

# Salidroside protects PC12 cells from A $\beta$ <sub>1-40</sub>-induced cytotoxicity by regulating the nicotinamide phosphoribosyltransferase signaling pathway

XUJIAO HUANG, SANLI XING, CHUAN CHEN, ZHIHUA YU and JIULIN CHEN

Geriatrics Laboratory, Shanghai Geriatric Institute of Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200031, P.R. China

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**Abstract.** Alzheimer's disease (AD) is the most common type of senile dementia, which often develops in elderly or presenile individuals. As one of the pathological features of AD, amyloid  $\beta$ -protein (A $\beta$ ) causes energy dysmetabolism, thereby inducing cellular damage and apoptosis. Salidroside is the main active component of the traditional Chinese medicine *Rhodiola*. Previous studies have demonstrated that salidroside exerts a regulatory role in energy metabolism. However, the role and the mechanism of action of salidroside in AD remain unclear. Therefore, the present study used A $\beta$ <sub>1-40</sub> to induce damage in PC12 cells, thereby establishing a cell model of AD. In addition, salidroside treatment was performed to investigate the protective effect of salidroside and the underlying mechanisms. A $\beta$ <sub>1-40</sub>-induced neuronal toxicity reduced cell viability and caused cellular damage. As a result, the expression level of nicotinamide phosphoribosyltransferase (NAMPT) decreased, the synthesis of nicotinamide adenine dinucleotide (NAD<sup>+</sup>; an energy metabolism-associated coenzyme) became insufficient, and the NAD<sup>+</sup>/nicotinamide adenine dinucleotide hydride ratio was reduced. Administration of salidroside alleviated A $\beta$ -induced cell damage and increased the expression level of the key protein NAMPT and the synthesis of NAD<sup>+</sup>. The results of the present study demonstrate that salidroside exerts a protective effect on A $\beta$ <sub>1-40</sub>-damaged PC12 cells. The underlying mechanism may be associated with the regulation of energy metabolism that relies predominantly on the NAMPT signaling pathway.

## Introduction

Alzheimer's disease (AD) is the most common form of dementia that affected an estimated 44 million individuals in 2015. This figure is expected to increase to 135 million by the year 2050 throughout the world (1). The main clinical symptoms of AD include memory impairment, decreased self-care ability, aphasia and visuospatial dysgnosia (2). Neuropathological studies have confirmed that extracellular senile plaque deposition, intracellular accumulation of neurofibrillary tangles and neuronal degeneration loss are the main pathological hallmarks of AD (3,4). Deposition of amyloid  $\beta$ -protein (A $\beta$ ) in the brain leads to the formation of senile plaques, which represent a primary pathological characteristic of AD (5). A $\beta$  refers to a class of amyloid precursor protein (APP)-derived polypeptides that are formed under the action of  $\beta$ - and  $\gamma$ -secretase. The common subtypes of A $\beta$  are A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub>, and each exert neurotoxic effects and induce apoptosis. Therefore, reducing A $\beta$ -induced neuron injury and intervening in AD progression are critical for the treatment of AD.

Studies have shown that energy dysmetabolism contributes significantly to the pathogenesis of a variety of ageing-associated diseases and degenerative diseases of the nervous system, including AD (6). Homeostatic imbalance of energy metabolism develops gradually in the brain with age, which promotes A $\beta$  production. A $\beta$  further induces energy dysmetabolism in the brain, resulting in impaired neuronal synaptic function (7,8). Nicotinamide adenine dinucleotide (NAD<sup>+</sup>) is an important coenzyme in the *in vivo* redox reaction. It participates in energy synthesis through glycolysis, the tricarboxylic acid cycle and mitochondrial oxidative phosphorylation (9). NAD<sup>+</sup> may be synthesized *de novo*, although the majority of NAD is synthesized via the salvage pathway from the conversion of nicotinamide into nicotinamide mononucleotide, which is later converted to NAD<sup>+</sup> (10). The NAD<sup>+</sup>/nicotinamide adenine dinucleotide hydride (NADH) ratio is a fundamental indicator of the cellular redox status (11). Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in the NAD<sup>+</sup> salvage pathway, and it has been demonstrated that NAMPT participates in energy metabolism and maintains the homeostasis of energy metabolism in the brain by regulating NAD<sup>+</sup> synthesis; thus, increased expression levels of NAMPT have

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*Correspondence to:* Dr Sanli Xing or Dr Chuan Chen, Geriatrics Laboratory, Shanghai Geriatric Institute of Chinese Medicine, Shanghai University of Traditional Chinese Medicine, 365C South Xiangyang Road, Shanghai 200031, P.R. China  
E-mail: xingsanli@163.com  
E-mail: article\_chenchuan@163.com

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been shown to positively regulate NAD<sup>+</sup> levels (12). Knockout of the NAMPT gene enhances the formation of A $\beta$  plaques in the brain (13). Therefore, recent studies have focused on regulation of NAMPT expression for the purpose of protecting against AD and other neurodegenerative diseases (13-15).

*Rhodiola rosea* is a perennial herb that belongs to the family Crassulaceae and genus *Rhodiola*. *Rhodiola* is a traditional herbal medicine commonly used across Asia and grows at altitudes of 1,800-2,500 m in cold, pollution-free zones. *Rhodiola* stimulates the nervous system, improves work efficiency, alleviates fatigue and prevents mountain sickness. Salidroside is the primary active ingredient of *Rhodiola*. Various studies have shown that salidroside inhibits the inflammatory response (16), reduces oxidative stress-induced damage (17) and exerts an anticancer effect (18). Certain studies demonstrated that salidroside has a neuroprotective effect, for example, salidroside inhibited A $\beta$ -induced neurotoxicity by regulating the phosphatidylinositol 3-kinase/Akt signaling pathway in primary cultured neurons (19). In a rat model of AD, salidroside improved memory impairment of rats (20), and salidroside reduced mitochondrial swelling and attenuated the injury in the central nervous system (21). However, whether the neuroprotective effects of salidroside involve regulation of the NAMPT/NAD<sup>+</sup> signaling pathway in PC12 cells remains unknown.

To gain further insights into the neuroprotective functions of salidroside, a PC12 model was used in the present study and the underlying mechanisms were investigated. Whether the neuroprotective effects of salidroside increasing NAD levels against A $\beta$ <sub>1-40</sub>-induced neurotoxicity in PC12 cells were elucidated in the current study. Furthermore, the possible underlying mechanisms were examined by investigating NAMPT expression levels.

## Materials and methods

**Preparation of A $\beta$ <sub>1-40</sub>.** The lyophilized powder of A $\beta$ <sub>1-40</sub> (cat no. A1075; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was diluted with sterile distilled water to a concentration of 200  $\mu$ mol/l. Subsequently, A $\beta$ <sub>1-40</sub> was incubated at 37°C for 7 days and stored at 4°C for further assays.

**Cell culture and treatment.** Highly differentiated PC12 rat adrenal pheochromocytoma cells (Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China) were maintained in Dulbecco's modified Eagle's medium (DMEM; cat no. 12100046) containing Gibco 10% fetal bovine serum (cat no. 10099-141) (both from Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 1% penicillin-streptomycin. The cells were maintained in a 37°C incubator in an atmosphere of 5% CO<sub>2</sub> and grew adherently. The culture medium was replaced daily. Upon reaching 80-90% confluence, the cells were trypsinized for 1-2 min. Once the cells became round, 2 ml of culture medium was added to the cells. The cells were then centrifuged, 1,500 x g for 5 min at room temperature, resuspended, and split into culture dishes. The cells were divided into the following main groups: Normal control group, the A $\beta$ <sub>1-40</sub> group, the A $\beta$ <sub>1-40</sub> + salidroside (cat no. B20504; Shanghai Yuanye Biological Technology Co., Ltd., Shanghai, China) group, and the salidroside group. The normal control

group was cultured conventionally in DMEM and did not receive any treatment. The A $\beta$ <sub>1-40</sub> group was subjected to 24 h of treatment with 5  $\mu$ mol/l A $\beta$ <sub>1-40</sub>. The A $\beta$ <sub>1-40</sub> + salidroside group was subjected to simultaneous A $\beta$ <sub>1-40</sub> (5  $\mu$ mol/l) and salidroside (100  $\mu$ mol/l) treatments for 24 h. The salidroside group was treated with 100  $\mu$ mol/l salidroside for 24 h.

**Cell viability assay.** PC12 cells were seeded into 96-well plates and cultured at 37°C in DMEM for 24 h. Once the cells attached stably to the culture surface, various concentrations of A $\beta$ <sub>1-40</sub> (1, 5 or 10  $\mu$ mol/l) or salidroside (12.5, 25, 50, 100 or 200  $\mu$ mol/l) were added to the cells. Following a 24-h treatment, each well of cells was overlaid with 10  $\mu$ l MTT solution (5 mg/ml; cat no. C0009) obtained from Beyotime Institute of Biotechnology (Haimen, China) and incubated at 37°C for 4 h. The supernatant was discarded, and 150  $\mu$ l dimethyl sulphoxide was added to each well of cells to fully dissolve the crystals. Subsequently, absorbance was measured at a wavelength of 490 nm using a PowerWave XS microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

**Lactate dehydrogenase (LDH) activity assay.** The cells were subjected to different treatments (medium only, 5  $\mu$ mol/l A $\beta$ <sub>1-40</sub>, 5  $\mu$ mol/l A $\beta$ <sub>1-40</sub> + 100  $\mu$ mol/l salidroside or 100  $\mu$ mol/l salidroside). Subsequently, the activity of released LDH was examined according to the instructions provided with the LDH Activity Assay kit (cat. no. G1780; Promega Corporation, Madison, WI, USA). Absorbance was measured at a wavelength of 490 nm. The LDH release was expressed as a percentage relative to the positive control group.

**NAD<sup>+</sup>/NADH analysis.** An NAD<sup>+</sup>/NADH quantification kit was used to determine NAD<sup>+</sup> and NADH levels (cat no. k337-100; BioVision, Inc., Milpitas, CA, USA) according to the manufacturer's instructions. The cells were washed with pre-cooled phosphate-buffered saline (PBS), homogenized in 400  $\mu$ l NAD<sup>+</sup>/NADH extraction buffer, and then centrifuged for 5 min at 18,000 x g and 4°C. The resulting supernatants were transferred to fresh Eppendorf (EP) tubes and labelled as NADt samples. Subsequently, 200  $\mu$ l NADt sample was transferred to fresh EP tubes and heated at 60°C for 30 min (all NAD<sup>+</sup> decomposed, leaving only NADH to be analyzed). After cooling on ice, the samples were quickly centrifuged 12,000 x g for 30 sec at 4°C, and the resulting pellets were discarded. The supernatants were collected and labelled as NADH samples for further assays. Standards (0, 2, 4, 6, 8 and 10  $\mu$ l) and 40  $\mu$ l samples were loaded into the wells of a microtiter plate, and appropriate volumes of NAD<sup>+</sup>/NADH Extraction Buffer were added to bring the volume to 50  $\mu$ l. Subsequently, 100  $\mu$ l enzyme reaction mix and 10  $\mu$ l NADH developer were added to each well. The mixtures were allowed to react at room temperature (RT) for 1-4 h, and the absorbance was measured using a microplate reader (wavelength, 450 nm).

**Immunofluorescence assay.** Following various treatments and interventions, all groups of cells were washed with PBS for 5 min. The cells were then fixed with 4% paraformaldehyde for 15 min, blocked with a blocking solution containing 10% calf serum for 45 min at RT, incubated with anti-NAMPT antibody (dilution, 1:200; cat no. ab45890; Abcam, Cambridge,

MA, USA) at 37°C for 1 h, and placed in a 4°C refrigerator overnight. After washing with PBS, the cells were incubated with fluorescein isothiocyanate-labelled goat anti-rabbit IgG (dilution, 1:500; cat no. A0562; Beyotime Institute of Biotechnology) for 1 h in a 37°C incubator. After 3 rinses with PBS, the cells were covered with DAPI staining solution (cat no. C1005; Beyotime Institute of Biotechnology) and incubated at RT for 5 min. The cells were rinsed 3 more times with PBS (5 min each time). Subsequently, the cells were mounted onto glass slides in PBS and glycerol (1:1). The fluorescence signal was examined using an Olympus BX51 biological microscope at a magnification of x200.

**Western blot analysis.** At the end of the treatment period, all groups of cells were collected. Following centrifugation 1,500 x g for 5 min at RT, the cells were lysed in lysis buffer. The resulting supernatants were extracted and the protein concentrations in the supernatants were determined using the bicinchoninic acid (BCA) method. Subsequently, the supernatants were mixed thoroughly with 5X sodium dodecyl sulphate (SDS) loading buffer, boiled at 100°C for 5 min, cooled on ice and then stored at -20°C. Immediately before the experiments, the protein samples were thawed at RT. The protein samples (50 µg each) were heated at 95°C for 5 min, then separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 2% bovine serum albumin for 1 h and then incubated with the primary antibody (anti-NAMPT antibody, dilution, 1:250; cat no. ab45890; Abcam) at 4°C overnight. Subsequently, the membranes were washed with PBS for 45 min, incubated with horseradish peroxidase-labelled secondary antibody (dilution, 1:2,000; cat no. SA00001-2; ProteinTech Group, Inc., Chicago IL, USA) at 37°C for 1 h, and washed again in PBS for 45 min. Protein bands were examined using an ImageQuant LAS 4000 mini chemiluminescence detector (GE Healthcare Life Sciences, Little Chalfont, UK). GAPDH (dilution, 1:1,000, cat no. 5174S; Cell Signaling Technology, Inc., Danvers, MA, USA) served as a loading control.

**Statistical analysis.** The results were statistically analyzed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). All data are expressed as the mean ± standard error. One-way analysis of variance was used to assess multiple groups of data and Student's t-test was used to compare between two groups and P<0.05 was considered to indicate a statistically significant difference.

## Results

**Impact of Aβ<sub>1-40</sub> treatment on the viability of PC12 cells and the protective effect of salidroside.** PC12 cells were treated with various concentrations of Aβ<sub>1-40</sub> (1, 5 or 10 µmol/l) for 24 h, which enabled the analysis of changes in PC12 cell viability under the action of different concentrations of Aβ<sub>1-40</sub>. The results demonstrated that cell viability declined as the Aβ<sub>1-40</sub> concentration increased. The viability of PC12 cells decreased by ~15% after treatment with 5 µmol/l Aβ<sub>1-40</sub>. Therefore, a concentration of 5 µmol/l Aβ<sub>1-40</sub> was selected to establish the cell model of AD (Fig. 1A).

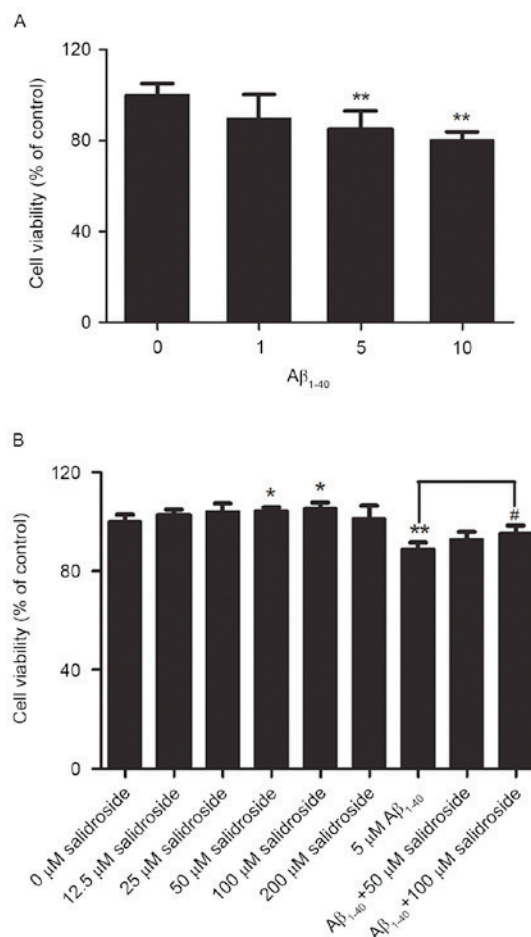


Figure 1. Impact of Aβ<sub>1-40</sub> treatment on the viability of PC12 cells and the effect of salidroside. (A) Effect of the cytotoxicity of Aβ<sub>1-40</sub> on PC12 cell viability. PC12 cells were treated with various concentrations of Aβ<sub>1-40</sub> (1, 5 and 10 µmol/l) for 24 h. (B) Effects of salidroside treatment on the viability of PC12 cells. PC12 cells were treated with various concentrations of salidroside (12.5, 25, 50, 100 and 200 µmol/l) for 24 h, and with different concentrations of salidroside (0, 50, and 100 µmol/l) and exposed to Aβ simultaneously. All data are expressed as the mean ± standard error from four independent experiments. \*P<0.05 and \*\*P<0.01 vs. control group; #P<0.05 vs. Aβ<sub>1-40</sub> group. Aβ, amyloid β-protein.

Before investigation of the protective effect of salidroside, the present study first examined whether salidroside would exert a neurotoxic effect on normal PC12 cells at certain concentrations. PC12 cells were treated with various concentrations of salidroside (12.5, 25, 50, 100 or 200 µmol/l). Compared with the normal control group, 24-h incubation with 12.5, 25 or 200 µmol/l salidroside exerted no significant effect on normal PC12 cells, whereas incubation with 50 and 100 µmol/l salidroside improved cell viability (P<0.05). Therefore, the two concentrations of salidroside (50 and 100 µmol/l) were selected for further investigation of the effect of salidroside on the viability of PC12 cells that were damaged by Aβ<sub>1-40</sub> exposure. The results indicated that cell viability was significantly decreased in the Aβ<sub>1-40</sub> group compared with the normal control group (P<0.01). Compared with the Aβ<sub>1-40</sub> group, treatment with 50 µmol/l salidroside failed to significantly improve the viability of Aβ<sub>1-40</sub>-damaged cells, whereas treatment with 100 µmol/l salidroside improved the viability of cells damaged by Aβ<sub>1-40</sub> (P<0.05). The results demonstrated

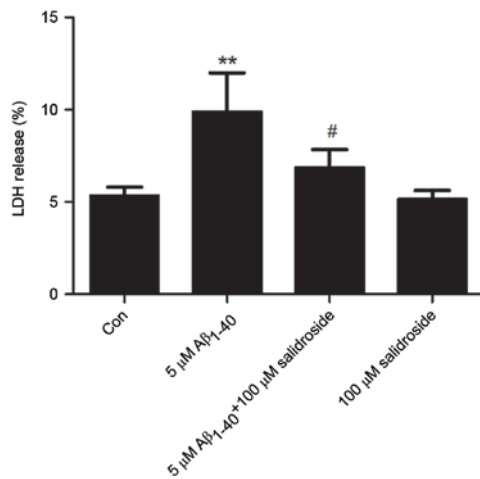


Figure 2. Effect of salidroside treatment on the LDH level of A $\beta_{1-40}$ -treated cells. PC12 cells were subjected to different treatments (medium only, 5  $\mu$ M A $\beta_{1-40}$ , 5  $\mu$ M A $\beta_{1-40}$  + 100  $\mu$ M salidroside, and 100  $\mu$ M salidroside) for 24 h. Subsequently, the cells were examined using an LDH Activity Assay kit. All data are presented as the mean  $\pm$  standard error from four independent experiments (n=4). \*\*P<0.01 vs. control group. #P<0.05 vs. A $\beta_{1-40}$  group. LDH, lactate dehydrogenase; A $\beta$ , amyloid  $\beta$ -protein; Con, control.

that 100  $\mu$ M salidroside exerted an effect on cell viability and alleviated the A $\beta$ -induced PC12 cell injury. Therefore, this

salidroside concentration (100  $\mu$ M) was selected for further experiments (Fig. 1B).

*Salidroside reduces the LDH level of A $\beta_{1-40}$ -treated cells.* The LDH level of a culture supernatant indirectly reflects the effects of A $\beta_{1-40}$  and salidroside on cells. As shown in Fig. 2, the A $\beta_{1-40}$  group displayed a significantly increased LDH level compared with that of the normal control group (P<0.01). By contrast, the LDH level was markedly reduced in the A $\beta_{1-40}$  + salidroside group compared with the A $\beta_{1-40}$  group (P<0.05). The results demonstrated that salidroside reduced the A $\beta_{1-40}$ -induced cellular damage.

*Salidroside increases the NAD<sup>+</sup> level and NAD<sup>+</sup>/NADH ratio of A $\beta_{1-40}$ -treated cells.* NAD<sup>+</sup> and NADH are coenzymes that are necessary for cellular energy metabolism. Under normal physiological conditions, the NAD<sup>+</sup>/NADH ratio reflects the cellular redox status and is in a constant state of dynamic equilibrium. Disorders of cellular energy metabolism are characterized by changes in NAD<sup>+</sup> and NADH levels, and an imbalance in the NAD<sup>+</sup>/NADH ratio (22). In the present study, the A $\beta_{1-40}$  group exhibited a decreased level of NAD<sup>+</sup> compared with that of the normal control group (P<0.01; Fig. 3A). However, the NADH level did not change significantly in the A $\beta_{1-40}$  group compared with the normal control group (Fig. 3B). As a result, the NAD<sup>+</sup>/NADH ratio was reduced in the A $\beta_{1-40}$

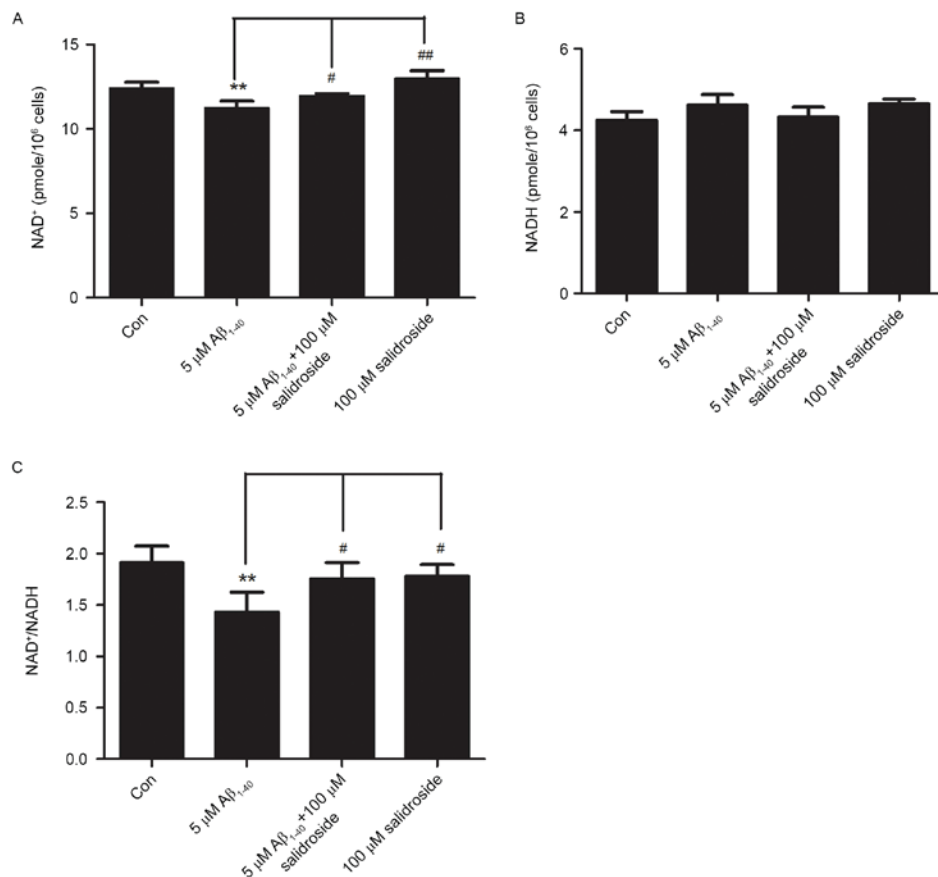


Figure 3. Effect of salidroside treatment on the NAD<sup>+</sup> level and NAD<sup>+</sup>/NADH ratio of A $\beta_{1-40}$ -treated PC12 cells. (A) Effect of salidroside treatment on the NAD<sup>+</sup> level in the PC12 cells damaged by A $\beta_{1-40}$  exposure. (B) Effect of salidroside treatment on the NADH level in the PC12 cells. (C) Effect of salidroside treatment on the NAD<sup>+</sup>/NADH ratio in A $\beta_{1-40}$ -induced damaged PC12 cells. All data are expressed as the mean  $\pm$  standard error from four independent experiments (n=4). \*\*P<0.01 vs. control group. #P<0.05 and ##P<0.01 vs. A $\beta_{1-40}$  group. NAD<sup>+</sup>, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide hydride; A $\beta$ , amyloid  $\beta$ -protein; Con, control.



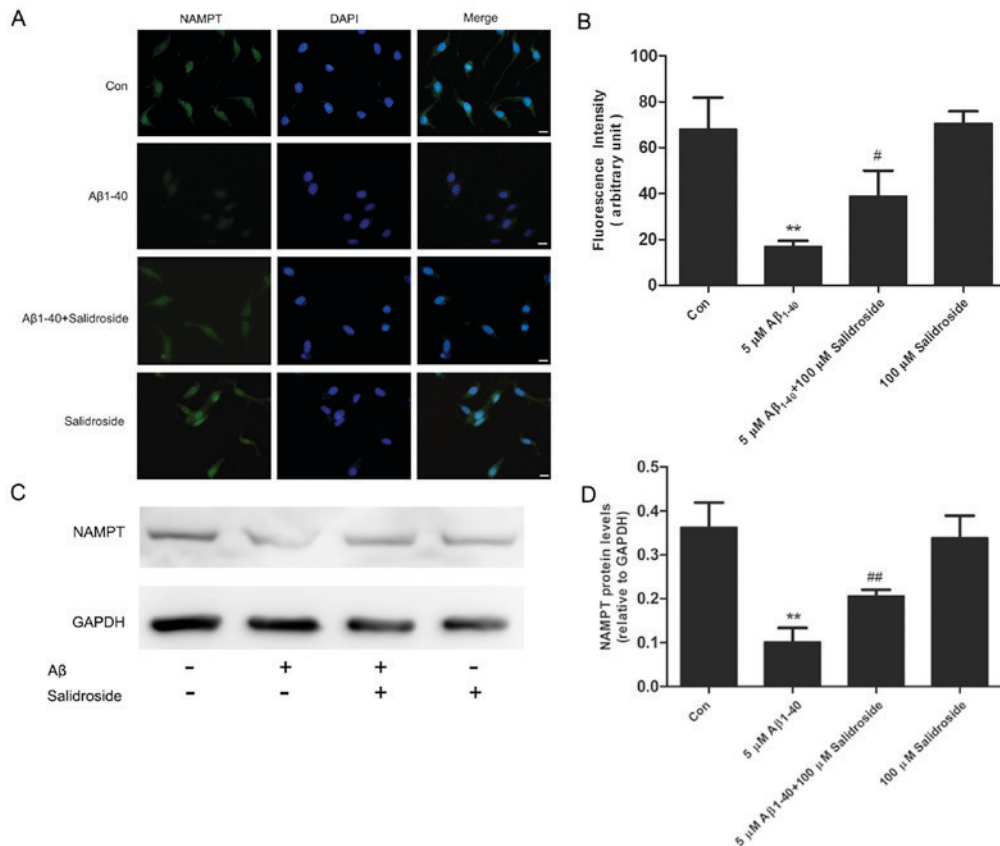


Figure 4. Salidroside upregulates the expression level of NAMPT. (A) NAMPT were detected by immunofluorescence staining and a representative photo-micrograph is presented (scale bar, 10  $\mu$ m). (B) The fluorescence intensity of the NAMPT protein. All data are expressed as the mean  $\pm$  standard error from three independent experiments. \*\* $P$ <0.01 vs. control group. # $P$ <0.05 vs. A $\beta$ <sub>1-40</sub> group. (C) Western blot analyses of PC12 lysates from the control (without any treatment), A $\beta$ <sub>1-40</sub> (with 5  $\mu$ mol/l A $\beta$ <sub>1-40</sub> treatment), A $\beta$ <sub>1-40</sub> + salidroside (5  $\mu$ mol/l A $\beta$ <sub>1-40</sub> and 100  $\mu$ mol/l salidroside simultaneously) and salidroside (100  $\mu$ mol/l salidroside only) groups. (D) Quantitation of NAMPT protein expression levels. Data are presented as the mean  $\pm$  standard error of three independent experiments. \*\* $P$ <0.01 vs. control group. ## $P$ <0.01 vs. A $\beta$ <sub>1-40</sub> group. NAMPT, nicotinamide phosphoribosyltransferase; A $\beta$ , amyloid  $\beta$ -protein; Con, control.

group ( $P$ <0.01; Fig. 3C). No significant difference was identified in the NADH level between the A $\beta$ <sub>1-40</sub> + salidroside group and the A $\beta$ <sub>1-40</sub> group (Fig. 3B). By contrast, the A $\beta$ <sub>1-40</sub> + salidroside group demonstrated a significant increase in the NAD<sup>+</sup> level ( $P$ <0.05; Fig. 3A) and the NAD<sup>+</sup>/NADH ratio ( $P$ <0.05; Fig. 3C).

**Salidroside upregulates the expression level of NAMPT.** NAMPT is the rate-limiting enzyme in the salvage pathway of NAD<sup>+</sup> biosynthesis and is important in regulating the homeostasis of energy metabolism. The results of an immunofluorescence assay demonstrated that the NAMPT-derived fluorescence signal was reduced following 24 h of A $\beta$ <sub>1-40</sub> treatment, whereas salidroside treatment increased the NAMPT fluorescence signal (Fig. 4A and B). In addition, western blot analysis demonstrated that NAMPT expression was decreased in the A $\beta$ <sub>1-40</sub> group compared with the normal control group. Salidroside treatment significantly increased the expression level of NAMPT (Fig. 4C and D). Thus, the results indicate that salidroside regulates NAMPT protein expression.

## Discussion

AD is the most common cause of dementia in the elderly, but the exact pathogenetic mechanisms underlying AD remain

uncertain. In previous studies, one extensively investigated mechanism is A $\beta$ -mediated neurotoxicity; the aggregates of A $\beta$  peptides exhibit cytotoxicity *in vivo* and *in vitro* (23). A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> are two major forms of A $\beta$ , although A $\beta$ <sub>1-42</sub> is markedly more prone to aggregation and more toxic to neurons than A $\beta$ <sub>1-40</sub>; however, A $\beta$ <sub>1-40</sub> was the predominant isoform of the most abundant cleaved form of APP (~90%) and exerts a toxic effect on neurons in the AD brain (24). In addition, the deposition of A $\beta$ <sub>1-40</sub> is required for the development of mature amyloid plaques from the initial deposition of A $\beta$ <sub>1-42</sub>, which was regarded as an early pathological process of AD, and A $\beta$ <sub>1-40</sub> is often used to establish the model of AD *in vivo* and *in vitro* (25). Injecting 5 mg/ml A $\beta$ <sub>1-40</sub> caused hippocampal neuronal loss or functional impairment and is thus considered as an indication of energy metabolism (26). As A $\beta$ <sub>1-40</sub> possesses neuronal toxicity and is capable of inducing neuronal neurotoxicity, PC12 cells treated with A $\beta$ <sub>1-40</sub> were used as the cell model of AD in the current study. The neuronal toxicity of A $\beta$ <sub>1-40</sub> is reflected in its ability to cause energy dysmetabolism, and induce cell damage and death (27,28). A number of previous studies used PC12 cells to investigate the molecular mechanisms underlying the development of degenerative diseases of the nervous system (29-32). Therefore, the present study employed the PC12 cells that were damaged by A $\beta$ <sub>1-40</sub> exposure as the cell model of AD and the significance of NAMPT in AD was evaluated.

The present study demonstrated that A $\beta$ <sub>1-40</sub> treatment reduced the NAD<sup>+</sup> expression level. The underlying reason may be associated with decreased NAMPT expression. As the rate-limiting enzyme in the NAD<sup>+</sup> salvage pathway, NAMPT promotes the production of NAD<sup>+</sup> (33). Previous studies demonstrated that an elevated NAMPT level reduces cell death (34,35), which is consistent with the results of the present study, which indicate that salidroside increased NAMPT expression and improved cell survival. In addition, the present study identified that salidroside significantly reduced the level of LDH released when compared with that in the A $\beta$ <sub>1-40</sub> group. The results of the LDH assay were consistent with those of the MTT assay, demonstrating that salidroside treatment attenuated the A $\beta$ <sub>1-40</sub>-induced PC12 cell injury and exerted a protective effect on PC12 cells.

The expression level of A $\beta$  increases as AD progresses, which causes mitochondrial dysfunction and energy metabolism disorders. The elevated A $\beta$  expression level represents one of the reasons behind neuronal damage and apoptosis (36). NAD<sup>+</sup> participates in energy metabolism. Furthermore, loss of NAD<sup>+</sup> homeostasis leads to decreased sirtuin-1 (SIRT1) activity. Consequently, SIRT1-mediated deacetylation of signaling molecules (including transcription factors and enzymes) is reduced (37,38). Peroxisome proliferator-activated receptor  $\gamma$  coactivator 1- $\alpha$  (PGC-1 $\alpha$ ) is a coactivator of the nuclear hormone receptor peroxisome proliferator-activated receptor  $\gamma$ , which also undergoes SIRT1-mediated deacetylation and participates in mitochondrial biosynthesis and energy homeostasis (39). The decreased SIRT1 activity in the brain affects the function of the downstream protein PGC-1 $\alpha$  in energy synthesis, resulting in a brain energy crisis (40). As NAMPT is important in NAD<sup>+</sup> synthesis, the present study investigated whether salidroside exerts its regulatory effect on energy metabolism pathways via NAMPT. Namely, salidroside attenuates the neuronal toxicity of A $\beta$  by further regulating NAD<sup>+</sup>/NADH levels. Studies have demonstrated that the expression level of NAMPT decreases gradually with age, with the decrease in NAMPT level particularly evident in the brain of AD mice compared with aged healthy mice. Such a decrease exacerbates neuronal apoptosis (34,41,42). By contrast, increased NAMPT expression levels in the hippocampus and cortex improve cognitive function (35). Therefore, novel treatment strategies for AD may involve the administration of salidroside to regulate NAMPT expression and reduce the severity of AD.

The present study demonstrated that salidroside exerted a protective effect on PC12 cells damaged by A $\beta$ <sub>1-40</sub> exposure. An AD model was established by A $\beta$ <sub>1-40</sub> treatment. The viability of PC12 cells decreased and the level of LDH release significantly increased. In addition, the protein expression of NAMPT, the level of NAD<sup>+</sup> and the ratio of NAD<sup>+</sup>/NADH were decreased. The above-mentioned effects of A $\beta$ <sub>1-40</sub> were attenuated following salidroside treatment. In addition, the present study demonstrated that the decrease in NAMPT/NAD<sup>+</sup> levels may represent one of the molecular pathological mechanisms of AD.

In conclusion, the present study demonstrated that salidroside exerted a protective effect on PC12 damaged by A $\beta$ <sub>1-40</sub> treatment. The protective effect of salidroside may reside in its regulatory effect in energy metabolism, such as the increases in NAMPT expression levels and NAD<sup>+</sup> production. Therefore,

NAMPT and NAD<sup>+</sup> are considered to be key factors in AD, as well as other neurodegenerative diseases. Thus, regulation of NAMPT and NAD<sup>+</sup> may serve as novel therapeutic strategies for the treatment of AD.

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