MicroRNA-3148 inhibits glioma by decreasing *DCUN1D1* and inhibiting the NF-kB pathway

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Abstract. Glioma, which originates in the brain, is the most aggressive tumor of the central nervous system. It has been shown that microRNA (miRNA) controls the proliferation, migration and apoptosis of glioma cells. The objective of the present study was to measure microRNA-3148 (miR-3148) expression and investigate its impact on the pathogenetic mechanism of glioma. In the present study, reverse transcription-quantitative real-time PCR was employed to detect miR-3148 expression levels in glioma tissues and cell lines. Cell Counting Kit-8 assay, 5-ethynyl-2'-deoxyuridine assay, and Transwell migration assay were performed to assess the influence of miR-3148 on the malignant biological behavior of glioma cells. The biological functions of miR-3148 in glioma were examined via a xenograft tumor growth assay. Furthermore, the association between miR-3148 and *DCUN1D1* was investigated via immunohistochemistry, dual-luciferase reporter assay and western blotting. It was observed that miR-3148 was expressed at low levels in glioma cells, and this represented a poor survival rate. In addition, an increased level of miR-3148 in cells and animal models inhibited glioma cell migration and proliferation. Moreover, miR-3148 decreased *DCUN1D1* and curbed the nuclear factor κ enhancer binding protein (NF-κB) signaling pathway, thus decreasing the growth of glioma. Thus, miR-3148 is expressed within glioma tissues at low levels where it suppresses glioma by curbing the NF-κB pathway and lowering *DCUN1D1*.

Introduction

Glioma is considered to be the most aggressive tumor among the primary central nervous system tumors. It is classified as astrocytoma, oligodendroglioma, ependymoma, medulloblastoma, and glioblastoma (1,2). The tumor is clinically treated primarily via surgical resection, chemoradiotherapy, gene therapy and sometimes, also by using Chinese medicine (3-5), while considering the patient's health condition, age, tumor type, location, size, and clinical grade. Nonetheless, as glioma is resistant to traditional chemotherapy, the mortality and relapse rates remain high. The in-depth pathogenesis of glioma, including aberrant activation of proto-oncogenes and inactivation of tumor suppressors, remains to be elucidated (6-8). Thus, probing the molecular mechanism and developing efficacious methods for diagnosing and treating gliomas are important.

MicroRNAs (miRNAs/miRs), measuring 18 to 26 nucleotides, are a class of non-coding RNA molecules in eukaryotes (9) that trigger the RNA-induced silencing complex to degrade messenger RNAs (mRNAs) or curb translation by pairing with their target genes (10). miRNAs have been highly conserved during species evolution and those discovered in plants, animals and fungi are expressed in specific tissues and at specific developmental stages only. The tissue and timing-associated specificities of miRNAs determine the distinctive functions of tissues and cells, indicating the multiple roles that miRNAs play in controlling developmental processes (11-14).

It has been verified by recent studies that miRNAs have an indispensable impact on the pathogenesis of gliomas (15); for example, intercellular transfer of miRNAs via gap junctions influences glioma cell proliferation (16). Mesenchymal stem cell-derived exosomal miR-133b targets enhancer of zeste homolog 2 via the Wnt/β-catenin signaling pathway (17) to curb glioma progression. In addition, miR-3148 has been reported to hinder proliferation and boost apoptosis in cervical cancer cells (18). Liu *et al* (19) pointed out that miR-3148 is significantly downregulated in human glioma stem cells compared with that in human neural stem cells. However, whether miR-3148 regulates glioma remains unknown.

In the current study, the downregulation of miR-3148 in human glioma tissues and cell lines was verified and the aim was to investigate the role of miR-3148 in glioma.

Materials and methods

Ethical compliance. The present study was approved by the Ethics Committee of the First People's Hospital of Jingmen

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(Hubei, China). All population-based studies were carried out in accordance with the World Medical Association's Declaration of Helsinki, and all subjects provided written informed consent.

Specimen collection and processing. Forty-eight surgically resected glioma specimens and non-tumor tissues (2 cm away from the tumor) were collected from patients (male=27, female=21; I/II 23, III/IV 25) at the First People's Hospital of Jingmen between March 2012 and March 2014. The median age of the patients was 46 years (range, 30 to 72 years). These patients without tumors of other classes, autoimmune diseases, viral hepatitis and those not undergoing preoperative chemoradiotherapy before operation. Following surgical resection under aseptic conditions, all specimens were immediately frozen in liquid nitrogen and preserved at -80°C until further use.

Taking the average expression level of miR-3148 as the cutoff, 48 patients were split into high and low expression groups for survival analysis. Data were analyzed by Fisher's exact test.

Cell culture. U87 MG (glioblastoma of unknown origin, BNCC100646), SHG-44, U251 and H4 glioma cell lines, and HEB normal cells (BeNa Culture Collection) were cultured in an incubator containing Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (FBS, Sigma-Aldrich; Merck KGaA), at 37°C and 5% CO_2. The cell lines were authenticated using STR profiling.

In total, 50 nM miR-3148 mimics (Sense, 5'-UGGAAA AAACUGGUGUGUGCUU-3'; Antisense, 5'-GCACACACC AGUUUUUUCCAUU-3') and the negative controls (Sense, 5'-UUCUCCGAACGUGUCACGUTT-3'; Antisense, 5'-ACG UGACACGUUCGGAGAATT-3') (Shanghai GenePharma Co., Ltd.) were used to overexpress miR-3148 in U251 cells. 50 nM miR-3148 inhibitors (5'-AAGCACACACCA GUUUUUUCCA-3') and the equivalent NC (5'-CAGUAC UUUUGUGUAGUACAA-3') (Shanghai GenePharma Co., Ltd.) were used to knock down miR-3148 in U87 MG cells. Lipofectamine 2000® (Invitrogen; Thermo Fisher Scientific, Inc.) was utilized to transfect these plasmids into U251 or U87 MG cells ($5x10^6$ cells/well) following the manufacturer's instructions. Following 48 h of transfection at 37°C, cells were collected (centrifugation at 4°C at 12,000 x g for 15 min) and the transfection efficiency was determined using RT-qPCR. Subsequent experiments were performed 48 h after transfection.

Reverse transcription quantitative PCR (RT-qPCR). Sequestering of total RNA was achieved by means of RNAzol reagent (Vigorous Biotechnology Beijing Co., Ltd.) as per the manufacturer's instructions. Then, Moloney murine leukemia virus reverse transcriptase (Promega Corporation) was used to reverse transcribe total isometric RNA (2 μg), with the transcription level normalized to the 18S rRNA level. Subsequently, RT-qPCR was performed using an ABI 7300 RT-PCR system with SYBR® Green RT-PCR Master mix (Toyobo Life Science). The qPCR was conducted at 95°C for 10 min followed by 40 cycles of 95°C for 30 sec and 60°C for 1 min. The primers for *CRNDE*, miR-3148 and *DCUN1D1*

were purchased from Guangzhou RiboBio Co., Ltd. The primer sequences that were used are as follows: miR-3148 forward (F), 5'-TGGAAAAAACTGGTGTGTGCTT-3'; miR-3148 reverse (R), 5'-GCTGTCAACGATACGCTACCTA-3'; *DCUN1D1* F, 5'-AGGATCATTGGACAGGAAGAAGT-3'; *DCUN1D1* R, 5'-TGCCAGGTCATCACAGAACTG-3'; GAPDH F, 5'-AGA AGGCTGGGGCTCATTTG-3'; and GAPDH R, 5'-AGGGGC CATCCACAGTCTTC-3' was used as an endogenous control for mRNA. The expression of miRNA was normalized to U6 F, 5'-CTCGCTTCGGCAGCACA-3'; and U6 R, 5'-AAC GCTTCACGAATTTGCGT-3'. Finally, the relative content of specimens was examined using the $2^{-\Delta\Delta Cq}$ method (20). Each assay was averaged over three performances.

Cell proliferation. After culturing cells at 37°C for 24 h in a 96-well plate at a density of $2x10^3$ cells/well, U87 MG and U251 cell lines were incubated at 37°C for 2 h with 10 μl Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.) solution. Then, the optical density at 450 nm was measured and recorded.

The role of miR-3148 in glioma cell proliferation was evaluated using Click-iT® EdU Imaging Kits (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. U87 MG and U251 cells were cultured in 96-well plates ($8x10^3$ cells/well) and incubated at room temperature with 10 μl of EdU reagent for 3 h. At room temperature, the cells were fixed with 4% formaldehyde for 20 min and washed with formaldehyde in PBS. The cells were then incubated at room temperature in 0.5% Triton X-100 (Sigma-Aldrich; Merck KGaA) for 5 min. The nuclei were then stained with a 4',6-diamidino-2-phenylindole solution (1 ml; Sigma-Aldrich; Merck KGaA), which was added to each well and incubated for 25 min at room temperature in the dark. The staining solution was then removed by washing thrice with PBS. Finally, the stained cells were imaged and counted under a fluorescence microscope (magnification, x100) (CKX41-F32FL; Olympus, Beijing, China). Each assay was performed in triplicates.

Cell migration. After being inoculated on a six-well plate, the cells were transfected with miR-3148 mimics, inhibitors and NCs. Approximately 100 μl serum-free medium containing cell suspension was added to the upper chamber, and 600 μl medium containing 10% FBS was added to the lower chamber. Following 36 h of transfection, the cells were stained with 0.1% crystal violet staining solution (Beyotime Institute of Biotechnology) at room temperature for 15 min and counted; 6 fields of view were selected in each well for imaging under x40 magnification using a light microscope. Each experiment was performed in triplicates.

Immunohistochemistry (IHC). Tissues were sectioned at 5 μm and collected on microscope slides (SuperFrost plus; Thermo Fisher Scientific, Inc.). Sections were then re-hydrated and deparaffinized by immersion in xylene (100% x2) followed by immersion in a graded alcohol series (100% ethanol for 1 min twice; 95% ethanol for 1 min and 70% ethanol for 1 min twice), ending with treatment distilled water. Heat-induced antigen retrieval was performed in citrate buffer, pH 6.0 (10 mM sodium citrate) containing 0.05% Tween-20 (Sigma-Aldrich; Merck KGaA) for 10 min at 90°C, followed by immersion in distilled

water for 10 min and in PBS (thrice for 3 min). Sections were then incubated in PBS containing 0.3% H_2O_2 at room temperature for 10 min, followed by rinses in PBS (thrice for 3 min). Sections were incubated in PBS containing 0.05% Triton X-100 (AppliChem GmbH) and 1% bovine serum albumin (BSA; Sigma-Aldrich; Merck KGaA; PBS-TX-BSA) for 30 min at room temperature. Incubation was performed in primary antibodies made in rabbit against *DCUN1D1* (PA5-83298; 1:50; Thermo Fisher Scientific, Inc.) for 16 h at 4˚C. After rinsing in PBS (thrice for 3 min), sections were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-rabbit; cat. no. A0208; 1:1,000; Beyotime Institute of Biotechnology) for 45 min at room temperature. Following rinses in PBS (thrice for 3 min), sections were incubated for 10 min in PBS containing 3,3'-diaminobenzidine (DAB, 25 mg/ml) and 0.05% H_2O_2. Sections were then rinsed in PBS (thrice for 3 min) and counterstained with hematoxylin (Histolab Products AG).

In vivo xenograft tumor model. 6 BALB/c athymic nude mice (female; 4-5 weeks old; 16-22 g) (National Laboratory Animal Center) were fed in sterile housing cages at 25˚C, with a humidity of 45-55% and 12-h light/dark cycle. The feed was sterilized by high temperature and high pressure, and mice had free access to food and water. All the nude mice were hypodermically injected with $1x10^6$ U251 cells (miR-3148 mimics or miR-NC). Fifteen days later, mice were euthanized via cervical dislocation, and the tumor tissues were harvested and tumor weight was measured with an electronic scale. Experiments in the present study were approved by the Ethics Committee for Experimental Animals at First People's Hospital of Jingmen and were conducted in the SPF Animal Laboratory at the Medical College of Hubei University of Arts and Science.

H&E staining. Tumors were fixed in 4% paraformaldehyde overnight at 4˚C. For staining, tissues were washed with water for several hours, and then dehydrated in 70, 80 and 90% ethanol, xylene, and other mixture for 15 min, and then in xylene I for 15 min, II for 15 min, until transparent. Samples were then placed in a mixture of xylene and paraffin (1:1) for 15 min, and then paraffin was added for 50-60 min. The tissues were then paraffin-embedded and sections (4-μm thick) were mounted on slides, dewaxed and rehydrated. Then, the sections were stained with hematoxylin for 4 min and eosin for 90 sec at room temperature and observed by an Olympus BX51 light microscopy (Olympus Corporation).

Western blotting. Cells were lysed in RIPA buffer (Beyotime Institute of Biotechnology) containing a protease inhibitor. Protein concentration was measured using a Bicinchoninic Acid Protein Assay kit (Thermo Fisher Scientific, Inc.). Next, the protein extracts were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (40 μg/lane) and transferred to polyvinylidene fluoride) membranes (Sangon Biotech Co. Ltd.). The membranes were blocked with 5% defatted milk, followed by overnight incubation at 4˚C with primary antibodies [anti-*DCUN1D1* antibody (cat. no. ab181233; 1:10,000; Abcam), anti-nuclear factor κ enhancer binding protein (NF-κB) p50 antibody

(cat. no. ab32360; 1:5,000; Abcam), anti-NF-κB p65 antibody (cat. no. ab28856; 1:1,000; Abcam)] and later with HRP-conjugated secondary antibodies (goat anti-rabbit, A0208; 1:1,000; Beyotime Institute of Biotechnology) at room temperature for 2 h. The protein levels were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH; cat. no. ab8245; 1:1,000; Abcam).

Dual-luciferase reporter gene assay. The binding sites between miR-3148 and DCUN1D1 were predicted using miRDB (http://mirdb.org/custom.html). The 3'-UTR sequence of *DCUN1D1* interplayed with miR-3148 and pGL3 promoter vectors (Promega Corporation) were injected with full-length *DCUN1D1* or a mutant sequence with the predicted target sites. After seeding on a 24-well plate, the cells were co-transfected with 5 ng pRL-SV40 (Promega Corporation), a *Renilla* luciferase vector, using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 48 h. 48 h after transfection, Dual-Luciferase® Reporter Assay System (Promega Corporation) was used for activity measurement.

Statistical analysis. SPSS 20.0 (IBM Corp.) and GraphPad Prism 5 (GraphPad Software, Inc.) statistical software were used for data assessment. For normally distributed data with equal variance, the difference was evaluated by two-tailed Student's t-test (two group comparisons) or one-way ANOVA followed by the Bonferroni post hoc test (multigroup comparisons) as appropriate. The paired t-test was only performed to detect the differential expression of miR-3148 and DCUN1D1 in cancer tissues compared with adjacent non-malignant tissues. The unpaired Student's t test were performed for assessing the significance of other between-group differences. For non-normally distributed data or data with unequal variances, the difference was evaluated by a nonparametric Mann-Whitney U test (two group comparisons) or the Kruskal-Wallis test followed by the Bonferroni post hoc test (multigroup comparisons). The average expression level was used as the cutoff and log-rank analysis was used for survival analysis. Data are presented as the mean ± SD. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics and expression of miR-3148 in glioma. Liu *et al* (19) have reported that miR-3148 is evidently decreased in glioma. However, no studies have reported whether miR-3148 plays a role in glioma. Here, 48 clinical specimens of human glioma were collected to examine miR-3148 expression levels in cancerous and non-cancerous tissues. miR-3148 was expressed in glioma tissues at a lower level compared with that in non-tumor tissues (Fig. 1A). Additionally, miR-3148 was expressed in glioma cells at a notably lower level compared with that in the HEB cell line. miR-3148 expression reached the maximum in the U87 MG cell line and was the minimum in the U251 cell line (Fig. 1B); therefore, these two cell lines were selected as research models for the subsequent assays. Furthermore, patients with high miR-3148 expression had a higher survival rate compared with those with low miR-3148 expression (Fig. 1C). The average expression level was used as the cutoff. These results suggest
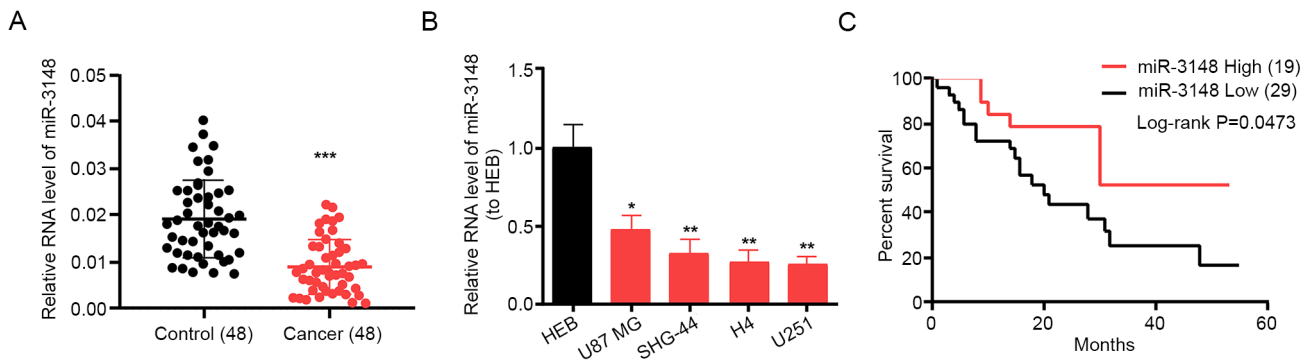
Figure 1. Characteristics and expression levels of miR-3148 in glioma. (A) Reverse transcription-quantitative PCR results for miR-3148 levels in glioma tissues (n=48) and non-tumor tissues (n=48). (B) miR-3148 is expressed at a lower level in glioma cell lines compared with that in the HEB cell line. (C) Kaplan-Meier survival analysis of miR-3148 showed that patients with a high miR-3148 expression had a higher survival rate compared with those with a lower miR-3148 expression level. Data are shown as mean ± SD. *P<0.05; **P<0.01; ***P<0.001. miR, microRNA.

that miR-3148 is downregulated in gliomas and is associated with survival and prognosis.

Biological changes post treatment with miR-3148 mimics or inhibitors. miRNA mimics were utilized to increase miR-3148 expression in U251 cell lines and miRNA inhibitors were utilized to decrease miR-3148 expression in U87 MG cell lines (Fig. 2A). CCK-8 and EdU assays showed that overexpression of miR-3148 inhibited glioma cell proliferation, while downregulated miR-3148 expression level boosted it (Fig. 2B and C). In addition, the Transwell assay revealed that cell migration was boosted by lowering miR-3148 expression level and was impeded by overexpression of miR-3148 (Fig. 2D). Hence, miR-3148 suppressed the proliferation and migration of glioma cells.

miR-3148 inhibits glioma growth in vivo. An *in vivo* xenograft tumor model was constructed to verify the functions of miR-3148 in glioma development. Post miR-3148 mimic transfection, U251 cells were subcutaneously inoculated, and miR-3148 overexpression was observed to impede tumor growth *in vivo*. The tumor volume and weight in the miR-3148 mimic group were significantly lower compared with those in the miR-NC group (Fig. 3A and B). H&E staining of tumor cells in the miR-NC and miR-3148 mimic groups verified that miR-3148 lowered the rate of occurrence of lung metastasis in nude mice (Fig. 3C). These data revealed that miR-3148 inhibits tumor growth *in vivo*.

miR-3148 targets DCUN1D1 and regulates its expression. Using miRDB, *DCUN1D1* was identified as the target gene of miR-3148. Importantly, *DCUN1D1* has been reported to promote glioma formation and malignant progression in mice (21). Using RT-qPCR assay, increased expression levels of *DCUN1D1* were detected in glioma tissues and cell lines (Fig. 4A and B). The luciferase reporter gene assay showed that miR-3148 did bind to *DCUN1D1* (Fig. 4C). Additionally, the mRNA and protein expression levels of *DCUN1D1* decreased following miR-3148 overexpression in U251 cells (Fig. 4D and E). *DCUN1D1* expression was also found to be inhibited by miR-3148 overexpression by immunohistochemical staining of *DCUN1D1* in xenograft tumor tissues

(Fig. 4F). The aforementioned results indicate that miR-3148 targets and regulates *DCUN1D1* expression.

miR-3148 blocks the NF-κB pathway. The aberrantly activated NF-κB pathway has been implicated in glioma development (22-24). Following upregulation of miR-3148 expression, the mRNA and protein expression levels of p50 was markedly decreased (Fig. 5A-C), and those of p65 showed the same trend (Fig. 5D-F). Thus, miR-3148 can block the NF-κB pathway, impeding the development of glioma. Furthermore, the upregulation of the expression of *DCUN1D1* stimulates the NF-κB pathway (Fig. S1).

Discussion

In the process of cancer development, miRNAs are dysregulated and function as oncogenes or tumor suppressors by promoting or impeding tumor cell proliferation and invasion, respectively (25-27). miRNA deregulation is also known to be a key mechanism in glioma pathogenesis (28-30).

The present study aimed to determine the function of miR-3148 in the onset of glioma using tissue specimens. Decreased miR-3148 expression was observed in glioma tissues compared with non-tumor tissues. The lower the expression level of miR-3148, the lower the overall survival rate of patients with glioma. Thereafter, glioma cells were transfected with miR-3148 mimics and inhibitors, and functional bioassays were used to examine the role of miR-3148. The present study showed that miR-3148 inhibits the proliferation and migration of tumor cells.

The mechanisms of action of miRNAs can be divided into two categories. Firstly, miRNAs bind closely to the open reading frame of mRNA to form a double-stranded structure, which leads to the degradation of mRNA. Secondly, miRNAs bind to the 3'-UTR of mRNA, which decreases the stability of mature mRNA and inhibits the post transcriptional translation of mRNA (31).

Bioinformatics analysis and the dual-luciferase reporter assay were performed to confirm that *DCUN1D1* was a target gene of miR-3148, which was highly expressed in tumor cells and tissues. *DCUN1D1* expression was decreased when miR-3148 was upregulated. A previous study demonstrated
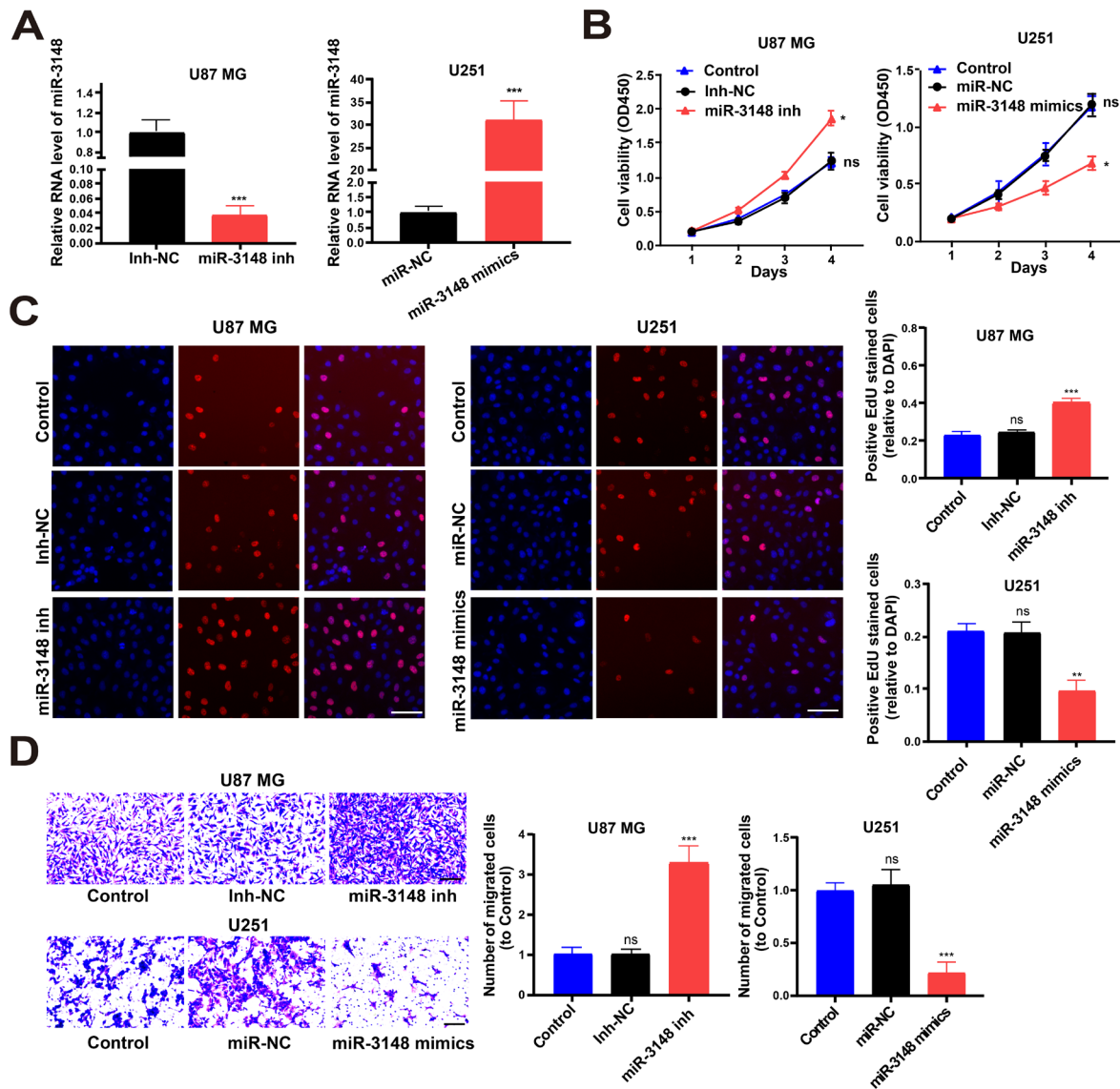
Figure 2. Cell biological variations after treatment with miR-3148 mimics or inhibitors. (A) Transfection efficiency of miR-3148 mimics and miR-3148 inhibitors. (B) The results of the Cell Counting Kit-8 assay revealed that cell proliferation could be boosted by lowering miR-3148 and suppressed by miR-3148 overexpression. (C) The results of the EdU assay revealed that decreased miR-3148 expression promoted cell proliferation, whereas elevating miR-3148 suppressed it (scale bar, 50 µm). (D) The results of the Transwell assay revealed that cell migration was boosted by down-regulating miR-3148 and was curbed via the overexpression of miR-3148 (scale bar, 100 µm). Data are shown as mean ± SD. *P<0.05; **P<0.01; ***P<0.001; ns, no significance; miR, microRNA; inh, inhibitor; NC, negative control.
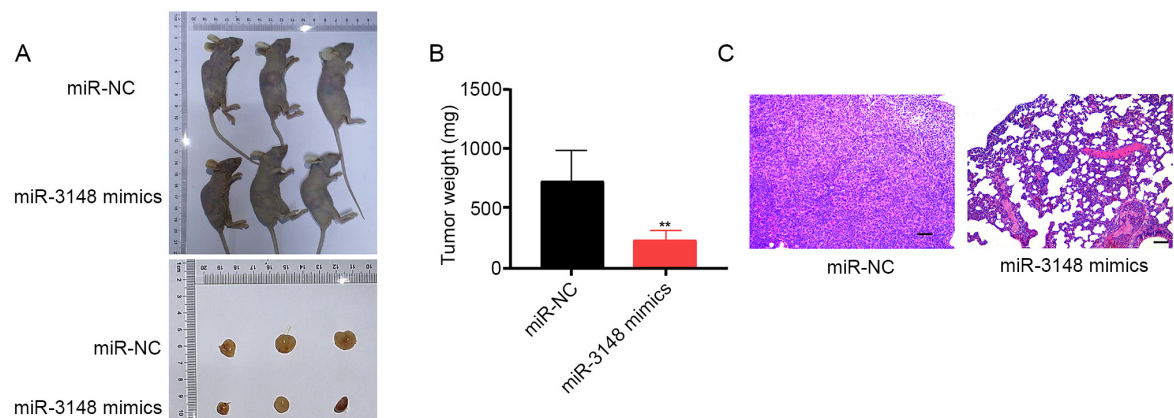

Figure 3. miR-3148 impedes glioma growth *in vivo*. (A) Typical images of tumors from indicated orthotopic xenografts. (B) Weight of orthotopic xenografts. (C) Hematoxylin and eosin staining images of lung tissues after U251 cell lines were injected through tail veins (scale bar, 100 µm; original magnification, x200). Data are shown as mean ± SD. **P<0.01. miR, microRNA; NC, negative control.
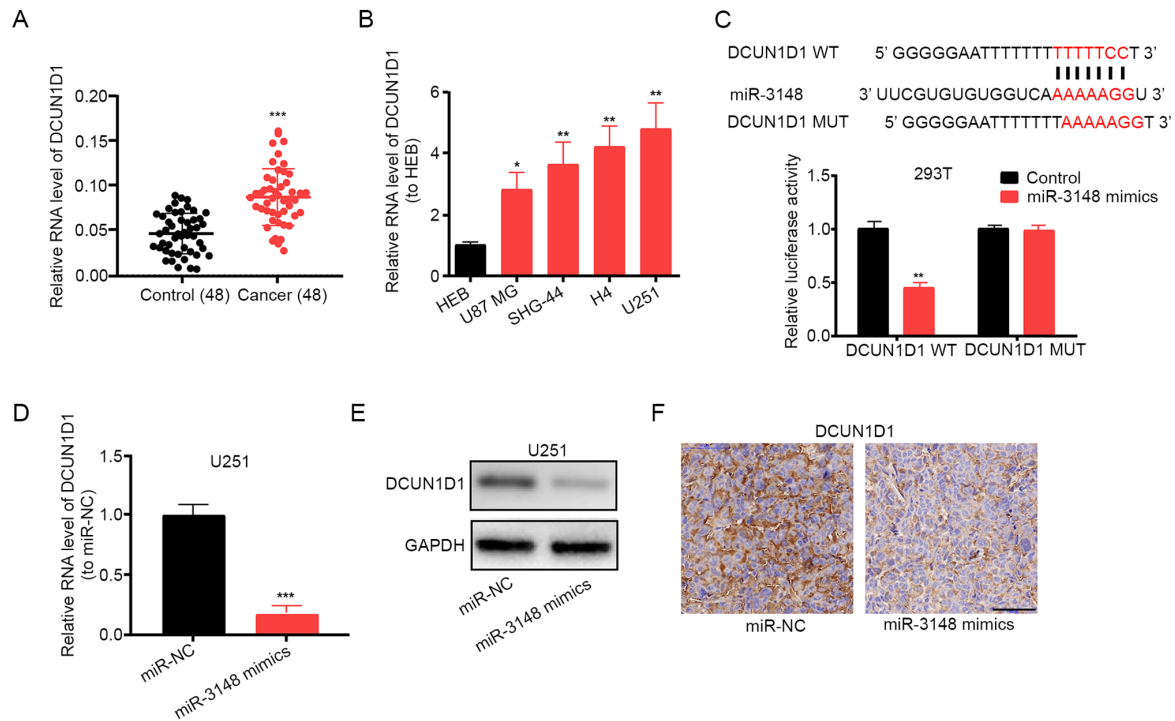
Figure 4. miR-3148 targets *DCUN1D1*. (A) *DCUN1D1* expression in glioma tissues and non-tumor tissues was compared and was found to be elevated in glioma tissues. (B) *DCUN1D1* was expressed at a lower level in glioma cell lines compared with that in the HEB cell line. (C) The putative miRNA binding sites in the *DCUN1D1* sequence. Dual-luciferase reporter was used to confirm the direct targeting between *DCUN1D1* and miR-3148. (D) miR-3148 mimics were transfected into U251 cells, after which the mRNA level of *DCUN1D1* was assessed using reverse transcription-quantitative PCR. (E) DCUN1D1 protein levels in U251 cells treated with miR-3148 mimics or NC were analyzed using western blotting, with GAPDH as control. (F) DCUN1D1 protein level in xenograft tumors was analyzed via immunohistochemistry (scale bar, 50 μm). Data are shown as mean ± SD. *P<0.05; **P<0.01; ***P<0.001. miR, microRNA; NC, negative control; WT, wild type; MUT, mutant.
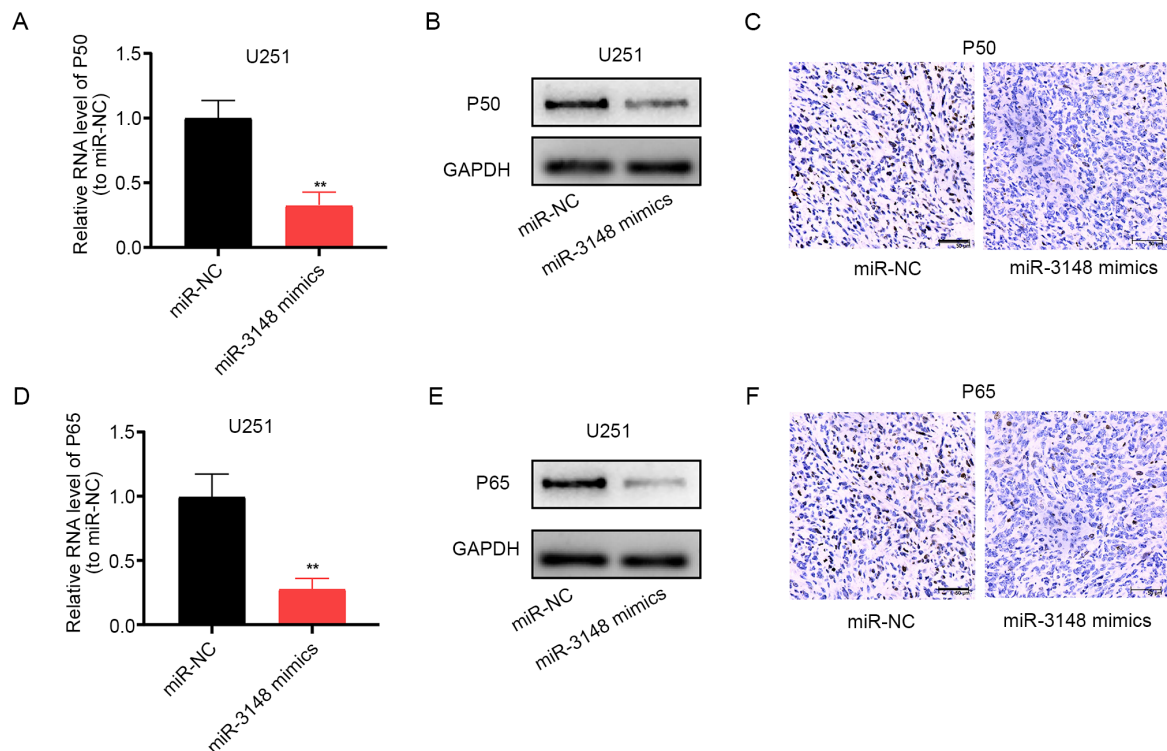

Figure 5. Overexpression of miR-3148 hinders the nuclear factor κ enhancer binding protein pathway. (A) miR-3148 mimics were transfected into U251 cells and the mRNA level of P50 was assessed using RT-qPCR. (B) P50 protein levels, after U251 cells were treated with miR-3148 mimics, were examined via western blotting, with GAPDH as control. (C) P50 protein levels in xenograft tumors were examined via IHC. (D) miR-3148 mimics were transfected into U251 cells and the mRNA level of P65 was assessed using RT-qPCR. (E) P65 protein levels in U251 cells treated with miR-3148 mimics were analyzed via western blotting, with GAPDH as control. (F) P65 protein levels in xenograft tumors were examined via IHC (scale bar, 50 μm). The data are indicated as mean ± SD. **P<0.01. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; IHC, immunohistochemistry.

that *DCUN1D1* is an important component of the neddylation E3 complex (32) and can promote the nuclear translocation and assembly of this complex (33). Several studies have also suggested that *DCUN1D1* has oncogenic activity in human cancer. For instance, *DCUN1D1* is highly conserved and is activated by its amplification in squamous cell carcinomas (34). *DCUN1D1* can also induce extracellular matrix invasion by activating matrix metalloproteinase 2, and thus, it is important for cancer metastasis (35). Recently, Broderick *et al* (21) showed that *DCUN1D1* promotes glioma formation and malignant progression in mice. These findings indicate that *DCUN1D1* is targeted and downregulated by miR-3148, and this function of miR-3148 could lead to the possible inhibition of glioma progress.

The NF-κB/Rel class of proteins include NF-κB2 p52/p100, NF-κB1 p50/p105, c-rel, RelA/p65 and RelB proteins. The NF-κB/Rel protein can act as polymerization transcription factor 2, control gene expression and affect biological processes, including B cell and lymphoid organ formation, inflammation, innate and adaptive immunity, and stress reactions (36). The activation of the NF-κB pathway has recently been determined to be closely associated with the onset of glioma (37-39). The present study proved that miR-3148 was involved in the mechanism of glioma by modulating the NF-κB pathway.

The present study had several limitations, for which the following measures are suggested for future work. Firstly, a stereotactic method *in situ* is required to validate the conclusion. Secondly, more target genes should be used that interact with miR-3148. Thirdly, further studies are required to determine whether other factors affect glioma cell proliferation and migration.

In conclusion, miR-3148 is expressed in glioma tissues at a low level and is associated with overall survival. Furthermore, high miR-3148 expression potentially curbs the onset of glioma by modulating *DCUN1D1* and suppresses the NF-κB pathway.

Acknowledgements

Availability of data and materials

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

Authors' contributions

QX and XC performed the experiments and generated data. BC and QX made substantial contributions to the conception and design of the present study. QX and XC conducted data analysis and interpretation of data. All authors contributed to the drafting and revision of the manuscript. All authors read, revised and approved the manuscript and agreed to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved. QX and BC confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the First People's Hospital of Jingmen (Hubei, China; approval no. 2012018). All population-based studies were carried out in accordance with the World Medical Association's Declaration of Helsinki, and all subjects provided written informed consent.

Animal experiments in the present study were approved by the Ethics Committee for Experimental Animals at First People's Hospital of Jingmen and were conducted in the SPF Animal Laboratory at the Medical College of Hubei University of Arts and Science.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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