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MiRNA-92a promotes cell proliferation and invasion through binding to KLF4 in glioma

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Abstract. – **OBJECTIVE:** Glioma is one of the most frequent brain tumors in adults, and it has a low 5-year survival rate. MicroRNA-92a (miR-92a) has been reported to be upregulated and acted as an oncogene in many cancers. The purpose of this study was to explore the molecular mechanisms of miR-92a and kruppel-like factor 4 (KLF4) in glioma.

PATIENTS AND METHODS: Western blotting assay and quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) were applied to calculate the relative expression of interest proteins and mRNAs. Luciferase ability assay was conducted to evaluate whether miR-92a was targeting to KLF4.

RESULTS: A higher expression of miR-92a was observed in glioma tissues compared with the corresponding adjacent non-tumor tissues. The upregulation of miR-92a predicted poor prognostic characteristics of glioma. The overexpression miR-92a significantly promoted cell proliferation an invasion, while the knockdown of miR-92a presented the opposite results. MiR-92a bound to KLF4 and mediated the expression of KLF4 in glioma cells. The knockdown of miR-92a inhibited cell invasion-mediated EMT. Furthermore, the knockdown of miR-92a suppressed cell proliferation through the KLF4/AKT/mTOR signal pathway.

CONCLUSIONS: MiR-92a promoted the proliferation through the KLF4/AKT/mTOR signal pathway in glioma. The newly identified miR-92a/KLF4/AKT/mTOR axis provides novel insight into the pathogenesis of glioma.

Key Words
MiR-92a, Glioma, KLF4, Invasion, Proliferation.

Introduction

Glioma is one of the most frequent brain tumors in adults, which originated in precursors and glial cells[1,2]. The 5-year overall survival rate of glioma is less than 10%, due to the extensive intracranial invasion and aggressiveness[3,4]. The effect of traditional treatments is still poor; therefore it is necessary to explore the mechanisms and potential pathways for glioma prevention and clinical treatment.

MicroRNAs (miRNAs), a type of endogenous short non-coding RNA, could degrade or inhibit mRNA translation by binding to the complementary sequences on the 3'-untranslated region (3'UTR) of target mRNA at post-transcriptional level[5,6]. Accumulating evidence indicated that miRNA acted as an oncogene or tumor suppressor, and regulated tumor process in various sides including cell proliferation, differentiation and apoptosis[7,8]. MiR-92a has been reported to be an oncogene in multiple of tumors, including nasopharyngeal carcinoma, non-small cell lung cancer, cervical and endometrial cancers[9-12]. In non-small cell lung cancer, miR-92a has been reported to upregulate and promote the cell proliferation[13]. Furthermore, miR-92a has been reported to significantly promote the cell viability and invasion in cervical cancer[14]. Thus, we strongly believe that miR-92a might play a great role in tumorigenesis and metastasis.

Kruppel-like factor 4 (KLF4) belongs to the Kruppel family; it is thought to control the G1-to-

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S transition of the cell cycle following DNA damage by mediating the tumor suppressor gene p53[15]. In colorectal cancer, KLF4 has been reported to downregulate and inhibit the cell proliferation through the NDRG2 signaling[16]. In non-small cell lung cancer, KLF4 could activate the c-Met/Akt signaling pathway by decreasing the inhibition of β-Catenin on phosphorylation of c-Met to prevent blockade by gefitinib[17].

Patients and Methods

Patients and Tissue Samples

Glioma tissues and corresponding adjacent non-tumor tissues were obtained from 50 glioma patients who has been treated with surgery at the Affiliated Yantai Yuhuangding Hospital of Qingdao University from January 2015 to December 2017. All the patients did not accept any treatment before the surgical operation. All the specimens were snapped frozen in liquid nitrogen and stored at -80°C. The study protocol was approved by the Ethics Committee of the Affiliated Yantai Yuhuangding Hospital of Qingdao University and all the patients provided informed consent before specimen collection.

Cell Line and Cell Culture

The normal immortalized gliocyte HEB and two glioma cells (LN18 and LN229) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Grand Island, NY, USA) were conducted to culture all the cells, which were supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) stored at 37°C containing 5% CO_2.

Vectors and Cell Transfection

MiR-92a mimic, miR-92a inhibitor and corresponding miRNA control oligo were purchased from GenePharma (Shanghai, China). LN229 cells were seeded into 6-well plate and cultured 12 h with 80% confluence. The transfection reagent and vectors were diluted by Opti-MEM/Reduced serum medium (Thermo Fisher Scientific, Waltham, MA, USA) and mixed. The mixture was then added in the 6-well plate and cultured at 37°C incubator. The transfections were performed using Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The cells with transiently transfection miR-92a mimic or inhibitor were harvested after culturing for 48 h. Whereas, the stable transfection cells were transfected with the plasmid containing miR-92a mimic or control fragment and then selected by Geneticin (G418; Thermo Fisher Scientific, Waltham, MA, USA). The sequences for miR-92a were as follows: mimic sense, UAUUGCACUUGUCCCGGCCUG; inhibitor CAG GCC GGGACA AGU GCA AUA.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was conducted to extract total RNA, which contains miRNA and mRNA. PrimeScript RT Reagent Kit (TaKaRa, Otsu, Shiga, Japan) was applied to carry out the first cDNA chain synthesis. QuantMir RT Kit (System Biosciences, Mountain View, CA, USA) and Real-time PCR Mixture Reagent (TaKaRa, Otsu, Shiga, Japan) were employed to perform qPCR of miR-92a and KLF4 expression under ABI 7900 Sequence Detection System (Life Technologies, Gaithersburg, MD, USA) with enzyme activation at 95°C for 5 min, followed by 40 cycles at 95°C for 5 s and 60°C for 20 s, and a final extension at 65°C for 15 s. The internal reference of miRNAs and mRNAs were U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), respectively. The relative expression was calculated in accordance with the $2^{-\Delta\Delta Ct}$ method. The primers are: miR-92a forward: 5'-CACCTATATTGCACTTGTCC-3', reverse: 5'-TGCGTGTCGTGGAGTC-3'; U6 forward: 5'-CGCTTCGGCAGCACATATAC-3', reverse: 5'-TTCACGAATTTGCGTGTCAT-3'; KLF4 Forward TTCCCATCTCAAGGCACAC, Reverse : GGTCGCATTTTTGGCACT; GAPDH forward, 5'-GGTGGTCTCCTCTGACTTCAACA-3' and reverse 5'-GTGGTCGTTGAGGGCAATG-3'.

Protein Extraction and Western Blot

Radioimmunoprecipitation assay (RIPA) Lysis Buffer (ProMab Biotechnology, Richmond, CA, USA) containing protease inhibitor (Thermo Fisher Scientific, Waltham, MA, USA) was used to isolate and extract total proteins on ice. After being centrifuged at 12,000 g for 20 min, the protein lysate with equal amount was separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. The proteins were electro-transferred onto polyvinylidene difluoride (PVDF) membranes (Roche, Basel, Switzerland), and then blocked in 5% bovine serum albumin

(BSA) for 1 h at room temperature. Subsequently, the membranes were incubated with antibodies against KLF4 (1:1000, Abcam, Cambridge, MA, USA; ab75486), p-AKT (1:1000, Abcam; ab8933), AKT (Cell Signaling Technology, Danvers, MA, USA; #9272), p-mTOR (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, USA; sc-293133), mTOR (1:1000, Abcam, Cambridge, MA, USA; ab32028), E-cadherin (1:1000, Abcam, Cambridge, MA, USA; ab15148), N-cadherin (1:1000, Abcam, Cambridge, MA, USA; ab18203) and Vimentin (1:1000, Abcam, Cambridge, MA, USA; ab8978) at 4°C overnight. Then, the anti-rabbit IgG, horseradish peroxidase (HRP) conjugated-linked antibody (Cell Signaling Technology, Danvers, MA, USA; #7074) incubated the membranes for 2h at room temperature. Enhanced Chemiluminescence Kit (ECL; Thermo Fisher Scientific, Waltham, MA, USA) was conducted to perform the interest proteins and exposed on Molecular Imager ChemiDoc XRS System (Bio-Rad, Hercules, CA, USA).

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide) Assay

MTT and dimethyl sulfoxide (DMSO) solutions (Sigma-Aldrich, St. Louis, MO, USA) were employed to calculate the cell proliferative ability. Specific cells of 2×10^4 were seeded into 96-well plates. The sterile MTT solution 10 µL was added in the 96-well plate after cells culturing for 24 h, 48 h, 72 h, and 96 h. After incubation for 4 h at 37°C, the medium was removed and was added 150 µL DMSO into each well. The absorbance at 490 nm was measured on a microplate reader (BioTek, Winooski, VT, USA).

Transwell Assays

Transwell inserts (Corning Incorporated, Corning, NY, USA) covered with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) were placed in 24-well plate and conducted lower and upper two chambers. A total of 5×10^5 LN229 cells resuspended by 200 µL RPMI-1640 medium free FBS were seeded into the upper chambers. The lower chambers were filled with 500 µL normal RPMI-1640 medium containing 15% FBS served as chemoattractant. After incubating 48 h at 37°C humidified incubator with 5% CO_2, the non-invaded cells were removed by cotton swabs, whereas invaded cells were fixed by paraformaldehyde and stained by crystal violet. The number of invaded cells was counted under a light microscope (Olympus, Tokyo, Japan).

Vector Construction and Luciferase Activity Assay

The binding sequence of miR-92a on KLF4 3’-UTR, which was predicted by TargetScan (http://www.targetscan.org/vert_71/), was mutated from GUGCAAU to CACGUUA using a Quick Change

Table I. Correlation of miR-92a expression with the clinicopathological characteristics of the glioma patients.

Clinicopathological features	Cases (n=50)	miR-92a expression		*p*-value
		High (n=27)	Low (n=23)	
Age (years)				0.9819
> 60	26	14 (53.8)	12 (46.2)	
≤ 60	24	13 (54.2)	11 (45.8)	
Gender				0.8110
Male	27	15 (55.6)	12 (44.4)	
Female	23	12 (52.2)	11 (47.8)	
Lymph node metastasis				0.0470*
YES	25	17 (68.0)	8 (32.0)	
NO	25	10 (40.0)	15 (60.0)	
Tumor size (cm)				0.0745
≥ 5.0	22	15 (68.2)	7 (31.8)	
< 5.0	28	12 (42.9)	16 (57.1)	
WHO grade				0.0119*
I-II	27	19 (67.9)	8 (32.1)	
III-IV	23	8 (36.4)	15 (63.6)	
KPS				0.0354*
< 80	21	15 (71.4)	6 (28.6)	
≥ 80	29	12 (41.4)	17 (58.6)	

WHO: World Health Organization; KPS: Karnofsky performance scale. *Statistically significant.

Multi Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA). The wild-type and the mutant 3'-UTR of KLF4 mRNA were inserted into the psiCHECK-2 vector (Promega, Madison, WI, USA), which were designated as psiCHECK2-KLF4-WT and psiCHECK2-KLF4-MUT. LN229 cells co-transfected miR-92a or miR-NC and psiCHECK2-KLF4-WT or psiCHECK2-KLF4-MUT using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) was performed to evaluate the firefly Luciferase ability with Renilla Luciferase as the internal reference.

Xenograft Tumor Formation Assay

The nude mice of four weeks were purchased from Charles River Laboratories (Beijing, China). LN229 cells (2×10^6) were subcutaneous injected into one side of the axillae of the nude mice to establish xenograft. The length and the width of the xenograft tumor were evaluated and recorded every 3 days after the transplant tumor model was completed. The volume of the xenografts was calculated as the square of length multiplied by the width divided by two. The experiment was terminated after culturing 26 days and the xenografts were incised. All animal experiments were performed in the Animal Laboratory Center of Qingdao University and were approved by the Qingdao University Animal Care and Use Committee.

Statistical Analysis

Statistical analyses were performed by GraphPad Prism 5.0 software (San Diego, CA, USA) and Statistical Product and Service Solutions (SPSS) 16.0 (Chicago, IL, USA); the results are expressed as the mean ± standard deviation (SD). Student's paired two-tailed *t*-tests and one-way ANOVA followed by a Tukey post-hoc test were performed for statistical analysis between two samples and more groups respectively. A *p*-value less than 0.05 was considered statistically significant.

Results

MiR-92a Was Upregulated in Glioma Tissues and the Upregulation of MiR-92a Predicted Poor Prognosis

To determine the possible functions of miR-92a in glioma, the mRNA levels of miR-92a were measured in cancer and corresponding adjacent non-tumor samples. We found that the expression of miR-92a was higher in all 50 glioma tissues than the adjacent normal non-tumor tissues ($p<0.0001$) (Figure 1A).

Furthermore, miR-92a had an inverse connection with lymph node metastasis ($p=0.0470$), WHO stage ($p=0.0119$) and Karnofsky performance scale ($p=0.0354$). In addition, Kaplan-Meier estimation was applied to analyze the clinical survival information. MiR-92a expressed at higher levels in glioma patients was related to worse

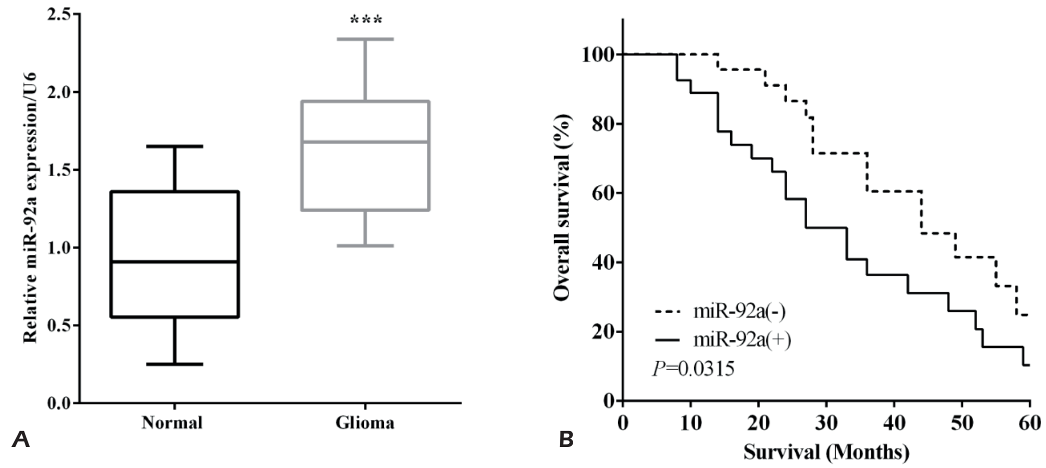

Figure 1. MiR-92a was upregulated in glioma tissues and the upregulation of miR-92a predicted poor prognosis. ***A,*** the average expression of miR-92a was higher in glioma tissues than in the adjacent normal non-tumor tissues. ***B,*** miR-92a expressed at higher levels in glioma patients was related to worse overall survival in glioma patients. ***$p<0.001$.

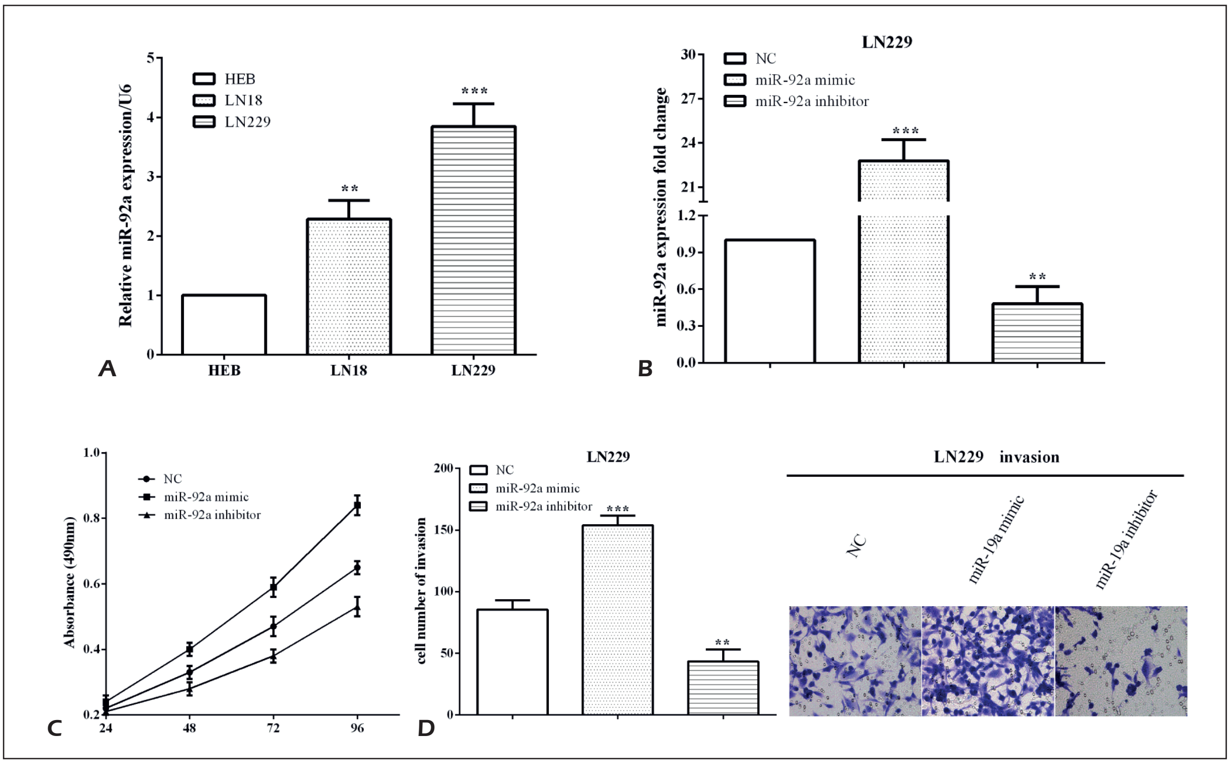

Figure 2. MiR-92a promoted the proliferation and invasion in glioma. ***A,*** miR-92a mRNA level was lower in immortalized gliocyte HEB than glioma cells LN18 and LN229. ***B,*** the miR-92a expression was increased by the miR-92a mimic, while it was reduced by the miR-92a inhibitor compared with the control in LN229 cells. ***C,*** The miR-92a mimic increased the proliferative ability whereas the miR-92a inhibitor repressed that in LN229 cells. ***D,*** the miR-92a mimic increased the invasive ability, whereas it was restrained by the miR-92a inhibitor. **$p<0.01$; ***$p<0.001$.

overall survival in glioma patients ($p=0.0315$) (Figure 1B). The results indicate miR-92a may act as a biomarker to predict the prognosis of glioma.

MiR-92a Promoted the Proliferation and Invasion in Glioma

As the results show, the expression of miR-92a was calculated in glioma cell lines LN18 and LN229 and normal immortalized gliocyte HEB. As expected, the mRNA level of miR-92a was lower in immortalized gliocyte HEB than that in glioma cells LN18 ($p=0.0021$) and LN229 ($p=0.0002$) (Figure 2A).

To explore the effects of miR-92a in glioma cells, we chose LN229 cells as the cell models according to the expression of miR-92a. MiR-92a mimic and miR-92a inhibitor were employed to up- or down-regulate the mRNA levels of miR-92a in LN229 cells that were confirmed by qRT-PCR. After transfection, the expression of miR-92a was increased ($p<0.0001$) by miR-92a mimic while reduced ($p=0.0031$) by miR-92a inhibitor *vs.* the control in LN229 cells (Figure 2B).

To determine its effect on proliferative and invasive abilities, the MTT and transwell assays were performed in LN229 cells. MTT assay was performed to examine the LN229 cells proliferative ability when exogenous altered miR-92a. Compared with the control group, the proliferative ability was increased ($p=0.0128$, 0.0080, 0.0008) by the miR-92a mimic whereas it was repressed ($p=0.0376$, 0.0124, 0.0045) by the miR-92a inhibitor in LN229 cells at 48 h, 72 h, 96 h, respectively (Figure 2C).

The transwell assay detected the invasive capacity after alteration of miR-92a in LN229 cell. Similar results with proliferation were found; the invade cell number was increased ($p=0.0004$) by the miR-92a mimic, but it was restrained ($p=0.0041$) by the miR-92a inhibitor (Figure 2D). The data indicated that miR-92a may play important roles in promoting cell proliferation and invasion.

KLF4 Was Identified as a Direct Target Gene of MiR-92a in Glioma Cells

The target genes of miR-92a were predicted by TargetScan, and we discovered KLF4 was a potential target of miR-92a. To verify miR-

92a binding to KLF4, we mutated the binding sequences from GUGCAAU to CACGUUA, as shown in Figure 3A. Both the wide-type and the mutant 3’-UTR of KLF4 mRNA, which contains the predicted binding site of miR-92a, were inserted into pmirGlo Luciferase vector (named as WT and MUT respectively). Subsequently, the Luciferase activities were calculated after being cotransfected miR-92a mimic and WT or MUT into LN229 cells. Compared to the control, miR-92a mimic could reduce (p=0.0010) the Luciferase activity of wide-type KLF4 mRNA 3’-UTR, whereas exhibited no inhibitory effects (p=0.4749) on the Luciferase activity of mutant KLF4 mRNA 3’-UTR in LN229 cells (Figure 3B). Furthermore, KLF4 mRNA level was downregulated (p=0.0019) by miR-92a mimic, while it was increased (p=0.0030) by miR-92a inhibitor in LN229 cells (Figure 3C). In short, the results indicated that miR-92a mediated KLF4 expression by binding to the 3’-UTR in glioma cells.

MiR-92a Enhanced Glioma Cell Proliferation and Invasion Through the AKT/mTOR Signal Pathway

The expression of KLF4 was calculated in 50 pairs of glioma and their corresponding adjacent non-tumor tissues. Contrary to the expression of miR-92a, KLF4 was significantly lowly expressed in glioma tissues compared to the corresponding adjacent non-tumor tissues (p<0.0001) (Figure 4A). Moreover, the mRNA levels of KLF4 were evaluated in both glioma cells and normal immortalized gliocyte. The expression of KLF4 was significantly lower in glioma cells LN18 (p=0.0115) and LN229 (p=0.0012) than HEB cells (Figure 4B).

To explore the mechanism of miR-92a mediated glioma cell proliferation and invasion, the EMT markers and mTOR pathway proteins were measured by Western blot. The proteins levels of KLF4 and E-cadherin were improved while the N-cadherin and Vimentin were reduced after the knockdown of miR-92a in LN229 cells (Figure

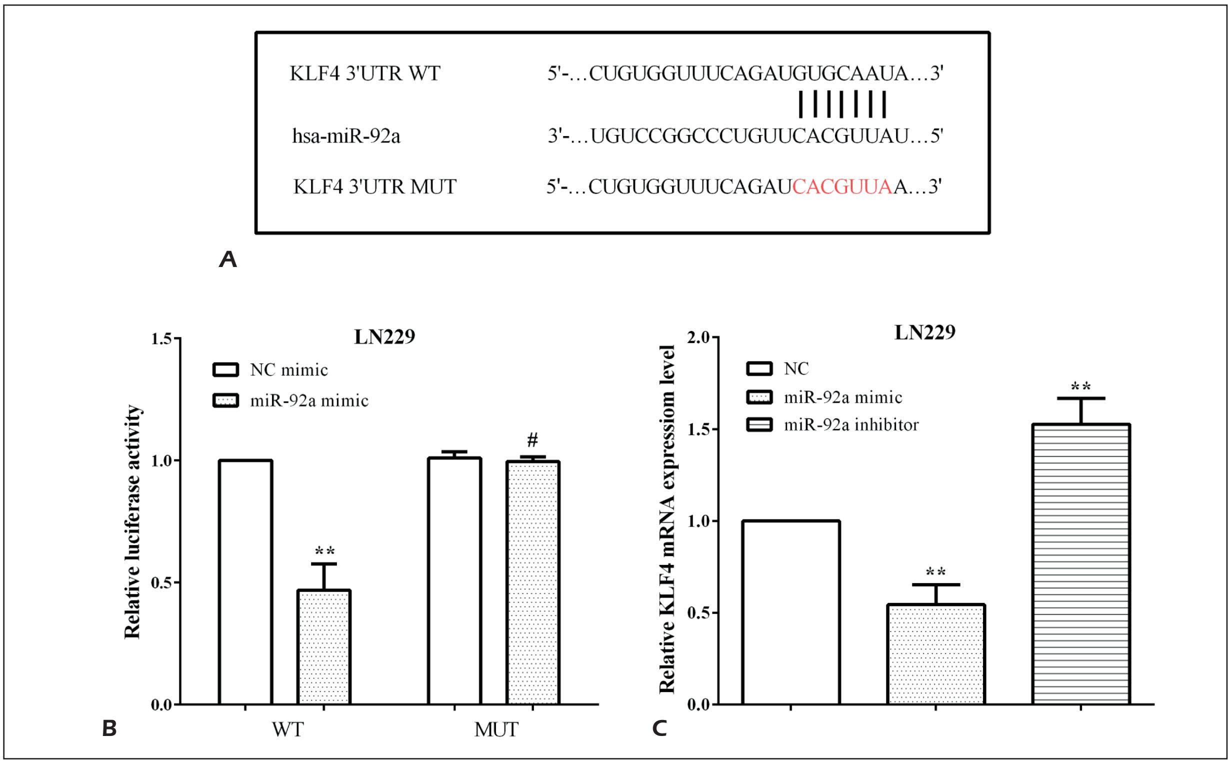

Figure 3. MiR-92a mediated the KLF4 expression by binding to the 3'-UTR in glioma cells. ***A,*** TargetScan database predicted the putative miR-92a binding sites in the 3'-UTR of KLF4 mRNA. ***B,*** miR-92a mimic could reduce the Luciferase activity of LN229 cell of wide-type KLF4 3'-UTR, whereas exhibited no inhibitory effects on the Luciferase activity of mutant KLF4 3'-UTR. ***C,*** KLF4 mRNA level was downregulated by miR-92a mimic, while increased by miR-92a inhibitor in LN229 cells. **p<0.01; $^{\#}p$>0.05; WT, wild-type of KLF4 mRNA 3'-UTR; MUT, mutant of KLF4 mRNA 3'-UTR.

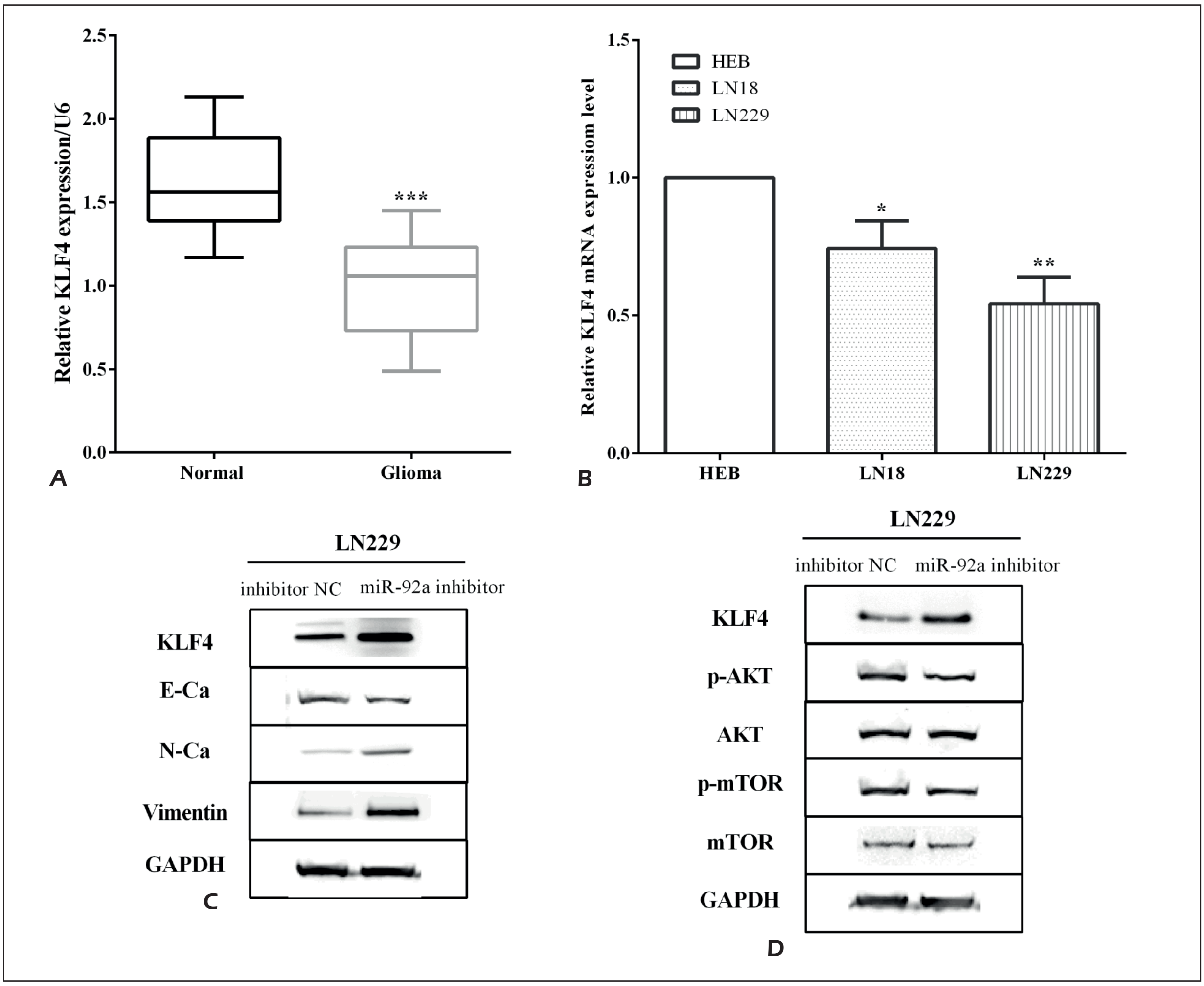

Figure 4. MiR-92a enhanced glioma cell proliferation and invasion through the AKT/mTOR signal pathway. ***A,*** KLF4 was lowly expressed in glioma tissues vs. adjacent non-tumor tissues. ***B,*** The expression of KLF4 was lower in glioma cells LN18 and LN229 than that in HEB cells. ***C,*** The knockdown of miR-92a inhibited cell EMT in LN229 cells. ***D,*** The knockdown of miR-92a inhibited mTOR signal pathway in LN229 cells. *p<0.05, **p<0.01, ***p<0.001.

4C). Moreover, the knockdown of miR-92a could inhibit the expression of p-AKT and p-mTOR, whereas the expression of AKT and mTOR had no alteration, which elucidated that the knockdown of miR-92a suppressed mTOR signal pathway (Figure 4D). The knockdown of miR-92a inhibited the invasion-mediated EMT and the proliferation through the AKT/mTOR signal pathway in LN229 cells.

MiR-92a Promoted the Xenograft Growth In Vivo

LN229 cells that stably transfected miR-92a inhibitor plasmid or control plasmid were subcutaneously injected into the nude mice. After culturing the mice and calculating the volumes of nude mice, the results showed that the xenograft volumes of nude mice injected with miR-92a inhibitor were 301.95 and 345.42 mm^3, whereas the sizes of the control group were 542.72 and 584.61 mm^3. The knockdown of miR-92a had a remarkable smaller tumor volume compared to the control group (p=0.0298) (Figure 5A). The volume of the xenograft tumors was calculated every 3 days and the growth rate was slower in the knockdown of the miR-92a group than the control group (p=0.0298), which elucidated that miR-92a promoted the growth of glioma xenograft (Figure 5B).

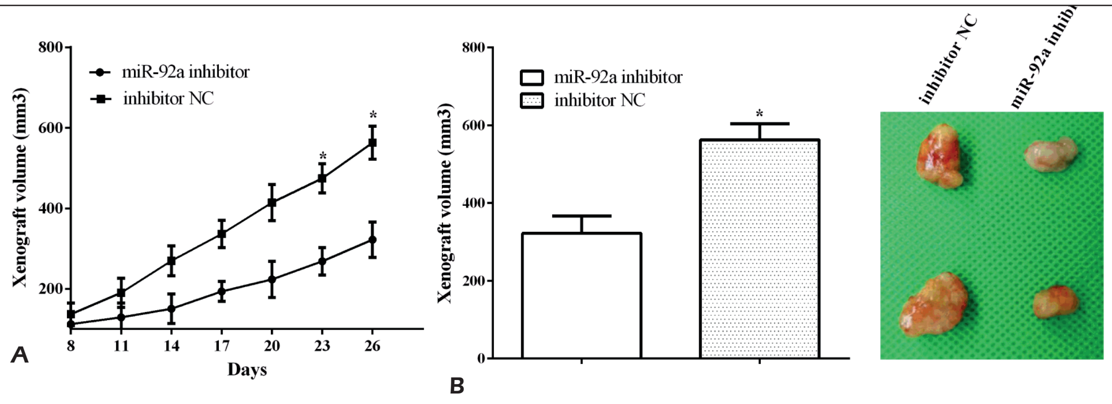

Figure 5. The knockdown of miR-92a suppressed the xenograft growth *in vivo*. ***A,*** The xenograft growth rate was significantly slower in the miR-92a inhibitor-transfected group than the control group. ***B,*** The knockdown of miR-92a had a remarkable smaller tumor volume compared to the control group. $^*p<0.05$.

Discussion

Glioma, originated in precursors and glial cells, has a low 5-year survival rate due to the extensive intracranial invasion and aggressiveness[3,4]. It is critical to explore the mechanisms and potential pathways for the prevention and clinical of glioma treatment.

MiRNAs may act as oncogenes or tumor suppressors and it had been reported to regulate various sides of the tumor process including cell proliferation, differentiation and apoptosis[7,8]. MiR-92a has been reported to act as an oncogene in several tumors[9-12]. In colorectal cancer, Zhang et al[18] indicated that the miR-92a acted as an oncogene and promoted the proliferation, migration, invasion and xenograft growth. Similarly, miR-92a has been reported to promote the growth, migration and invasion through the PI3K/AKT signal pathway in non-small cell lung cancer[19]. Our results were consistent with all the findings; miR-92a was discovered to be overexpressed in glioma and high level of miR-92a predicted poor prognosis in glioma. In glioma cells, we found that miR-92a promoted cell proliferation, migration, invasion and the growth of xenograft. Furthermore, we discovered that KLF4 was a direct target gene of miR-92a, which was consistent with the findings of Lv et al[20] in colorectal cancer.

KLF4 could control the G1-to-S transition of the cell cycle following DNA damage by mediating the tumor suppressor gene p53[15]. KLF4 was reported to be downregulated and it inhibited the proliferation through the NDRG2 signaling in colorectal cancer[16]. Furthermore, it also reported that KLF4 inhibited cell migration, invasion, EMT and inhibited xenograft growth in pancreatic cancer [21]. Consistent with all the findings, we indicated that KLF4 was downregulated in glioma tissues and cells. KLF4 could mediate by miR-92a by direct targeting it, which was consistent with Lv et al[20]. We first propose that the knockdown of miR-92a enhanced cell EMT by targeting to KLF4 in glioma. Moreover, miR-92a promoted glioma cell proliferation by activating the AKT/mTOR signaling, which was consistent with Lu et al[22]. In addition, we first propose that the knockdown of miR-92a promoted the growth of glioma cell xenograft.

Conclusions

We dicovered that the expression level of miR-92a was higher in glioma tissues than in the corresponding adjacent non-tumor tissues. MiR-92a upregulation predicted poor prognosis in glioma. MiR-92a overexpression promoted cell proliferation and invasion, while knockdown suppressed. MiR-92a directly bound to the 3'-UTR of KLF4 mRNA and mediated the expression of KLF4 in glioma cells. The knockdown of miR-92a inhibited cell invasion-mediated EMT. Furthermore, the knockdown of miR-92a suppressed cell proliferation through the KLF4/AKT/mTOR signal pathway.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Kouhkan F, Mobarra N, Soufi-Zomorrod M, Keramati F, Hosseini Rad SM, Fathi-Roudsari M, Tavakoli R, Hajarizadeh A, Ziaei S, Lahmi R, Hanif H, Soleimani M. MicroRNA-129-1 acts as tumor suppressor and induces cell cycle arrest of GBM cancer cells through targeting IGF2BP3 and MAPK1. J Med Genet 2016; 53: 24-33.
2) Xu J, Sun T, Hu X. microRNA-513c suppresses the proliferation of human glioblastoma cells by repressing low-density lipoprotein receptor-related protein 6. Mol Med Rep 2015; 12: 4403-4409.
3) Zhao WH, Wu SQ, Zhang YD. Downregulation of miR-124 promotes the growth and invasiveness of glioblastoma cells involving upregulation of PPP1R13L. Int J Mol Med 2013; 32: 101-107.
4) Munthe S, Halle B, Boldt HB, Christiansen H, Schmidt S, Kaimal V, Xu J, Zabludoff S, Mollenhauer J, Poulsen FR, Kristensen BW. Shift of microRNA profile upon glioma cell migration using patient-derived spheroids and serum-free conditions. J Neurooncol 2017; 132: 45-54.
5) van den Berg A, Mols J, Han J. RISC-target interaction: cleavage and translational suppression. Biochim Biophys Acta 2008; 1779: 668-677.
6) Siomi H, Siomi MC. Posttranscriptional regulation of microRNA biogenesis in animals. Mol Cell 2010; 38: 323-332.
7) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
8) Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15: 509-524.
9) Yu Y, Zuo J, Tan Q, Zar TK, Li P, Zhu M, Yu M, Fu Z, Liang C, Tu J. Plasma miR-92a-2 as a biomarker for small cell lung cancer. Cancer Biomark 2017; 18: 319-327.
10) Su Z, Yang H, Zhao M, Wang Y, Deng G, Chen R. MicroRNA-92a promotes cell proliferation in cervical cancer via inhibiting p21 expression and promoting cell cycle progression. Oncol Res 2017; 25: 137-145.
11) Torres A, Kozak J, Korolczuk A, Wdowiak P, Domanska-Glonek E, Maciejewski R, Torres K. In vitro and in vivo activity of miR-92a-locked nucleic acid (LNA)-inhibitor against endometrial cancer. BMC Cancer 2016; 16: 822.
12) Zhang H, Cao H, Xu D, Zhu K. MicroRNA-92a promotes metastasis of nasopharyngeal carcinoma by targeting the PTEN/AKT pathway. Onco Targets Ther 2016; 9: 3579-3588.
13) Zhu Q, Zang Q, Jiang ZM. Enhanced expression of non coding miR 92a expression is implicated in the development of lung cancer. Eur Rev Med Pharmacol Sci 2018; 22: 1028-1034.
14) Luo S, Li N, Yu S, Chen L, Liu C, Rong J. MicroRNA-92a promotes cell viability and invasion in cervical cancer via directly targeting Dickkopf-related protein 3. Exp Ther Med 2017; 14: 1227-1234.
15) Shie JL, Chen ZY, Fu M, Pestell RG, Tseng CC. Gut-enriched Kruppel-like factor represses cyclin D1 promoter activity through Sp1 motif. Nucleic Acids Res 2000; 28: 2969-2976.
16) Ma Y, Wu L, Liu X, Xu Y, Shi W, Liang Y, Yao L, Zheng J, Zhang J. KLF4 inhibits colorectal cancer cell proliferation dependent on NDRG2 signaling. Oncol Rep 2017; 38: 975-984.
17) Feng W, Xie Q, Liu S, Ji Y, Li C, Wang C, Jin L. Kruppel-like factor 4 promotes c-Met amplification-mediated gefitinib resistance in non-small-cell lung cancer. Cancer Sci 2018; 109: 1775-1786.
18) Zhang G, Zhou H, Xiao H, Liu Z, Tian H, Zhou T. MicroRNA-92a functions as an oncogene in colorectal cancer by targeting PTEN. Dig Dis Sci 2014; 59: 98-107.
19) Ren P, Gong F, Zhang Y, Jiang J, Zhang H. MicroRNA-92a promotes growth, metastasis, and chemoresistance in non-small cell lung cancer cells by targeting PTEN. Tumor Biol 2016; 37: 3215-3225.
20) Lv H, Zhang Z, Wang Y, Li C, Gong W, Wang X. MicroRNA-92a promotes colorectal cancer cell growth and migration by inhibiting KLF4. Oncol Res 2016; 23: 283-290.
21) Zhu Z, Yu Z, Wang J, Zhou L, Zhang J, Yao B, Dou J, Qiu Z, Huang C. Kruppel-like factor 4 inhibits pancreatic cancer epithelial-to-mesenchymal transition and metastasis by down-regulating caveolin-1 expression. Cell Physiol Biochem 2018; 46: 238-252.
22) Lu C, Shan Z, Hong J, Yang L. MicroRNA-92a promotes epithelial-mesenchymal transition through activation of PTEN/PI3K/AKT signaling pathway in non-small cell lung cancer metastasis. Int J Oncol 2017; 51: 235-244.