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miR-200a-3p promotes β-Amyloid-induced neuronal apoptosis through down-regulation of SIRT1 in Alzheimer's disease

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The aberrantly expressed microRNAs (miRNAs) including miR-200a-3p have been reported in the brains of Alzheimer's disease (AD) patients in recent researches. Nevertheless, the role of miR-200a-3p in AD has not been characterized. The purpose of this study was to examine whether miR-200a-3p regulated β-Ameyloid (Aβ)-induced neuronal apoptosis by targeting SIRT1, a known anti-apoptotic protein. An increased level of miR-200a-3p and a decreased level of SIRT1 in the hippocampus of APPswe/PSΔE9 mice (a model for AD) were observed. To construct an *in vitro* cell model of AD, PC12 cells were cultured in presence of $A\beta_{25\text{-}35}$. The results of flow cytometry analysis showed that the apoptosis rate and cleaved-caspase-3 expression in PC12 cells exposed to $A\beta_{25\text{-}35}$ were remarkably increased, but the apoptosis rate and cleaved-caspase-3 activity were decreased when cells were transfected with anti-miR-200a-3p. On the other hand, MTT assay showed that the cell survival rate was increased in the $A\beta_{25\text{-}35}$ + anti-miR-200a-3p group compared with the $A\beta_{25\text{-}35}$ + anti-miR-NC group. Dual-luciferase reporter gene assay validated the predicted miR-200a-3p binding sites in the 3′-UTR of SIRT1 mRNA. In addition, downregulation of SIRT1 promoted $A\beta_{25\text{-}35}$-induced neuronal apoptosis and cleaved-caspase-3 level in PC12 cells, whereas anti-miR-200a-3p reversed these effects. Knockdown of SIRT1 decreased the inhibitory effect of $A\beta_{25\text{-}35}$ on cell viability, while anti-miR-200a-3p attenuated this effect. Overall, the results suggest that suppression of miR-200a-3p attenuates $A\beta_{25\text{-}35}$-induced apoptosis in PC12 cells by targeting SIRT1. Thus, miR-200a-3p may be a potential therapeutic target for treatment of AD.

Keywords. Alzheimer's; apoptosis; miR-200a-3p; SIRT1; β-amyloid

1. Introduction

Alzheimer's disease (AD), the most common progressive neurodegenerative disease, is characterized by cognitive impairment, learning and memory deficits and loss of language skills. It has been estimated that about 34 million people currently suffered from AD around the world (Alzheimer's 2015). Unfortunately, up to now, there are no curative therapeutic strategies for AD. The primary neuropathological feature in AD brain is the existence of insoluble β-Amyloid (Aβ) aggregate and soluble Aβ oligomers, both of which are toxic to neurons and synapses (Benilova *et al.* 2012). Synthetic Aβ has been demonstrated to destroy synaptic transmission and induce synaptic degeneration, resulting in neuronal dysfunction (Shankar and Walsh 2009). Furthermore, the mitochondrial fission/fusion balance and transport could be disrupted by oligomeric Aβ (Wang *et al.* 2008b). $A\beta_{25\text{-}35}$ oligomers, a fragment of full length $A\beta_{1\text{-}42}$ peptide, are easier to spread in brain due to its smaller size. Serious cognitive impairment and a loss of neurons in the cerebral cortex and the hippocampus were observed in mice subjected to intracerebroventricular injection of $A\beta_{25\text{-}35}$ oligomers (Klementiev *et al.* 2007). Therefore, the exploitation of novel drug targets based on Aβ is the key point to cure AD.

microRNAs (miRNAs) are a class of single-stranded non-coding RNAs that are usually 18 to 25 nucleotides long. miRNAs suppress gene expression at the post-transcriptional level by pairing with the 3′-untranslated region (3′-UTR) of target messenger RNAs (mRNAs), ultimately leading to either mRNA degradation or translational suppression (Gao *et al.* 2010). miRNAs have been demonstrated to modulate multiple biological and cellular processes that are implicated in various kinds of human diseases (Izaurralde 2015). Many studies have suggested that the miRNA gene expression could be affected by a variety of stimulus including toxicant, hypoxia, superoxide, and so on (Chen *et al.* 2010; Lema and Cunningham 2010; Liu *et al.* 2009). miR-29a and miR-29b-1 were found to be down-regulated in the brain of sporadic AD patients. The *in vitro* study showed that miR-29a and miR-29b-1 can modulate β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) activity and Aβ generation by

binding to 3′-UTR of BACE1 mRNA (Hébert *et al.* 2008). A previous study found that miR-200a-3p was up-regulated in the hippocampus of late-onset AD patients, suggesting that miR-200a-3p may be implicated in the pathologic process of AD (Lau *et al.* 2013).

Silent information regulator transcript-1 (SIRT1), an NAD^+-dependent nuclear histone deacetylase, modulates multiple important cellular processes, including cell metabolism, proliferation, apoptosis and differentiation (Bordone and Guarente 2005). SIRT1 has been found to possess the capabilities of epigenetic modifications and gene expression regulation at transcriptional level by deacetylating histone and non-histone proteins, respectively (Denu 2005). SIRT1 protected cells against oxidative stress injury by deacetylating FOXO transcription factor FOXO3 (Brunet *et al.* 2004). An *in vivo* study showed that SIRT1 was highly expressed in the nucleus of cardiomyocytes in the failing hearts of TO-2 hamsters. Furthermore, *in vitro* experiments suggested that nuclear SIRT1 reduced oxidative stress and promotes cell survival of in chronic heart failure by contributing to the generation of manganese superoxide dismutase (Mn-SOD) (Tanno *et al.* 2010). miR-34a suppressed the expression of SIRT1 protein by binding to the 3′-UTR of SIRT1 mRNA, resulting in an up-regulation of p21 and PUMA, both of which were transcriptional targets of p53, that regulate the cell cycle and apoptosis, respectively (Yamakuchi *et al.* 2008). Sun *et al.* have found that activation of SIRT1 by curcumin alleviated the neurotoxicity of $A\beta_{25\text{-}35}$ and inhibited the expression of Bax in rat cortical neurons exposed to $A\beta_{25\text{-}35}$ (Sun *et al.* 2014). According to the previously published literature, it is speculated that up-regulation of miR-200a-3p might promote $A\beta_{25\text{-}35}$-induced neuronal apoptosis at least partially by targeting SIRT1.

In this study, it is found that SIRT1 expression is regulated by miR-200a-3p. It is demonstrated a vital role of miR-200a-3p in $A\beta_{25\text{-}35}$-induced neuronal apoptosis and investigated its underlying molecular mechanism.

2. Materials and methods

2.1 *AD transgenic mice*

APPswe/PSΔE9 mice were obtained from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences & Comparative Medical Center (Beijing, China). The animal experiments were approved by the ethics committee of Huaihe Hospital of Henan University.

2.2 *Cells and cell culture*

The PC12 cells (Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco) at 37 °C in a humidified incubator with 5% CO_2.

2.3 *Quantitative real-time PCR (qRT-PCR)*

Total RNA was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. The reverse transcription reaction was conducted by means of a TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. qRT-PCR was carried out with the Brilliant II Fast SYBR green QPCR master mix (Agilent Technologies, Santa Clara, CA, USA) and MyiQ Real-Time PCR Detection System (Bio-Rad, Richmond, CA, USA) in accordance with the manufacturer's protocol. Sequences of primers (Sangon Biotech, Shanghai, China) were as follows: miR-200a-3p forward, 5′-GGCTAACACTGTCTGGTAACGATG-3′; reverse, 5′-GTGCAGGGTCCGAGGT-3′; U6 forward, 5′-CTCGCTTCGGCAGCACA-3′; reverse, 5′-AACGCTTCACGAATTTGCGT-3′. The relative expression analysis was performed using the comparative C_T method ($2^{-\Delta\Delta CT}$). The nuclear RNA U6 was used to normalize the miRNA qRT-PCR data.

2.4 *Western blot*

At 48 hours after transfection, cells were collected and then total cell proteins were lysed with RIPA lysis buffer (Beyotime Institute of Biotechnology, Jiangsu, China). The protein concentrations were measured using a Protein Assay Reagent (Thermo Scientific, Rockford, IL, USA), according to the manufacturer's instruction. Equal amount of protein was loaded on SDS-polyacrylamide gels and separated by gel electrophoresis, and then transferred onto a polyvinylidene fluoride (PVDF) membrane (Merck Millipore, Billerica, MA, USA). Membranes were incubated in blocking buffer (5% non-fat dried milk in tris-buffered saline containing 0.1% Tween-20, TBST) for 1 h at room temperature. Blots were immunolabeled with primary antibodies including the anti-SIRT1 antibody (Abcam, Cambridge, MA, USA), anti-caspase-3 antibody (Abcam) or anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibody overnight at 4 °C. β-actin was used as an internal control. After three washes with TBST, the membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Following three washes with TBST, immunoreactive bands were visualized using enhanced chemi-luminescence (ECL) detection regent (Pierce, Rockford, IL, USA) and exposed to

X-Ray film. The densitometric analysis of band intensities was carried out by using the Image J software (National Institutes of Health, Bethesda, MD, USA).

2.5 *MTT assay*

Cell proliferation was assessed by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyl tetrazolium bromide (MTT) cell proliferation assay kit (Sigma-Aldrich, St. Louis, MO, USA). Briefly, PC12 cells were collected and seeded in a 96-well plate at a density of 5000 cells per well. After culture for 12 h, cells were transfected with miR-200a-3p mimics or inhibitors, and then incubated with 20 μM $A\beta_{25-35}$ for 48 h. After treatment, cells were incubated with 0.5 mg/mL MTT for 4 h at 37 °C in a humidified incubator with 5% CO_2. Next, the MTT solution was removed and 100 μL dimethyl sulfoxide (DMSO) was added into each well to dissolve the MTT formazan crystals. The absorbance of each sample was measured on a microplate reader (Becton Dickinson, Mountain View, CA, USA) at 490 nm.

2.6 *Flow cytometry*

Cell apoptosis assay was conducted by using an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) double staining apoptosis detection kit (BD Biosciences Pharmingen, San Jose, CA, USA) according to the manufacturer's instructions. In brief, PC12 cells were transfected with miR-200a-3p mimics or inhibitors using Lipofectamine 2000 (Invitrogen) according to the instruction, and then incubated with 20 μM $A\beta_{25-35}$ for 48 h. After treatment, cells were digested with 0.05% trypsin (Sigma-Aldrich) at 37 °C and then centrifuged at 200*g* for 3 min. Cells were washed twice with pre-cold 0.01 M PBS and then resuspended in 1× binding buffer at a concentration of 1×10^6 cells/mL. Subsequently, cells were stained with Annexin V-FITC and propidium iodide (PI) for 20 min in the dark at room temperature. Finally, apoptotic cells were identified by a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) and data were analysed using the Cell Quest software (Becton Dickinson).

2.7 *Dual-luciferase reporter assay*

miR-200a-3p mimics and scramble mimics (negative control; miR-NC) were synthesized by Genepharma (GenePharma, Shanghai, China). Luciferase reporter plasmids containing either wild-type (WT) or mutant (Mut) SIRT1 3′-UTR were purchased from Promega (Madison, WI, USA). For the luciferase assay, PC12 cells were seeded into a 24-well plate (4×10^4 cells/well) and cultured for 24 h. PC12 cells were cotransfected with 0.5 μg luciferase reporter plasmid and 50 nM miR-200a-3p mimics or miR-NC using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. After incubation for 48 hours, the luciferase assay using a Dual Luciferase Reporter Assay Kit (Promega) was carried out according to the protocol provided by manufacturer. The luminescence was measured with a 96-well plate reader (Berthold Detection System, Oak Ridge, TN, USA). The experiment was repeated three times, each time in triplicate.

2.8 *Statistical analysis*

All experiments were repeated at least three times. Comparisons between two groups were carried out using the unpaired Student's *t*-test. One-way analysis of variance (ANOVA) was used to compare the means of three or more groups. The statistical analyses were performed using the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). A *P*-value <0.05 was considered statistically significant.

3. Results

3.1 *miR-200a-3p is up-regulated and SIRT1 is down-regulated in the hippocampus of APPswe/PSΔE9 mice*

Six APPswe/PSΔE9 transgenic (Tg) mice and an equal number of age-matched controls were used to determine the expression levels of miR-200a-3p and SIRT1. The results of qRT-PCR showed that the expression level of miR-200a-3p was increased in 6-month-old APPswe/PSΔE9 mice compared with age-matched controls (figure 1A). More importantly, the SIRT1 mRNA and protein expression were decreased in the hippocampus of APPswe/PSΔE9 mice (figure 1B and C).

3.2 *Inhibition of miR-200a-3p attenuates $A\beta_{25-35}$-induced neuronal toxicity*

PC12 cells were treated with 1, 10 or 20 μM $A\beta_{25-35}$ for 48 h, and then the expression of miR-200a-3p was evaluated by qRT-PCR. The results showed that $A\beta_{25-35}$ treatment in cells lead to an increase in miR-200a-3p expression, which was positive correlation with the concentration $A\beta_{25-35}$. To study the role of miR-200a-3p in neuronal toxicity, PC12 cells were transfected with miR-200a-3p mimics. The results revealed that miR-200a-3p mimics resulted in an obvious increase in cell apoptosis rate (figure 2B) and cleaved caspase-3 level (figure 2C), while an apparent decrease in cell viability in P12 cells (figure 2D). Then, an *in vitro* cell model of $A\beta_{25-35}$-induced damage was constructed in PC12

cells, and the effect of miR-200a-3p and anti-miR-200a-3p on PC12 cells treated with $A\beta_{25-35}$ was assessed. As shown in figure 2B-D, the apoptosis rate and leaved caspase-3 expression were remarkably increased, and cell viability was significantly dropped in the $A\beta_{25-35}$ group compared with the DMSO (control) group. Moreover, miR-200a-3p aggravated $A\beta_{25-35}$-induced cell apoptosis and cleaved caspase-3 level in PC12 cells, while promoted the inhibitory effect of $A\beta_{25-35}$ oncell viability. However, the cell apoptosis and leaved caspase-3 level were evidently reduced, and cell viability was augmented in the $A\beta_{25-35}$ + anti-miR-200a-3p group compared with the $A\beta_{25-35}$ + anti-miR-NC group (figure 2D). Taken together, these findings suggest that down-regulation of miR-200a-3p attenuates $A\beta_{25-35}$-induced neuronal toxicity.

3.3 *miR-200a-3p suppresses SIRT1 expression by directly targeting SIRT1 mRNA 3′-UTR*

To identify potential miRNAs that directly target SIRT1 gene, several target gene prediction websites were searched including Targetscan (*http://www.targetscan.org/*), PicTar (*http://pictar.mdc-berlin.de/*), and Mirbase (*http://www.mirbase.org/*). Based on bioinformatics analysis, it is found that miR-200a-3p seed region was highly conserved among different species. The predicted miR-200a-3p-binding sites in human SIRT1 3′-UTR and the Mut SIRT1 3′-UTR with modified binding sequence were shown in figure 3A. To determine whether miR-200a-3p directly targets the predicated sites of the SIRT1 3′-UTR, dual luciferase reporter gene assays was conducted. The luciferase activity of constructs with WT SIRT1 3′-UTR in the miR-200a-3p mimics group was obviously decreased when compared with the miR-NC group, but no decrease was observed when the miR-200a-3p-binding site is in the potential target 3′-UTR were mutated (figure 3B). In addition, the expression level of SIRT1 mRNA was decreased in PC12 cells transfected with miR-200a-3p mimics, and increased in PC12 cells transfected with anti-miR-200a-3p compared with respective controls (figure 3C). Consistently, miR-200a-3p mimics notably inhibited the expression of SIRT1 (figure 3D), while anti-miR-200a-3p elevated SIRT1 protein level (figure 3E). These results implied that miR-200a-3p negatively regulated the transcription of SIRT1 by directly binding to SIRT1 3′-UTR region.

3.4 *Down-regulation of miR-200a-3p reverses the promotive effect of SIRT1 knockdown on Aβ25-35-induced neuronal toxicity*

To further explore the mechanism underlying the neuroprotective effects of SIRT1 against $A\beta_{25-35}$, a plasmid vector, pcDNA3.1-SIRT1 that highly expressed SIRT1 protein was conducted. PC12 cells were transfected with pcDNA3.1-SIRT1 or si-SIRT1 or co-transfected si-SIRT1 with anti-200a-3p. The expression level of SIRT1 protein was increased in the pcDNA3.1-SIRT1 group, and decreased in the si-SIRT1 group (figure 4A). And then transfected cells were exposed to $A\beta_{25-35}$ for 48 hours. Flow cytometry and western blot were performed to detect cell apoptosis and cleaved casepase-3 expression, respectively. The results showed that

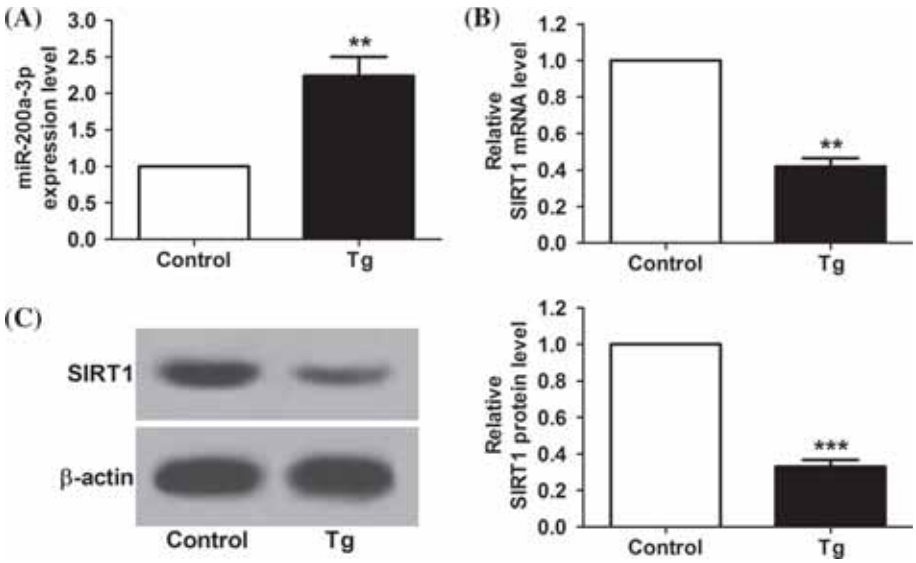

Figure 1. The expression of miR-200a-3p in the hippocampus of APPswe/PSΔE9 transgenic (Tg) mice and control mice. (**A**, **B**) qRT-PCR was performed to examine the expressions of miR-200a-3p and SIRT1 mRNA in the hippocampus of APPswe/PSΔE9 mice and control mice. U6 snRNA was used as an internal control. (**C**) The protein expression of SIRT1 was determined by Western blot. The data are expressed as the means ± SD. $^{**}P < 0.01$, $^{***}P<0.001$, n = 6.

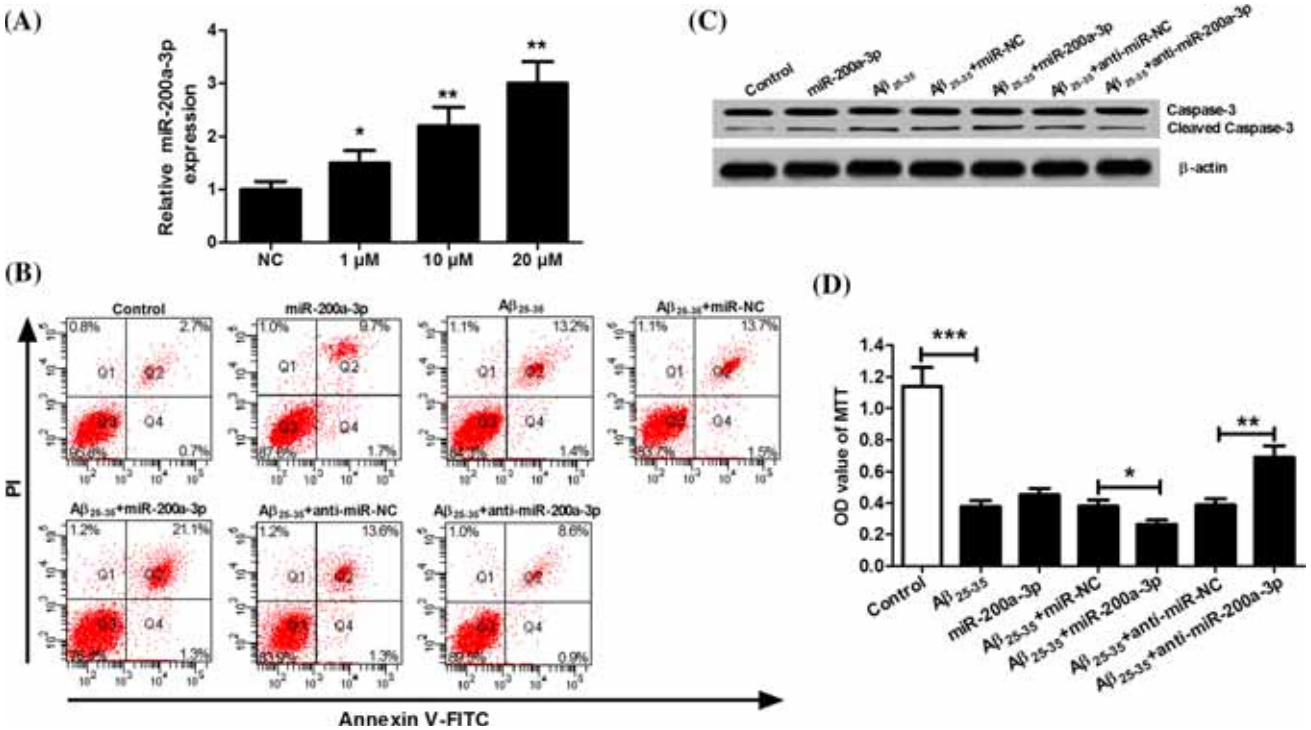

Figure 2. The effect of miR-200a-3p mimics on $A\beta_{25\text{-}35}$-induced neuronal toxicity. (**A**) PC12 cells were treated with 1, 10 or 20 μM $A\beta_{25\text{-}35}$ for 48 h, and then the expression of miR-200a-3p was evaluated by qRT-PCR. (**B**) PC12 cells were transfected with miR-200a-3p mimics or anti-miR-200a-3p and then exposed to 20 μM $A\beta_{25\text{-}35}$ for 48 h. Flow cytometry was performed to detect cell apoptosis rate. For each plot: lower left quadrant, viable cells; upper right quadrant, late-stage apoptotic cells; upper left quadrant, end stage apoptotic or necrotic cells; lower right quadrant, early-stage apoptotic cells. (**C**) Caspase-3 activity was analysed by Western blot. (**D**) Cell viability was evaluated by MTT assay. The data are presented as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001, n = 3.

overexpression of SIRT1 inhibited $A\beta_{25\text{-}35}$-induced cell apoptosis and cleaved casepase-3 activity in PC12 cells. Moreover, it is found that the apoptosis rate in the si-SIRT1 group was increased compared with si-NC group; consistently, the level of cleaved casepase-3 in the si-SIRT1 group was also increased compared with the si-NC group (figure 4B and C). MTT assays to assess the effect of SIRT1 on the $A\beta_{25\text{-}35}$-induced decrease in cell viability were performed. The results showed that the cell survival rate was increased in the $A\beta_{25\text{-}35}$ +SIRT1 group compared with the $A\beta_{25\text{-}35}$ + si-NC group (figure 4C). Furthermore, compared with si-SIRT1 group, the co-transfection group resulted in an increase of SIRT1 protein level and cell survival rate, and a decrease of apoptosis rate and casepase-3 activity. These findings demonstrated that miR-200a-3p promotes β-Amyloid-induced neuronal apoptosis through down-regulation of SIRT1.

4. Discussion

The overproduction of Aβ is considered as the leading cause of synaptic and neuronal loss, which lead to the development of dementia in AD (Benilova *et al.* 2012). The therapeutic interventions on the processing and metabolism of Aβ peptides in AD patients have failed up to the present day. The etiological factors of AD are very complicated and the underlying molecular pathological mechanism is not understood well. Several miRNAs have been reported to play important roles in AD and may be developed as potential therapeutic targets for AD (Lukiw 2007). In this study, an *in vitro* neuronal cell injury model was established, which provided evidences on the inhibitory effect of miR-200a-3p on Aβ-induced neurotoxicity in PC12 cells.

The expression level of miR-107 was decreased in AD patients with the earliest stages of pathology and may contribute to disease progression via regulation of β-Site amyloid precursor protein-cleaving enzyme 1 (BACE1) (Wang *et al.* 2008a). miR-299-5p, which was down-regulated in AD patients, inhibited autophagy-related apoptosis by suppressing autophagy protein 5 in primary hippocampal neurons and improved cognitive capacity in the APPswe/PS1dE9 mouse model of AD (Zhang *et al.* 2016). Takehiro *et al.* found that plasma miR-34a and miR-146a expression levels, and cerebrospinal fluid (CSF) miR-34a, miR-125b, and miR-146a expression levels in AD patients were obviously lower than those in control subjects. Reversely, CSF miR-29a and miR-29b expression were up-regulated in AD

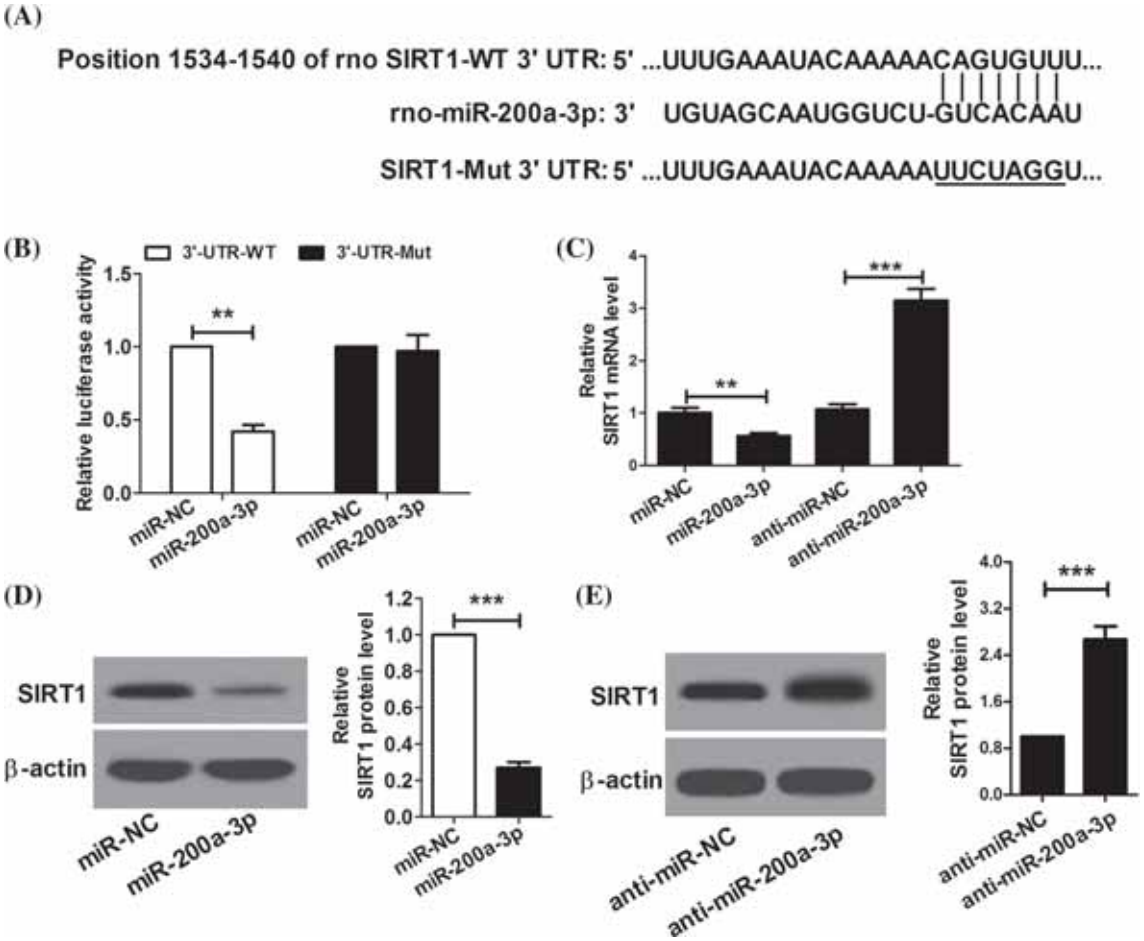

Figure 3. SIRT1 is a target gene of miR-200a-3p. (**A**) A schematic representation of putative miR-200a-3p binding sites on the 3′-UTR of SIRT1 in *Rattus norvegicus* (rno). (**B**) PC12 cells were transfected with WT or Mut SIRT1 3′-UTR recombined luciferase reporter vector along with miR-200a-3p mimics or miR-NC. At 48 h post-transfection, the relative luciferase activities were determined according to the protocol provided by manufacturer. PC12 cells were transfected with miR-200a-3p mimics, miR-NC, anti-miR-200a-3p mimics or anti-miR-NC. At 48 h after transfection, The mRNA (**C**) and protein expression (**D** and **E**) of SIRT1 were analysed. The data are presented as the mean ± SD. $^{**}P<0.01$, $^{***}P<0.001$, n = 3.

patients. miRNAs in plasma and CSF may be able to serve as effective biomarkers for early diagnosis of AD (Kiko *et al.* 2014). In breast cancer, miR-200a modulates activity of transcription factor NF-E2-related factor by suppressing the expression of Kelch-like ECH-associated protein 1 at a posttranscriptional level, thereby inhibiting the anchorage-independent growth of breast cancer cells (Eades *et al.* 2011). miR-200a-3p has been shown to be aberrantly expressed in the hippocampus of late-onset AD patients (Lau *et al.* 2013). However, the role of elevated miR-200a in AD has not been reported in the literature so far. In this study, qRT-PCR was conducted to determine the expression level of miR-200a-3p in APPswe/PSΔE9 double transgenic mouse models of AD. The results showed that miR-200a-3p was up-regulated in hippocampus of APPswe/PSΔE9 mice, indicating that miR-200a-3p may be implicated in the disease progression of AD. In addition, it is found that the addition of $A\beta_{25-35}$ to PC12 cells suppressed cell growth. Importantly, the inhibitory effect of $A\beta_{25-35}$ on cell viability was reversed by transfection with anti-miR-200a-3p in PC12 cells.

SIRT1 is thought to be a vital regulator of cell survival and apoptosis via its interaction with nuclear proteins (Pfister *et al.* 2008). Increasing evidence from mouse models has showed that SIRT1 acts as an effective protector from ageing-related pathologies, including diabetes, stroke, neurodegeneration and, multiple kinds of cancer (Revollo and Li 2013). Up-regulation of SIRT1 suppressed both Smad7- and transforming growth factor β-induced cell apoptosis in glomerular mesangial cells through deacetylation of Smad7 by directly interacting with the N terminus of Smad7, but SIRT1 knockdown contributed to this apoptosis (Kume *et al.* 2007). In lipotoxic cardiomyopathy, activation of SIRT1 inhibited palmitate-induced apoptosis in cultured neonatal

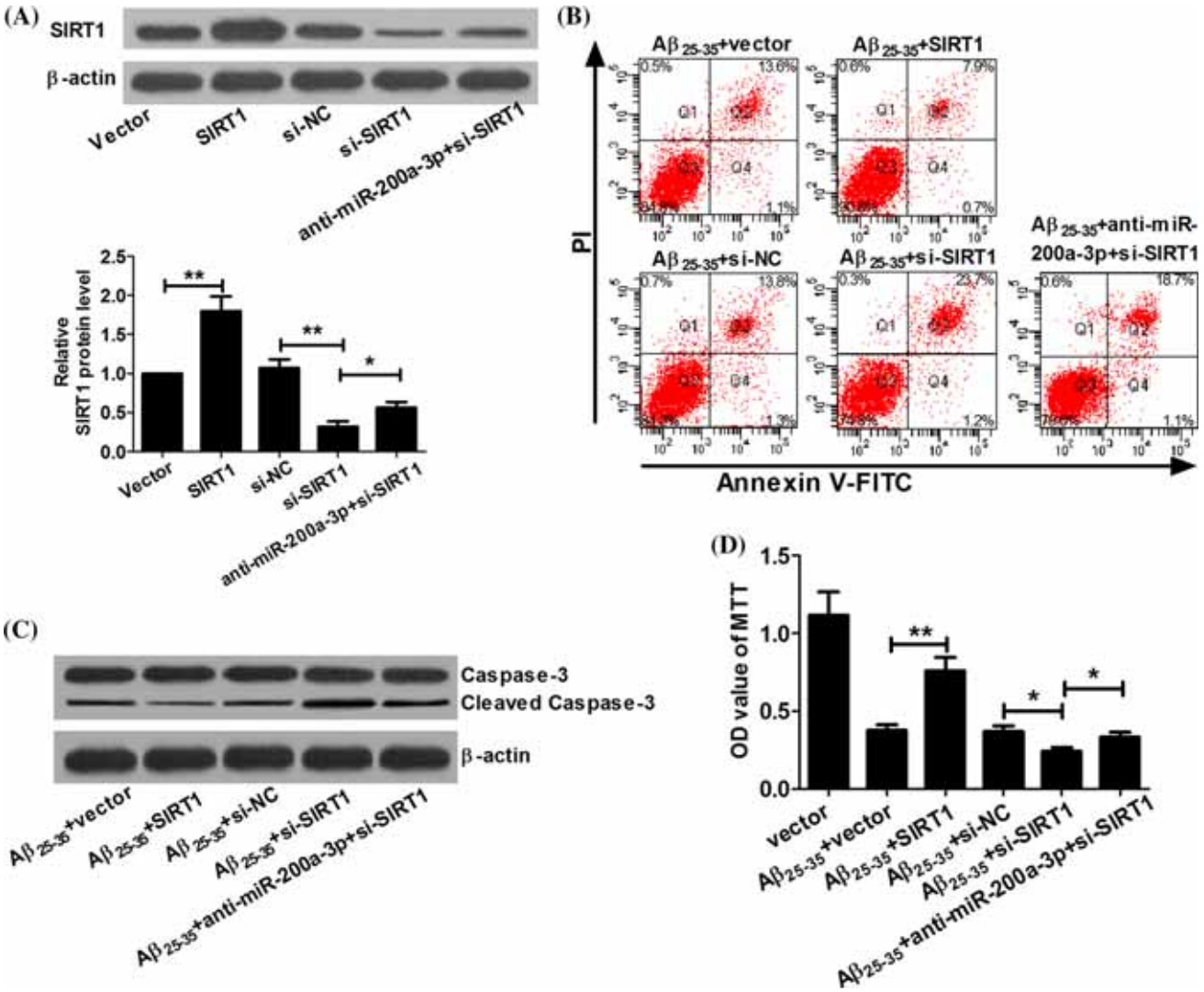

Figure 4. Effect of SIRT1 overexpression on $A\beta_{25\text{-}35}$-induced neuronal toxicity. PC12 cells were transfected with SIRT1 or si-SIRT1 or si-SIRT1 + anti-miR-200a-3p. (**A**) western blot analysis was performed to assess the expression of SIRT1 protein. (**B**) The apoptosis rate in transfected PC12 cells were evaluated by flow cytometry analysis after exposing to $A\beta_{25\text{-}35}$ for 48 h. (**C**) Caspase-3 activity in transfected PC12 cells was analysed by western blot after exposing to $A\beta_{25\text{-}35}$ for 48 h. (**D**) Cellular viability of transfected PC12 cells was evaluated by MTT assay after exposing to $A\beta_{25\text{-}35}$ for 48 hours. The data are presented as the mean ± SD.*P<0.05, **P<0.01, n = 3.

mouse cardiomyocytes. miRNA-195 was demonstrated to promote palmitate-induced apoptosis in cardiomyocytes by negatively regulating the expression of SIRT1 (Zhu *et al.* 2011). Here, it is revealed that inhibition of miR-200a-3p attenuated $A\beta_{25\text{-}35}$-induced neuronal toxicity. To further make clear the mechanism by which anti-miR-200a-3p reduces $A\beta_{25\text{-}35}$-induced neuronal toxicity, a dual luciferase experiment was carried out to determine whether miR-200a-3p could target the 3′-UTR of SIRT1 mRNA. The results showed that SIRT1 expression was negatively regulated by miR-200a-3p. On the other hand, PC12 cells were transfected with plasmid vectors harboring the SIRT1 gene. It is found that the pro-apoptotic effect of $A\beta_{25\text{-}35}$ on PC12 cells was reversed by overexpressing SIRT1. Moreover, PC12 cells were co-transfected with mir-200a-3p inhibitor and si-SIRT1, and the results showed that down-regulation of SIRT1 attenuates Aβ25-35-induced neuronal toxicity, however, the addition of anti-miR-200a-3p reversed these effects, which strongly confirmed our conclusion that miR-200a-3p promotes β-Amyloid-induced neuronal apoptosis through down-regulation of SIRT1.

Taken together, it is demonstrated that down-regulation of miR-200a-3p protected PC12 cells from $A\beta_{25\text{-}35}$-induced neurotoxicity and inhibited the cell apoptosis. Moreover, it is showed that SIRT1 was a target gene of miR-200a-3p and exerted a neuroprotective effect against $A\beta_{25\text{-}35}$-induced

toxicity in PC12 cells. These findings suggested that an inhibitory strategy against miR-200a-3p might be of great help for treatment of AD.

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