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Research on miR-126 in glioma targeted regulation of PTEN/PI3K/Akt and MDM2-p53 pathways

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Abstract. – OBJECTIVE: To study the correlations of micro ribonucleic acid (miR)-126 expression with pathogenesis and prognosis of glioma, and to screen potential biological targets for the diagnosis, treatment and prognosis of glioma.

PATIENTS AND METHODS: miR-126 expression in cancer tissues, normal brain tissues, U87MG cells and normal astrocytes in glioma patients was quantitatively analyzed via quantitative reverse transcription-polymerase chain reaction (qRT-PCR). U87MG cells were transfected with miR-126 mimics or miR-126 inhibitor, followed by verification via qRT-PCR. The cell proliferation, apoptosis, migration and invasion after transfection were analyzed using methyl thiazolyl tetrazolium (MTT) assay, Annexin V/propidium iodide (PI) assay, wound healing assay and transwell assay, respectively. The expression levels of proteins related to phosphatase and tensin homolog deleted on chromosome ten/phosphatidylinositol 3-kinase/protein kinase B (PTEN/PI3K/Akt) pathway and double mouse minute 2 homolog (MDM2)-p53 pathway were detected via Western blotting. Moreover, the prognostic analysis was performed using the Kaplan-Meier method and log-rank test.

RESULTS: Results of qRT-PCR showed that the miR-126 expression in highly malignant glioma tissues and U87MG cells were significantly lower than those in normal brain cells, and its expression level was significantly higher or lower than that in negative control group after transfection with miR-126 mimics or inhibitor. Analyses of cell proliferation, apoptosis, migration and invasion revealed that the up-regulation of miR-126 could remarkably inhibit the *in-vitro* proliferation, migration and invasion and promote apoptosis of glioma cells, and vice versa. Results of Western blotting manifested that after overexpression of miR-126, PI3K, p-Akt and MDM2 protein levels in U87MG cells were significantly decreased compared with those in control group, but PTEN and p53 protein expressions were significantly increased, and vice versa. Besides, according to prognostic analysis, the prognosis of patients with a low miR-126 level was poorer.

CONCLUSIONS: The miR-126 expression is abnormally low in glioma cells, and miR-126 inhibits the course of glioma through targeted regulation of PTEN/PI3K/Akt and MDM2-p53 pathways, which, therefore, can be used as a new potential biomarker for the diagnosis, treatment and prognosis of glioma.

Key Words:
miR-126, Glioma, PTEN/PI3K/Akt pathway, MDM2-p53 pathway, Prognosis.

Introduction

Glioma is the most common kind of brain tumors, accounting for about 80% of all malignant brain tumors. Due to the high postoperative recurrence rate, poor prognosis and median survival time of patients with its subtype glioblastoma of only 14 months, glioma seriously threatens human life and health[1,2]. The pathogenic process of glioma is very complicated, and studies have found that asthma, serum immunoglobulin E and allergy are all related to the occurrence and development of glioma[3]. The pathological mechanism of glioma often involves inhibiting T cell proliferation and promoting T cell apoptosis[4]. In addition, deoxyribonucleic acid (DNA) repair genes (XPD and XRCC1) are significantly correlated with the increased risk of glioma[5], and the activation of a variety of signaling pathways is involved in the proliferation of glioma. At present, chemotherapy and radiotherapy are dominated in the treatment of glioma, but cancer cells still cannot be completely killed, and there are toxic and side effects[6]. Therefore, it is urgently necessary to deeply understand the internal mechanism of glioma, so as to find potential therapeutic targets.

A micro ribonucleic acid (miRNA) is a kind of non-coding RNA with about 22 nt in length.

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Studies have demonstrated that it regulates the target messenger RNA (mRNA) expression at the post-transcriptional level through complementary pairing with the 3'-untranslated region (UTR) of the target gene. At the same time, miRNAs are involved in various cellular processes, including signal transduction, cell cycle progression, cell metabolism, proliferation and apoptosis of cancer cells, etc.,[7] and there is also abnormal expression of miRNA in glioma cells, indicating that it can regulate the occurrence and development of glioma[8]. With the rise of high-throughput sequencing and computational biology, up to hundreds of miRNA families have been identified, among which miR-126, located in the intron of the epidermal growth factor-like domain-containing protein 7 (EGFL7), has been proved to be able to inhibit proliferation, migration and invasion of a variety of cancer cells (such as lung cancer, oral cancer and breast cancer). This anti-cancer effect is often realized via regulating signaling pathways, thereby inhibiting the expressions of various oncogenes[9,10]. In addition, miR-126 possibly affects the motility and migration of glioma cells through cytoskeleton-associated proteins, thus inhibiting the malignant progression of glioma[11]. In conclusion, miR-126 may be a novel biomarker for the prevention and diagnosis of a variety of cancers.

This study aims to investigate the correlation between miR-126 expression and glioma pathology, and the internal molecular mechanism of miR-126 in affecting the proliferation, apoptosis, migration and invasion of glioma cells through regulating phosphatase and tensin homolog deleted on chromosome ten/phosphatidylinositol 3-kinase/protein kinase B (PTEN/PI3K/Akt) and mouse double minute 2 homolog (MDM2)-p53 signaling pathways. The prognostic analysis was performed, so as to provide a theoretical basis for screening potential therapeutic and prognostic biological targets of glioma.

Patients and Methods

Patients

A total of 44 patients diagnosed with glioma in Zhangzhou Affiliated Hospital of Fujian Medical University from March 2011 to July 2014 were collected, including 24 males and 20 females with an average age of (50.12±12.63) years old. All patients had no history of other diseases and received no radiotherapy, chemotherapy or hormone therapy. All cancer tissue samples were histopathologically confirmed and divided into grade I (n=6, fibrous astrocytoma), II (n=14, diffuse astrocytoma, oligodendroglioma, protoplasmic astrocytoma, CNS cytoma and ependymoma), III (n=15, anaplastic astrocytoma, anaplastic cytoma and anaplastic ependymoma) and IV (n=9, neuroblastoma and medulloblastoma) by three pathologists according to the World Health Organization (WHO) classification. For the convenience of the study, samples in grade I and II were classified into low-grade malignancy group (LG) and high-grade malignancy group (HG). In addition, 20 cases of normal brain tissues from the deaths due to cerebral trauma were selected as control group, and the samples were collected and quickly stored in liquid nitrogen. All collection processes were agreed by patients and our study was Ethically approved by our institution; further, all patients signed the confidentiality agreement.

Main Reagents

Glioma U87MG cell line (astrocytoma and glioblastoma, grade-IV malignant tumor) and normal human astrocyte cell line (NHA) were preserved in our laboratory. Roswell Park Memorial Institute (RPMI)-1640 medium and fetal bovine serum (FBS) (Rockville, MD, USA), TRIzol Reagent, Prime Script® RT reagent Kit with gDNA Eraser and SYBR®Premix Ex Taq™ II (TaKaRa, Dalian, China), miR-126 mimics/control and miR-126 inhibitor/control (Ambion, Thermo Fisher, Waltham, MA, USA), Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA), methyl thiazolyl tetrazolium (MTT) (Sigma-Aldrich, St. Louis, MO, USA), Annexin V-fluorescein isothiocyanate (FITC) apoptosis assay kit (eBioscience, San Diego, CA, USA), transwell artificial basement membrane (Pharmacia-Biotech, Uppsala, Sweden), mouse anti-human PTEN, PI3K, p-Akt, MDM2, p53 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibodies, horseradish peroxidase (HRP) rabbit anti-mouse secondary antibodies (CST, Danvers MA, USA), and primers (Sangon Biotech, Shanghai, China).

Methods

Cell Culture and Transfection

Glioma U87MG and NHA cell lines were cultured in the RPMI-1640 medium containing 10% FBS and 1% penicillin-streptomycin double antibodies in an incubator with 5% CO_2 and 95% humidity at 37°C. At 1 d before transfection,

U87MG cells in the logarithmic phase were inoculated into a culture dish until 50-70% cells were fused. The medium was replaced with the RPMI-1640 medium without antibody and serum in the first 2 h. The miR-126 mimics, miR-negative control mimics (NC mimics), miR-126 inhibitor and miR-negative control inhibitor (NC inhibitor) were mixed with liposome and added into the culture dish, respectively. Next, the mixture was placed into the incubator, and the medium was replaced with normal RPMI-1640 medium after 6 h. The control group without any treatment was also set up. Cells were collected after 48 h or 72 h for subsequent experiments.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted from tissues or cells, complementary deoxyribonucleic acid (cDNA) was synthesized and qRT-PCR was performed according to instructions of the TRIzol Reagent, Prime Script® RT reagent Kit with gDNA Eraser and SYBR® Premix Ex Taq™ II kits, respectively, followed by detection using the CFX-96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). After amplification, data were collected and processed using the comparative cycle threshold method with β-actin as the internal reference. Primer sequences are shown in Table I.

Cell Proliferation Analysis via MTT Assay

After transfection for 48 h, cells in each group were digested with 0.25% trypsin and cultured in a 96-well plate (1.0×10^4 cells/well) containing Dulbecco's modified Eagle's medium (DMEM) and 10% fetal bovine serum (FBS). After 12, 24, 48 and 72 h, MTT solution was added for incubation for 4 h. Next, the supernatant was discarded, and dimethyl sulfoxide (DMSO) was added and shaken for 10 min. Finally, the absorbance of each well was measured at a wavelength of 570 nm.

Apoptosis Analysis

After stable transfection for 48 h, cells were subjected to Annexin V-FITC/propidium iodide (PI) staining according to the apoptosis assay kit, and the early and late apoptotic rates were analyzed using a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Table I. qRT-PCR primer sequences.

Primer	Sequence (5′-3′)
miR-126-F	CGCGCCGTACCGTGAGTAA
miR-126-R	GTGCAGGGTCCGAGGT
ACTB-F	ACTCGTCATACTCCTGCT
ACTB-F	GAAACTACCTTCAACTCC

Wound Healing Assay

A marker pen and a ruler were used to scratch the back of the 6-well plate evenly and horizontally at an interval of 0.5-1 cm, and at least 3 horizontal lines were made per well. After transfection for 48 h, cells were cultured in the 6-well plate, and horizontal lines were scratched perpendicularly using a spearhead on the next day. The plate was washed with phosphate-buffered saline (PBS) for 3 times to remove the fallen cells, and the serum-free medium was added for culture, followed by microphotography every 24 h.

Transwell Invasion Analysis

After transfection for 24 h, cells were collected and resuspended using the serum-free medium until the density of 5×10^5/mL. 200 mL cell suspension was added into the upper chamber of the basement membrane, while the lower chamber was filled with medium containing 10% fetal bovine serum (FBS), followed by incubation for 24 h. The non-invasive cells were wiped off with a cotton swab, and those invading the lower membrane were fixed in paraformaldehyde for 20 min and stained with 0.1% crystal violet. Six fields of view were randomly selected in each well for microscopic observation and counting of stained cells.

Western Blotting

After transfection for 72 h, cells were collected and lysed with RIPA, and the total protein was extracted and quantified. An equal amount of 50 μg protein was separated via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), electro-transferred onto a polyvinylidene fluoride (PVDF) membrane and sealed at room temperature for 1 h, followed by incubation using PTEN, PI3K, p-Akt, MDM2, p53 and GAPDH primary antibodies (1:1000) at 4°C overnight. On the next day, the membrane was washed with Tris-buffered saline and Tween 20 (TBST) for 3 times, followed by incubation with HRP-coupled secondary antibody (1:2000) at room temperature for 2 h. After the membrane was washed again with TBST, the color was developed using the ECL detection system (Thermo Fisher Scientific, Waltham, MA, USA), and the gray value of

band was calculated using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). Target protein/β-actin gray scale indicated the protein level.

Prognostic Analysis

According to the median expression level of miR-126, 44 glioma patients were divided into high-expression group (n=22, miR-126 expression level >median) and low-expression group (n=22, miR-126 expression level <median). Patients were followed-up for 5 years and the survival condition was recorded. Kaplan-Meier survival analysis and log-rank test were performed to analyze the correlation between miR-126 expression level and prognosis of glioma patients.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data were presented as mean ± standard deviation. One-way analysis of variance and two-tailed t-value test were performed between groups and the post-hoc test was LSD test. $p<0.05$ suggested that the difference was statistically significant.

Results

Expression of miR-126 was Abnormally Low in Glioma Cells

The expression level of miR-126 in cancer tissues of glioma patients and normal brain tissues, NHA and U87MG cells, was detected via qRT-PCR. Results showed that the expression of miR-126 was abnormally low in glioma patients. There was no significant difference in LG group compared with that in control group, but the expression was significantly decreased in HG group compared with that in control group. Besides, the expression level of miR-126 in glioma U87MG cells was significantly lower than that in NHA (Figure 1 A, B).

To further investigate the correlation between miR-126 and pathological mechanism of glioma, U87MG cells were transfected with miR-126 mimics and inhibitor and its NC, and the miR-126 expression was quantified via qRT-PCR. Results revealed that the miR-126 expression was significantly increased and decreased compared with that in NC group after transfection with miR-126 mimics and inhibitor, respectively (Figure 1 C, D).

MiR-126 Inhibited in-vitro Proliferation of Glioma Cells

The *in-vitro* proliferation of U87MG cells after transfection was detected via MTT assay. Results revealed that cell proliferation was obviously inhibited compared with that in NC group after up-regulation of miR-126 expression, but it was significantly promoted compared with that in NC group after down-regulation of miR-126 expression (Figure 2).

MiR-126 Induced Apoptosis of Glioma Cells

Annexin V/PI double staining was performed to detect the apoptosis of U87MG cells at 48 h after transfection. Results manifested that the apoptotic rate of U87MG cells after overexpression of miR-126 was significantly higher than that in NC group, but it was significantly reduced compared with that in NC group after down-regulation of miR-126 expression (Figure 3).

MiR-126 Inhibited in-vitro Migration of Glioma Cells

Wound healing assay was performed to detect the *in-vitro* migration of U87MG cells after transfection. Results demonstrated that the *in-vitro* migration rate of U87MG cells was remarkably reduced compared with that in NC group after up-regulation of miR-126 expression, but it was remarkably increased compared with that in NC group after down-regulation of miR-126 expression (Figure 4).

MiR-126 Inhibited in-vitro Invasion of Glioma Cells

In-vitro invasion of U87MG cells after transfection was detected via transwell invasion assay. Results showed that the number of U87MG cells passing through the membrane was significantly decreased compared with that in NC group after up-regulation of miR-126 expression, but it was significantly increased compared with that in NC group after down-regulation of miR-126 expression (Figure 5).

MiR-126 Inhibited Glioma via Targeted Regulation of PTEN/PI3K/Akt and MDM2-p53 Pathways

Levels of related proteins in U87MG cells after transfection were detected via Western blotting. Results displayed that in the PTEN/PI3K/Akt pathway, PI3K and p-Akt protein expressions were remarkably decreased compared with those in NC

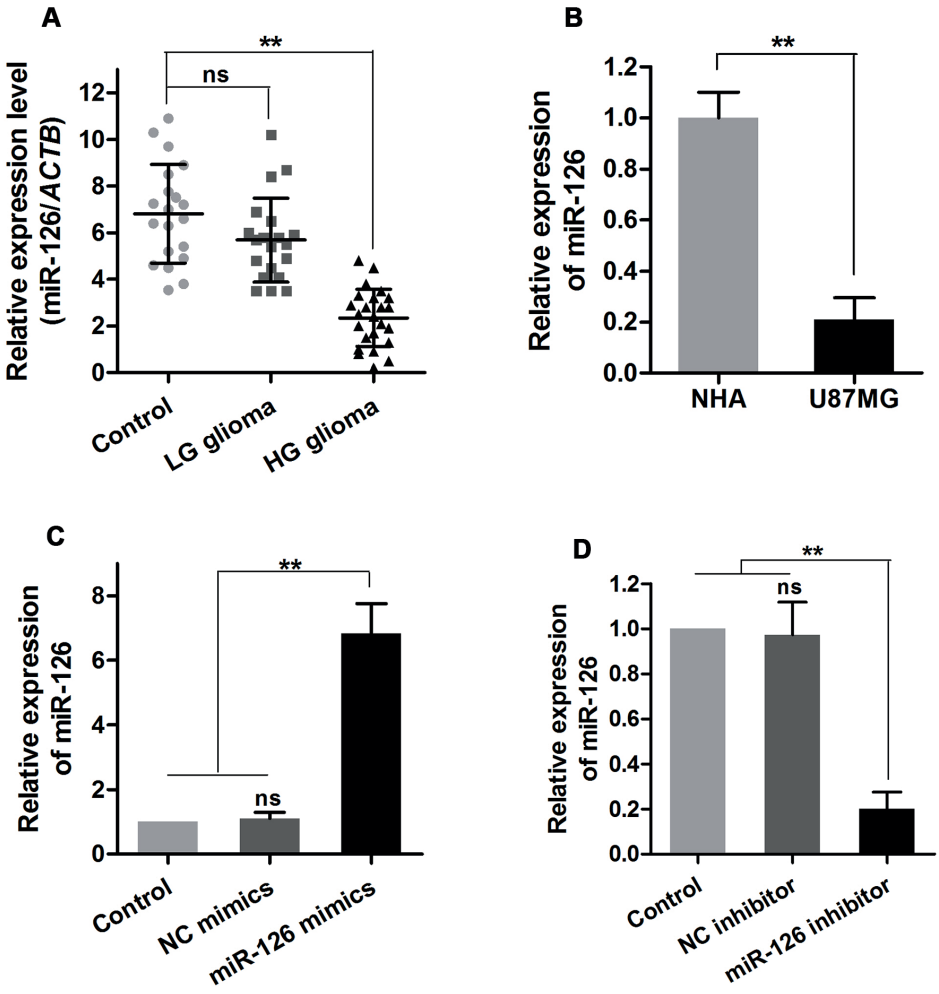

Figure 1. Expression level of miR-126 in glioma tissues and cells, and its expression after transfection. ***A,*** miR-126 level in tissues of patients with different degrees of glioma, ***B,*** miR-126 level in U87MG cells and NHA, ***C,*** miR-126 level after transfection with mimics, ***D,*** miR-126 level after transfection with inhibitor. Results of qRT-PCR show that the miR-126 expression in HG group and U87MG cells is significantly lower than that in control group. After transfection with mimics and inhibitor, the expression level of miR-126 is significantly increased and decreased compared with that in NC group, respectively. ***p*<0.01 *vs.* Control and NC, nsp>0.05 *vs.* Control.

group after overexpression of miR-126, but the PTEN protein level was remarkably increased. In the MDM2-p53 pathway, the MDM2 protein level was obviously decreased, but the p53 protein expression was obviously increased. The opposite results were obtained after down-regulation of miR-126 expression, and there were extremely significant differences (Figure 6).

Prognosis of Patients with a Low miR-126 Level Was Poor

The correlation between miR-126 expression and prognosis of glioma patients was analyzed using Kaplan-Meier survival analysis and log-rank test. Results demonstrated that the survival rate of patients with a low expression level of miR-126 was lower than that of patients with a high expression level of miR-126, and there was a significant difference between the two groups, so the miR-126 level was a factor affecting the prognosis of patients (Figure 7).

Discussion

Glioma, especially glioblastoma, is the most common tumor in the central nervous system, which is characterized by extremely high morbi-

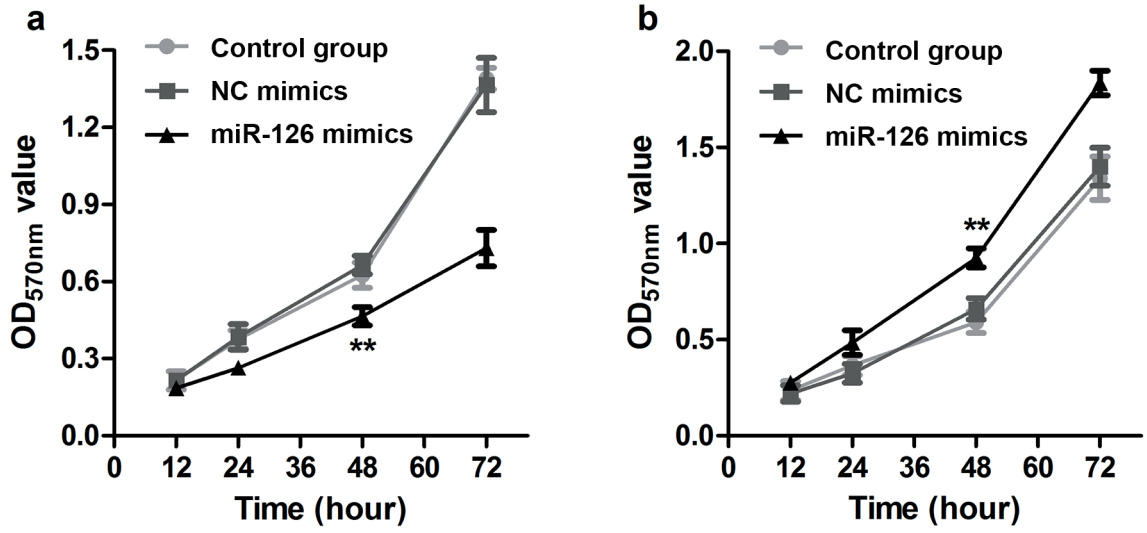

Figure 2. Effect of transfection with miR-126 mimics or inhibitor on in-*vitro* proliferation of U87MG cells. ***A***, U87MG proliferation after transfection with miR-126 mimics, ***B***, U87MG proliferation after transfection with miR-126 inhibitor. Results of MTT assay reveal that up-regulation or down-regulation of miR-126 expression can significantly inhibit or promote the *in-vitro* proliferation of glioma U87MG cells. ** $p<0.01$ *vs.* Control and NC.

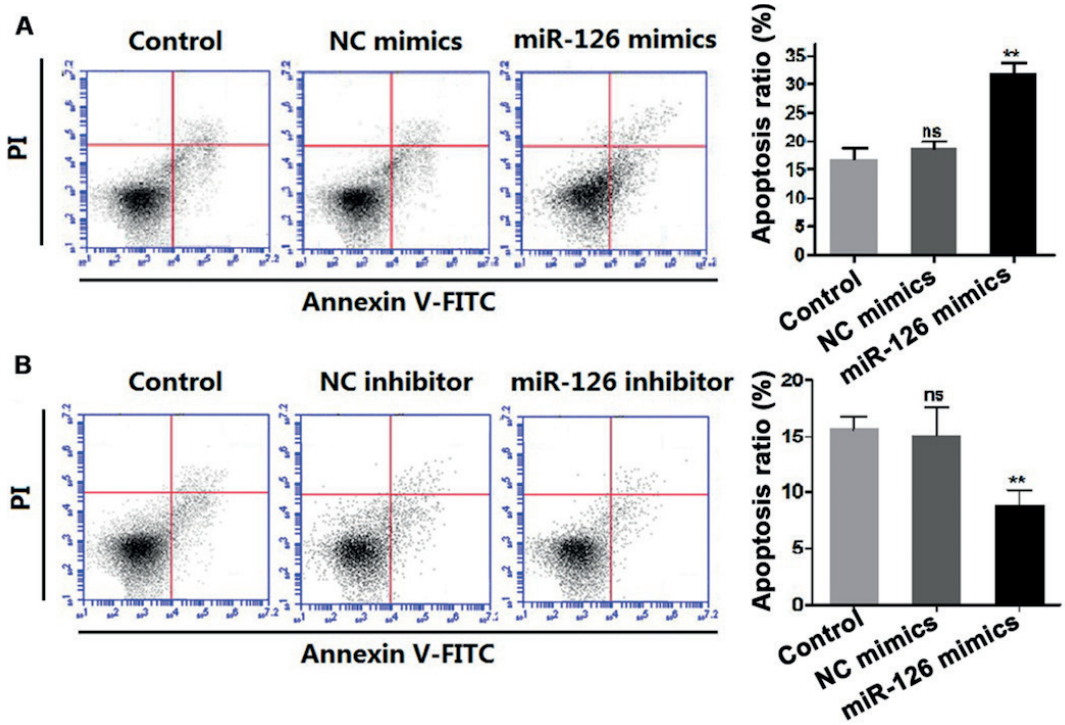

Figure 3. Effect of transfection with miR-126 mimics or inhibitor on apoptosis of U87MG cells. ***A***, apoptosis of U87MG cells after transfection with miR-126 mimics, ***B***, apoptosis of U87MG cells after transfection with miR-126 inhibitor. Results of apoptosis assay manifest that overexpression or down-regulation of miR-126 expression can significantly induce or decrease the apoptosis of glioma U87MG cells. ** $p<0.01$, *vs.* Control and NC, ns $p>0.05$ *vs.* Control.

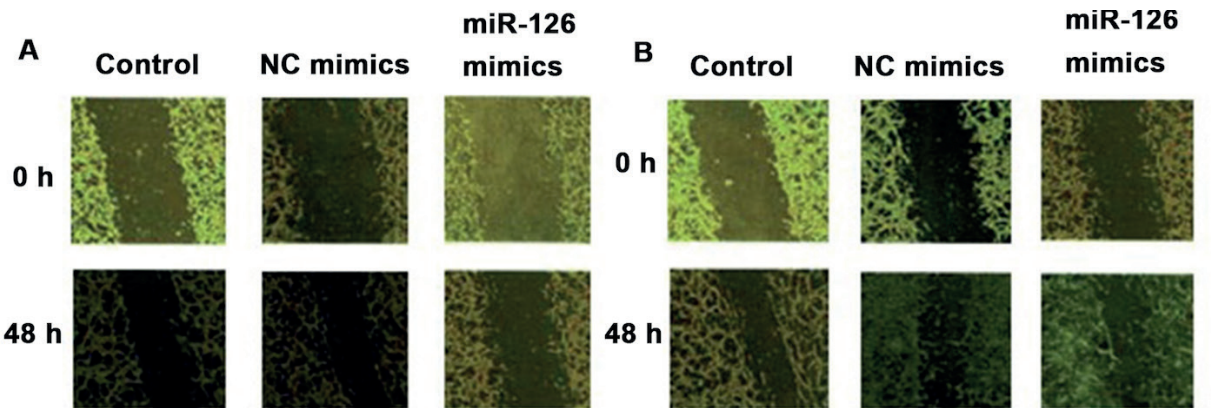

Figure 4. Effect of transfection with miR-126 mimics or inhibitor on migration of U87MG cells. ***A,*** migration of U87MG cells after transfection with miR-126 mimics, ***B,*** migration of U87MG cells after transfection with miR-126 inhibitor. Results of wound healing assay manifest that overexpression or down-regulation of miR-126 expression can significantly inhibit or promote the migration of glioma U87MG cells.

dity and mortality rates, poor prognosis and low sensitivity to radiotherapy/chemotherapy[12]. Furnari et al[13] have demonstrated that the high mortality rate of glioma is mainly due to its strong capacities of invasion and migration, so it is easy to infiltrate and migrate in a large area to surrounding brain tissues, thus accelerating the course of the disease. Patients with the most malignant glioblastoma can only survive for 14 months on average, so the internal molecular mechanism of the occurrence, progression and invasion of glioma is already clarified, helping the development of more effective treatment strategies, which is a research hotspot currently. A miRNA is a kind of

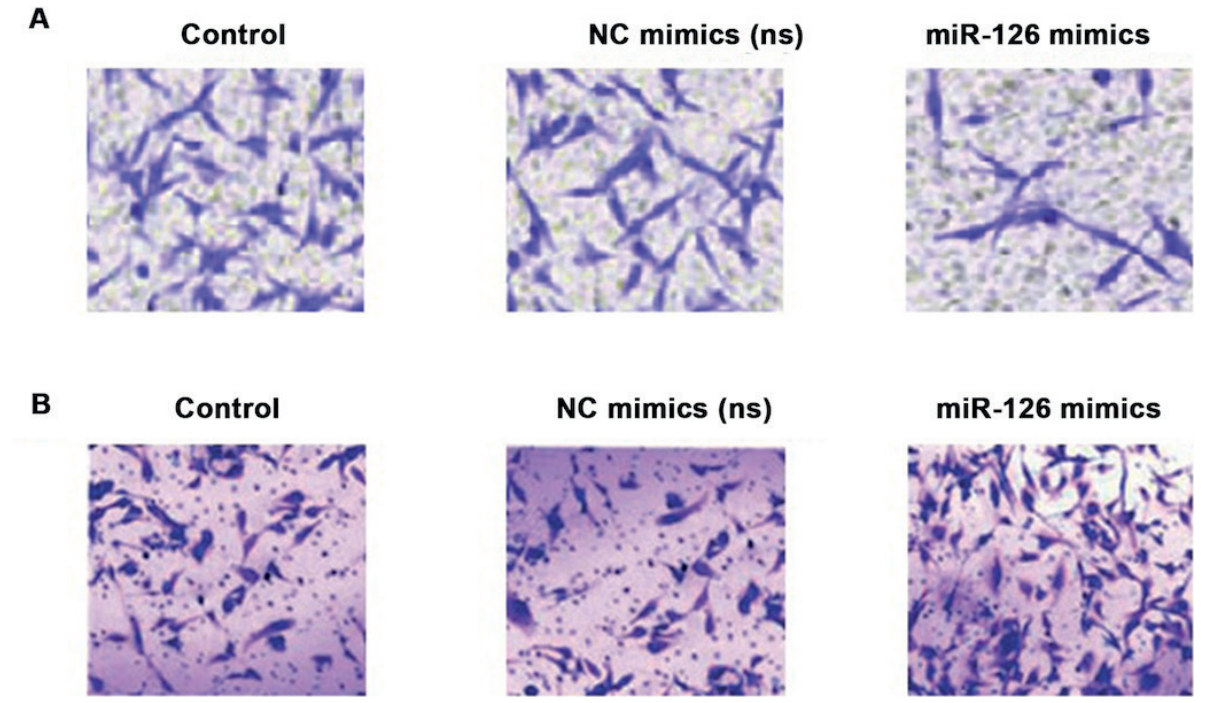

Figure 5. Effect of transfection with miR-126 mimics or inhibitor on invasion of U87MG cells. ***A,*** invasion of U87MG cells after transfection with miR-126 mimics, ***B,*** invasion of U87MG cells after transfection with miR-126 inhibitor. Results of cell invasion assay display that up-regulation or down-regulation of miR-126 expression can significantly inhibit or promote the invasion of glioma U87MG cells.

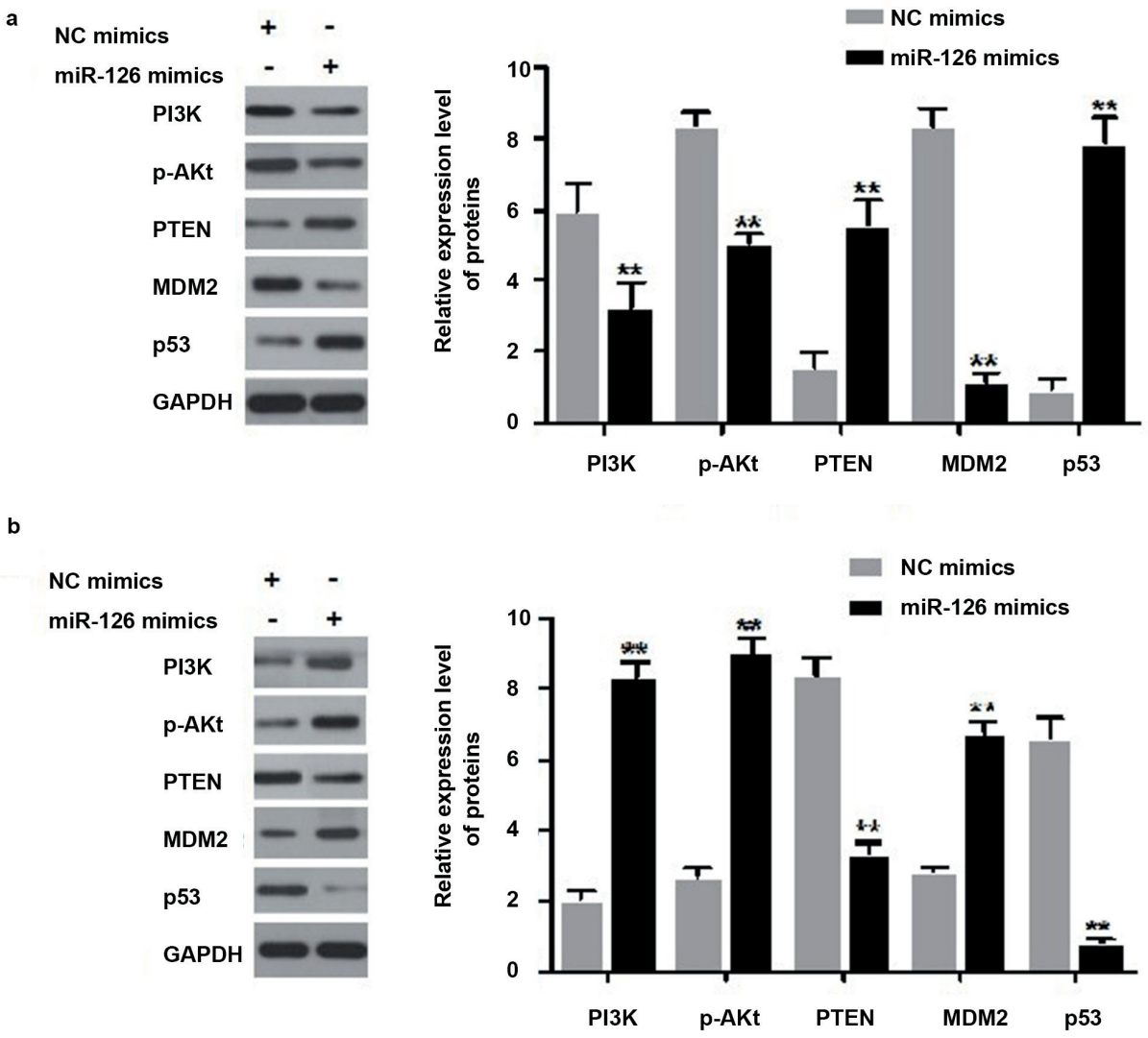

Figure 6. Effect of transfection with miR-126 mimics or inhibitor on related protein levels in U87MG cells. ***A,*** changes in protein levels after transfection with miR-126 mimics, ***B,*** changes in protein levels after transfection with miR-126 inhibitor. Western blotting results reveal that up-regulation of miR-126 expression can significantly reduce PI3K, p-Akt and MDM2 protein expressions and significantly increase PTEN and p53 protein levels, and *vice versa*. ** $p<0.01$ *vs.* NC.

novel regulatory molecule, which has been proved to be widely involved in the development, differentiation, apoptosis and proliferation of various human tumor cells. MiRNAs can serve as both tumor suppressor gene and oncogene, and they often bind to target genes to regulate the activity of a variety of signaling pathways, thereby regulating the cancer cell events[14]. It is reported that the expression of miR-126 is abnormally low in a variety of human cancer cells, which also exerts a potential tumor-inhibiting effect. Moreover, miR-126 can negatively regulate the cancer cell proliferation, migration and invasion through binding to specific genes, including VEGF, insulin receptor substrate 1 and PIK3R2[15,16]. Signaling pathways are important information transmission pathways in signal transduction, gene expression, cell metabolism and activity, whose abnormal activation will lead to abnormal expressions of downstream target genes that play important roles in the occurrence and development of tumors. According to researches, miR-126 can affect the activity of PTEN/PI3K/Akt pathway through negatively regulating PIK3R2 gene, thereby inhibiting *in-vitro* proliferation, migration and invasion capacities of non-small cell lung cancer[17]. Besides, miR-126, throu-

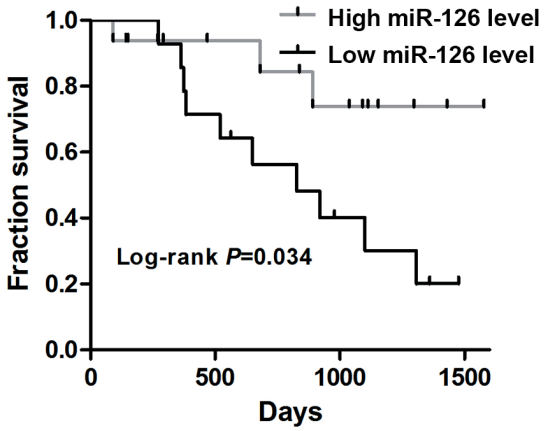

Figure 7. Kaplan-Meier survival curve. Prognostic analyses show that the prognosis of patients with a low expression level of miR-126 is poorer.

gh targeted binding to 3'-UTR of KRAS, can also regulate the ERK pathway to inhibit proliferation and invasion of glioma cells[18]. PTEN/PI3K/Akt and MDM2-p53 are important pathways in regulating cell events, among which PTEN can inhibit the activity of PI3K/Akt pathway and the activation of cancer cells through the dephosphorylation of PI3P, and p53 has the tumor suppressive activity and it is inactivated in a variety of cancer cells via point mutation. Besides, MDM2 is a kind of oncogene playing an important role in cancer progression. Researches showed that overexpression of MDM2 can inactivate p53, both of which regulate tumor proliferation and apoptosis synergistically[19-21]. There are a large number of studies indicating that miRNAs can regulate the occurrence and development of various tumors and cell events through PTEN/PI3K/Akt and MDM2-p53 pathways, but there have been no reports on miR-126 in regulating the progression of glioma through this pathway. In this study, it was found that the miR-126 expression was significantly down-regulated in tissues and cells of patients with malignant glioma, which is consistent with results in previous studies. The miR-126 expression was up-regulated and down-regulated in U87MG cells, and the *in-vitro* proliferation, apoptosis, migration and invasion capacities were detected. At the same time, related protein levels in PTEN/PI3K/Akt and MDM2-p53 pathways were detected. Results revealed that the up-regulation of miR-126 level could obviously inhibit *in-vitro* proliferation, migration and invasion of glioma cells, and significantly induce *in-vitro* apoptosis. Meanwhile, PI3K, p-Akt and MDM2 protein levels in U87MG cells were significantly decreased after overexpression of miR-126, but PTEN and p53 protein expressions were remarkably increased, indicating that miR-126 can inhibit *in-vitro* proliferation and migration and promote apoptosis of glioma cells through targeted regulation of PTEN/PI3K/Akt and MDM2-p53 pathways. In addition, prognostic analysis manifested that the prognosis of patients with a low miR-126 level was poorer, suggesting that the difference in miR-126 level is a prognostic marker in the clinical diagnosis of glioma.

Conclusions

We demonstrated that the miR-126 expression was abnormally low in glioma cells, and miR-126 inhibited the course of glioma through targeted regulation of PTEN/PI3K/Akt and MDM2-p53 pathways, which, therefore, can be used as a new potential biomarker for the diagnosis, treatment and prognosis of glioma.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SC and WC contributed equally to perform the data analyses and wrote the manuscript. XD was responsible for qRT-PCR. AG helped with MTT assay. HC performed apoptosis analysis. GL and RL analyzed and interpreted Western blotting and prognostic analysis. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

The study was approved by the Ethics Committee of Zhangzhou Affiliated Hospital of Fujian Medical University and informed consents were signed by the patients and/or guardians.

Consent for publication

Informed consents were signed by the patients and/or guardians.

References

1) Goodenberger ML, Jenkins RB. Genetics of adult glioma. Cancer Genet 2012; 205: 613-621.
2) Koshy M, Villano JL, Dolecek TA, Howard A, Mahmood U, Chmura SJ, Weichselbaum RR, McCarthy BJ. Improved survival time trends for glioblastoma using the SEER 17 population-based registries. J Neurooncol 2012; 107: 207-212.
3) Zhao H, Cai W, Su S, Zhi D, Lu J, Liu S. Allergic conditions reduce the risk of glioma: a meta-analysis based on 128,936 subjects. Tumour Biol 2014; 35: 3875-3880.
4) Su HY, Lin ZY, Peng WC, Guan F, Zhu GT, Mao BB, Dai B, Huang H, Hu ZQ. MiR-448 downregulates CTTN to inhibit cell proliferation and promote apoptosis in glioma. Eur Rev Med Pharmacol Sci 2018; 22: 3847-3854.
5) Adel Fahmideh M, Schwartzbaum J, Frumento P, Feychting M. Association between DNA repair gene polymorphisms and risk of glioma: a systematic review and meta-analysis. Neuro Oncol 2014; 16: 807-814.
6) Wang F, Huang Q, Zhou L Y. Analysis of the treatment of gliomas with SEC therapy combined with radiochemotherapy. Eur Rev Med Pharmacol Sci 2015; 19: 2400-2405.
7) Wu L, Belasco JG. Let me count the ways: mechanisms of gene regulation by miRNAs and siRNAs. Mol Cell 2008; 29: 1-7.
8) Gabriely G, Wurdinger T, Kesari S, Esau CC, Burchard J, Linsley PS, Krichevsky AM. MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators. Mol Cell Biol 2008; 28: 5369-5380.
9) Ebrahimi F, Gopalan V, Smith RA, Lam AK. miR-126 in human cancers: clinical roles and current perspectives. Exp Mol Pathol 2014; 96: 98-107.
10) Wang S, Wang X, Guo Q, Wang G, Han X, Li X, Shi ZW, He W. MicroRNA-126 overexpression inhibits proliferation and invasion in osteosarcoma cells. Technol Cancer Res Treat 2016; 15: NP49-59.
11) Xu Y, Xu W, Lu T, Dai Y, Liang W. miR-126 affects the invasion and migration of glioma cells through GATA4. Artif Cells Nanomed Biotechnol 2017; 45: 1-7.
12) Luan Y, Zuo L, Zhang S, Wang G, Peng T. MicroRNA-126 acts as a tumor suppressor in glioma cells by targeting insulin receptor substrate 1 (IRS-1). Int J Clin Exp Pathol 2015; 8: 10345-10354.
13) Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C, Chin L, DePinho RA, Cavenee WK. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev 2007; 21: 2683-2710.
14) Croce CM, Calin GA. miRNAs, cancer, and stem cell division. Cell 2005; 122: 6-7.
15) Liu B, Peng XC, Zheng XL, Wang J, Qin YW. MiR-126 restoration down-regulate VEGF and inhibit the growth of lung cancer cell lines in vitro and in vivo. Lung Cancer 2009; 66: 169-175.
16) Crawford M, Brawner E, Batte K, Yu L, Hunter MG, Otterson GA, Nuovo G, Marsh CB, Nana-Sinkam SP. MicroRNA-126 inhibits invasion in non-small cell lung carcinoma cell lines. Biochem Biophys Res Commun 2008; 373: 607-612.
17) Song L, Li D, Gu Y, Wen ZM, Jie J, Zhao D, Peng LP. MicroRNA-126 targeting PIK3R2 inhibits NSCLC A549 cell proliferation, migration, and invasion by regulation of PTEN/PI3K/AKT pathway. Clin Lung Cancer 2016; 17: e65-e75.
18) Li Y, Li Y, Ge P, Ma C. MiR-126 regulates the ERK pathway via targeting KRAS to inhibit the glioma cell proliferation and invasion. Mol Neurobiol 2017; 54: 137-145.
19) Liu YZ, Wu K, Huang J, Liu Y, Wang X, Meng ZJ, Yuan SX, Wang DX, Luo JY, Zuo GW, Yin LJ, Chen L, Deng ZL, Yang JQ, Sun WJ, He BC. The PTEN/PI3K/Akt and Wnt/beta-catenin signaling pathways are involved in the inhibitory effect of resveratrol on human colon cancer cell proliferation. Int J Oncol 2014; 45: 104-112.
20) Zhang Z, Li M, Wang H, Agrawal S, Zhang R. Antisense therapy targeting MDM2 oncogene in prostate cancer: effects on proliferation, apoptosis, multiple gene expression, and chemotherapy. Proc Natl Acad Sci U S A 2003; 100: 11636-11641.
21) Toledo F, Wahl GM. Regulating the p53 pathway: *in vitro* hypotheses, in vivo veritas. Nat Rev Cancer 2006; 6: 909-923.