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ORIGINAL ARTICLE

miR-3175 and miR-134 affect proliferation, invasion and apoptosis of glioma cells through PI3K/AKT signaling pathway

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Summary

Purpose: *This study aimed to investigate the expressions of microRNA (miR)-3175 and miR-134 in gliomas and their effects on the proliferation and invasion of U251 glioma cells.*

Methods: *Tumor tissues of 42 patients with glioma and non-tumor tissues of 10 patients with severe craniocerebral injury were collected. These patients were diagnosed in Jinan City People's Hospital from March 2010 to April 2012. qRT-PCR was used to detect differences of miR-3175 and miR-134 expressions. Kaplan-Meier method was used to generate survival curves and Log-rank test to evaluate differences between miR-3175, miR-134 and 5-year survival of the patients. Western blot was used to detect levels of p-PI3K in PI3K signaling pathway.*

Results: *Proliferative activity of the cells in miR-3175 mimic group and miR-control group at different time points was significantly better than that of the cells in miR-3175 inhibitor group ($p<0.05$). The proliferative activity of the cells in miR-134 mimic group and miR-control group at different time points was significantly worse than that of the cells in miR-134 inhibitor group ($p<0.05$). Apoptosis rate of the cells in miR-3175 mimic group and miR control group was significantly lower than that of the cells in miR-3175 inhibitor group ($p<0.05$). Apoptosis rates of the cells in miR-134 mimic group and miR-control group were significantly higher than that of the cells in miR-134 inhibitor group ($p<0.05$).*

Conclusion: *Downregulating expression of miR-3175 or facilitating expression of miR-134 could inhibit the proliferative and invasive activity of glioma cells and facilitate their apoptosis by inhibiting the activation of PI3K signaling pathway.*

Key words: *apoptosis, cell invasion, cell proliferation, glioma, miR-134, miR-3175, PI3K/AKT signaling pathway*

Introduction

Glioma is a common primary tumor of the central nervous system (CNS), and its morbidity accounts for about 30% among tumors in the CNS and 80% among intracephalic malignant tumors [1]. The prognosis of patients with glioma is poor, and the higher the degree of malignancy, the worse the prognosis of patients [2,3]. It has been reported [4] that the median survival time of patients with glioblastoma multiforme is only one year, and 5-year survival rate of the patients is less than 5%. Glioma is one of malignant tumors that have a high mortality rate. Although patients with glioma receive some treatments actively, such as resection, radiotherapy and chemotherapy, their prognosis is

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still poor [5]. The development of molecular targeted therapy uncovers increasing evidence that molecular heterogeneity is a key barrier to improve clinical outcomes of glioma [6]. Therefore, it is imperative to elucidate the molecular mechanism of proliferation and invasion of glioma cells to find effective therapeutic targets.

miRs, widely expressed in eukaryotic cell organisms, are short (18 to 25 nucleotides long) and single-stranded RNA molecules. miRs regulate the expressions of genes by combining with 3'-untranslated region of target mRNAs and regulate some biological processes, such as proliferation, invasion, apoptosis and differentiation of cells [7,8]. Studies have reported that miRs are closely related to glioma [9,10]. The function of miR-3175 and miR-134 in tumors was found in many studies. Fomicheva et al [11] found that the expression level of miR-3175 increased when they detected miR spectrum of prostate cancer. Zhang et al [12] also found that the expression level of miR-3175 increased when they preliminarily screened miR spectrum of gastric cancer. However, at present, reports about the specific effect of miR-3175 on tumors are few. The expression of miR-134 was reported to be downregulated in renal cell carcinoma. Overexpression of miR-134 can use MAPK/ERK pathway that is related to KRAS as a tumor inhibiting factor, inhibit the migration and invasion of cells by blocking epithelial mesenchymal transition (EMT), as well as inhibit the proliferation of cells by blocking cell cycle [13]. In the study of Sun et al [14], it was reported that the expression of miR-134 was downregulated in non-small cell lung cancer, and its overexpression could inhibit the proliferation, migration and invasion of cells and facilitate apoptosis by inhibiting the expression of CCND 1. However, reports about the functions of miR-3175 and miR-134 in glioma are few, and specific functions of miR-3175 and miR-134 in tumors need to be verified. PI3K/AKT is a signaling pathway that is closely related to glioma. PI3K/Akt/mTOR signaling pathway may be an important way to improve targeted therapeutic effect of glioma [15]. Moreover, some studies have reported that upregulation of miR-134 expression can be considered as a promoter of PI3K/Akt activation in lung cancer cells [16]. Nevertheless, reports about miR-3175 and PI3K/AKT signaling pathway are scarce.

Therefore, in this study, the expressions of miR-3175 and miR-134 in glioma were detected, and the effects of both miRs on PI3K/AKT signaling pathway and on the proliferation and invasion of cells were analyzed, in order to provide a potential therapeutic target for glioma.

Methods

Study subjects

Tumor tissues of 42 patients with different grades of glioma and non-tumor tissues of 10 patients were collected. Non-tumor tissues were taken from patients with severe craniocerebral injury, part of brain tissues needed to be resected. These patients were diagnosed in Jinan City People's Hospital Affiliated to Taishan Medical University from March 2010 to April 2012. Their ages ranged between 20 and 40 years. All tumor specimens were pathologically diagnosed as glioma and were classified and graded histologically according to World Health Organization CNS classification guidelines. There were 14 patients with grade I, 13 patients with grade II, 10 patients with grade III and 5 patients with grade IV. The patients with glioma did not receive radiotherapy and chemotherapy before operation. No patient had abnormal bleeding or coagulation abnormalities, and their medical records and data of follow-up visits were complete. The study followed principles of the Declaration of Helsinki. Patients with other benign or malignant tumors were excluded. Patients with a history of tumors were excluded. Pregnant or lactating women were excluded. This study was approved by medical ethics committee of Jinan City People's Hospital. The patients and their family members were informed by telephone or letters, and provided signed informed consent.

U251 glioma cells were purchased from Shanghai Enzyme Research Biotechnology Co., Ltd.. DMEM complete medium (Shanghai Zhongqiao Xinzhou Biotechnology Co., Ltd.) containing 10% fetal bovine serum (FBS) was used. Culture conditions included a constant temperature of 37°C, 5% of CO_2 and 95% relative humidity.

Establishment of expression vectors of miR-3175 and miR-134

Expression vectors of miR-3175 mimic, miR-3175 inhibitor, miR-134 mimic, miR-134 inhibitor and miR-control were constructed and synthetized by Shanghai GenePharma Biotechnology Co., Ltd. The cells were digested by trypsin 24 h before transfection. Expression vectors of the cells were transfected when the confluence was about 80%. Specific steps were carried out according to the instructions of kits. The cells were cultured in an incubator (37°C and 5% of CO_2) for 48 h and the medium was replaced every 6 h. Transfection results were detected by qRT-PCR. LipofectamineTM 2000 transfection kit was purchased from Shanghai Yanjin Biotechnology Co., Ltd.

qRT-PCR

The concentration of cell suspension was adjusted to 1×10^7/mL. Glioma tissue cells and U251 human glioma cells were extracted by TRIzol (Guangzhou LabGene Biotechnology Co., Ltd.). Extraction procedure of total RNA was referred to the instructions of kits. Micro-ultraviolet spectrophotometer DanoProp1000 (Thmorgan Biotechnology Co., Ltd.) was used to analyze the concentration and purity of RNA that was extracted and 3% of agarose gel electrophoresis was used to analyze

its integrity (gel electrophoresis kits were purchased from Shanghai Jingke Chemical Technology Co., Ltd.). A260/A280 value between 1.8 and 2.1 was considered to meet the experimental requirements. After RNA was extracted, qRT-PCR was carried out. Reverse transcription reaction system was: 5×PrimerScript Buffer 2 μL, PrimerScript RT Enzyme Mix 0.5 μL, Random 6 mers (100 μM) 0.5 μL, Oligo dT Primer (50 μM) 0.5 μL, total RNA 2 μg. Non-ribonuclease distilled water was added up to 10 μL. Reverse transcription reaction was carried out at 37°C for 15 min. Inactivation reaction of reverse transcriptase was carried out at 85°C for 5 sec. The reaction was finished at 4°C. After reverse transcription reaction was finished, PCR amplification was carried out. PCR amplification system was: 4 μL of cDNA template, 25 μL of SYBR Green Mix (2x), 1 μL of upstream primer and 1 μL of downstream primer, 1 μL of Reference Dye (optional), double distilled water was added up to 50 μL. After pre-denaturation was carried out at 95°C for 3 min, denaturation was carried out at 95°C for 30 sec, annealing was carried out at 55°C for 30 sec, extension was carried out at 72°C for 60 sec, 30 cycles were carried out. Extension was carried out at 72°C for 5 min after the cycles were finished. U6 was used as a reaction internal reference. Three repeated wells were established in all samples. The results were analyzed by $2^{-\Delta Ct}$ assay. Primer sequences were designed and synthesized by He Peng (Shanghai) Biological Co., Ltd. (Table 1).

MTT cell proliferation assay in vitro

Cell suspension with a concentration of 4×10^6 cells/mL was prepared by U251 cells, which were single-arrayed. The cells were routinely inoculated and cultured in 96-well cell culture plates. After 6 h, 20 μL of MTT solution (5 mg/mL) were added into the cell culture plates. Then the cells were cultured for 4 h at 37°C. The supernatant containing impurities was removed, then dimethyl sulfoxide was added into the cell culture plates which were placed on a horizontal vibrator for 10 min. Finally, the absorbance at 570 nm was measured by an ultraviolet-visible spectrophotometer in 12 h, 24 h, 48 h and 72 h (Dongguan Specimen Experimental Equipment Technology Co., Ltd., SPCC). MTT test kits were purchased from Shanghai LMAI Bioengineering Co., Ltd.

Transwell invasion assay in vitro

U251 cell suspension and the cell suspension in the negative group were inoculated in Transwell chambers. Matrigel was preliminarily laid in Transwell chambers. 100 μL of the cell suspension in which the cells were single-arrayed was inoculated in Transwell chambers. RPMI1640 medium and 10% FBS as well as 100 μL of cell suspension were added into the upper chamber. RPMI1640 medium and 20% FBS as well as 500 μL of cell suspension were added into the lower chamber. Then, the cells were cultured for 48 h, in 37°C and 5% CO_2. After this, the cells were stained with aniline violet and the number of transmembrane cells was calculated. Six chambers were counted and 6 visual fields were counted in each chamber. Thre parallel assays were carried out simultaneously. Transwell chambers and reagents were purchased from Shanghai Yuanzi Biotechnology Co., Ltd.

TUNEL apoptosis assay

U251 cell suspension with a concentration of 5×10^7 cells/mL, which were cultured for 48 h, were fixed with 4% of neutral formaldehyde at indoor temperature for 10 min. Then, the residual liquid was removed. U251 cells were washed by PBS twice, and the duration was 5 min each time. PBS containing 2% of hydrogen peroxide was disposed at indoor temperature for 5 min. Then, the residual liquid was removed. U251 cells were washed by PBS twice, and the duration was 5 min each time. The cells were stained according to the instructions of TUNEL kits (Shanghai Runwell Industrial Co., Ltd.). The number of TUNEL positive cells in a 400-time visual field was counted by Image-proPlus5.0, and 5 400-time visual fields needed to be counted. Integral optical density meant the total number of TUNEL positive cells. The above steps were carried out for three times.

Western blot

Protein extracting solution (Beibo-Bestbio, Shanghai, BB-3531) was used to extract the protein in U251 cells, which was then was separated by polyacrylamide gel electrophoresis, and the initial voltage was 90V. After polyacrylamide gel electrophoresis was finished, the membrane was transferred with a constant voltage of 100V for 100 min and was sealed at 37°C for 60 min. Then, the membrane was sealed and antibody hybridization was carried out. Next, the membrane was incubated with the primary antibody overnight at 4°C, and in the next day the membrane was washed by PBS for three times, for 10 min each time. Then, the membrane was incubated with HRP-labeled goat anti-mouse IgG secondary antibody (Shenzhen Otwo Biotechnology Co., Ltd., PL03-0375R) for 2 h. After this step was finished, ECL luminescence reagent was used to develop and fix the cells. The band scanned by the film was statistically analyzed by Quantity One software. Relative expression level of the protein=band gray value/internal reference gray value. Polyacrylamide gel electrophoresis buffer solution was purchased from Xiamen Huijia Biotechnol-

Table 1. Primer sequences

	Forward primer	*Reverse primer*
miR-3175	GATACTCACGGGGAGAGAACGCAG	GTGCAGGGTCCGAGGT
miR-134	AACTGCAGAGCTGTGGTTCTGT	CGCGGATCCCGTGTCATCGCA
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

ogy Co., Ltd., article number: orb154330. Western blot test kits were purchased from Shanghai Junrui Biotechnology Co., Ltd., article number: UFC04948. p-PI3K, p-AKT antibody were purchased from Xiamen Huijia Biotechnology Co., Ltd., article numbers were PV4789 and sc-16646.

Statistics

SPSS19.0 (IBM) was used. The measurement data were expressed as percents. The ratio was compared by x^2 test. The count data were expressed as mean ± standard deviation (mean±SD). T-test was used for comparison between two groups. Analysis of variance (ANOVA) was used for comparison between groups. Kaplan-Meier method was used to analyze the relationship among miR-3175, miR-134 and 5-year survival of patients. P<0.05 was considered to be statistically significant.

Results

Expression levels of miR-3175 and miR-134 in glioma tissues

qRT-PCR showed that the relative expression level of miR-3175 in gliomas was 1.744±0.127. The relative expression level of miR-3175 in non-tumor tissues was 0.973±0.053. The relative expression level of miR-3175 in gliomas was significantly higher than that in non-tumor tissues (p<0.05). The relative expression level of miR-134 in gliomas was 0.665±0.025 and the relative expression level of miR-134 in non-tumor tissues was 1.500±0.121. The relative expression level of miR-134 in gliomas was significantly lower than that in non-tumor tissues (p<0.05) (Figure 1).

The relationship among miR-3175, miR-134 and survival prognosis of patients with glioma

According to the relative expression levels of miR-3175 and miR-134 in tumor tissues of patients with glioma, the medians of relative expression levels of miR-3175 and miR-134 were 1.756 and 0.664, respectively. The medians were used as critical values, and the high and low expression groups were distinguished by the critical values. Kaplan-Meier survival analysis was carried out, and the results showed that 5-year survival rate of the patients was 19.05% (4 cases) in miR-3175 high expression group. The 5-year survival rate of the patients was 57.14% (12 cases) in the miR-3175 low expression group. The 5-year survival rate of the patients in the miR-3175 low expression group was higher than that of the patients in the miR-3175 high expression group (p<0.05). The 5-year survival rate of the patients was 57.14% (12 cases) in the miR-134 high expression group, while the 5-year survival rate of the patients was 19.05% (4 cases) in the miR-134 low expression group. The 5-year survival rate of the patients in the miR-134 low expression group was lower than that of the patients in miR-134 high expression group (p<0.05) (Figure 2).

Transfection results of miR-3175 and miR-134 in U251 cells

The differences among relative expression level of miR-3175 in the cells in miR-3175 mimic group, miR-3175 inhibitor group and miR-control group

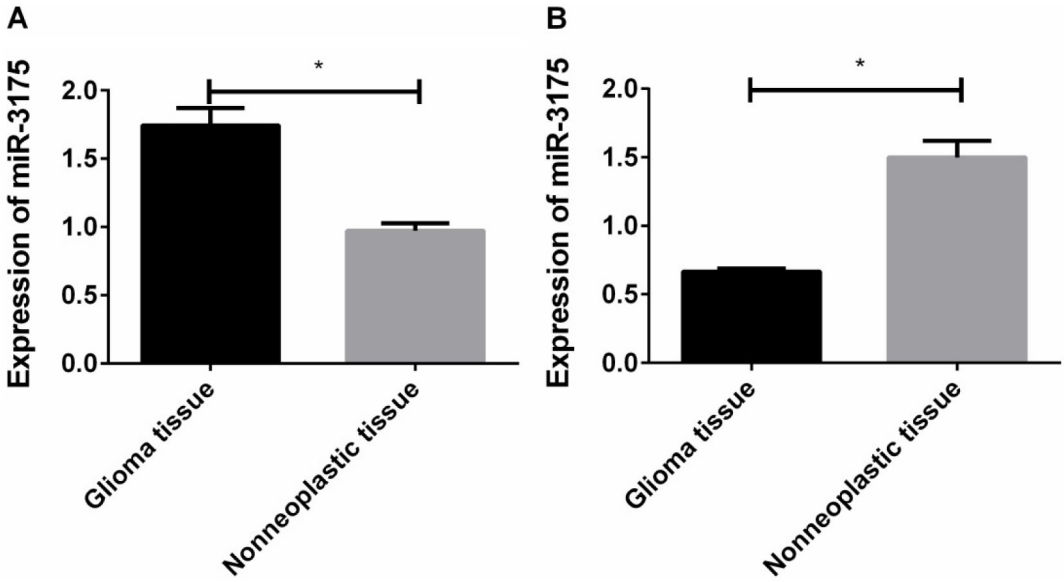

Figure 1. Expression levels of miR-3175 and miR-134 in glioma tissues. **A:** Expression level of miR-3175 in glioma tissues. The result of qRT-PCR showed that the relative expression level of miR-3175 was significantly higher than that in non-tumor tissues. **B:** Expression level of miR-134 in glioma tissues. The result of qRT-PCR showed that the relative expression level of miR-134 was significantly lower than that in non-tumor tissues (*p<0.05).

were statistically significant (p<0.05). The relative expression level of miR-3175 in the cells in miR-3175 mimic group was significantly higher than that in miR-3175 inhibitor group and miR-control group (p<0.05). The relative expression level of miR-3175 in the cells in miR-3175 inhibitor group was lower than that in miR-control group (p<0.05). The differences among the relative expression level of miR-134 in the cells in miR-134 mimic group, miR-134 inhibitor group and miR-control group were statistically significant (p<0.05). The relative expression level of miR-134 in the cells in miR-134 mimic group was significantly higher than in the miR-134 inhibitor group and miR-control group (p<0.05). The relative expression level of miR-134 in the cells in the miR-134 inhibitor group was lower than that in miR-control group (p<0.05) (Figure 3).

The effects of miR-3175 and miR-134 on the proliferation of U251 cells

The results of MTT cell proliferation assay *in vitro* showed that the difference among the proliferation rate of the cells in miR-3175 mimic group,

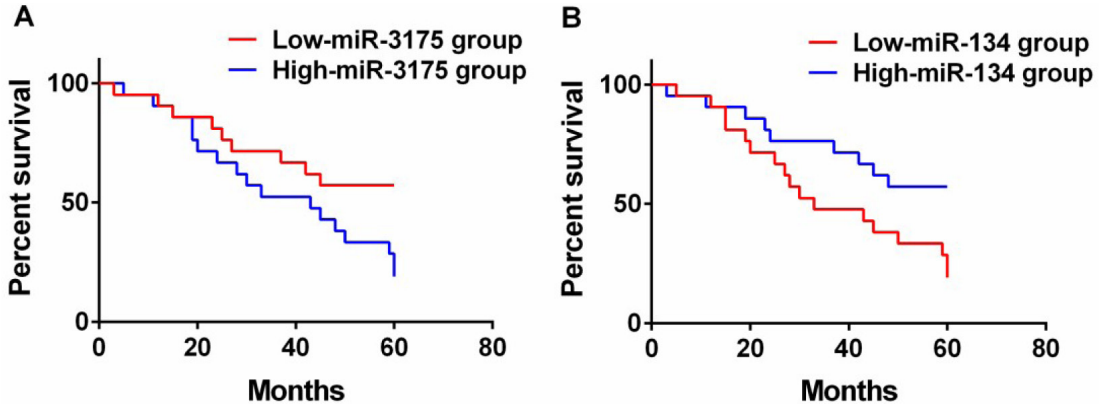

Figure 2. The relationship among miR-3175, miR-134 and prognosis of survival of patients with glioma. **A:** The relationship between miR-3175 and prognosis of survival of patients with glioma. Kaplan-Meier survival curve showed that 5-year survival rate of the patients in miR-3175 low expression group was higher than that of the patients in miR-3175 high expression group (p<0.05). **B:** The relationship between miR-134 and prognosis of survival of patients with glioma. Kaplan-Meier survival curve showed that 5-year survival rate of the patients in miR-134 low expression group was lower than that of the patients in miR-134 high expression group (p<0.05).

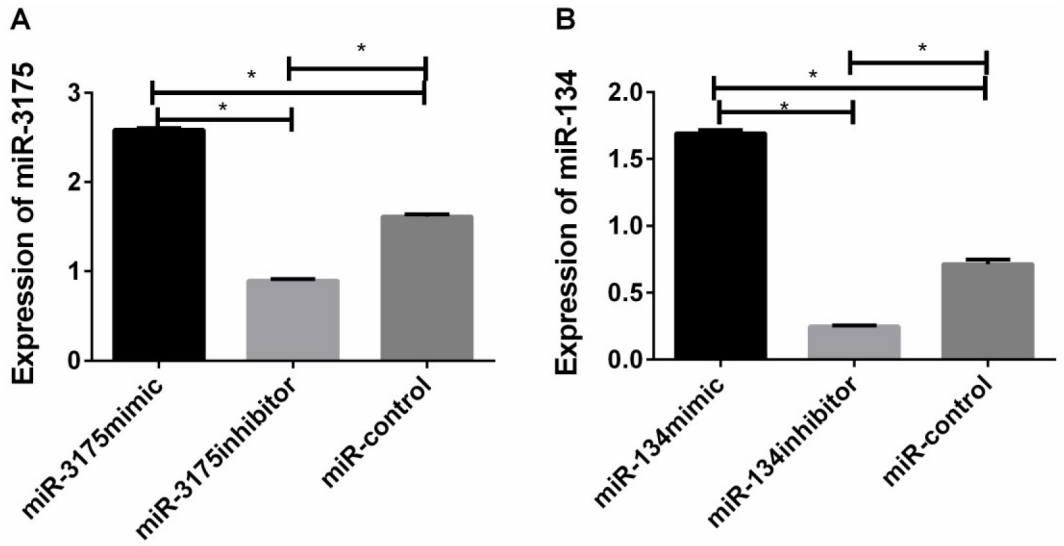

Figure 3. Transfection results of miR-3175 and miR-134 in U251 cells. **A:** Transfection result of miR-3175 in U251 cells. The result of qRT-PCR showed that the relative expression level of miR-3175 in the cells in miR-3175 mimic group was significantly higher than that in miR-3175 inhibitor group and miR-control group. The relative expression level of miR-3175 in the cells in miR-3175 inhibitor group was lower than that in miR-control group. **B:** Transfection result of miR-134 in U251 cells. The result of qRT-PCR showed that the relative expression level of miR-134 in the cells in miR-134 mimic group was significantly higher than that in miR-134 inhibitor group and miR-control group. The relative expression level of miR-134 in the cells in miR-134 inhibitor group was lower than that in miR-control group (*p<0.05).

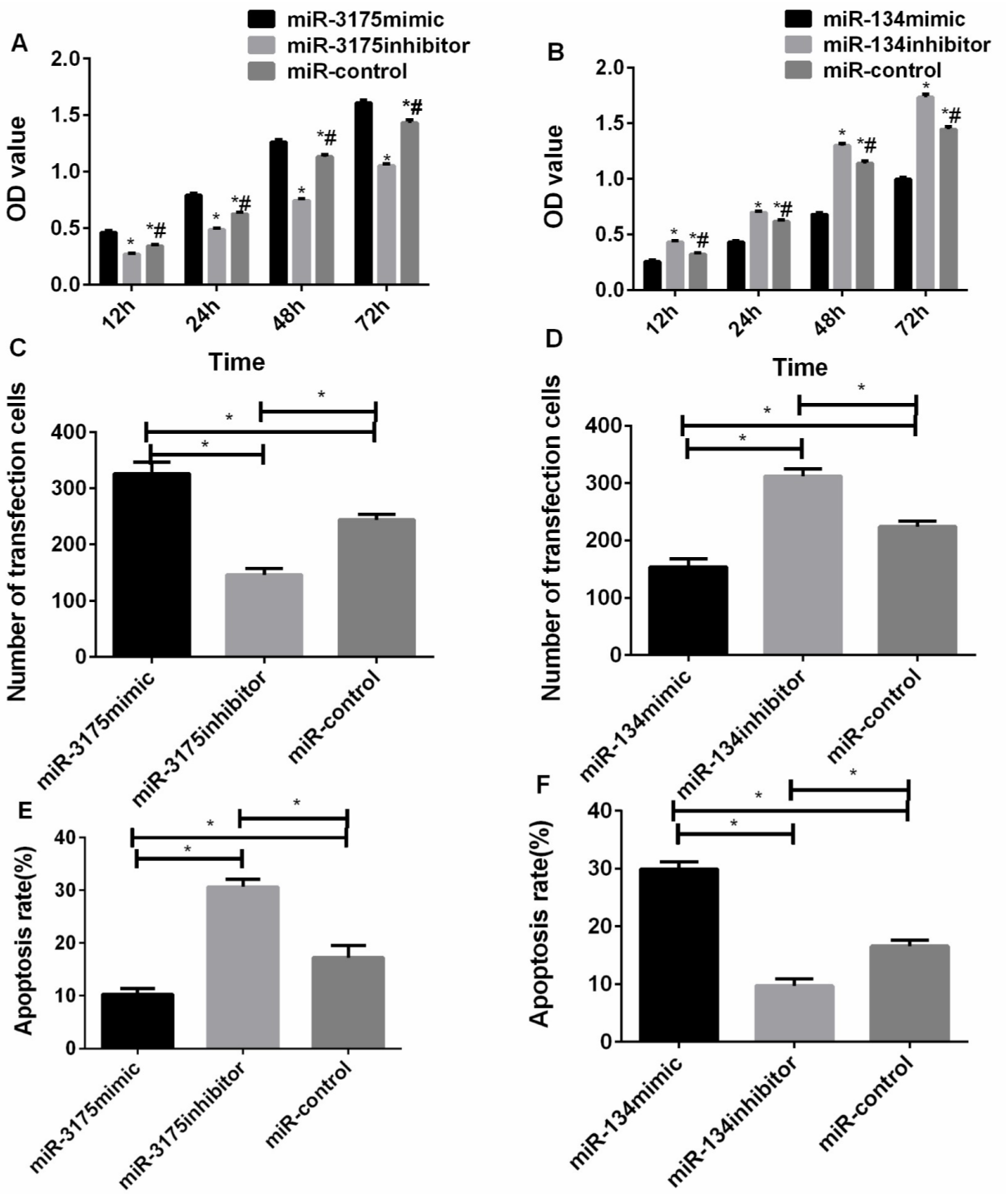

Figure 4. The effects of miR-3175 and miR-134 on proliferation, invasion and apoptosis of U251 cells. **A:** The result of MTT cell proliferation assay *in vitro* showed that the difference among absorbance value of the cells in miR-3175 mimic group, miR-3175 inhibitor group and miR-control group was statistically significant (*p<0.05). **B:** The result of MTT cell proliferation assay *in vitro* showed that the difference in absorbance value of the cells between miR-134 mimic group, miR-134 inhibitor group and miR-control group was statistically significant (*p<0.05), compared to miR-3175 mimic group at the same time point (p<0.05, # compared to miR-3175 inhibitor group at the same time point (p<0.05). **C:** The result of MTT cell proliferation assay *in vitro* showed that the difference among absorbance value of the cells in miR-3175 mimic group, miR-3175 inhibitor group and miR-control group was statistically significant (*p<0.05). **D:** The result of MTT cell proliferation assay *in vitro* showed that the difference in absorbance value of the cells between miR-134 mimic group, miR-134 inhibitor group and miR-control group was statistically significant (*p<0.05), compared to miR-3175 mimic group at the same time point (*p<0.05). #, compared to miR-3175 inhibitor group at the same time point (*p<0.05). **E:** The result of TUNEL apoptosis assay showed that the difference in apoptosis rate of the cells between miR-3175 mimic group, miR-3175 inhibitor group and miR-control group was statistically significant (*p<0.05). **F:** The result of TUNEL apoptosis assay showed that the difference in apoptosis rate of the cells between miR-134 mimic group, miR-134 inhibitor group and miR-control group was statistically significant (*p<0.05).

miR-3175 inhibitor group and miR-control group was statistically significant (p<0.05). The proliferation rate of the cells in miR-3175 mimic group and miR-control group at each time point was significantly higher than that of the cells in miR-3175 inhibitor group (p<0.05). The proliferation rate of the cells in miR-3175 mimic group at each time point was significantly higher than that of the cells in miR-control group (p<0.05). The difference among proliferation rate of the cells in miR-134 mimic group, miR-134 inhibitor group and miR-control group was statistically significant (p<0.05). The proliferation rate of the cells in miR-134 mimic group and miR-control group at each time point was significantly lower than that of the cells in miR-134 inhibitor group (p<0.05). The proliferation rate of the cells in miR-134 mimic group at each time point was significantly lower than that of the cells in miR-control group (p<0.05) (Figures 4A and 4B).

The effects of miR-3175 and miR-134 on invasion of U251 cells

The results of Transwell invasion assay *in vitro* showed that the difference among the numbers of transmembrane cells in miR-3175 mimic group, miR-3175 inhibitor group and miR-control group were statistically significant (p<0.05). The number of transmembrane cells in miR-3175 mimic group and miR-control group was significantly higher than that in miR-3175 inhibitor group (p<0.05). The difference in the number of transmembrane cells between miR-134 mimic group, miR-134 inhibitor group and miR-control group was statistically significant (p<0.05). The number of transmembrane cells in miR-134 mimic group and miR-control group was significantly lower than that in miR-134 inhibitor group (p<0.05). The number of transmembrane cells in miR-134 mimic group was significantly lower than that in miR-control group (p<0.05) (Figures 4C and 4D).

The effects of miR-3175 and miR-134 on apoptosis of U251 cells

The results of TUNEL apoptosis assay showed that the difference among apoptosis rate of the cells in the miR-3175 mimic group, miR-3175 inhibitor group and miR-control group was statistically significant (p<0.05). The apoptosis rate of the cells in the miR-3175 mimic group and miR-control group was significantly lower than that in the miR-3175 inhibitor group (p<0.05). The apoptosis rate of the cells in the miR-3175 mimic group was significantly lower than that in the miR-control group (p<0.05). The difference in apoptosis rate of the cells between miR-134 mimic group, miR-134 inhibitor group and miR-control group was statistically significant (p<0.05). The apoptosis rate of the cells in the miR-134 mimic group and miR-control group was significantly higher than that in miR-134 inhibitor group (p<0.05). The apoptosis rate of the cells in the miR-134 mimic group was significantly higher than that in the miR-control group (p<0.05) (Figures 4E and 4F).

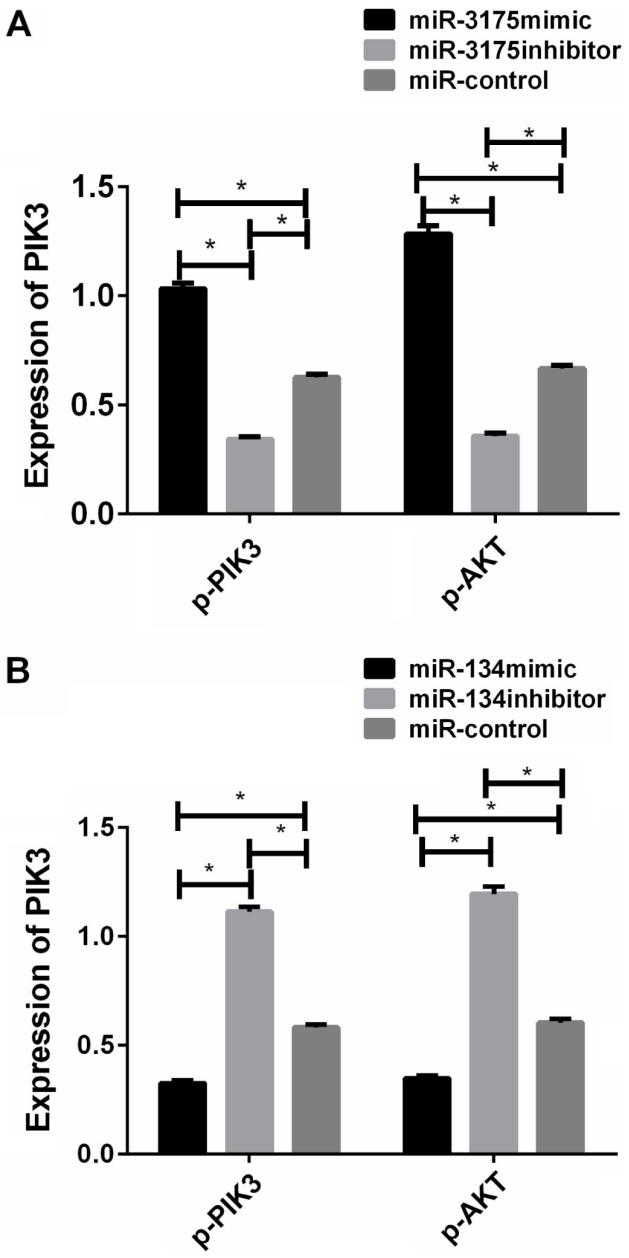

Figure 5. The effects of miR-3175 and miR-134 on levels of p-PI3K and p-AKT in U251 cells. **A:** The result of Western Blot showed that the difference in the relative expression levels of p-PI3K and p-AKT between the cells in miR-3175 mimic group, miR-3175 inhibitor group and miR-control group was statistically significant (*p<0.05). **B:** The result of Western Blot showed that the difference in the relative expression levels of p-PI3K and p-AKT in the cells between miR-134 mimic group, miR-134 inhibitor group and miR-control group was statistically significant (*p<0.05).

The effects of miR-3175 and miR-134 on the levels of p-PI3K and p-AKT in U251 cells

The results of Western Blot showed that the difference in the relative expression levels of p-PI3K and p-AKT in the cells between miR-3175 mimic group, miR-3175 inhibitor group and miR-control group was statistically significant ($p<0.05$). The relative expression levels of p-PI3K and p-AKT in miR-3175 mimic group were significantly higher than those in the miR-3175 inhibitor group and miR-control group ($p<0.05$). The relative expression level of miR-3175 in the cells in the miR-3175 inhibitor group was lower than in the miR-control group ($p<0.05$). The difference in the relative expression levels of p-PI3K and p-AKT in the cells between miR-134 mimic group, miR-134 inhibitor group and miR-control group was statistically significant ($p<0.05$). The relative expression levels of p-PI3K and p-AKT in the miR-134 mimic group were significantly lower than those in the miR-134 inhibitor group and the miR-control group ($p<0.05$). The relative expression levels of p-PI3K and p-AKT in the cells in the miR-134 inhibitor group were significantly higher than that in the miR-control group ($p<0.05$). (Figure 5).

Discussion

The occurrence and development of glioma involve a multi-step and multi-molecular pathological process. Infinite proliferative ability, high invasive ability and high metastatic ability are important biological characteristics of glioma cells [17,18]. With the development of molecular biology and therapeutic methods, an ever growing number of researches pay attention to the functions of miRs in connection with the occurrence and development of tumors. The expressions of miR-3175 and miR-134 in gliomas and their effects on the biological behavior of glioma cells were investigated in this study to provide an experimental basis for finding molecular therapeutic targets in this disease.

Firstly, the expressions of miR-3175 and miR-134 in tumor tissues of 40 patients with glioma were detected. Compared with non-tumor tissues, the relative expression level of miR-3175 was significantly higher than that in non-tumor tissues, while the relative expression level of miR-134 was significantly lower than that in non-tumor tissues. The results of survival analysis showed that the 5-year survival rate of the patients in miR-3175 low expression group was higher than that of the patients in miR-3175 high expression group, and the 5-year survival rate of the patients in miR-134 low expression group was lower than that of the patients in miR-134 high expression group, suggesting that miR-3175 might play a role as a proto-oncogene in gliomas, and that miR-134 might play a role as a tumor suppressor gene in this disease. To verify this hypothesis, the effects of miR-3175 and miR-134 on the biological behavior of U251 cells were analyzed and the results showed that upregulated expression of miR-3175 could facilitate the proliferation and invasion of U251 cells and inhibit their apoptosis, and that downregulated expression of miR-3175 had the opposite effect, while upregulated expression of miR-134 can inhibit the proliferation and invasion of U251 cells and facilitate their apoptosis. Downregulated expression of miR-134 can facilitate the proliferation and invasion of U251 cells and inhibit their apoptosis. These results verify the hypothesis that miR-3175 might play a role as a proto-oncogene in gliomas, while miR-134 might play a role as tumor suppressor gene in this malignancy.

There are few reports about the expression of miR-3175 and miR-134 in gliomas. In the study of Han et al [19], the expression of miR-3175 was upregulated in glioma cells, and downregulating its expression could targetedly increase the expression level of HOXB1 and inhibit the proliferative and invasive capacity of U87 glioma cells. In the study of Zhou et al [20], grading of gliomas was negatively correlated with the expression of miR-3175, survival time and prognosis of patients. Their findings are similar to the findings of this study. Although different brain glioma cell lines were used in our study, it is still demonstrated that miR-3175 plays a role as a proto-oncogene in gliomas. In the study of Niu et al [21], the expression of miR-134 was decreased in glioma tissues, and the increasing expression of miR-134 could inhibit the proliferative and invasive capability of U87 glioma cell lines. The relationship between miR-134 and prognosis of patients with gliomas has not been reported in studies. However, in the study of Liu et al [22], it was reported that miR-134 was an independent prognostic factor for patients with head and neck squamous cell carcinoma. In the study of Zhang et al [23], it was reported that miR-134 could inhibit the promotion effect of tyrosine kinase receptor on glioblastoma by regulating Kras and Stat5b. These reports help increase the credibility of the findings of this study and indicate that miR-134 plays a role as tumor suppressor in gliomas.

Reports about the mechanism of miR-3175 and miR-134 in tumors are few. In the study of El-Daly et al [24], it was reported that miR-134 could targetedly regulate PI3K signaling pathway and its downstream signal molecules in colorectal cancer cells, and that after miR-134 mimics transfection,

the expression of p-AKT and p-mTOR were significantly decreased and proliferation, colony formation, migration and invasion of cells were inhibited. Therefore, PI3K signaling pathway was used in this study to analyze the specific mechanism of miR-3175 and miR-134 in glioma cells. The results showed that downregulating the expression of miR-3175 or upregulating the expression of miR-134 inhibited the activation of PI3K signaling pathway. These results indicated that miR-3175 and miR-134 had opposite effects in glioma cells. However, at present, no research on this topic has been reported yet, so this result still needs to be proved by experimental evidence.

In summary, miR-3175 is highly expressed in U251 glioma cells. The 5-year survival rate of patients with high miR-3175 expression is low. miR-134 is lowly expressed in gliomas. The 5-year survival rate of patients with low expression of miR-134 is low. Downregulating the expression of miR-3175 or facilitating the expression of miR-134 could inhibit the proliferative and invasive capability of glioma cells and facilitate their apoptosis by inhibiting the activation of PI3K signalling pathway.

Conflict of interests

The authors declare no conflict of interests.

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