EXPRESSION OF MIR-98 IN GLIOMA AND THE PROMOTION EFFECT ON THE INVASION OF GLIOMA CELLS BY MIR-98 DOWNREGULATION

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ABSTRACT

Objective*: To detect the expression of miR-98 in glioma tissues and to investigate the effect of miR-98 on the invasion and migration of the human glioma cell line U251.*

Methods*: The expressions of miR-98 in glioma tissues and in normal brain tissues were detected by real-time fluorescent quantitative PCR. After cotransfection with the miR-98 inhibitor and the wild-type inhibitor of nuclear factor kappa B kinase epsilon (IKBKE) 3'-untranslated region (UTR) or the mutation type IKBKE 3'-UTR recombination vector, the specific binding ability of miR-98 to 3'-UTR in the IKBKE gene was examined by the luciferase gene reporter system. The expression levels of miR-98, IKBKE mRNA and IKBKE protein in glioma cell line U251 after transfection with the miR-98 inhibitor were measured by real-time fluorescent quantitative PCR and Western blotting, respectively. The abilities of migration and the invasion of U251 cells after transfection with the miR-98 inhibitor were detected by Transwell assay. The expression of miR-98 in U251 cells after transfection with IKBKE siRNA was detected by real-time fluorescent quantitative PCR.*

Results*: The expression of miR-98 in glioma tissues was lower than that in normal brain tissues ($P < 0.05$). The fluorescence intensity of U251 cells cotransfected with the miR-98 inhibitor and wild-type IKBKE recombination vector was improved ($P < 0.05$). The expression of miR-98 was downregulated, and the expression levels of IKBKE mRNA and protein were upregulated in U251 cells after transfection with the miR-98 inhibitor (all $P < 0.05$). While the abilities of migration and the invasion of U251 cells after transfection with the miR-98 inhibitor were increased (both $P < 0.05$), there was no change in the expression of miR-98 in U251 cells after inhibition of the IKBKE expression ($P > 0.05$).*

Conclusions*: The expression of miR-98 is low in glioma tissues. The miR-98 inhibitor may promote the invasion and migration of glioma U251 cells by regulation of the IKBKE expression.*

Keywords*: Glioma, MicroRNAs, Cell migration assays, Neoplasm invasiveness.*

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Introduction

In recent years, cardiovascular diseases (CVD) havGlioma, a common primary tumor of the central nerve system, is characterized by invasive growth and recurrence[(1-2)]. With invasive and rapid growth, even if glioma can be removed by surgery and with interventions by several regimens, such as radiotherapy and chemotherapy, it will be recurrent and metastasize distantly in a short time, thereby threatening the patient's life; thus, glioma has been a difficult issue in neurosurgery. Glioma is a polygenic abnormal disease in nature. Overexpressed proto-oncogenes and the mutation or deficiency of anti-oncogenes make tumor cells escape from the regulation mechanism of normal growth[(3)].

Currently, therapies focusing on genetic abnormality related to glioma development have become a heavily researched subject.

Studies have revealed that microRNA (miR)-98, a member of the Let-7 family, is downregulated in multiple cancers (such as prostate cancer, esophageal squamous cell carcinoma, and recurrent nasopharyngeal carcinoma)[(4-6)]. It was reported that miR-98 inhibits the proliferation, migration and invasion of hepatocellular cancer cells through targeting CTHRC1[(7)]. miR-98 controls the growth and metastasis of oral squamous cell carcinoma by restraining the expression of the IGF1R protein[(8)]. Functionally, miR-98 inhibits the proliferation, invasion and epithelial-mesenchymal transition of tumor cells through important targeting regulatory factors such as SALL4, IGF1R and PAK1[(9, 10)].

However, the role and mechanism of miR-98 in glioma is not clear to date. This study investigates the expression of miR-98 in glioma tissues and its effect on the migration and invasion of glioma U251 cells to provide a reference for further research on the pathogenesis and new therapeutic targets of glioma.

Materials and methods

Sample collection

A total of 94 glioma samples, which were surgically removed from 94 glioma patients and were confirmed by pathological examination, and 13 normal brain tissues, which were removed from 13 patients with traumatic brain injury in the Department of Neurosurgery of the Second Affiliated Hospital of Nanchang University from August 2017 to August 2018, were collected for the study. Among glioma patients, there were 53 males and 41 females, aged 21 to 74 years old with a mean age of 42.38±9.28 years. The patients were graded according to the WHO (2007) classification of neurological tumors: 41 patients with low-grade glioma, including 29 low-grade astrocytoma (WHOI~II), 12 oligodendroglioma (WHO class II), and 53 high-grade glioma, including 22 anaplastic astrocytoma (WHO III) and 31 glioblastoma (WHOIV). None of the patients received chemotherapy before surgery. This study obtained informed consent from all participants and was approved by the medical ethics committee of the Second Affiliated Hospital of Nanchang University.

Main reagents

Fetal bovine serum was purchased from HyClone, USA and DMEM from Gibco, USA. The TRIzol reagent, reverse transcription kit, real-time PCR assay kit and Lipofect AMINE 2000 were purchased from TaKaRa, Japan. The PCR primer, miR-98 inhibitor (5'-AACAAUACAACUUACUA CCUCA-3') and its negative inhibitor control (5'-CAGUACUUU-UGUGUAGUACAA-3'), internal control plasmid pc DNA3/EGFP containing enhanced green fluorescent protein (EGFP), recombinant vector pc DNA3/EGFP-wt IKBKE 3'-UTR containing the wild-type (wt) inhibitor of the nuclear factor kappa B kinase epsilon (IKBKE) gene 3'-end noncoding region (untranslated region, UTR) sequence, the mutation type (mut) IKBKE gene 3'-UTR recombinant vector pc DNA3/EGFP-mut IKBKE 3'-UTR, and the luciferase reporter gene assay kits were purchased from Guangzhou Ruibo Biotechnology Co., Ltd.

The total cell protein extraction kit and the BCA protein quantification kit were purchased from Shanghai Jikai Biotechnology Co., Ltd., rabbit anti-human IKBKE monoclonal antibody and rabbit anti-human β-actin (internal control) polyclonal antibody from American CST company, and horseradish peroxidase-labeled goat anti-rabbit Ig G (secondary antibody) and chemiluminescence kit from Wuhan Boster Biotechnology Co., Ltd. The Transwell chamber and the Transwell chamber containing Matrigel were purchased from Corning, USA. IKBKE siRNA (5'-GCUGAACCACCA-GAACAUCTT-3') and its negative control siRNA control (5'-GTGGTTACCAUTATTUGAA-3') were provided by Wuhan Boster Biotechnology Co., Ltd.

miR-98 expression in glioma tissues detected by real-time fluorescent quantitative PCR

Total RNA was extracted from glioma tissues and normal brain tissues by the TRIzol method. The absorbance at D260 nm and D280 nm were detected by UV spectrophotometer, and the concentration and purity of total RNA were determined. RNA was reverse transcribed into cDNA by using a reverse transcription kit, and the PCR was amplified following the instructions of a real-time fluorescent quantitative PCR detection kit with a templet of this c DNA. The upstream primer sequence of miR-98 was 5'-GGGGTGAGGTAGTAAGTT-GT-3', and the downstream primer sequence was 5'-TGCGTGTCGT-GGAGTC-3'. The upstream primer sequence of internal control U6 was 5'-CTCGCTTCGGCAGCACA-3', downstream, and its downstream primer sequence was 5'-AACGCTTCACGAATTTGCGT-3'. The PCR reaction conditions: 95 °C for 30 s, 95 °C for 5 s, and 60 °C for 34 s, with 40 cycles in total. The relative expression level of miR-98 was expressed as a 2-ΔΔCt value.

Cells and cell culture

Human glioma U251 cells were preserved in our laboratory and were cultured in a DMEM medium containing 10% fetal bovine serum and

were placed in a 5% CO2 and 95% humidity incubator at 37 °C.

Regulation of miR-98 to the IKBKE gene detected by the luciferase reporter detection system

Glioma U251 cells in the logarithmic growth phase were seeded in 6-well plates (5 x 104 cells/well). The cells were cultured in DMEM without antibiotics but with 10% fetal bovine serum. When cell confluence was approximately 60%, the miR-98 inhibitor and pc DNA3/EGFP-wt IKBKE, inhibitor control and pc DNA3/EGFP-wt IKBKE 3'-UTR, miR-98 inhibitor and pc DNA3/EGFP-mut IKBKE3'-UTR, and inhibitor control and pc DNA3/EGFP-mut IKBKE 3'-UTR were cotransfected to U251 cells following the instructions of Lipofect AMINE2000, respectively. Forty-eight hours after transfection, the relative fluorescence intensity values of the above four groups of cells were measured following the instructions of the luciferase reporter gene assay kit.

Expression of miR-98 and IKVKE mRNA in glioma U251 cells after miR98 inhibitor transfection detected by real-time fluorescent quantitative PCR

U251 cells in the logarithmic growth phase were seeded in 6-well cell culture plates (5 x 104 cells/well). The cells were cultured in DMEM without antibiotics but with 10% fetal bovine serum. When cell confluence was approximately 60%, the miR-98 inhibitor and the negative inhibitor control were transfected to U251 cells following the instructions of Lipofect AMINE2000.

The untransfected U251 cells were used as a blank control group. The cells in each group after 36 h of transfection were collected, and their miR-98 and IKBKE mRNA expression levels were detected following the methods in section 1.3.

The upstream primer sequence of the IKBKE gene was 5'-TGCGT-GCAGAAGTATCAAGC-3', and its downstream primer sequence was 5'-TACAGCAGCCACAGAACA G-3'.

The upstream primer sequence of the GAPDH gene (internal reference) was 5'-GTTGGAGGTCGGAGTCAACG G-3', and its downstream primer sequence was 5'-GAGGGATCTCGCTCCT-GGAGGA-3'.

The primer sequences of miR-98 and internal control U6 were identical to those of section 1.3.

Expression of IKBKE protein in glioma U251 cells after miR-98 inhibitor transfection was detected by Western blotting

U251 cells after transfection with the miR-98 inhibitor and the negative inhibitor control for 48 h in section 1.6 were collected, and the untransfected U251 cells were used as the blank control group. After centrifugation, the supernatant was discarded, and the total protein of each group was extracted by the total protein extraction kit. The protein concentration was determined by the BCA method, and 10 μg/well of protein was taken for 10% SDS-PAGE. The protein separated by electrophoresis was transferred to a PVDF membrane and reacted with a blocking solution containing 5% skim milk powder at 4 °C overnight. The mixture was washed with TBST 3 times, and rabbit anti-human IKBKE monoclonal antibody and rabbit anti-human β-actin polyclonal antibody were added (internal control, the volume dilution ratio was 1:800) and reacted overnight at 4 °C. After the TBST was rinsed 3 times again, the horseradish peroxidase-labeled goat anti-rabbit Ig G (secondary antibody, volume dilution ratio 1:5 000) was added and reacted at room temperature for 2 h. After washed with TBST 3 times, the mixture was developed with a chemiluminescence kit. The gray value of the protein band was determined and analyzed by ImageJ2x software, and the relative expression level of the IKBKE protein was represented by the ratio of the gray value of the target protein band to that of the internal control protein band.

Effect of the miR-98 inhibitor on the invasion and migration of glioma U251 cells detected in the Transwell assay.

The Transwell chamber with Matrigel was prewarmed to 37 °C and placed in a 24-well plate. DMEM containing 10% fetal bovine serum, 600 μL, was added to the lower chamber. After miR-98 inhibitor or negative control inhibitor transfection for 48 h of control, the glioma U251 cells were processed as a single cell suspension using the serum-free medium and was added to the upper chamber of the Transwell chamber (1 x 105 cells/chamber).

After the cells were cultured for 24 h, the culture in the upper chamber was discarded, and the cells failing to pass through the membrane on the upper chamber were wiped with a cotton swab. The cells were fixed in a 4% methanol solution for 30 min at room temperature and were stained with a 0.1% crystal violet solution at room temperature for 20 min after being dried. The cells were observed under an inverted light microscope, 10 visual fields (magnification 200 times) were randomly selected, and the number of cells passing through the membrane in each visual field was counted.

The cell migration experiment was the same as the above invasion experiment, except that the upper chamber of the Transwell had no Matrigel.

Expression of IKBKE mRNA and miR-98 in glioma U251 cells after IKBKE siRNA transfection was detected by real-time fluorescent quantitative PCR

IKBKE siRNA and its negative siRNA control were transfected to U251 cells, and the untransfected U251 cells were used as a blank control group. The expression of IKBKE mRNA and miR-98 in each cell group was determined 36 h after transfection. The transfection and detection methods were consistent with those mentioned in section 1.6.

Statistical analysis

Each experiment was repeated 4 times in this study, independently. The experimental data were statistically analyzed using SPSS 20.0 software. The measurement data were expressed as x±s and ANOVA was used for a comparison on the mean of multiple groups. The SNK-q test was used for intragroup pairwise comparison. The ratio was compared using a χ^2 test. When P was < 0.05, the difference was considered to be statistically significant.

Results

Expression level of miR-98 was lower in high-grade glioma tissues than in normal brain tissues

The relative expression levels of miR-98 in high-grade glioma tissues, low-grade glioma tissues and normal brain tissues were detected by real-time quantitative PCR.

The results (Table 1) revealed that the expression level of miR-98 was considerably lower in high-grade glioma tissues than those in low-grade glioma tissues and in normal brain tissues (P<0.001).

High-grade glioma tissues (53)	Low-grade glioma tissues (41)	Normal brain tissues (13)	F	P
0.26±0.06^{ab}	0.54±0.08^{a}	1.03±0.12	557.65	<0.001

Table 1: Expression of miR-98 in glioma tissues and in normal brain tissues detected by real-time quantitative PCR.
a vs. normal group P<0.05; b vs. low-grade group P<0.05

miR-98 can regulate the expression of IKBKE

The miR-98 inhibitor and pc DNA3/EGFP-wt IKBKE 3'-UTR, inhibitor control and pc DNA3/EGFP-wt IKBKE 3'-UTR, miR-98i inhibitor and pc DNA 3/EGF Pm ut IKBK E3 '-UTR, and inhibitor control and pc DNA3/EGFP-mut IKBKE 3'-UTR were separately cotransfected into U251 cells, which were detected by the luciferase reporter gene assay kit. The detection findings (Table 2) revealed that the fluorescence intensity was higher in the R-98 inhibitor and pc DNA3/EGFP-wt IKBKE 3'-UTR cotransfected group than that in the inhibitor control and pc DNA3/EGFP-wt IKBKE 3'-UTR cotransfected group (P<0.05), and there was no significant difference in the fluorescence intensity between the miR-98 inhibitor and pc DNA3/EGFP-mut IKBKE 3'-UTR cotransfected group and the inhibitor control and pc DNA3/EGFP-mut IKBKE 3'-UTR cotransfected group (P>0.05), which suggested that miR-98 can regulate the expression of IKBKE.

Transfection methods	Fluorescence intensity	Transfection methods	Fluorescence intensity
miR-98 inhibitor and pc DNA3/EGFP-wt IKBKE 3'-UTR(n=4)	2.18±0.34	mi R-98 inhibitor and pc DNA3/EGFP-mut IKBKE 3'-UTR(n=4)	1.08±0.21
inhibitor control and pc DNA3/EGFP-wt IKBKE 3'-UTR(n=4)	0.91±0.13	inhibitor control and pc DNA3/EGFP-mut IKBKE 3'-UTR(n=4)	1.05±0.16
t	6.98	t	0.23

Table 2: Regulation of miR-98 to IKBKE detected by the luciferase reporter gene assay kit.

miR-98 inhibitor expression in miR-98 downregulated U251 cells and in the expression of miR-98 with upregulated IKBKE mRNA

To further verify the regulation of miR-98 on IKBKE gene expression, the miR-98 inhibitor was transfected to glioma U251 cells, and the expression of miR-98 and IKBKE mRNA in cells was detected by real-time fluorescent quantitative PCR. The results (Table 3) showed that the expression level of miR-98 was significantly lower in the miR-98 inhibitor transfection group than those in the untransfected blank control group and in the negative inhibitor control group (P<0.05). This result indicated that the miR-98 inhibitor was successfully transfected and successfully inhibited the expression of miR-98. However, the expression level of IKBKE mRNA was considerably higher in the transfection group than those in the untransfected blank control group and in the negative inhibitor control group (P<0.05). This finding suggested that the expression of miR-98 in downregulated glioma U251 cells can significantly upregulate the expression level of IKBKE mRNA.

	miR-98 inhibitor transfection group (n=4)	Blank control group (n=4)	Negative control group (n=4)	F	P
Number of cell migration	46.28±6.82ab	17.43±3.52	19.44±2.98	45.93	<0.001
Number of cell invasion	42.73±8.19 ab	16.26±2.64	11.33±2.16	43.37	<0.001

Table 3: Expression of miR-98, IKBKE, and IKBKE protein in glioma U251 cells transfected with the mi R-98 inhibitor detected by real-time fluorescent quantitative PCR and Western blotting were used to detect.
a vs. blank group P<0.05; b vs. negative group P<0.05

Expression of IKBKE protein in U251 cells with miR-98 inhibitor upregulation

After the miR-98 inhibitor was transfected to glioma U251 cells, the expression of IKBKE protein in the cells was detected by Western blotting. The results (Table 3) revealed that the expression level of IKBKE protein was significantly higher in the mi R-98 inhibitor transfection group than those in the untransfected blank control group and in the negative inhibitor control transfection group (P<0.05). This result was consistent with the findings of real-time fluorescent quantitative PCR.

Migration and invasion of U251 cells promoted by the miR-98 inhibitor

After the miR-98 inhibitor was transfected to glioma U251 cells, the migration and invasion capacities of U251 cells were detected by using the Transwell method.

The results (Table 4) showed that the number of transmembrane cells was significantly higher in the miR-98 inhibitor transfection group than those in the untransfected blank control group and in the negative inhibitor control transfection group (P<0.05). This result indicated that the miR-98 inhibitor can promote the migration and invasion of U251 cells.

	miR-98 inhibitor transfection group (n=4)	Blank control group (n=4)	Negative control group (n=4)	F	P
Number of cell migration	46.28±6.82ab	17.43±3.52	19.44±2.98	45.93	<0.001
Number of cell invasion	42.73±8.19 ab	16.26±2.64	11.33±2.16	43.37	<0.001

Table 4: Effect of the miR-98 inhibitor on the migration and invasion of glioma U251 detected by the Transwell method.
a vs. blank group P<0.05; b vs. negative group P<0.05

KBKE siRNA does not affect the expression of miR-98 in U251 cells

After IKBKE siRNA was transfected to glioma U251 cells, and the expression levels of IKBKE mRNA and miR-98 were detected by real-time fluorescent quantitative PCR. The results (Table 5) revealed that the expression level of IKBKE m RNA was much lower in the IKBKE siRNA transfection group than those in the untransfected blank group and in the negative siRNA control transfection group (P<0.05). It was suggested that IKBKE siRNA was successfully transfected to U251 cells. There was no significant difference in the expression level of miR-98 between the IKBKE siRNA transfection group and the untransfected blank group, as well as the negative siRNA control transfection group (P>0.05), which indicated that miR-98 has one-way regulation on the IKBKE gene.

	IKBKE siRNA transfection group	Blank group	Negative control group	F	P
IKBKE mRNA	0.54±0.03ab	1.03±0.04	1.25±0.09	149.55	<0.001
miR-98	1.04±0.07	0.99±0.06	1.10±0.05	3.31	0.084

Table 5: Expression of IKBKE mRNA and miR-98 in glioma U251 after IKBKE siRNA transfection detected by real-time fluorescent quantitative PCR..
a vs. blank group P<0.05; b vs. negative group P<0.05;

Discussion

miRNA is a kind of endogenous noncoding small RNA of 19~25 nt in length, which promotes the degradation of target mRNA or inhibits its translation process by acting on the 3'-UTR of

the target gene mRNA, negatively regulates the posttranscriptional level of gene, and affects a variety of physiological and pathological processes, such as apoptosis, proliferation and differentiatio[11-15]. Studies have shown that miRNAs play a role similar to the proto-oncogene or anti-oncogene in the development of tumors[16-17].

Reinhart et al.[18] discovered and confirmed the existence of the let-7 family. There are 13 members in the human let-7 family, and miR-98 is an important member of the family, which binds to the 3'-UTR of the target mRNA in a complete or incomplete form to degrade the target mRNA or inhibit its translation process, thus playing a negative regulatory role. Recent studies have found that miR-98 is downregulated in a variety of tumors. Wang Jin, et al[19]) detected the expression of miR-98 in the tumor central tissues and their adjacent normal paracancerous tissues of 32 patients with gastric cancer using real-time fluorescent quantitative PCR and found 87.5% (28/32) of gastric cancer central tissues has lower miR-98 expression than their adjacent paracancerous tissues. In addition, the higher the gastric cancer grade, the lower the expression level of miR-98. The expression level of mi R-98 in gastric cancer epithelial cells is considerably lower than that in normal gastric epithelial cells. Alajez, et al[20] found that the expression of miR-98 in nasopharyngeal carcinoma cells is significantly and negatively related to the expression of the EZH2 protein, and miR-98 can target the 3'-UTR of EZH2 mRNA, thus greatly restraining the expression of the EZH2 protein, and thereby significantly inhibits the metastasis of nasopharyngeal carcinoma cells. Siragam, et al[21], pointed out that the overexpression of miR-98 can depress the proliferation and invasion of breast cancer cells in a highly aggressive breast cancer model.

Conversely, the inhibited expression of miR-98 boosts the malignant biological phenotype of breast cancer cells. miR-98 acts on 3'-UTR of activin receptor-like kinase 4 (ALK4) and matrix metalloprotein-11 (MMP-11) and controls their expressions. Restoring the expression of ALK4 and MMP-11 can reverse the anti-proliferative and anti-invasive effects of miR-98. Wendler, et al.[22] discovered that miR-98 acts on the 3'-UTR of progesterone receptor membrane components 1 (PGRMC1) and restrains the expression of miR-98. To some extent, miR-98 affects the proliferative ability of ovarian cancer, lung cancer and breast cancer.

In this study, we used real-time fluorescent quantitative PCR to detect the expression of miR-98 in glioma tissues and found that the expression level of miR-98 is much lower in glioma tissues than in normal brain tissues, and the expression of miR-98 is also much lower in high-grade glioma than in low-grade glioma. The miR-98 inhibitor depresses its action by specifically binding to the adult miR-98 molecules and weakens the gene silencing effect caused by miR-98 in the cell.

In this study, the miR-98 inhibitor is transfected to human glioma U251 cells to obtain U251 cells with a reduced expression of miR-98. Furthermore, the findings from the Transwell assay revealed that the migration and invasion of U251 cells were enhanced after transfection with the miR-98 inhibitor.

To further investigate the mechanism on the inhibition of the miR-98 expression enhancing the migration and invasion of U251 cells, we examined the effect of miR-98 on IKBKE mRNA and protein expression in this study. IKBKE is a member of the inhibitor of e nuclear factor kappa-B kinase (IKK) family, also known as the inhibitor of the nuclear factor inducible kappa-B kinase (IKK-i). Boehm, et al[23], used immunohistochemistry to detect breast cancer tissue and found that IKBKE can promote nuclear factor kappa-B (NF-κB) transposition. Currently, there are few studies on IKBKE in the field of glioma. Guan, et al[24], confirmed that IKBKE is expressed in the tumor tissues of approximately 96% of glioma patients. With immunohistochemistry, they found that 53.5% of gliomas have high IKBKE expression and confirmed that IKBKE may advance the transposition of NF-κB from the cytoplasm to the nucleus, thereby enhancing the anti-apoptotic effect of glioma cells. With real-time fluorescent quantitative PCR and Western blotting, Li, et al[25], found that the expression level of IKBKE is negatively correlated with pathological grade glioma. The higher the malignant degree of glioma, the lower the expression level of IKBKE. After the expression of IKBKE is depressed using IKBKE siRNA, the transposition of NF-κB p65 is significantly reduced, which inhibits the transcription and expression of downstream-related proteins and restrains biological behaviors, such as cell proliferation and invasion. Consequently, IKBKE may play a role in regulating the malignant

phenotype of tumor cells and is closely associated with the development of glioma.

At present, the upstream regulatory factors of IKBKE are not fully clear. In this study, the findings of the luciferase reporter gene detection system revealed that the miR-98 inhibitor can greatly promote the fluorescence activity of transfected wt IKBKE 3'-UTR recombinant expression vector cells, indicating that IKBKE is directly regulated by miR-98 and that IKBKE is a downstream target gene of miR-98. The results of real-time fluorescent quantitative PCR and Western blotting also have suggested that the expression levels of IKBKE mRNA and protein are much higher in the miR-98 inhibitor transfection group than those in the untransfected blank control group and in the negative control groups, which indicated that the miR-98 inhibitor can significantly upregulate the expression of IKBKE. Further studies have revealed that there is no significant change in the expression level of miR-98 after depressing the IKBKE expression, which indicated that miR-98 has a one-way regulation effect on IKBKE. The above results indicate that miR-98 may affect malignant biological behaviors, such as the migration and invasion of glioma cells, by regulating the expression of IKBKE.

To summarize, miR-98 is expressed at a low level in glioma tissues, and the miR-98 inhibitor can strongly enhance the migration and invasion of glioma U251 cells, which may be achieved by regulating the IKBKE expression. Therefore, miR-98 may be one of the major targets for glioma treatment, and the findings of this study provide new ideas for the molecular targeted therapy of glioma.

References

1) Zhang J, Wu Y, Lin YH, Guo S, Ning PF, et al. Prognostic value of hypoxia-inducible factor-1 alpha and prolyl 4-hydroxylase beta polypeptide overexpression in gastric cancer. World J Gastroenterol 2018; 24: 2381-2391.
2) Guo E, Liu H, Liu X. Overexpression of SCUBE2 Inhibits Proliferation, Migration, and Invasion in Glioma Cells. Oncol Res 2017; 25: 437-444.
3) Qin YC, Jia D, Yu QW. Effects of Lentivirus-mediated Cullin3 Silencing on Human Glioma Cells. Neural Inj Funct Reconstr 2017; 12: 9-13.
4) Ankasha SJ, Shafiee MN, Wahab NA, Ali RAR, Mokhtar NM. Post-transcriptional regulation of microRNAs in cancer: From prediction to validation. Oncol Rev 2018; 12: 344.
5) Rizos E, Siafakas N, Skourti E, Papageorgiou C, Tsoporis J. miRNAs and their role in the correlation between schizophrenia and cancer. Mol Med Rep 2016; 14: 4942–4946.
6) Su JZ, Liang QM. Influence on Proliferation and Invasion of Human Breast Carcinoma Cell Line MDA-MB-231 by Using MicroRNA Interference Technique to Suppress MTDH Expression. J Med Res 2017; 46: 84-90.
7) Wang CY, Zhang JJ, Hua L, Yao KH, Chen JT, et al. MicroRNA-98 suppresses cell proliferation, migration and invasion by targeting collagen triple helix repeat containing 1 in hepatocellular carcinoma. Mol Med Rep 2016; 13: 2639-2644.
8) Du Y, Li Y, Lv H, Zhou S, Sun Z, et al. miR-98 suppresses tumor cell growth and metastasis by targeting IGF1R in oral squamous cell carcinoma. Int J Clin Exp Pathol 2015; 8: 12252–12259.
9) Zhou W, Zou B, Liu L, Cui K, Gao J, et al. MicroRNA-98 acts as a tumor suppressor in hepatocellular carcinoma via targeting SALL4. Oncotarget 2016; 7: 74059-74073.
10) Yang G, Zhang X, Shi J. MiR-98 inhibits cell proliferation and invasion of non-small cell carcinoma lung cancer by targeting PAK1. Int J Clin Exp Med 2015; 8: 20135–20145.
11) Zeng YB, Liang XH, Zhang GX, Jiang N, Zhang T, et al. mi RNA-135a promotes hepatocellular carcinoma cell migration and invasion by targeting forkhead box O1. Cancer Cell Int 2016; 16: 63.
12) Wu B, Lei D, Wang L, Yang X, Jia S, et al. Mi RNA-101 inhibits oral squamouscell carcinoma growth and metastasis by targeting zinc finger E-box binding homeobox 1. Am J Cancer Res 2016; 6: 1396-1407.
13) Luo W, Lin Y, Meng S, Guo Y, Zhang J, et al. mi RNA-296-3p modulates chemosensitivity of lung cancer cells by targeting CX3CR1. Am J Transl Res 2016; 8: 1848-1856.
14) Sahoo S, Meijles DN, Al Ghouleh I, Tandon M, Cifuentes-Pagano E, et al. MEF2C-MYOCD and leiomodin 1 suppression by miR NA-214 promotes smooth muscle cell phenotype switching in pulmonary arterial hypertension. PLOS One 2016; 11: 153780.
15) Surmiak M, Hubalewska-Mazgaj M, Wawrzycka-Adamczyk K, Musiał J, Sanak M. Neutrophil Mi RNA-128-3p is decreased during active phase of granulo-matosis with polyangiitis. Curr Genomics 2015; 16: 359-365.
16) Jin B, Liu Y, Wang H. Antagonism of mi RNA-21 sensitizes human gastric cancer cells to paclitaxel. Cell Biochem Biophys 2015; 72: 275-282.
17) Boo L, Ho WY, Ali NM, Yeap SK, Ky H, et al. Mi RNAtranscriptome profiling of spheroidenriched cells with cancer stem cell properties in human breast MCF-7 cell line. Int J Biol Sci 2016; 12: 427-445.
18) Wang D, Hou L, Nakamura S, Su M, Li F, et al. LIN-28 balances longevity and germline stem cell number in Caenorhabditis elegans through let-7/AKT/DAF-16 axis. Aging Cell 2017; 16: 113-124.
19) Wang J, Zhou JP, Yang QY. Down-regulation of micro RNA-98 expression in gastric cancer and its regulation on gastric cancer cell growth. Chin J Exp Surg 2013; 30: 1245-1247.

20) Hwang CF, Huang HY, Chen CH, Chien CY, Hsu YC, et al. Enhancer of zeste homolog 2 overexpression in nasopharyngeal carcinoma: an independent poor prognosticator that enhances cell growth. Int J Radiat Oncol Biol Phys 2012; 82: 597-604.
21) Ye W, Wang L, Cheng C, Chu X. New insights into the regulatory role of microRNA in tumor angiogenesis and clinical implications. Mol Cancer 2018; 17: 22.
22) Mattos F, Canavessi A, Wiltbank MC, Bastos MR, Lemes AP, et al. Investigation of mechanisms involved in regulation of progesterone catabolism using an overfed versus underfed ewe-lamb model. J Anim Sci 2017; 95: 5537-5546.
23) Hafner M, Niepel M, Chung M, Sorger PK. Growth rate inhibition metrics correct for confounders in measuring sensitivity to cancer drugs. Nat Methods 2016; 13: 521-527.
24) Wang L, Guo S, Zhang H. MiR-98 Promotes Apoptosis of Glioma Cells via Suppressing IKBKE/NF-κB Pathway. Technol Cancer Res Treat 2017; 16: 1226-1234.
25) Gu G, Wang L, Zhang J, Wang H, Tan T, et al. MicroRNA-384 inhibits proliferation migration and invasion of glioma by targeting at CDC42. Oncotargets & Ther 2018; 11: 4075-4085.

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