS1 promotes the tumorigenesis of glioma through sponging microRNA-138-2-3p and activating the TRIM24/Wnt/β-catenin axis. J Exp Clin Cancer Res 2020; 39: 63.

[2] Zi JJ, Wang WS, Sun MT, Mei W, Li SF, Li B, Xiao Y, Fei ZY, Zhang RP, Yu M and Xiong W. A high expression of MTERF3 correlates with tumor progression and predicts poor outcomes in patients with brain glioma. Int J Clin Exp Pathol 2019; 12: 1909-1920.

[3] Lin WW, Ou GY, Lin JZ, Yi SJ, Yao WC, Pan HC and Zhao WJ. Neuregulin 1 enhances cell adhesion molecule L1 like expression levels and promotes malignancy in human glioma. Oncol Lett 2020; 20: 326-336.

[4] Teodora AA and Iosif M. Alterations of contralateral white matter in glioma and meningioma patients: a numerical diffusion-weighted imaging approach. Int J Clin Exp Med 2019; 12: 2575-2582.

[5] Schulte C, Barwari T, Joshi A, Zeller T and Mayr M. Noncoding RNAs versus protein biomarkers in cardiovascular disease. Trends Mol Med 2020; 26: 583-596.

[6] Wasson CW, Abignano G, Hermes H, Malaab M, Ross RL, Jimenez SA, Chang HY, Feghali-Bostwick CA and Galdo FD. Long non-coding RNA HOTAIR drives EZH2-dependent myofibroblast activation in systemic sclerosis through miRNA 34a-dependent activation of NOTCH. Ann Rheum Dis 2020; 79: 507-517.
[7] Miao TT, Si QZ, Wei Y, Fan RH, Wang JJ and An XL. Identification and validation of seven prognostic long non-coding RNAs in oral squamous cell carcinoma. Oncol Lett 2020; 20: 939-946.
[8] Wu ZY, Wang SG, Li Q, Zhao QS and Shao MM. Identification of 10 differently expressed lncRNAs as prognostic biomarkers for prostate adenocarcinoma. Math Biosci Eng 2019; 17: 2037-2047.
[9] Xie Y and Cheng Y. LINC01198 facilitates gliomagenesis through activating PI3K/AKT pathway. RNA Biol 2020; 17: 1040-1052.
[10] Huang W, Shi Y, Han B, Wang QL, Zhang B, Qi CJ and Liu F. LncRNA GAS5-AS1 inhibits glioma proliferation, migration, and invasion via miR-106b-5p/TUSC2 axis. Hum Cell 2020; 33: 416-426.
[11] Huang C, Wu MH, Tang YL, Li XL, Ouyang J, Xiao L, Li D and Li GY. NAG7 promotes human nasopharyngeal carcinoma invasion through inhibition of estrogen receptor alpha and up-regulation of JNK2/AP-1/MMP1 pathways. J Cell Physiol 2009; 221: 394-401.
[12] Li G, Wang CM, Wang YB, Xu B and Zhang WZ. LINC00312 represses proliferation and metastasis of colorectal cancer cells by regulation of miR-21. J Cell Mol Med 2018; 22: 5565-5572.
[13] Min X, Liu K, Zhu HT and Zhang J. Long noncoding RNA LINC003121 inhibits proliferation and invasion of thyroid cancer cells by suppression of the phosphatidylinositol-3-kinase (PI3K)/akt signaling pathway. Med Sci Monit 2018; 24: 4592-4601.
[14] Jiang Y, Zhou JP, Zhao JS, Hou DQ, Zhang HY, Li L, Zou D, Hu JF, Zhang Y and Jing ZT. MiR-18a downregulated RORA inhibits the proliferation and tumorigenesis of glioma using the TNF-α-mediated NF-κB signaling pathway. EBioMedicine 2020; 52: 102651.
[15] Xu R, Zhou FQ, Yu TF, Xu GH, Zhang JX, Wang YY, Zhao L and Liu N. MicroRNA-940 inhibits epithelial-mesenchymal transition of glioma cells via targeting ZEB2. Am J Transl Res 2019; 11: 7351-7363.
[16] Wang P, Liu YH, Yao YL, Li Z, Li ZQ, Ma J and Xue YX. Long non-coding RNA CASC2 suppresses malignancy in human gliomas by miR-21. Cell Signal 2015; 27: 275-282.
[17] Grube S, Dünisch P, Freitag D, Klausnitzer M, Sakr Y, Walter J, Kalff R and Ewald C. Overexpression of fatty acid synthase in human gliomas correlates with the WHO tumor grade and inhibition with Orlistat educes cell viability and triggers apoptosis. J Neurooncol 2014; 118: 277-287.
[18] Zhang ML, Huang NN, Yang XS, Luo JY, Yan S, Xiao FZ, Chen WP, Gao XY, Zhao K, Zhou HK, Li ZQ, Ming L, Xie B and Zhang N. A novel protein encoded by the circular form of the SHPRH gene suppresses glioma tumorigenesis. Oncogene 2018; 37: 1805-1814.
[19] Li Jun and Zhou L. Overexpression of lncRNA DANCR positively affects progression of glioma via activating Wnt/β-catenin signaling. Biomed Pharmacother 2018; 102: 602-607.
[20] Disney-Hogg L, Cornish AJ, Sud A, Law PJ, Kinnersley B, Jacobs DI, Ostrom QT, Labreche K, Eckel-Passow JE, Armstrong GN, Claus EB, Il›yasova D, Schildkraut J, Barnholtz-Sloan JS, Olson SH, Bernstein JL, Lai RK, Schoemaker MJ, Simon M, Hoffmann P, Nöthen MM, Jöckel KH, Chanock S, Rajaraman P, Johansen C, Jenkins RB, Melin BS, Wrensch MR, Sanson M, Bondy ML and Houlston RS. Impact of atopy on risk of glioma: a Mendelian randomisation study. BMC Med 2018; 16: 42.
[21] Sun G, Cao Y, Xu YT, Huai D, Chen P, Guo J, Li M and Dai YY. Overexpression of Hsc70 promotes proliferation, migration, and invasion of human glioma cells. J Cell Biochem 2019; 120: 10707-10714.
[22] da Silva RA, Ferreira MR, Gomes AM and Zambuzzi WF. LncRNA HOTAIR is a novel endothelial mechanosensitive gene. J Cell Physiol 2020; 235: 4631-4642.
[23] Zheng YY, Wang ZY, Zhu YB, Wang W, Bai M, Jiao Q, Wang YR, Zhao SJ, Yin XB, Guo D and Bai WL. LncRNA-000133 from secondary hair follicle of Cashmere goat: identification, regulatory network and its effects on inductive property of dermal papilla cells. Anim Biotechnol 2020; 31: 122-134.
[24] Zhang YL, Zhang DH, Lv J, Wang S and Zhang Q. LncRNA SNHG15 acts as an oncogene in prostate cancer by regulating miR-338-3p/FKBP1A axis. Gene 2019; 705: 44-50.
[25] Jiang JX, Wang XL, Gao GZ, Liu XX, Chang H, Xiong R, Lu J and Sun ZY. Silencing of lncRNA HOXA11-AS inhibits cell migration, invasion, proliferation, and promotes apoptosis in human glioma cells via upregulating microRNA-125a: in vitro and in vivo studies. Am J Transl Res 2019; 11: 6382-6392.
[26] Chen YJ, Qiu FH, Huang LC, Liu WP, Li LQ, Ji CH, Zeng XQ, Qiao LL, Liu MQ and Gong XQ. Long non-coding RNA LINC00312 regulates breast cancer progression through the miR-9/CDH1 axis. Mol Med Rep 2020; 21: 1296-1303.
[27] Peng ZZ, Wang J, Shan B, Li B, Peng W, Dong YP, Shi WW, Zhao WY, He D, Duan MH, Cheng YD, Zhang CF and Duan CJ. The long noncod-

ing RNA LINC00312 induces lung adenocarcinoma migration and vasculogenic mimicry through directly binding YBX1. Mol Cancer 2018; 17: 167.
[28] Zhang ZY, Wang S, Ji DJ, Qian WW, Wang QY, Li J, Gu JO, Peng W, Hu T, Ji B, Zhang Y, Wang SJ and Sun YM. Construction of a ceRNA network reveals potential lncRNA biomarkers in rectal adenocarcinoma. Oncol Rep 2018; 39: 2101-2113.
[29] Zhao YJ, Wang H, Wu CJ, Yan MN, Wu HJ, Wang JZ, Yang XX and Shao QX. Construction and investigation of lncRNA-associated ceRNA regulatory network in papillary thyroid cancer. Oncol Rep 2018; 39: 1197-1206.
[30] Kong XL, Duan Y, Sang YT, Li YM, Zhang HW, Liang YR, Liu Y, Zhang N and Yang QF. LncRNA-CDC6 promotes breast cancer progression and function as ceRNA to target CDC6 by sponging microRNA-215. J Cell Physiol 2019; 234: 9105-9117.
[31] Luo HR, Xu CD, Le W, Ge BJ and Wang TR. lncRNA CASC11 promotes cancer cell proliferation in bladder cancer through miRNA-150. J Cell Biochem 2019; 120: 13487-13493.
[32] Cui BZ, Li BS, Liu Q and Cui YQ. lncRNA CCAT1 promotes glioma tumorigenesis by sponging miR-181b. J Cell Biochem 2017; 118: 4548-4557.
[33] Loh CY, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, Chong PP and Looi CY. The E-Cadherin and N-Cadherin Switch in epithelial-to-mesenchymal transition: signaling, therapeutic implications, and challenges. Cells 2019; 8: 1118.
[34] Noh MG, Oh SJ, Ahn EJ, Kim YJ, Jung TY, Jung S, Kim KK, Lee JH, Lee KH and Moon KS. Prognostic significance of E-cadherin and N-cadherin expression in Gliomas. BMC Cancer 2017; 17: 583.
[35] Satelli A and Li SL. Vimentin in cancer and its potential as a molecular target for cancer therapy. Cell Mol Life Sci 2011; 68: 3033-3046.
[36] Strouhalova K, Přechová M, Gandalovičová A, Brábek J, Gregor M and Rosel D. Vimentin intermediate filaments as potential target for cancer treatment. Cancers (Basel) 2020; 12: 184.
[37] Wang TT, Wang XM and Zhang XL. Circulating vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1): relationship with carotid artery elasticity in patients with impaired glucose regulation (IGR). Ann Endocrinol (Paris) 2019; 80: 72-76.
[38] Sun XF, Shao YB, Liu MG, Chen Q, Liu ZJ, Xu B, Luo SX and Liu H. High-concentration glucose enhances invasion in invasive ductal breast carcinoma by promoting Glut1/MMP2/MMP9 axis expression. Oncol Lett 2017; 13: 2989-2995.
[39] Li YN, Dai DW, Lu Q, Fei MY, Li MM and Wu X. Sirt2 suppresses glioma cell growth through targeting NF-κB-miR-21 axis. Biochem Biophys Res Commun 2013; 441: 661-667.
[40] Guo XF, Qiu W, Liu QL, Qian MY, Wang SB, Zhang Z, Gao X, Chen ZH, Xue H and Li G. Immunosuppressive effects of hypoxia-induced glioma exosomes through myeloid-derived suppressor cells via the miR-10a/Rora and miR-21/Pten pathways. Oncogene 2018; 37: 4239-4259.
[41] Chai C, Song LJ, Han SY, Li XQ and Li M. MicroRNA-21 promotes glioma cell proliferation and inhibits senescence and apoptosis by targeting SPRY1 via the PTEN/PI3K/AKT signaling pathway. CNS Neurosci Ther 2018; 24: 369-380.
[42] Yang F, Wang TC, Du P, Fan HT, Dong XS and Guo H. M2 bone marrow-derived macrophage-derived exosomes shuffle microRNA-21 to accelerate immune escape of glioma by modulating PEG3. Cancer Cell Int 2020; 20: 93.