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MicroRNA-409-3p Represses Glioma Cell Invasion and Proliferation by Targeting High-Mobility Group Nucleosome-Binding Domain 5

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Emerging evidence has suggested that aberrantly expressed microRNAs (miRNAs) are associated with glioma development and progression. The aberrant expression of miR-409-3p has been reported in several human cancers. However, little is known about the function of miR-409-3p in gliomas. The aim of this study was to investigate the specific role and molecular mechanism of miR-409-3p in gliomas. In the present study, we found that miR-409-3p was downregulated in glioma tissue and cell lines. Overexpression of miR-409-3p inhibited glioma cell invasion and proliferation, whereas suppression of miR-409-3p promoted glioma cell invasion and proliferation. High-mobility group nucleosome-binding domain 5 (HMGN5), a well-known oncogene in gliomas, was identified as a functional target of miR-409-3p using bioinformatics, dual-luciferase reporter assay, real-time quantitative polymerase chain reaction, and Western blot analysis. Furthermore, miR-409-3p was found to regulate the expression of matrix metalloproteinase 2 and cyclin D1. Restoration of HMGN5 expression significantly reversed the inhibitory effects of miR-409-3p overexpression on glioma cell invasion and proliferation. Taken together, our results suggest that miR-409-3p inhibits glioma cell invasion and proliferation by targeting HMGN5, representing a potential therapeutic target for glioma.

Key words: Glioma; High-mobility group nucleosome-binding domain 5 (HMGN5); miR-409-3p; Invasion; Proliferation

INTRODUCTION

Glioma is one of the most common brain tumors and the leading cause of cancer-related death associated with the central nervous system[1,2]. Despite advances in cancer therapy over the past decades, glioma-related mortality remains high[3]. Improving the survival rate of glioma patients remains a challenge for clinical medicine. Efforts have been made to explore novel therapeutic targets in order to improve the diagnosis, prognosis, and treatment of glioma[4]. However, the molecular pathogenesis of glioma remains largely unknown.

MicroRNAs (miRNAs) are small, noncoding RNAs with a length of ~22 nucleotides, which are emerging as novel regulators of gene expression[5,6]. miRNAs inhibit translation by binding to the 3′-untranslated region (3′-UTR) of mRNAs[5,6]. Thus, miRNAs are involved in many cellular biological processes, including cell proliferation, apoptosis, differentiation, migration, and invasion[7]. A growing body of evidence has shown that aberrantly expressed miRNAs are associated with cancer development and progression[8,9]; consequently, miRNAs have been investigated as potential therapeutic targets for the development of cancer therapies[10,11]. Studies have also shown that miRNAs play an essential role in glioma development and progression[12,13]. Target-specific miRNAs represent a promising approach for inhibiting glioma cell progression[14–16]. Therefore, a better understanding of the role of miRNAs in glioma progression will provide novel biomarkers for diagnosis and prognosis, as well as for identifying novel targets for treatment.

High-mobility group nucleosome-binding domain 5 (HMGN5), also known as nucleosome-binding protein 1, has been identified as an oncogene in many human cancers[17]. HMGN5 is a ubiquitous protein that plays a key role in DNA repair, replication, recombination, and transcription[18–20]. However, dysregulation of HMGN5 is associated with tumorigenesis[17]. A high expression of HMGN5 has been reported in numerous cancer types, including

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renal cell carcinoma[21], prostate cancer[22], and bladder cancer[23]. HMGN5 participates in tumorigenesis by regulating a series of tumor-associated genes, including those that encode matrix metalloproteinase (MMP) 2/9, cyclin B1, cyclin D1, and Bcl-2[21]. Studies have also shown that HMGN5 is highly expressed in glioma tissue and is associated with glioma cell proliferation[24]. Therefore, HMGN5 may be a promising target for the treatment of glioma.

Aberrant expression of miR-409-3p has been reported in many human cancers[25,26]. A recent study suggested that miR-409-3p may be involved in glioma progression[27]. However, the clinical significance and function of miR-409-3p in glioma are not entirely clear. Thus, this study focused on investigating the association between miR-409-3p and glioma. We found that miR-409-3p was downregulated in glioma tissue and cell lines. Overexpression of miR-409-3p inhibited glioma cell invasion and proliferation. HMGN5 was identified as a functional target of miR-409-3p in glioma cells. Furthermore, miR-409-3p was found to regulate the expression of MMP2 and cyclin D1, which are associated with cancer metastasis and malignancy. Taken together, our results suggest that miR-409-3p inhibits glioma cell invasion and proliferation by targeting HMGN5. These findings provide novel insight into our understanding of the molecular pathogenesis of glioma and suggest a potential therapeutic target.

MATERIALS AND METHODS

Clinical Samples and Cell Lines

Glioma tissue samples were obtained from 20 glioma patients who underwent surgery in Tangdu Hospital. Normal brain tissue samples were obtained from patients undergoing internal decompression surgery, which served as the control samples. All samples were collected after receiving the patients' informed consent and following approval from the Institutional Human Experiment and Ethics Committee of Tangdu Hospital. Experiments were carried out in accordance with the Helsinki Declaration. Glioma cell lines (A172, SHG44, U251, and U87) and normal human astrocytes (NHAs) were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, P.R. China). Cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in a humidified incubator at 37°C with 5% CO_2.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from clinical samples and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) or mirVana™ miRNA isolation kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturers' instructions. To detect mRNA expression, cDNA was obtained using M-MLV reverse transcriptase (BioTeke, Beijing, P.R. China). To detect miRNA expression, cDNA was obtained using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems). RT-qPCR was performed with SYBR Green PCR Master Mix (Applied Biosystems) with specific primers. GAPDH served as the internal control for the normalization of mRNA expression, and U6 served as the internal control for normalization of miRNA expression. Data were analyzed using the $2^{-\Delta\Delta}$Ct method. The primer sequences were as follows: HMGN5, 5′-GGTTGTCTGCTATGCTTGTG-3′ (forward) and 5′-ACTGCTTCTTGCTTGGTTTC-3′ (reverse); cyclin D1, 5′-GCTGCGAAGTGGAAACCATC-3′ (forward) and 5′-CCTCCTTCTGCACACATTTGAA-3′ (reverse); MMP2, 5′-AGGCCAAGTGGTCCGTGTGA-3′ (forward) and 5′-TAGGTGGTGGAGCACCAGAG-3′ (reverse); GAPDH, 5′-GAAGGTGAAGGTCGGAGTC-3′ (forward) and 5′-GAAGATGGTGATGGGATTTC-3′ (reverse); miR-409-3p, 5′-GAATGTTGCTCGGTGAACCCCT-3′ (forward) and 5′-TGGTGTCGTGGAGTCG-3′ (reverse); U6, 5′-GCTTCGGCAGCACATATACTAAAAT-3′ (forward) and 5′-CGCTTCACGAATTTGCGTGTCAT-3′ (reverse).

Cell Transfection

The miR-409-3p mimics, inhibitor, and negative control (NC) were purchased from GenePharma (Shanghai, P.R. China). Cells were grown to 80% confluence prior to transfection. Transfection of the miR-409-3p mimics or inhibitor into glioma cells was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. HMGN5 was cloned into pcDNA vector (Invitrogen), and then pcDNA/HMGN5 vector was transfected into cells using Lipofectamine 2000 (Invitrogen). An empty vector served as the control. At 48 h posttransfection, transfection efficacy was measured by RT-qPCR or Western blot.

Cell Invasion Assay

The Transwell inserts were coated with 1 mg/ml Matrigel matrix (BD Biosciences, San Jose, CA, USA). After 48 h of transfection, cells (1×10^4) that had been suspended in 0.5 ml of medium without FBS were added to the inserts, and 0.5 ml of medium containing 10% FBS was added to the lower chamber. Cells were allowed to grow for 24 h at 37°C. The cells that remained on the upper surface of the membrane were discarded, and cells that had invaded the lower surface of the inserts were fixed and stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA). Cells were counted under a light microscope.

Water-Soluble Tetrazolium Salt (WST) Assay

Cells were seeded onto a 96-well plate (1×10^4 cells/well) and cultured overnight. After transfection of the miR-409-3p mimics or inhibitor for 48 h, cells were treated with 10 μl

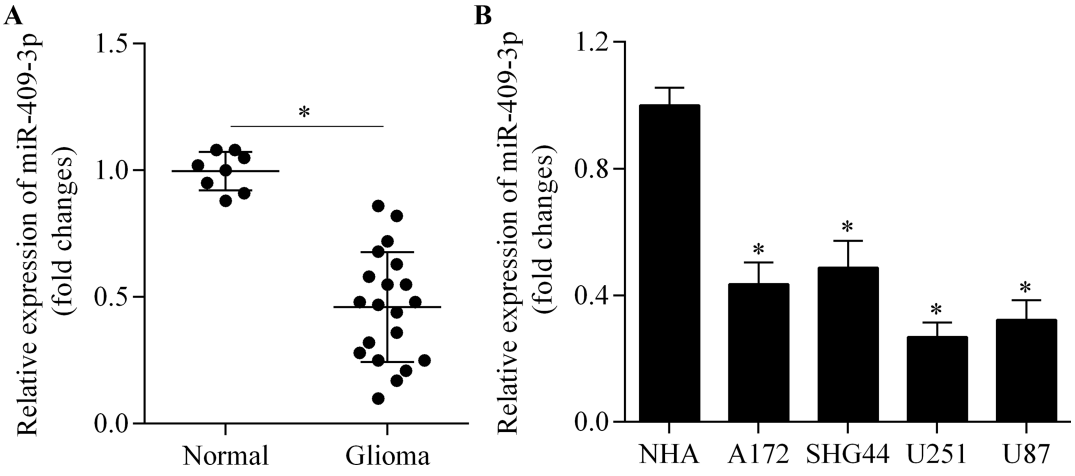

Figure 1. Expression of miR-409-3p in glioma tissue and cell lines. (A) miR-409-3p expression in glioma tissue ($n=20$) and normal brain tissue ($n=8$) samples, as determined by real-time quantitative polymerase chain reaction (RT-qPCR). *$p<0.05$. (B) miR-409-3p expression in A172, SHG44, U251, and U87 cells and normal human astrocytes (NHAs), as determined by RT-qPCR. *$p<0.05$ versus NHA.

of cell counting kit solution (Sigma-Aldrich) and incubated for 4 h. The absorbance at 450 nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

Colony Formation Assay

The transfected cells were plated into six-well plates at a density of 100 cells/well and grown in 0.4% agar medium for 14 days at 37°C. The colonies were then visualized with 0.1% crystal violet (Sigma-Aldrich) and counted under a microscope.

Cell Cycle Assay

Cells were serum starved for 24 h to synchronize the cell cycle before transfection. After 48 h of transfection,

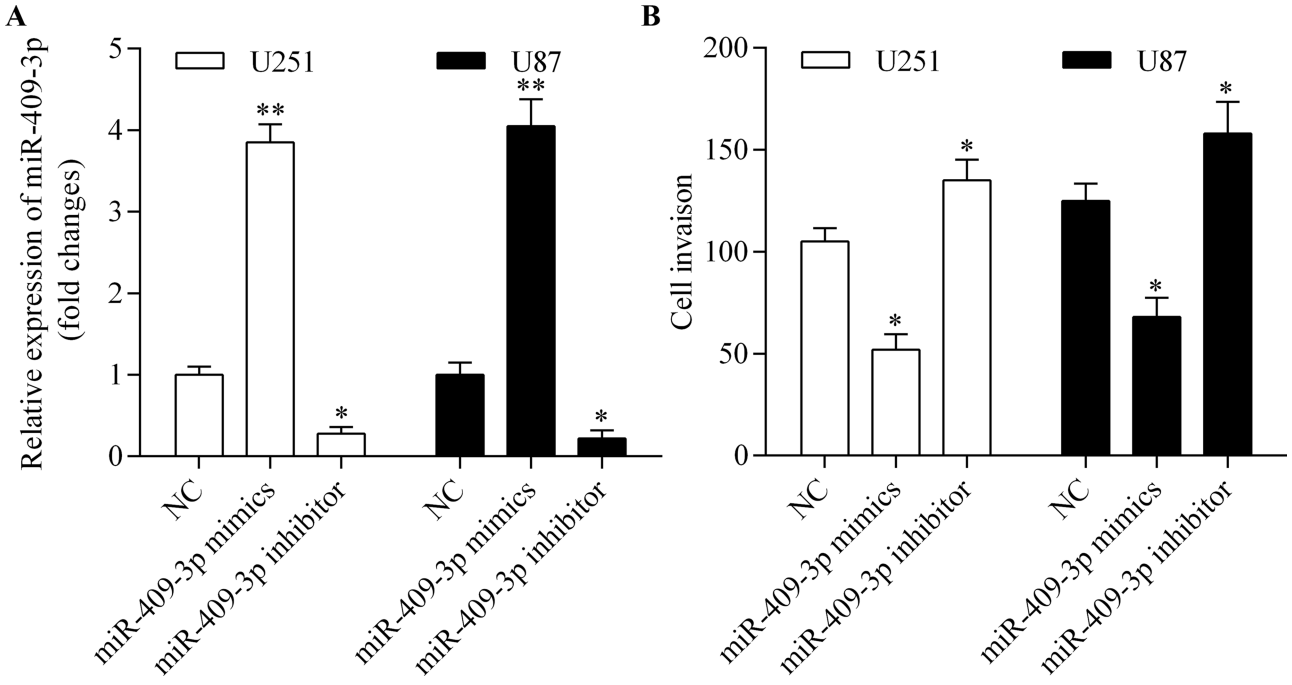

Figure 2. miR-409-3p suppresses glioma cell invasion. U251 and U87 cells were transfected with miR-409-3p mimics or inhibitor for 48 h. (A) Expression of miR-409-3p measured by RT-qPCR. (B) Cell invasion measured using a Transwell invasion assay. *$p<0.05$ and **$p<0.01$ versus negative control (NC).

cells were harvested with trypsin, washed with phosphate-buffered saline, and fixed with 70% ethanol. Afterward, cells were treated with 100 μg/ml of propidium iodide (Sigma-Aldrich) in the presence of 10 μg/ml of RNase A and incubated for 30 min in the dark. Cell distribution was then detected using a FACSCalibur flow cytometer (BD Biosciences) and analyzed with CellQuest software.

Dual-Luciferase Reporter Assays

The HMGN5 3′-UTR, containing the seed-matched or mutated sequences of miR-409-3p, was cloned into pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA). Cells were plated into 24-well plates and transfected with the miR-409-3p mimics or inhibitor, in addition to the luciferase reporter. Cells were harvested after 48 h of transfection and then detected using the Dual-GLO Luciferase Assay Kit (Promega).

Western Blot Analysis

Equivalent quantities (30 μg) of protein were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel and then transferred to a polyvinylidene fluoride membrane (Millipore, Boston, MA, USA). The membrane was blocked with 5% nonfat milk, followed by incubation with primary antibodies (anti-HMGN5 and anti-GAPDH; Abcam, Cambridge, UK) at appropriate concentrations overnight at 4°C. The membrane was then incubated with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:2,000 dilution for 1 h. Protein bands were visualized using a Pierce ECL Western Blotting Kit (Pierce, Rockford, IL, USA). The protein band intensity was determined using the Image-Pro Plus 6.0 software (Media Cybernetics Inc., Rockville, MD, USA).

Data Analysis

Results are expressed as mean ± standard deviation. Statistical analyses were performed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) with Student's *t*-test or one-way analysis of variance, followed by Bonferroni's post hoc test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Decreased Expression of miR-409-3p in Glioma Tissues and Cell Lines

To investigate miR-409-3p expression in glioma, we first detected the expression pattern of miR-409-3p in glioma tissue by RT-qPCR. miR-409-3p showed a lower expression in glioma tissue compared with normal brain tissue (Fig. 1A). We further investigated miR-409-3p expression in a series of human glioma cell lines. Consistent with the

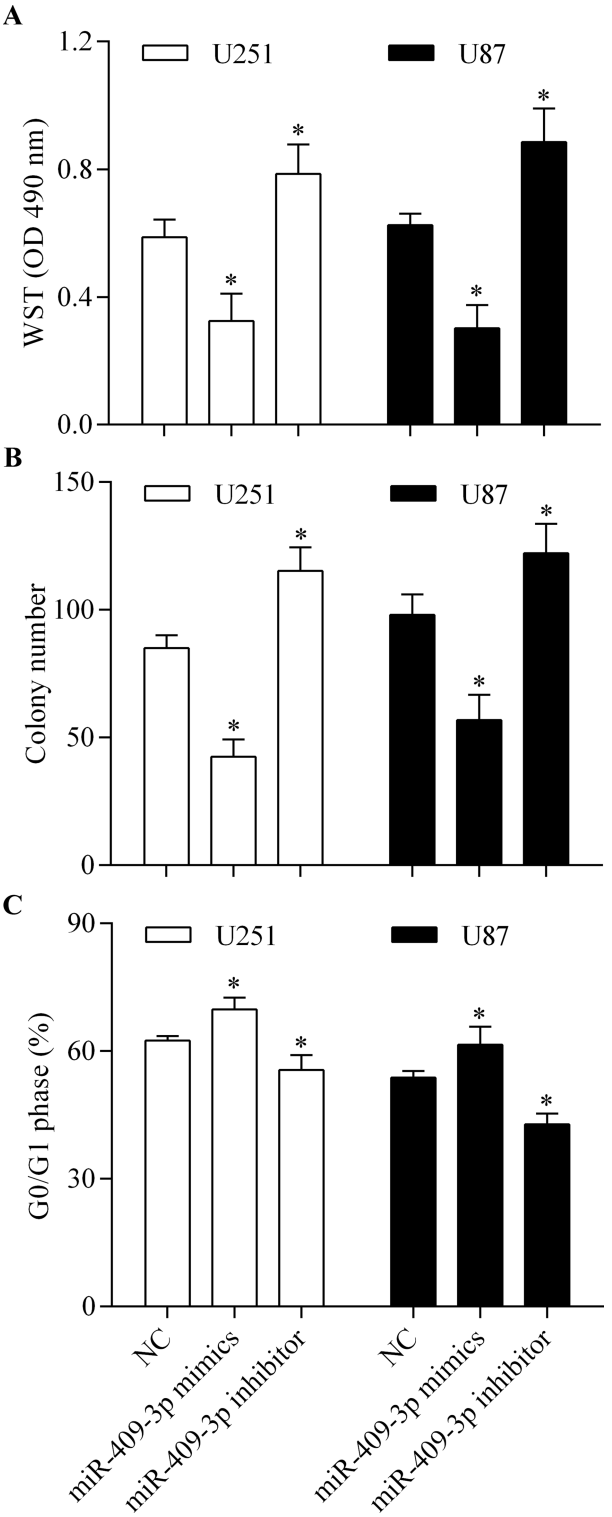

Figure 3. miR-409-3p suppresses glioma cell proliferation. U251 and U87 cells were transfected with miR-409-3p mimics or inhibitor for 48 h and then used for detection. (A) Cell proliferation detected by water-soluble tetrazolium salt (WST) assay. (B) Colony-forming capacity of glioma cells assessed using a colony formation assay. (C) Number of cells in the G_0/G_1 phase, detected by flow cytometry. *$p < 0.05$ versus NC.

results in brain tissue, miR-409-3p was significantly downregulated in the glioma cell lines compared with normal human astrocytes (Fig. 1B). Our data suggest that miR-409-3p may function as a tumor suppressor in glioma.

miR-409-3p Suppresses Glioma Cell Invasion

To investigate the antitumor activity of miR-409-3p in glioma, we performed gain-of-function and loss-of-function experiments using miR-409-3p mimics and inhibitor, respectively, in U251 and U87 cells (Fig. 2A). We then detected the role of miR-409-3p in the regulation of glioma cell invasion using a Transwell invasion assay. The results showed that overexpression of miR-409-3p significantly inhibited glioma cell invasion, while suppression of miR-409-3p promoted glioma cell invasion (Fig. 2B). These results suggest that miR-409-3p inhibits glioma cell invasion.

miR-409-3p Inhibits Glioma Cell Proliferation

To further investigate the role of miR-409-3p in glioma, we examined the role of miR-409-3p in the regulation of glioma cell proliferation. The results showed that overexpression of miR-409-3p markedly inhibited cell proliferation (Fig. 3A) and colony formation (Fig. 3B) of glioma cells and induced cell cycle arrest (Fig. 3C) in the G_0/G_1 phase. In contrast, suppression of miR-409-3p showed the opposite effects. Collectively, these data suggest that miR-409-3p suppresses glioma cell proliferation.

HMGN5 Is a Target Gene of miR-409-3p in Glioma Cells

To investigate the underlying mechanism by which miR-409-3p inhibits glioma cell invasion and proliferation, we performed bioinformatics analysis to identify potential targets of miR-409-3p. We found that HMGN5 was a putative target gene of miR-409-3p. The predicted seed-matched sequences are presented in Figure 4A. To confirm binding of miR-409-3p with the HMGN5 3′-UTR, we carried out dual-luciferase assays. The results showed that the luciferase activity of the reporter vector containing the wild-type HMGN5 3′-UTR was markedly decreased as a result of miR-409-3p overexpression (Fig. 4B). Furthermore, suppression of miR-409-3p enhanced the luciferase activity of the reporter vector containing the wild-type HMGN5 3′-UTR (Fig. 4B). However, these effects were abolished by mutation of the binding sites (Fig. 4B), indicating that miR-409-3p directly targets the 3′-UTR of HMGN5. To further confirm that HMGN5 is the target gene of miR-409-3p, we examined the regulatory effect of miR-409-3p on HMGN5. We found that overexpression of miR-409-3p significantly inhibited the mRNA (Fig. 5A and B) and protein (Fig. 5C and D) expression of HMGN5 in glioma cells, whereas suppression of miR-409-3p promoted HMGN5 expression. Overall, these results indicate that HMGN5 is a direct target gene of miR-409-3p.

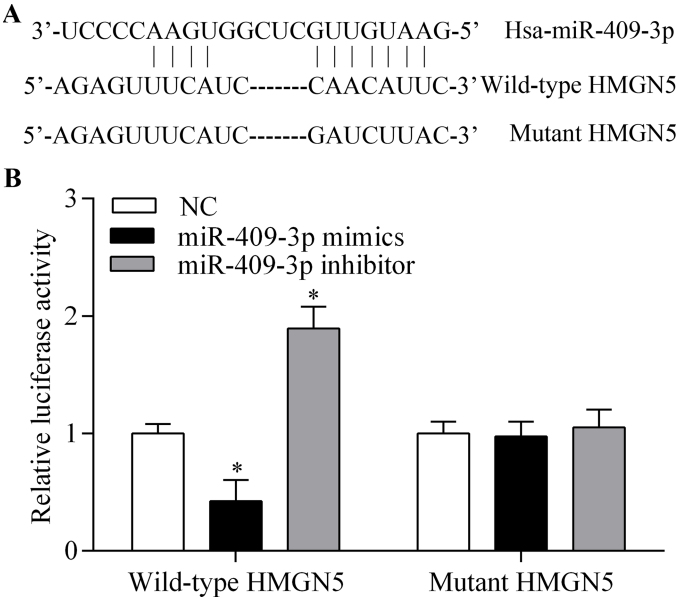

Figure 4. miR-409-3p targets the 3′-untranslated region (3′-UTR) of high-mobility group nucleosome binding domain 5 (HMGN5). (A) Schematic of the miR-409-3p-binding sites in the HMGN5 3′-UTR. (B) Dual-luciferase assays of miR-409-3p and HMGN5 3′-UTR. Wild-type or mutated 3′-UTR of HMGN5 was cloned into luciferase reporter vectors. U251 cells were transfected with miR-409-3p mimics or inhibitor in addition to the luciferase reporter vector and incubated for 48 h. *$p < 0.05$ versus NC.

miR-409-3p Inhibits the Expression of MMP2 and Cyclin D1

To further investigate the molecular basis of miR-409-3p in the regulation of glioma cell invasion and proliferation, we measured the expression of MMP2 and cyclin D1, both of which are associated with cancer cell metastasis and proliferation. The results showed that overexpression of miR-409-3p significantly suppressed the mRNA expression of MMP2 (Fig. 6A) and cyclin D1 (Fig. 6B) in glioma cells. In contrast, suppression of miR-409-3p markedly promoted the expression of MMP2 (Fig. 6A) and cyclin D1 (Fig. 6B). These results indicate that miR-409-3p inhibits glioma cell invasion and proliferation, which are associated with suppression of cyclin D1 and MMP2.

Restoration of HMGN5 Expression Abrogates Antitumor Effects of miR-409-3p

To investigate whether miR-409-3p inhibits glioma cell invasion and proliferation by targeting HMGN5, we performed rescue experiments by overexpressing HMGN5. Glioma cells were transfected with miR-409-3p mimics along with the pcDNA/HMGN5 vector without the 3′-UTR. The results showed that transfection of the pcDNA/HMGN5 vector significantly restored HMGN5 expression (Fig. 7A–D). We then detected the effect of restoring HMGN5 expression on miR-409-3p-mediated cell invasion and proliferation. The results showed that restoration of HMGN5 expression significantly reversed the inhibitory effect of miR-409-3p overexpression on glioma cell invasion (Fig. 8A) and proliferation (Fig. 8B). Taken together, these results suggest that miR-409-3p inhibits glioma cell invasion and proliferation by regulating HMGN5.

DISCUSSION

In recent years, accumulating evidence has confirmed the critical role of miRNAs in glioma[28,29]. Although the miRNA signatures in glioma have been well characterized,

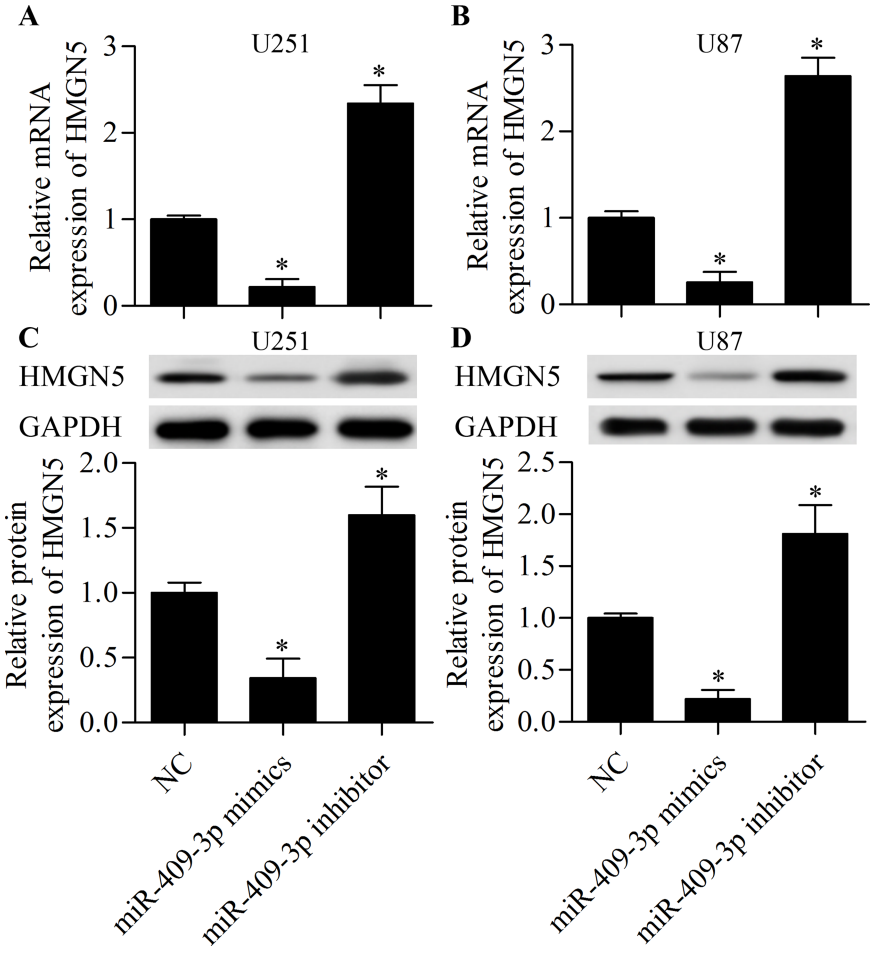

Figure 5. miR-409-3p suppresses HMGN5 expression. U251 and U87 cells were transfected with miR-409-3p mimics or inhibitor for 48 h prior to detection. The mRNA expression of HMGN5 in U251 (A) and U87 (B) cells as detected by RT-qPCR. The protein expression of HMGN5 in U251 (C) and U87 (D) cells as detected by Western blot. *$p < 0.05$ versus NC.

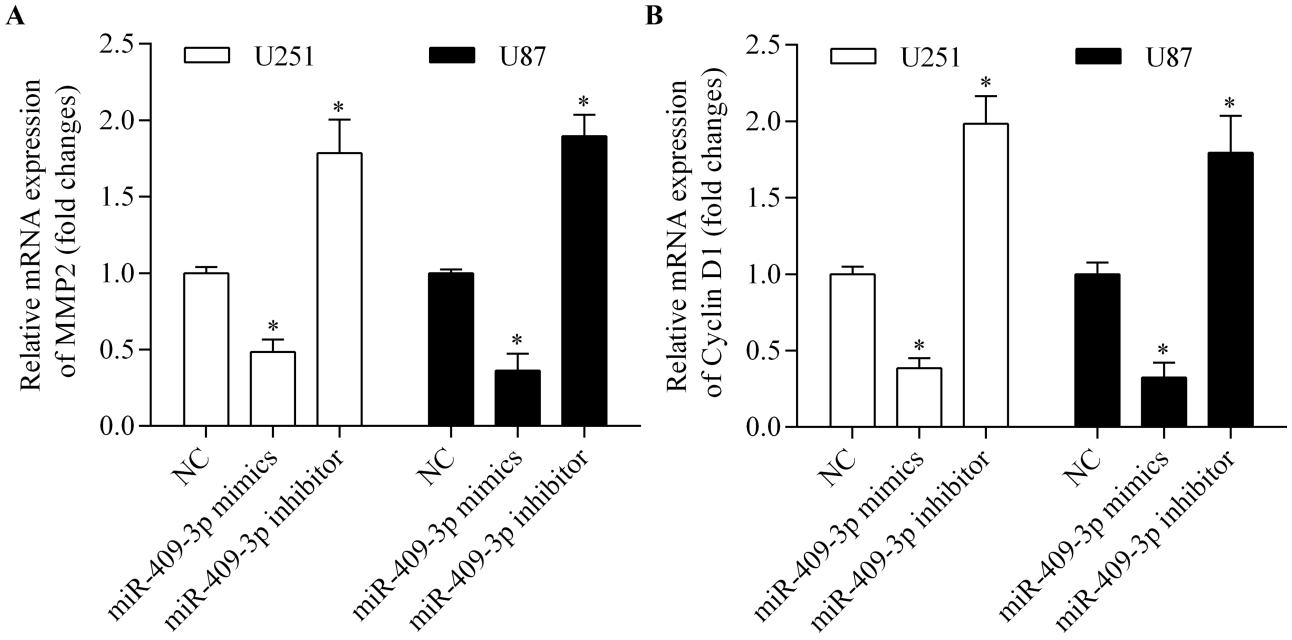

Figure 6. miR-409-3p inhibits the expression of matrix metalloproteinase 2 (MMP2) and cyclin D1. U251 and U87 cells were transfected with miR-409-3p mimics or inhibitor for 48 h prior to detection. The mRNA expression of MMP2 (A) and cyclin D1 (B) in cells as detected by RT-qPCR. $*p<0.05$ versus NC.

the role of dysregulated miRNAs on glioma progression and development remains largely unknown. A recent study reported that miR-409-3p expression may predict the expression of O^6-methylguanine-DNA methyltransferase in glioma patients undergoing alkylating chemotherapy[27]. However, the clinical significance and function of miR-409-3p in glioma were not entirely clear. In the present study, we identified miR-409-3p as a novel dysregulated miRNA in glioma. We found that miR-409-3p was decreased in glioma tissue and cell lines, and overexpression of miR-409-3p inhibited glioma cell invasion and proliferation, indicating a tumor suppressor role of miR-409-3p in glioma.

Previous studies have shown that miR-409-3p is dysregulated in many types of human cancers, where it participates in tumor development and progression[25,26]. Low expression of miR-409-3p has been reported in breast cancer specimens, associated with an advanced TNM stage, lymph node metastasis, and poorer pathological differentiation[30]. Venkatadri et al. reported that miR-409-3p functions as an apoptosis-related miRNA in resveratrol-induced breast cancer cell death[31]. Other studies have shown that miR-409-3p inhibits breast cancer cell growth and metastasis by targeting zinc-finger E-box-binding homeobox 1[26] and Akt1[32]. The expression of miR-409-3p was found to be downregulated in colorectal cancer tissue, and overexpression of miR-409-3p suppresses cell proliferation, migration, and invasion of colorectal cancer cells by targeting GRB2-associated-binding protein 1[25] and nemo-like kinase[33]. Moreover, miR-409-3p sensitizes colon cancer cells to oxaliplatin-induced cell death by suppressing Beclin-1-mediated autophagy[34]. It has also been reported that miR-409-3p inhibits gastric cancer cell growth and metastasis by targeting PHD-finger protein 10[35] and radixin[36] and that overexpression of miR-409-3p suppresses osteosarcoma cell migration and invasion by inhibiting catenin-δ1[37]. Furthermore, miR-409-3p inhibits the progression and development of fibrosarcoma[38], lung adenocarcinoma[39], and bladder cancer[40] by targeting different oncogenes. These reports suggest that miR-409-3p functions as a tumor suppressor; however, an oncogenic role of miR-409-3p has also been reported. Josson et al. reported a high expression of miR-409-3p in human prostate cancer tissue and bone metastatic prostate cancer cell lines[41], and that overexpression of miR-409-3p promotes tumorigenesis and metastasis of prostate cancer[41]. Thus, the precise role of miR-409-3p needs to be further investigated. In the current study, we observed a low expression pattern of miR-409-3p in glioma tissue and cell lines. Overexpression of miR-409-3p suppressed glioma cell invasion and proliferation, supporting a tumor-suppressor role for miR-409-3p.

In this study, we identified HMGN5 as a potential target gene of miR-409-3p in glioma cells. HMGN5 is a well-known oncogene, recognized in many types of cancers[17]. The silencing of HMGN5 in prostate cancer cells has been shown to suppress cell proliferation and

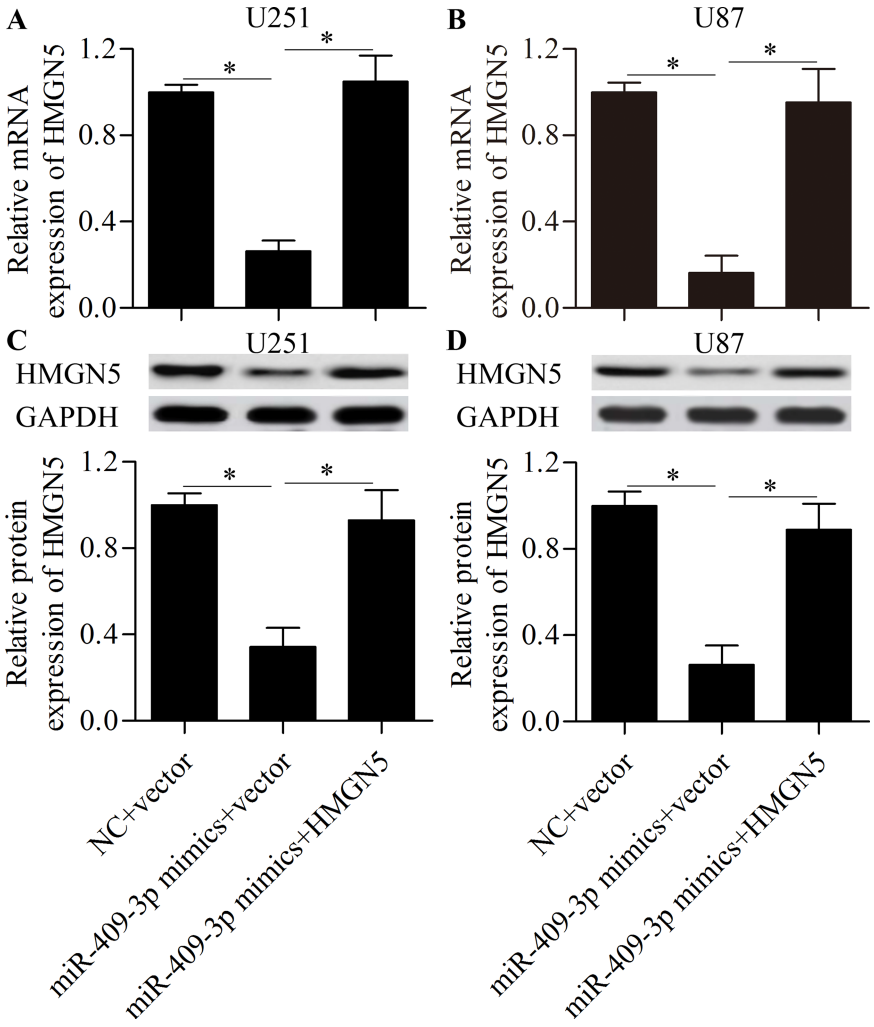

Figure 7. Restoration of HMGN5 expression in miR-409-3p-transfected glioma cells. U251 and U87 cells were transfected with miR-409-3p mimics along with the pcDNA/HMGN5 vector without the 3′-UTR and incubated for 48 h. The mRNA expression of HMGN5 in U251 (A) and U87 (B) cells as detected by RT-qPCR. The protein expression of HMGN5 in U251 (C) and U87 (D) cells as detected by Western blot. *$p<0.05$.

promote cell apoptosis[22,42]. Furthermore, a high expression of HMGN5 has been reported in clear cell renal cell carcinoma tissue, and knockdown of HMGN5 was found to induce cell cycle arrest and apoptosis, also inhibiting cell invasion[21]. HMGN5 is highly expressed in breast cancer and promotes breast cancer cell proliferation and invasion[43]. A high expression of HMGN5 has also been reported in bladder cancer[23,44], osteosarcoma[45], and lung cancer[38], also promoting tumorigenesis through cell proliferation and metastasis. The molecular basis of HMGN5 in tumorigenesis is associated with the regulation of various genes, including cyclin B1, cyclin D1, Bcl-2, MMP2/9, and vascular endothelial growth factor, which regulates cell cycle, proliferation, apoptosis, metastasis, and angiogenesis[21–23,42,44,45]. Moreover, HMGN5 promotes chemotherapy and radiotherapy resistance of prostate cancer[46,47] and osteosarcoma[48] cells. A high expression of HMGN5 has been reported in glioma tissue[24], and silencing of HMGN5 was found to induce cell cycle arrest in the G_0/G_1 phase, in addition to delaying cell proliferation and promoting apoptosis of glioma cells[24], indicating an oncogenic role for HMGN5 in glioma. In this study, we identified HMGN5 as a direct target gene of miR-409-3p in glioma cells. Suppression of HMGN5 by overexpression of miR-409-3p markedly repressed the invasion and proliferation of glioma cells, associated with the inhibition of cyclin D1 and MMP2 expression. Our results suggest that miR-409-3p/HMGN5 plays an important role in glioma tumorigenesis.

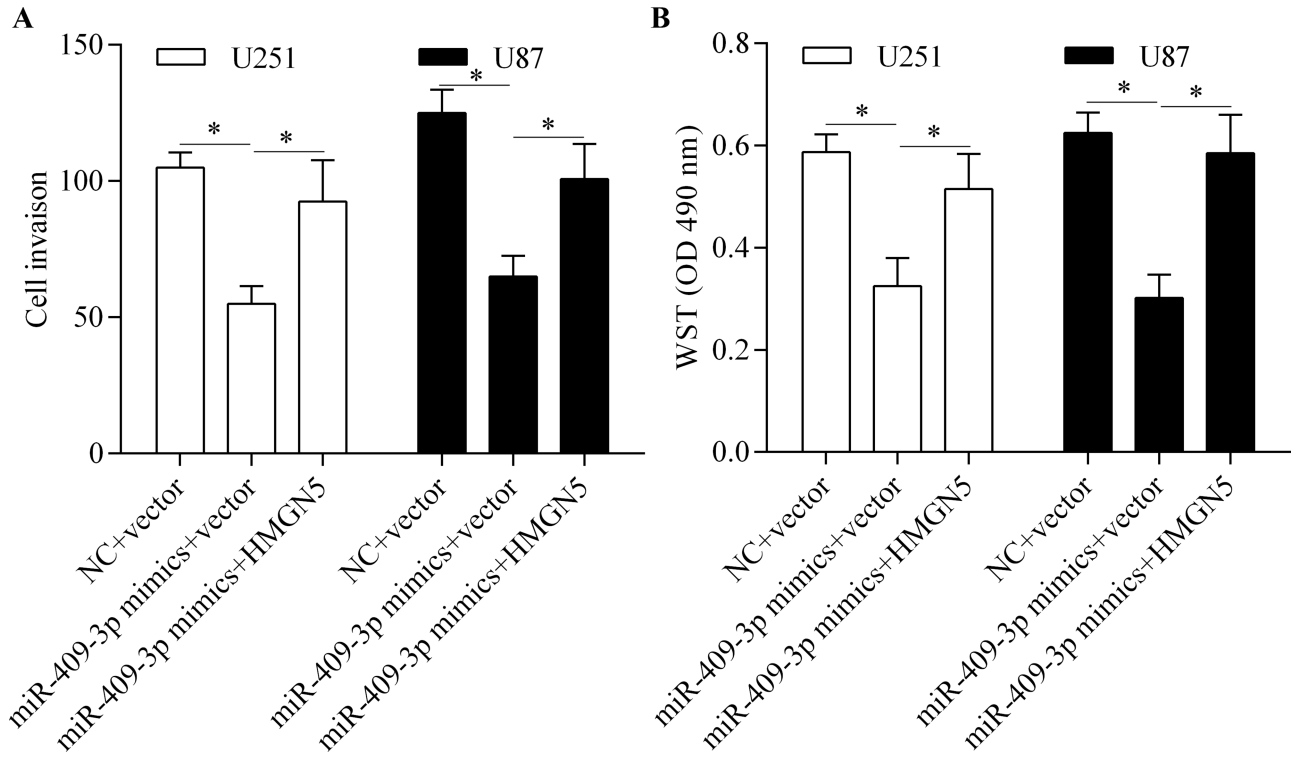

Figure 8. Restoration of HMGN5 expression abrogates antitumor effects of miR-409-3p. U251 and U87 cells were transfected with miR-409-3p mimics along with the pcDNA/HMGN5 vector without the 3′-UTR and incubated for 48 h. (A) Cell invasion detected by the Transwell assay. (B) Cell proliferation assessed by the WST assay. $*p < 0.05$.

HMGN5 has been found to be regulated by several miRNAs, including miR-326[49], miR-340[50], and miR-186[51], and suppression of HMGN5 by these miRNAs significantly represses the proliferation and metastasis of cancer cells[49–51]. These studies suggest that HMGN5-related tumor progression is epigenetically regulated by miRNAs. However, it was still unclear whether HMGN5 undergoes epigenetic regulation by miRNAs in glioma. Our study revealed that miR-409-3p specifically targeted and modulated HMGN5 in glioma cells, suggesting that this miRNA is a novel inhibitor of HMGN5. Therefore, targeting HMGN5 by miR-409-3p may show promise for the treatment of glioma.

Overall, our study reported a tumor-suppressor role for miR-409-3p in glioma. We observed decreased miR-409-3p expression in glioma, and overexpression of miR-409-3p repressed glioma cell invasion and proliferation by targeting HMGN5, an oncogene associated with glioma[24]. These findings add to our understanding of the molecular pathogenesis of glioma and suggest a potential therapeutic approach for the treatment of glioma by inhibiting HMGN5 with miR-409-3p.

ACKNOWLEDGMENT: The authors declare no conflicts of interest.

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