MicroRNA-125b regulates Alzheimer's disease through SphK1 regulation

YAN JIN[1,2], QIUYUN TU[1,2] and MIN LIU[1,2]

Departments of [1]Operation Center and [2]Gereology,
The Third Xiangya Hospital of Central South University, Changsha, Hunan 410013, P.R. China

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Abstract. The present study aimed to investigate the expression of microRNA (miR)-125b in patients with Alzheimer's disease (AD) and to determine its potential role in AD. Mouse neuroblastoma Neuro2a APPSwe/Δ9 cells were used to generate an *in vitro* AD model. The results demonstrated that the expression levels of miR-125b were markedly increased in patients with AD compared with in the normal group. In addition, overexpression of miR-125b significantly inhibited cell proliferation, induced apoptosis, and enhanced inflammation and oxidative stress in an *in vitro* model of AD model. Furthermore, overexpression of miR-125b significantly promoted amyloid precursor protein and β-secretase 1 expression and β-amyloid peptide production, and suppressed sphingosine kinase 1 (SphK1) protein expression *in vitro*. These findings suggested that miR-125b may regulate AD, and neuronal cell growth and apoptosis, via the regulation of inflammatory factors and oxidative stress by SphK1; therefore, miR-125b may be involved in the development of AD.

Introduction

Alzheimer's disease (AD) is a common type of neurodegenerative disease, the main clinical manifestation of which is progressive dementia (1). AD-associated pathological alterations include the presence of senile plaques in the brain, neurofibrillary tangles (NFTs) and the loss of neurons (1). As the aged population increases, the incidence of AD continues to rise. According to recent statistics, there are >20 million patients with AD, and this number increases by 4.6 million annually in China (1). In addition, for individuals >65 years old, the risk of AD doubles every 5 years, and in people ≥85 years old, almost half suffer from AD (1). Due to progressive decline in the cognitive function of patients with AD, not only is patient quality of life decreased, but the care of patients with AD is considered a burden to families and society. AD is a major social problem worldwide; therefore, research regarding the pathogenesis and prevention of AD has garnered attention in recent years (1).

Sphingosine kinase 1 (SphK1) is a key enzyme in the regulation of ceramide/sphingosine-1-phosphate (S1P). Due to their contrasting functions, the balance between ceramide and S1P is associated with cell death and survival (2). The balance between these two factors is mainly regulated by SphK1, which is an enzyme that can convert sphingomyelin into S1P (3). When it is overexpressed, SphK1 induces the transformation of ceramide into S1P (3). Conversely, the downregulation of SphK1 results in accumulation of ceramide, which is associated with anticancer therapy-induced cell death (4).

S1P is a biologically active lipid molecule, which has recently garnered attention. The synthesis and degradation of S1P is regulated by various enzymes, and its generation is regulated by SphK1 (5). SphK catalyzes the first carbon atom of sphingosine to connect with the ethyl phosphate group; this is essential for the generation of S1P (6). S1P has a dual role inside and outside the cell; as a G protein-coupled receptor ligand, S1P can regulate numerous physiological activities by activating these receptors, including cell migration, angiogenesis, vascular maturation, cardiac development and nerve axon functions. In addition, S1P can be used as a second messenger to regulate intracellular calcium ion levels for stability, cell proliferation promotion and apoptosis inhibition (3).

S1P regulates cell death and survival, apoptosis, calcium balance, blood vessel maturation and angiogenesis, and participates in various biochemical processes in the central nervous system; therefore, it has attracted wide attention (6). Adjusting the expression of the key enzyme SphK1, which is associated with the S1P/ceramide balance, may therefore be considered a potential treatment for certain neurological disorders (7). In a previous study, high expression of SphK1 was associated with a reduction in the survival rate of patients with primary glioblastoma multiforme with the strongest invasive capabilities (3).

MicroRNAs (miRNAs/miRs) are non-coding single-stranded small RNA molecules, ~22 nucleotides in length (8,9). Mature miRNAs originate from a precursor transcript, which has a hairpin structure containing ~70 nucleotides,

Correspondence to: Mrs. Min Liu, Department of Operation Center, The Third Xiangya Hospital of Central South University, 138 Tongzi Road, Changsha, Hunan 410013, P.R. China
E-mail: mr864337667@126.com

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by the Dicer enzyme (8). Mature miRNA molecules complementarily bind to the untranslated region of target mRNAs, thus leading to mRNA degradation or translational inhibition post-transcription, so as to regulate the expression of immediate early genes (9). miRNAs exist in a wide variety of species and are highly conserved; miRNAs serve an important role in the regulation of gene expression and have garnered much attention in recent years (8).

It has been reported that miRNAs serve an important role in the central nervous system and its disorders (8). The specific expression of miRNAs in various cell types has been reported in detail, including miR-23, miR-26 and miR-29 in astrocytes, miR-124 and miR-128 in neurons, and the let-7 family in hippocampal neurons (10). Cell specialization is also associated with particular miRNAs, and it has been reported that, in zebrafish, the specific miRNA in neural precursor cells is miR-92b, the specific miRNAs in mature neurons are miR-124, miR-181 and miR-222, the specific miRNA in motor neurons is miR-218a, and the specific miRNA in dendrites is miR-134 (11). Similarly, in the rat hippocampus, neuronal cell bodies and projections contain specific miRNAs: miR-124 and miR-26a, respectively. In addition, in the human frontal cortex, miR-30a is highly expressed in pyramidal cells (12). Therefore, tissue- and cell-specific miRNA expression levels in the human central nervous system may help further the understanding regarding their functions (12). Ma *et al* revealed that miR-125b enhances neuronal apoptosis and Tau phosphorylation in patients with Alzheimer's e disease (13). Therefore, the present study aimed to investigate the expression of miR-125b in patients with AD, and to determine its potential role in AD.

Patients and methods

Patients and ethics. An initial pilot study was performed using AD samples and healthy volunteers from the Department of Gereology, The Third Xiangya Hospital of Central South University (Changsha, China) from April 2014 to October 2014. A total of 24 patients with AD (77-82 years age) were included in the present study. All cerebrospinal fluid (CSF) samples of participants were collected by lumbar puncture in the L3/L4 or L4/L5 interspace at a standardized time point between 8:00 and 9:00 a.m. (14). Healthy volunteers (n=24, 22-26 years age) were designated as not exhibiting neurological disease symptoms. The present study was approved by the Institutional Review Board of The Third Xiangya Hospital of Central South University; written informed consent was obtained from all of the patients.

miRNA expression analysis. Total RNA was harvested from CSF samples using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Total RNA (5 ng) was reverse transcribed into cDNA using a PrimeScript™ II 1st Strand cDNA Synthesis kit (Takara Bio, Inc., Otsu, Japan). RT-quantitative polymerase chain reaction (qPCR) was performed using an ABI 7500 instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) and Terra qPCR Direct SYBR Premix (Takara Bio, Inc.). The primer sequences were as follows: Mouse miR-125b-5p forward, 5'-TCCCTGAGACCC TAACTTGT-3' and reverse, 5'-CTCGCTTCGGCAGCACAC A-3'; mouse U6 forward, 5'-UUCUCCGAACGUGUCACG UTT-3' and reverse, 5'-GTCATTGATGGCAACAATATC CACT-3'; human miR-125b-5p forward, 5'-TCCCGAGACCCT AACTTGTGA-3'; human U6 forward, 5'-CTTCGGCAGCAC ATATACTAAAAT-3' and reverse, 5'-CAGGGGCCATGCTA AATCTTC-3'. qPCR was conducted as follows: 60 sec at 95˚C; 40 cycles at 95˚C for 15 sec, 60˚C for 15 sec, 72˚C for 45 sec and 4˚C for 1 min. Relative gene expression was determined using the $2^{-\Delta\Delta Cq}$ method (15).

Cell culture. Mouse neuroblastoma Neuro2a APPSwe/Δ9 cells were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal calf serum (FCS; Gibco; Thermo Fisher Scientific, Inc.), and were seeded into 6-well plates at a concentration of $1.5\text{-}2x10^5$ cells/well the day prior to transfection at 37˚C in 5% CO_2.

Transfection. miR-125b (5'-GACGCAAACTTGCTGATG TT-3' and 5'-CTGCGTTTGAACGATACAA-3') and negative mimics (5'-CCCCCCCCCCCCCCCC-3' and 5'-CCC CCCCCCCCCCCCCCC-3') were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). In the control group, Neuro2a APPSwe/Δ9 cells group were cultured with DMEM at 37˚C; in the negative control group, negative control Neuro2a APPSwe/Δ9 cells were cultured with 100 ng of negative mimics; and in the miR-125b group, Neuro2a APPSwe/Δ9 cells were cultured with 100 ng of miR-125b. Neuro2a APPSwe/Δ9 cells were seeded into 6-well plates ($1\text{-}2x10^5$ cells/well) were transfected with 100 ng of miR-125b or 100 ng of negative mimics (Sangon Biotech Co., Ltd.) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. A total of 4 h post-transfection, the medium was replaced with fresh DMEM supplemented with 10% FCS.

Cell proliferation and apoptosis assays. Post-transfection with miR-125b or negative mimics for 48 h, cells were seeded into 96-well plates at $1\text{-}2x10^3$ cells/well. Cells were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 5 mg/ml) for 4 h at 37˚C. Subsequently, dimethyl sulfoxide was added to the cells and proliferation was measured using a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) at 492 nm.

Post-transfection with miR-125b or negative mimics for 48 h, cells were seeded into 6-well plates at $1\text{-}2x10^5$ cells/well at 37˚C. Cells were stained with 5 μl propidium iodide (PI) and 5 μl fluorescein isothiocyanate-labeled Annexin V (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China) according to the manufacturer's protocol. The samples were assessed by flow cytometry within 1 h using the BD FACSCanto II system and Image-ProPlus 6.0 software (BD Biosciences, Franklin Lakes, NJ, USA).

ELISA. Post-transfection with miR-125b or negative mimics for 48 h, total protein was extracted from Neuro2a cells using radioimmunoprecipitation assay (RIPA, Beyotime Institute of Biotechnology, Nanjing, China) buffer, and was quantified using a bicinchoninic acid (BCA) assay (Thermo Fisher

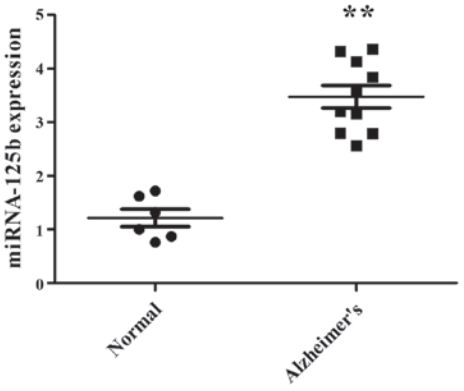

Figure 1. miRNA-125b expression in patients with Alzheimer's disease compared with in normal individuals. **P<0.01 vs. normal group. miRNA-125b, microRNA-125b.

Scientific, Inc.). Subsequently, 10 ng total proteins were incubated with reagents from ELISA kits to detect tumor necrosis factor (TNF)-α (cat. no. PT512; Beyotime Institute of Biotechnology), interleukin (IL)-1β (cat. no. PI301), IL-6 (cat. no. PI326; both Beyotime Institute of Biotechnology), IL-10 (cat. no. H009; Nanjing Jiancheng Biology Engineering Institute, Nanjing, China), superoxide dismutase (SOD; cat. no. S0101), malondialdehyde (MDA; cat. no. S0131; both Beyotime Institute of Biotechnology) and Aβ (cat. no. H229; Nanjing Jiancheng Biology Engineering Institute) peptide production, according to the manufacturer's protocol.

Western blot analysis. Post-transfection with miR-125b or negative mimics for 48 h, total protein was extracted from Neuro2a cells using RIPA buffer and was quantified using a BCA assay (Thermo Fisher Scientific, Inc.). Equal amounts of protein (50 ng) were separated by 8-12% SDS-PAGE and were transferred to nitrocellulose membranes (Bio-Rad Laboratories, Inc., Hercules, CA, USA). After blocking with 5% non-fat milk in Tris-buffered saline containing 0.1% Tween (TBST) for 1 h at 37˚C, membranes were incubated with the following primary antibodies: Amyloid precursor protein (APP; cat. no. sc-9129; 1:500), β-secretase 1 (BACE1; cat. no. sc-10748; 1:500), Tau1 (Tau1; cat. no. sc-5587; 1:500), SphK1 (cat. no. sc-48825; 1:500), p-extracellular signal-regulated kinase (ERK; cat. no. sc-23759-R; 1:1,000), ERK (cat. no. sc-292838; 1:500; all Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and GAPDH (cat. no. AF1186; 1:2,000; Beyotime Institute of Biotechnology) overnight at 4˚C. Membranes were then washed three times with TBST and were incubated with anti-rabbit horseradish peroxidase-conjugated secondary antibody (cat. no. D110058; 1:5,000; Sangon Biotech Co., Ltd.) for 1 h at 37˚C. Membranes were developed using enhanced chemiluminescence solution (Thermo Fisher Scientific, Inc.) and blotting was analyzed by densitometry using Quantity One software 3.0 (Bio-Rad Laboratories, Inc.).

Statistical analysis. Data are presented as the mean ± standard error of the mean, and all experiments were performed in triplicate. Statistical significance was determined using Student's t-test, or one analysis of variance (ANOVA) or two-way ANOVA followed by Tukey post hoc test. SPSS 17.0 software (IBM Corp., Armonk, NY, USA) was used to analyze the data. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-125b expression in patients with AD. The present study used CSF samples from patients with AD to analyze miR-125b expression. In CSF samples from patients with AD, the expression levels of miR-125b were significantly increased compared with in samples from the normal participants (Fig. 1). These results indicated that miR-125b expression is altered in samples from patients with AD and may be associated with AD.

Overexpression of miR-125b inhibits cell proliferation and induces apoptosis. Mouse neuroblastoma Neuro2a APPSwe/Δ9 cells were used in the present study; cells were transfected with miR-125b mimics and miR-125b overexpression was confirmed by qPCR (Fig. 2A). Post-transfection with miR-125b mimics, cell viability of Neuro2a APPSwe/Δ9 cells was significantly inhibited compared with the negative control group (Fig. 2B). In addition, miR-125b over-expression significantly enhanced the apoptotic rate of Neuro2a APPSwe/Δ9 cells compared with the negative control group (Fig. 2C).

Overexpression of miR-125b enhances the expression of inflammatory factors. The present study aimed to determine whether overexpression of miR-125b affected the expression of inflammatory factors. TNF-α, IL-1β, IL-6 and IL-10 activity levels were detected in response to miR-125b overexpression. As shown in Fig. 3A-C, TNF-α, IL-1β and IL-6 activities were significantly increased in the AD *in vitro* model transfected with miR-125b compared with the negative control group. Conversely, IL-10 activity levels were significantly reduced in the AD *in vitro* model, in which miR-125b was overexpressed, compared with in the negative control group (Fig. 3D).

Overexpression of miR-125b enhances oxidative stress. The present study employed miR-125b mimics to verify oxidative stress in AD. SOD and MDA levels were detected in cells post-transfection with miR-125b mimics. As presented in Fig. 4A, SOD levels were significantly inhibited in the *in vitro* AD model group, in which miR-125b was overexpressed, compared with in the negative control group. As presented in Fig. 4B, MDA levels were significantly enhanced in the *in vitro* AD model group, in which miR-125b was overexpressed, compared with in the negative control group.

Overexpression of miR-125b promotes APP, BACE1 and Tau1 protein levels. In order to determine whether overexpression of miR-125b affects APP protein expression, the protein expression levels of APP were detected by western blotting. As shown in Fig. 5, compared with in the negative control group, APP protein expression was significantly increased in Neuro2a APPSwe/Δ9 cells post-transfection with miR-125b mimics.

Overexpression of miR-125b suppresses SphK1 protein expression. The present study detected SphK1 protein

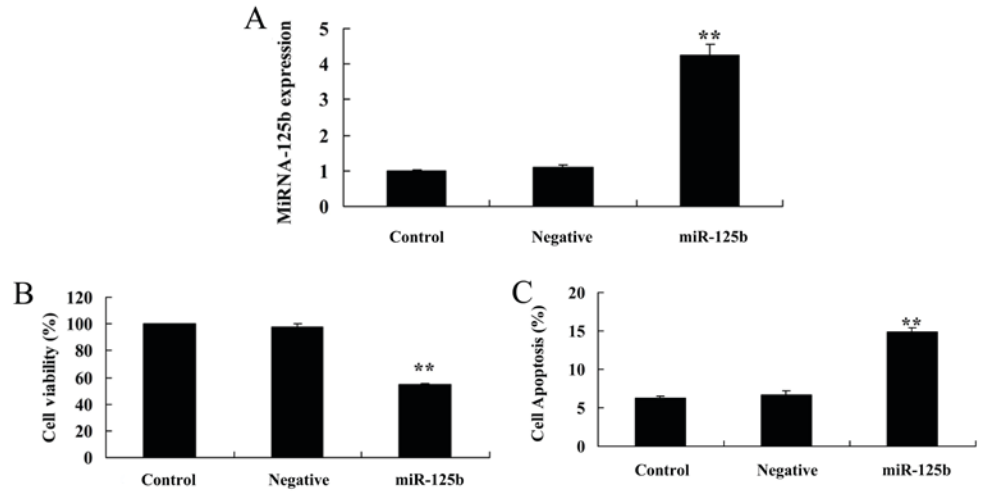

Figure 2. Overexpression of miR-125b inhibits cell proliferation and induces apoptosis. (A) miR-125b overexpression was confirmed in mimics-transfected Neuro2a APPSwe/Δ9 cells by quantitative polymerase chain reaction. Overexpression of miR-125b (B) inhibited cell proliferation and (C) induced apoptosis. **P<0.01 vs. negative control group. Control, control group; miR-125b, microRNA-125b group; negative, negative control group.

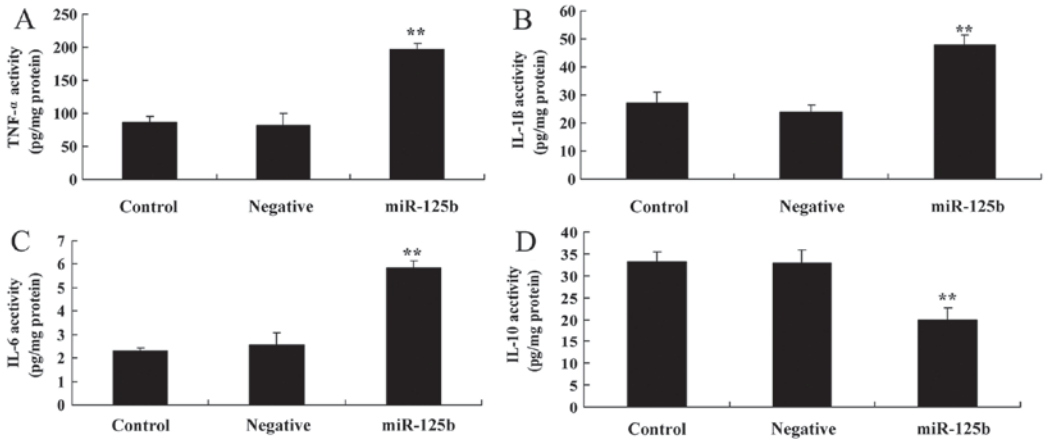

Figure 3. Overexpression of miR-125b enhances the expression of inflammatory factors. Overexpression of miR-125b enhanced (A) TNF-α, (B) IL-1β and (C) IL-6 activity levels, and (D) decreased IL-10 activity levels. **P<0.01 vs. negative control group. IL, interleukin; control, control group; miR-125b, microRNA-125b group; negative, negative control group; TNF-α, tumor necrosis factor-α.

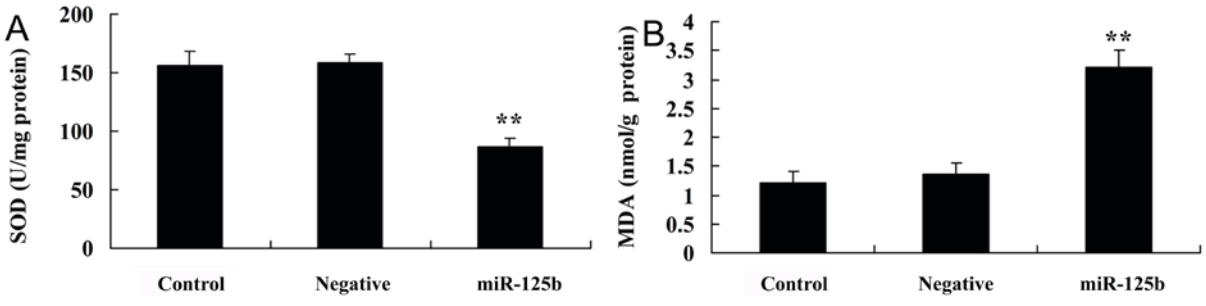

Figure 4. Overexpression of miR-125b enhances oxidative stress. (A) Overexpression of miR-125b suppressed SOD activity. (B) Overexpression of miR-125b enhanced MDA activity. **P<0.01 vs. negative control group. MDA, malondialdehyde; control, control group; miR-125b, microRNA-125b group; negative, negative control group; SOD, superoxide dimutase.

expression in an AD *in vitro* model, in which miR-125b was overexpressed. As shown in Fig. 6, overexpression of miR-125b significantly inhibited SphK1 protein expression in Neuro2a APPSwe/Δ9 cells compared with the negative control group.

Overexpression of miR-125b promotes Aβ peptide production. To investigate the underlying mechanism of miR-125b in AD, an ELISA analysis was used to detect Aβ peptide production in the *in vitro* AD model. Overexpression of miR-125b significantly increased Aβ peptide production in Neuro2a

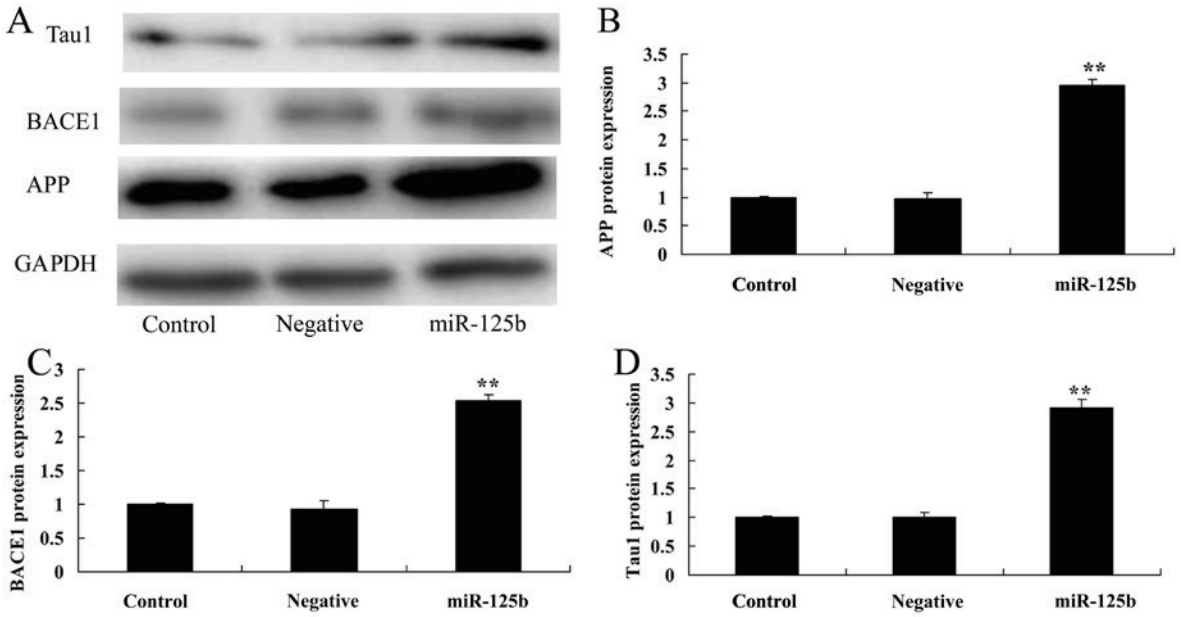

Figure 5. Overexpression of miR-125b promotes APP, BACE1 and Tau1 protein expression. Overexpression of miR-125b promoted APP, BACE1 and Tau1 protein expression, as determined by (A) western blotting and (B-D) semi-quantitative analysis. **P<0.01 vs. negative control group. APP, amyloid precursor protein; control, control group; miR-125b, microRNA-125b group; negative, negative control group.

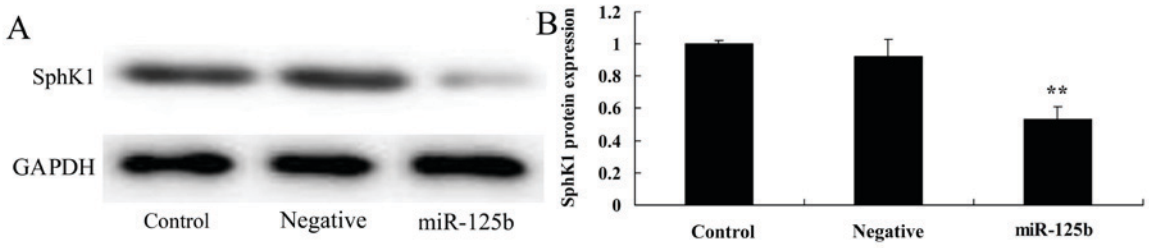

Figure 6. Overexpression of miR-125b suppresses SphK1 protein expression. Overexpression of miR-125b inhibited SphK1 protein expression, as determined by (A) western blotting and (B) semi-quantitative analysis. **P<0.01 vs. negative control group. Control, control group; miR-125b, microRNA-125b group; negative, negative control group; SphK1, sphingosine kinase 1.

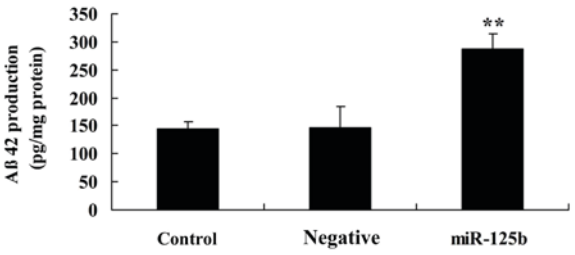

Figure 7. Overexpression of miR-125b promotes Aβ peptide production. **P<0.01 vs. negative control group. Aβ, β-amyloid; control, control group; miR-125b, microRNA-125b group; negative, negative control group.

APPSwe/Δ9 cells compared with in the negative control group (Fig. 7).

Overexpression of miR-125b promotes p-ERK protein expression. To explore the effects of miR-125b on ERK protein expression, western blotting was performed. The protein expression levels of p-ERK were significantly promoted by overexpression of miR-125b in Neuro2a APPSwe/Δ9 cells compared with in the negative control group (Fig. 8).

Discussion

AD is a degenerative disease of the central nervous system; >20 million people suffer from this disease globally, among which 15% are >65 years old and 50% are >85 years old (16). As well as progressive memory loss and cognitive dysfunction, the main clinical symptoms associated with AD include brain atrophy, and neuronal and synaptic reduction, and less common symptoms include the presence of neuritic plaques and NFTs (17). At present, the exact etiology and pathogenesis of AD have not been fully elucidated; therefore, an effective therapeutic strategy is lacking. Further insights into the mechanisms underlying neuronal degeneration and death are required, in order to identify drugs that may delay or block these processes (17). There are numerous hypotheses regarding the pathogenesis of AD, including Aβ aggregation, generation of phosphorylated tau protein, genetic mutations, oxidative stress, genetic factors and alterations in lipid metabolism (18).

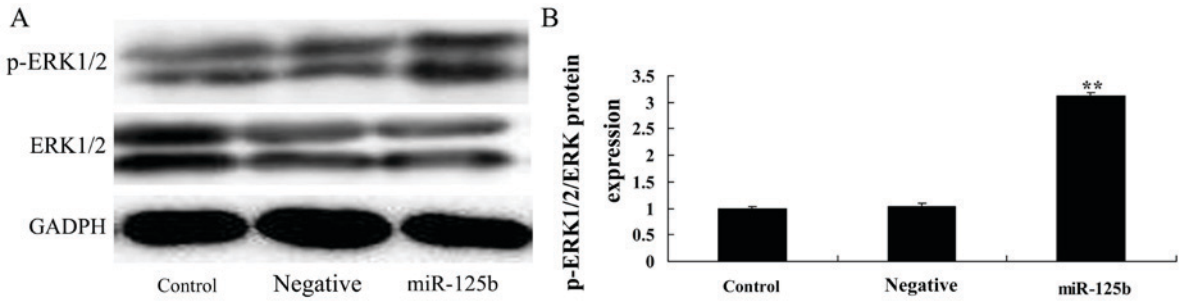

Figure 8. Overexpression of miR-125b promotes p-ERK protein expression. Overexpression of miR-125b promoted p-ERK protein expression, as determined by (A) western blotting and (B) semi-quantitative analysis. **P<0.01 vs. negative control group. ERK, extracellular signal-regulated kinase; p-ERK, phosphorylated-ERK; control, control group; miR-125b, microRNA-125b group; negative, negative control group.

The results of the present study demonstrated that the expression of miR-125b was markedly increased in patients with AD compared with in the normal group. In addition, overexpression of miR-125b significantly inhibited cell proliferation and induced apoptosis, enhanced inflammatory factors and MDA levels, and suppressed SOD levels in an *in vitro* model of AD. miR-9, miR-34a, miR-125b, miR-146a and miR-155 have been suggested to be associated with the neuropathology of common, age-related inflammatory neurodegeneration of the human central nervous system (19).

Aβ is the major component of senile plaques in the AD brain, which is generally composed of 39-43 amino acid residues, and overexpression of Aβ42 and Aβ40 has been demonstrated to induce AD (20). The secondary structure of Aβ is made up of β-sheets, hence why it is known as Aβ (21). It has previously been reported that Aβ is derived from a larger precursor protein, which is known as APP (21). The results of the present study suggested that overexpression of miR-125b significantly increased Aβ peptide production in Neuro2a APPSwe/Δ9 cells.

The APP gene is located in the long arm of human chromosome 21, which is widely present in many cell membranes of the body; in particular, APP is abundant in human neurons and astrocytes, and is mainly located in the synapse and neuronal cell membrane (22). However, the function of APP is currently unclear. A previous study demonstrated that cultured hippocampal neurons with a lack of APP exhibited enhanced neuronal synaptic transmission (23). The present study demonstrated that the protein expression levels of APP were significantly increased in Neuro2a APPSwe/Δ9 cells in response to miR-125b overexpression.

The core component of senile plaques in patients with AD is Aβ, which is produced by the hydrolysis of APP. Although various cells and cell lines can synthesize APP, neurons are the main source of APP, and only brain cells are able to process APP (24). APP is mainly hydrolyzed by α-secretase, which hydrolyzes APP within the Aβ domain and completely blocks Aβ generation, resulting in the generation of APPs and C83, which is further degraded into P342 or P340 under the role of γ-secretase; this pathway is the predominant pathway of APP metabolism, and the release of extracellular α-soluble APP has a neuroprotective effect (25). The other metabolic pathway is known as the Aβ-generated pathway; APP is initially hydrolyzed by BACE to generate βAPPs and C99, which results in the generation of Aβ42 or Aβ40 (24). In the present study, the results support the hypothesis that overexpression of miR-125b significantly increases Aβ peptide production in AD brains.

Aβ formation and deposition may induce toxic effects and mitochondrial injury, leading to an overload of Ca^{2+}, which can activate Ca^{2+}/calmodulin-dependent protein kinase II, further leading to Tau hyperphosphorylation and inhibition of the microtubule assembly-promoting activity of Tau (26). When microtubules cannot be properly assembled, NFTs are generated, which eventually leads to neuronal dysfunction and even death (27). AD-associated dementia caused by Tau gene mutations is not associated with amyloid deposition, even if severe NTFs appear in the brain, thus suggesting that NFTs are generated following the metabolic alterations associated with Aβ. Therefore, the Aβ cascade theory has been hypothesized, which suggests that abnormal or oversecretion of Aβ can induce other pathological alterations associated with AD (28). Collectively, these results suggested that miR-125b may regulate BACE1 and Tau1 protein expression, and affect Aβ levels, resulting in AD-associated alterations.

Taul proteins are microtubule-associated proteins, the main functions of which are associated with microtubule assembly, stable microtubule formation, the establishment of cellular polarity and axonal transport maintenance in neuronal cells (29). When Taul proteins are excessively phosphorylated and accumulate in cells, they lose their functions, thus resulting in damage to microtubules (30). In AD, excessively phosphorylated Taul proteins form paired helical filaments, thus reducing their affinity to microtubules (31). A previous study indicated that neuronal death and cognitive dysfunction are associated with excessively phosphorylated Taul proteins. The results of the present study demonstrated that miR-125b overexpression significantly promoted Tau1 protein expression. Collectively, these results suggested that miR-125b may regulate BACE1 and Tau1 protein expression, and affect Aβ levels, resulting in AD-associated alterations.

Upregulation of SphK1 can significantly improve learning and memory, and reduce the deposition of amyloid proteins in the brains of APP/presenilin 1 (PS1) transgenic mice (32), thus indicating that high SphK1 expression may serve a protective role in APP/PS1 transgenic mice (33). In addition, alterations in the expression levels and the regulation of SphK1 may effectively improve pathological alterations associated with AD, and may be used to generate effective treatments for patients with AD (34). In the present study, the results suggested that overexpression of

miR-125b significantly suppressed SphK1 protein expression and enhanced the levels of p-ERK protein *in vitro*. However, the present study did not determine the effects of SphK1 inhibition on AD. In future studies we aim to investigate the effects of SphK1 inhibitors and small interfering RNA-SphK1.

In conclusion, the present study demonstrated that overexpression of miR-125b significantly inhibited cell proliferation and induced apoptosis, enhanced the expression of inflammatory factors and oxidative stress, promoted APP and BACE1 expression, Aβ peptide production, and suppressed SphK1 protein expression *in vitro*. Based on these results, it may be hypothesized that miR-125b is associated with the pathogenesis of AD. However, further studies are required to clarify the roles of miR-125b and SphK1 in AD.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

ML designed the experiment; YJ and QT performed the experiments; ML and YJ analyzed the data; ML wrote the manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of The Third Xiangya Hospital of Central South University. Written informed consent was obtained from all of the patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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