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Overexpression of MicroRNA-216a Suppresses Proliferation, Migration, and Invasion of Glioma Cells by Targeting Leucine-Rich Repeat-Containing G Protein-Coupled Receptor 5

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Increasing studies have suggested that microRNAs (miRNAs) are involved in the development of gliomas. MicroRNA-216a has been reported to be a tumor-associated miRNA in many types of cancer, either as an oncogene or as a tumor suppressor. However, little is known about the function of miR-216a in gliomas. The present study was designed to explore the potential role of miR-216a in gliomas. We found that miR-216a was significantly decreased in glioma tissues and cell lines. Overexpression of miR-216a significantly suppressed the proliferation, migration, and invasion of glioma cells. Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) was identified as a target gene of miR-216a in glioma cells by bioinformatics analysis, dual-luciferase reporter assay, real-time quantitative polymerase chain reaction, and Western blot analysis. Moreover, miR-216a overexpression inhibited the Wnt/β-catenin signaling pathway. The restoration of LGR5 expression markedly reversed the antitumor effect of miR-216a in glioma cells. Taken together, these findings suggest a tumor suppressor role for miR-216a in gliomas, which inhibits glioma cell proliferation, migration, and invasion by targeting LGR5. Our study suggests that miR-216a may serve as a potential therapeutic target for future glioma treatment.

Key words: Glioma; Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5); Proliferation; Migration; Invasion

INTRODUCTION

Gliomas are the most prevalent and malignant brain tumor with an increasing incidence worldwide[1]. The diagnosis and prognosis of glioma remain poor[2]. Although treatment approaches have made certain progress, overall survival has not effectively improved[3]. Glioma remains a refractory disease because of the unrestricted proliferation and extensive metastasis of tumors[4]. Therefore, an improved understanding of the molecular pathogenesis of glioma is essential for the development of effective treatment.

MicroRNAs (miRNAs), a group of small, endogenous, and noncoding RNAs (~22 nucleotides in length), play a vital role in various life activities and diseases[5]. miRNAs negatively modulate gene expression on a transcription level by targeting the 3′-untranslated region (3′-UTR) of mRNA[6,7]. Many studies have shown that miRNAs regulate various cellular processes, including cell proliferation, differentiation, migration, invasion, and apoptosis[8]. Aberrantly expressed miRNAs in cancer development and progression have been suggested as oncogenes or tumor suppressors[9,10]. Emerging evidence has suggested that miRNAs are promising targets for cancer treatment[11]. In recent years, increasing studies have reported that miRNAs participate in glioma tumorigenesis[12]. Targeting specific miRNAs inhibits the tumorigenesis of glioma cells[13,14]. Thus, a better understanding of miRNAs in glioma may provide novel insights for treatment development.

The leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5), also known as G-protein coupled

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receptor 49, has been recognized as an oncogene in several types of cancers[15,16]. LGR5 is identified as an orphan G protein-coupled receptor encompassing a seven-transmembrane domain and an extracellular domain with 17 leucine-rich repeats[17]. LGR5 is a somatic stem cell marker and plays an important role during development[18,19]. LGR5 is highly expressed during embryonic development, but its expression is restricted in stem cells in postnatal tissues[20,21]. However, aberrantly expressed LGR5 is associated with tumorigenesis[15,16]. LGR5 is correlated with the clinical grade of glioma, and depletion of LGR5 promotes apoptosis of brain cancer stem-like cells[22]. Silencing of LGR5 inhibits the proliferation of glioma cells[23]. Several studies have shown that LGR5 is an important regulator of the Wnt/β-catenin signaling pathway, a critical oncogenic signaling pathway in cancers[24]. LGR5 may therefore represent a potential therapeutic target for glioma.

Several studies have shown that miR-216a is involved in tumorigenesis in some types of human cancers such as pancreatic cancer[25], oral squamous cell carcinoma[26], and liver cancer[27]. However, little is known about the function of miR-216a in glioma. In the present study, we demonstrated an important role for miR-216a in glioma. We found that miR-216a was significantly decreased in glioma tissues and cell lines. Overexpression of miR-216a significantly suppressed the proliferation, migration, and invasion of glioma cells. Interestingly, LGR5 was identified as a target gene of miR-216a in glioma cells. Moreover, miR-216a overexpression inhibited the Wnt/β-catenin signaling pathway in glioma cells. In addition, the restoration of LGR5 expression markedly reverses the antitumor effect of miR-216a in glioma cells. Taken together, these findings suggest a tumor suppressor role for miR-216a in glioma, which inhibits glioma cell proliferation, migration, and invasion by targeting LGR5. Our study suggests that miR-216a/LGR5 may play an important role in the progression of glioma, serving as a potential therapeutic target for future glioma treatment.

MATERIALS AND METHODS

Human Tissue Samples

Human glioma tissue samples were collected from 15 patients who underwent surgical resection in The No.1 Hospital of Xi'an. Normal brain tissue samples were collected from five patients who underwent traumatic brain injuries. All patients who participated in this study signed an informed consent prior to sample collection. The experiment was approved by the Institutional Human Experiment and Ethics Committee of The No.1 Hospital of Xi'an and was performed in accordance with the Helsinki Declaration.

Cell Lines

Human glioma cell lines (U251MG, U87MG, U118, and A172) and normal human astrocytes (NHA) were purchased from Type Culture Collection of the Chinese Academy of Sciences (Shanghai, P.R. China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin mix (Sigma-Aldrich, St. Louis, MO, USA) and were incubated in 5% CO_2 at 37°C.

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

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Dusseldorf, Germany) and reverse transcribed into cDNA using M-MLV reverse transcriptase (TaKaRa, Dalian, P.R. China) and the miRcute miRNA cDNA kit (TIANGEN, Beijing, P.R. China) according to the manufacturers' instructions. RT-qPCR was carried out with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and the appropriate primers: miR-216a, 5′-ATCCAGTGCGTGTCGTG-3′ (forward) and 5′-TGCTTAATCTCAGCTGGCA-3′ (reverse); U6, 5′-GCTTCGGCAGCACATATACTAAAAT-3′ (forward) and 5′-CGCTTCACGAATTTGCGTGTCAT-3′ (reverse); LGR5, 5′-GAGGATCTGGTGAGCCTGAGAA-3′ (forward) and 5′-CATAAGTGATGCTGGAGCTGGTAA-3′ (reverse); cyclin D1, 5′-AACTACCTGGACCGCTTC CT-3′ (forward) and 5′-CCACTTGAGCTTGTTCAC CA-3′ (reverse); c-myc, 5′-TCAAGAGGTGCCACGTCT CC-3′ (forward) and 5′-TCTTGGCAGCAGGATAGTC CTT-3′ (reverse); GAPDH, 5′-GAAGGTGAAGGTCG GAGTC-3′ (forward) and 5′-GAAGATGGTGATGGG ATTTC-3′ (reverse). U6 and GAPDH served as internal controls. Relative gene expression was calculated using the $2^{-\Delta\Delta}$Ct method.

Cell Transfection

The miR-216a mimics and negative control (NC) were purchased from GenePharma (Shanghai, P.R. China). The cDNA of LGR5 without 3′-UTR was cloned into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA) to generate the pcDNA3.1-LGR5 vector. Cells were allowed to grow to 80% cell confluence before transfection. Cell transfection of miRNAs or the vector was conducted using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocols. The transfection efficacy was measured by RT-qPCR or Western blot after 48 h of transfection.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay

Cells were plated into 96-well plates at a density of 1×10^4 cells/well. After transfection of miR-216a mimics

for 48 h, cells were incubated with 5 mg/ml MTT (Sigma-Aldrich; 20 µl/well) for 4 h. Thereafter, the medium was discarded from the wells by aspiration, and the formazan crystals in living cells were dissolved by 200 µl/well dimethyl sulfoxide (DMSO; Sigma-Aldrich). The absorbance value at 490 nm was measured with an enzyme-linked immunosorbent assay (ELISA) plate reader (Bio-Rad, Hercules, CA, USA).

Colony Formation Assay

Cells were transfected with miR-216a mimics for 48 h and then seeded into six-well plates (1,000 cells/well) in 0.4% agar medium for 2 weeks. Colonies were stained with 0.1% crystal violet (Sigma-Aldrich) and counted under a microscope (Olympus, Tokyo, Japan).

Cell Cycle Assay

The cell cycle was synchronized by serum starvation for 24 h and then transfected with miR-216a mimics for 48 h. Cells were collected with trypsin, washed with ice-cold phosphate-buffered saline, and fixed with 70% ethanol at 4°C overnight. Afterward, cells were treated with 50 µg/ml RNase A for 30 min at room temperature and then treated with 100 µg/ml of propidium iodide (Sigma-Aldrich) for 30 min in the dark. Cell cycle distribution was detected by a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA), and the data were analyzed by CellQuest software.

Cell Migration and Invasion Assays

For the migration assay, transfected cells (1×10^4 cells) were suspended in 0.5 ml of serum-free medium and seeded into the upper chambers of 24-well Transwell plates (Corning Inc., Corning, NY, USA). The lower chambers were filled with 0.5 ml of growth medium containing 10% FBS. Cells were cultured at 37°C and allowed to migrate for 24 h. After migration, cells in the top chambers were removed by a cotton swab, and the cells in the bottom chambers were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet (Sigma-Aldrich). The stained cells were counted under a microscope (Olympus). For the invasion assay, a similar protocol was followed except that the top chamber of the Transwell plate was precoated with Matrigel (BD Biosciences).

Dual-Luciferase Reporter Assay

The cDNA fragment of LGR5 3′-UTR harboring the seed-matched sequences or mutated sequences of miR-216a was inserted into pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA). Cells were cotransfected with miR-216a mimics and pmirGLO luciferase reporter for 48 h. Then cell lysates were harvested, and luciferase activity was measured by the Dual-Luciferase Reporter Assay System (Promega).

Western Blot Analysis

Equivalent amounts of protein (50 µg) were separated by 10% sodium dodecyl sulfate polyacrylamide gels and transferred to a nitrocellulose membrane (Millipore, Boston, MA, USA). The membrane was blocked with 3% nonfat milk and then probed with primary antibodies (anti-LGR5, anti-β-catenin, and anti-GAPDH; Abcam, Cambridge, UK) with the recommended concentrations at 4°C overnight. Horseradish peroxidase-conjugated secondary antibody (1:2,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was then used to probe the membrane for 1 h at room temperature. Blots were developed with the Pierce ECL Western Blotting Kit (Pierce, Rockford, IL, USA). The intensity of the membrane was analyzed by Image-Pro Plus 6.0 software.

Data Analysis

Data were expressed in the form of means ± standard deviation. SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Comparisons between the two groups ($n=2$) were performed using the Student's *t*-test. Comparisons among multiple groups ($n\geq3$) were performed using one-way analysis of variance followed by the Bonferroni test. Values of $p<0.05$ were regarded as statistically significant.

RESULTS

Expression of miR-216a Is Decreased in Glioma Tissues and Cell Lines

To investigate the potential role of miR-216a in glioma, we first detected its expression profile in glioma tissue specimens by RT-qPCR. The data showed that miR-216a expression in glioma tissues was much lower than that in normal brain tissues (Fig. 1A). We then examined the expression of miR-216a in human glioma cell lines. We found that miR-216a expression was significantly downregulated in glioma cell lines including U251MG, U87MG, U118, and A172 compared with NHA (Fig. 1B). These results suggest a tumor-suppressive role for miR-216a in glioma.

Overexpression of miR-216a Suppresses the Proliferation of Glioma Cells

To investigate the exact biological role of miR-216a in glioma cells, we overexpressed miR-216a in U251MG and U87MG cells by transfection of miR-216a mimics and detected its effect on glioma cell proliferation. The expression of miR-216a was significantly upregulated by transfection of miR-216a mimics (Fig. 2A). The MTT assay showed that overexpression of miR-216a

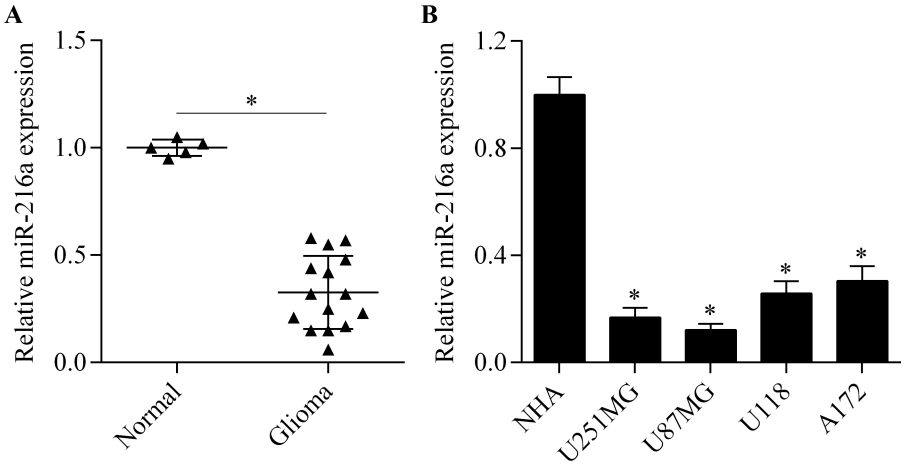

Figure 1. Decreased expression of miR-216a in glioma. (A) Real-time quantitative polymerase chain reaction (RT-qPCR) analysis of miR-216a expression in human glioma specimens and normal brain tissues. *$p<0.05$. (B) RT-qPCR analysis of miR-216a expression in human glioma cell lines (U251MG, U87MG, U118, and A172) and normal human astrocytes (NHA). *$p<0.05$ versus NHA.

significantly inhibited glioma cell proliferation (Fig. 2B). The colony-forming capacity of glioma cells was also markedly repressed by miR-216a overexpression, as detected by the colony formation assay (Fig. 2C). Moreover, overexpression of miR-216a induced cell cycle arrest in the G_0/G_1 phase (Fig. 2D). Taken together, these results suggest that miR-216a functions as a tumor suppressor by inhibiting glioma cell proliferation.

Overexpression of miR-216a Inhibits the Migration and Invasion of Glioma Cells

To further investigate the antitumor effect of miR-216a in glioma cells, we detected the effect of miR-216a overexpression of cell migration and invasion by Transwell assays. The results showed that both the migration (Fig. 3A) and invasion (Fig. 3B) abilities of glioma cells were significantly reduced by miR-216a overexpression. These data indicate that miR-216a suppresses glioma cell migration and invasion.

miR-216a Directly Targets LGR5 in Glioma Cells

To investigate the underlying mechanism of miR-216a in regulating glioma cell proliferation, migration, and invasion, we adopted bioinformatics analysis to predict the potential targets of miR-216a in glioma cells. We found that the 3′-UTR of LGR5, an oncogene of glioma[23], contains binding sites of miR-216a (Fig. 4A). To investigate whether miR-216a directly binds to the 3′-UTR of LGR5, we constructed a luciferase reporter vector containing the 3′-UTR of LGR5. We found that the luciferase activity in the luciferase reporter vector containing the 3′-UTR of LGR5 was significantly decreased by miR-216a overexpression (Fig. 4B and C). However, the luciferase activity in the luciferase reporter vector containing the mutant 3′-UTR of LGR5 was not obviously affected by miR-216a overexpression (Fig. 4B and C). Furthermore, both mRNA (Fig. 5A) and protein (Fig. 5B) levels were significantly decreased upon miR-216a overexpression. Overall, these results suggest that LGR5 is a direct target gene of miR-216a in glioma cells.

Overexpression of miR-216a Inhibits the Wnt/β-Catenin Signaling Pathway

To further elucidate the molecular basis of miR-216a in regulating glioma cell proliferation, migration, and invasion, we detected the effect of miR-216a on the Wnt/β-catenin signaling pathway. The results showed that overexpression of miR-216a significantly inhibited the protein expression of β-catenin (Fig. 6A). Moreover, the transcription of target genes of the Wnt/β-catenin signaling pathway, including cyclin D1 (Fig. 6B) and c-myc (Fig. 6C), was also significantly reduced by miR-216a overexpression. Overall, these results suggest that the antitumor effect of miR-216a is associated with suppression of the Wnt/β-catenin signaling pathway.

Overexpression of LGR5 Abolishes the Antitumor Effects of miR-216a

To confirm that LGR5 contributes to miR-216a-induced antitumor effects, we performed a rescue assay. U251MG and U87MG cells were cotransfected with miR-216a mimics and LGR5-expressing vector. The results showed that transfection with the LGR5-expressing vector significantly restored LGR5 expression in U251MG- and U87MG-miR-216a-transfected cells (Fig. 7A and B). The restoration of LGR5 expression significantly reversed the inhibitor effects of miR-216a overexpression on glioma cell proliferation (Fig. 8A), migration (Fig. 8B), and

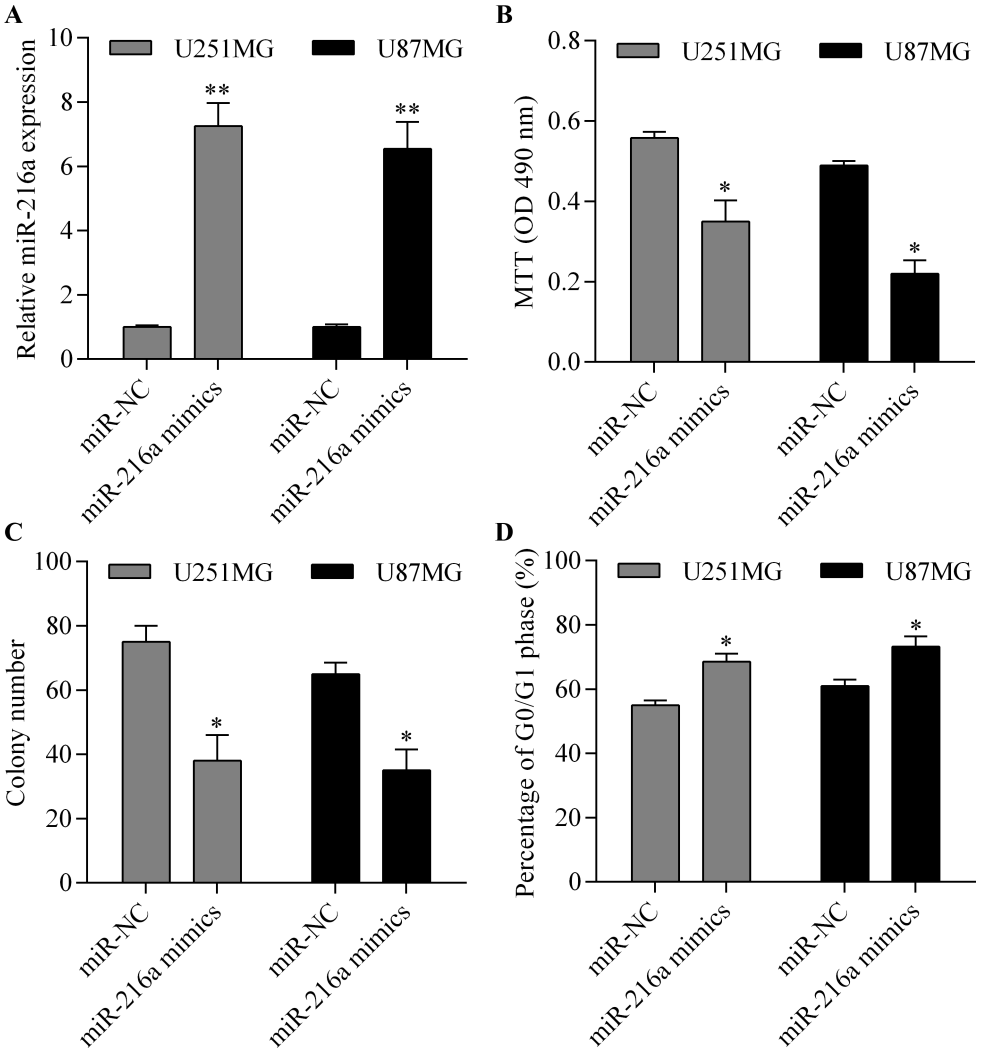

Figure 2. miR-216a suppresses glioma cell proliferation. U251MG and U87MG cells were transfected with miR-216a mimics or miR-NC for 48 h and then subjected to the following detections: (A) the expression of miR-216a in U251MG and U87MG cells was examined by RT-qPCR analysis; (B) the cell proliferation of U251MG and U87MG cells was assessed by the MTT assay; (C) the colony-forming capacity of U251MG and U87MG cells was evaluated by the colony formation assay; (D) the cell cycle in the G_0/G_1 phase of U251MG and U87MG cells was detected by flow cytometry. *$p<0.05$; **$p<0.01$.

invasion (Fig. 8C). Overall, these results suggest that miR-216a inhibits glioma cell proliferation, migration, and invasion through suppression of LGR5.

DISCUSSION

A growing body of evidence suggests the importance of miRNAs in glioma[28,29]. However, the precise function of dysregulated miRNAs in glioma progression and development is still poorly understood. In this study, we report a tumor-suppressive role for miR-216a in glioma cells. We found that miR-216a was decreased in glioma tissues and cell lines and that overexpression of miR-216a inhibited glioma cell proliferation and invasion. Our study suggests that dysregulated miR-216a expression contributes to glioma tumorigenesis.

miR-216a, a member of the miR-216 family, is widely expressed in many species[30]. It has been reported to play a critical role in the pathogenesis of kidney disorders[31], pancreatitis[32], diabetic nephropathy[33], and endothelial dysfunction[34]. Interestingly, the role of miR-216a in tumorigenesis has also been extensively studied. The expression of miR-216a is decreased in pancreatic cancer[35,36], and decreased expression of miR-261a in feces may serve as a biomarker for pancreatic cancer[37]. Overexpression of miR-216a inhibits the tumor growth of pancreatic cancer through targeting Janus kinase 2[25,38]. Moreover,

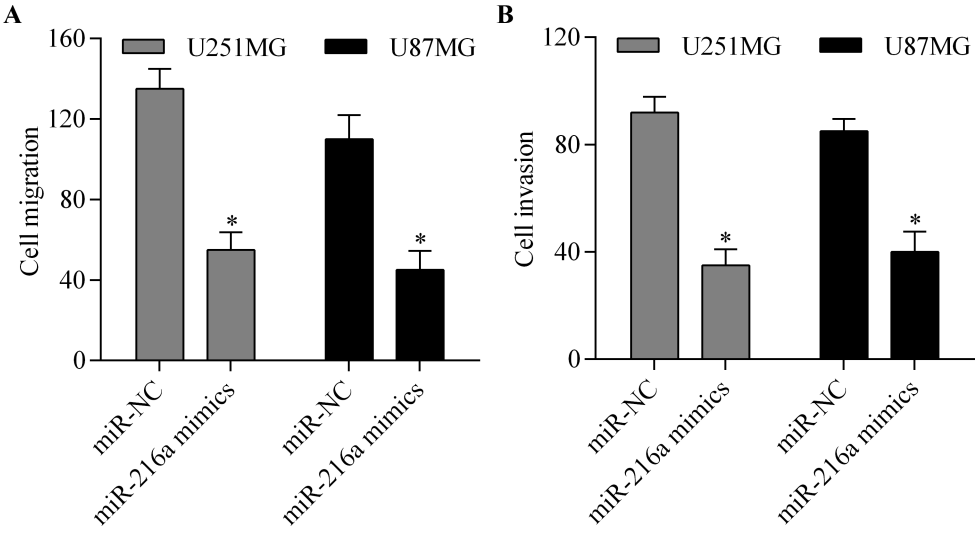

Figure 3. miR-216a suppresses glioma cell migration and invasion. The migration (A) and invasion (B) of U251MG and U87MG cells were determined by Transwell assays. U251MG and U87MG cells transfected with miR-216a mimics or miR-NC for 48 h were subjected to Transwell assays. After being cultured for 24 h in Transwell plates, the migrated or invaded cells were stained and counted under a microscope. $*p<0.05$.

miR-261a promotes the radiosensitivity of pancreatic cancer cells through suppression of Beclin-1-mediated autophagy[39]. Li et al. reported that miR-216a suppressed the growth, migration, and invasion of oral squamous cell carcinoma cells by targeting eukaryotic translation initiation factor 4B[26]. miR-216a may inhibit the tumorigenesis and angiogenesis of breast cancer cells by targeting the 3′-UTR of CD44[30]. All of these reports demonstrate a tumor suppressive role for miR-216a. However, an oncogenic role for miR-216a has also been found in other cancers. The increased expression of miR-216a induced by the androgen pathway inhibits the tumor suppression in the lung cancer-1 gene in early hepatocarcinogenesis[40]. miR-216a induces the epithelial–mesenchymal transition of hepatocellular carcinoma cells through inhibiting the phosphatase and tensin homolog and mothers against

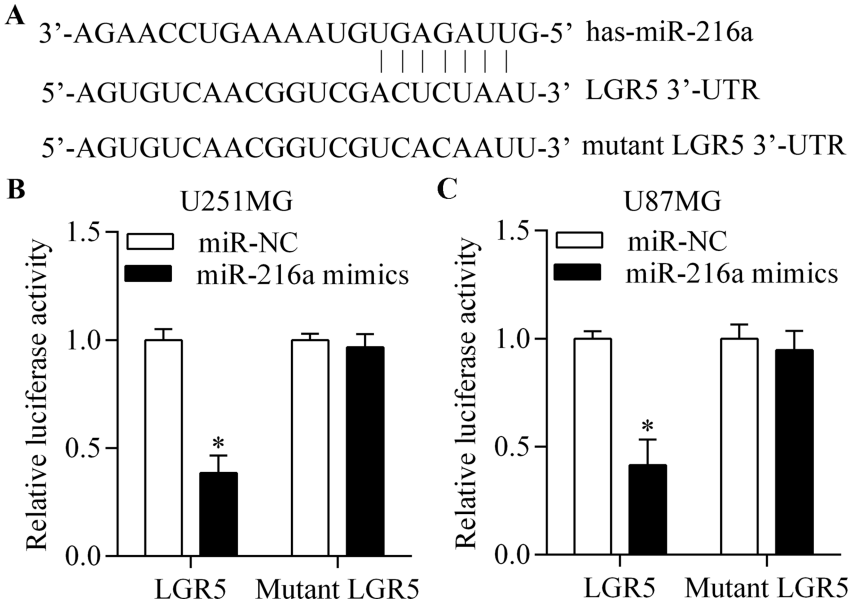

Figure 4. miR-216a targets the LGR5 3′-untranslated region (3′-UTR). (A) Predicted miR-216a target sequences in 3′-UTR of LGR5. Dual-luciferase reporter assay in U251MG (B) and U87MG (C) cells cotransfected with miR-216a mimics and luciferase reporters containing either the predicted miR-216a target sites in LGR5 3′-UTR or its corresponding mutant form. $*p<0.05$.

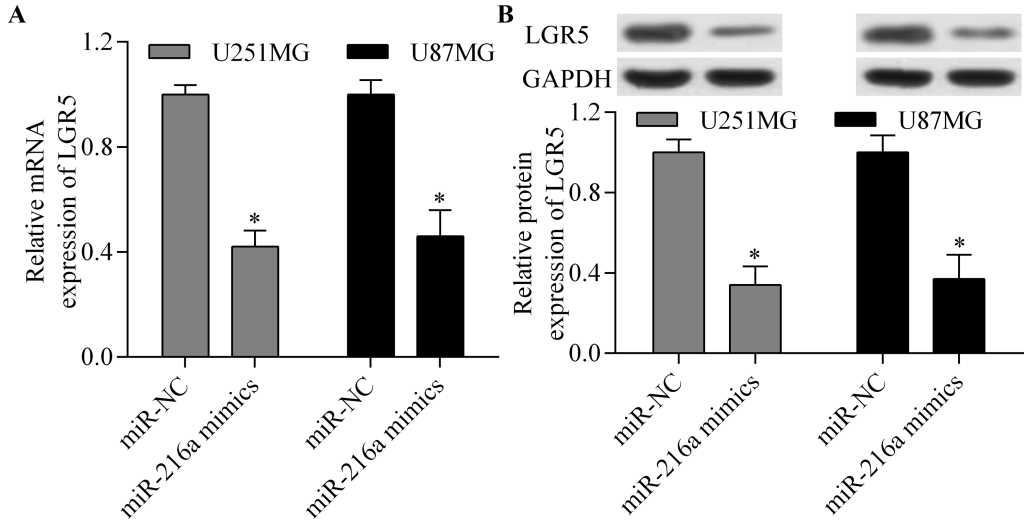

Figure 5. miR-216a inhibits LGR5 expression. U251MG and U87MG cells were transfected with miR-216a mimics or miR-NC for 48 h and then subjected to detection. (A) The mRNA expression of LGR5 was detected by RT-qPCR. (B) The protein expression of LGR5 was examined by Western blot analysis. *$p<0.05$.

decapentaplegic homolog 7[27]. Overexpression of miR-216a suppresses bicalutamide-mediated growth suppression in prostate cancer cells[41]. In our study, we found that miR-216a was decreased in glioma, and overexpression of miR-216a inhibits glioma cell proliferation and invasion, supporting a tumor-suppressive role for miR-216a. However, the precise role of miR-216a in tumorigenesis needs to be further studied.

LGR5, an orphan G protein-coupled receptor, has been suggested as a molecular marker for self-renewing stem cells[18,19]. The dysregulation of LGR5 has been found in numerous cancers, including gastric cancer, hepatocellular carcinoma, and colorectal cancer[15,16,42,43]. Increased expression of LGR5 is correlated with lymphatic invasion and poor patient survival[15,44,45]. LGR5 promotes tumor formation and cell proliferation in basal cell carcinoma cells[46]. LGR5 also plays a role in glioma. High expression of LGR5 is correlated with the clinical grade of glioma, and depletion of LGR5 promotes apoptosis of brain cancer stem-like cells[22]. LGR5 is regulated by the proneural factor, which maintains the stem-like cells in glioma[47]. LGR5 is highly expressed in glioma tissues and cell lines, and silencing of LGR5 inhibits the proliferation of glioma cells in vivo and in vitro[23,48]. LGR5 induced by

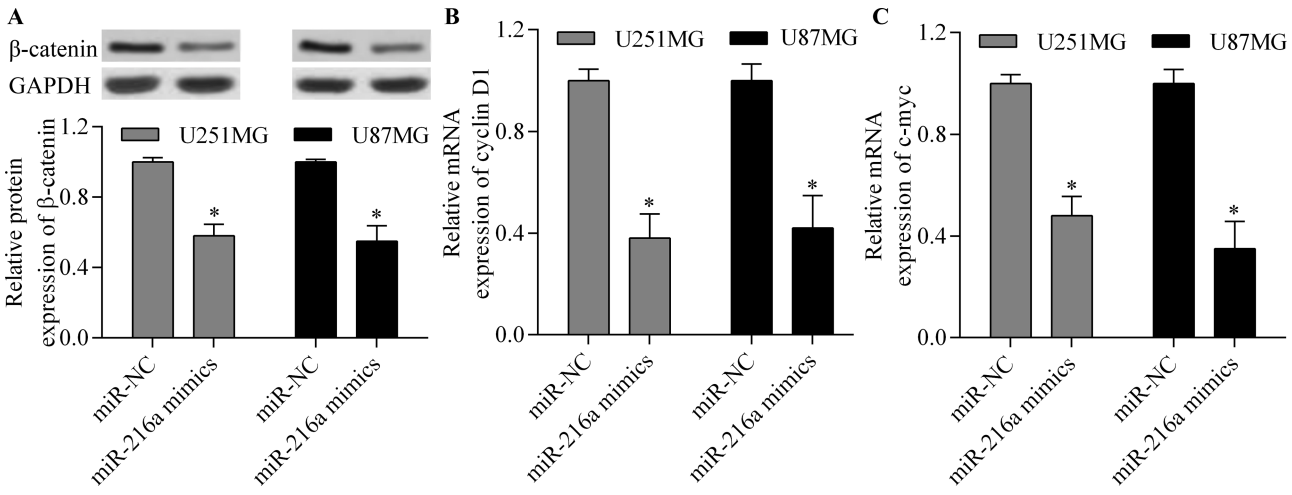

Figure 6. miR-216a inhibits the Wnt/β-catenin signaling pathway. U251MG and U87MG cells were transfected with miR-216a mimics or miR-NC for 48 h and then subjected to analysis. (A) Protein expression of β-catenin was detected by Western blot assay. The mRNA expression of cyclin D1 (B) and c-myc (C) was detected by RT-qPCR. *$p<0.05$.

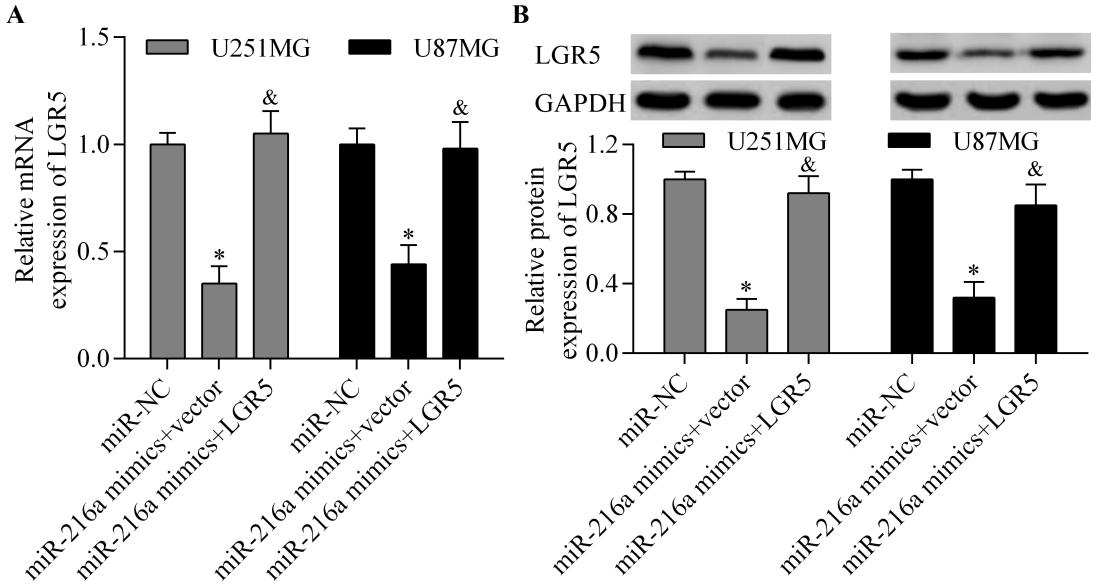

Figure 7. Restoration of LGR5 in glioma cells. U251MG and U87MG cells were cotransfected with miR-216a mimics and the pcDNA3.1-LGR5 vector and incubated for 48 h. (A) The mRNA expression of LGR5 was detected by RT-qPCR. (B) The protein expression of LGR5 was examined by Western blot analysis. *$p<0.05$ versus miR-NC. &$p<0.05$ versus miR-216a mimics + vector.

SOX9 contributes to the proliferation and tumorigenicity of glioma cells[49]. All of these findings suggest that LGR5 is a novel and potential therapeutic target for glioma. In this study, we demonstrated that LGR5 is a target gene of miR-216a. The decreased expression of miR-216a may contribute to the high expression of LGR5 in glioma cells. We also showed that inhibition of LGR5 by miR-216a overexpression significantly suppressed the proliferation and invasion of glioma cells. Our study indicates that inhibition of LGR5 by miR-216a may be a promising strategy for the treatment of glioma.

LGR5 has been suggested to be an important regulator of the Wnt/β-catenin signaling pathway, a critical oncogenic signaling pathway in cancers[24]. R-spondins have been identified as ligands for LGR5, and their engagement can activate the Wnt/β-catenin signaling

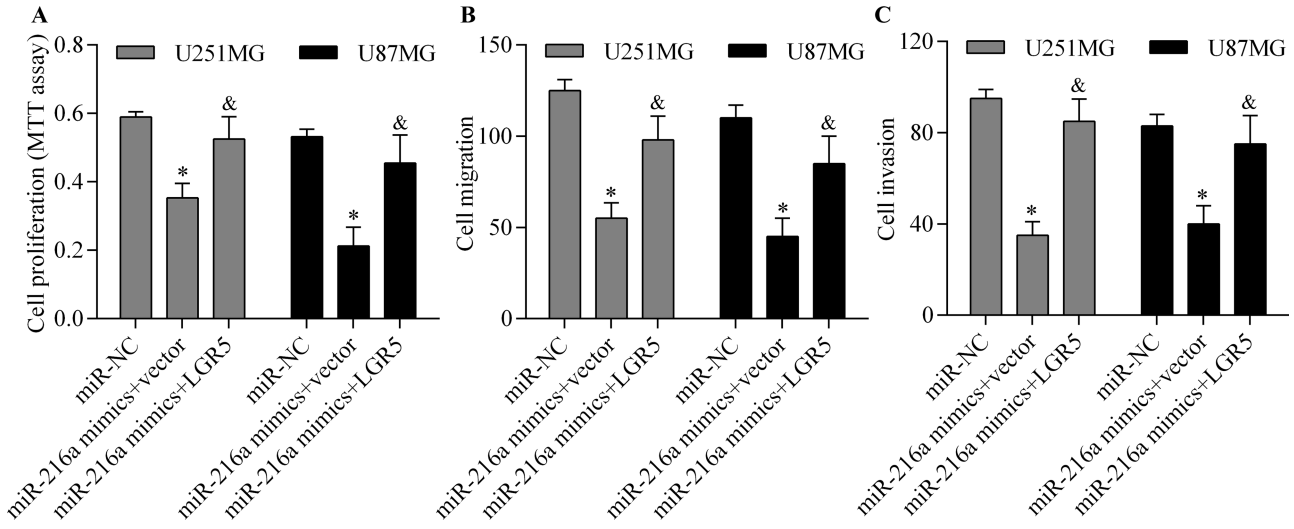

Figure 8. Restoration of LGR5 abolishes the antitumor effects of miR-216a. U251MG and U87MG cells were cotransfected with miR-216a mimics and the pcDNA3.1-LGR5 vector for 48 h and then subjected to the following detection. (A) Cell proliferation was detected by MTT assay. Cell migration (B) and invasion (C) were determined by Transwell assays. *$p<0.05$ versus miR-NC. &$p<0.05$ versus miR-216a mimics + vector.

pathway[19,50]. LGR5 promotes cancer cell progression by activation of the Wnt/β-catenin signaling pathway in neuroblastoma[51], hepatocellular carcinoma[43], colon cancer[52], and breast cancer[53]. In this study, we showed that inhibition of LGR5 by miR-216a reduced β-catenin protein levels as well as the transcription of cyclin D1 and c-myc. In line with our findings, a recent study reported that suppression of LGR5 by trichosanthin inhibits the proliferation of glioma cells by suppression of the Wnt/β-catenin signaling pathway[54]. These findings confirm that LGR5 promotes the tumorigenicity of glioma cells associated with the activation of the Wnt/β-catenin signaling pathway.

Evidence has reported that LGR5 is targeted by miR-142-3p and miR-100 in colon cancer cells[55,56], indicating that an epigenetic regulation of LGR5 contributes to tumorigenesis. In this study, we identified that miR-216a was a novel miRNA that targeted and inhibited LGR5 expression. LGR5 epigenetically regulated by miR-216a may contribute to the pathogenesis of glioma. Targeting LGR5 by miR-216a may represent a novel strategy for glioma treatment.

In conclusion, our study reports a tumor suppressive role for miR-216a in glioma. We showed that miR-216a inhibited glioma cell proliferation and invasion by targeting LGR5. miR-216a/LGR5 may play an important role in the pathogenesis of glioma and may serve as potential therapeutic targets for glioma treatment.

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