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MiR-449b-5p inhibits human glioblastoma cell proliferation by inactivating WNT2B/Wnt/β-catenin signaling pathway

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Abstract. – OBJECTIVE: As the most common primary brain cancer in adults, glioblastoma shows an extremely poor prognosis. Glioblastoma-associated deaths account for approximately 3%-4% of all malignancy-associated deaths. Numerous microRNAs (miRNAs) play important roles in the occurrence and progression of solid tumors. Herein, identifying functional miRNAs and the central molecular mechanisms would provide novel proofs for the development of targeted cancer therapies. In this study, we described the role of miR-449b-5p in restraining ontogenesis and progression of glioblastoma.

PATIENTS AND METHODS: Human glioblastoma tissues were provided by our hospital. Human U251 glioblastoma cells were infected with lentivirus induced miR-449b-5p mimics or miR-449b-5p siRNA. Real-time qPCR was carried out to determine miRNA expression. Tumor spheres formation, MTT assay, and BrdU cell proliferation assay were used to evaluate the growth ability of U251 cells. Western blot assay was performed to measure protein expression. ChIP was used to detect the capacity of β-catenin to recruit its downstream genes. Dual-Luciferase assay was conducted to detect the ability of miR-449b-5p to regulate the 3'UTR (untranslated regions) of WNT2B. TOP/FOP ratio was used to evaluate the activity of Wnt/β-catenin signaling pathway.

RESULTS: Down-regulation of miR-449b-5p expression was found in both human glioblastoma tissues and cell lines, which was negatively associated with the clinical stages. Up-regulation of miR-449b-5p inhibited tumor spheres formation, cell viability and proliferation ability of glioblastoma cells. The expression levels of WNT2B and nuclear β-catenin were negatively associated with miR-449b-5p levels in glioblastoma cells. MiR-449b-5p inhibited Wnt/β-catenin signaling by targeting WNT2B.

CONCLUSIONS: MiR-449b-5p acts as a tumor suppressor and retards the oncogenesis of glioblastoma, which is achieved via inactivation of Wnt/β-catenin signaling by directly targeting WNT2B.

Key Words:
MiR-449b-5p, WNT2B, Proliferation, Wnt/β-catenin.

Abbreviations

FBS = fetal bovine serum; qRT-PCR = RNA extraction reverse transcription quantitative real-time; MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMEM = Dulbecco's Modified Eagle's Medium; SDS-PAGE = dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis.

Introduction

As a most prevalent primary brain malignancy in adults, glioblastoma multiforme (GBM) is a fatal and the most aggressive form of astrocytoma among the adult brain tumors, which is characterized by rapid progression and particularly poor prognosis. Regardless of the aggressive treatments, such as maximal surgical exeresis, radiation therapy, and chemotherapy, GBM keeps constantly lethal, and presents a median life span of 12-15 months. As a consequence, GBM-associated deaths account for approximately 3%-4% of all malignancy-associated deaths[1-5].

Due to its aggressive nature and the availability of tissues for molecular examination, GBM is one of the first cancers to be characterized by the Cancer Genome Atlas[6]. Studies have repor-

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ted that GBM progression is related to disordered gene regulatory networks[7]. Other than abnormal gene expression in GBM, many reports have confirmed the functions of non-coding RNAs (ncRNAs), characterized by no protein-coding ability due to the lack of an open reading frame, in GBM progression[8]. Among these ncRNAs, microRNAs (miRNAs) are the single strand RNAs with 22 nucleotides in length and have been demonstrated to down-regulate various protein-encoding target genes through binding to the 3' untranslated region (UTR), thus act as the negative gene regulators at the post-transcriptional level[9,10].

Growing evidences[11,12] have revealed that miRNAs exert important functions in different biological processes. Aberrant miRNAs have been detected in various malignancies including breast cancer, bladder cancer, gastric cancer, liver cancer, lung cancer, prostate cancer, and glioblastoma. MiRNAs powerfully regulate many tumorigenic progressions, such as proliferation, differentiation, apoptosis, angiogenesis and invasion in cells[13-15]. Therefore, the miRNAs have currently been considered as potential treatment targets.

MiR-449b-5p has been revealed to be a cancer-related miRNA, which plays a central role in several cancers[16-19]. However, little is identified about the function of miR-449b-5p in GBM. In this study, we investigated the expression level of miR-449b-5p in GBM tissues and cell lines, also revealing its biological function and mechanism in GBM cells.

Patients and Methods

Tissue Samples and Ethics Statement

The Sixth Affiliated Hospital of Guangzhou Medical University provided the glioblastoma patient tissues. Normal brain tissues were obtained from car accident victims without any pre-existing diseases.

This investigation was approved by the Human Research Ethics Committee of the Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan City People's Hospital. This study was performed in accordance with the principles of the Declaration of Helsinki. Each participant provided written informed consent.

Cells and Culture Conditions

The normal human astrocytes (NHAs) and the human glioblastoma cell lines (U87, SNB19, LN444, U251, U118, A172) were bought from American Type Culture Collection (ATCC, Manassas, VA, USA). NHAs were cultured under the condition as the manufacturer instructed. Glioblastoma cells were routinely cultured in DMEM medium (Invitrogen, Carlsbad, CA, USA) in the presence of 10% FBS (HyClone, South Logan, UT, USA).

RNA Separation and Reverse ranscription Quantitative-PCR (RT-qPCR) Assay

Total RNAs were separated from cells using the TRIzol reagent (Invitrogen, Rockville, MD, USA) following the instructions of the manufacturer. The cDNA was reverse transcribed with 2 µg of total RNA from each sample, using the Primescript RT reagent kit (TaKaRa Bio Inc, Otsu, Shiga, Japan). The mRNA expression level was detected using SYBR Green PCR Kit (TaKaRa Bio Inc, Otsu, Shiga, Japan).

The primers are: c-Myc, forward, TCAAGAGGCGAACACACAAC, and reverse, GGCCTTTTCATTGTTTTCCA; Nanog, forward, GATTTGTGGGCCTGAAGAAA, and reverse, ATGGAGGAGGGAAGAGGAGA; FGF4, forward, CTCTGGGTGGCTCACCAAAC, and reverse, TGGAGGTCAAGGCCACAATC; OCT4, forward, GTGGAGGAAGCTGACAACAA, and reverse, GGTTCTCGATACTGGTTCGC; CCND1, forward, TCCTCTCCAAAATGCCAGAG, and reverse, GGCGGATTGGAAATGAACTT. RNA expression level was normalized by the geometric mean of the housekeeping gene GAPDH (forward, 5'-GACTCATGACC ACAGTCCATGC-3', and reverse, 5'-AGAGGCAGGGATGATGTT CTG-3') to control the variability and calculated as $2^{-[(Ct^{of\ target}) - (Ct^{of\ GAPDH})]}$, where the Ct represented the threshold cycle for each transcript.

Reverse transcription and quantification of miR-449b-5p were performed with the RiboBio miRNA kit (RiboBio, Guangzhou, China). The Ct was used to calculate the expression level of miR-449b-5p. The relative miR-449b-5p expression was calculated by 2-[(Ct value of miR-449b-5p) – (Ct value of U6)] after normalized by the internal reference expression of U6 small nuclear RNA.

Sphere Formation Examination

Cells were cultured in a 6-well (1×10^3 cells/well) or a 24-well (about 10 cells/well) ultra low cluster plate (Corning, Corning, NY, USA) for 13 days. Spheres were grown in serum-free DMEM/F12 medium (Gibco, Grand Island, NY, USA) supplied with 2% B27 (Gibco, Grand Island, NY, USA), 20 ng/ml of basic fibroblast growth fac-

tor (bFGF; PeproTech, Offenbach, Germany), 20 ng/ml of epidermal growth factor (EGF; PeproTech, Offenbach, Germany), 5 µg/ml insulin (Sigma-Aldrich, St. Louis, MO, USA), and 0.4% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA).

MTT Assay

Cell viabilities were assessed by MTT assay. The 3-(4, 5-dimetrylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was got from Sigma-Aldrich (St. Louis, MO, USA). In brief, wild type, miR-449b-5p over-expressed or silenced U251 glioblastoma cells (1×10^5 cells/ml) were seeded in 96-well plates and incubated overnight. At the indicated time, the optical density (OD) values were measured at a wavelength of 570 nm.

BrdU Cell Proliferation Assay

5-Bromo-2-deoxy Uridine (BrdU) cell proliferation assay kit (Kit #6813) was obtained from Cell Signaling Technology (Danvers, MA, USA). The 1×wash buffer was made via dilution of the 20×wash buffer using purified water; 1×detection antibody solution was made by dilution of the BrdU detection antibody 1:100 using the detection antibody diluent (green); 1× HRP-conjugated secondary antibody solution was made via dilution of the anti-mouse IgG and HRP-linked antibody (1:100) using HRP-linked antibody diluent (red); 10× BrdU solution was made by dilution of BrdU (1:100) using cell culture medium. Incorporation of BrdU: the wild type, the miR-449b-5p over-expressed or the silenced U251 glioblastoma cells were seeded in the 96-well cell culture plates (5000 cells/ml) and incubated overnight for attachment. Prepared 10× BrdU solution was added into each well to a final 1× concentration and incubated for 4 hours in an incubator at 37°C with 5% CO_2. After the medium was removed, the Fixing/Denaturing solution was added into the plate (100 µl/well) and incubated for 30 min at room temperature. The solution was removed and 100 µl of 1× detection antibody solution was added in each well, incubated for 1 hour at room temperature. Removed the solution and washed the plates for 3 times using 1×wash buffer. A 1× HRP-conjugated secondary antibody solution was added into the wells (100µl/well), incubated for 30 min at room temperature. The solution was removed and the plates were washed 3 times using 1× Wash Buffer, then 100 µl of TMB substrate was added, incubated at room temperature for 30 min and added 100 µl stop solution. The images and numbers of the BrdU positive cells were obtained under the fluorescence microscope.

Western Blotting Analysis

Wild type, miR-449b-5p over-expressed or silenced U251 glioblastoma cells were collected and rinsed using pre-cold PBS. The cell lysis buffer (CST, Danvers, MA, USA) was added and kept on ice for 30 min to isolate the total proteins. The concentrations of the proteins were measured by bicinchoninic acid (BCA) assay. Equal amount protein (10-20 µg) samples were denatured, applied to SDS-PAGE and transferred onto the polyvinylidene difluoride (PVDF) membranes for identification of the protein expression levels. Five percent of non-fat dry milk was dissolved in washing buffer (0.05% Tween-20 TBST) and used to block proteins on the PVDF membranes at room temperature. One hour later, the membranes were incubated with the specific primary antibody for 24 hours at 4°C. The primary antibodies used were as follows: β-catenin (ab32572), p84 (ab487) and WNT2B (ab50575) (Abcam, Cambridge, UK). The membranes were washed in TBST washing buffer for 3 times, 10 minutes/time. Then, the membranes were incubated with the horseradish peroxidase-conjugated secondary antibody. The membranes were also washed in TBST washing buffer for 3 times. Proteins were then visualized by ECL reagents, which were bought from Thermo Fisher Scientific Inc (Waltham, MA, USA).

TOP/FOP Flash Reporter Examination

The pRL-TK plasmid (10 ng/well) together with FOP flash plasmid or TOP flash plasmid (200 ng/well) were co-transfected into the wild type, miR-449b-5p overexpressed or silenced U251 glioblastoma cells. The luminescence intensity was evaluated with a Dual-Luciferase Reporter Assay System (Promega Corporation, Madison, WI, USA) after 36-48 h. The ratio between the activities of TOP Flash and FOP Flash was calculated.

Cytosolic/Nuclear Protein Extraction

Nuclear/Cytosol Fractionation Kit (BioVision, Milpitas, CA, USA) was used to separate the nuclear and cytosolic proteins of wild type, miR-449b-5p overexpressed or silenced U251 glioblastoma cells following the instructions. Protein expression in each fraction was evaluated by Western blotting assay. GAPDH and nuclear protein

p84 were acted as the cytosolic and nuclear markers respectively.

ChIP Assay

ChIP assay was performed as follows. In brief, wild type, miR-449b-5p over-expressed or silenced U251 glioblastoma cells were crosslinked for 10 min with 1% formaldehyde followed by quenching in glycine (125 mM). The nuclei pellet was dissolved in the lysis buffer with 0.1% SDS to obtain the fragments of 200-600 bp by sonication. Protein G magnetic beads and ChIP grade β-catenin antibody were applied for analysis. IgG protein was used as the negative control. RT-qPCR was used to confirm the enrichment.

Statistical Analysis

SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses of all data, which were presented as mean ± standard deviation. To estimate the statistical significance of data differences between groups, a 2-tailed Student's *t*-test was applied; ANOVA was performed using the data analysis tools provided by the software. $p<0.05$ was indicated a statistical significance.

Results

MiR-449b-5p Expression Was Down-Regulated in Human Glioblastoma

To identify whether miR-449b-5p is essential during the pathogenesis of glioblastoma, miR-449b-5p expression was compared between the 115 GBM tissues and the 26 normal brain tissues by RT-qPCR. It was identified that miR-449b-5p expression was significantly down-regulated in the GBM tissues than the normal brain tissues (Figure 1A). Furthermore, the down-regulated miR-449b-5p expression in human glioblastoma tissues was confirmed in different clinical stages of glioblastoma tissues, which showed a correlation with the grade of clinical stage, the higher grade of clinical stage showed the lower miR-449b-5p expression level ($p< 0.05$, Figure 1B). In the meantime, down-regulated miR-449b-5p expression was also confirmed in 6 different glioblastoma cell lines (U87, SNB19, LN444, U251, U118, A172) than the normal human astrocytes (NHA) (Figure 1C). Together, these data indicated that miR-449b-5p showed a down-regulation, which might be associated with human glioblastoma development.

MiR-449b-5p Inhibited the Growth of U251 Human Glioblastoma Cells

As shown in Figure 1, miR-449b-5p expression was down-regulated in human glioblastoma tissues. We supposed that miR-449b-5p may be associated with the pathogenesis of glioblastoma. RT-qPCR confirmed that miR-449b-5p expression was either successfully over-expressed or knocked down in U251 human glioblastoma cells induced by lentivirus infection (Figure 2A). Furthermore, miR-449b-5p over-expression greatly weakened the tumor spheres formation (approximately three-fold fewer with a ~2-4-fold

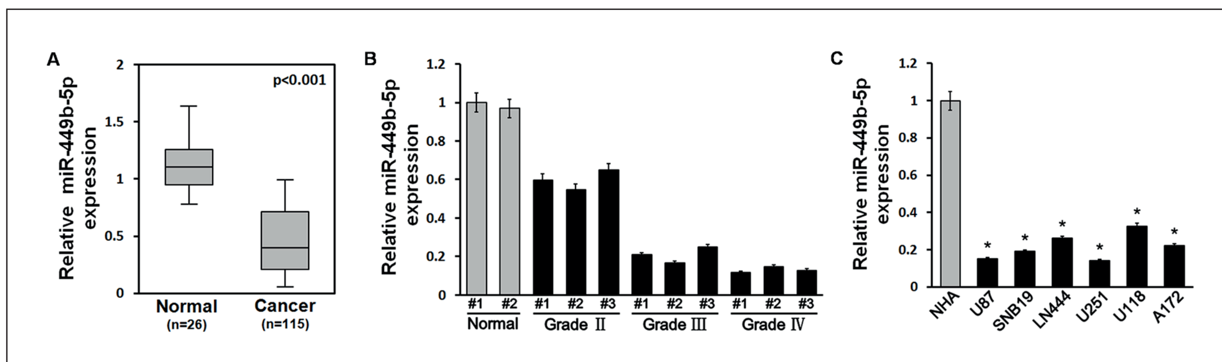

Figure 1. MiR-449b-5p was down-regulated in human glioblastoma tissues and cell lines. **A**, Expression of miR-449b-5p in freshly-frozen human glioblastoma tissues was statistically significantly lower than that in the normal tissues (Normal, n = 26; GBM, n = 115). $p < 0.001$. **B**, Down-regulated expression of miR-449b-5p was further confirmed in 9 different clinical stages of human glioblastoma tissues, including 3 samples of clinical stage II, stage III and stage IV individually, *vs.* the two normal tissues. **C**, Down-regulated expression of miR-449b-5p was further confirmed in 6 different glioblastoma cell lines *vs.* the normal human astrocytes (NHA) $*p < 0.05$.

lower cell number) of the cultured glioblastoma cells. Conversely, miR-449b-5p-silenced cells formed more tumor spheres (~two-fold more with a ~2-5-fold higher cell number) than the control cells (Figure 2B). Afterward, MTT assay confirmed that miR-449b-5p mimics apparently declined, miR-449b-5p inhibitor significantly elevated U251 cell viability after 96 h culture (Figure 2C). Comparably, the BrdU cell proliferation assay indicated that miR-449b-5p knockdown stimulated the proliferation ability of the U251 cells, while miR-449b-5p over-expression decreased the proliferation ability of U251 cells (Figure 2D). These data together pointed out that miR-449b-5p over-expression inhibited the growth of U251 cells.

MiR-449b-5p Inhibited Wnt/β-Catenin Signaling Pathway

Wnt/β-catenin signaling pathway is very important in GBM development. Therefore, we aimed to find out whether miR-449b-5p had the potential to regulate the activity of the Wnt/β-catenin signaling pathway in this work. Our results showed that the ratio of TOP/FOP was notably decreased in U251 cells when the miR-449b-5p was over-expressed. Conversely, the ratio of TOP/FOP was increased when the miR-449b-5p inhibitor was infected into the U251 cells (Figure 3A). Furthermore, we studied the effect of miR-449b-5p on the nuclear translocation of β-catenin, which is the center of Wnt/β-catenin signaling pathway activation. Western blotting assay was applied to measure the β-catenin level in the extracted nucleus proteins. Our results discovered that the nuclear β-catenin was apparently inhibited with miR-449b-5p over-expression, suggesting that miR-449b-5p blocked the β-catenin to translocate into the nucleus during the oncogenesis of GBM (Figure 3B). The RT-qPCR assay showed that several stem cell and cell proliferation associated genes (c-Myc, Nanog, FGF4, CD44, OCT-4, and CCND1) were significantly decreased

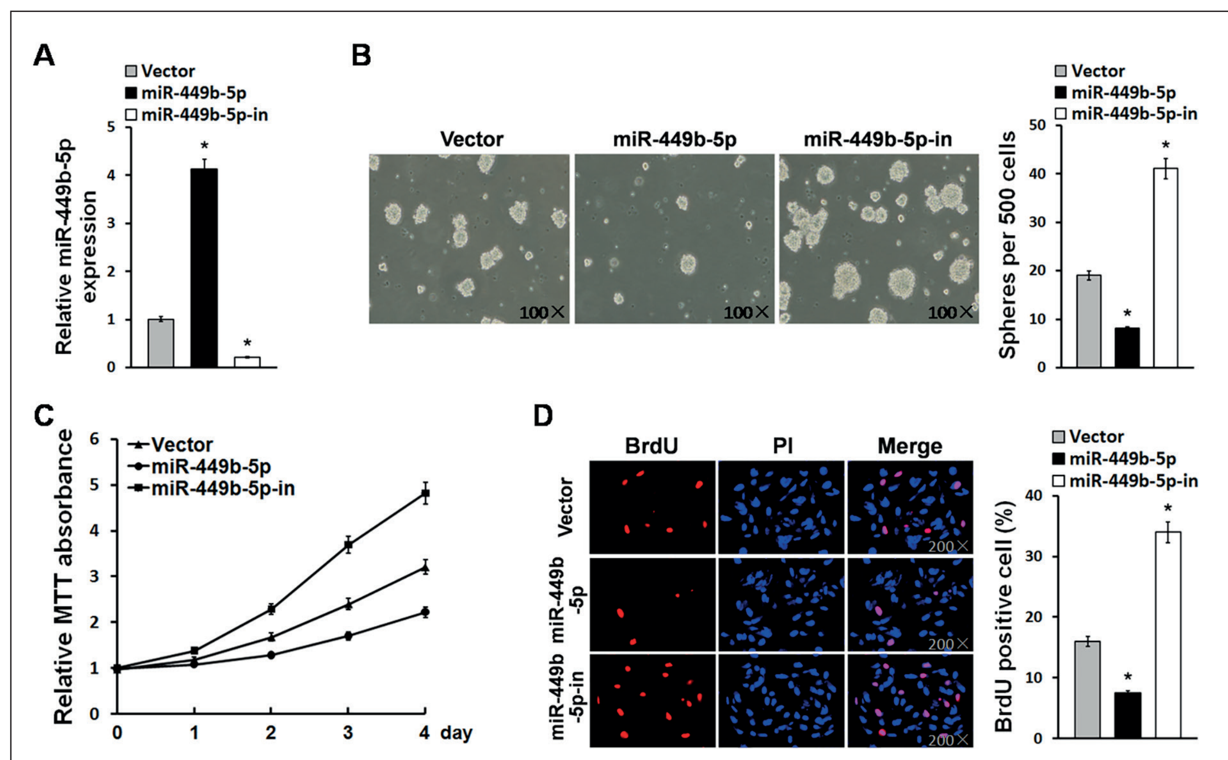

Figure 2. Over-expression of miR-449b-5p inhibited glioblastoma cell growth. **A**, The RT-qPCR analysis confirmed that miR-449b-5p was successfully over-expressed or knocked down with miR-449b-5p or miR-449b-5p-in containing lentivirus infection. **B**, Representative micrographs of neural spheres and calculated sphere numbers per 500 cells of tumor sphere formation for the indicated cells (100×). **C**, MTT assays discovered that miR-449b-5p over-expression inhibited the viability, while miR-449b-5p silence promoted the viability of U251 cells. **D**, BrdU cell proliferation assay, cells were seeded (5000 cells/well) in a 96-well plate and incubated overnight. A total of 10 μM BrdU was added into the cells and incubated for 4 h. The pictures (left panel) and numbers (right panel) of the BrdU positive cells were acquired under the fluorescence microscope (200×). *$p < 0.05$.

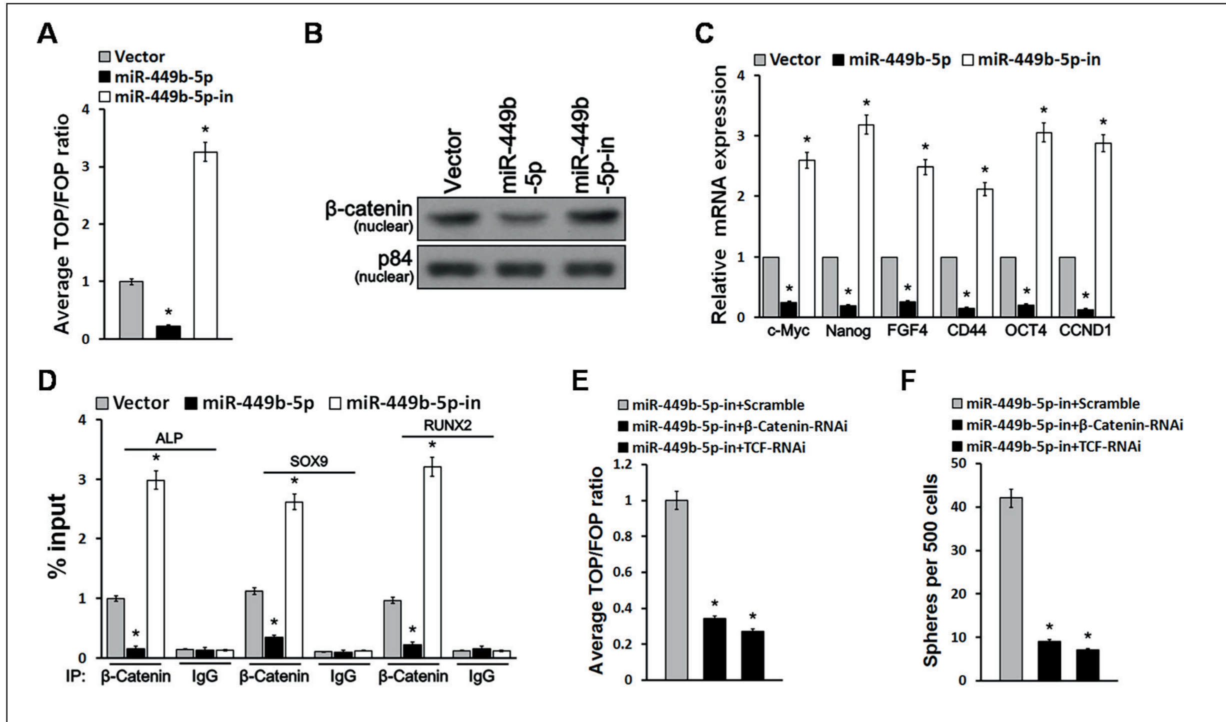

Figure 3. Up-regulated miR-449b-5p blocked Wnt/β-catenin signaling. **A**, The activity of Wnt/β-catenin signaling pathway in miR-449b-5p or miR-449b-5p-in transfected U251 cells was determined by the TOP/FOP ratio. **B**, Nuclear β-catenin protein expression was identified by Western blotting assay. **C**, Real-time PCR detection of stem cell and proliferation-related gene expressions, including c-Myc, Nanog, FGF4, CD44, OCT-4 and CCND1 in miR-449b-5p or miR-449b-5p-in transfected U251 cells. **D**, ChIP assays were performed, in miR-449b-5p, miR-449b-5p-in and vector control cells, with anti-β-catenin antibody to identify β-catenin recruitment in the promoters of ALP, SOX9, and RUNX2. IgG was a negative control. **E**, TOP/FOP ratio determining the activity of Wnt/β-catenin signaling pathway in U251 cells transfected by miR-449b-5p-in together with β-catenin siRNA or TCF-siRNA. **F**, Calculated sphere numbers (per 500 cells) of tumor sphere formation in the U251 cells transfected by miR-449b-5p-in plus β-catenin siRNA or TCF- siRNA. *$p < 0.05$.

in U251 cells when miR-449b-5p was over-expressed (Figure 3C). The chromatin immunoprecipitation assay (ChIP) was used to detect the ability of β-catenin to recruit its downstream genes of ALP, Sox9 and RUNX2, which showed that miR-449b-5p over-expression blocked nuclear β-catenin to recruit its downstream genes of ALP, Sox9, and RUNX2, while this recruitment ability was amplified with miR-449b-5p knockout (Figure 3D). Meanwhile, the TOP/FOP ratios were all inhibited in U251 glioblastoma cells after being infected by miR-449b-5p-in together with β-catenin siRNA or TCF-siRNA (Figure 3E). The calculated sphere numbers per 500 cells of tumor sphere formation were also inhibited in the U251 glioblastoma cells after being infected by miR-449b-5p-in plus β-catenin siRNA or TCF-siRNA (Figure 3F). These results all suggested that down-regulated miR-449b-5p promoted human glioblastoma pathogenesis by activation of Wnt/β-catenin signaling pathway.

MiR-449b-5p Inhibited Wnt/β-Catenin Signaling Pathway by Targeting WNT2B

Our data mentioned above showed that down-regulated miR-449b-5p induced glioblastoma pathogenesis by activation of the Wnt/β-catenin signaling pathway. To further explore the specific mechanism, we investigated the target molecules during this process. Moreover, by searching the miRNA database, WNT2B was found to be the potential targets of miR-449b-5p (Figure 4A). Luciferase activity examination displayed that the mRNA 3'UTR level of WNT2B was reduced in miR-449b-5p over-expressed U251 cells, and it was increased with miR-449b-5p knockout (Figure 4B). However, the Luciferase activity in U251 cells transfected with WNT2B UTR-mutation remained unchanged (Figure 4C), suggesting that down-regulated miR-449b-5p participated glioblastoma pathogenesis by activation of WNT2B via directly binding with its 3'UTR. Western

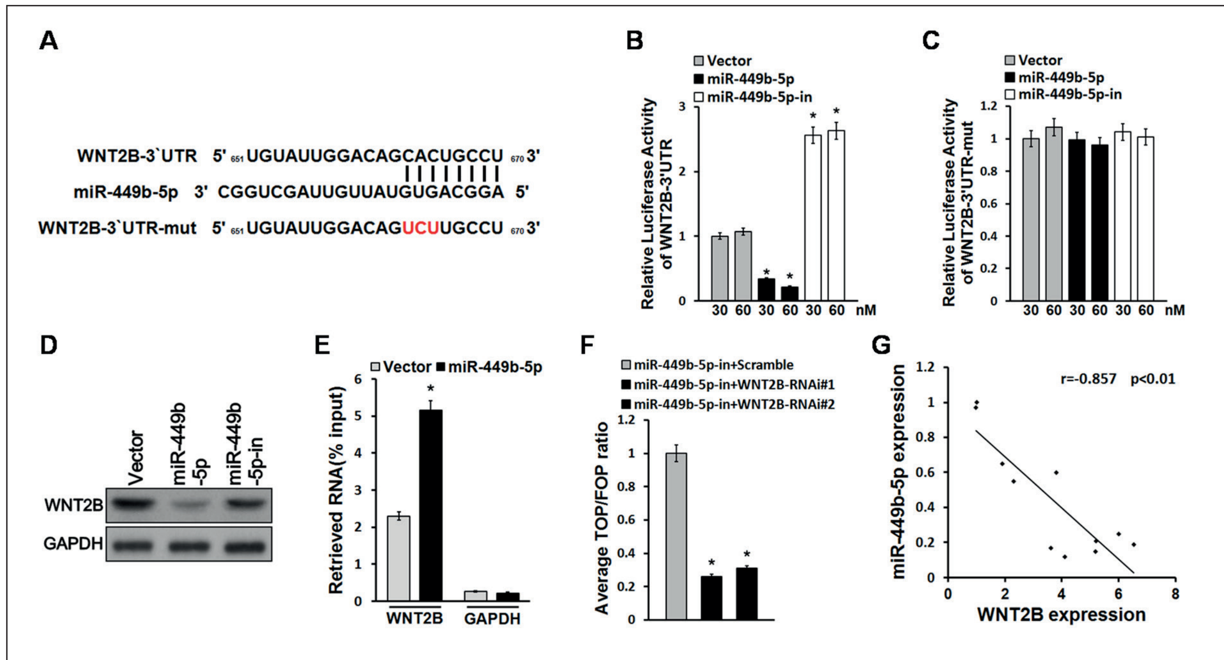

Figure 4. MiR-449b-5p directly targeted WNT2B. **A**, The predicted target sequence of miR-449b-5p in the 3'UTR of WNT2B. **B**, Dual-Luciferase reporter assay of the U251 cells with the transfection of WT-WNT2B-3'UTR reporter and miR-449b-5p or miR-449b-5p-in. **C**, Dual-Luciferase reporter assay of the U251 glioblastoma cells with the transfection of the mutated WNT2B-3'UTR reporter and miR-449b-5p or miR-449b-5p-in. **D**, WNT2B expression, in U251 glioblastoma cells with miR-449b-5p or miR-449b-5p-in transfection, checked by Western blot assay. **E**, Microribonucleoprotein (miRNP) assay for the association between miR-449b-5p and WNT2B transcripts in U251 cells. GAPDH served as a negative control. **F**, TOP/FOP ratio determining the activity of Wnt/β-catenin signaling pathway in U251 cells transfected by miR-449b-5p-in plus WNT2B siRNAs. **G**, Correlation analysis of miR-449b-5p and WNT2B expression in U251 cells. ** $p<0.01$, *vs.* the control.

blot assay showed that miR-449b-5p over-expression inhibited WNT2B expression in U251 glioblastoma cells, while miR-449b-5p knockout up-regulated WNT2B expression (Figure 4D).

Microribonucleoprotein (miRNP) immunoprecipitation (IP) assay revealed a specific association of miR-449b-5p with WNT2B, but not with GAPDH (Figure 4E). Meanwhile, the TOP/FOP ratio was also decreased in U251 cells co-transfected with miR-449b-5p-in and WNT2B-siRNAs (Figure 4F). This suggested that WNT2B-siRNA abolished miR-449b-5p inhibition-mediated Wnt/β-catenin pathway activity. Correlation analysis demonstrated that WNT2B expression was negatively related to miR-449b-5p expression in glioblastoma tissues (Figure 4G), indicating that miR-449b-5p and WNT2B were closely related during the oncogenesis of glioblastoma.

Discussion

Studies have provided evidence that the changed miRNA expression in carcinoma tissues and cells indicates great molecular and clinical implications[20,21]. Profiling studies have shown that miRNAs are differentially expressed in brain cancer tissues compared with the normal tissues, and abnormal miRNAs have potential diagnostic and prognostic implications[22]. Some miRNA expressions involved in the clinical outcomes of certain GBM patients[23,24] and many abnormal miRNAs had been described independently about their role and targets in GBM[24,25].

In the current work, our results presented that miR-449b-5p was down-regulated in glioblastoma tissues and cell lines, which was negatively associated with the clinical stages of the glioblastoma patients. Further studies exposed that miR-449b-5p over-expression suppressed the tumor sphere formation, cell viability and proliferation of the U251 cells. As a result, our study demonstrated that miR-449b-5p could serve as a tumor suppressor during the GBM development.

The Wnt/β-catenin signaling pathway has attracted the research consideration in recent years, and many reports have suggested that this is one of the most important oncogenic pathways[26].

The β-catenin is a signal transducer of the Wnt/β-catenin signaling pathway in cells and has been recognized to play a very important role in the cadherin protein complexes, which is the center to activate the Wnt/β-catenin signaling pathway during embryonic development and tumorigenesis[27-29]. WNT proteins, including WNT2B, are one group of the members in the secretory glycoprotein family, and key regulators to mediate the oncogenesis[30]. They work by binding to the membrane receptors containing Frizzled (Fzd) family, serpentine receptors and low-density lipoprotein receptor-related protein 5/6(LRP5/LRP6), and are necessary to recruit cytoplasmic phosphoprotein disheveled (Dsh/Dvl). Dsh/Dvl, the main intermediate during this process, is activated to deliver signals from the Wnt/β-catenin receptor to the glycogen synthase kinase 3β (GSK-3β) destruction complex and axin, thus to inhibit the β-catenin phosphorylation[31-33]. The communication of Wnt and its receptor results in the recruitment of unphosphorylated β-catenin in the cytoplasm. This will recruit the β-catenin to translocate into the nucleus, which later activates the downstream genes and other transcriptional factors[34,35]. Furthermore, the Wnt signaling pathway regulates many cellular functions, such as cell growth, apoptosis, invasion and migration, which are related to WNT associated carcinogenesis procedures[36,37].

However, the role and potential regulatory mechanism of WNT2B in GBM occurrence have not been discovered. Because of the fundamental role of Wnt/β-catenin signaling pathways in the oncogenesis, invasion and development of cancers, it was predicted that this pathway is the main mechanism by which WNT2B works in human GBM.

In the current study, we found that WNT2B was a direct target of miR-449b-5p via activation of Wnt/β-catenin signaling in glioblastoma cells, and WNT2B expression was negatively associated with miR-449b-5p expression in glioblastoma tissues and cells.

In the future, it would be significant to explore the participation and specific mechanism of miR-449b-5p in animal models and patients with glioblastoma. At the meantime, the association between WNT2B and Wnt/β-catenin signaling pathway was only confirmed in one of the glioblastoma cells. More studies are still necessary to confirm these findings, which will provide strong evidence supporting the function of miR-449b-5p and WNT2B in glioblastoma oncogenesis, proliferation and development.

Conclusions

We indicated that miR-449b-5p expression was down-regulated in human glioblastoma. The viability, proliferation and tumor sphere formation of glioblastoma cells were significantly inhibited after over-expression of miR-449b-5p. Moreover, WNT2B functions as a target of miR-449b-5p via activation of Wnt/β-catenin signaling during glioblastoma progression, suggesting that miR-449b-5p is a tumor suppressor miRNA in glioblastoma. It would be a prospective strategy to up-regulate the expression of miR-449b-5p or inhibit the WNT2B degradation for glioblastoma therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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