

# Long non-coding RNA MIR22HG inhibits glioma progression by downregulating microRNA-9/CPEB3

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**Abstract.** Glioma is one of the most common and aggressive malignant intracranial tumors worldwide. Recently, non-coding RNAs have been found to play critical roles in the development of glioma. However, the exact mechanisms have not been fully elucidated. In the present study, reverse transcription-quantitative PCR was used to determine the expression level of the long non-coding RNA MIR22HG and microRNA (miR)-9, while western blot analysis was used to detect the protein expression level of CPEB3. The potential binding sites were predicted using the StarBase v2.0 online tool and the hypothesis was verified using a luciferase reporter assay. A Cell Counting Kit-8 assay was used to assess cell viability, while wound healing and Matrigel assays were used to determine the migration and invasion ability of glioma cancer cells. The results showed that MIR22HG expression level was decreased but miR-9 expression level was elevated in glioma tissues and cell lines. Furthermore, MIR22HG was found to sponge miR-9, while CPEB3 was the direct target of miR-9 in the glioma cell line. Functionally, MIR22HG regulated the proliferation, invasion and migration of the glioma cell line by targeting miR-9. CPEB3 may be involved in the progression of the glioma cell line. Taken together, these findings confirmed that MIR22HG suppressed glioma development by inhibiting the miR-9/CPEB3 axis and provides a novel therapeutic strategy for glioma treatment.

## Introduction

Glioma is one of the most aggressive and common malignant intracranial tumors (1-3) and is characterized by a high invasive ability and a high lethality rate. In 2014, 56,000 patients

died as a result of glioma in China (4-6). According to the morphological characteristics of glioma, it can be classified as either astrocytoma, glioblastoma, oligodendroglioma, mixed oligoastrocytomas or ependymoma (7). Despite the development of active therapies (including surgery, radiotherapy, and chemotherapy), the prognosis of patients with glioma is still poor (8). A previous study reported that the median survival time for patients with high-grade glioma does not exceed 15 months (9). Therefore, elucidating the underlying mechanism of glioma pathogenesis is beneficial for diagnosis and treatment.

Long non-coding (lnc)RNAs are defined as a group of RNAs, with a total length of >200 nucleotides and a lack of protein-coding ability (10). lncRNAs, as tumor suppressor genes or oncogenes, play essential roles in vital cell functions, such as cell growth, proliferation, and invasion (11,12). Currently, increasing evidence suggests that lncRNAs function as competitive endogenous RNAs in cancer progression by sponging and inhibiting the expression of microRNAs (miRNA/miR) (13). For example, the lncRNA MIR503HG inhibited cell migration and invasion by sponging miR-103 in triple-negative breast cancer (14), while the lncRNA MEG3 repressed cell growth in glioma by modulating the expression level of miR-96-5p (15). For colorectal cancer (CRC), the lncRNA MFI2-AS1 facilitated CRC cell proliferation, migration and invasion by sponging miR-574-5p (16).

The lncRNA MIR22HG is located in the 17p13.3 region; it primarily exists in the cytoplasm and is frequently hypermethylated and degraded (17). It has been hypothesized that the lncRNA MIR22HG could be involved in the development and progression of different types of tumor, including hepatocellular carcinoma (18). Previous research has shown that the lncRNA MIR22HG contributed to the inhibition of gastric cancer by attenuating NOTCH2 signaling (19). In endometrial carcinoma, the apoptosis of endometrial carcinoma cells was strengthened by MIR22HG, which negatively regulated miR-141-3p and enhanced death-associated protein kinase 1 protein expression (20). In addition, the lncRNA MIR22HG plays a tumor suppressor role in lung cancer by downregulating the oncogenes, Y-box binding protein 1, MET proto-oncogene receptor tyrosine kinase, and p21 (21).

The cytoplasmic polyadenylation element-binding protein (CPEB) family includes RNA-binding proteins that mediate

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translation through cytoplasmic polyadenylation (22,23). The CPEB family typically consists of four members, CPEB-1, -2, -3 and -4 in vertebrates (24). CPEB3 primarily exists in the cytoplasm and plays a vital role in synaptic plasticity and memory (25,26). In recent years, it has been reported that CPEB3 participates in regulating various types of cancer (27,28). In CRC, the lncRNA SUMO1P3 induced cell proliferation and the cell cycle, and restricted apoptosis by epigenetically silencing CPEB3 (29).

Recently, the upregulation of CPEB3 was found to inhibit cell proliferation and growth in a glioma cell line (30). Furthermore, miR-9 was found to be a positive regulator in glioma tumorigenesis and angiogenesis (31). However, the association between miR-9 and CPEB3 is unclear. In addition, previous findings indicated that the lncRNA MIR22HG participated in the development of a variety of different cancers including prostate cancer, colorectal cancer and glioma (32-34). However, the exact role and detailed mechanism of the lncRNA MIR22HG in the development of glioma is unclear.

In the present study, we aimed to explore the expression of lncRNA MIR22HG, miR-9 and CPEB3. We tried to investigate the effect of MIR22HG in the progression of glioma and elucidate the underlying molecular. Our study intended to uncover the molecular pathological mechanism and provided potential therapeutic targets for glioma.

## Materials and methods

**Clinical specimens.** All tissues, including 25 human glioma tissues and 15 normal brain tissues (tissues from patients with traumatic brain injury) were obtained from patients who underwent surgical excision at the Neurosurgery Department of Tangdu Hospital from January 2017 to January 2018. The mean ages of the patients with glioma and control patients were 48.5 and 46.8 years, respectively. Patients with glioma composed of 14 males and 11 females. The control patients composed of eight males and 7 females. All tissue samples were pathologically evaluated by senior pathologist according to the fourth edition of the World Health Organization classification of tumors of the central nervous system (2016) (35) and stored in liquid nitrogen at -80°C. The study protocol was approved by the Ethical Committee of Tangdu Hospital of the Fourth Military Medical University, and written informed consent was provided from all the patients.

**Cell culture.** The human glioma cell lines U251 (TCHu 58), U87 (TCHu138; glioblastoma of unknown origin) and the HA1800 normal human astrocyte cell line (JNO171-101) were purchased from the Cell Resource Center of the Shanghai Institute of Biological Sciences. To confirm the identity of the cells over long culture periods, the U87 cell line was genotyped using short tandem repeat analysis and the cells were confirmed to be the U87MG cell line from American Type Culture Collection. All the cell lines were cultured in DMEM, supplemented with 10% FBS and 100 U/ml penicillin/streptomycin at 37°C in a humidified incubator with 5% CO<sub>2</sub>.

**Starbase and TargetScan analysis.** The relationship between MIR22HG and miR-9 was analyzed using online software

Starbase (<http://starbase.sysu.edu.cn/>). The miRNA-LncRNA tool was utilized to analyze the relationship. The target miRNA was miR-9 and the predicted lncRNAs were listed in the software. Similarly, the relationship between miR-9 and CPEB3 was also analyzed using TargetScan ([http://www.targetscan.org/mamm\\_31/](http://www.targetscan.org/mamm_31/)). The method we used was miRNA- tool and the target miRNA was also miR-9.

**Reverse transcription-quantitative PCR (RT-qPCR).** Total RNA from glioma tissues or cell lines was extracted using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.). The isolated RNA was reverse transcribed into complementary DNA using a PrimeScript™ RT master mix (Takara Biotechnology, Inc.) and TaqMan advanced miRNA assay kit (Thermo Fisher Scientific, Inc.) at 42°C for 15 min. A 20-μl reaction system was used: 0.5 μl Sense and antisense primers, 50 ng RT product and 10 μl 2X SYBR TransStar Green PCR Super mix (cat. no. B21202; <https://www.bimake.com/product/sybr-green-qpcr-master-mix.html>) in an IQ™ 5 Real-Time PCR Detection system (Bio-Rad Laboratories, Inc.). The thermocycling conditions were: 95°C For 4 min for denaturation, followed by 40 cycles for 23 sec, and annealing for 28 sec at 60°C and extension at 72°C for 35 sec. miR-103 and GAPDH served as normalizing controls and the 2<sup>-ΔΔC<sub>q</sub></sup> method was used for quantification (36,37). The sequences of the primers were as follows: MIR22HG forward, 5'-CGGACGCAGTGATTTGCT-3' and reverse, 5'-GCTTTA GCTGGGTCAGGACA-3'; miR-9 forward, 5'-GAGGCCCGT TTCTCTCTTTG-3' and reverse, 5'-AGCTTTATGACGGCT CTGTG-3'; CPEB3 forward, 5'-TCAACACAACGACATTGA CAAA-3' and reverse, 5'-CCCTGACACTCGTCACACAT-3'; GAPDH forward, 5'-AAATCCCATCACCATCTTCCAG-3' and reverse, 5'-TGATGACCCTTTTGGCTCCC-3'; miR-103 forward, 5'-GCCGAGCTGCCTTGTGGAATC-3' and reverse, 5'-CTGCCCTGTGGAATCACAT-3'.

**Cell transfection.** The pcDNA3 empty vector and pcDNA3-MIR22HG overexpression vector, and the miR-9 mimic and negative control (NC) mimic were constructed by Shanghai GenePharma, Co., Ltd.. The cells were transfected with 3 μg control or overexpression vector in one well of 6-well plate. The working concentration of miR-9 mimic was 50 nM. Then, according to the manufacturer's instructions, the U87 and U251 cell lines were transfected with the pcDNA3 empty vector, pcDNA3-MIR22HG, miR-9 mimic, or NC mimic using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). After transfection for 48 h, the cells were used for subsequent experiments.

**Luciferase reporter assay.** The luciferase reporter assay was used to determine the association between miR-9 and MIR22HG, and CPEB3. Wild-type (WT) and mutant (MUT) MIR22HG or CPEB3 3'-untranslated region sequences were inserted into the pmirGLO luciferase reporter vectors (Promega Corporation) to construct a luciferase reporter vector. In total, 50 ng WT and MUT luciferase vectors or 50 nM miR-9 mimic were transfected into U87 or U251 cells using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 20 min at room temperature. After 48 h, the luciferase activities were detected using a dual-luciferase reporter assay kit

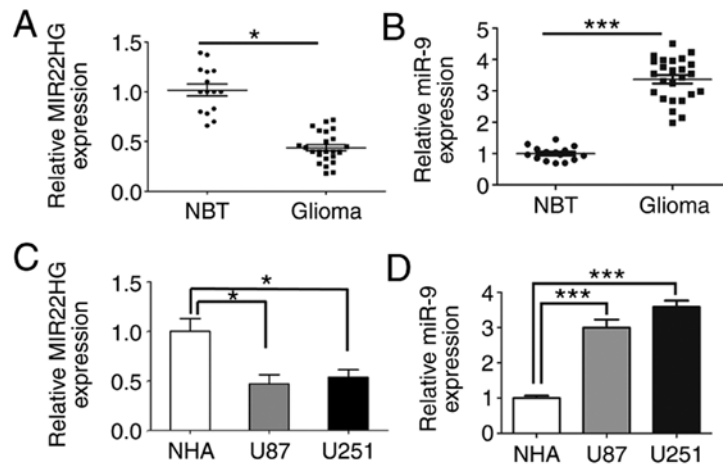


Figure 1. Expression level of MIR22HG and miR-9 in glioma tissues and cell lines. (A) MIR22HG expression level was decreased in glioma tissues compared with that in NBT. The data was compared using a Student's t-test.  $n=40$ .  $^*P<0.05$ . (B) miR-9 expression level was increased in glioma tissues compared with that in NBT. The data was compared using a Student's t-test.  $n=40$ .  $^{***}P<0.001$ . (C) The expression level of MIR22HG was decreased in the U87 and U251 cell lines compared with that in the NHA cell line. The data was compared using one-way ANOVA, followed by Tukey's post hoc test.  $n=3$ .  $^*P<0.05$ . (D) The expression level of miR-9 was increased in the U87 and U251 cell lines compared with that in the NHA cell line. The data was compared using one-way ANOVA, followed by Tukey's post hoc test.  $n=3$ .  $^{***}P<0.001$ . NBT, normal brain tissue; miR, microRNA; NHA, normal human astrocyte.

(Promega Corporation). The firefly luciferase was normalized using *Renilla* luciferase as the internal control.

**Western blot assay.** The collected cells was lysed in the RIPA lysis buffer (Beyotime Institute of Biotechnology) on the ice for 30 min. Total protein was extracted from the glioma cell lines and the protein concentration was determined using a BCA protein concentration kit (Beyotime Institute of Biotechnology). Then, 20  $\mu\text{g}$  protein was separated per lane using 10% SDS-PAGE and transferred to PVDF membranes (EMD Millipore). Following which, the PVDF membranes were blocked with 5% skimmed milk for 2 h, at room temperature, then incubated with anti-CPEB3 (cat. no. ab10883, 1:1,000 dilution) and  $\beta$ -actin antibodies (cat. no. ab8226, 1:2,000 dilution both from Abcam,) overnight at 4°C. The membranes were washed with TBST for 5 min three times at room temperature. After washing with 1X TBST, the membranes were then incubated with HRP-conjugated goat anti-rabbit IgG H&L (cat. no. as014) or goat anti-mouse IgG H&L secondary antibodies (cat. no. as003; 1:5,000) (both ABclonal Biotech Co., Ltd.) for 2 h at room temperature. The signals were visualized using an enhanced chemiluminescence kit (EMD Millipore).

**Cell Counting Kit-8 (CCK-8) assay.** The kit was used according to the instruction of the manufacturer. U87 and U251 cells transfected with MIR22HG or/and miR-9 were seeded in a 96-well plate, at a density of 5,000 cells/well. In total, 10  $\mu\text{l}$  CCK-8 solution (Beyotime Institute of Biotechnology) was added to each well according to the manufacturer's instructions. The absorbance was examined at 450 nm in Multiskan™ FC microplate reader with Skan It software 2.4.2 (both Thermo Fisher Scientific, Inc.).

**Matrigel assay.** The diluted Matrigel was used to cover the upper chamber of the Transwell inserts for 5 h at 37°C. Then, 200  $\mu\text{l}$  serum-free medium containing  $3 \times 10^4$  U87 or

U251 cells was added into the upper chamber of the Transwell inserts, while 500  $\mu\text{l}$  10% FBS-containing complete medium was added to the lower chamber. The cells were incubated for 24 h (at 37°C in a humidified incubator with 5%  $\text{CO}_2$ ). After 24 h, the cells were fixed with 4% paraformaldehyde for 0.5 h at room temperature, then stained with crystal violet staining solution for 0.5 h at room temperature. The number of stained cells in five random fields of view was counted using a light phase contrast microscope with x10 magnification.

**Wound healing assay.** The U87 and U251 cell lines were plated in a 6-well plate until they had reached 90% confluence. The glioma cells were scraped using a 20- $\mu\text{l}$  pipette tip to generate a wound. Then, the cells were washed three times with PBS and cultured with serum-free culture medium for 24 h. Cell migration was observed using a light phase contrast microscope at x10 magnification. Images were captured and analyzed with FL Color Cell Imaging systems version 1.4. (Thermo Fisher Scientific, Inc.).

**Statistical analysis.** An unpaired Student's t-test was used to analyze differences between two groups, while one-way ANOVA followed by Tukey's post hoc test to determine the differences between three or more groups. SPSS v19.0 (SPSS, Inc.) was used for the analysis. The data are presented as the mean  $\pm$  SEM of at least three different experiments.  $P<0.05$  was considered to indicate a statistically significant difference.

## Results

**MIR22HG is downregulated and miR-9 is increased in human glioma tissues and glioma cell lines.** To determine the role of MIR22HG in human glioma tissues, the expression level of MIR22HG was measured using RT-qPCR and the results showed that the expression level of MIR22HG significantly decreased in human glioma tissues compared with that in adjacent normal tissues (Fig. 1A). However, the expression

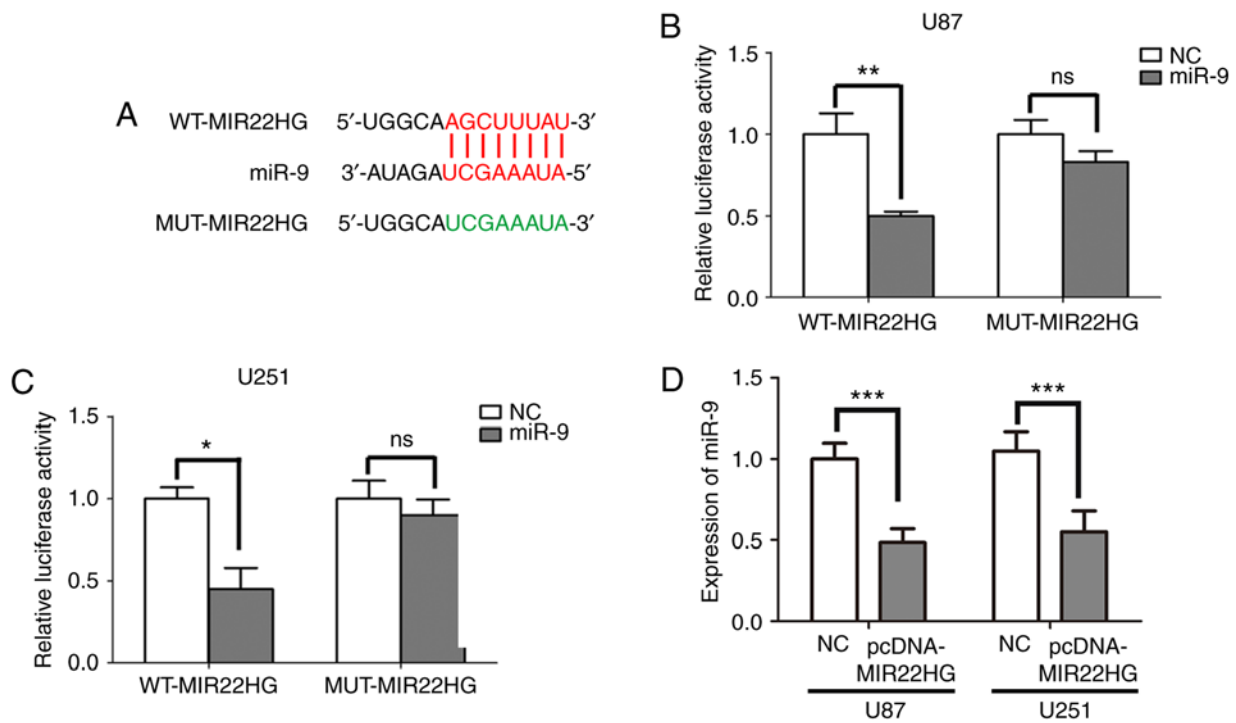


Figure 2. MIR22HG directly targets miR-9 in the glioma cell lines. (A) The predicted binding sites between miR-9 and MIR22HG using bioinformatics tools. Luciferase activity was decreased in the (B) U251 and (C) U87 glioma cells co-transfected with miR-9 mimic and MIR22HG-WT. The data was compared using a Student's t-test.  $n=3$ . \* $P<0.05$ , \*\* $P<0.01$ . (D) The expression level of miR-9 in the U251 and U87 glioma cells transfected with MIR22HG overexpression vector. The data was compared using a Student's t-test.  $n=3$ . \*\*\* $P<0.001$ . miR, microRNA; WT, wild-type; MUT, mutant; NC, negative control; ns, non-significant.

level of miR-9 was increased in human glioma tissues, which suggested that MIR22HG may be associated with miR-9 (Fig. 1B). Therefore there may be a regulatory relationship between them. Furthermore, the expression level of MIR22HG and miR-9 in the glioma cell lines was also detected using RT-qPCR and the results showed that the expression level of MIR22HG was decreased in the glioma cell lines compared with that in the normal human astrocytes (Fig. 1C). Conversely, miR-9 expression level was also increased in the glioma cell lines compared with that in the normal cell lines (Fig. 1D).

**MIR22HG sponges miR-9 in the glioma cell lines.** Previous studies have confirmed that lncRNAs could act as sponges to regulate miRNAs (38-40). It was found that MIR22HG contained a potential binding site for miR-9 using the bioinformatics database StarBase v2.0 (Fig. 2A). To further investigate whether MIR22HG could directly bind to miR-9 in the glioma cell lines, the U251 or U87 cells were co-transfected with miR-9 mimic and WT or MUT MIR22HG. The effect of miR-9 mimic in the U87 and U251 cells was confirmed (Fig. S1A and B). Then, the luciferase activity of the cells was detected. The results indicated that the luciferase activity was significantly reduced when U87 or U251 cells were transfected with the miR-9 mimic and MIR22HG-WT. In contrast, the luciferase activity did not significantly change when U87 or U251 cells were transfected with the miR-9 mimic and MIR22HG-MUT (Fig. 2B and C). These results verified that miR-9 was a direct target of MIR22HG. Furthermore, MIR22HG was overexpressed in the U87 and U251 cell lines and the expression level of miR-9 was detected

using RT-qPCR. The results confirmed that MIR22HG was increased when transfected with pcDNA-MIR22HG (Fig. S1C and D). Furthermore, upregulating MIR22HG expression suppressed the expression level of miR-9 in the glioma cell lines (Fig. 2D).

**CPEB3 is the target molecule of miR-9 in the glioma cell lines.** The present study revealed that miR-9 is the direct target of MIR22HG in the glioma cell lines; however, the potential regulatory mechanisms remain unclear. The target gene of miR-9 was predicted using bioinformatics tools (TargetScan) (Fig. 3A). CPEB3 was identified as the downstream target of miR-9. The luciferase reporter assay showed that the luciferase activity was significantly decreased in CPEB3-WT, while there was no significant difference for CPEB3-MUT (Fig. 3B and C). Then, the mRNA and protein expression levels of CPEB3 were determined using RT-qPCR and western blot analysis, respectively. Transfection with MIR22HG overexpression vector increased the mRNA and protein expression level of CPEB3, while the mRNA and protein expression levels of CPEB3 was reduced when the U87 and U251 cell lines were co-transfected with miR-9 mimic and MIR22HG overexpression vector (Fig. 3D and E). In summary, CPEB3 was a direct target of miR-9, and MIR22HG regulated the expression of CPEB3 via miR-9.

**MIR22HG regulates the proliferation, invasion and migration of the glioma cells through miR-9.** It has been reported that MIR22HG is a tumor suppressor in a variety of different cancer types (33,41,42). However, the role of MIR22HG in

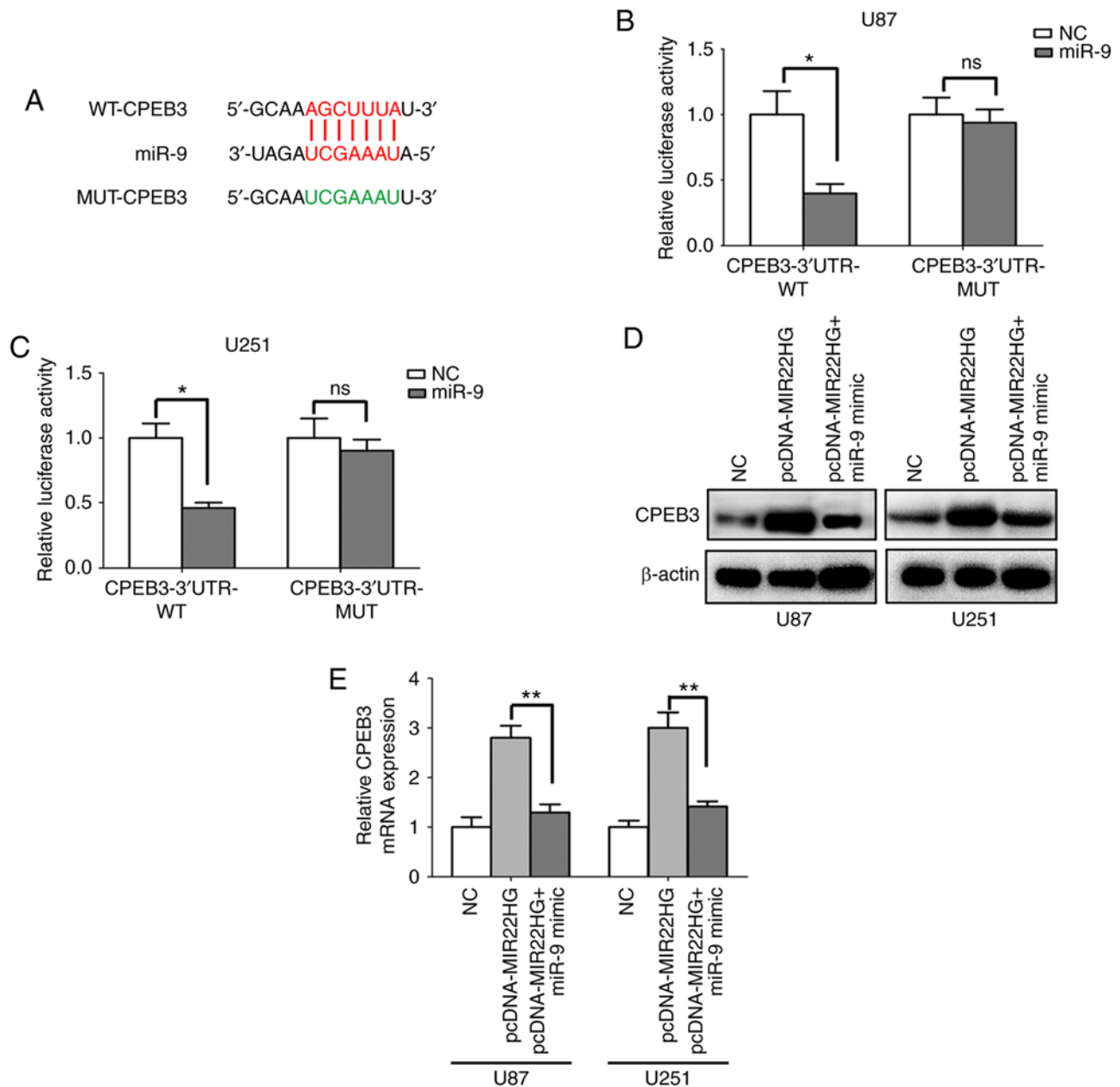


Figure 3. CPEB3 is the target of miR-9 in the glioma cell lines. (A) The potential binding site between miR-9 and CPEB3 was predicted using bioinformatics analysis. Luciferase activity was decreased in (B) U251 and (C) U87 glioma cells co-transfected with miR-9 mimic and WT-CPEB3. The data was compared using a Student's t-test.  $n=3$ . \* $P<0.05$ . (D) The protein and (E) expression levels of CPEB3 were detected using reverse transcription-quantitative PCR and western blot analysis, respectively, following co-transfection with pcDNA-MIR22HG and with and without miR-9 mimic in the U251 and U87 glioma cell lines. The data was compared with one-way ANOVA, followed by Tukey's post hoc test.  $n=3$ . \*\* $P<0.01$ . miR, microRNA; WT, wild-type; MUT, mutant; NC, negative control; ns, non-significant; UTR, untranslated region.

glioma is unknown. To investigate the function of MIR22HG in the glioma cell lines, MIR22HG overexpressing vector was transfected into the cell lines with or without miR-9 mimic. The CCK-8 assay showed that upregulated MIR22HG expression restricted glioma cell proliferation, but the miR-9 mimic reversed the inhibitory effects of MIR22HG overexpression (Fig. 4A and B). In addition, the overexpression of MIR22HG repressed glioma cell migration and invasion and the effects were abrogated by transfection with the miR-9 mimic (Fig. 4C-D). In summary, the aforementioned data suggested that MIR22HG regulated the proliferation, invasion and migration of glioma cells through miR-9.

## Discussion

Glioma is a malignant brain tumor with high incidence rates, that can immensely impair public health (43). Despite improvements in glioma therapy, the 5-year survival rate of patients is <6% in 2016 (44). Therefore, clarifying the detailed molecular mechanisms of glioma would assist with discovering novel therapeutic targets. As showed in Fig. 5, it was found that MIR22HG expression level was decreased while miR-9 expression level was increased in glioma tissues and cell lines. Then, MIR22HG was proven to directly bind to and negatively regulate miR-9 in the glioma cell lines.



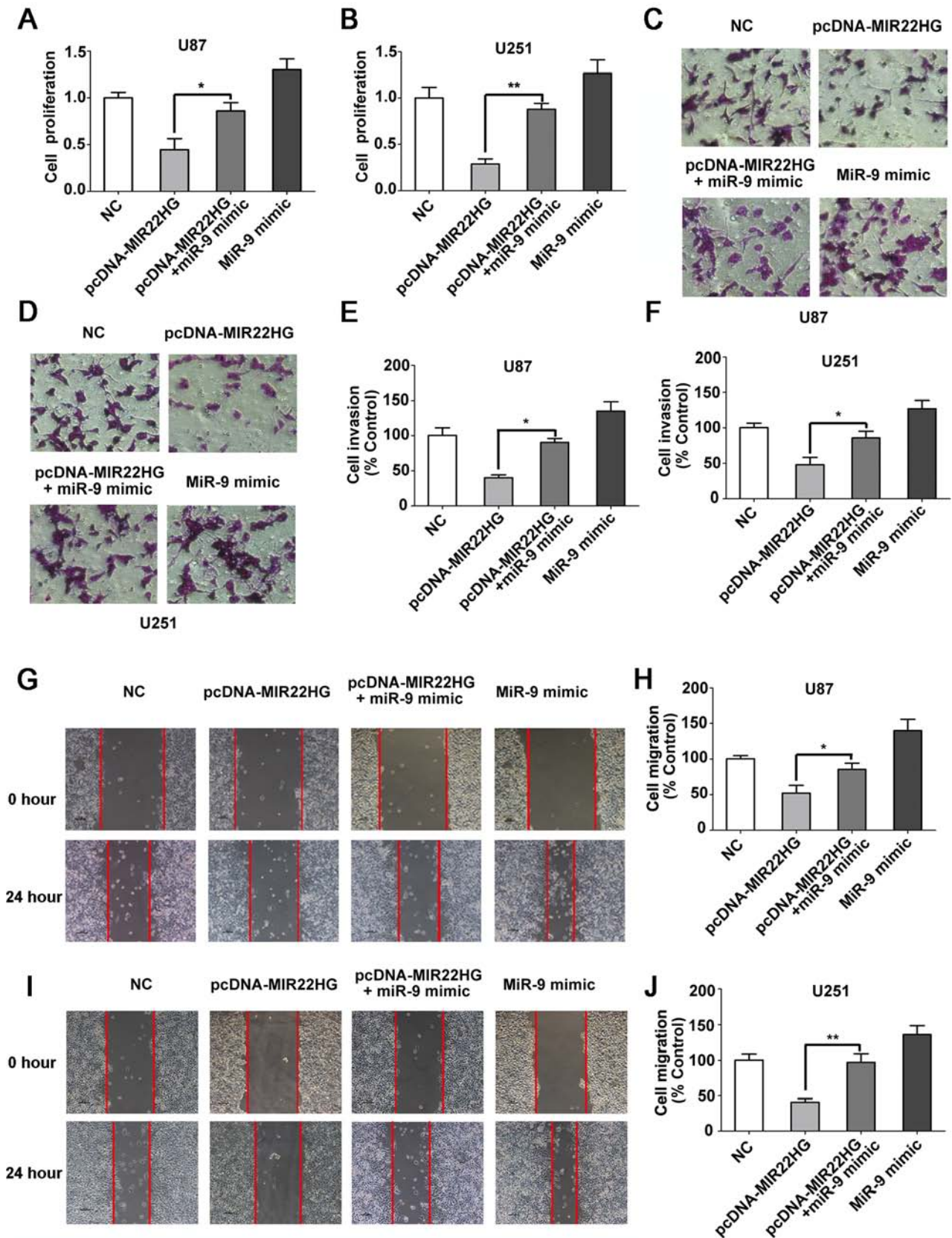


Figure 4. MIR22HG regulates the proliferation, invasion and migration of the glioma cells through miR-9. Cell proliferation was detected in (A) U87 and (B) U251 cell lines transfected with NC, pcDNA-MIR22HG, pcDNA-MIR22HG + miR-9 mimic, or with miR-9 mimic using Cell Counting Kit-8 assay. The cell viability of NC group was set as the control. The data was compared using one-way ANOVA, followed by Tukey's post hoc test.  $n=3$ . \* $P<0.05$ . \*\* $P<0.01$ . Cell invasion was examined in the (C) U87 and (D) U251 cell lines transfected with NC, pcDNA-MIR22HG, pcDNA-MIR22HG+miR-9 mimic, or miR-9 mimic using Matrigel assay, and the data was subsequently quantified in (E) U87 and (F) U251 cell lines. The data was compared using one-way ANOVA, followed by Tukey's post hoc test.  $n=3$ . \* $P<0.05$ . \*\* $P<0.01$ . Cell migration was determined in (G, H, I and J) U87 and U251 cell lines transfected with NC, pcDNA-MIR22HG, pcDNA-MIR22HG + miR-9 mimic, or miR-9 mimic. The data was compared using one-way ANOVA, followed by Tukey's post hoc test.  $n=3$ . \* $P<0.05$ . \*\* $P<0.01$ . miR, microRNA; WT, wild-type; MUT, mutant; NC, negative control.

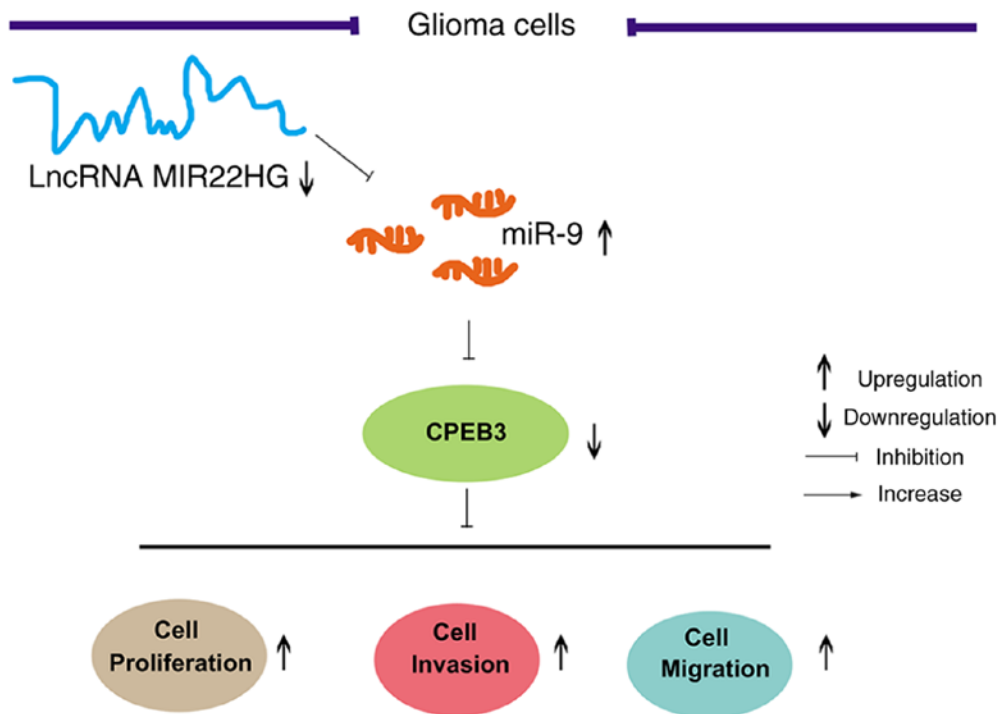


Figure 5. Graphical presentation of the molecular mechanisms in the present study. LncRNA MIR22HG negatively modulated miR-9 and miR-9 negatively regulated CPEB3. As a result, MIR22HG inhibited the proliferation, invasion and migration of glioma via modulating the miR-9/CPEB3 axis. MIR22HG was cloned into the pcDNA3.1 overexpression vector. Lnc, long non-coding; miR, microRNA.

Furthermore, it was found that CPEB3 was the target molecule of miR-9. Functionally, MIR22HG negatively regulated glioma cell proliferation, invasion and migration by modulating the miR-9/CPEB3 axis.

In the present study, we identified that MIR22HG expression level was significantly decreased in human glioma tissues compared with that in adjacent normal tissues. In contrast, the expression level of miR-9 was increased. It has been reported that the lncRNA MIR22HG functions as an antioncogene (45) and the expression level was decreased in multiple types of cancer. For example, the expression level of the lncRNA MIR22HG was decreased in lung cancer tissues, and was associated with poor patient survival (21). In thyroid cancer, decreased MIR22HG expression level was associated with the degree of malignancy of tumors, which indicated that MIR22HG could be a new diagnostic marker for thyroid cancer (46). miR-9 has been found to be an oncogene or a tumor suppressor in several types of cancer. In prostate cancer cell lines, miR-9 inhibitor transfection could suppress cell proliferation, invasion and migration (47). Furthermore, a previous research showed that miR-9 expression level was increased in glioma tissues and cell lines (48).

Then, the association between MIR22HG and miR-9 was investigated. The bioinformatics database StarBase v2.0 showed that MIR22HG contained a potential binding site for miR-9. A luciferase reporter assay was used to confirm that MIR22HG directly regulated miR-9 in the glioma cell lines. Next, we investigated the target gene of miR-9 using TargetScan. CPEB3 was found to be the downstream target of miR-9 and the luciferase activity confirmed that CPEB3 was the target molecule of miR-9 in the glioma cell lines. Moreover, MIR22HG was proved to regulate the and protein

expression level of CPEB3 via miR-9. CPEB3, a member of the CPEB family, was discovered to monitor cell translation by adjusting cytoplasmic polyadenylation. It has also been reported that CPEB3 was aberrantly expressed in several types of cancer (23,30,49). In a study of ovarian cancer cells, miR-301b-3p increased cell invasion and migration by targeting CPEB3 (50). In addition, CPEB3 plays a crucial role in glioma pathogenesis. The protein level of CPEB3 was increased by the lncRNA HCG11, which inhibited glioma cell proliferation and arrested the cell cycle (30). Thus, MIR22HG and miR-9 may regulate the development of glioma via targeting CPEB3.

However, the effect of MIR22HG in glioma remains unclear. To investigate the effect of MIR22HG in the glioma cell lines, a CCK-8 assay was used to measure cell proliferation. The results showed that upregulated MIR22HG expression restricted glioma cell proliferation, but miR-9 mimic reversed the inhibitory effects of MIR22HG. In addition, the overexpression of MIR22HG reduced cell migration and invasion. The MIR22HG-induced effects were abrogated by transfection with miR-9 mimic. It has been demonstrated that MIR22HG interacts with multiple signaling pathways and affects the progression of multiple tumors, including thyroid cancer, gastric cancer, and hepatocellular carcinoma (19,46,51). The results from the present study indicated that MIR22HG inhibited the progression of glioma via negatively modulating the downstream target miR-9.

MIR22HG was decreased in glioma tissue and cell lines in the present study, and that the miR-9/CPEB3 axis was the downstream pathway. However, little is known how MIR22HG expression level is reduced in glioma. Circular (circ)RNAs have been reported to regulate the expression of lncRNAs and

function as a diagnostic or prognostic biomarker in laryngeal squamous cell carcinoma and bladder cancer (52,53). In glioma, it is unknown whether MIR22HG is modulated by circRNAs, and it also remains unknown which circRNA directly regulates MIR22HG. This will be investigated in future studies.

In summary, it was found that MIR22HG expression level and CPEB3 were reduced while miR-9 expression level was increased in glioma tissues and cell lines. Then, MIR22HG was found to negatively modulate miR-9 in the glioma cell lines. In addition, CPEB3 was proven to be the target molecule of miR-9 in the glioma cell lines. In addition, upregulated MIR22HG expression level inhibited the proliferation, invasion and migration of glioma cells by inhibiting miR-9 expression level. The present study indicated that MIR22HG regulated the proliferation, invasion and migration of glioma cells through miR-9 and that CPEB3 is involved in this mechanism.

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### Availability of data and materials

All the data generated and/or analyzed during the present study are included in this published article.

### Author's contributions

XZ and YH conceived and designed the experiments. YH, HN, LY, TM, MM, BT and SG performed the experiments. YH, HN and LY collected and analyzed the data. XZ and YH interpreted the results and wrote the manuscript. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

This study was performed in accordance with standard guide-lines and was approved by the Ethics Committee of The Second Affiliated Hospital of the Fourth Military Medical University. All patients provided written informed consent prior to the study.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### References

1. Lv D, Li Y, Zhang W, Alvarez AA, Song L, Tang J, Gao WQ, Hu B, Cheng SY and Feng H: TRIM24 is an oncogenic transcriptional co-activator of STAT3 in glioblastoma. *Nat Commun* 8: 1454, 2017.
2. Liu X, Zheng J, Xue Y, Qu C, Chen J, Wang Z, Li Z, Zhang L and Liu Y: Inhibition of TDP43-mediated SNHG12-miR-195-SOX5 feedback loop impeded malignant biological behaviors of glioma cells. *Mol Ther Nucleic Acids* 10: 142-158, 2018.
3. Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, Pekmezci M, Schwartzbaum JA, Turner MC, Walsh KM, *et al*: The epidemiology of glioma in adults: A 'state of the science' review. *Neuro Oncol* 16: 896-913, 2014.
4. Zhou S, Ding F and Gu X: Non-coding RNAs as emerging regulators of neural injury responses and regeneration. *Neurosci Bull* 32: 253-264, 2016.
5. Van Meir EG, Hadjipanayis CG, Norden AD, Shu HK, Wen PY and Olson JJ: Exciting new advances in neuro-oncology: The avenue to a cure for malignant glioma. *CA Cancer J Clin* 60: 166-193, 2010.
6. Yuan X, Curtin J, Xiong Y, Liu G, Waschmann-Hogiu S, Farkas DL, Black KL and Yu JS: Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene* 23: 9392-9400, 2004.
7. Lino MM, Merlo A and Boulay JL: Notch signaling in glioblastoma: A developmental drug target? *BMC Med* 8: 72, 2010.
8. Schwartzbaum JA, Fisher JL, Aldape KD and Wrensch M: Epidemiology and molecular pathology of glioma. *Nat Clin Pract Neurol* 2: 494-503, 1-516, 2006.
9. Ostrom QT, Gittleman H, Stetson L, Virk SM and Barnholtz-Sloan JS: Epidemiology of gliomas. *Cancer Treat Res* 163: 1-14, 2015.
10. Huarte M: The emerging role of lncRNAs in cancer. *Nat Med* 21: 1253-1261, 2015.
11. Wang Y and Lee CG: MicroRNA and cancer-focus on apoptosis. *J Cell Mol Med* 13: 12-23, 2009.
12. Wang X, Yu H, Sun W, Kong J, Zhang L, Tang J, Wang J, Xu E, Lai M and Zhang H: The long non-coding RNA CYTOR drives colorectal cancer progression by interacting with NCL and Sam68. *Mol Cancer* 17: 110, 2018.
13. Kopp F and Mendell JT: Functional classification and experimental dissection of long noncoding RNAs. *Cell* 172: 393-407, 2018.
14. Fu J, Dong G, Shi H, Zhang J, Ning Z, Bao X, Liu C, Hu J, Liu M and Xiong B: LncRNA MIR503HG inhibits cell migration and invasion via miR-103/OLFM4 axis in triple negative breast cancer. *J Cell Mol Med* 23: 4738-4745, 2019.
15. Zhang S and Guo W: Long noncoding RNA MEG3 suppresses the growth of glioma cells by regulating the miR965p/MTSS1 signaling pathway. *Mol Med Rep* 20: 4215-4225, 2019.
16. Li C, Tan F, Pei Q, Zhou Z, Zhou Y, Zhang L, Wang D and Pei H: Non-coding RNA MFI2-AS1 promotes colorectal cancer cell proliferation, migration and invasion through miR-574-5p/MYCBP axis. *Cell Prolif* 52: e12632, 2019.
17. Zhao X, He M, Wan D, Ye Y, He Y, Han L, Guo M, Huang Y, Qin W, Wang MW, *et al*: The minimum LOH region defined on chromosome 17p13.3 in human hepatocellular carcinoma with gene content analysis. *Cancer Lett* 190: 221-232, 2003.
18. Zhang DY, Zou XJ, Cao CH, Zhang T, Lei L, Qi XL, Liu L and Wu DH: Identification and functional characterization of long non-coding RNA MIR22HG as a tumor suppressor for hepatocellular carcinoma. *Theranostics* 8: 3751-3765, 2018.
19. Li H and Wang Y: Long noncoding RNA (lncRNA) MIR22HG suppresses gastric cancer progression through attenuating NOTCH2 signaling. *Med Sci Monit* 25: 656-665, 2019.
20. Cui Z, An X, Li J, Liu Q and Liu W: LncRNA MIR22HG negatively regulates miR-141-3p to enhance DAPK1 expression and inhibits endometrial carcinoma cells proliferation. *Biomed Pharmacother* 104: 223-228, 2018.
21. Su W, Feng S, Chen X, Yang X, Mao R, Guo C, Wang Z, Thomas DG, Lin J, Reddy RM, *et al*: Silencing of long noncoding RNA MIR22HG triggers cell survival/death signaling via oncogenes YBX1, MET, and p21 in lung cancer. *Cancer Res* 78: 3207-3219, 2018.
22. Huang YS, Kan MC, Lin CL and Richter JD: CPEB3 and CPEB4 in neurons: Analysis of RNA-binding specificity and translational control of AMPA receptor GluR2 mRNA. *EMBO J* 25: 4865-4876, 2006.
23. Liu H, Wang Y, Chen B, Shen X and Li W: Effects of lidocaine-mediated CPEB3 upregulation in human hepatocellular carcinoma cell proliferation in vitro. *Biomed Res Int* 2018: 8403157, 2018.
24. Theis M, Si K and Kandel ER: Two previously undescribed members of the mouse CPEB family of genes and their inducible expression in the principal cell layers of the hippocampus. *Proc Natl Acad Sci USA* 100: 9602-9607, 2003.



25. Chao HW, Tsai LY, Lu YL, Lin PY, Huang WH, Chou HJ, Lu WH, Lin HC, Lee PT and Huang YS: Deletion of CPEB3 enhances hippocampus-dependent memory via increasing expressions of PSD95 and NMDA receptors. *J Neurosci* 33: 17008-17022, 2013.
26. Berger-Sweeney J, Zearfoss NR and Richter JD: Reduced extinction of hippocampal-dependent memories in CPEB knockout mice. *Learn Mem* 13: 4-7, 2006.
27. Zhang Y, Yu R and Li L: LINC00641 hinders the progression of cervical cancer by targeting miR-378a-3p/CPEB3. *J Gene Med* 22: e3212, 2020.
28. Waku T, Katayama H, Hiraoka M, Hatanaka A, Nakamura N, Tanaka Y, Tamura N, Watanabe A and Kobayashi A: NFE2L1 and NFE2L3 complementarily maintain basal proteasome activity in cancer cells through CPEB3-mediated translational repression. *Mol Cell Biol* 40: e00010-20, 2020.
29. Lin H, Guo Q, Lu S, Chen J, Li X, Gong M, Tang L and Wen J: LncRNA SUMO1P3 promotes proliferation and inhibits apoptosis in colorectal cancer by epigenetically silencing CPEB3. *Biochem Biophys Res Commun* 511: 239-245, 2019.
30. Chen Y, Bao C, Zhang X, Lin X, Huang H and Wang Z: Long non-coding RNA HCG11 modulates glioma progression through cooperating with miR-496/CPEB3 axis. *Cell Prolif* 52: e12615, 2019.
31. Du P, Liao Y, Zhao H, Zhang J, Muyiti, Keremu and Mu K: ANXA2P2/miR-9/LDHA axis regulates Warburg effect and affects glioblastoma proliferation and apoptosis. *Cell Signal* 74: 109718, 2020.
32. Shen H, Weng XD, Yang D, Wang L and Liu XH: Long noncoding RNA MIR22HG is down-regulated in prostate cancer. *Math Biosci Eng* 17: 1776-1786, 2019.
33. Xu J, Shao T, Song M, Xie Y, Zhou J, Yin J, Ding N, Zou H, Li Y and Zhang J: MIR22HG acts as a tumor suppressor via TGFbeta/SMAD signaling and facilitates immunotherapy in colorectal cancer. *Mol Cancer* 19: 51, 2020.
34. Han M, Wang S, Fritah S, Wang X, Zhou W, Yang N, Ni S, Huang B, Chen A, Li G, *et al*: Interfering with long non-coding RNA MIR22HG processing inhibits glioblastoma progression through suppression of Wnt/beta-catenin signalling. *Brain* 143: 512-530, 2020.
35. Wen PY and Huse JT: 2016 World Health Organization Classification of central nervous system tumors. *Continuum (Minneapolis)* 23: 1531-1547, 2017.
36. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
37. Inada K, Okoshi Y, Cho-Isoda Y, Ishiguro S, Suzuki H, Oki A, Tamaki Y, Shimazui T, Saito H, Hori M, *et al*: Endogenous reference RNAs for microRNA quantitation in formalin-fixed, paraffin-embedded lymph node tissue. *Sci Rep* 8: 5918, 2018.
38. Xu YL, Liu Y, Cai RP, He SR, Dai RX, Yang XH, Kong BH, Qin ZB and Su Q: Long non-coding RNA CASC7 is associated with the pathogenesis of heart failure via modulating the expression of miR-30c. *J Cell Mol Med* 24: 11500-11511, 2020.
39. Liu B, Liu Y, Zhou M, Yao S, Bian Z, Liu D, Fei B, Yin Y and Huang Z: Comprehensive ceRNA network analysis and experimental studies identify an IGF2-AS/miR-150/IGF2 regulatory axis in colorectal cancer. *Pathol Res Pract* 216: 153104, 2020.
40. Wu T, Wang S, Wang L, Zhang W, Chen W, Lv X, Li Y, Hussain Z and Sun W: Long noncoding RNA (lncRNA) CTTN-IT1 elevates skeletal muscle satellite cell proliferation and differentiation by acting as ceRNA for YAP1 through absorbing miR-29a in Hu Sheep. *Front Genet* 11: 843, 2020.
41. Shu J and Wang D: Functional characterization of the long noncoding RNA MIR22HG as a tumour suppressor in cervical cancer by targeting IGF2BP2. *Eur Rev Med Pharmacol Sci* 24: 7953-7962, 2020.
42. Tang Q, Jiang X, Ma S, Wang L, Li R and Ma J: MIR22HG regulates miR-486/PTEN axis in bladder cancer to promote cell proliferation. *Biosci Rep* 40: BSR20193991, 2020.
43. Lapointe S, Perry A and Butowski NA: Primary brain tumours in adults. *Lancet* 392: 432-446, 2018.
44. Shergalis A, Bankhead AR III, Luesakul U, Muangsin N and Neamati N: Current challenges and opportunities in treating glioblastoma. *Pharmacol Rev* 70: 412-445, 2018.
45. Chen H, Ali M, Ruben A, Stelmakh D and Pak M: E2F6-mediated downregulation of MIR22HG facilitates the progression of laryngocarcinoma by targeting the miR-5000-3p/FBXW7 Axis. *Mol Cell Biol* 40: e00496-19, 2020.
46. Qin L, Luo JZ, Tang XL and Han CG: Identification of long noncoding RNA MIR22HG as a Novel biomarker in thyroid cancer. *Pathol Oncol Res* 25: 703-710, 2019.
47. Chen L, Hu W, Li G, Guo Y, Wan Z and Yu J: Inhibition of miR-9-5p suppresses prostate cancer progress by targeting StarD13. *Cell Mol Biol Lett* 24: 20, 2019.
48. Chen X, Yang F, Zhang T, Wang W, Xi W, Li Y, Zhang D, Huo Y, Zhang J, Yang A and Wang T: MiR-9 promotes tumorigenesis and angiogenesis and is activated by MYC and OCT4 in human glioma. *J Exp Clin Cancer Res* 38: 99, 2019.
49. Zhong Q, Fang Y, Lai Q, Wang S, He C, Li A, Liu S and Yan Q: CPEB3 inhibits epithelial-mesenchymal transition by disrupting the crosstalk between colorectal cancer cells and tumor-associated macrophages via IL-6R/STAT3 signaling. *J Exp Clin Cancer Res* 39: 132, 2020.
50. Liu F, Zhang G, Lv S, Wen X and Liu P: miRNA-301b-3p accelerates migration and invasion of high-grade ovarian serous tumor via targeting CPEB3/EGFR axis. *J Cell Biochem* 120: 12618-12627, 2019.
51. Wu Y, Zhou Y, Huan L, Xu L, Shen M, Huang S and Liang L: LncRNA MIR22HG inhibits growth, migration and invasion through regulating the miR-10a-5p/NCOR2 axis in hepatocellular carcinoma cells. *Cancer Sci* 110: 973-984, 2019.
52. Lin G, Sheng H, Xie H, Zheng Q, Shen Y, Shi G and Ye D: circLPAR1 is a novel biomarker of prognosis for muscle-invasive bladder cancer with invasion and metastasis by miR-762. *Oncol Lett* 17: 3537-3547, 2019.
53. Zhao R, Li FQ, Tian LL, Shang DS, Guo Y, Zhang JR and Liu M: Comprehensive analysis of the whole coding and non-coding RNA transcriptome expression profiles and construction of the circRNA-lncRNA co-regulated ceRNA network in laryngeal squamous cell carcinoma. *Funct Integr Genomics* 19: 109-121, 2019.



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