miR-181a-5p inhibits the proliferation and invasion of drug-resistant glioblastoma cells by targeting F-box protein 11 expression

XUEYAN WEN[1-3*], SONGRONG LI[1,2*], MENGCHAN GUO[1,2*], HONGZHAN LIAO[1-3], YONGMIN CHEN[1,2], XI KUANG[1,2], XIAOPING LIAO[1,2], LIN MA[1,2] and QIFU LI[1,2]

[1]Department of Neurology, The First Affiliated Hospital of Hainan Medical University, Haikou, Hainan 570102; [2]Key Laboratory of Brain Science Research and Transformation in Tropical Environment of Hainan Province, Haikou, Hainan 571199; [3]Department of Neurosurgery, Affiliated Hospital of Guilin Medical University, Guilin, Guangxi 541001, P.R. China

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Abstract. Glioblastoma (GBM) is the most common malignant primary tumor in the human central nervous system. The present study aimed to explore the molecular mechanism by which microRNA (miR)-181a-5p targets the F-box protein 11 (FBXO11) in glioma cells to inhibit cell proliferation and invasion. Reverse transcription-quantitative (RT-q)PCR was performed to detect the expression levels of miR-181a-5p in U251TR cells, U251 cells, primary GBM tissues and relapsed GBM tissues in order to determine the association between miR-181a-5p and the chemoresistance of GBM cells. The expression levels of miR-181a-5p in GBM cells were modulated via transfecting miR-181a-5p mimics and inhibitors. Cell Counting Kit-8 assays were undertaken to assess the effects of miR-181a-5p on drug sensitivity and proliferation of GBM cells. Wound healing assays were performed to examine the effects of miR-181a-5p on the migratory ability of GBM cells. Furthermore, the effects of miR-181a-5p on the invasive ability of GBM cells were analyzed using an *in vitro* invasion assay. Flow cytometry analysis was carried out to determine whether overexpression of miR-181a-5p can promote the apoptotic rate of GBM cells. RT-qPCR and western blotting were employed to detect the effects of miR-181a-5p on mRNA and protein expression of FBX011. miR-181a-5p exhibited low expression in resistant GBM cell lines and recurrent tumor tissues. Dual-luciferase reporter assays were utilized to detect luciferase activity to verify the targeted regulatory association between miR-181a-5p and FBXO11. Upregulation of miR-181a-5p promoted the sensitivity of GBM cells to temozolomide (TMZ), increased the apoptotic rate of GBM cells and significantly inhibited the invasive and migratory capacities of GBM cells. In drug-resistant glioma cells, compared with the miR-negative control group and the blank group, the expression of miR-181a-5p was significantly upregulated ($P<0.01$), while the expression of FBXO11 protein was downregulated. miR-181a-5p increased the sensitivity of GBM cells to TMZ. miR-181a-5p significantly inhibited the migratory and invasive capacities of GBM cells. miR-181a-5p may become a novel effective target for the treatment of GBM. The results of dual-luciferase reporter assays indicated that miR-181a-5p could target the 3'-untranslated region of FBXO11. The underlying mechanism may be targeted inhibition of *FBXO11* gene expression, or may be associated with apoptosis.

Introduction

Glioblastoma (GBM) is the most common primary malignant brain tumor, comprising 16% of all primary brain and central nervous system neoplasms (1). According to a 2007-2011 statistical report from the Central Brain Tumor Registry of the United States, it accounts for 15.4% of primary brain tumors and 45.6% of primary malignant brain tumors (1). Among malignant tumors, the incidence of glioblastoma is ~3.19 per 100,000 population, and its risk increases with age, mainly between the ages of 75 and 84 years old (1). GBM is characterized with a high mortality and recurrence rate, as well as low survival rate, with the 5-year survival rate of patients being ~5% (1). GBM mainly occurs at the frontal lobe and temporal lobe regardless of an individual's age (2). Chemotherapy is a critical process in the postsurgical treatment of glioma, and temozolomide (TMZ) is currently a major chemotherapeutic drug (3). However, GBM exhibits high resistance to TMZ, which can result in relapse, as well as treatment failure (4). MicroRNAs (miRNAs/miRs) are short

Correspondence to: Dr Lin Ma or Dr Qifu Li, Department of Neurology, The First Affiliated Hospital of Hainan Medical University, Haikou, Hainan 570102, P.R. China
E-mail: malin0116@163.com
E-mail: lee-chief@163.com

*Contributed equally

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non-coding RNA sequences, typically 19-25 nucleotides in length, first identified in 1993 (5). As important regulatory factors, miRNAs participate in cell proliferation, differentiation, apoptosis, cell cycle progression and other biological processes, playing vital regulatory roles in biological development and tumorigenesis (6). The miR-181 family has notable effects on the biological processes of range of tumor cells. Liu *et al* (7) demonstrated that low miR-181b expression in nervous glioma cells has a role as a tumor suppressor gene, inhibiting the proliferation and migration of tumor cells, as well as inducing apoptosis. F-box protein is a type of protein that is widely found in eukaryotes and contains an F-box domain. In the ubiquitin-proteasome pathway, it specifically recognizes substrate proteins and participates in cell cycle regulation, transcription regulation, cell apoptosis, cell signal transduction and other cellular activities (8). Previous studies have revealed that miRNAs indirectly participate in cancer progression via mediating different F-box proteins (8,9). For example, miR-25, miR-27a, miR-92a, miR-182, miR-223 and miR-503 can promote cancer development by inhibiting F-box and WD repeat domain-containing 7 in colon, esophageal, stomach, liver and other types of cancer, while miR-7, miR-30, miR-203 and miR-340 can inhibit cancer by inhibiting Skp2. The expression levels of miRNAs of the miR-30 family are upregulated in cancer cell lines, such as non-small cell lung cancer and melanoma cells (9). However, the underlying mechanisms by which miR-181a-5p is involved in the drug resistance of glioma cells have not been resolved. The present study investigated the effects of miR-181a-5p on the invasion and resistance of glioma cells to TMZ, and reported that miR-181a-5p represents a potential target for overcoming drug resistance in GBM cells.

Materials and methods

Glioma tissue samples and cells. Glioma tissue samples collected between November 2016 and June 2017 used in the present study were obtained from Nanfang Hospital and Zhujiang Hospital (Southern Medical University, Guangzhou, China), and Guangdong 999 Brain Hospital (Guangzhou, China) after pathological diagnosis, which included 22 grade I-II tumors, 58 grade III-IV tumors (according to the 2007 World Health Organization Classification of Tumours of the Central Nervous System) (10) and 16 relapsed tumors that had undergone TMZ chemotherapy for 6 months. All glioma specimens have been examined and diagnosed by pathological examination at The Department of Pathology of Zhujiang Hospital and Guangdong 999 Brain Hospital. Tissue samples were immediately aliquoted into separate test tubes, frozen on dry ice and stored at -80˚C until analysis. Follow-up data was obtained from reviews of patients' medical records. Prior to surgery, no patients had received radiation or chemotherapy. The same procedure occurred for the patients who relapsed. This study was approved by the Ethics Committee of Hainan Medical University (Haikou, China; approval no. 201818). The present study was conducted in accordance with the regulations of the Hainan Institutional Review Board (affiliated to Hainan Provincial Organization Office). All enrolled patients provided written informed consent form before craniotomy. Human nervous glioma cell lines U251 and TMZ-resistant GBM cells (U251TR) were collected from The Department of Pathology, Nanfang Hospital, Southern Medical University. In order to verify that miR-296-3p may at least partially serve a role in multi-drug resistance in glioblastoma by targeting ether-à-go-go, the TMZ-resistant GBM U251TR cell line has been previously constructed from a U251 cell line (11) and stored in the laboratory of the Department of Laboratory of Southern Hospital.

Cell culture and transfection. The glioma cell lines, U251 and U251TR, were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS; HyClone; Cytiva) and 10 μg/ml TMZ (Sigma-Aldrich; Merck KGaA), and were incubated at 37˚C in a humidified 5% CO_2-enriched atmosphere. Human embryonic kidney 293T cells (purchased from the American Type Culture Collection) were also cultured in the same way. The glioma cells were transiently transfected using Lipofectamine® 3000 Transfection reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. The concentrations of miR-181a-5p mimics, miR-181a-5p inhibitors and controls were all 100 nmol/l. RNA fragments (miR-181a-5p mimics/inhibitors) used in transfection assay were purchased from Shanghai GenePharma Co., Ltd. Cells were divided into 6 groups, including: Untreated cells as the control group (2 groups, one for each cell line); cells transfected with miR-181-5p negative control (NC) as the NC group (2 groups, one for each cell line); drug-resistant GBM cells (U251TR) transfected with miR-181-5p mimics as the miR-mimics group; and GBM cells (U251) transfected with miR-181-5p inhibitors as the miR-inhibitors group. Additionally, U251 cells were transfected with the mimic to see whether there was an apoptotic effect. A total of $2x10^5$ cells/well were seeded in a 6-well culture plate and cultured until the cell density reached 70% at 37˚C. According to the manufacturer's protocol, the transfection reagent was added into serum-free DMEM, which was added to the culture plate to replace the complete medium and cultured for 48 h at 37˚C. The transfection efficiency was observed under a fluorescence microscope (data not shown). The sequences of RNA oligo ribonucleotides were as follows: miR-181a-5p mimics forward, 5'-AACAUUCAACGCUGUCGGUGAGU-3' and reverse, 5'-UCACCGACAGCGUUGAAUGUUUU-3'; miR-181a-5p mimics-NC forward, 5'-UUCUCCGAACCUGUCACGUTT-3' and reverse, 5'-ACGUGACACGGUCGGAGAATT-3'; miR-181a-5p inhibitors, 5'-ACUCACCGACAGCGUUGAAUGUU-3'; miR-181a-5p inhibitors-NC, 5'-CAGUACUUUUGUGUAGUACAA-3'.

Reverse transcription-quantitative (RT-q)PCR analysis. Total RNA was extracted and isolated from tissues and cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was reverse transcribed. The RT mixed reaction solution was prepared on ice, including the following reagents: 2 μl 5X PrimeScript® Buffer (Takara Biotechnology Co., Ltd.), 0.5 μl PrimeScript RT Enzyme Mix I, 0.5 μl oligo dT primer (50 μM), 0.5 μl random 6-nucleotide primers (100 μM), template RNA ($4x10^5$ copies), RNase-free double-distilled water up to 10 μl. The reaction conditions were as follows: 37˚C for 15 min for the reverse transcription reaction, and

85˚C for 5 sec to inactivate the reverse transcriptase. The synthesized cDNA was stored at -20˚C until subsequent use. The expression levels of miR-181a-5p were detected using the SYBR Green Hairpin-it™ miRNAs qPCR Quantitation kit (Shanghai GenePharma Co., Ltd.), in a Fast Real-time PCR 7500 System (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. The reaction conditions were as follows: Pre-denaturation at 95˚C for 30 sec, followed by 30-40 cycles of denaturation at 95˚C for 5 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 10 sec. The *U6* gene was used as an internal reference. Each assay was performed in triplicate and repeated three times. The relative expression levels were calculated and analyzed using the $2^{-\Delta\Delta Cq}$ method (12). Primers used in qPCR assays were synthesized by Shanghai GenePharma Co., Ltd. (miR-181a-5p, cat. no. 91151N61; U6, cat. no. 8301151105).

Chemotherapeutic drug sensitivity of glioma cells. Cells ($1x10^4$) were seeded into 96-well plates and divided into six groups: Two blank control groups, two NC groups, miR-181a-5p mimic group and miR-181a-5p inhibitor group, after transient transfection using Lipofectamine as aforementioned. After 24 h, the cells were treated with different concentrations of TMZ, ranging from 10 to 60 μg/ml for 48 h. The assay was undertaken in five replicate wells for each sample. The spectrophotometric absorbance of the samples was calculated as an average. Subsequently, the half maximal inhibitory concentration (IC_{50}) values of TMZ were respectively calculated.

Cell Counting Kit (CCK)-8 assay. A 96-well plate was used, at a density of $5x10^4$ cells/well. At 24, 48 and 72 h after transfection, 10 μl of CCK-8 solution (Pierce; Thermo Fisher Scientific, Inc.) was added according to the manufacturer's protocol. After 1 h, optical density (OD) at 450 nm value was measured using a microplate reader.

Wound healing assay. Cells were seeded into a 6-well plate at a density of $5x10^5$ cells/well and cultured for 24 h to create a confluent monolayer. The cells were serum-starved during this assay. The confluent monolayer was scraped with a 200-μl pipette tip along a sterile measuring rule, and washed three times in PBS. The corresponding images under a light microscope (magnification, x4) were captured at 0 and 24 h. Finally, ImageJ software v1.52a (National Institutes of Health) was used for quantification.

Cell invasion and Transwell migration assays. Cell invasion assays were performed using Transwell chambers following the manufacturer's instructions. Briefly, Matrigel (precoating at 37˚ for 15 min) was diluted using serum-free DMEM. A total of 200 μl of glioma cell ($1x10^5$ cells) suspension was seeded into the upper chamber. Subsequently, 600 μl of media supplemented with 20% FBS was added into the lower chamber, followed by incubation for 24 h. A cotton-tipped swab was used to wipe off the cells that did not migrate to the bottom chamber. Cells were stained with 0.1% Crystal Violet Staining Solution (Pierce; Thermo Fisher Scientific, Inc.) at room temperature for 15 min. The chambers were then observed, images were captured and the number of migrated cells were counted under a light microscope (magnification, x10) in six random fields of view. All assays were independently repeated for three times.

Cell apoptosis assays. Cells ($1x10^5$) were trypsinized and collected by centrifugation at 4˚C at 150 x g for 8 min, and apoptosis was analyzed using an Apoptosis Detection kit (Pierce; Thermo Fisher Scientific, Inc.). A total of 250 μl binding buffer was added to the suspended cells, 100 μl of suspension was added into a 1.5-ml Eppendorf tube, along with 5 μl Annexin V-PE and 10 μl 7-AAD, the samples were gently mixed in the dark and left to incubate at room temperature for 15 min before the addition of 400 μl of binding buffer, and analyzed using a FASCAria II (BD Biosciences) flow cytometer and FlowJo software (v7.6.1; FlowJo, LLC) within 1 h.

Western blot assay. Following cell lysis with RIPA lysis buffer (Beyotime Institute of Biotechnology), a BCA protein concentration assay kit (Biosharp Life Sciences) and Synergy HTX (BioTek Instruments, Inc.) microplate reader were used to determine the protein concentration. Protein loading buffer (5X; Beijing Zoman Biotechnology Co., Ltd.), was used for equalization of protein loading. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% SDS-PAGE) Gel Quick Preparation kit (Beyotime Institute of Biotechnology) was used for gel production. Samples were boiled at 100˚C for 10 min to denature the proteins. Protein samples (100 μg/lane) were separated via SDS-PAGE, and transferred onto polyvinylidene difluoride membranes (EMD Millipore). The membranes were blocked with 5% skimmed milk powder for 2 h at room temperature and then rinsed with TBS-Tween (1% Tween-20) for 3 times (10 min each time). The membrane was incubated with primary rabbit antibodies against human F-Box protein 11 (FBXO11; 1:500; cat. no. ab181801; Abcam) and GAPDH (1:1,000; cat. no. AF1186; Beyotime Institute of Biotechnology) for 24 h on a shaker at 4˚C, followed by incubation with a HRP-labeled goat anti-rabbit secondary antibody (1:4,000; cat. no. A2028; Beyotime Institute of Biotechnology) at room temperature for 1 h. Signal was detected using enhanced chemiluminescence detection reagent (Beyotime Institute of Biotechnology). Finally, the 4600SF chemiluminescent imaging system (Tanon) was used to semi-quantify the bands. Three independent experiments were performed for each analysis.

Bioinformatics analysis. TargetScan (www.targetscan.org) was utilized to predict biological targets for miR-181a-5p. Of all these hypothetical targets, FBXO11 was selected for further research.

Dual-luciferase reporter assay. The 3'-untranslated region (UTR) and mutant 3'-UTR of wild-type FBXO11 were amplified by Wuhan GeneCreate Biological Engineering Co., Ltd., and integrated into the psichenk2.0 (Wuhan Servicebio Technology Co., Ltd.) vector containing the luciferase gene. The plasmids were tested and co-transfected into 293T cells with miR-181a-5p mimics or miR-NC. After 48 h of transfection, the cells were lysed using a Dual-Luciferase® Reporter Assay kit (Promega Corporation). In the case of firefly luciferase as the internal control, the relative light unit (RLU)

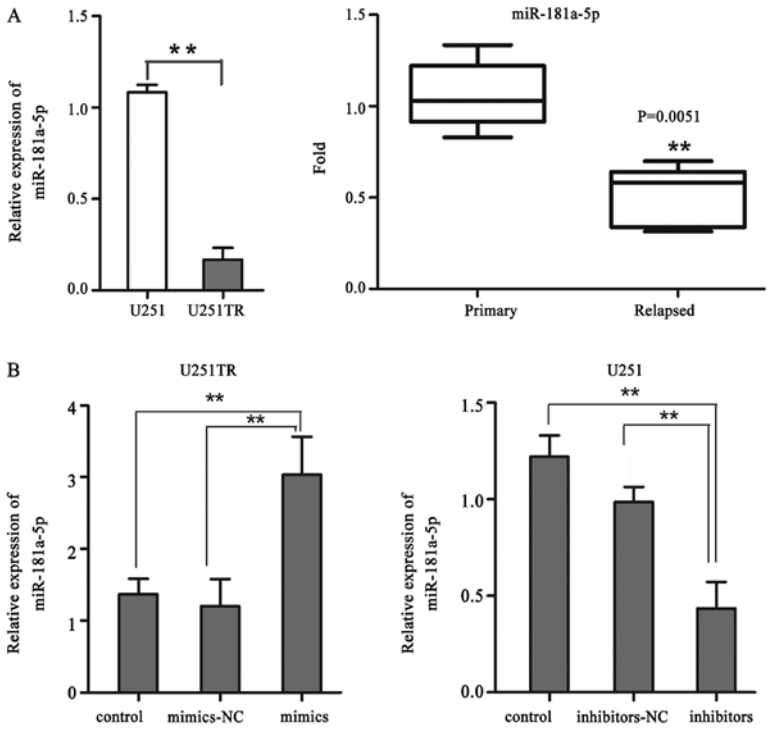

Figure 1. Expression of miR-181a-5p in normal and drug-resistant glioma cells. (A) RT-qPCR was conducted to assess the miR-181a-5p expression levels in U251 and U251TR cell lines. **P<0.01 vs. U251. RT-qPCR was also conducted to examine the miR-181a-5p expression levels in primary GBM tissues and relapsed GBM tissues. The data are shown as the mean ± SEM. **P<0.01 vs. the primary GBM tissues. (B) RT-qPCR was performed to detect the miR-181a-5p expression levels in glioma U251TR and U251 cells transfected with miR-181a-5p mimics or inhibitors, respectively. **P<0.01 vs. the NC and untransfected control. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; GBM, glioblastoma; NC, negative control.

value measured by *Renilla* luciferase was divided by the RLU value measured by firefly luciferase. According to the obtained ratio, the inhibitory effect of miRNA on the target gene was compared, and the luciferase activity was analyzed using a Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Inc.).

Statistical analysis. All statistical analyses were conducted using SPSS 19.0 (IBM Corp.) and GraphPad Prism 8.0 software (GraphPad Software, Inc.). Each experiment was performed in triplicate and repeated for three times. Data were presented as mean ± standard deviation. Independent-samples t-tests were used to analyze significant differences between two groups, while one-way analysis of variance was utilized to estimate significant differences among multiple groups. The least significant difference method was used for multiple comparisons when the variance was equal. The Dunnett's corrective test was employed when the variance was not equal (in the chemotherapeutic drug sensitivity experiments of glioma cells). P<0.05 was considered to indicate a statistically significant difference.

Results

miR-181a-5p expression is upregulated in normal glioma cells and downregulated in drug-resistant glioma cells. RT-qPCR was employed to detect miR-181a-5p expression levels in U251 and U251TR cells and 51 glioma tissues containing primary and relapsed tissues. The data revealed that miR-181a-5p expression was significantly decreased in U251TR cells compared with expression in U251 cells (P<0.01; Fig. 1A). It was also revealed that miR-181a-5p expression was significantly reduced in relapsed GBM tissues after chemotherapy compared with primary GBM tissues (0.5080±0.072 vs. 1.06±0.082, respectively; P<0.01; Fig. 1A). Untransfected U251TR and U251 cells were then used as controls in addition to the mimics NC. miR-181a-5p expression levels were markedly increased in U251TR cells transfected with miR-181a-5p mimics compared with those in the NC group (P<0.01). However, the miR-181a-5p expression levels were significantly decreased in U251 cells transfected with the miR-181a-5p inhibitors compared with the untransfected control group (P<0.01) (all Fig. 1B).

miR-181a-5p can inhibit the proliferation and invasion of glioma cells and promotes the apoptosis of glioma cells. The present study sought to investigate the biological functions of miR-181a. The CCK-8 assays indicated that the miR-181a-5p simulation group had a significantly enhanced sensitivity to TMZ, with a decreased IC_{50} value in transfected U251TR cells compared with untransfected cells (P<0.01). In contrast, the IC_{50} value of TMZ in U251 cells transfected with miR-181a-5p inhibitors was notably elevated compared with the control group (P<0.01) (Fig. 2A). The CCK-8 assay demonstrated that the proliferation of drug-resistant glioma cells transfected with the miR-181a-5p mimic was significantly lower compared with that in the NC group and drug-resistant glioma cells (P<0.05; Fig. 2B). The proliferation rate of normal glioma cells transfected with miR-181a-5p inhibitor was higher compared with that in the NC group (P<0.05; Fig. 2B).

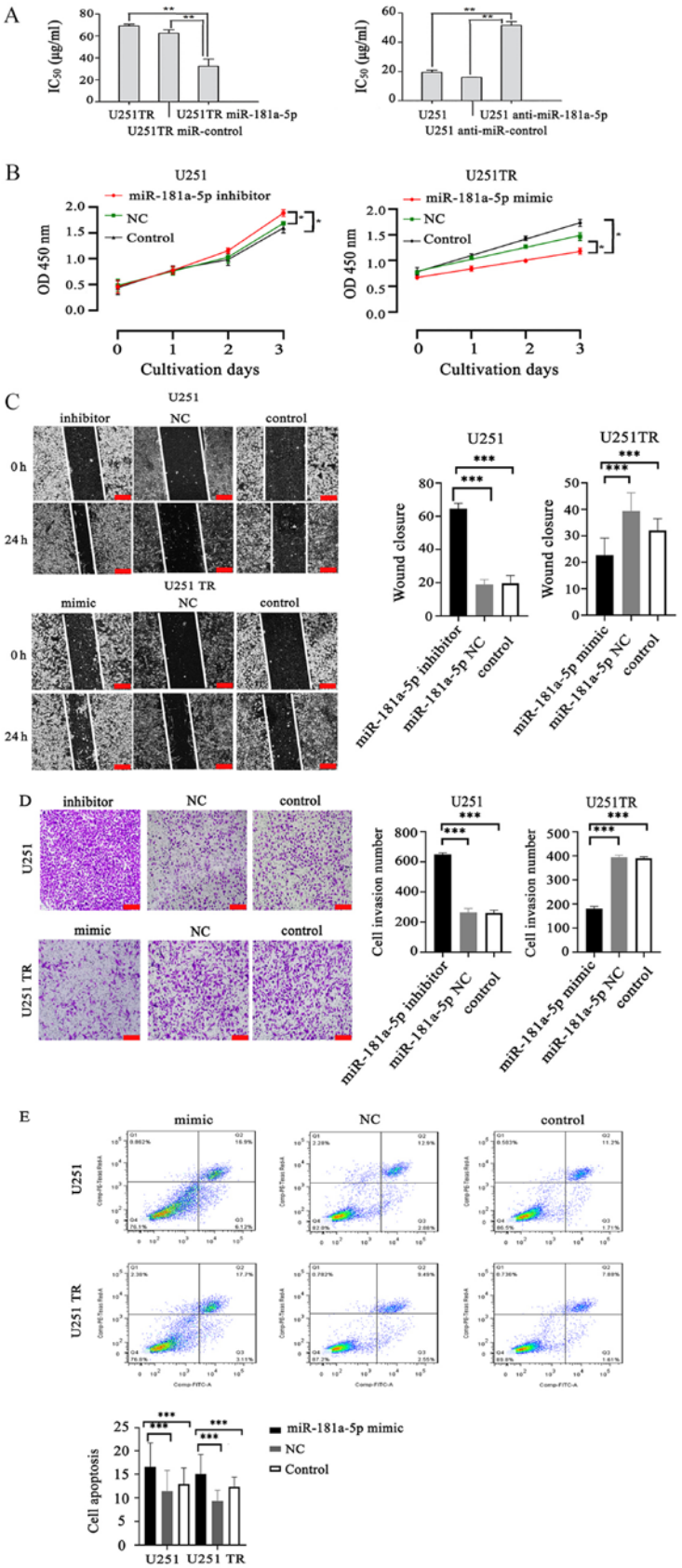

Figure 2. Effects of miR-181a-5p on the proliferation, invasion and apoptosis of glioma cells. (A) The IC_{50} values of TMZ in glioma U251TR and U251 cells transfected with miR-181a-5p mimics or inhibitors. **P<0.01. (B) miR-181a-5p mimics/inhibitors can inhibit or promote the proliferation of drug-resistant glioma cells/normal glioma cells, respectively, compared with the NC and untransfected control group. *P<0.05. (C) Wound healing assay was carried out to determine the migration of glioblastoma U251TR and U251 cells transfected with miR-181a-5p mimics/inhibitors, respectively. ***P<0.001. Scale bar, 200 μm. (D) Transwell migration assays were performed to determine the effects of miR-181a-5p on the invasive capacity of the glioma cells. ***P<0.001. Scale bar, 200 μm. (E) miR-181a-5p induced apoptosis in U251 and U251TR cells. Flow cytometry analysis showed that overexpression of miR-181a-5p enhanced apoptosis in U251 and U251TR cells. Analysis indicated that the apoptosis rate was upregulated in U251 and U251TR cells. ***P<0.001 vs. control. miR, microRNA; TMZ, temozolomide; IC_{50}, half maximal inhibitory concentration; NC, negative control.

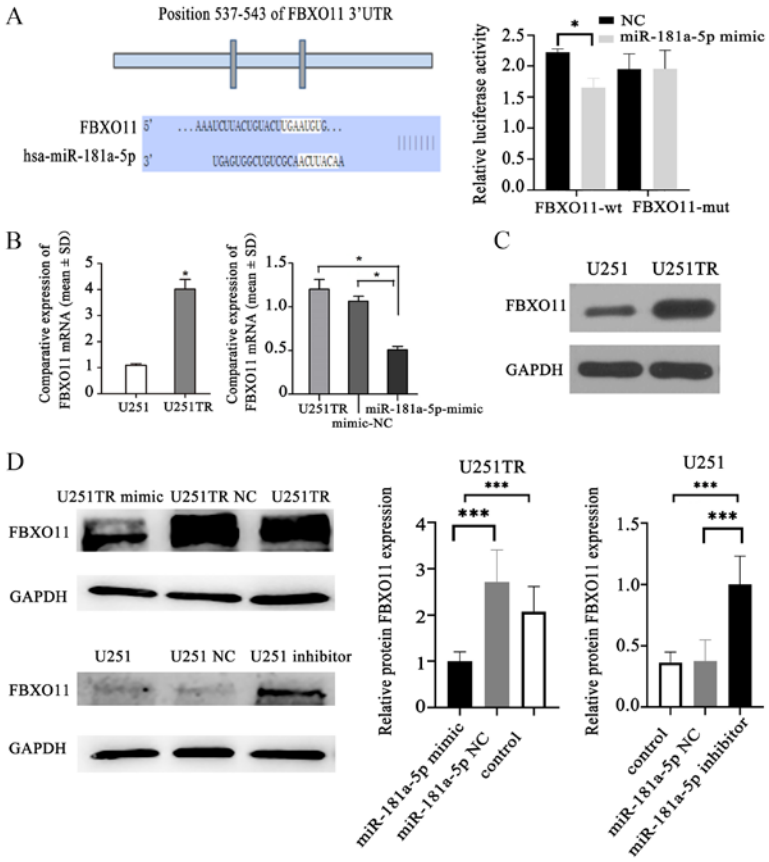

Figure 3. miR-181a-5p targeting FBXO11. (A) Map of FBXO11 and miR-181a-5p target sites. In the 3'UTR FBXO11 non-coding region. The luciferase activity of U251 cells co-transfected with miR-181a-5p mimic and wt FBXO11 3'UTR was lower compared with that of NC cells in FBXO11-wt group. *P<0.05. (B) The left panel shows the expression of *FBXO11* mRNA in glioma and drug-resistant cell lines. The right panel shows that upregulation miR-181a-5p inhibits the expression levels of *FBXO11* mRNA in glioma-resistant cells. *P<0.05. (C and D) Western blot assays were performed to determine (C) FBXO11 expression in normal glioma cells and drug-resistant glioma cells, and (D) miR-181A-5P targeting FBXO11. FBXO11 expression was downregulated in drug-resistant glioma cells transfected with miR-181a-5p mimic. FBXO11 expression was upregulated in glioma cells transfected with miR-181a-5p inhibitor. ***P<0.001. miR, microRNA; UTR, untranslated region; FBXO11, F-Box protein 11; NC, negative control; wt, wild-type.

The wound healing assay revealed that, compared with the NC and untransfected control group, the U251 cells transfected with the miR-181a-5p inhibitor had a significantly faster 24-h wound closure rate (P<0.001). However, compared with the NC group, the 24 h wound closure rate of U251TR cells transfected with the miR-181a-5p mimic group was significantly less (P<0.001; Fig. 2C). Results of Transwell migration assays revealed that the miR-181a-5p mimics had significantly fewer invasive cells compared with the NC group (P<0.001). In contrast, U251 treatment with miR-181a-5p inhibitor resulted in a significantly higher number of invasive cells compared with the NC group (P<0.001; Fig. 2D). Flow cytometry results showed that compared with the NC and untransfected control group, the percentage of apoptotic U251 cells was significantly higher in the miR-181a-5p mimic group (P<0.001; Fig. 2E). For this experiment, U251 cells were also transfected with the mimic to see whether there was an apoptotic effect. Additionally, similar results were observed in U251TR cells (P<0.001; Fig. 2E). The aforementioned results indicated the inhibitory effects of miR-181a on glioma cells, especially drug-resistant glioma cells.

FBXO11 is a downstream target of miR-181a-5p. According to the TargetScan prediction website, miR-181a-5p was predicted to bind to *FBXO11*, the base complementary region is located at 537-543 of the non-coding region of *FBXO11* gene. In the presence of miR-181-5p mimic, the luciferase activity of the FBXO11-wt group was significantly lower than that of the NC group, indicating that miR-181a-5p can target the 3'UTR end of *FBXO11* (P<0.05; Fig. 3A). In glioma-resistant cells, the expression of FBXO11 was significantly higher compared with in ordinary glioma cells (P<0.05). When the expression levels of miR-181a-5p were upregulated, the expression levels of *FBXO11* in glioma-resistant cells were suppressed compared with untransfected cells (P<0.05) (Fig. 3B). Western blotting demonstrated that FBXO11 expression was markedly upregulated in drug-resistant cells compared with non-resistant cells (Fig. 3C). Additionally, FBXO11 expression was downregulated in drug-resistant glioma cells transfected with miR-181a-5p mimic, while it was upregulated in glioma cells transfected with miR-181a-5p inhibitor compared with in the controls (P<0.001; Fig. 3D).

Discussion

GBM is a common malignant tumor in the brain, accounting for ~45% of all brain tumors between 2011 and 2014 globally (13,14). Gliomas pose a notable threat to human life and

health, which can be manifested as mental disorders, such as dementia and brain damage as a result of brain tumor hemorrhage, leading to sudden brain herniation, and even hemiplegia or death, secondary epilepsy and blindness (15). To date, comprehensive approaches, including surgical excision, radiotherapy, chemotherapy and immunotherapy, are major treatment strategies for GBM worldwide. However, although postoperative patients undergo chemoradiotherapy, the overall survival time of patients is only ~12 months, owing to the strong migratory and invasive capabilities of GBM cells (16). Glioma is characterized by common recurrence after surgery, drug resistance after chemotherapy, a high mortality rate and an extremely low survival rate (17). Among malignant tumors, the incidence of glioblastoma is ~3.19 per 100,000 population, and its risk increases with age, mainly between the ages of 75 and 84 years old (1). The 5-year survival rate of patients is ~5% (1). In addition, high-grade malignancy, short survival period and drug resistance during postoperative chemotherapy often lead to the treatment failure (18). Therefore, understanding the specific molecular mechanisms underlying the resistance of glioma to TMZ and understanding of its downstream regulatory networks have research attracted attention.

TMZ is the most widely used anticancer drug for chemotherapy of GBM, mainly through influencing various division phases of tumor cells, especially late G_1 phase and early S phase (19). TMZ can induce the apoptosis and sensitize tumor tissues to chemotherapy by interfering with DNA replication and the repair of tumor cells (19). Nonetheless, the curative effect of TMZ gradually declines in patients with GBM, indicating that patients develop a resistance to chemotherapeutics (20). At present, the acknowledged molecular mechanisms of glioma resistance to TMZ, such as the overexpression of drug transporter protein, stronger cell repair ability, repressed apoptosis and obstruction of the blood-brain barrier, are considered to be involved (21). Recently, studies have shown that there is a close correlation between miRNAs and chemotherapy resistance of tumor cells. For instance, miR-221 is overexpressed in hepatocellular cancer cells resistant to sorafenib, and then targets caspase-3 to induce drug resistance of tumor cells through anti-apoptotic pathways (22). However, low expression of miR-424(322)/503 results in the upregulation of Bcl-2 and the insulin-like growth factor 1 receptor in breast cancer, which further induces resistance of breast cancer cells to chemotherapy (23). In addition, the abnormal expression of miRNAs modulate the expression of downstream target genes, and contributes to critical changes in the sensitivity of tumor cells to chemotherapeutic drugs (24).

miRs are endogenous non-coding RNAs that are 20-25 nucleotides in length. Dysregulation of miRNAs in brain tumors result in chromosomal aberration, epigenetic modifications and mutations (25). miRNAs possess special biological characteristics and an important function (22-24). For example, in hepatocellular carcinoma, miR-221 regulates sorafenib resistance by inhibiting caspase-3-mediated apoptosis (22). miR-424 is a breast cancer suppressor, and loss of miR-424 expression can enhance resistance to chemotherapy (23,24). Thus, they not only could be specific diagnostic markers for certain malignant tumors and hereditary diseases, but also indicators of invasion, migration, drug resistance or prognosis of malignant tumors (26,27). A previous study analyzed the results of a glioma-related GeneChip and found that there are ~22 differentially expressed miRNAs, and that some miRNAs promote the proliferation of GBM cells (28). miR-181a is a highly conserved member of the miR-181 family and acts as an early brain tumor marker, as well as target-regulating factor (29).

Glioma is the most common malignant tumor of the central nervous system, and the prognosis of patients is still very limited. miRNAs have been a research hotspot in recent years, and the mechanism of miR-181a-5p regulating the drug resistance of glioma remains unknown. The present study demonstrated that miR-181a-5p could regulate glioma resistance to TMZ and inhibit the migration and invasion capacities of glioma cells. Moreover, the results indicated that the expression of FBXO11 was reduced in U251TR cells via overexpression of miR-181a-5p, which indicated that increased expression of miR-181a-5p can downregulate FBXO11 expression. However, in U251 cells with depleted miR-181a-5p expression, FBXO11 expression was increased, demonstrating that increased miR-181a-5p expression could upregulate FBXO11 expression. In an experiment that analyzed the expression levels of miR-181a-5p in glioma resistant cells and relapsed glioma tissues after chemotherapy, the average expression levels of miR-181a-5p were significantly lower after chemotherapy in relapsed glioma cells compared with the tumor tissue. Further studies are required to determine if miR-181a-5p expression is higher in patients who relapse compared with patients who do not relapse. It would be valuable to understand the differences between patients that had relapsed with different grades. The present study suggested that miR-181a-5p and FBXO11 serve an important role in regulating the resistance to chemotherapy in glioma; however, research on miR-181a-5p and glioma drug resistance remains unclear. Future studies should determine the association between miR-181a-5p and glioma drug resistance, clarify its specific mechanism of action and clinical significance, improve the theoretical basis of glioma drug resistance and provide new targets for glioma drug resistance treatment.

Furthermore, the present results suggested that miR-181a-5p increased the chemotherapeutic sensitivity of glioma probably by affecting apoptosis. Drug resistance, similar to tumorigenesis and progression, is a complex process involving altered expression of several genes, multipath changes and multi-step accumulation (30). Hence, molecular mechanisms of drug resistance should be further explored. According to the current experimental results, the mechanism of apoptosis could be further explored to look for pathway genes associated with apoptosis genes.

At present, application of miRNA in the drug-resistant treatment of GBM has become an important research avenue and has an extensive clinical prospect. In summary, miR-181a-5p inhibits the proliferation and invasion of drug-resistant GBM cells. At present, the treatment of GBM cells mainly depends on chemotherapy, and the increase of drug resistance is a global problem (4). Several studies have revealed that miRNA is involved in the process of glioma resistance (23-25). Collectively, the present findings demonstrated that miR-181a-5p is a tumor suppressor gene in GBM, potentially providing a theoretical basis for treatment of drug-resistant GBM in clinical practice.

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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

XW, LM and QL conceived and designed the study. SL, MG and XL designed the experiments. HL and YC performed the experiments and analyzed the data. XW and XK performed the pathological analysis. XW and SL wrote, edited and revised the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Hainan Medical University (Haikou, China; approval no. 201818). This study was conducted in accordance with the regulations of the Hainan Institutional Review Board (affiliated to Hainan Provincial Organization Office). All enrolled patients signed the written informed consent form before surgery.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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