MicroRNA-634 alters nerve apoptosis via the PI3K/Akt pathway in cerebral infarction

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Abstract. In the present study, the role and mechanism of microRNA-634 (miRNA-634) in the adjustment of nerve inflammation and apoptosis in cerebral infarction were investigated. In a cerebral infarction rat model, the expression of miRNA-634 was increased, compared with that in the normal control group. The upregulated expression of miRNA-634 in an *in vitro* model of cerebral infarction increased cell apoptosis and the protein expression of capsase-3/B-cell lymphoma 2-associated X protein (Bax) via inactivation of the phosphoinositide 3-kinase (PI3K)/Akt pathway. The down-regulation of miRNA-634 enhanced cell growth and inhibited cell apoptosis in the *in vitro* model of cerebral infarction through induction of the PI3K/Akt pathway. Subsequently, a PI3K inhibitor was used to inhibit the expression of PI3K in the *in vitro* model of cerebral infarction via the downregulation of miRNA-634, which showed that cell apoptosis and the protein expression of capsase-3/Bax were also increased. A PI3K agonist reduced the effects of the upregulation of miRNA-634 in the *in vitro* model of cerebral infarction. In conclusion, the data obtained demonstrated the possible future use of miRNA-634 as a therapeutic target in cerebral infarction through the PI3K/Akt pathway.

Introduction

Cerebral thrombosis is an ischemic cerebrovascular disease (1). It is an important component type of atherosclerosis, accounting for 70-80% of all patients with stroke. In cerebral thrombosis, ~90% of cases develop on the basis of cerebral atherosclerosis (2). Therefore, it is frequently referred to as atherosclerotic cerebral thrombosis. Atherosclerosis is a chronic progressive disease. It develops during childhood but clinical symptoms present in middle- and old-aged individuals.

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The pathological changes of atherosclerosis mainly involve the aorta and medium-sized elastic arteries (3). Among these, the coronary artery and cerebral arteries are the most dominant (3). Furthermore, the lesion predominantly involves multiple organs at the same time (2). With the increase in detailed investigations on atherosclerosis over the last century, several representative theories have formed successively. These include lipid infiltration, thrombosis, smooth muscle cell clones, injury response and chronic inflammation (4).

MicroRNAs (miRNAs) are a class of small, endogenous, non-coding single-strand RNAs. They are one of the most important gene expression regulatory factors (5). miRNAs interact with the specific sequence of a target gene and inhibits target gene activity or promotes its degradation at the post-transcriptional level (5). In addition, miRNAs regulate target gene expression, and are involved in biological processes that include cell proliferation, differentiation, apoptosis and metabolism (6). The expression of brain-specific miRNAs in the central nervous system is rich, whereas no or minimal expression is observed in the majority of other organs (6). miRNAs are important in nervous system development and function. It is reported that miRNA may be passively leaked out from damaged tissue cells or infiltrated cells, similar to other materials (7). Alternatively, it may be actively secreted from the damaged tissue cells into the blood circulation. The latter is considered to be the major mechanism (6).

The phosphoinositide 3-kinase (PI3K)/Akt signal transduction pathway is an important pathway for the intracellular transduction of membrane receptor signals (8). It is key in maintaining cell survival and inhibiting cell apoptosis. In addition, it can affect the activation process of effector molecules, including downstream apoptosis-related proteins and cell cycle regulating proteins. Therefore, it is crucial in inhibiting apoptosis and promoting proliferation in cells (9). The aim of the present study was to examine the effects of plasma miRNA-634 on adjusting nerve inflammation and apoptosis in cerebral infarction, and investigate its possible mechanism.

Materials and methods

Middle cerebral artery occlusion (MCAO) model. Male Sprague-Dawley (SD) rats (170-210 g) were purchased for animal experiments from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All rats

were housed at 22-23°C with a 55-60% humidity, 12/12 h light/dark cycle and free access to food and water. Cerebral ischemia-reperfusion was induced by MCAO. The SD rats were anesthetized with 35 mg/kg of pentobarbital (i.p) and randomly assigned into two groups: Control and MCAO model group. A midline neck incision was made and the left common and external carotid arteries was isolated, and was ligated with a microvascular clip (cat. no. FE691, Aesculap, Tuttlingen, Germany). An 8-0 nylon monofilament with silicon resin (180-190 μm) was introduced through a small incision into the common carotid artery and advanced 9 mm distal to the carotid bifurcation to induce MCAO. In the control group, the rats were anesthetized with 35 mg/kg of pentobarbital only without MCAO. After 1 h, reperfusion was initiated by withdrawal of the monofilament. After 1 day, the SD rats were anesthetized with 35 mg/kg of pentobarbital and sacrificed for subsequent investigations.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Brain tissue samples were subsequently obtained and hippocampal tissues were isolated. Total RNA from the hippocampal tissues samples was isolated using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The cDNA was reverse transcribed using the M-MLV Reverse Transcription system (Takara Biotechnology Co., Ltd., Dalian, China). The qPCR analysis was performed using 100 ng cDNA on an Applied Biosystems 7500 Fast Real-Time PCR system with the SYBR-Green PCR kit (TransGen, Beijing, China). The following primers were used for qPCR: miR-634 forward, 5'-CAGTCTCAAACCAGCACC-3' and reverse, 5'-TATGGTTGTTCACGACTCCTTCAC-3'; and U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'. The qPCR analysis was performed by denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec. Relative expression levels of mRNA were calculated by the $2^{-\Delta\Delta Cq}$ method (10).

Gene expression microarrays. RNA (500 ng) from hippocampal tissues was amplified using fluorescent complementary RNA and a RNA Labeling kit (Arraystar Inc., Rockville, MD, USA) in accordance with the manufacturer's protocol. Array hybridization was performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technologies, Inc., Santa Clara, CA, USA) and subsequent washing was performed using a mRNA-ONLY™ Eukaryotic mRNA Isolation kit (Epicentre; Illumina, Inc., San Diego, CA, USA). cDNA samples were labeled with Cy3 using the SureTag DNA Labeling kit (Agilent Technologies, Inc.). Scanning of the microarrays was performed using Feature Extraction software (v.10.7.3.1; Agilent Technologies, Inc.).

Transient transfection of cells. BV2 cells were purchased from the Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), glutamine (2 mmol/l, Invitrogen; Thermo Fisher Scientific, Inc.), penicillin (200 U/ml; Hyclone; GE

Healthcare Life Sciences, Logan, UT, USA) and streptomycin (100 μg/ml; Hyclone; GE Health Care Life Sciences) at 37°C with 5% CO_2. The miRNA (miR)-634 inhibitor mimics (100 nM; Sangon Biotech Co., Ltd., Shanghai, China) and negative mimics (100 nM; Sangon Biotech Co., Ltd.) were transfected into BV2 cells using Lipofectamine 2000 according to the manufacturer's protocol (Invitrogen; Thermo Fisher Scientific, Inc.). A total of 4 h post-transfection, BV2 cells were incubated in DMEM medium without glucose or serum in hypoxic conditions (5% CO_2 and 95% N_2) for 4 h at 37°C followed by incubation under normoxic conditions for 14 h at 37°C.

Cell proliferation and cell apoptosis assays. The cells (1×10^3 per well) were grown in 96-well plates and analyzed with an MTT assay for 4 h at 37°C. DMSO was added to dissolve crystals for 20 min at 37°C. The luminescence was then measured using a microplate reader (BioTek Instruments, Inc., Vinooski, VT, USA) at 492 nm.

For the analysis of apoptosis, the cells were proliferated in 6-well plates for 48 h following transfection, following which the cells were washed once in PBS and resuspended with binding buffer. The cells were stained with 5 μl fluorochrome-conjugated Annexin V and 5 μl PI (BD Biosciences, Franklin Lakes, NJ, USA). Apoptosis was detected using flow cytometry (FACSCanto; BD Biosciences).

Enzyme-linked immunosorbent assay (ELISA). The cell supernatants were collected and protein content was measured using a BCA assay. The levels of capsase-3, capsase-8 and capsase-9 were quantified using commercially available ELISA kits.

Western blot analysis. Total protein extracts were obtained from the cultured cells using RIPA assay and centrifuged at 10,000 g for 5 min at 4°C. Protein content was measured using a BCA assay. The protein samples (50 μg) were separated with 8-12% SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were then blocked with 5% BSA in TBST and incubated overnight at 4°C with the following primary antibodies: B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax; cat. no. sc-6236; 1:1,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), PI3K (cat. no. sc-1331; 1:1,000; Santa Cruz Biotechnology, Inc.), phosphorylated (p-)Akt (cat. no. sc-7985-R; 1:1,000; Santa Cruz Biotechnology, Inc.), MDM2 proto-oncogene (MDM2; cat. no. sc-812; 1:1,000; Santa Cruz Biotechnology, Inc.), p53 (cat. no. sc-47698; 1:1,000; Santa Cruz Biotechnology, Inc.) and GAPDH (cat. no. sc-293335; 1:2,000; Santa Cruz Biotechnology, Inc.). The membranes were then washed with TBST and incubated for 1 h at 37°C with HRP-conjugated goat anti-rabbit IgG (cat. no. sc-2004; 1:5,000; Santa Cruz Biotechnology, Inc.) or HRP-conjugated goat anti-mouse IgG secondary antibodies (cat. no. sc-2005; 1:5,000; Santa Cruz Biotechnology, Inc.). The protein bands were visualized using ECL western blotting detection reagents (Thermo Fisher Scientific, Inc.).

Statistical analysis. All data are expressed as the mean ± standard error of the mean using SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). All statistical
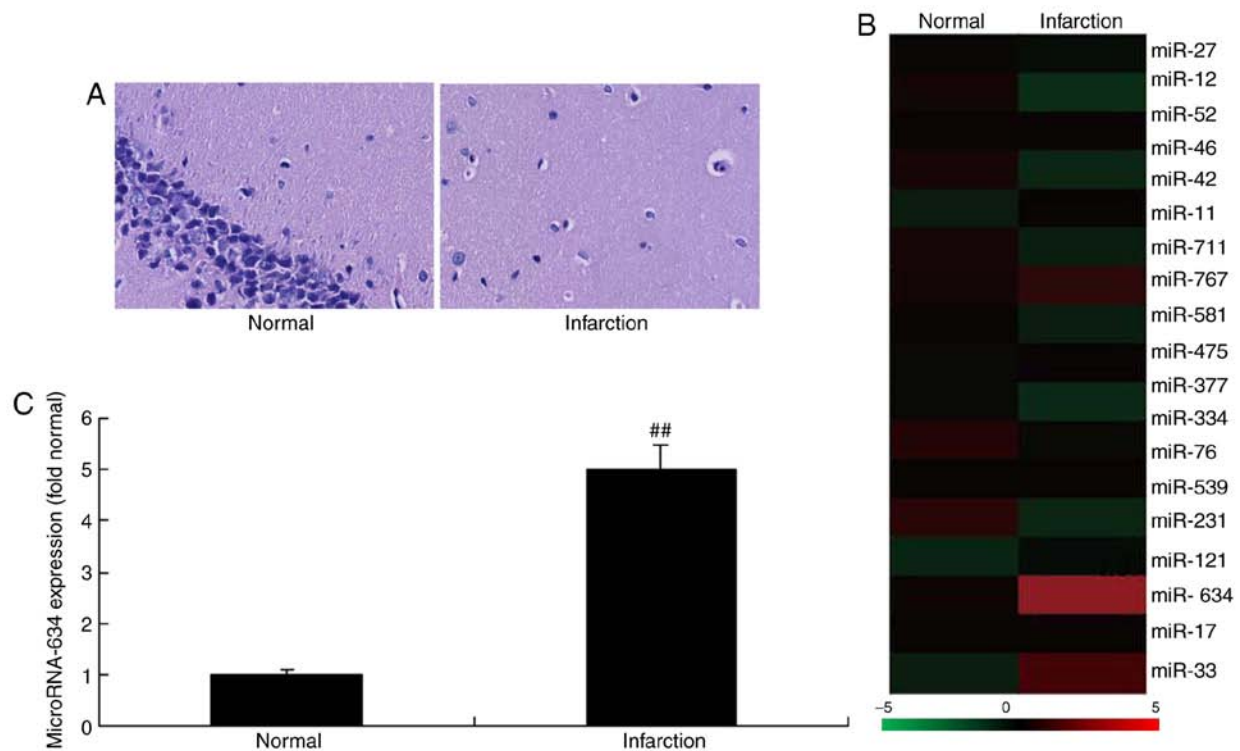
Figure 1. Expression of miRNA-634 in the cerebral infarction rat model. (A) Neurocyte death was apparent in the cerebral infarction group (magnification, x200). (B) Gene chip (green color indicates downregulated miRs and red color indicates upregulated miRs) and (C) reverse transcription-quantitative polymerase chain reaction analyses were performed to analyze the expression levels of miRNA-634. Normal, control rat group; cerebral infarction, cerebral infarction rat group. [##]P<0.01, vs. control. miR/miRNA, microRNAs.

significance was determined using one-way analysis of variance with Student's t-test or one-way analysis of variance and Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of miRNA-634 in the cerebral infarction rat model. To determine the expression level of miRNA-634 in the cerebral infarction rat model, RT-qPCR and gene chip analyses were performed to analyze the expression level of miRNA-634. As shown in Fig. 1A, neurocyte death was apparent in the cerebral infarction rat model, compared with the normal group. The expression level of miRNA-634 in the cerebral infarction rat group was increased compared with that in the normal control group (Fig. 1B and C).

Upregulation of the expression of miRNA-634 increases cell apoptosis in vitro. To investigate the effects of mature miRNA-634 on nerve inflammation and apoptosis in cerebral infarction, miRNA-634 mimics were used to increase the expression of miRNA-634. As shown in Fig. 2A-E, the miRNA-634 mimics significantly increased the expression of miRNA-634, inhibited cell proliferation and induced apoptosis in the *in vitro* model of cerebral infarction, compared with the control negative group. There were also significant increases in the levels of capsase-3, capsase-8 and capsase-9 in the *in vitro* model of cerebral infarction with upregulated miRNA-634, compared with levels in the negative control group (Fig. 2F-H).

Downregulation of the expression of miRNA-634 suppresses cell apoptosis in vitro. The present study also determined the function of the downregulation of miRNA-634 on cell apoptosis in the *in vitro* model of cerebral infarction. The downregulation of miRNA-634 promoted cell proliferation and inhibited apoptosis in the *in vitro* model of cerebral infarction, compared with the control negative group (Fig. 3A-E). The downregulation of miRNA-634 decreased the levels of capsase-3, capsase-8 and capsase-9 in the *in vitro* model of cerebral infarction with downregulated miRNA-634, compared with levels in the negative control group (Fig. 3F-H).

miRNA-634 regulates the MDM2/p53/Bax pathway in vitro. The present study also aimed to validate the effect on the 53/Bax pathway in the *in vitro* model of cerebral infarction following miRNA-634 exposure. As shown in Fig. 4A-D, the upregulation of miRNA-634 suppressed the protein expression of MDM2, and induced the protein expression of p53 and Bax in the *in vitro* model of cerebral infarction, compared with expression in the negative control group. The downregulation of miRNA-634 induced the protein expression of MDM2, and suppressed the protein expression of p53 and Baxin the *in vitro* model of cerebral infarction, compared with the expression in the negative control group (Fig. 4E-H).

miRNA-634 regulates the PI3K/Akt pathway in vitro. To determine the mechanism underlying the effect of miRNA-634 on nerve apoptosis of cerebral infarction, the protein expression levels of PI3K and p-Akt were measured using western blot analysis and immunofluorescence. As
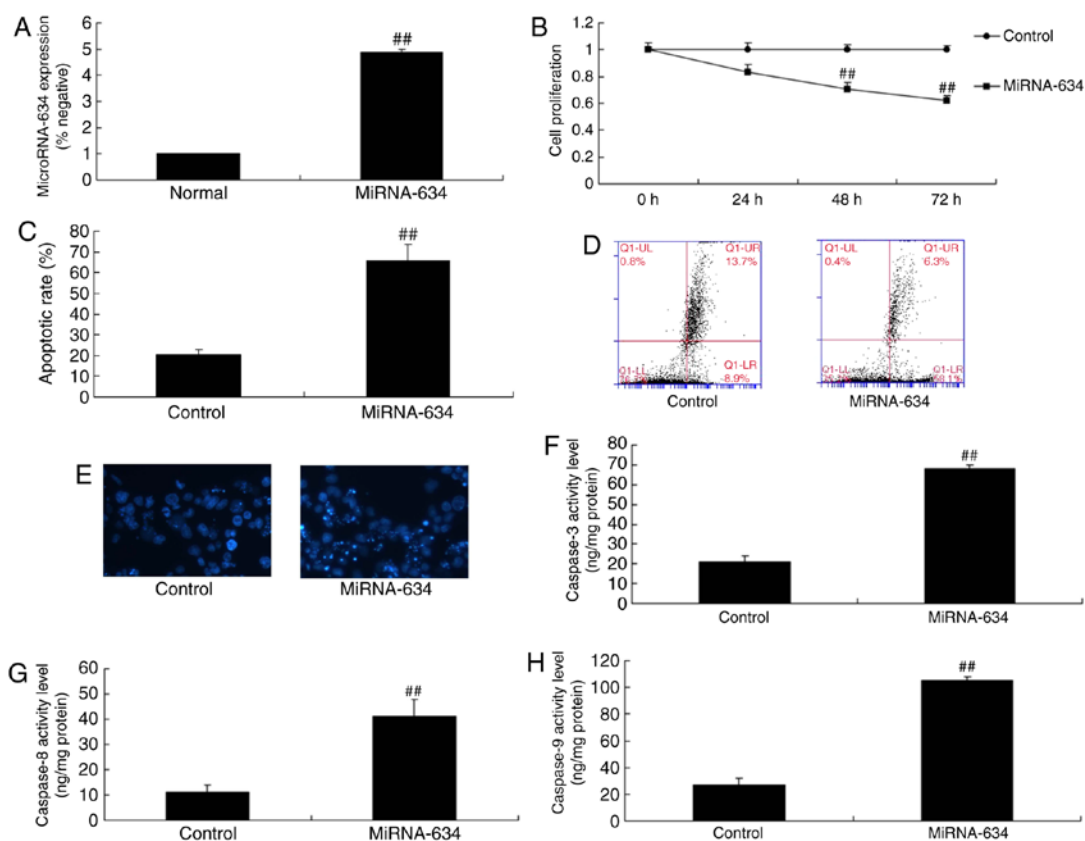
Figure 2. Upregulation of the expression of miRNA-634 increases cell apoptosis *in vitro*. (A) Expression of miRNA-634; (B) cell proliferation, (C) quantifica-tion of cell apoptosis from (D) flow cytometry; (E) DAPI staining (magnification, x100). Activity of (F) caspase-3, (G) caspase-8 and (H) caspase-9 in the *in vitro* model. Control group, negative control group; miRNA-634, overexpression of miRNA-634 group. ##P<0.01, vs. control. miRNA, microRNA.
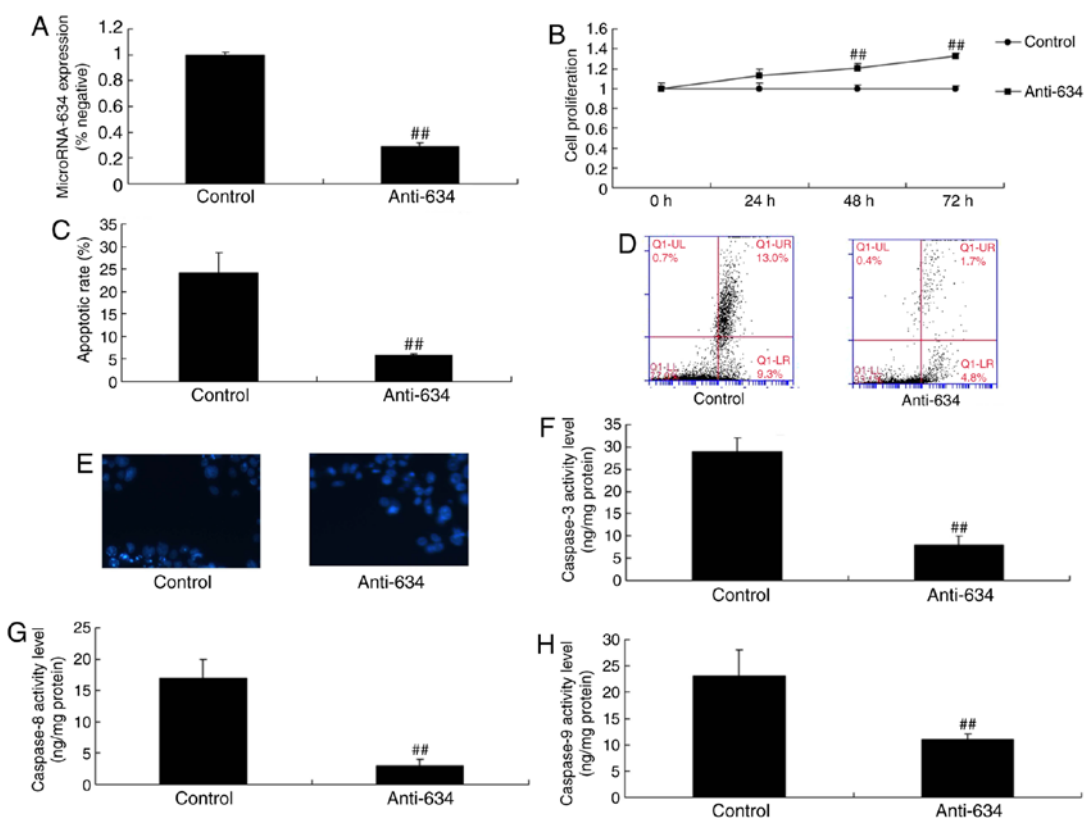

Figure 3. Downregulation of the expression of miRNA-634 suppresses cell apoptosis *in vitro*. (A) Expression of miRNA-634; (B) cell proliferation; (C) quanti-fication of (D) results of cell apoptosis; (E) DAPI staining (magnification, x100); activity levels of (F) caspase-3; (G) caspase-8 and (H) caspase-9 in the *in vitro* model. Control, negative control group; antii-634, downregulation of miRNA-634 group. ##P<0.01, vs. control negative group. miRNA-634, microRNA-634.
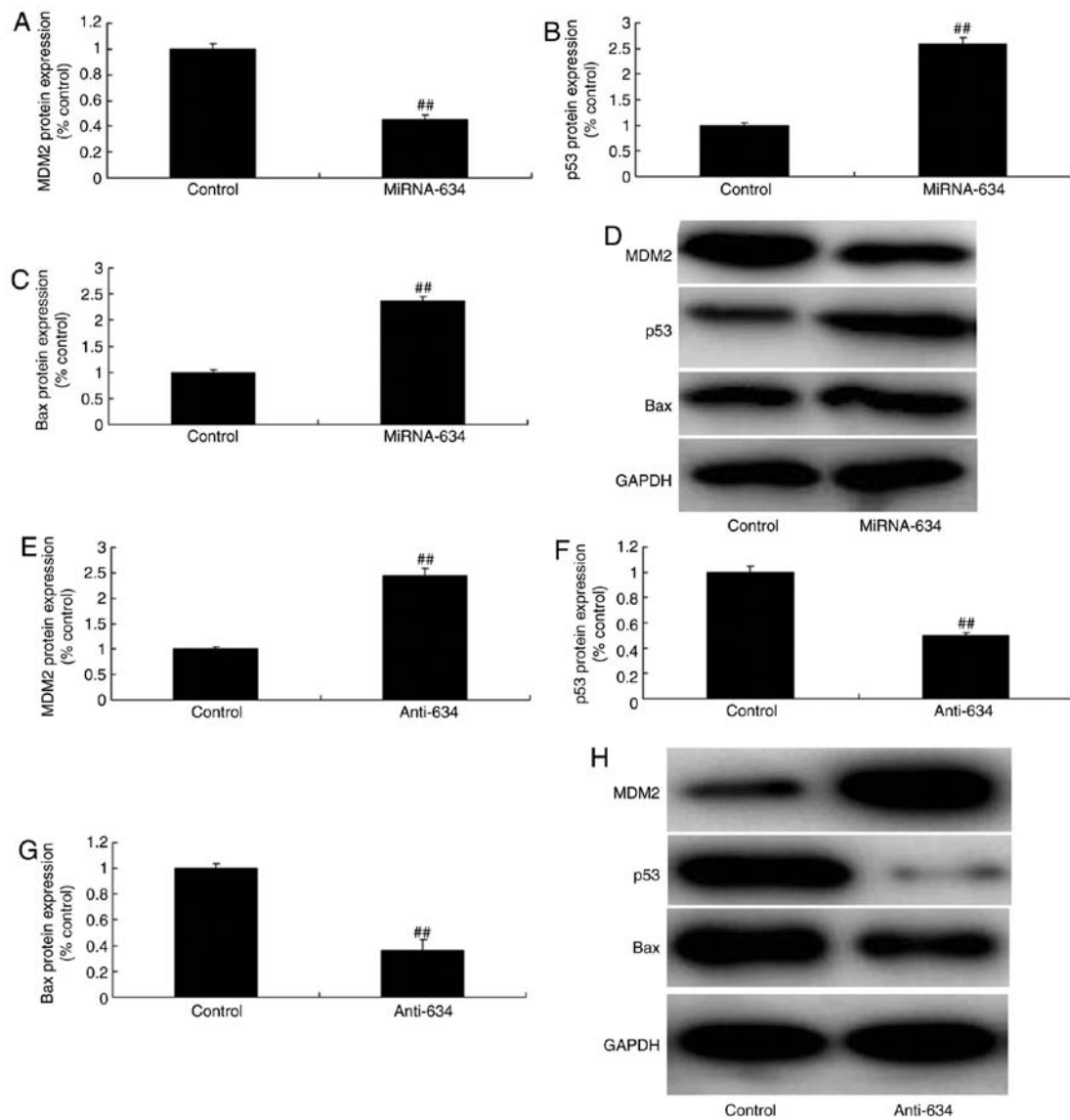
Figure 4. miRNA-634 regulates the MDM2/p53/Bax pathway *in vitro*. Statistical analysis of the protein expression levels of (A) MDM2, (B) p53 and (C) Bax from (D) western blot analysis in the *in vitro* model overexpressing miRNA-634. Statistical analysis of protein expression of (E) MDM2, (F) p53 and (G) Bax from (H) western blot analysis in the *in vitro* model of downregulated miRNA-634. Control, negative control group; miRNA-634, overexpression of miRNA-634 group; anti-634, downregulation of miRNA-634 group. ##P<0.01, vs. control. miRNA, microRNA; MDM2, MDM2 proto-oncogene; Bax, B-cell lymphoma 2-associated X protein.

the schematic in Fig. 5A shows, the 3' untranslated region of PI3K contains the miRNA-634 seed sites. The results of the immunofluorescence showed that miRNA-634 suppressed the protein expression of PI3K in the *in vitro* model of cerebral infarction, compared with that in the negative control group (Fig. 5B). As shown in Fig. 5C-E, the upregulated expression of miRNA-634 suppressed the protein expression of PI3K and p-Akt in the *in vitro* model of cerebral infarction, compared with the expression in the negative control group. The down-regulation of miRNA-634 induced the protein expression of PI3K and p-Akt in the *in vitro* model of cerebral infarction, compared with the expression in the negative control group (Fig. 5F-H).

Promotion of PI3K reduces the effects of miRNA-634 in vitro. Additionally, the present study assessed the role of PI3K in the effects of microRNA-634 in the *in vitro* model. As shown

in Fig. 6, the PI3K agonist (10 ng/ml of 1,3-dicaffeoylquinic acid) induced the expression of PI3K. The protein expression of p-Akt in the *in vitro* model of cerebral infarction was also induced by miRNA-634 and the PI3K agonist, compared with that in the miRNA-634 group (Fig. 6A-C). The PI3K agonist induced the protein expression of MDM2, and suppressed the protein expression of p53 and Bax in the *in vitro* model of cerebral infarction by anti-miRNA-634, compared with the anti-miRNA-634 group (Fig. 6D-G). The PI3K agonist was shown to reduce the effects of miRNA-634 on cell prolifera-tion, apoptosis and caspase-3/8/9 activity levels in the *in vitro* model of cerebral infarction by miRNA-634, compared with levels in the miRNA-634 group (Fig. 7A-G).

Inhibition of PI3K reduces the effects of anti-miRNA-634 in vitro. To assess the mechanism underlying the effect of miRNA-634 on apoptosis in the *in vitro* model of cerebral
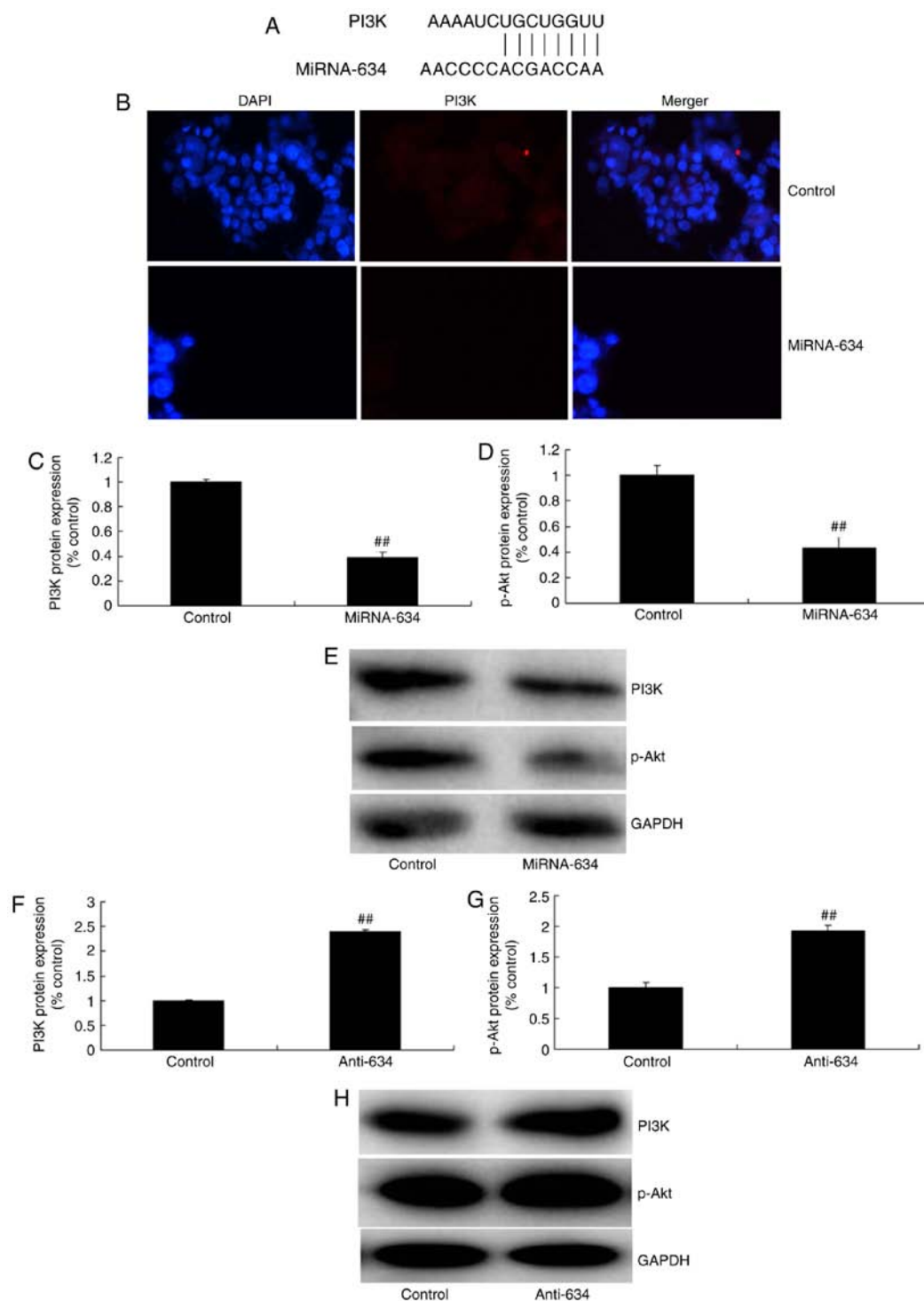
Figure 5. miRNA-634 regulates the PI3K/Akt pathway *in vitro*. (A) Schematic showing that the 3' untranslated region of PI3K contains the miRNA-634 seed sites. (B) Results of immunofluorescence showed the protein expression of PI3K (magnification, x100). Statistical analysis of a the protein expression of (C) PI3K and (D) p-Akt from (E) western blot analysis in the *in vitro* model overexpressing miRNA-634. Statistical analysis of a the protein expression of (F) PI3K and (G) p-Akt from (H) western blot analysis in the *in vitro* model with downregulated miRNA-634. ##P<0.01, vs. control. Control, negative control group; miRNA-634, overexpression of miRNA-634 group; anti-634, downregulated expression of miRNA-634 group. miRNA, microRNA; PI3K, phosphoinositide 3-kinase; p-, phosphorylated.

infarction, the PI3K inhibitor (20 nM of NVP-BAG956), was used to inhibit the protein expression of PI3K. The results of western blot analysis showed that the PI3K inhibitor suppressed the protein expression of PI3K and p-Akt in the *in vitro* model of cerebral infarction by anti-miRNA-634, compared with the anti-miRNA-634 group (Fig. 8A-C). The PI3K inhibitor

suppressed the protein expression of MDM2, and induced the protein expression of p53 and Bax in the *in vitro* model of cerebral infarction by anti-miRNA-634, compared with the anti-miRNA-634 group (Fig. 8D-G). The PI3K inhibitor also reduced the effects of anti-miRNA-634 on cell proliferation, apoptosis and caspase-3/8/9 activity levels in the *in vitro* model
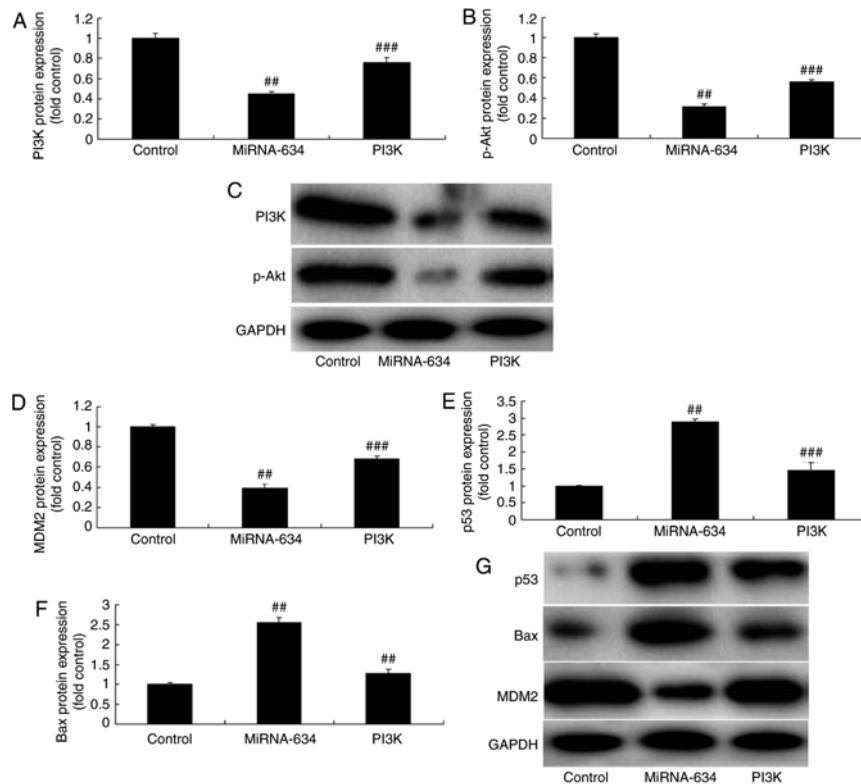
Figure 6. Promotion of PI3K reduces the effects of miRNA-634 on the PI3K/Akt pathway *in vitro*. Statistical analysis of protein expression levels of (A) PI3K and (B) from (C) western blot analysis. Statistical analysis of protein expression levels of (D) MDM2, (E) p53 and (F) Bax from (G) western blot analysis in the *in vitro* model. ##$P<0.01$, vs. control; ###$P<0.01$, vs. overexpression of miRNA-634 group. Control, negative control group; miRNA-634, overexpression of miRNA-634 group; PI3K, overexpression of miRNA-634 and 1,3-dicaffeoylquinic acid group. miRNA, microRNA; PI3K, phosphoinositide 3-kinase; p-, phosphorylated; MDM2, MDM2 proto-oncogene; Bax, B-cell lymphoma 2-associated X protein.
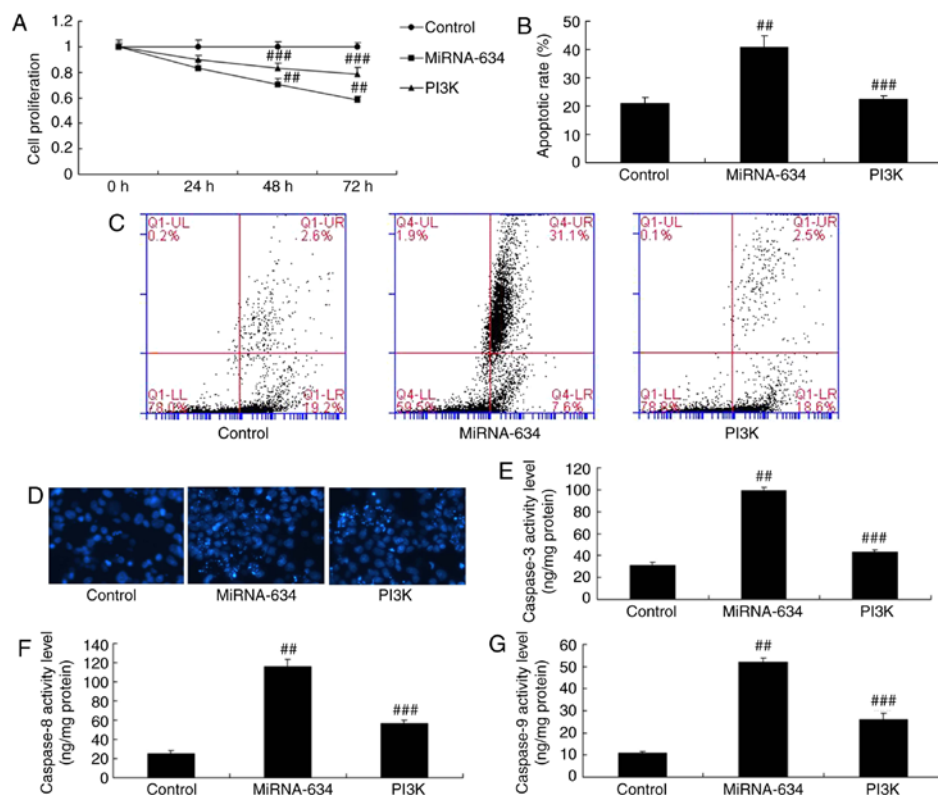

Figure 7. Promotion of PI3K reduces the effects of miRNA-634 on cell apoptosis *in vitro*. (A) Cell proliferation; (B) quantification of cell apoptosis from (C) results of flow cytometry; (D) DAPI staining (magnification, x100); activity levels of (E) caspase-3, (F) caspase-8 and (G) caspase-9 in the *in vitro* model. ##$P<0.01$, vs. control; ###$P<0.01$, vs. miRNA-634 group. Control, negative control group; miRNA-634, overexpression of miRNA-634 group; PI3K, overexpression of miRNA-634 and 1,3-dicaffeoylquinic acid group; miRNA, microRNA; PI3K, phosphoinositide 3-kinase.
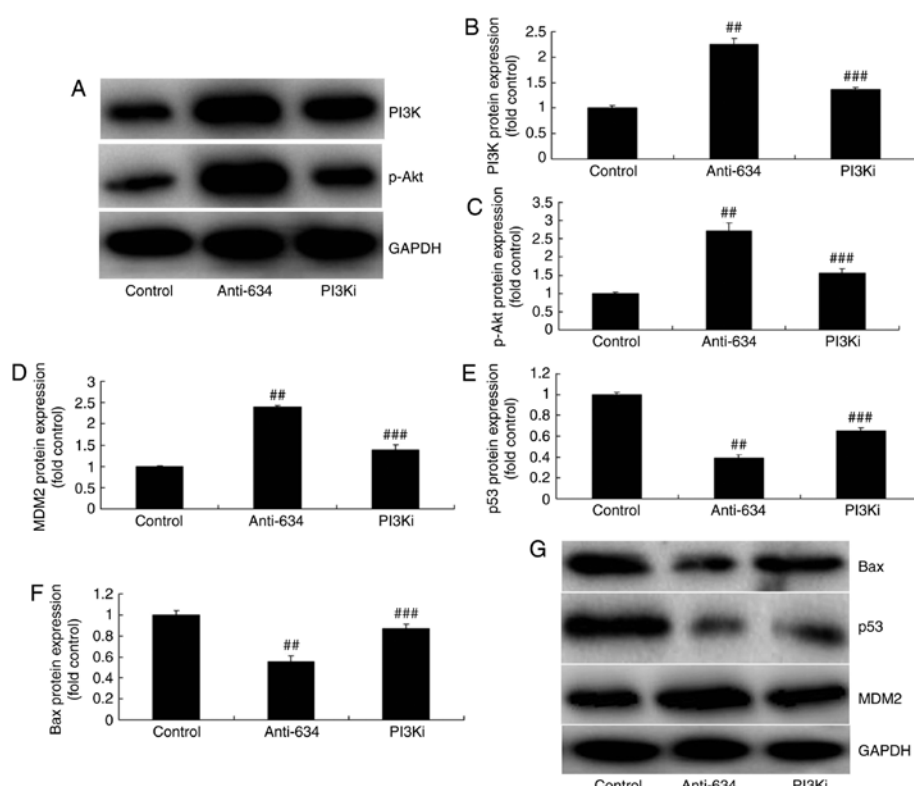
Figure 8. Inhibition of PI3K reduces the effects of anti-miRNA-634 on the PI3K/Akt pathway *in vitro*. (A) Western blot analysis of the protein expression of (B) PI3K and (C) p-Akt. Statistical analysis of the protein expression of (D) MDM2, (E) p53 and (F) Bax from (G) western blot analysis in the *in vitro* model. ##P<0.01, vs. control; ###P<0.01, vs. downregulation of miRNA-634 group. Control, negative control group; anti-634, downregulation of miRNA-634 group; PI3Ki, downregulation of miRNA-634 and NVP-BAG956 group; miRNA, microRNA; PI3K, phosphoinositide 3-kinase; MDM2, MDM2 proto-oncogene; Bax, B-cell lymphoma 2-associated X protein; p-, phosphorylated.
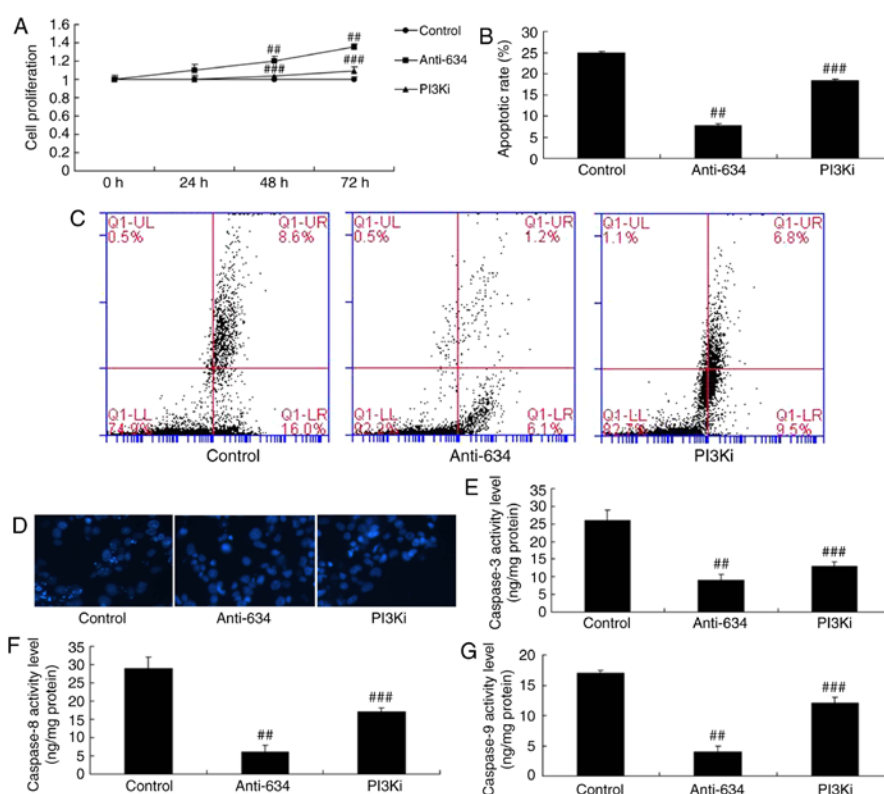

Figure 9. Inhibition of PI3K reduces the effects of anti-miRNA-634 on cell apoptosis *in vitro*. (A) Cell proliferation; (B) quantification of cell apoptosis from (C) flow cytometry; (D) DAPI staining (magnification, x100), activity levels of (E) caspase-3, (F) caspase-8 and (G) caspase-9 in the *in vitro* model. ##P<0.01, vs. control; ###P<0.01, vs. downregulation of miRNA-634 group. Control, negative control group; anti-634, downregulation of miRNA-634 group; PI3Ki, downregulation of miRNA-634 and NVP-BAG956 group; miRNA, microRNA; PI3K, phosphoinositide 3-kinase.
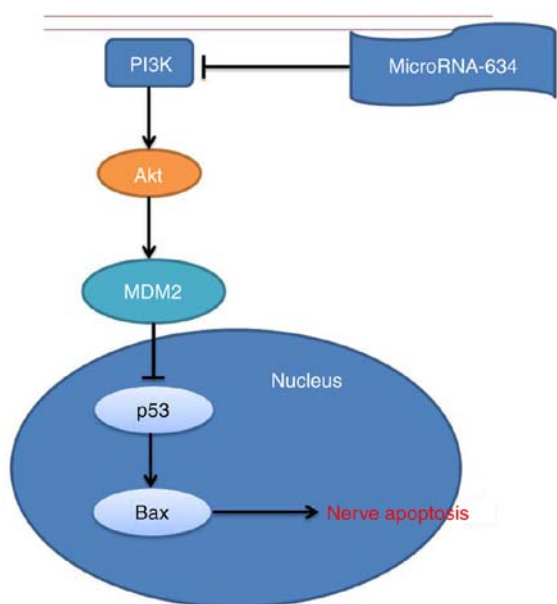
Figure 10. MicroRNA-634 alters nerve apoptosis via the PI3K/Akt pathway in cerebral infarction. PI3K, phosphoinositide 3-kinase; MDM2, MDM2 proto-oncogene; Bax, B-cell lymphoma 2-associated X protein.

of cerebral infarction by anti-miRNA-634, compared with the anti-miRNA-634 group (Fig. 9A-G).

Discussion

Acute cerebrovascular disease is a common and frequently occurring disease in middle-aged and elderly individuals (11). It is associated with high rates of disability and mortality. The morbidity rate of atherosclerosis has shown an increasing and younger trend year on year with the population aging, and changes to living and social environments. Therefore, it is a serious threat to human health and life (3). In the present study, it was showed that the expression level of miRNA-634 in a cerebral infarction rat model was increased, compared with that in the normal control group. Jeansonne *et al* (12) showed that the expression of miRNA-634 was upregulated in glioblastoma.

The detection of miRNAs in human tissues is either difficult to achieve or repeatedly used in clinic. It has been found in previous studies that there are stable miRNA molecules in the peripheral blood (13). Additionally, different diseases are associated with different miRNA expression profiles. This finding has led to novel thinking in adopting peripheral blood miRNA for the non-invasive diagnosis of disease (7). It is found that peripheral blood miRNA with specific expression changes can be observed in multiple diseases, including tumors, diabetes, myocardial infarction, Parkinson's disease and Alzheimer's disease (14). In addition, the present study found that miRNA-634 mimics significantly inhibited cell proliferation and induced apoptosis in an *in vitro* model of cerebral infarction. Cong *et al* (15) showed that miRNA-634 also decreases cell proliferation and induces apoptosis in cervical cancer cells.

The PI3K/Akt signal transduction pathway is a pro-survival signal. Its activation is important in ischemic-hypoxic neuronal injury, and it has attracted increasing attention (16). The PI3K/Akt signal transduction pathway is an important pathway for the intracellular transduction of membrane receptor signals. It is key in maintaining cell survival and inhibiting cell apoptosis (17). It can affect the activation of effector molecules, including downstream apoptosis-related protein and cell cycle regulatory protein (17). Consequently, it is important in inhibiting apoptosis and promoting proliferation in cells (9). PI3K/Akt has a definite anti-apoptotic protective effect on the brain in cerebral ischemia. Inhibiting such a pathway can aggravate cerebral ischemia-induced nerve cell apoptosis (9). The findings of the present study suggested that downregulation of the expression of miRNA-634 significantly suppressed the protein expression of PI3K and p-Akt in the *in vitro* model of cerebral infarction. Cui *et al* (18) suggested that the overexpression of miRNA-634 suppresses survival and matrix synthesis by targeting PIK3 regulatory subunit 1 in human osteoarthritic chondrocytes.

Caspase-3 is the most important apoptotic protease during apoptosis. Its activation is dependent on the release of cytochrome *c* (19). The Bcl-2 and Bax genes of the Bcl-2 family are the most important regulatory genes involved in cell apoptosis known at present. They can mediate the release of substances, including cytochrome *c*, through the mitochondrial pathway (20). A number of previous studies have found that Bcl-2 and Bax can serve as the upstream regulatory mechanism of caspase-3 and are involved in regulating the activity of caspase-3 (20). In addition, they can be treated as the direct substrate of caspase-3 to act on the downstream caspase-3. The two correlate with and restrain each other during cell apoptosis transduction (21). In the present study, it was shown that the inhibition of PI3K increased the effect of the downregulation of miRNA-634 on cell apoptosis and capsase-3/Bax protein expression in the *in vitro* model of cerebral infarction.

In conclusion, the findings of the present study suggested that the plasma miRNA-634 adjusted nerve apoptosis in cerebral infarction through the MDM2/p53/Bax pathway by PI3K/Akt (Fig. 10). miRNA-634 may be used as a potential therapeutic target in the treatment of cerebral infarction for clinical use in the future.

Availability of data and materials

The analysed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

QS designed the study; YC, WH, SL, ZY, QW and XL performed the experiments; QS and YC analyzed the data; and QS wrote the manuscript.

Ethics approval and consent to participate

The study was approved by the Animal Experimental Ethics Committee of Tangshan, Worker Hospital (Tangshan, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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