Int J Clin Exp Pathol 2016;9(10):9715-9726
www.ijcep.com /ISSN:1936-2625/IJCEP0028010

Review Article

MiR-34a induces apoptosis of glioma stem cells via the inhibition of SIRT1

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Received March 11, 2016; Accepted July 12, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Neuroglioma is one common intracranial tumor with high incidence and mortality. The role of microRNA (miR)-34a in glioma has been recognized, but its biological property in glioma as well as tumor stem cells remains unknown. This study aimed to investigate the expression of miR-34a in neuroglioma and stem cells, along with its biological function and possible intrinsic mechanism, in order to provide novel methods for targeted treatment of glioma. Glioma and adjacent tissue samples were collected from 80 patients with neuroglioma in the department of neurosurgery in our hospital. MiR-34a and SIRT1 expression was quantified. CD133 magnetic beads were used to enrich primary glioma and stem cells from U87 cell line. Expression of CD133 and Nestin was determined by immunofluorescence. $CD133^+$ U87 cells were then transfected with miR-34a mimics to test the neurosphere formation, self-renewal, differentiation toward astrocyte, cell apoptosis and migration potencies. In tumor tissue, miR-34a was significantly depressed and SIRT1 expression was elevated, both of which were correlated with pathology grade. $CD133^+$ U87 cells had features of neural stem cells, as it co-expressed CD133 and nestin. These cells showed down regulation of miR-34a and up regulation of SIRT1 expression. Over-expression of miR-34a remarkably decreased SIRT1 protein expression, inhibited tumor sphere formation and self-renewal potency, and decreased the migration ability of $CD133^+$ U87 cells, thus inducing its apoptosis or differentiation towards astrocytes. MiR-34a could inhibit glioma stem cell proliferation, self-renewal and migration via targeting SIRT1, and induce tumor cell apoptosis and differentiation.

Keywords: Glioma, stem cell, MiR-34a, SIRT1

Introduction

Neural glioma is one type of common intracranial tumor, with high incidence (6/100,000 in adults) and mortality (5-year survival rate between 20% and 30%) [1]. Currently, surgery is still recognized as the primary treatment method. However, due to tumor's profiles of rapid progress, infiltration, proliferation, high malignancy and invasion, it is difficult to be completely removed, thus leads to high recurrence and inefficacy in clinics. Study has revealed the existence of glioma stem cells, which are similar with neural stem cells (NSCs) in side glioma tissues, in addition to those tumor cells with strong proliferation and invasion potency. These glioma stem cells play a crucial role in occurrence, progression and recurrence of glioma [2]. MicroRNA (miR) is closely related with tumor occurrence via its abnormally high expression or low expression, as it can exert pro- or anti-tumor effects via post-transcriptional regulation on target gene expression [3]. The expression and abnormal function of miR in neural glioma or glioma stem cells have been suggested by various studies [4, 5]. MiR-34a is one member of miR-34 family (including miR-34a, miR-34b and miR-34c). As a downstream molecule of p53 gene, miR-34a mainly exerts anti-tumor effect, as it can inhibit the proliferation of various tumor/tumor stem cells to induce their apoptosis or significantly weaken the invasion potency [6, 7]. The abnormal expression of miR-34a has been proved to be related with multiple tumors including pulmonary cancer [8], colon cancer [9] and breast carcinoma [10]. With advancement in study, the role of miR-34a in glioma has been recognized,

but its biological property in neural glioma cell and glioma stem cell is still unclear. This study thus investigated the expression, biological function and possible intrinsic mechanism of miR-34a in neuroglioma and stem cells, in order to provide novel methods for targeted treatment of glioma.

Materials and methods

Reagent and materials

Human glioma cell line U87 was obtained from Shanghai Institute of Cell Biology, Chinese Academy of Science. DMEM/F12 and DMEM-high glucose were purchased from Gibco (US). Lipofactamin RNAi MAX was purchased from Invitrogen (US). Mouse anti-human SIRT1, mouse anti-humn Nestin and rabbit anti-human GFAP were purchased from Abcam (US). Rabbit anti-human CD133 was obtained from Abnova (US). CD133 sorting magnetic beads were purchased from Miltenyi (Germany). MiR-34a mimics and NC mimics were synthesized by Ribobio (China). Reverse transcription and fluorescence qPCR kits were purchased from Takara (China). Transwell chamber was obtained from Corning (US).

Sample collection

Tumor tissue samples were collected from a total of 80 glioma patients in the department of neurosurgery in our hospital from June 2014 to August 2015. Among all patients there were 23, 28 and 29 cases belonging to WHO pathology grade II, grade III and grade IV, respectively. Tumor samples were collected without cyst, necrosis or electric coagulation. Tumor adjacent tissues were simultaneously extracted. This study has obtained written consents from patients and families.

Primary culture of glioma cells

Tumor tissues were in serum-free NSC medium (DMEM/F12 medium containing 20 ng/mL bFGF, 20 ng/mL EGF, 20 ng/mL LIF and 2% B27 additives) for three times. Tissues were cut into small pieces and digested in 0.25% trypsin for 10 min, followed by serum quenching. After washing 2~3 times, serum-free NSC medium was added to prepare single-cell suspension, which was inoculated into culture flask at 3×10^5/mL. Cells were incubated in a humidified chamber with 5% CO_2 at 37°C. After seven days of incubation, clonal sphere was formed in suspension. When the single colony reached 0.1 mm size, attached cells were discarded. Supernatants were collected, centrifuged, and re-suspended. Cells were passed at 1:4 ratio. Further passage was performed every 3~4 days, when the size of single clonal sphere reached 0.1 mm, followed by centrifugation purification for 4~5 times.

U87 cell culture

U87 cells were inoculated into high-glucose DMEM medium in a humidified chamber with 5% CO_2 at 37°C. Medium was changed every 2~3 days. Cells were passed every 3~4 days.

Enriching of CD133$^+$ cells

Cells were collected and re-suspended in 80 μL MACS buffer (for 10^7 cells). 20 μ FcR blocking reagent and 20 μL CD133 magnetic beads were added for 4°C incubation for 14 min. Excess beads were washed in 2 mL MACS buffer twice. Cells were re-suspended in 0.5 mL MACS buffer. The sorting column was placed in the magnetic field. After activated by 0.5 mL MACS buffer, the sorting column was added with cell suspension for elution. The column was then washed in 0.5 mL MACS buffer for three times. 1 mL MACS buffer was then used to wash away CD133$^+$ cells for collection. FITC mouse anti-human CD133 antibody was used to detect the cell purity after magnetic bead sorting. Enriched CD133$^+$ U87 cells were immediately transferred into serum-free NSC medium (DMEM/F12 medium containing 20 ng/mL bFGF, 20 ng/mL EGF, 20 ng/mL LIF and 2% B27 additives). Clonal sphere can be formed after 2~3 days. Those spheres with satisfactory activity were used for immunofluorescence.

Immunofluorescent assay for specific markers

Those CD133$^+$ U87 spheres with satisfactory activity were mounted on polylysine pre-coated glass slides. Excess culture medium was washed away by PBS. Cells were fixed in 4% paraformaldehyde for 40 min, followed by PBS rinsing for three times. Blocking was performed under room temperature using 10% goat serum. Mouse anti-human nestin monoclonal antibody (1:200) or rabbit anti-human CD133 monoclonal antibody (1:400) was added for

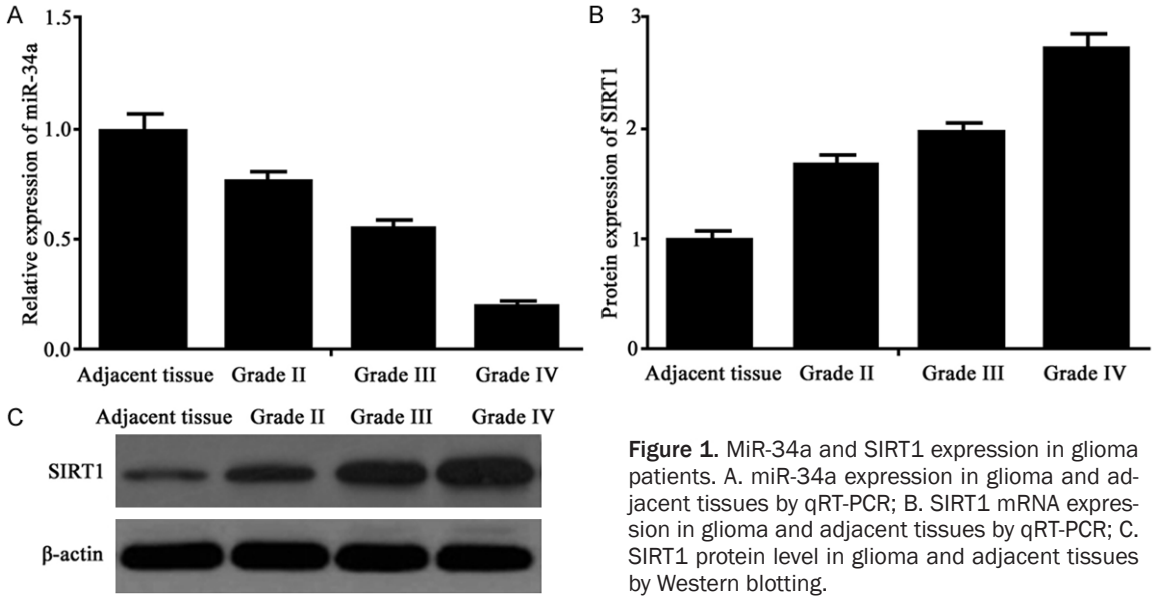

Figure 1. MiR-34a and SIRT1 expression in glioma patients. A. miR-34a expression in glioma and adjacent tissues by qRT-PCR; B. SIRT1 mRNA expression in glioma and adjacent tissues by qRT-PCR; C. SIRT1 protein level in glioma and adjacent tissues by Western blotting.

4°C overnight incubation. Excess primary antibody was washed away, followed by the addition of Alexa fluor 488-labelled goat anti-mouse (1:200) or Alexa fluor 594-labelled goat anti-rabbit (1:200) antibody for 60 min incubation at room temperature. DAPI was added for 1 min staining after washing. Slides were observed under a fluorescent microscope.

Cell transfection

Serum free Opti-MEM medium was used to dilute miR-34a mimics or NC mimics, which were mixed with Lipofectamin RNAi MAX. Serum-free cultured $CD133^+$ U87 cells were removed for old medium and replenished with fresh NSC medium without double antibiotics. Mimics and Lipo RNAi MAX mixture was added into the culture dish. Final concentration for mimics transfection concentration was 100 nmol/L. After 6 h, fresh serum-free NSC medium was added for continuous culture.

Clonal formation and single cell clonal assay

Based on the potency of sphere forming of glioma stem cells, this study investigated the effect of miR-34a on clonal formation ability of $CD133^+$ U87 glioma stem cells. In brief, 1×10^5 glioma stem cells transfected with miR-34a (5'-UGGCA GUGUC UUAGC UGGUU GU-3') or NC mimics (5'-UUCUC CGAAC GUGUC ACGUT T-3') or NC mimics (5'-UUCUC CGAAC GUGUC ACGUT T-3') were inoculated in 24-well plate containing 1 mL serum-free NSC medium. After 3 days culture, cell sphere can be observed. Five randomly selected fields were observed to compared number and size of cell spheres. Glioma cell spheres were then digested to prepare single cell suspensions and adjusted to 1000 cells per mL. 2 μL cell suspensions were added into 96-well plate, with the addition of 100 μL serum-free NSC medium. The number of cells in each well was recorded after 4 hours. Those wells with only single cell were annotated. Cell growth was observed every other day. The clonal formation rate was counted after 7 days.

Differentiation assay

Clonal sphere with single cell origin under serum-free culture was digested and inoculated into 24-well plate with poly-lysine pre-coated coverslips, using DMEM/F12 medium containing 10% FBS. After 5~7 days, cells were detected for differentiation specific markers including CD133, Nestin and glial fibrillary acidic protein (GFAP), when they had elongated perturbations or polygon, star-like or spindle shapes.

Cell apoptotic assay

Cells were transfected as abovementioned and incubated in a humidified chamber with 5% CO_2 at 37°C for 48 h. Cells were then digested by trypsin and collected. After quenching, cells were washed in PBS, and were centrifuged at

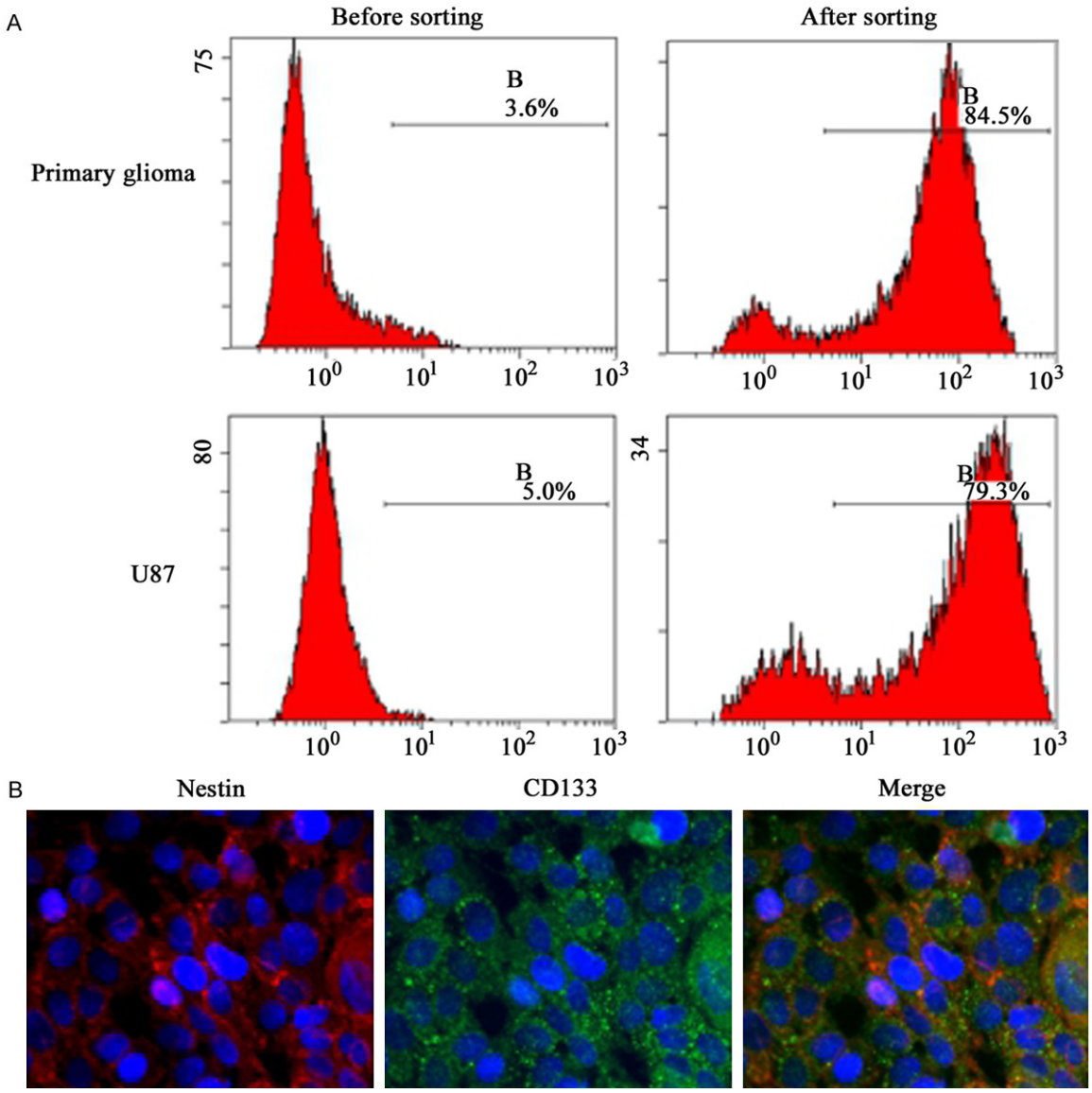

Figure 2. Soring and immunofluorescence of CD133⁺ glioma stem cells. A. Flow cytometry identification of CD133⁺ before and after sorting; B. Identification of NSC marker (Nestin) on CD133⁺ cells by immunofluorescence (200×).

1000 g for 5 min. Supernatants were discarded, followed by the addition of 195 μL Annexin V-FITC buffer for re-suspension. 5 μL Annexin V-FITC solution was then added, followed by the addition of 10 μL PI dye for mixture gently. With dark incubation at room temperature for 10~20 min, the mixture was placed on ice for flow cytometry assay. Data were collected for statistical analysis.

In vitro cell migration assay

1×10^5 CD133⁺ U87 glioma stem cell transfected with miR-34a mimics or NC mimics were re-suspended in 100 μL serum-free medium, and were inoculated into the upper chamber of Transwell, whose lower chamber was filled with 600 μL high-glucose DMEM medium containing 10% FBS. After 24 h incubation, Transwell chamber was collected to wash away medium. With washing in PBS twice, methanol was used to fix cells for 30 min, followed by 0.1% crystal violet staining for 20 min. Five randomly selected fields were observed under 200× magnification to count cell numbers.

qRT-PCR for gene expression assay

Trizol reagent was used to extract total RNA, which was used as the template to synthesize

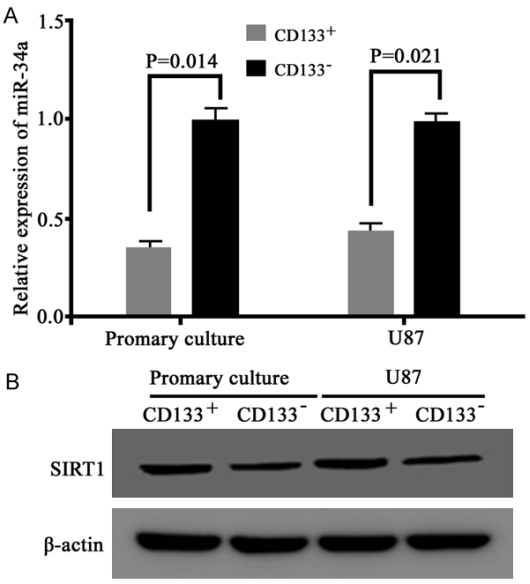

Figure 3. MiR-34a and SIRT1 expression in $CD133^+$ glioma cells. A. qRT-PCR showed miR-34a expression in $CD133^+$ and $CD133^-$ glioma cells; B. Western blotting revealed SIRT1 expression in $CD133^+$ and $CD133^-$ glioma cells.

cDNA by reverse transcription with specific primers. Using cDNA as the template, PCR amplification reaction was performed using Taq DNA polymerase. PCR primers were: miR-34a-P_F: 5'-ACCTG GCAGT GTCTT AGCTG GT-3'; miR-34a-P_R: 5'-AATCC ATGAG AGATC CCTAC CG-3'; U6P_F: 5'-CTCGC TTCGG CAGCA CA-3'; U6P_R: 5'-AACGC TTCAC GAATT TGCGT-3'; SIRT1P_F: 5'-TGGCA AAGGA GCAGA TTAGT AGG-3'; SIRT1P_R: 5'-CTGCC ACAAG AACTA GAGGA TAAGA-3'; β-actin P_F: 5'-GCACT CTTCC AGCCT TCC-3'; β-actin P_R: 5'-AGAAA GGGTG TAACG CAACT AAG-3'. The PCR system contains 4.5 μL 2XSYBR Green Mixture, 0.5 μL forward/reverse primers (5 μm/L); 1 μL cDNA and 3.5 μL ddH_2O. The reaction conditions were: 95°C denature for 5 min, followed by 40 cycles each containing 95°C 15 sec and 60°C 1 min. PCR was performed on ABI ViiA7 fluorescent qPCR cycler. Data were analyzed by $2^{-\Delta\Delta Ct}$ method. Expression level of microRNA and mRNA was normalized against U6 and β-actin internal reference genes, respectively. Each sample was tested in triplicates (N=3).

Western blotting

Total proteins were extracted from cells, and were separated in SDS-PAGE. After transfer, the membrane was blocked for 60 min at room temperature using 5% defatted milk powder. Mouse anti-human SIRT1 antibody was added for 4°C overnight incubation. Unbounded primary antibody was washed away, followed by incubation in secondary antibody for 60 min. ECL chemoillumence was applied to expose the membrane. Under X-ray, the film was developed and scanned.

Statistical processing

SPSS 18.0 software was used to collect and analyze all data, of which measurement data were presented as mean ± standard deviation (SD) while enumeration data were presented as percentage. Student t-test or analysis of variance (ANOVA) was employed to compare means of measurement data, while chi-square test was used to compare enumeration data. A statistical significance was defined when $P<0.05$.

Results

MiR-34a and SIRT1 expression in glioma patients

QRT-PCR test results showed that, compared to adjacent tissues, miR-34a expression level in tumor tissues of glioma patients was significantly decreased. With advanced WHO pathology grade, miR-34a expression level was gradually decreased (**Figure 1A**). Oppositely, SIRT1 mRNA and protein levels in glioma tissues were significantly higher than those of adjacent tissues, and were gradually increased with advanced pathology grade (**Figure 1B** and **1C**).

$CD133^+$ glioma stem cell sorting and immunofluorescent assay

Using magnetic beads sorting technique, $CD133^+$ glioma cells were selected from either primary cultured glioma cells or U87 cell line. Flow cytometry was performed after sorting and we found that the elevation of $CD133^+$ percentage grew from 3.6% to 84.5% in primary glioma cells and from 5.0% to 79.3% in U87 cells, respectively (**Figure 2A**). Using immunofluorescent identification, we observed that U87-derived $CD133^+$ glioma cells also expressed Nestin, which is one of the NSC markers, suggesting the nature of neural glioma stem cells (**Figure 2B**).

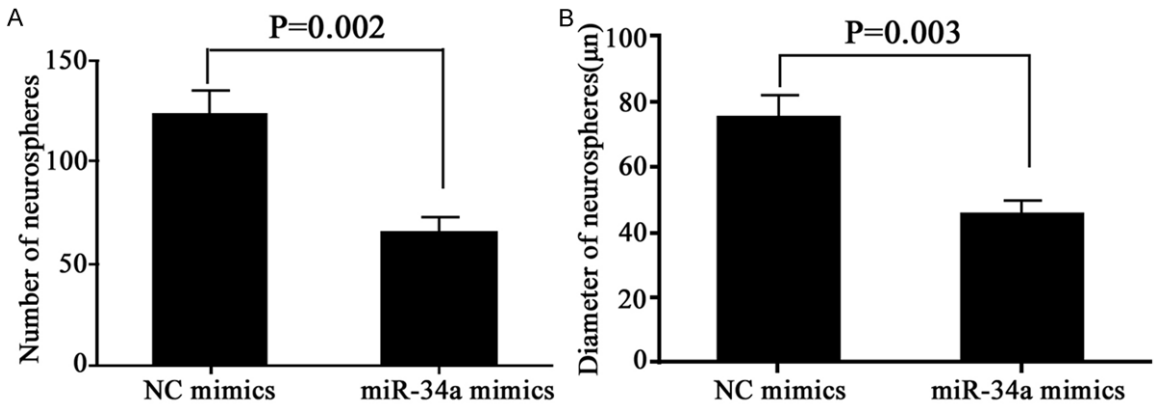

Figure 4. Self-renewal of glioma stem cells under miR-34a over-expression. A. Cell sphere number; B. Cell sphere diameter.

Down-regulation of miR-34a in CD133⁺ glioma cells

Fluorescent qPCR results showed lower miR-34a in CD133⁺ glioma cells from either primary cultured glioma (t=7.872, P=0.014) or U87 cell line (t=6.258, P=0.021), compared to CD133⁻ glioma cells (**Figure 3A**). SIRT1 expression, however, was higher in CD133⁺ glioma cells compared to those in CD133⁻ glioma cells (**Figure 3B**).

Inhibition of self-renewal ability of glioma stem cells by over-expressing miR-34a

As CD133⁺ glioma cells have the potency to form clonal sphere, we further tested such potency under the over-expression of miR-34a. Results demonstrated that the transfection of miR-34a mimics significantly decreased sphere formation potency of CD133⁺ U87 glioma stem cells, as shown by lower sphere number (t=7.417, P=0.002) and diameter (t=6.480, P=0.003, **Figure 4A** and **4B**). MiR-34a transfected cells or control cells were cultured for observing sphere formation ability after 7 days. With normalized against NC mimics (100%), the clonal formation rate of miR-34a mimics transfected cells was only 39.56%±9.19% (t=12.573, P<0.001).

Facilitation of glioma stem cell differentiation by over-expressing miR-34a

Glioma stem cells have the potency to be differentiated towards GFAP+ astrocytes. In this study, we incubated transfected glioma stem cells in medium containing 10% FBS for 7 days, and tested the expression level of differentiation markers. Results showed that, after 7 days of differentiation, CD133 and Nestin expression levels were significantly depressed in both groups of cells. MiR-34a mimics transfected U87 stem cells had significantly lower CD133 and Nestin level compared to negative control group, suggesting that miR-34a effectively down-regulate CD133 and Nestin expression levels in U87 stem cells (**Figure 5A**). After 7 days of differentiation, visible perturbation can be observed in the edge of cells, which transform into polygon, star-like or spindle shapes. Immunofluorescence results showed significantly elevated GFAP expression level in miR-34a mimics transfected group, suggesting that miR-34a could facilitate the differentiation of CD133⁺ U98 glioma stem cells, and induce their transformation towards astrocytes (**Figure 5B**).

Facilitation of glioma stem cell apoptosis by miR-34a

Fluorescent qPCR results showed significantly elevated miR-34a expression in CD133⁺ U87 cells after transfected with miR-34a mimics, as it can reach 9.4-fold of those in NC mimics transfected group (t=107.4, P<0.001), suggesting high efficiency of transfection (**Figure 6A**). Meanwhile, SIRT1 protein expression level in CD133⁺ U87 cells was remarkably decreased (**Figure 6B**). Flow cytometry results exhibited significantly increase of cell apoptosis (apoptotic rate 13.4%) in miR-34a mimics transfected glioma stem cells, suggesting that the over-expression of mi-34a could induce apoptosis of glioma stem cells.

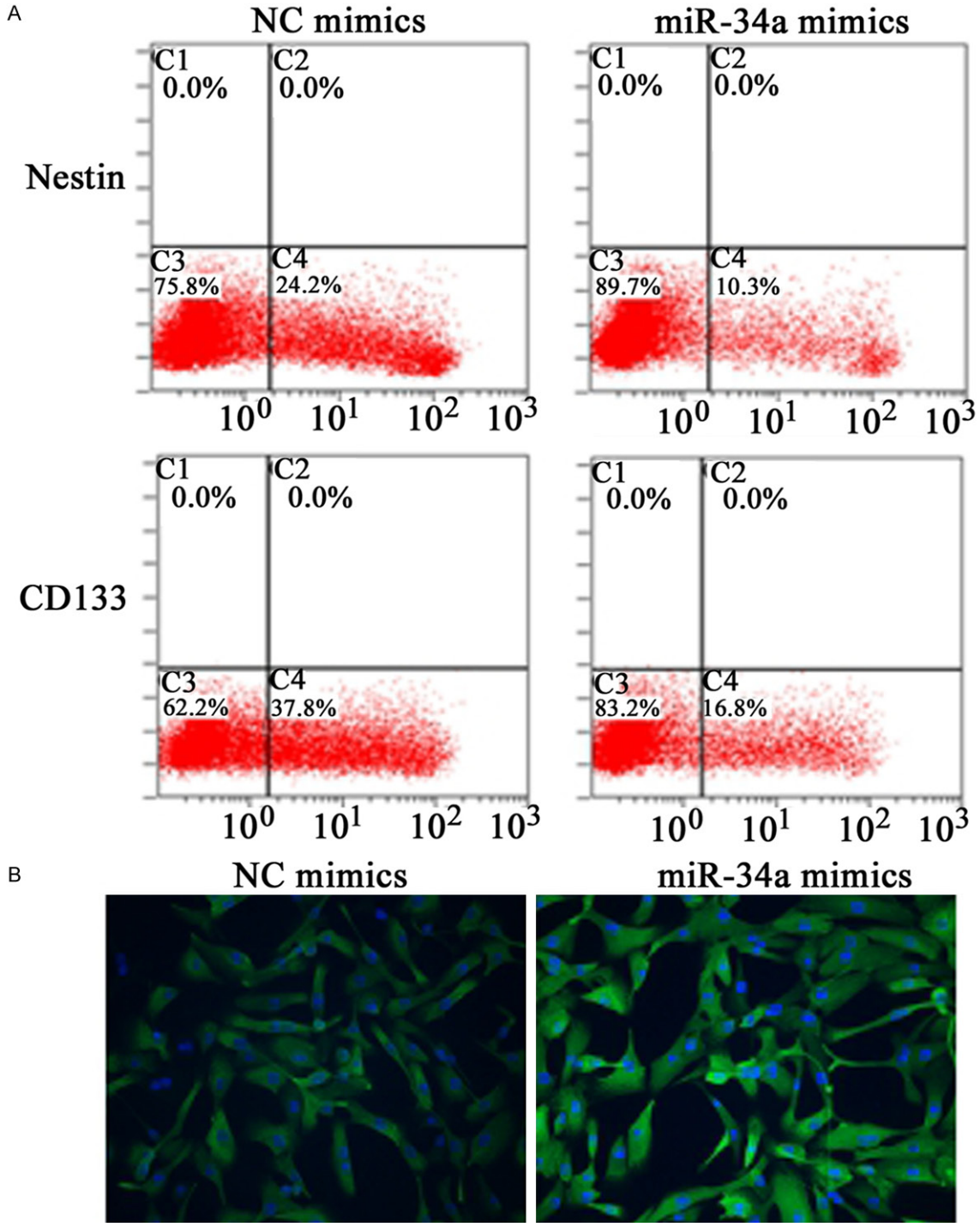

Figure 5. MiR-34a facilitates the neural glioma stem cell differentiation. A. CD133 and Nestin expression by flow cytometry after 7-day differentiation; B. GFAP expression of glioma stem cells during the differentiation towards astrocytes (200×).

Inhibition of in vitro migration by miR-34a

Transwell assay illuminated significantly decreased migration potency of $CD133^+$ U87 cells after over-expressing miR-34a (**Figure 7A**), as the number of perforated cells decreased from 110.5±9.7 to 56.5±8.5 (**Figure 7B**).

Discussion

As the most common CNS tumor, glioma has high invasiveness, migration and proliferation potency, leading to unfavorable prognosis, as patients' median survival period was only 1~2 years [11]. A major challenge in clinical treat-

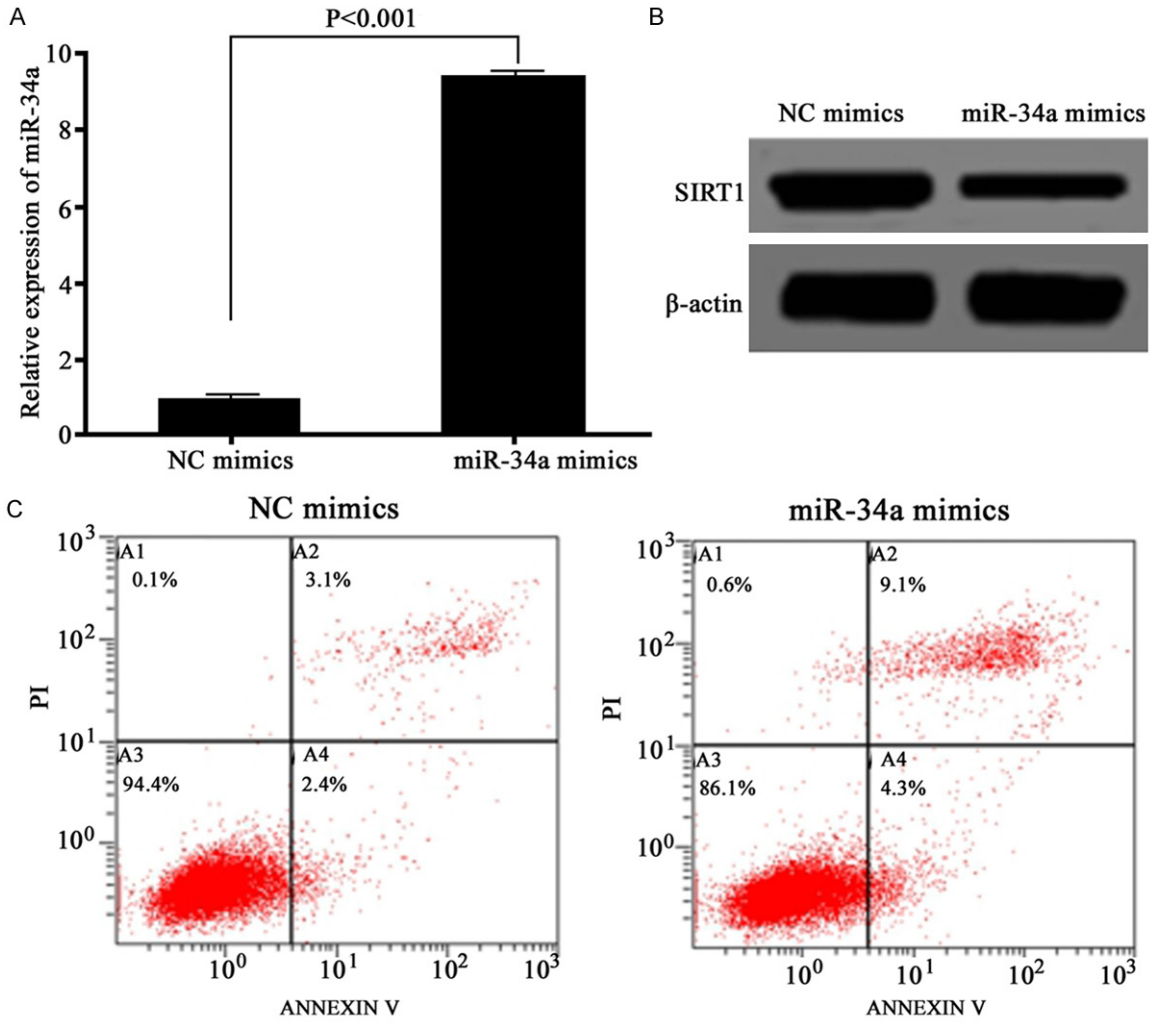

Figure 6. MiR-34a facilitated apoptosis of glioma cells. A. qRT-PCR for miR-34a expression in glioma cells; B. Western blotting for SIRT1 protein expression; C. Flow cytometry for cell apoptosis.

ment is tumor recurrence, which is related with invasive growth and unlimited proliferation of glioma cells. The original of tumor is one fundamental question. Recent studies found the existence of one sub-population of tumor cells with stem cell properties including self-renewal, unlimited proliferation and pluripotent differentiation, thus naming them cancer stem cells (CSCs) [12], which play an important role in tumor formation and maintenance [13]. The discovery of CSCs provides novel insights for tumor treatment. Singh et al recognized the critical role of glioma stem cells (GSCs) in tumor occurrence, progression and recurrence via their independent oncogenic, anti-apoptotic and invasive/migration potencies [14]. Tumor pathogenesis is one delicate process which requires complicated regulation for gene expression at both transcription and post-transcription levels. MicroRNA is one family of newly discovered non coding single stranded small molecule RNA with 19~25 nucleic acids. As one important gene expression mediator, microRNA plays a central role in human gene expression regulation, for mediating transcription/translation of about one third of total human genes [15]. MicroRNA mainly binds with 3'-untranslated region (3'-UTR) of target mRNA via complementary binding, leading to the degradation of target mRNA or the inhibition of post-transcriptional translation, thus participating in the mediation of cell growth, differentiation, apoptosis and migration [16]. MicroRNA down-regulates various target genes including oncogene

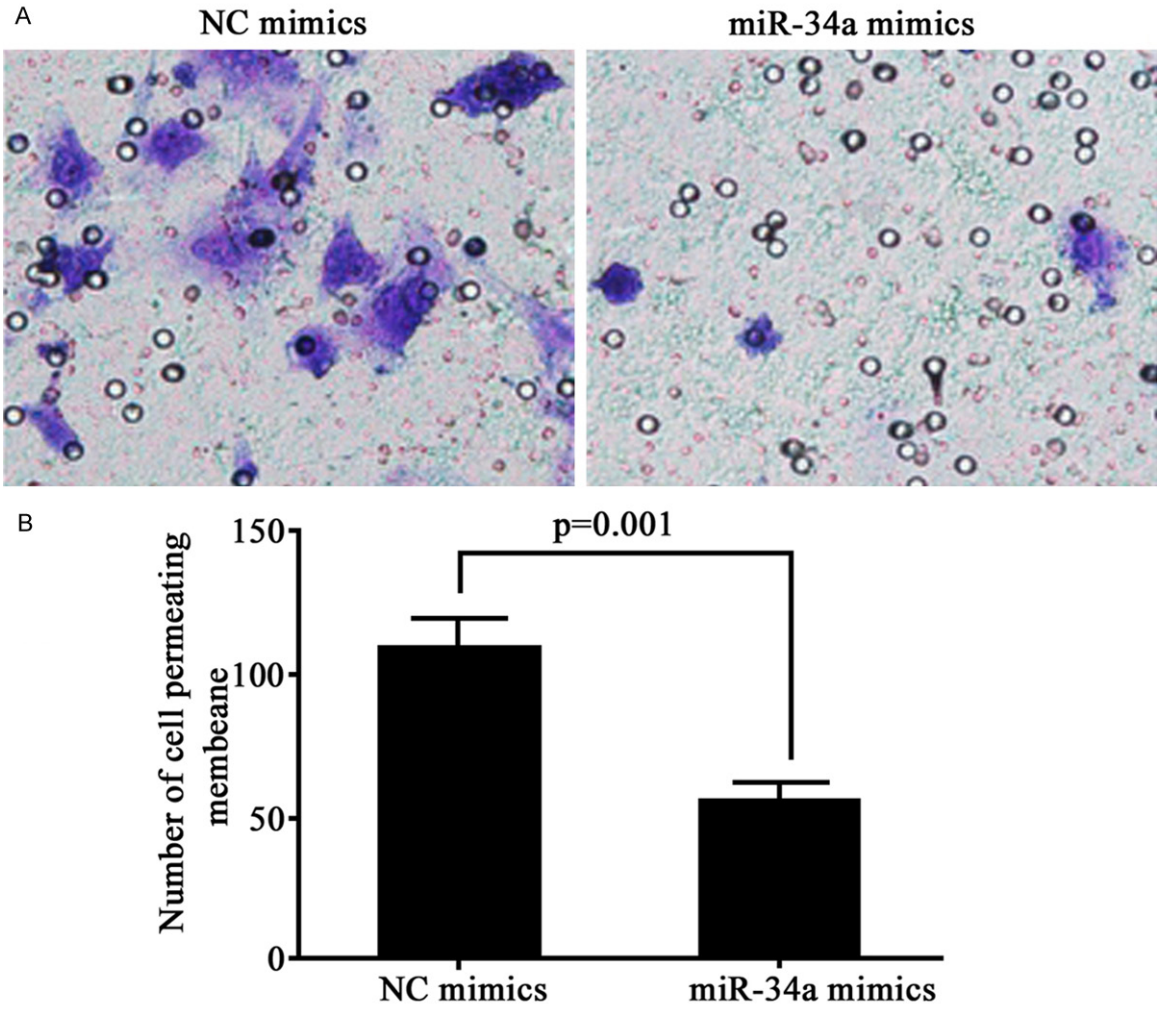

Figure 7. MiR-34a inhibited *in vitro* migration of glioma stem cells. A. Transwell assay testing *in vitro* cell migration; B. Number of perforated cells.

and tumor-suppressor gene. Therefore, microRNA can exert both tumor-inhibition and facilitation roles [17, 18]. Increasing number of studies showed the existence of abnormal expression of microRNA in neural glioma cells, indicating the possibly important role of microRNA expression and dysfunction in glioma pathogenesis [19, 20]. Glioma occurrence is correlated with malignant transformation. Such cells may also acquire stem cell potency, leading to over-proliferation for facilitating tumor formation or recurrence. Besides glioma cells, the abnormal expression and function of microRNA in GSCs have also been described [4, 5]. MicroRNA can regulate both malignant transformation of normal NSCs toward GSCs [21], and can regulate the differentiation of GSCs towards neural cells [22], thus participating in glioma occurrence and progression.

As a downstream molecule of P53 gene, miR-34a is in the regulation of P53. It can inhibit tumor cell proliferation and induce cell apoptosis of various tumor cells, along with the weakening of invasiveness, thus mainly playing a tumor inhibitor function [23-25]. Sirtuin 1 (SIRT1) is a nicotinamide adenine dinucleotide-dependent deacetylase, and plays an important role in mediating cell apoptosis [26]. SIRT1 mainly exerts its anti-apoptotic function via inhibiting acetylation and transcription of P53 gene, thus inhibiting the expression of pro-apoptotic gene PUMA [27]. As an anti-apoptotic gene, the abnormally high expression of SIRT1

has been found to be related with various tumors such as colorectal carcinoma [28], breast cancer [29] and gastric carcinoma [30, 31]. SIRT1 is a target gene of miR-34a, which can inhibit transcription and translation of SIRT1, and can exert anti-apoptotic and anti-tumor potencies via indirect facilitation of transcription/translation of P53 and downstream signals [27]. The abnormal expression of miR-34a has been found to be related with the occurrence of multiple tumors. With advanced research, the role of miR-34a in glioma has been gradually recognized, but leaving its biological properties in neural glioma cells and GSCs largely unknown. This study collected both tumor and adjacent tissues from glioma patients, and found significantly lower miR-34a expression in glioma tissues compared to adjacent tissues. In patients with advanced grade of tumors, miR-34a level was further down-regulated, suggesting that miR-34a might works as one predictive index for the malignancy of glioma, and the role of miR-34a as one tumor suppressor gene in glioma pathogenesis. In contrast, the target gene of miR-34a, SIRT1, had significantly elevated expression in patients with advanced pathology grade, suggesting the involvement of miR-34a/SIRT1 abnormality in the occurrence of glioma. Residual CSCs are major reason for tumor recurrence. The targeted treatment for GSCs thus require the sorting of GSCs. The flow cytometry or immune magnetic beads based on specific markers (CD133, Nestin) are commonly used for sorting out GSCs. In this study, those cells sorted by magnetic beads had CD133 positive rate above 80% by flow cytometry assay, qualifying further experiments. Immunofluorescence results showed the co-expression of CD133 and NSC marker Nestin in those sorted glioma cells, confirming their GSCs nature. Based on the important role of miR-34a in glioma, we further tested the expression of miR-34a in GSCs and found significant down-regulation of miR-34a in $CD133^+$ GSCs compared to those in $CD133^-$ GSCs, endowing unique biological features on GSCs in contrast to normal glioma cells, as consistent with Sun et al [32]. Meanwhile, SIRT1 was up-regulated in the formation of GSCs. Previous study has found that miR-34a could inhibit tumor cell proliferation via targeted inhibition on the expression of oncogenes c-Met, Notch1 and Notch2 in GSCs [33]. The formation of neurosphere can reflect the proliferation and self-renewal ability of GSCs to certain extents. In this study we found that over-expression of miR-34a could significantly inhibit the formation of U87 stem cell sphere, indicating that miR-34a inhibited self-renewal ability of GSCs. Meanwhile, we also found expressions of CD133 and Nestin in U87 stem cells remarkably decreased after over-expressing miR-34a. This may be due to the expression inhibition by miR-34a via unknown pathways, or may be attributed to the inhibition of U87 GSCs growth for decreasing cell number and thus lower expression of CD133 and Nestin, as reported by Guessous et al [32]. By the help of immunofluorescence, we observed elevated GFAP expression in the process of NSCs differentiation towards astrocytes under miR-34a over-expression, indicating the potency of miR-34a for inducing the differentiation of CSCs into mature astrocytes. SIRT1 is one important molecule for miR-34a to exerting anti-apoptosis. This study found significant down-regulation of SIRT1 protein in GSCs with miR-34a over-expression, indicating that miR-34a could facilitate GSCs apoptosis via targeted inhibition on SIRT1 expression in GSCs. These were consistent with Sotillo et al, who reported the induction of cell cycle arresting and facilitated apoptosis by miR-34a [33]. The migration ability of tumor cells is critical for its invasion and distal metastasis. In GSCs with miR-34a over-expression, we observed significantly weakened *in vitro* migration ability, this might be another possible mechanism for miR-34a-directed anti-tumor effects in glioma.

In summary, miR-34a could inhibit proliferation, self-renewal and migration, and induce apoptosis and differentiation in GSCs via targeted inhibition on SIRT1. This study provides further evidences for both basic study and clinical development of treatment method or target selection of glioma. However, the study of miR-34a in GSCs is still at the early phase, as its modulatory effects on biological property of GSCs are still unclear and are worth further investigations.

Acknowledgements

Research supported by the Science foundation of shandong province (NO. ZR2009CL022 and 2013ZRB14142).

Disclosure of conflict of interest

None.

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