MicroRNA-9 enhances chemotherapy sensitivity of glioma to TMZ by suppressing TOPO II via the NF-κB signaling pathway

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Abstract. Glioma is the most common primary tumor of the central nervous system (CNS) that develops chemotherapy resistance. The microRNA (miRNA) miR-9 is a tissue-specific miRNA of the CNS that may serve a key role in the modulation of chemotherapy sensitivity. The aim of the present study was to investigate the effect of miR-9 on glioma chemotherapy sensitivity by altering the expression of miR-9 in U251 glioma cells by viral transfection and subsequently treating with gradient concentrations of temozolomide (TMZ). Cell viability, apoptosis and the cell cycle were examined, and drug resistance genes were analyzed by western blotting. The role of nuclear factor κB (NF-κB) in this regulation was also examined. The results revealed that the susceptibility of glioma cells to TMZ was enhanced by miR-9 overexpression. When miR-9 and TMZ were applied together, the apoptotic rate and percentage of cells arrested at the G2/M stage were significantly higher compared with either treatment alone. Topoisomerase II expression was suppressed by miR-9 via the NF-κB signaling pathway, which may be responsible for the sensitization. The results of the present study suggested that miR-9 may be a potential target for glioma chemotherapy.

Introduction

Glioma, which has a poor prognosis, is the most common malignant primary brain tumor; it often displays unique biological features, including strong propensity for proliferation and metastasis (1). Despite the use of the most effective treatments available, including surgery and chemoradiotherapy, the median survival time of patients with glioblastoma has no optimistic estimate of being extended (1). Glioma often develops resistance to a number of chemotherapy drugs, such as nitrosourea. There are several approaches for glioma to obtain resistance, including reduced drug absorption, increased drug discarding, enhanced ability to decompose anticancer drugs, increased proliferation and reduced apoptosis by stimulation of cytokine excretion to alter the microenvironment (2). Drug resistance genes, including O6-methylguanine-DNA methyltransferase (*MGMT*), topoisomerase II (*TOPO II*) and multiple drug resistance 1, are involved in these activities (3). Temozolomide (TMZ) has been used extensively for the treatment of glioblastomas (GBMs) over the past two decades; it is an alkylating agent that causes DNA mismatches resulting in cell apoptosis in GBMs (4,5). Nevertheless, TMZ does not increase life span significantly, possibly due to high-grade glioma developing resistance to the majority of chemotherapy drugs, including TMZ. The underlying mechanisms require investigation and solutions to the problem of drug resistance are being sought.

MicroRNAs (miRNAs) are involved in gene expression regulation by binding to mRNA 3'-untranslated region to suppress translation or induce cleavage of the target mRNA directly to regulate protein expression (6). They serve important roles in all physiological functions; for example, cellular differentiation, proliferation, cell cycle control, cell death and organ development (7). miRNAs are also involved in a variety of human diseases, including inflammation and cancer (8). Our previous study demonstrated that miRNA-9 (miR-9) inhibited vasculogenic mimicry and modulated migration by suppressing stathmin expression (9), and the silencing of stathmin was able to enhance chemotherapeutic sensitivity of glioma to TMZ (10). However, the effects of miR-9 on chemotherapeutic sensitivity of glioma to TMZ and the underlying mechanisms are unknown.

In the present study U251 glioma cells were transfected with lentiviral (LV) vectors carrying a miR-9 mimic or inhibitor and subsequently treated with gradient concentrations of TMZ. Cell viability, cell apoptosis, cell cycle and proteins responsible for apoptosis and drug resistance were examined. The role of nuclear factor κB (NF-κB) in the regulation of sensitivity to chemotherapy was also investigated. The aim of the present study was to evaluate the effects of miR-9 on chemotherapy of glioma to provide a target for glioma chemotherapy.

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Materials and methods

Cell culture and miR-9 interference. The malignant glioma cell line U251 was obtained from the American Type Culture Collection (Manassas, VA, USA) and was cultured in complete Dulbecco's modified Eagle's medium (DMEM) at 37˚ with 5% CO_2. The Hsa-miR-9 sequence was obtained from the miRbase database (www.miRbase.org). The miR-9 mimic sequence (5'-TCTTTGGTTATCTAGCTGTATGA-3') and inhibitor sequence (5'-TCATACAGCTAGATAACCAAA GA-3') were synthesized, inserted into green fluorescent protein (GFP)-containing lentivirus vectors (GV280 for inhibitor and GV369 for mimic and negative control), and then embedded in lentiviral particles by Shanghai GeneChem Co., Ltd. (Shanghai, China). The cells ($5x10^4$ per well of a 6-well plate) were transfected with lentivirus particles using Lentifectin™ transfection reagent (ABM Inc., Richmond, BC, Canada) as per the protocol provided by the lentivirus manufacturer (virus titer, $5x10^8$ transducing units/ml; multiplicity of infection, 5). Next, the cells were incubated in the cell incubator. miR-9 transfection efficiency was examined by fluorescence microscopy 72 h following transfection and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) 24 h following transfection. The cells transfected with lentivirus particles carrying empty vectors were used as negative controls (NC) and the cells treated with transfection reagent only were regarded as the mock group. For the following steps, cells were incubated for 120 h after transfection.

TMZ treatment. TMZ (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was dissolved in dimethyl sulfoxide (DMSO) to make a 10 mM stock solution that was serially diluted in DMEM to the following concentrations: 31.25, 62.5, 125, 250, 500 and 1,000 μM. All groups had the same final concentration of DMSO (1% v/v). A further control with DMSO only was named the 0 μM control. U251 cells were plated and treated with the all concentrations of TMZ to assess cell viability by MTT assay, or for apoptosis and cell cycle assays, with 100 μM TMZ, at which concentration cell viability is inhibited.

MTT assay. Glioma cells were plated in 96-well plates (6,000 cells/well) and transfected and/or treated with TMZ as aforementioned. Transfected and non-transfected cells were incubated for 24, 48 or 72 h. Then cell viability was analyzed by MTT colorimetric assay. MTT (500 mg) was dissolved in 100 ml PBS to make 5 mg/ml stock solution. This MTT solution (20 μl) was added to each well and incubated for 4 h, then the crystal was dissolved in 150 μl DMSO after the culture medium was discarded. The absorbance at a wave length of 490 nm was detected using a microplate reader (M2009PR; Tecan infinite; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Experiments were performed three times.

Apoptosis assay. Transfected cells were treated with 100 μM TMZ for 24 h, washed with Hank's D solution, harvested and counted. The eBioscience™ Annexin V Apoptosis Detection Kit APC (cat. no. 88-8007-72; Invitrogen; Thermo Fisher Scientific, Inc.) was used to measure apoptosis according to the manufacturer's protocol. A total of $1x10^5$ cells were resuspended in 100 μl binding buffer, and 10 μl of Annexin V and 5 μl of propidium iodide (PI) were added. The cells were then incubated in the dark for 15 min at room temperature, and subsequently analyzed using an Epics Altra II cytometer (Beckman Coulter Inc., Brea, CA, USA). The data were analyzed by Kaluza analysis software (version 1.3; Beckman Coulter Inc.) and the apoptotic rate (%) was determined by adding the cell population positive for PI and annexin V (late apoptosis) and the population positive for annexin V only (early apoptosis). The experiment was repeated three times.

NF-κB signaling pathway interference. Bay117082 (Sigma-Aldrich; Merck KGaA,), an inhibitor of the NF-κB signaling pathway, was dissolved in DMSO to make a 10 mM stock solution. U251 cells were plated at a concentration of $5x10^4$ cells/ml (100 μl for 96-well plates, and 1 ml for 6-well plates) and treated with 10 μM Bay117082 for 24 h at 37˚C in a cell incubator with 5% CO_2.

Western blot analysis. Total protein was extracted from cells in one well of a 6-well plate (~$1x10^6$ cells) following 24-h treatment with 100 μM TMZ as aforementioned and the concentrations were measured using a spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology, Shanghai, China). Protein at a concentration of >5 μg/μl (50 μg in total) was separated by 10% or 12% SDS-PAGE and was transferred onto polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA), followed by blocking with skimmed milk dissolved in TBS and 0.05% Tween-20 (TBST) for 1 h at room temperature. The membranes were incubated with primary antibodies (Table I) at 4˚C overnight, washed three times with TBST and were incubated in horseradish peroxidase-conjugated secondary antibodies at 1:5,000 dilution (sc-2004 and sc2005; Santa Cruz Biotech, Santa Cruz, CA, USA) for 1 h at room temperature after washing with TBST three times. Following washing, the protein bands were detected with ECL substrates or DAB Detection System (both OriGene Technologies, Inc., Beijing, China). GAPDH was used as a loading control, and all experiment were repeated three times. Quantitative analysis was performed using the Quantity One Software (version 4.6.2; Bio-Rad Laboratories, Inc.).

Cell cycle assay. Transfected cells were treated with 100 μM TMZ for 24 h in a cell incubator at 37˚C and subsequently harvested, washed with ice-cold PBS and fixed with 70% ethanol at 4˚C overnight. The ethanol was removed by centrifugation at 300 x g for 10 min at room temperature and ~$1x10^6$ cells were resuspended in PBS containing PI (50 μg/ml) and RNase A (50 μg/ml; both Sigma-Aldrich; Merck KGaA) for 30 min in the dark prior to analysis by flow cytometry (FACScalibur; BD Biosciences, San Jose, CA, USA). The data were analyzed by ModFit LT™ software (Verity Software House, Inc., Topsham, ME, USA) and the percentage of cells at G0/G1, S or G2/M phase was calculated. DMSO-treated cells were used as untreated controls. Experiments were repeated three times.

Table I. Primary antibodies used in western blot analysis.

Protein	Supplier	Cat. no.	Origin	Dilution	Molecular weight
Bax	Abcam	Ab32503	Rabbit	1:1,000	21 kDa
Caspase-3	Abcam	Ab4051	Rabbit	1:500	32 kDa
Bcl-2	Abcam	Ab692	Mouse	1:500	26 kDa
GAPDH	Santa Cruz	Sc-32233	Mouse	1:2,000	36 kDa
TOPO II	Abcam	Ab52934	Rabbit	1:1,000	174 kDa
MMP-2	Cell Signal Tech	13132	Rabbit	1:1,000	72 kDa
MMP-9	Cell Signal Tech	13667	Rabbit	1:1,000	92 kDa
NF-κB	Abcam	Ab32536	Rabbit	1:10,000	65 kDa
IκB	Santa Cruz	Sc52900	Mouse	1:1,000	36 kDa
Cyclin D	Santa Cruz	Sc450	Mouse	1:1,000	35 kDa
N-Cad	Cell Signal Tech	13116	Rabbit	1:1,000	140 kDa
R-Cad	Abcam	Ab109242	Rabbit	1:10,000	150 kDa

Bax, Bcl-2-associated apoptosis regulator; Bcl-2, Bcl-2 apoptosis regulator; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TOPO II, DNA topoisomerase II; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; IκB, inhibitor of κB; N-Cad, neural cadherin; R-Cad, retinal cadherin.

RT-qPCR. Cells of one well of 6-well plate (~1x10^6 cells) were lysed with TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following 24-h treatment with 100 μM TMZ as aforementioned and total mRNA was extracted. The mRNA was reverse-transcribed into cDNA in a reverse-transcription reaction with the following reagents: M-MLV reverse transcriptase (M1705); dNTPs (U1240; both Promega Corporation, Madison, WI, USA); and Oligo dT primer (Sangon Biotech Co., Ltd., Shanghai, China). For PCR analysis, cDNA was diluted to a final concentration of 10 ng/μl. qPCR was performed with a Universal Master Mix (Nantong Chem-Base Co. Ltd, Nantong, China). cDNA (50 ng) was used to determine the relative amounts of mRNA by qPCR using a MAX3000 Sequence-Detection System (Nantong Chem-Base Co. Ltd,) using specific primers with SYBR Green dye. The thermocycling conditions were as follows: An initial step of 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec, 60˚C for 30 sec, 95˚C for 15 sec, 55˚C for 30 sec and 95˚C for 15 sec. The primers used for PCR were as follows: miR-9 forward, 5'-GTGCAGGGTCCG AGGT-3' and reverse, 5'-GCGCTCTTTGGTTATCTAGC-3'. U6 was amplified as reference for miR-9 using the following primers: U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'. The experiment was performed three times, and the $2^{-\Delta\Delta Cq}$ method was used for determining relative expression levels of mRNA or microRNA (11).

Statistical analysis. The results were presented as mean ± standard deviation. Statistical analyses were performed using SPSS 16.0 statistical software (SPSS, Inc., Chicago, IL, USA). One-way or two-way analysis of variance (ANOVA) were used to compare the differences between the groups, and Dunnett's or Tukey post hoc test was used for multiple comparisons. $P<0.05$ was considered to indicate a statistically significant difference. The graphs were drawn using GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Verification of transfection. U251 cells were transfected with LV carrying miR-9 mimic or inhibitor. An empty virus was used as positive transfection control group, and cells incubated with transfection reagent only were used as an untreated control (mock). As the results demonstrated, green fluorescence was detected in >95% cells of the transfected groups (Fig. 1A). The levels of miR-9 expression in test groups were evaluated by RT-qPCR. The results revealed that there were significant differences between the mimic and the control groups, as well as the inhibitor and the control groups (Fig. 1B).

miR-9 enhances TMZ-induced inhibition of cell viability. When U251 cells were treated with TMZ (NC group), the cell viability decreased with increasing TMZ concentration. When the concentration of TMZ was >62.5 μM, cell viability was significantly decreased compared with the 0 μM group (Fig. 2A; 24 h, P=0.0469; 48 h, P=0.0322; 72 h, P=0.0013).

When miR-9 was overexpressed, the viability of glioma cells was significantly lower compared with the negative control group (NC) 24, 48 or 72 h following TMZ treatment (Fig. 2B). Conversely, when the miR-9 inhibitor was transfected, cell viability was significantly higher compared with the NC group 48 or 72 h following TMZ treatment (Fig. 2B). However, when TMZ concentration was >500 μM, there was no significant difference in cell viability compared to the NC group (500 μM at 72 h, inhibitor group; 1,000 μM at 72 h, inhibitor group and mimic group; 1,000 μM at 48 h, mimic group).

miR-9 overexpression increases the apoptotic rate and aggravates G2/M stage arrest induced by TMZ. Cell cycle and apoptotic rate were also analyzed. When treated with TMZ, the apoptotic rate of U251 cells increased significantly compared with the groups without TMZ (Fig. 3A). In miR-9 mimic-transfected cells, the apoptotic rate increased, whereas in the inhibitor-transfected cells, the apoptotic rate

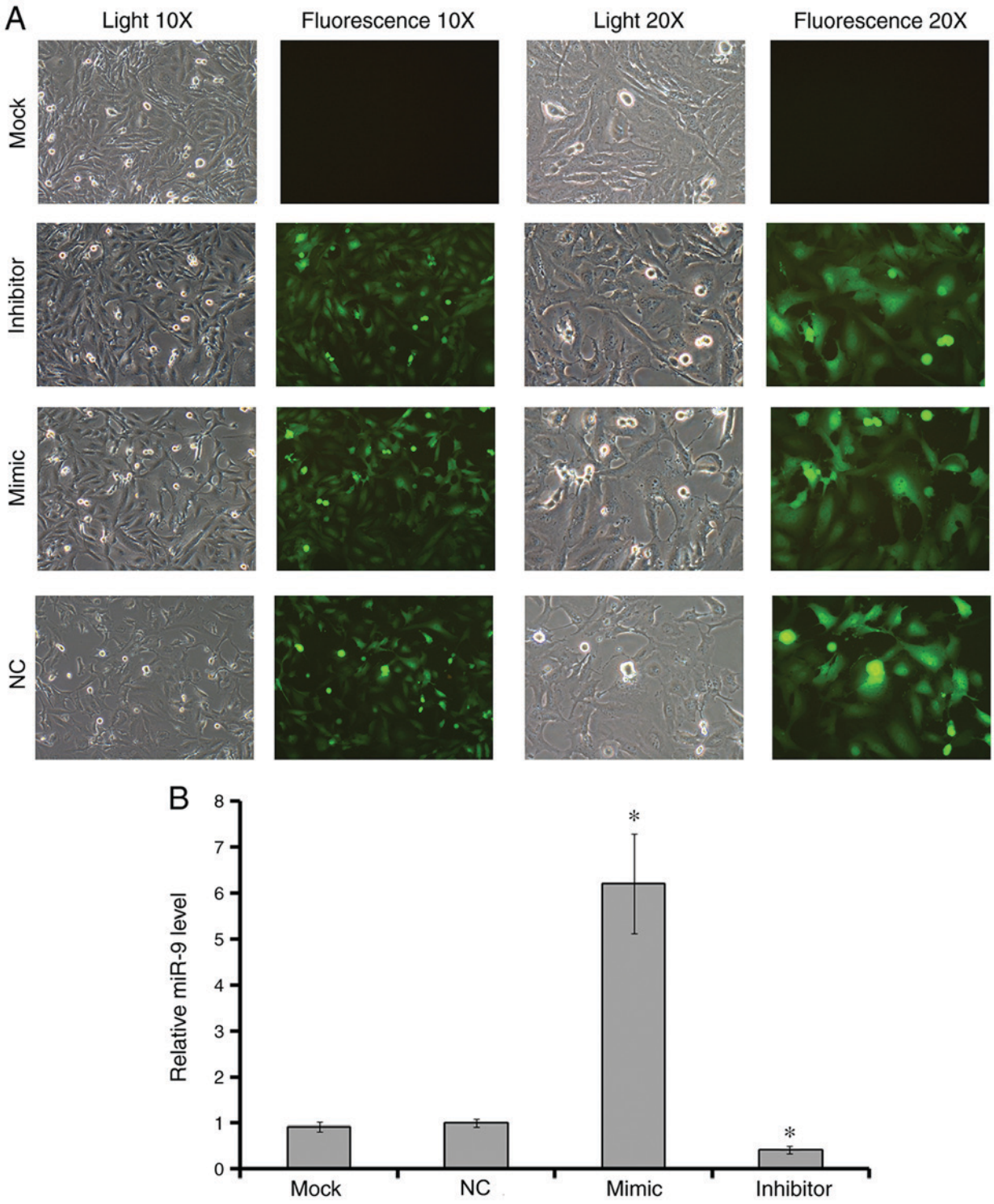

Figure 1. Verification of miR-9 transfection efficiency in U251 glioma cells. (A) Fluorescence identification; cells expressing green fluorescence were transfected successfully. A total of 5 fields were examined for each group, and the experiment was repeated three times. (B) miR-9 expression level analysis by reverse transcription-quantitative polymerase chain reaction; the data were presented as mean ± SD. There were no significant differences between the NC and mock groups. *P<0.05 vs. NC. miR-9, microRNA-9; NC, negative control.

reduced (Fig. 3A). In cells co-treated with TMZ and miR-9 mimic the apoptotic rate increased significantly compared with miR-9 alone.

When cells were treated with miR-9 or TMZ, caspase-3 and Bax expression increased while Bcl-2 decreased compared to the NC group. When co-treated with miR-9 and TMZ, Bax and caspase-3 protein expression levels markedly increased compared with miR-9- or TMZ-only treated cells; Bcl-2 expression further decreased compared to the miR-9 or TMZ groups (Fig. 3B). Furthermore, TMZ or miR-9 significantly induced G2/M stage arrest compared with the NC group, and when the treatments were combined, the rate of G2/M stage increased significantly compared to the miR-9 or TMZ groups (Fig. 3C).

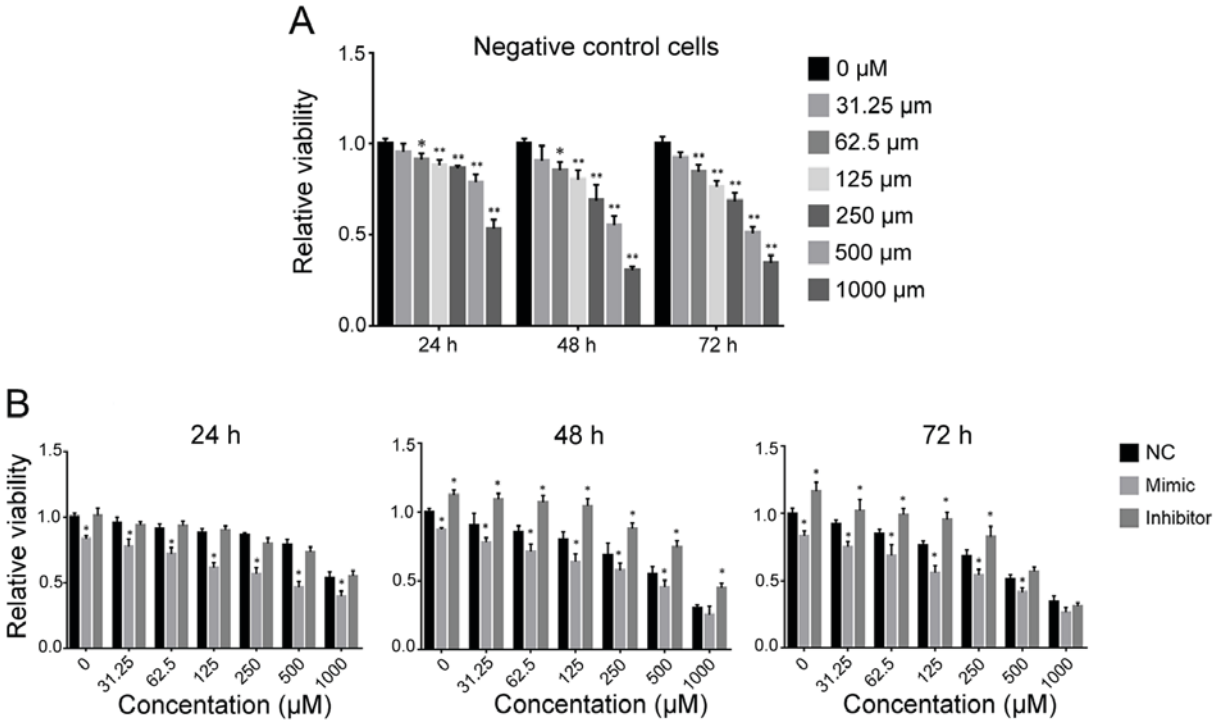

Figure 2. MTT analysis of cell viability. Each concentration was tested in three wells and the experiments were repeated three times. (A) Cells with empty virus transfected (NC group) treated with TMZ; *P<0.05 vs. no TMZ (0 μM), **P<0.01 vs. no TMZ (0 μM); data were analyzed with one-way ANOVA and Dunnett's post hoc test; TMZ (0 μM) was regarded as control in this analysis; (B) U251 cells transfected with miR-9 mimic or inhibitor and treated with TMZ at the indicated concentrations; data were analyzed with two-way ANOVA and Tukey post hoc test; *P<0.05 vs. NC. miR-9, microRNA-9; NC, negative control; TMZ, temozolomide.

miR-9 overexpression inhibits TOPO II expression via the NF-κB signaling pathway. Proteins responsible for drug resistance, including TOPO II, MGMT and p170, were analyzed. The expression of TOPO II was not significantly changed in inhibitor group (P=0.9977; Fig. 4A and B) and was notably downregulated in the mimic group compared to the NC group (P=0.0433; Fig. 4A and B).

The role of the NF-κB pathway in the modulation of chemotherapy sensitivity was investigated using an inhibitor of NF-κB activation, Bay117082. When miR-9 mimic or Bay117082 was applied, NF-κB expression was suppressed, as well as the downstream genes matrix metalloproteinase (MMP)-2, MMP-9 and cyclin D1 (Fig. 4). Proteins associated with drug resistance and invasion, including TOPO II, retinal (R)-cadherin and neural (N)-cadherin were also suppressed as NF-κB signal pathway was blocked. When the inhibitor was transfected, the expression of all proteins were not significantly altered except for R-cad, which was significantly upregulated (Fig. 4). IκB was slightly upregulated when Bay117082 was applied, but the change was not significant (P=0.1005; Fig. 4), and it was not altered in the mimic or inhibitor groups.

Discussion

miR-9 is a tissue-specific miRNA of the central nervous system (CNS) that is expressed in embryonic stages and serves vital roles during CNS development; it promotes neural stem cell differentiation and prevents neurons from mutating into gliocytes (12). In cerebral development, miR-9 promotes the formation of cortex construction by modulating the expression of forkhead box G1 (*FoxG1*) (13). Furthermore, miR-9 also serves important roles in spinal cord and peripheral neural system development (14). Function impairment of miR-9 results in severe malformation or deficiencies of the nervous system (15). miR-9 also participates in tumor development, proliferation, metastasis and invasion. For the tissue specificity of cancers, as well as the different target genes modulated, miR-9 acts as a tumor suppressor or a tumor-promoting factor depending on the situation. For example, miR-9 promotes breast cancer metastasis and invasion by inhibiting epithelial-cadherin expression (16) and enhances the invasion of endometrial cancer by suppressing the expression of *FoxO1* (17). However, miR-9 acts as tumor suppressor in certain other cancers. For example, overexpression of miR-9 in gastric adenoma, which expresses low levels of miR-9, stimulated the expression of phosphatase and tensin homolog and induced apoptosis, inhibiting proliferation, differentiation and invasion (18). In ovarian cancer, miR-9 inhibited tumor progression by suppressing the expression of fibroblast growth factor, BCL-2 and B-Raf proto-oncogene, serine/threonine kinase (19).

As miR-9 is specifically expressed in primary tumors of the nervous system, it may be regarded as a marker for differentiation between primary and metastatic tumors, tumor progression and prognosis for patients with glioma (20). The functions of miR-9 in glioma may be complicated and need further investigation, as its target genes include both tumor suppressors and tumor promoters (21,22). Our previous study demonstrated that miR-9 inhibited glioma cell proliferation,

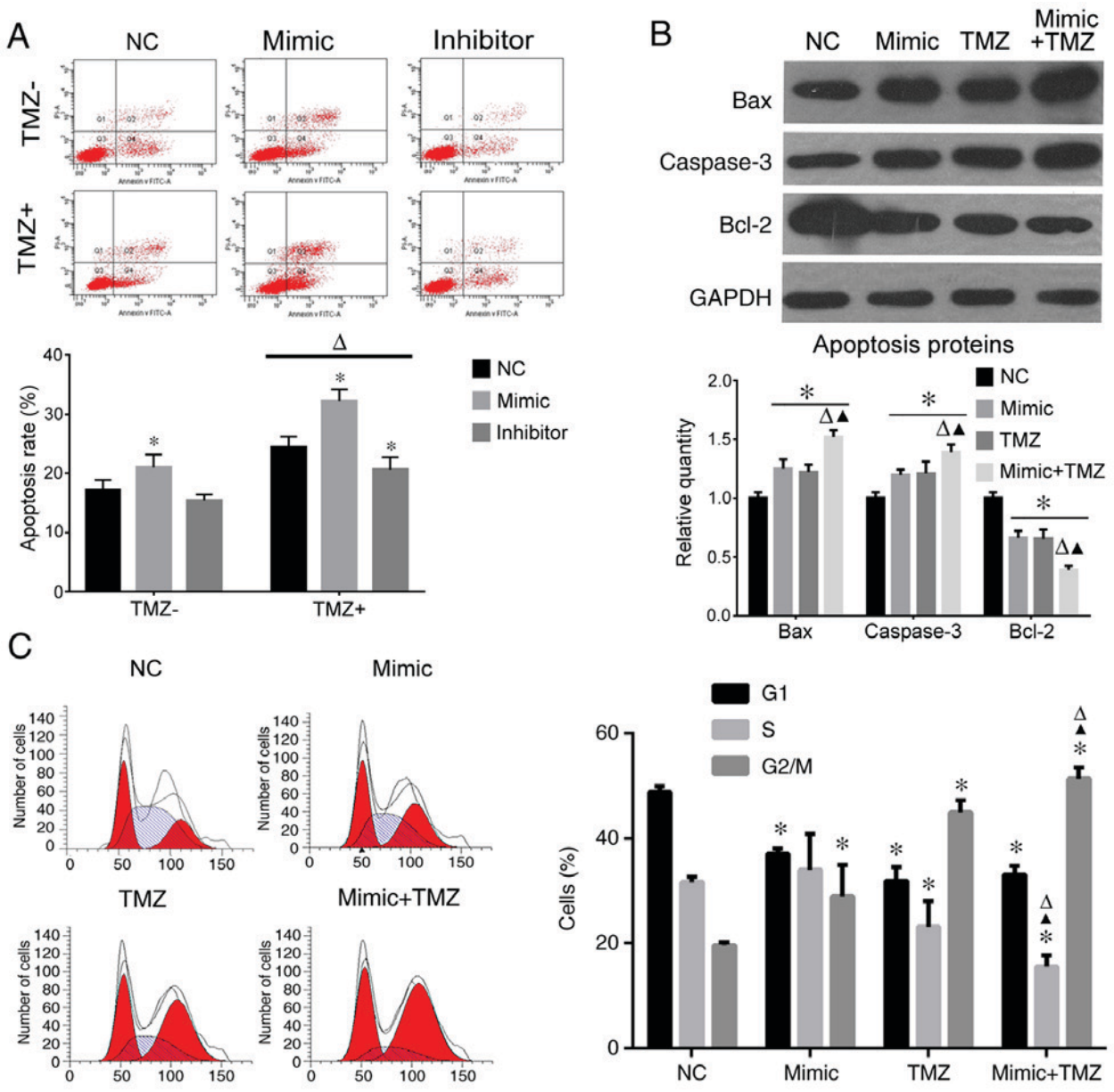

Figure 3. miR-9 increases TMZ-induced apoptosis and G2/M stage arrest. Experiments were repeated three times. (A) miR-9 overexpression induced apoptosis of U251 cells; the tendency was more substantial when TMZ was applied. miR-9 downregulation suppressed apoptosis. *P<0.05 vs. control; $^{\Delta}$P<0.05, TMZ^{+} vs. TMZ^{-}. Two-way ANOVA and Tukey post hoc test was applied. (B) Western blotting analysis of the expression levels of apoptosis-related proteins; GAPDH was used as a loading control; *P<0.05 vs. NC; $^{\blacktriangle}$P<0.05 mimic + TMZ vs. mimic alone; $^{\Delta}$P<0.05 mimic + TMZ vs. TMZ alone, one-way ANOVA and Tukey post hoc test was applied. (C) Cell cycle analysis, one-way ANOVA and Tukey post hoc test was applied; *P<0.05 vs. NC; $^{\blacktriangle}$P<0.05 mimic + TMZ vs. mimic alone; $^{\Delta}$P<0.05 mimic + TMZ vs. TMZ alone. miR-9, microRNA-9; NC, negative control; TMZ, temozolomide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

metastasis and vasculogenic mimicry both *in vitro* and *in vivo* through the suppression of stathmin expression (9). When stathmin-targeted small interfering (si)RNA was applied, chemotherapeutic sensitivity of glioma cells to TMZ was enhanced (10). miR-9 overexpression induced G2/M stage arrest; the same effect was observed when TMZ and stathmin-targeted siRNA were applied (23). Therefore, stathmin is involved in TMZ chemotherapeutic sensitivity. In the present study, miR-9 applied together with TMZ induced more substantial apoptosis and inhibition of viability by suppressing TOPO II expression. However, as miR-9 is an upstream modulating factor, it may enhance chemotherapy sensitivity through the regulation of a drug resistance gene, TOPOII, as the current study showed.

TOPO II, which is located on chromosome 17, is involved in DNA repair and replication, as well as chromosome segregation and replication (24). TOPO II is an essential nuclear enzyme that induces repair and replication of DNA by protecting the double helix, insuring stability and genomic integrity when DNA faces physical or chemical damage (24). As opposed to MGMT and excision repair associated protein ERCC that are abundant in normal tissue, TOPO II levels are higher in glioma tissue, correlating with an increased glioma grade (3). TOPO II may stimulate and promote tumor growth and metastasis and inhibit apoptosis, and they are involved in maintaining the glioma stem cell character (25). Silencing of TOPO II expression resulted in increased apoptosis and cell cycle arrest at G0/G1 (26). TOPO II is essential for TMZ resistance, as glioma

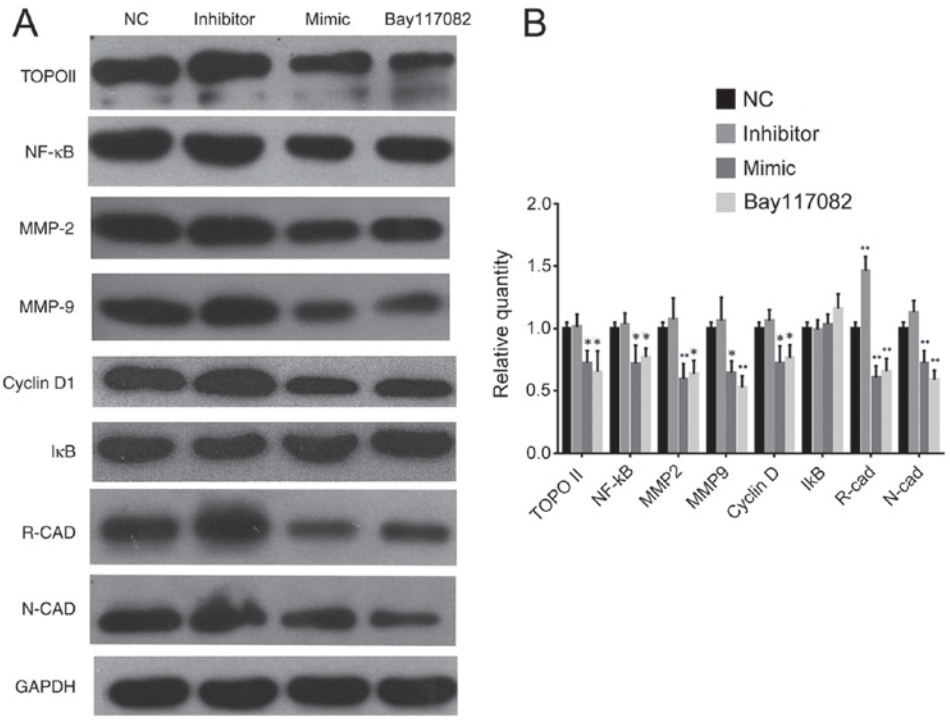

Figure 4. Western blot analysis of NF-κB and proteins associated with invasion. Experiments were repeated three times. (A) western blot images and (B) densitometric analysis of proteins; *P<0.05 vs. NC, **P<0.01 vs. NC. TOPO II, topoisomerase II; NF-κB, nuclear factor κB; MMP, metalloproteinase; IκB, inhibitor of κB; R-CAD, retinal cadherin; N-CAD, neural cadherin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NC, negative control; Bay117082, inhibitor of the NF-κB signaling pathway. Data for each protein were analyzed with one-way ANOVA and Dunnett's post hoc test.

cells resistant to TMZ have higher TOPO II expression (27). Downregulation of TOPO II expression by overexpression of leucine-rich repeats and immunoglobulin-like domains protein-1 in U251 cells resulted in hypersensitivity to TMZ (28). TOPO II may be a good target for glioma chemotherapy, as the strategy to interfere and generate enzyme-mediated DNA damage is proven to be effective for cancer chemotherapy (24). Results from the present study revealed that miR-9 suppressed TOPO II expression and induced apoptosis and sensitization to TMZ in glioma cells.

NF-κB serves an essential role in cancer development and is a direct target gene of miR-9 (16,18). The role of NF-κB in the regulation of chemotherapy sensitivity mediated by miR-9 has been studied. MMPs and cadherins, important for glioma metastasis, also serve vital roles in drug resistance (3). The present results demonstrated that when miR-9 was overexpressed or a signaling pathway inhibitor was applied, NF-κB expression was suppressed, and the expression levels of proteins regulated by NF-κB were also lower, including MMP-2, MMP-9, N-cadherin and R-cadherin. However, the expression of inhibitor of κB (IκB) had no significant change, which may be due to miR-9 potentially inhibiting NF-κB independently of IκB, and Bay110782 inhibiting IκB phosphorylation only. NF-κB also has strong interactions with TOPO II, as a previous study reported that some chemotherapy drugs suppressed TOPO II activity through the downregulation of NF-κB activity to achieve cell toxicity (29). TOPO II is essential for NF-κB activation in mitoxantrone-induced apoptosis (30), whereas miR-106a silencing modulated TOPO II and glutathione S-transferase π expression by inhibition of NF-κB activation and AKT expression (31). Results from the present study revealed that when the NF-κB signaling pathway was blocked, TOPO II expression was downregulated. These results provided further evidence for TMZ sensitization by miR-9, and the NF-κB signaling pathway serves an important role in that regulation.

Further elucidation of the mechanisms of tumor chemotherapy resistance may provide more precise and effective anticancer therapies. Results from the present study revealed that miR-9 enhanced chemotherapeutic sensitivity of glioma to TMZ by suppressing TOPO II via the NF-κB signaling pathway, which suggested that miR-9 may be used as a novel therapeutic target for glioma treatment.

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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

QL performed cell incubation and transfection. YC performed the western blot and PCR. LM performed the cell cycle and cell apoptosis assay. YS designed the experiment, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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