

miR-92b regulates glioma cells proliferation, migration, invasion, and apoptosis via PTEN/Akt signaling pathway

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Received: 25 October 2015 / Accepted: 3 February 2016 / Published online: 18 February 2016
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Abstract Glioblastoma (GBM) is a highly invasive malignant primary brain tumor with neoplastic growth. Despite the progresses made in surgery, chemotherapy, and radiation in recent decade, the prognosis of patients with gliomas remains poor and the average survival time of patients suffering from glioblastoma is still short. As a potential therapy strategy, microRNAs have been considered as new targets for possible cancer treatment. In this study, we found that the miR-92b inhibitors (miR-92b-I) could inhibit the proliferation, migration, invasion, and promote the apoptosis of glioma cells. As a predicted target of miR-92b, phosphatase and tensin homolog (PTEN), also elevated at both mRNA and protein levels. Moreover, the Akt phosphorylation was consistently inhibited. The rescue experiment with miR-92b and PTEN double knockdown resulted in partial reversion of miR-92b-I-induced phenotypes. Taken together, our findings indicated that miR-92b-I could restrain the proliferation, invasion, migration, and stimulate apoptosis of glioma cells by targeting PTEN/Akt

signaling pathway. Further investigations will focus on antitumor effect of miR-92b-I in glioma treatment.

Keywords miR-92b · Glioblastomas · PTEN · Akt · Carcinogenesis

Introduction

Glioblastoma (GBM) is one of the most common and malignant tumors of the central nervous system, characterized by well-adapted to poorly immunogenic and hypoxic conditions. Despite advances and progress in surgery, chemotherapy, and radiation, the average survival time of patients suffering from glioblastoma is only 9–12 months, and the progression-free survival (PFS) time is round 5–8 months, with 53.9 % as 6 months PFS and 26.9 % as 2-year overall survival (OS) rates [5–7]. Increasing evidence suggests that the major obstacle to effective treatment of gliomas is due to the highly proliferation, migration, and invasion nature of glioma cell [15]. However, the mechanisms controlling these are still poorly understood. Therefore, understanding the regulatory mechanisms involved in these biological characteristics of GBM cells is essential for developing novel and effective therapeutic approaches.

MicroRNAs (miRNAs) are a class of small endogenous non-coding RNA molecules found in eukaryotic organisms, which regulate gene expression via translational suppression or target degradation

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by base-pairing with complementary sequences, ordinarily in the 3'-untranslated region (UTR) of target mRNAs [3]. Numerous evidences denoted that miRNAs played roles in diversity biologic processes such as cell proliferation, development, differentiation, migration, invasion, and apoptosis [2, 12, 19]. Recently, cumulative studies have indicated that miRNAs act as crucial regulators in the development of glioma cells. For example, miR-136 can modulate glioma cell sensitivity to temozolomide by targeting astrocyte elevated gene-1 [33]. miR-218 was also reported to be markedly upregulated in the glioma cell line and targeting tumor suppressor *Bmi1*, suggesting a possible function of miR-218 in cancer development and progression [29]. miR-92b is mainly expressed in brain primary tumors and can be used to distinguish primary and metastatic brain tumors [23]. Recently, it was reported that miR-92b acts as a potential oncogene by targeting *Smad3* in glioblastomas [32]. However, the role of miR-92b in tumorigenic processes of GBM is not fully understood.

Phosphatase and tensin homolog (PTEN) is always defined as a multifunctional tumor suppressor which is downregulated in different kinds of tumors [26]. By negatively regulating the PI3K/Akt signaling pathway through dephosphorylating phosphatidylinositol-3,4,5-trisphosphate (PIP3), PTEN regulates the proliferation, migration, invasion, apoptosis, cell cycle, and metastasis of cancer cells [22]. Recently, it has been demonstrated that PTEN were regulated by miRNAs in numerous types of cancers, including colon cancer [30], breast cancer [35], cervical cancer [27], hepatocellular carcinoma [16], osteosarcoma [11, 17, 25], and bladder cancer [10]. Especially, Xie et al. found that miR-221 mediates epithelial-mesenchymal transition-related gene expression by targeting PTEN [34].

Here, we found that the inhibition of miR-92b could reduce the proliferation, migration, and invasion of glioma cells, and promote the apoptosis of glioma cells. In addition, we demonstrated that PTEN was the direct target of miR-92b and reduce of PTEN expression partially rescued the inhibitory effects of miR-92b inhibitor on the glioma cells. Our data also suggested that the role of miR-92b in glioma cell might involve the regulation of PTEN/Akt signaling pathway.

Materials and methods

Cell culture

U251 and LN229 human glioma cell lines were purchased from the Cell Bank of Chinese Academy of Sciences. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco BRL, Grand Island, NY, USA) with high glucose and sodium pyruvate, supplemented with 10 % fetal bovine serum (Invitrogen, Carlsbad, CA, USA) and 1 % PS (100 units/ml penicillin, 100 mg/ml streptomycin). Cells were habitually passaged at 3–4-day intervals in a humidified atmosphere of 5 % CO₂ at 37 °C.

Total RNA extraction, reverse-transcription, and quantitative real-time PCR

Total RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol, and 1 µg of RNA was employed to synthesis cDNA by using HiScript First Strand cDNA Synthesis Kit (Vazyme, Nanjing, Jiangsu, China). Quantitative real-time PCR (qRT-PCR) was performed with SYBR[®] Green Real-time PCR Master Mix (Vazyme) in triplicate in ABI StepOnePlus[™] real-time PCR System (Foster City, CA, USA). For miRNA analysis, the sequences of RT primers were miR-92b: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGGAGGCC-3' U6: 5'-AAAATA TGAACGCTCACGAATTTG-3'. The sequences of quantitative PCR primers were miR-92b, 5'-GGGGCAGTTATTGCACTTGTC-3' (forward) and 5'-CCAGTGCAGGGTCCGAGGTA-3' (reverse). U6 small nuclear RNA, 5'-GTGCTCGCTTCGGCAGCACATATAC-3' (forward) and 5'-AAAATATGAACGCTCACGAATTTG-3' (reverse); PTEN, 5'-CCCAGTCAGAGGCGCTATGTGTAT-3' (forward) and 5'-GTTCCGCCACTGAACATTGG-3' (reverse); beta-actin, 5'-AAAGACCTGTACGCCAACAC-3' (forward) and 5'-GTCATACTCCTGCTTGCTGAT-3' (reverse). U6 and beta-actin were used as endogenous control. The 2^{-ΔΔCt} method was applied to calculate the relative expression levels of miR-92b and PTEN. All data were expressed as the means ±SD and as representative of an average of three measurements.

Oligonucleotide synthesis and plasmid construction

miR-92b inhibitor (5'-GGAGGCCGGGACGA GUGCAAUA-3') and negative control (5'-UCUACUCUUUCUAGGAGGUUGUGA-3') oligonucleotides were synthesized from GenePharma (Shanghai, China). The wild-type and mutant 3'UTR of human PTEN containing the target site for miR-92b was chemically synthesized and inserted into the pGL3 control vector (Promega, Madison, WI, USA). Cell transfection was performed with Lipofectamine 2000 Transfection Reagent (Invitrogen) in serum-free medium according to the manufacturer's recommendation.

Knockdown of PTEN by siRNA

Two small interfering RNA (siRNA) oligonucleotides for human PTEN (5'-GGCGUAUACAGGAA CAAUA-3' and 5'-AAGAUCUUGACCAAUGG CUA-3') and negative control (5'-UUCUCCGAAC GUGUCACGU-3') were synthesized from GenePharma (Shanghai, China), then transfected into LN229 cells using Lipofectamine[®] RNAiMAX transfecting reagent (Invitrogen) in serum-free medium according to the manufacturer's instruction.

CCK-8 assay

The CCK-8 cell counting kit (Vazyme) was used to determine the cell viability. Cells were seeded in the 96-well culture plates at 2000 cells per well. Cells transfected with miR-92b inhibitors (100 nM), negative control (100 nM), and PTEN siRNAs (100 nM) were cultured for additional 5 days. CCK-8 assays were conducted immediately after the medium replacement every 24 h till 5 days. CCK-8 solution (10 μ l) was added into each well and incubated for 3 h. Absorbance at 450 nm was recorded using the SpectraMax M5 microplate reader (Molecular Devices, Silicon Valley, CA, USA).

Wound-healing assays

Equal numbers of cells were seeded in 24-well plates; a homogeneous wound was made manually on the cell monolayer 24 h after transfection (about 90 % confluence) with a 200- μ L pipette tip. To remove the floating cells, plates were washed three times with phosphate-buffered saline. Cells that migrated into the wound were measured at two different time points (0 and 24 h) by a

light microscope (Olympus, Tokyo, Japan) at $\times 100$ magnification ($\times 10$ ocular with $\times 10$ objective). The percentage of the area with migrated cells compared to the initial wound region was defined as a migration rate (setting the gap area as 0 % at 0 h).

Invasion assay

Glioma cells (2×10^5) were plated into the top side of the polycarbonate Transwell filters (Millipore, Billerica, MA, USA, diameter 12 mm, pore size 8 mm). For the invasion assay, the upper surface of polycarbonate filters was coated with Matrigel which can then be used as the extracellular matrix for tumor cell invasion analysis. Glioma cells were suspended in serum-free medium in the upper chamber; medium supplemented with serum was used as a chemoattractant in the bottom chamber. The cells were incubated at 37 °C for 48 h before removing the medium from the top chamber. The non-migratory or non-invasive cells were scraped off by a cotton swab. Cells on the lower surface were fixed in 100 % methanol for at least 10 min, then dried and stained with crystal violet for 20 min. Images were collected under an inverted microscope (Olympus) at $\times 200$ magnification. Finally, the dye was dissolved by acetic acid and the absorbance at 570 nm was documented using the SpectraMax M5 microplate reader (Molecular Devices). Each assay was repeated three times. The values obtained were presented as invasion percentage normalized by control group.

Apoptosis assay

Cells (10^5 /well) were plated in 6-well plates, 48 h after transfection with miR-92b inhibitor (100 nM) or negative control (100 nM); cell apoptosis was detected using annexin V-FITC-labeled Apoptosis Detection Kit (Invitrogen) according to the manufacturer's protocol by flow cytometry.

Bioinformatics prediction and dual luciferase activity assay

The luciferase reporter vectors carrying wild-type or mutant miR-92b targeting sites were cotransfected with miR-92b inhibitor (100 nM) or negative control (100 nM) into U251 and LN229 cells seeded in 24-well plates using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. The renilla/

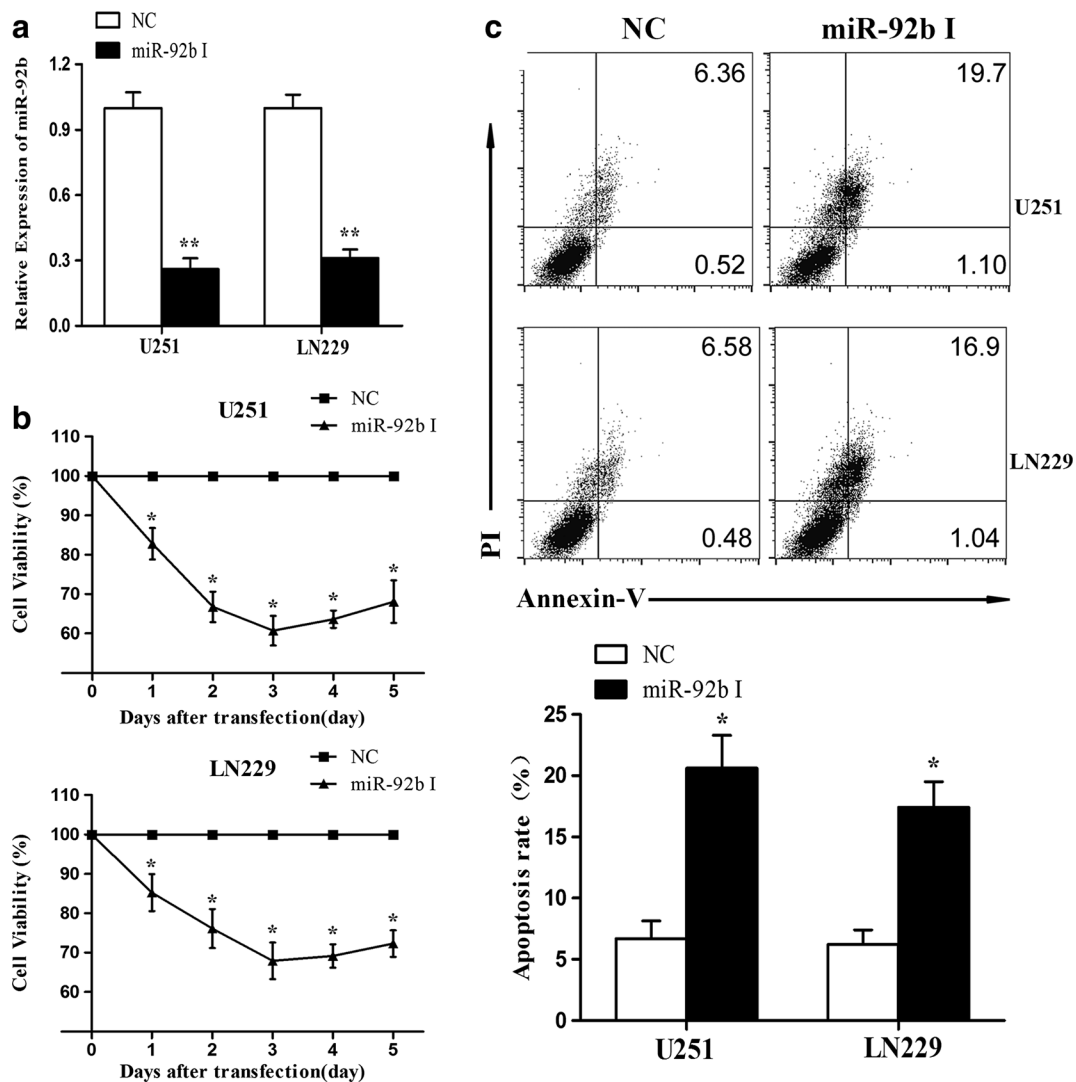


Fig. 1 Downregulation of miR-92b impeded cell viability while promoted apoptosis in glioma cells. **a** The relative expression of miR-92b was assessed by qRT-PCR analysis in U251 and LN229 cells. The average miR-92b expression was normalized to U6 expression. **b** The rates of cell viability were measured using

CCK-8 assay. The absorbance at 450 nm was measured. **c** Forty-eight hours after transfection, the apoptosis was assessed using Annexin V-FITC analysis. All experiments were performed at least three times and the mean values \pm SD were used. * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control

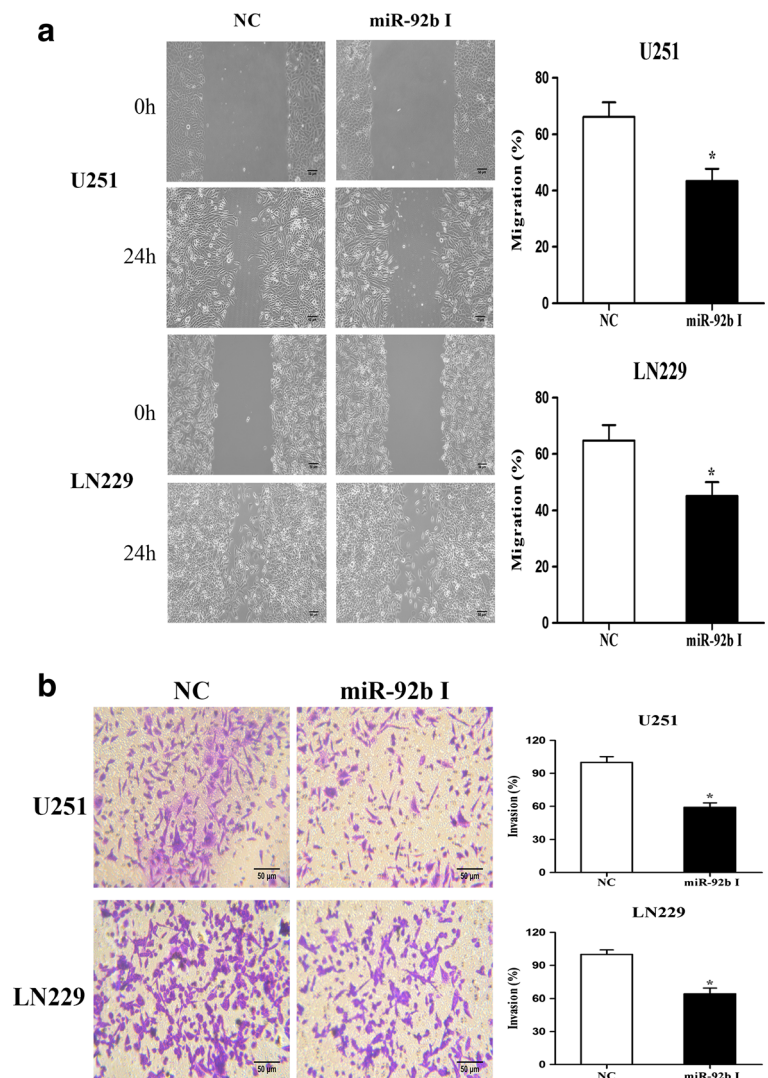
firefly luciferase activity was measured by the Dual Luciferase Reporter Assay System (Promega) after 24 h. The value for the wild-type PTEN 3'UTR with microRNA negative control cotransfected group was set as 100 %.

Western blot

Proteins from cells (40 μ g/lane) were separated by 10 % SDS-PAGE gel before transferring onto a polyvinylidene fluoride (PVDF) membrane

(Millipore). Membranes were blocked in 5 % non-fat dry milk diluted in Tris-buffered saline Tween-20 (TBST) (20 mM Tris-HCl, 150 mM NaCl, pH 7.5, and 0.1 % Tween 20) at room temperature for 1 h, and then incubated overnight at 4 $^{\circ}$ C with primary antibody. The primary antibodies and dilutions used were as follows: beta-actin, PTEN, phospho-Akt (p-Akt-ser307), and total-Akt (t-Akt) were obtained from Cell Signaling Technology Inc (1:1000 diluted; Danvers, MA, USA). After three rinses with slow shaking for 15 min each time by TBST, incubation for 2 h with the goat anti-

Fig. 2 Knockdown of miR-92b on migration and invasion in glioma cells. **a** Twenty-four hours after transfection, wound-healing assay was employed to detected cell migration rate. **b** The invasion ability was measured using a Transwell assay. All cells stained with crystal violet were dissolved by acetic acid. Absorbance at 570 nm was detected. *Scale bar* represents 50 μm . Values represent the mean \pm SD ($n = 3$ replicates). * $p < 0.05$ vs. control



rabbit or anti-mouse IgG conjugated to horseradish peroxidase (1:1000 diluted; Santa Cruz Biotechnology, Santa Cruz, USA) was performed following by three times 15-min washes. The membrane was then visualized by the SuperSignal West Femto kit (Pierce, Rockford, IL, USA) with an enhanced chemiluminescence system (DuPont, Boston, MA, USA). Beta-actin was used as the loading control in the Western blots.

Statistical analysis

All data were expressed as mean \pm S.D. Student's *t* test was used to evaluate the significance for differences among groups. Statistic significance will be considered when *p* value is < 0.05 .

Result

Suppression of miR-92b impeded cell viability and promoted apoptosis

To investigate the role of miR-92b in glioma cells, miR-92b inhibitor, a 22-bp RNA fragment complementary to miR-92b was applied to glioma cells with comparison of a random sequence as negative control. miR-92b inhibitor and random fragments were transiently transfected into U251 and LN229 cell lines respectively. Forty-eight hours later, qPCR assay for miR-92b revealed that the miR-92b transcripts were reduced significantly both in U251 and in LN229 cells (Fig. 1a). Then, we used CCK-8 assays to test the effects of miR-92b on cell viability. As shown in

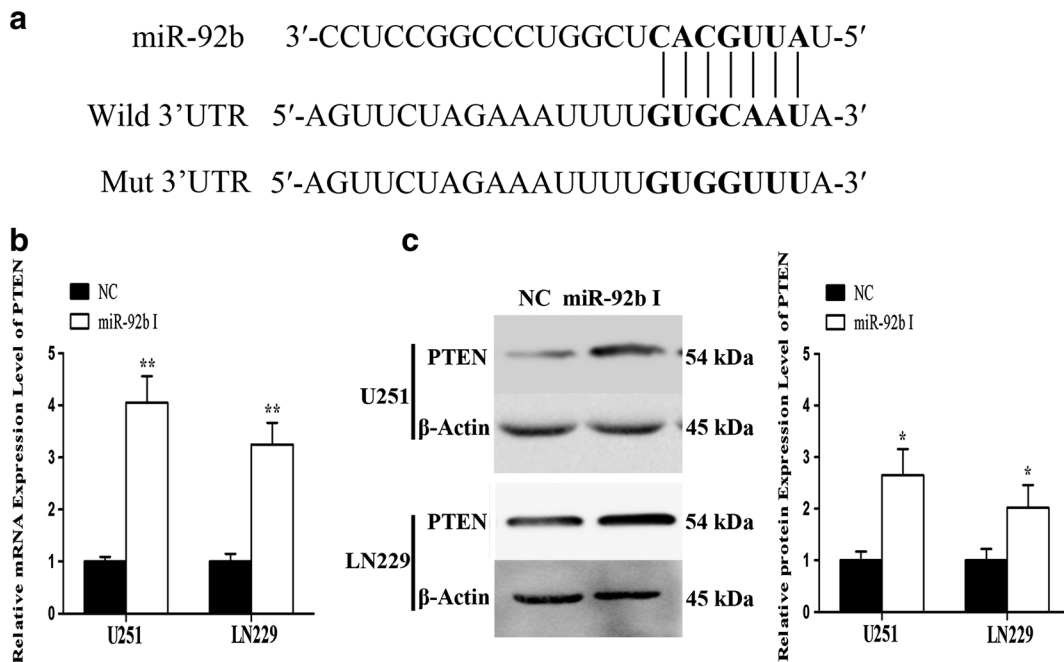


Fig. 3 miR-92b regulated the mRNA and protein level of PTEN in glioma cells. **a** Binding sites of miR-92b on the PTEN mRNA 3' UTR. PTEN wild and PTEN mutant luciferase report plasmids were constructed. **b** The mRNA expression level of PTEN was measured by qRT-PCR 48 h posttransfection of miR-92b inhibitor

in U251 and LN229 cells. **c** Western blotting for PTEN at 48 h after transfection of miR-92b inhibitor in U251 and LN229 cells. The data were expressed as the mean \pm SD and were representative of an average of three independent experiments. * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control

Fig. 1b, the cell viability was decreased in the miR-92b inhibitor-transfected group compared with the control from 2 to 5 days after transfection ($p < 0.05$ for each). These results indicated that downregulation of miR-92b could inhibit the viability of glioma cells.

Because inhibition of miR-92b could impede cell viability, we then suspect that miR-92b might also be involved in apoptosis process in glioma cells. Annexin V-FITC analysis was performed to measure the rate of apoptosis, as shown in Fig. 1c, wherein the decrease of miR-92b caused a significant increase in apoptosis rate (20.61 ± 2.69 and 17.45 ± 2.18 %) compared to the control group (6.69 ± 1.45 and 6.25 ± 1.27 %) in both U251 and LN229 cells.

Downregulation of miR-92b inhibits migration and invasion of glioma cells

Wound-healing assay was performed to investigate whether miR-92b regulates the migration of glioma cells. Approximately 24 h after wound scraping, the cells transfected with miR-92b inhibitor exhibited a slower migration compared to the control group. In U251 cells, (44.23 ± 3.12 %) area of the wound was filled by cells

from the miR-92b inhibitor-transfected group, which is significantly less than that from the control group (66.17 ± 4.68 %). In the LN229 cells, the migration rate was 48.18 ± 3.91 % with the miR-92b inhibitor vs. 64.84 ± 3.13 % of the control group (Fig. 2a).

To test whether miR-92b indeed participates in the invasion, we performed transwell assay with Matrigel to detect the invasion ability. As shown in Fig. 2b, the miR-92b inhibitor markedly lowered the invasion rates of glioma cells as shown in the photography and quantitative analysis. In U251 cells, the invasion rate in the miR-92b-I group was 59.13 ± 4.02 %, only half of that found in the control group. In LN229 cells, invasive activity was also inhibited in the miR-92b-I group to 64.16 ± 5.19 %. These above data showed that miR-92b inhibitor could repress the migration ability and invasion ability of glioma cells.

miR-92b binds to 3'UTR of PTEN and inhibits PI3K/Akt signaling

To determine the possible mechanisms of miR-92b in regulating glioma cells, we predicted its potential targets using miRBase (<http://www.mirbase.org/>) and TargetScan

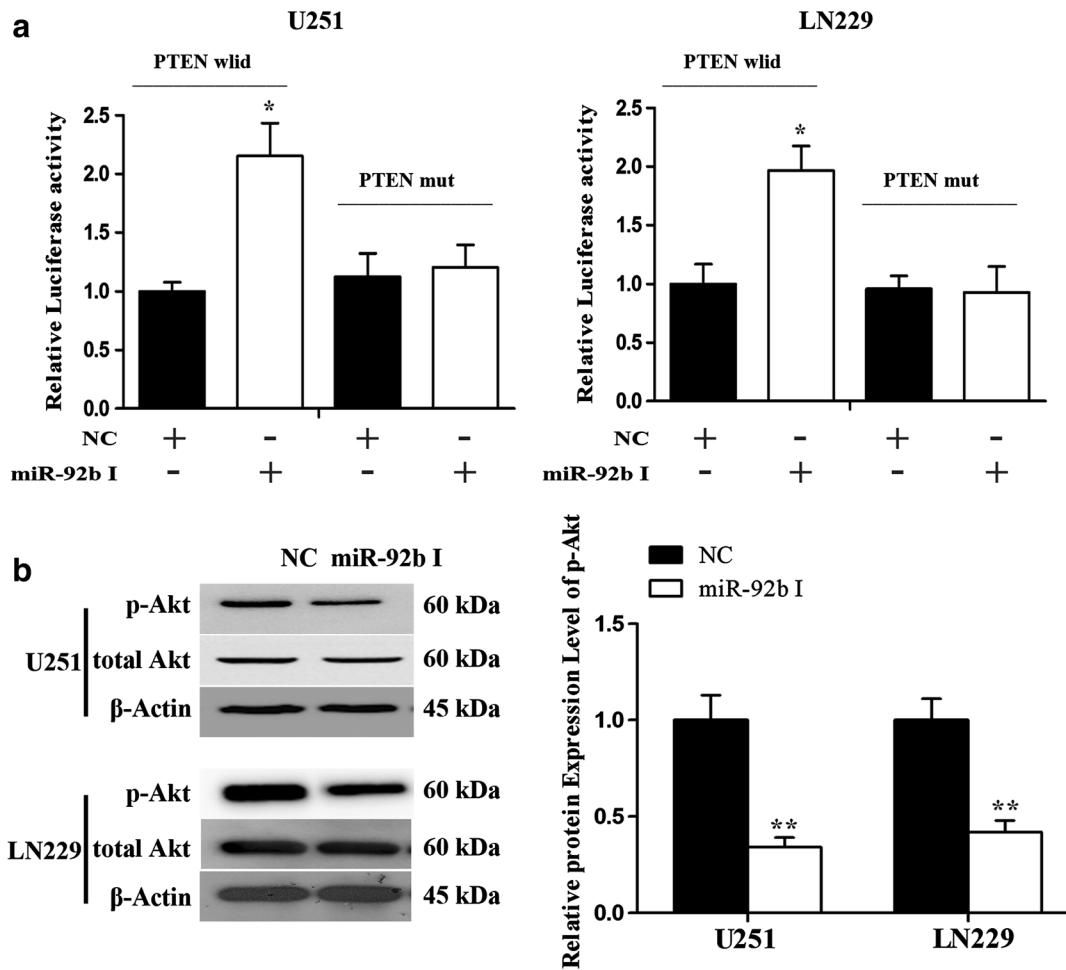


Fig. 4 miR-92b bound to the 3'UTR of PTEN and inhibited PTEN/Akt signaling pathway. **a** The relative luciferase activity was detected by Dual-Luciferase Reporter Assay System 48 h after transfection. **b** Total protein level of Akt and the phosphorylation

level of Akt were measured by western blotting 48 h after transfection of miR-92b inhibitor in U251 and LN229 cells. All data were obtained from three independent experiments. * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control

(<http://www.targetscan.org/>). We found that PTEN is a potential target of miR-92b, and there are seven conserved binding sites for miR-92b on the 3'UTR region of PTEN mRNA (Fig. 3a). Moreover, PTEN has been implicated in regulation of proliferation and migration of numerous cancer cells. Therefore, we analyzed the correlation of the expression of miR-92b and the mRNA and protein level of PTEN in U251 and LN229 cells. qRT-PCR and western blot analysis presented that there is a negative correlation between miR-92b and PTEN. As shown in Fig. 3b, c, knockdown of miR-92b dramatically increased PTEN mRNA and protein levels in glioma cells. In addition, when using wild-type 3'UTR sequence or the mutant-type 3'UTR (the seed region binding miR-92b was changed from GUGCAAU to GUGGUUU) of PTEN in

the luciferase assay, the wild-type PTEN 3'UTR caused significantly upregulated reporter activity 48 h after transfection with miR-92b inhibitor compared to the control. On the other hand, in the mutant vector, the stimulative effect of miR-92b inhibitor on the 3'UTR of PTEN was not observed (Fig. 4a).

Since PTEN has been reported to act as a negative regulator of PI3K/Akt signaling pathway by dephosphorylating PIP3 on 3' side and 5' side, resulting more PIP2 that consequently inhibits the phosphorylation level of Akt. Therefore, we hypothesized that miR-92b might play a role in regulating the activity of the PI3K-AKT pathway via PTEN. As shown in Fig. 4b, downregulation of miR-92b could suppress the phosphorylation level of Akt, whereas the total Akt level

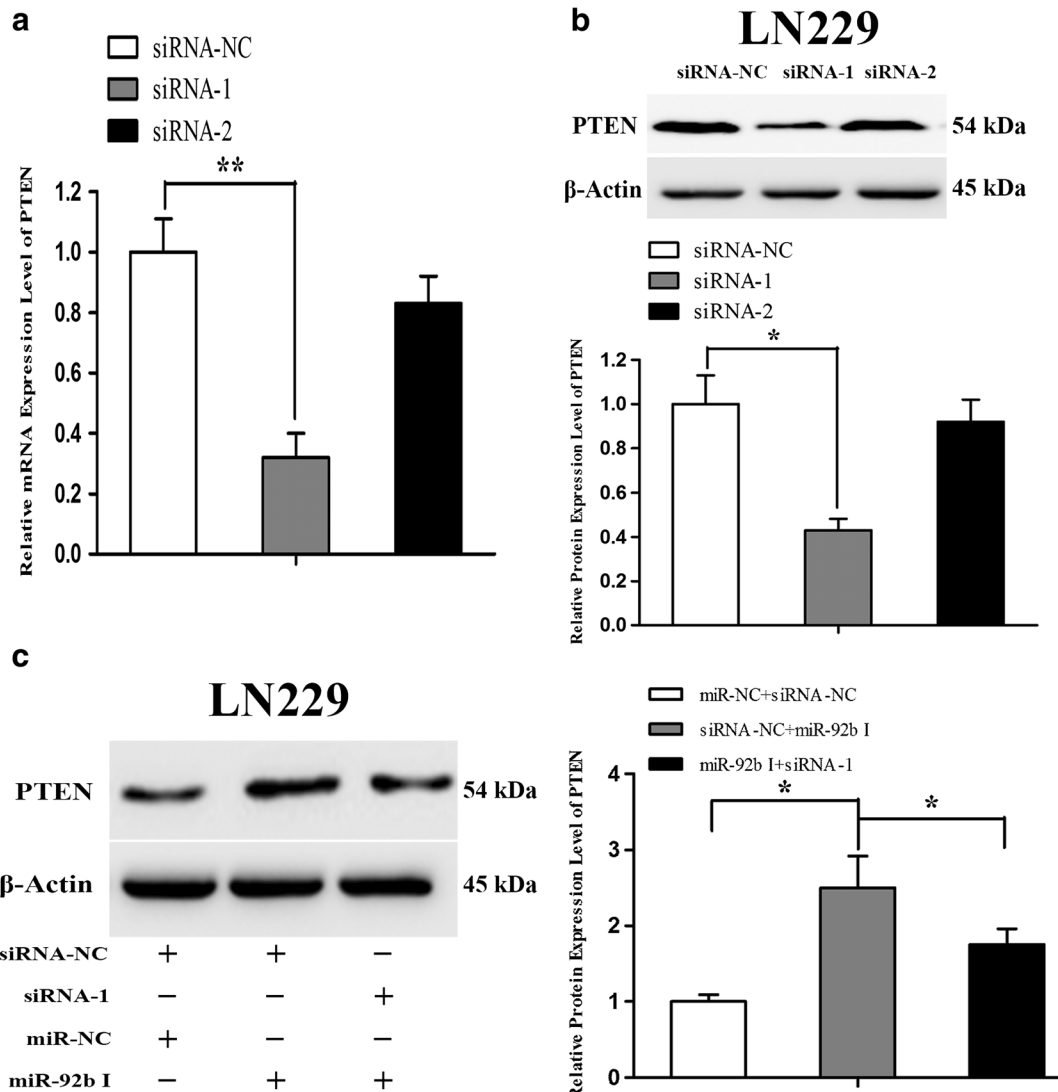


Fig. 5 Expression of PTEN was silenced by siRNAs in LN229 cells. **a** The mRNA expression level of PTEN in LN229 cells was assessed by qRT-PCR after transfection of siRNAs at 24 h. **b** Western blot analysis of PTEN in LN229 cells after transfection

of siRNAs at 24 h. **c** Western blotting analysis for PTEN protein level in LN229 cells. The results are means \pm SE of three different experiments. * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control

remains unchanged in glioma cells. Taking together, these data showed that the miR-92b could bind to PTEN 3'UTR and miR-92b is able to downregulate the activity of PI3K/Akt signal pathway.

Knockdown of PTEN largely rescued the inhibitory effects of miR-92b on GBM

We further examine whether miR-92b indeed exerts its function through its target PTEN. Firstly, two small interfering RNA (siRNA-1 and siRNA-2) targeting PTEN

mRNA were synthesized and transfected into LN229 cells. The inhibitory effects of siRNA were detected by qRT-PCR and western blot analysis. Knockdown effects of siRNA-1 are better than that of siRNA-2 (Fig. 5a, b). Therefore, siRNA-1 was used to inhibit PTEN expression (Fig. 5c) in GBM cells. LN229 cells were cotransfected with miR-92b inhibitor (miR-92b I) or its negative control (miR-NC) with PTEN siRNA-1 or its negative control (siRNA-NC). The results demonstrated that knockdown of PTEN partially reversed the repressive effect of miR-92b inhibitor on cell proliferation (Fig. 6a), migration

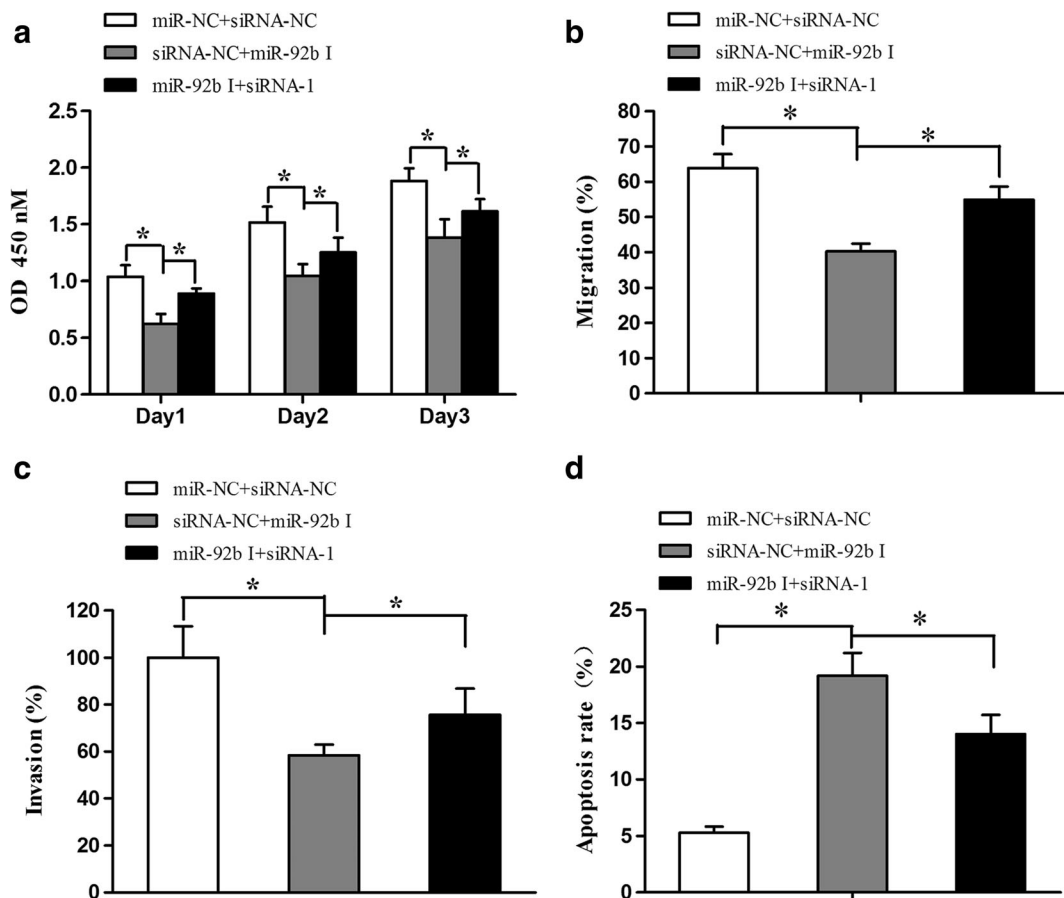


Fig. 6 Knockdown of PTEN partially rescued miR-92b inhibitor-induced inhibitory effects on glioma cells. **a** Knockdown of PTEN partially reversed miR-92b inhibitor-induced inhibition of proliferation in LN229 cells determined by CCK-8 assay. Knockdown of PTEN partially reversed miR-92b inhibitor-induced suppression of migration (**b**) and invasion (**c**) in LN229 cells detected by

wound-healing assay and Transwell assay, respectively. **d** Knockdown of PTEN partially reversed miR-92b inhibitor-induced apoptosis in LN229 cells detected by flow cytometry. These data were presented as mean \pm SD from three independent experiments. * $p < 0.05$ vs. control

(Fig. 6b), and invasion (Fig. 6c). Moreover, downregulation of PTEN partially suppressed the cell apoptosis promoted by miR-92b inhibitor (Fig. 6d). These data suggested that miR-92b inhibitor might alternate cell growth, migration, invasion, and apoptosis through its transcriptional modulation on PTEN.

Discussion

MicroRNAs play critical roles in diversity of biological processes and their dysregulation are usually associated with tumorigenesis [9]. Increasing evidence suggested that significantly upregulated miRNA genes in tumors may be oncogenes [14]. These miRNAs usually promote tumor development by negatively regulating

tumor suppressor genes that control biological processes. Therefore, these miRNAs may be used as prognostic marker, and decreasing their expression may be a valuable strategy for cancer treatment.

A number of studies have shown that miR-92b plays an important role in carcinogenesis. miR-92b is overexpressed in brain tumor tissues [23]. For example, it could control glioma proliferation and invasion while inhibiting apoptosis via targeting DKK3 [21]. As a potential oncogene, miR-92b could also promote glioblastomas by targeting Smad3 [32]. Through regulating Wnt/beta-catenin signaling via Nemo-like kinase, miR-92b controls glioma proliferation and invasion [31]. In our study, miR-92b inhibitor suppressed the proliferation, migration, and invasion of GMB cells. Moreover, it also promotes apoptosis of GMB cells.

The identification of target genes is a key step in assessing the role of aberrantly expressed miRNA in cancer. And, it may be valuable for further development of miRNA-based gene diagnosis and therapy. With the help of two bioinformatics software, miRBase and Targetscan, we predict the potential targets of miR-92b and found that PTEN has binding sites for miR-92b in its 3'UTR.

PTEN plays a critical role in regulation of cell migration, differentiation, apoptosis, cell cycle detention, and the sensitivity to cancer cells through interfering with multiple signaling pathways [4, 28]. Kurose et al. reported that PTEN gene could inhibit Akt activation (phosphorylation), which plays an unappreciated role in an outermost complex network of cell growth modulation that affects apoptosis, protein biosynthesis, and cell cycle arrest [18]. Recently, PTEN was reported as a direct target of miR-92b, involving in miR-92b-mediated effects on cell growth and cisplatin chemosensitivity in non-small cell lung cancer cell line [20]. This is particularly interesting that loss of PTEN function, including the decrease of PTEN protein expression, is one of the major factors contributing to glioma formation [1]. It has been also reported that downregulation of PTEN in colon cancer leads to the aberrant activation of the PI3K/Akt pathway, resulting colon tumorigenesis [24]. On the other hand, Graff et al. showed that the activation of the AKT pathway in malignant cells is a survival signal [13]. Besides proliferation, PI3K/AKT signaling pathway also regulates diverse cellular processes, including migration and invasion of glial cells [8]. Our findings suggested that in glioma cells, PTEN might have similar tumor-suppressive functions found in colon cancer via PI3K/Akt signaling.

In our experiment, inhibition of the miR-92b specifically increased the expression of the reporter gene that carries PTEN 3'UTR. In contrast, no inhibitory effect was observed when using plasmid carrying mutations in the seed sites. Downregulation of miR-92b could increase PTEN expression in both mRNA and protein levels and further decreased the phosphorylation level of Akt, whereas the total level of Akt retained unchanged. These results implied that the miR-92b directly targets PTEN who further suppresses PI3K/Akt signaling pathway in glioma cells.

To further confirm the mechanisms by which miR-92b regulates glioma formation and development, we applied PTEN siRNAs in LN229 cells to rescue the

phenotype induced by miR-92b-I. Indeed, the knock-down of PTEN partially reversed the oncogenic effects by miR-92b inhibitors. Therefore, it may highlight the significance of the interaction between microRNAs and mRNAs in tumorigenesis that miR-92b inhibitor suppressed malignancy of glioma at least partially by activating PTEN.

In conclusion, the present study presented that suppression of miR-92b exerts an inhibitory effect on proliferation, migration, and invasion of glioma cells while promotes apoptosis with a potential involvement of PTEN/AKT signaling pathway via regulation of PTEN expression, which may help improve our understanding of the pathogenesis and therapies of GBMs.

Acknowledgments The authors thank the staff at the department of Biochemistry of Southeast University.

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