

Simvastatin ameliorates cognitive impairments via inhibition of oxidative stress-induced apoptosis of hippocampal cells through the ERK/AKT signaling pathway in a rat model of senile dementia

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Abstract. Senile dementia is a degenerative disease of the nervous system associated with cognitive impairments, memory disorders, executive dysfunctions, cognitive decline and dementia. Previous reports suggested that simvastatin presents ameliorative effects in the progression of senile dementia. However, the mechanism underlying simvastatin-mediated improvements of cognitive competence during the progression of senile dementia remains to be elucidated. In the present study, a potential mechanism underlying simvastatin activity in hippocampal cells, was investigated. Results of the present study demonstrated that simvastatin significantly improved cognitive impairments, memory competence, amyloid plaques, loss of neurons and synapses, neurofibrillary tangles and oxidative damage in experimental rats. Results of the present study demonstrated that administration of simvastatin regulates superoxide dismutase, reactive oxygen species, catalase and glutathione in oxidative stress processes in hippocampal cells. Apoptosis of hippocampal cells was suppressed by simvastatin treatment in rats with senile dementia. Notably, the administration of simvastatin inhibited activating transcription factor-6-mediated extracellular signal-regulated kinase/AKT serine/threonine kinase (ERK/AKT) signaling pathway in hippocampal cells. Taken together, the preclinical results of the present study indicate that simvastatin is efficient in preventing memory lapse and inhibiting apoptosis of hippocampal cells via the ERK/AKT signaling pathway, which may in the future improve cognitive decline and dementia in patients.

Introduction

Senile dementia is a progressive neurodegenerative syndrome characterized by cognitive function disorders and decline caused by degeneration and apoptosis of nerve cells in the brain and nervous system (1). Clinical manifestations of senile dementia include memory impairment, aphasia, damage of visual spatial skill, executive dysfunction and personality and behavioral dysfunctions (2,3). Neurodegenerative disorders in patients with senile dementia frequently result in an increased mortality rate in the elderly population (4,5). Systematic reviews and meta-analyses have investigated the association between statins and senile dementia. Statin drugs reduce the plasma concentration of cholesterol and have been reported to reduce the risk of senile dementia (6-8).

Previous reviews have discussed the effectiveness of statins in the prevention of dementia, but there is insufficient evidence to recommend statins for the treatment of Alzheimer's disease, a condition that leads to the development of numerous forms of dementia (9,10). Pathogenesis of senile dementia mainly targets the hippocampal area associated with memory and cognition, and leads to cognitive function disorders and impairments of cognition including, memory, language and attention (11,12). Elevated rates of apoptosis and oxidative stress in hippocampal cells are frequently observed in patients with senile dementia (13).

Apoptosis of hippocampal cells is a symptom of senile dementia (14,15). Petit *et al* (16) reported that the prevention of basal and induced neuronal apoptosis abrogates mutations associated with Parkinson disease. Previous reports also indicated that apoptosis of hippocampal cells contributes to aggravation of senile dementia and leads to cognitive impairments, memory deterioration, formation of amyloid plaques, loss of neurons and synapses, and aggregation neurofibrillary tangles (17,18). These reports suggest that inhibition of apoptosis of hippocampal cells is beneficial for the suppression of cognitive impairments.

A previous study indicated that memory dysfunction and failure of energy metabolism induced by oxidative stress are associated with the development of neurodegenerative disorders (19). Biomarkers of oxidative stress in patients with

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vascular dementia have been observed in clinical patients with senile dementia (20). Furthermore, activation of the amyloidogenic pathway can cause oxidative stress, which leads to mitochondrial accumulation and apoptosis (21). Furthermore, protecting execution of hippocampal cells against apoptosis caused by oxidative stress in senile dementia disease was also clearly elaborated in previous study (22).

In the present study, the anti-apoptotic effects of simvastatin and potential molecular mechanism underlying the prevention of pathological processes of senile dementia in a rat model were investigated. A candidate signaling pathway triggered by simvastatin and leading to the improvement of cognitive impairment was evaluated in a rat model of senile dementia. The results of the present study indicated that simvastatin treatment significantly reduced amyloid plaques, loss of neurons and synapses and neurofibrillary tangles induced by oxidative stress, and apoptosis of hippocampal cells, through the extracellular signal-regulated kinase (ERK)/AKT serine/threonine kinase (AKT) signaling pathway in a rat model of senile dementia.

Materials and methods

Animal study. A total of 60 male Sprague Dawley rats (8 weeks old, 240-320 g) were purchased from the Chinese Academy of Sciences Institute of Biophysics (Beijing, China). All rats were intraperitoneally injected with scopolamine (1 mg/kg; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) to establish a model of senile dementia (23). The injection was repeated five times once every three days and rats were divided into two groups (30 rats each). Rats with senile dementia were treated with a single daily intravenous 10 mg/kg/day simvastatin injection for 60 days or with an equivalent amount of PBS. All rats were housed at 24-26°C with a 12 h light/dark cycle and fed *ad libitum*.

Ethical approval. The present study complied with the Guide for the Care and Use of Laboratory Animals of China. All experimental surgeries and animal operations were performed in accordance with the Ethics of Animal Experiments Defense Research. During all surgical operations and euthanasia, efforts were made to minimize suffering. The present study was approved by the Ethics Committee of the Traditional Chinese Medicine Hospital of Weifang (Weifang, China).

Cell culture. Hippocampal cells were isolated from the CA1 region of rats with senile dementia as previously described (24). Hippocampal cells were cultured in RPMI-1640 medium (Sigma-Aldrich; Merck KGaA) and the culture medium was supplemented with 10% fetal bovine serum (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 100 µg/ml penicillin and streptomycin (Sigma-Aldrich; Merck KGaA) and 10% glutamine (Invitrogen; Thermo Fisher Scientific, Inc.).

ATF-6 overexpression. Hippocampal cells (1×10^5) were cultured in a 6-well plate until 85% confluence; the media was then removed from the culture plate followed by 3 washes with PBS. Hippocampal cells were transfected by plentivirus-ATF-6 (ATFOP) using Lipofectamine 2000 (Sigma-Aldrich; Merck

KGaA) according to the manufacturer's protocols. After 48 h transfection, cells were used for further analysis.

Transfection of small interference RNA (Si-RNA). Hippocampal cells (4×10^5 cells/well) were seeded in a 6-well plate for 24 h at 37°C. The medium was removed and Opti-MEM (Invitrogen) added for 24 h at 37°C. The siRNA sequences corresponding to the gene were designed and synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). The siRNA sequences against ATF-6 (Si-ATF-6): 5'-GAAGGUAGUUGA AUGGUGCAUACAA-3' or siRNA-vector (Control): 5'-CUC GUCUCAUUGATGACAGTT-3'. After 48 h transfection, cells were used for further analysis.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from hippocampal cells using an RNAeasy Mini Kit (Qiagen, Inc., Valencia, CA, USA). Expression levels of superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) and inducible nitric oxide synthase (iNOS) in hippocampal cells were measured by an RT-qPCR kit (AB4104C; Invitrogen; Thermo Fisher Scientific, Inc.) with β -actin as an endogenous control (25). The PCR cycling conditions were performed at 95°C for 30 sec and 45 cycles of 95°C for 5 sec, 56.5°C for 10 sec and 72°C for 10 sec. All primers (Table I) were synthesized by Invitrogen (Thermo Fisher Scientific, Inc.). Relative mRNA expression changes were calculated by the $2^{-\Delta\Delta C_q}$ method (26). The results are expressed as a fold change compared with the β -actin control.

Western blotting. Hippocampal cells (1×10^6) were homogenized in lysate buffer containing protease-inhibitor and were centrifuged at $8,000 \times g$ at 4°C for 10 min. The supernatant was used for analyzing protein expression. Protein concentration was measured by a BCA protein assay kit (Thermo Scientific Fisher Scientific, Inc.). Protein samples (20 µg) were separated on 12% SDS-PAGE and transferred onto PVDF membranes (EMD Millipore, Billerica, MA, USA). Following blocking with 5% bovine serum albumin at 37°C for 1 h, the following primary antibodies were used in immunoblotting assays: Anti Bcl2-associated agonist of cell death (Bad; cat. no. ab32445; 1:1,000); apoptosis regulator BAX (Bax; cat. no. ab32503; 1:1,000); P53 (cat. no. ab1101; 1:1,000); Bcl-2 apoptosis regulator (Bcl-2; cat. no. ab32124; 1:1,000); SOD (cat. no. ab13533; 1:1,000); GSH (cat. no. ab94733; 1:1,000); iNOS (cat. no. ab15323; 1:500); CAT (cat. no. ab78292; 1:500); forkhead box protein P2 (Foxp2; cat. no. ab16046; 1:1,000); SHIP (cat. no. ab59338; 1:1,000); cAMP-response element binding protein (cREB; cat. no. ab33613; 1:1,000); activating transcription factor-6 (ATF-6; cat. no. ab122897; 1:1,000); ERK (cat. no. ab54230; 1:1,000); AKT (cat. no. ab8805; 1:1,000) and β -actin (cat. no. ab8226; 1:2,000; all from Abcam, Cambridge, UK) for 12 h at 4°C. Horseradish peroxidase-conjugated antibody (cat. no. HAF019; 1:5,000; Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used as a secondary antibody for 2 h at 37°C and detected using a western blotting Luminol reagent (cat. no. 12015218001; Sigma-Aldrich; Merck KGaA) for enhanced chemiluminescence. The density of the bands was analyzed by Quantity one software version 4.62 (Bio-Rad Laboratories, Inc.).

Table I. Primer sequences for reverse transcription-quantitative polymerase chain reaction.

Target gene	Forward primer	Reverse primer
SOD	5'-TCTGGATGGGTGTGGCTTGCTCT-3'	5'-GCATGCTCCCAAACATCGATC-3'
GSH	5'-GAAAGCCCAGTCTTCATTGC-3'	5'-TTGGAACCGTGCTAGTCTCA-3'
iNOS	5'-GACGAGACGGATAGGCAGAG-3'	5'-CACATGCAAGGAAGGGAAC-3'
CAT	5'-CGTGCTGAATGAGGAACAGA-3'	5'-AGTCAGGGTGGACCTCAGTG-3'
Foxp2	5'-AGCAACCAGCTCTTCAGGTTCC-3'	5'-ACGTTGTATTTGTCTGAGTACCG-3'
SHIP	5'-CCTCAAGATGCACATCCGAAG-3'	5'-AAAGTTTCAATGACCAAGC-3'
cREB	5'-GATACTCAGGCAGAGATGATCTACCC-3'	5'-AGACCAGGCACCAGACCAAAGA-3'
β-actin	5'-GTGGGCGCCCAGGCACCA-3'	5'-CTCCTTAATGTACGCACGATTT-3'

SOD, superoxide dismutase; GSH, glutathione; iNOS, inducible nitric oxide synthase; CAT, catalase; Foxp2, forkhead box protein P2; cREB, cAMP-response element binding protein.

Terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) assay. The TUNEL assay was used for the analysis of apoptosis of hippocampal neuron cells in experimental rats following simvastatin treatment (10 mg/kg/day; Sigma-Aldrich; Merck KGaA) or an equivalent dose of PBS. Procedures were performed as previously described (27). Briefly, cells (1×10^4) were cultured in a 6-well plate for at 37°C for 12 h. Then cells were fixed with 4% paraformaldehyde followed by permeabilization with 0.1% Triton X-100. Subsequently, apoptosis of cells were stained with TUNEL reaction mixture (Sigma-Aldrich; Merck KGaA) at 37°C for 2 h. Cells were washed 3 times in TBST. TUNEL assays were conducted using a TUNEL fluorescence FITC kit (Roche, Indianapolis, IN, USA) according to the manufacturer's instructions. Hippocampal neuronal cell images in 6 fields were captured using a Zeiss LSM 510 confocal microscope (Zeiss AG, Oberkochen, Germany) at a wavelength of 488 nm.

Cognitive tests. Cognitive competence of rats was determined by open field activity levels in a black Plexiglas box (60x60x25 cm) to analyze therapeutic effects of simvastatin. Rats were placed in the open black box for 10 min and their behavior was monitored and evaluated using an auto-tracking system (SmarTrack GPS Tracker; SmarTrack, Coalville, UK). The Morris water maze test was performed prior to and following the treatment with simvastatin to measure changes in the cognitive competence. The Morris water maze experiment was performed in a circular stainless-steel tank (155 cm diameter, 60 cm depth) filled with water to a depth of 40 cm ($27.0 \pm 1.0^\circ\text{C}$) that was made opaque by the addition of skim milk. Rats learned to find a hidden circular platform (10 cm diameter, 1.5 cm below the surface of the water) in a fixed area in one quadrant of the tank. A modified Rankin scoring (mRS) system (28) was used for assessing the therapeutic effects. Good outcomes and futile outcomes were defined as mRS scores ≤ 2 and 5-6, respectively. Rats tend to favor the closed arm when they feel strong anxiety (29).

Immunological staining. The effects of the simvastatin treatment on neuronal loss, amyloid plaques and neurofibrillary tangles were evaluated using immunohistochemical (IHC) staining for neuroprotection-associated proteins in hippocampi

from experimental rats. Staining was performed on cerebral neurons of hippocampi of randomly selected animals from simvastatin- or PBS-treated groups. IHC procedures were previously reported in detail (30). Brains were frozen and coronal sections were cut in a cryostat following perfusion, fixation using 95% alcohol for 15 min at 37°C and cryoprotection. Free-floating sections (4 μm) were rinsed and placed in a solution containing anti-p75 nerve growth factor (NGF) receptor antibody (cat. no. ab8874; 1:500; Abcam) or anti-homocysteine (Hcy) antibody (cat. no. ab15154; 1:500; Abcam). Following rinsing, sections were incubated in the presence of a biotinylated horse anti-rabbit antibody (cat. no. a0545; 1:500; Chemicon; Merck KGaA) for NGF or Hcy staining for 2 h at 37°C. Sections were washed and observed using fluorescent video microscopy (BZ-9000; Keyence Corporation, Osaka, Japan).

Thionin staining. The hippocampal area from experimental rats was post-fixed in Böhm-Sprenger fixative (methanol, formalin, and acetic acid, in a 16:3:1 volume ratio) for 1 h at 37°C, hydrolyzed for 1 h in 5N hydrochloric acid, immersed in the thionin (Sigma-Aldrich; Merck KGaA) staining solution for 1 h, and rinsed thrice in a bisulphite rinse solution [0.5% sodium bisulphite (w/v) in 0.05N hydrochloric acid], each separated by water rinses. Images was observed by light microscope (Olympus BX51; Olympus Corporation, Tokyo, Japan).

Statistical analysis. All data are expressed as the mean of triplicate experiments \pm standard deviation and analyzed using Student's t test or one-way analysis of variance followed by a Tukey honest significant difference post-hoc test. All data were analyzed using SPSS software (version 19.0; IBM Corp., Armonk, NY, USA), GraphPad Prism (version 5.0; GraphPad Software, Inc., La Jolla, CA, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Simvastatin demonstrates beneficial effects on cognitive competence of hippocampal network in rats with senile dementia. *In vivo* efficacy of simvastatin on the treatment of senile dementia in rats was analyzed using cognitive

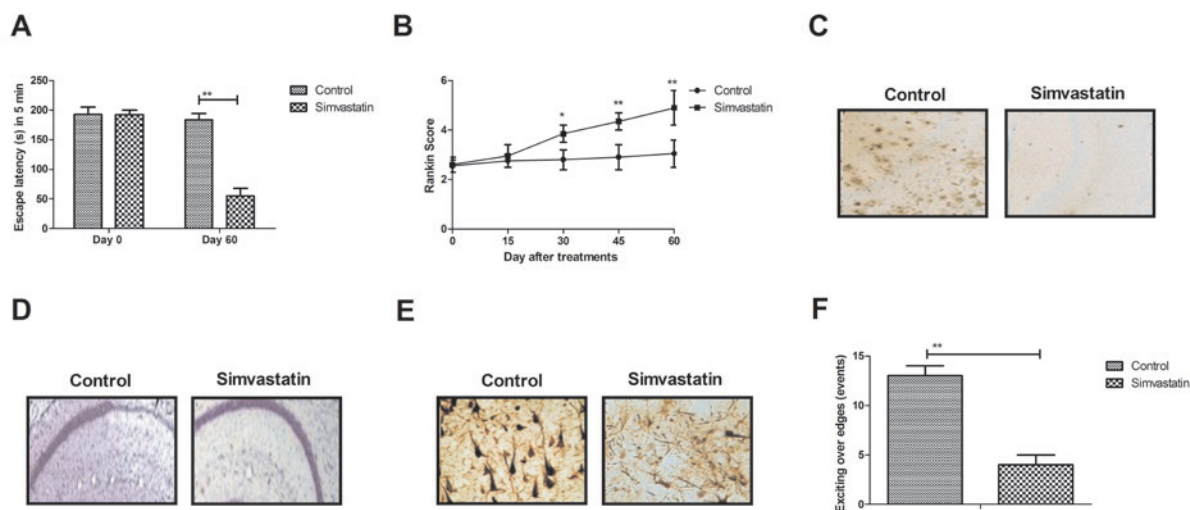


Figure 1. Simvastatin improves cognitive impairments of hippocampal network in the rat model of senile dementia. (A) Simvastatin treatment reduces the duration of escape latency in the treatment group compared with the control group. (B) Rankin scores of rats from the control and simvastatin treatment group. Immunohistochemical analysis of (C) amyloid plaques, (D) loss of neurons and (E) neurofibrillary tangles in the hippocampi of rats from the control and simvastatin treatment group. (F) Simvastatin improves anxiety in rats with senile dementia. * $P < 0.05$, ** $P < 0.01$ vs. the control group.

experiments. As presented in Fig. 1A, simvastatin treatment improved cognitive impairments determined by the escape latency during a 5-min study period. The memory competence, evaluated by the Rankin preclinical score of cognitive competences, was ameliorated by simvastatin treatment (Fig. 1B). Histological analysis of hippocampus demonstrated that amyloid plaques and loss of neurons were decreased in simvastatin-treated rats with senile dementia compared with the control (Fig. 1C and D). The results of the present study also demonstrated that the abundance of neurofibrillary tangles and anxiety was improved by simvastatin treatment in rats with senile dementia determined by Morris water maze (Fig. 1E and F). These results suggest that simvastatin treatment induces beneficial effects on cognitive competence of the hippocampal network.

Simvastatin inhibits apoptosis of hippocampal cells in the rat model of senile dementia. Apoptosis of hippocampal cells is associated with senile dementia. The efficacy of simvastatin in the prevention of apoptosis of hippocampal cells in the rat model of senile dementia was investigated. As determined by the TUNEL assay and presented in Fig. 2A, simvastatin (10 mg/kg/day) treatment inhibited apoptosis of hippocampal cells. The results of the present study demonstrated that simvastatin improved the viability of hippocampal cells compared with the control group (Fig. 2B). Western blotting demonstrated that Bad and Bax were downregulated, while P53 and Bcl-2 were upregulated in hippocampal cells from the CA1 region in experimental rats compared with the control group (Fig. 2C and D). These results indicate that simvastatin potentially inhibits apoptosis of hippocampal cells in the rat model of senile dementia through the regulation of apoptosis-associated protein expression.

Simvastatin ameliorates oxidative stress of hippocampal cells in the rat model of senile dementia. Oxidative stress serves an essential role in the programmed hippocampal cell death. As

presented in Fig. 3A and B, gene and protein expression levels of SOD and GSH were upregulated by simvastatin in hippocampal cells in the rat model of senile dementia. However, gene and protein expression levels of iNOS and CAT were downregulated in hippocampal cells in the rat model of senile dementia (Fig. 3C and D). These results indicate that simvastatin treatment ameliorates oxidative stress of hippocampal cells in the rat model of senile dementia.

Simvastatin improves neuroprotective protein expression in hippocampal cells in the rat model of senile dementia. The neuroprotective effect is an important aspect of anti-dementia drugs. Therefore, the present study analyzed changes in the expression of neuroprotective proteins in hippocampal cells in the rat model of senile dementia following treatment with simvastatin. As presented in Fig. 4A and B, simvastatin treatment increased gene and protein expression levels of Foxp2, SHIP and cREB in hippocampal cells. Immunohistochemistry demonstrated that simvastatin increased the expression of NGF and down-regulated Hcy expression levels in neurons (Fig. 4C). It was also observed that thionin staining of hippocampal area in simvastatin-treated rats demonstrated a marked difference in the dispersion of the pyramidal cell layer (Fig. 4D). These results indicate that simvastatin treatment can improve neuroprotective protein expression in hippocampal cells in the rat model of senile dementia.

Simvastatin regulates apoptosis of hippocampal cells through the ATF-6-mediated ERK/AKT signaling pathway. In order to analyze the potential mechanism underlying the activity of simvastatin in the progression of senile dementia, the ERK/AKT signaling pathway in hippocampal cells was investigated. As presented in Fig. 5A, expression levels of ATF-6, ERK and AKT were downregulated by simvastatin treatment in hippocampal cells compared with the control group. *In vitro* assays demonstrated that ATF-6 overexpression increased apoptosis in simvastatin-induced hippocampal

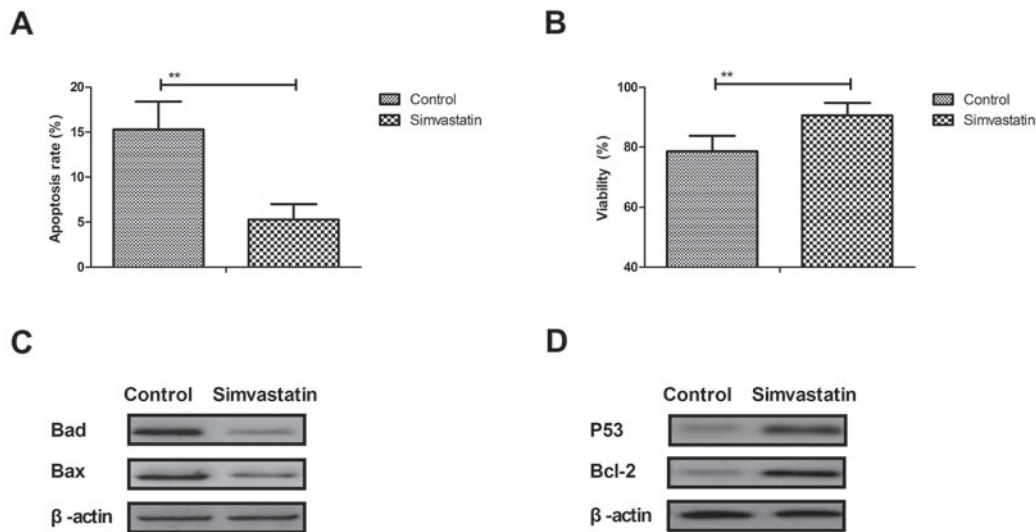


Figure 2. Simvastatin inhibits apoptosis of hippocampal cells in the rat model of senile dementia. (A) Simvastatin treatment inhibits hippocampal cell apoptosis determined by a TUNEL assay, compared with the control group. (B) Simvastatin increases the viability of hippocampal cells compared with the control group. Simvastatin treatment (C) downregulates Bad and Bax expression levels and (D) upregulates P53 and Bcl-2 expression levels in hippocampal cells of experimental rats compared with the control group. ** $P < 0.01$. Bad, Bcl2-associated agonist of cell death; Bax, apoptosis regulator BAX; Bcl-2, Bcl-2 apoptosis regulator.

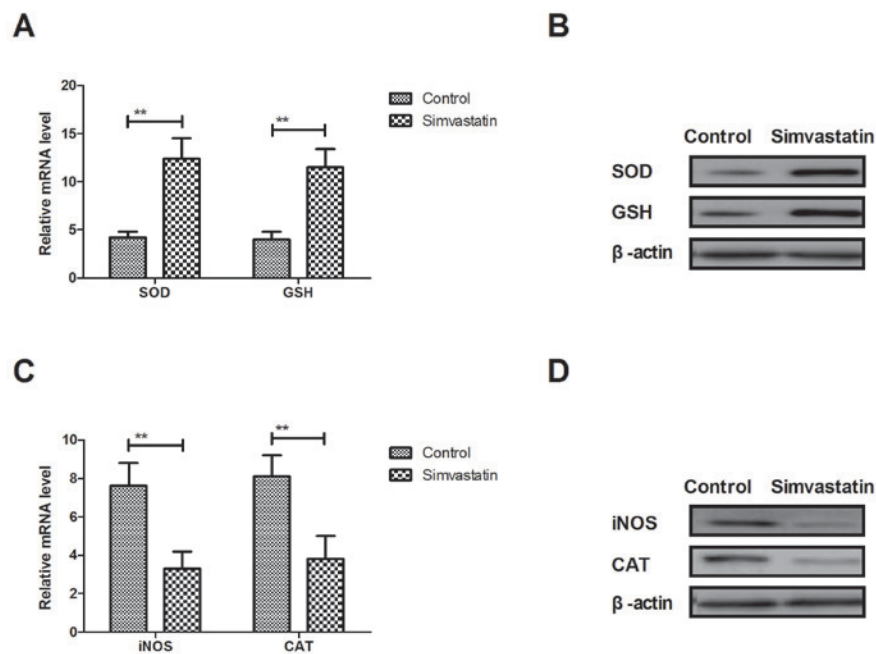


Figure 3. Simvastatin ameliorates oxidative stress of hippocampal cells in the rat model of senile dementia. Simvastatin upregulates (A) gene and (B) and protein expression level of SOD and GSH in hippocampal cells of the rat model of senile dementia, compared with the control group. Simvastatin upregulates (C) gene and (D) protein expression of iNOS and CAT in hippocampal cells of the rat model of senile dementia. ** $P < 0.01$. SOD, superoxide dismutase; GSH, glutathione; CAT, catalase.

cells (Fig. 5B). Expression and phosphorylation levels of ERK and AKT were also increased by ATF-6 overexpression in hippocampal cells (Fig. 5C). However, ATF-6 knockdown enhanced apoptosis of hippocampal cells and downregulation of ERK1/2 and AKT expression levels in hippocampal cells (Fig. 5D and E). ATF-6 overexpression blocked simvastatin-regulated oxidative stress in hippocampal cells (Fig. 5F). These results suggest that simvastatin regulates

apoptosis of hippocampal cells through ATF-6-mediated ERK/AKT signaling pathway.

Discussion

The majority of patients with senile dementia suffer from vascular dementia, which decreases the quality of their life (31). Senile dementia damages the nervous system and

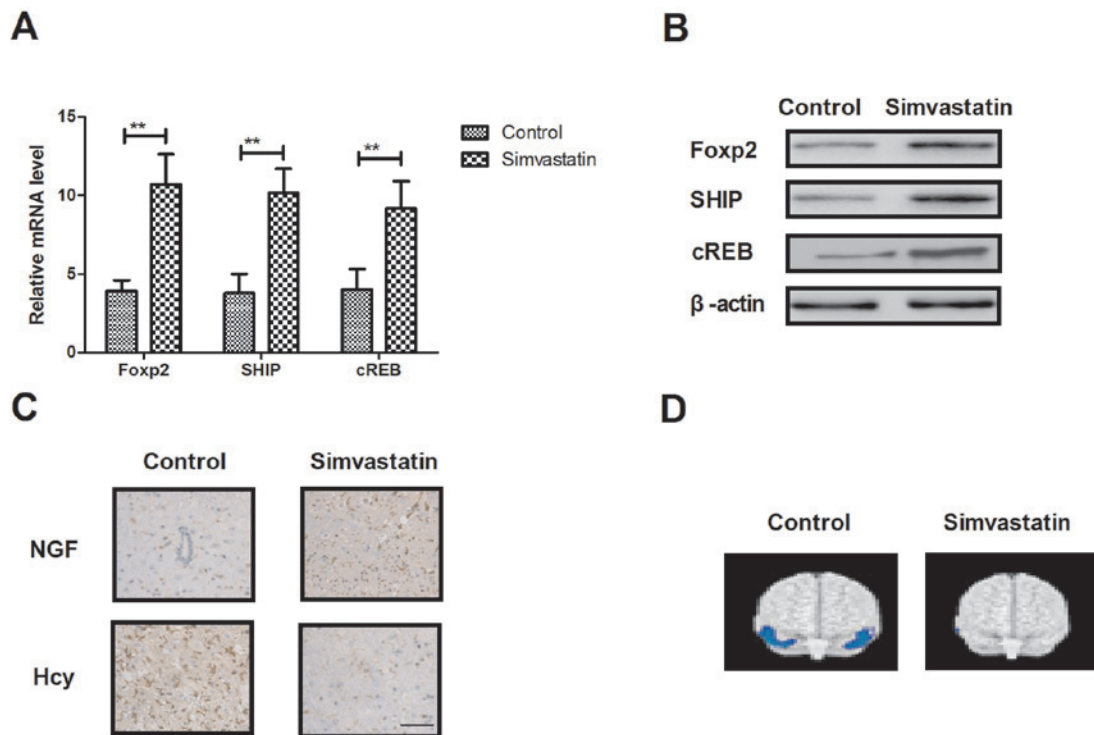


Figure 4. Simvastatin improves neuroprotective protein expression in hippocampal cells in the rat model of senile dementia. Simvastatin treatment increases (A) gene and (B) and protein expression levels of Foxp2, SHIP and cREB in hippocampal cells. (C) Simvastatin increases NGF and downregulated Hcy expression levels in neurons. (D) Simvastatin treatment improves dispersion of the pyramidal cell layer determined by thionin staining. ** $P < 0.01$. Fxop2, forkhead box protein P2; cREB, cAMP-response element binding protein; NGF, p75 nerve growth factor; Hcy, homocysteine. Magnification, 20x.

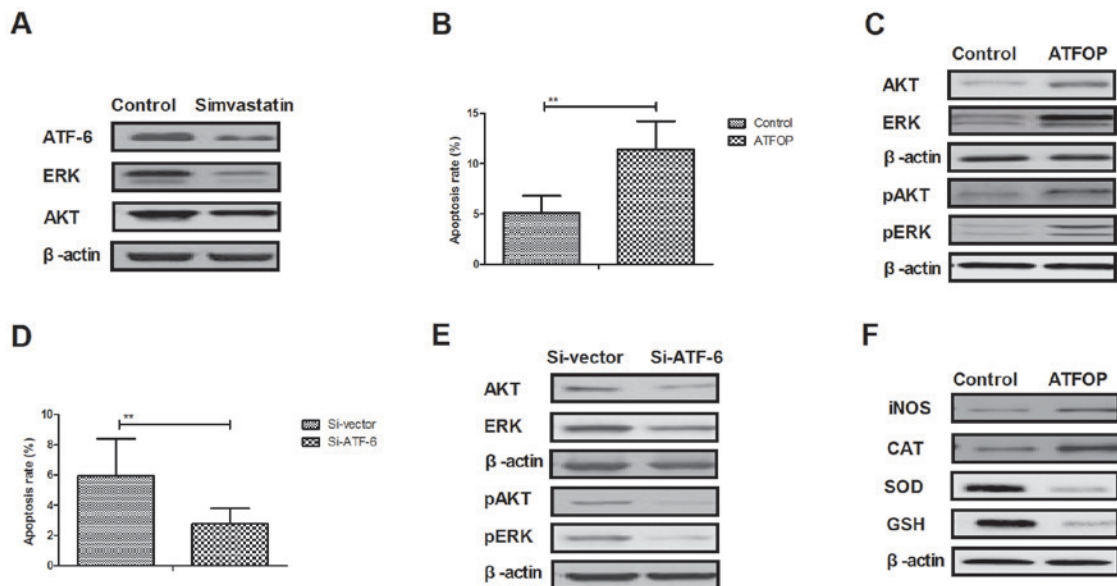


Figure 5. Simvastatin regulates apoptosis of hippocampal cells through ATF-6-mediated ERK/AKT signaling pathway. (A) Western blotting of expression levels of ATF-6, ERK and AKT in hippocampal cells. (B) ATF-6 overexpression abolishes simvastatin-inhibited apoptosis of hippocampal cells. (C) Expression and phosphorylation levels of pERK and pAKT were reversed by ATF-6 overexpression in hippocampal cells. (D) ATF-6 knockdown enhances simvastatin-inhibited apoptosis of hippocampal cells. (E) Si-ATF-6 downregulates of ERK1/2 and AKT expression levels in hippocampal cells. (F) ATF-6 overexpression blocks simvastatin-regulated oxidative stress in hippocampal cells. ERK, extracellular signal-regulated kinase; AKT, AKT serine/threonine kinase; ATF-6, activating transcription factor-6; ATFOP, ATF-6 overexpression; Si, small interfering RNA; p, phosphorylated; ROS, reactive oxygen species; CAT, catalase; SOD, superoxide dismutase; GSH, glutathione.

impairs the cognitive ability in the elderly (32). Hippocampal cell apoptosis is highly correlated with hypoxia, ischemia and injury evidenced by changes in the infarct volume during

chronic cerebral hypoperfusion (33). It has been demonstrated that simvastatin has a crucial role in apoptosis of hippocampal cells, memory recovery, cognitive rehabilitation and neuronal

survival (34). In addition, oxidative damage of brain regions may induce apoptosis of hippocampal cells, which could lead to cognitive dysfunction in patients with senile dementia (35). Furthermore, upregulation of the activity-dependent neuroprotective protein in the Tau mutation is regarded a novel marker for the onset of frontotemporal dementia (36). In the present study, the benefits of simvastatin for the treatment of senile dementia were further investigated and a potential mechanism of simvastatin-mediated anti-apoptosis in hippocampal cells was analyzed in a rat model of senile dementia.

Simvastatin is a statin that reduces hypercholesterolemia. Simvastatin has neuroprotective potential against 6-hydroxy-dopamine-induced Parkinson's disease-like symptoms (37). Neuronal marker recovery following simvastatin treatment of dementia in the rat brain was investigated *in vivo* by magnetic resonance (38). El-Dessouki *et al* (39) revealed that the neuroprotective effect of simvastatin is mediated via regulation of neurotransmitters and activity of acetylcholinesterase in L-methionine-induced vascular dementia. In addition, a previous study proposed a novel mechanism of simvastatin-mediated neuroprotection in neurodegenerative diseases through the modulation of seladin-1-associated metabolic regulation (40). A previous molecular analysis demonstrated that the neuroprotective effect of simvastatin is derived from the improvement of endoplasmic reticulum stress response in a rat model of global forebrain ischemia/reperfusion (41). In the present study, simvastatin improved cognitive impairments, memory competence, reduced the number of amyloid plaques and diminished the loss of neurons and synapses, neurofibrillary tangles and oxidative damage in experimental rats.

Apoptosis of hippocampal cells aggravates the degree of dementia and damages the cranial nerve system in patients with senile dementia (17). In the present study, simvastatin treatment suppressed apoptosis of hippocampal cells in CA1 regions and contributed to the improvement of cognitive competence. Previous research indicated that simvastatin exerts beneficial outcomes on patients with senile dementia due to the anti-neoplastic and anti-apoptotic effects in a number of cell types (42). In addition, attenuation of apoptosis, inflammation and oxidative stress in the blood and brain tissues is beneficial for the treatment of scopolamine-induced dementia in rats (23). A previous study suggested that ATF-6 is associated with apoptosis of dopaminergic neurons and accumulates in the core of Lewy bodies in Parkinson's disease (43). The results of the present study suggest that simvastatin treatment inhibits ATF-6-mediated ERK/AKT signaling pathway in hippocampal cells in CA1 regions in the rat model of senile dementia.

Expression of ERK is upregulated in hippocampal neurons of mice with vascular dementia (44). Hu *et al* (45) demonstrated that autophagy and AKT/cREB signaling are involved in the neuroprotective effects of nimodipine in a rat model of vascular dementia. Yao *et al* (46) demonstrated that ATF-6 mediates oxidized low-density lipoprotein-induced cholesterol accumulation and apoptosis in macrophages by upregulating DNA damage-inducible transcript 3 protein expression. In the present study, simvastatin treatment downregulated ATF-6, ERK and AKT expression in CA1 hippocampal cells in the rat model of senile dementia, which is consistent with a previous report (47). Endogenous expression of ATF-6 abolished the protective effects of simvastatin-inhibited neuronal damage in

hippocampal cells in the CA1 region through the modulation of the activity of the ERK/AKT signal pathway.

In conclusion, the present study indicates that simvastatin may be an efficient agent for the treatment of senile dementia. Simvastatin treatment protects hippocampal neurons against apoptosis that further repairs the nervous system in the brain. The results of the present study indicate that simvastatin treatment suppresses apoptosis of hippocampal cells in the CA1 region through ATF-6-mediated ERK/AKT signaling pathway. These results suggest that simvastatin is a promising agent for the treatment of senile dementia.

References

1. Zeng L, Zou Y, Kong L, Wang N, Wang Q, Wang L, Cao Y, Wang K, Chen Y, Mi S, *et al*: Can Chinese Herbal medicine adjunctive therapy improve outcomes of senile vascular dementia? Systematic review with meta-analysis of clinical trials. *Phytother Res* 29: 1843-1857, 2015.
2. Song MH, Hamada H and Mimura M: Semiological differences between late-life schizophrenia and senile dementia. *Keio J Med* 63: 34-38, 2014.
3. Kawahara M, Mizuno D, Koyama H, Konoha K, Ohkawara S and Sadakane Y: Disruption of zinc homeostasis and the pathogenesis of senile dementia. *Metallomics* 6: 209-219, 2014.
4. Disse M, Reich H, Lee PK and Schram SS: A Review of the association between parkinson disease and malignant melanoma. *Dermatol Surg* 42: 141-146, 2016.
5. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A and Logroscino G: Accuracy of clinical diagnosis of Parkinson disease: A systematic review and meta-analysis. *Neurology* 86: 566-576, 2016.
6. Vos E and Nehrlich HH: Use of statins and incidence of dementia and cognitive impairment without dementia in a cohort study. *Neurology* 73: 406-407, 2009.
7. Wilson PW and Vega GL: Counterpoint: Lipoproteins and dementia: Is there compelling evidence to treat Alzheimer's patients with statins? *J Clin Lipidol* 2: 394-396, 2008.
8. Horsdal HT, Olesen AV, Gasse C, Sørensen HT, Green RC and Johnsen SP: Use of statins and risk of hospitalization with dementia: A Danish population-based case-control study. *Alzheimer Dis Assoc Disord* 23: 18-22, 2009.
9. McGuinness B, O'Hare J, Craig D, Bullock R, Malouf R and Passmore P: Statins for the treatment of dementia. *Cochrane Database Syst Rev*: CD007514, 2010.
10. McGuinness B, Craig D, Bullock R and Passmore P: Statins for the prevention of dementia. *Cochrane Database Syst Rev*: CD003160, 2009.
11. Pettigrew C, Soldan A, Zhu Y, Wang MC, Brown T, Miller M and Albert M; BIOCARD Research Team: Cognitive reserve and cortical thickness in preclinical Alzheimer's disease. *Brain Imaging Behav* 11: 357-367, 2017.
12. Fritz NE, Kegelmeyer DA, Kloos AD, Linder S, Park A, Kataki M, Adeli A, Agrawal P, Scharre DW and Kostyk SK: Motor performance differentiates individuals with Lewy body dementia, Parkinson's and Alzheimer's disease. *Gait Posture* 50: 1-7, 2016.
13. Morimoto S, Kuzuhara S and Kokubo Y: Increased oxidative stress in patients with amyotrophic lateral sclerosis/Parkinsonism-dementia complex in the Kii peninsula, Japan. *Mov Disord* 24: 123-126, 2009.
14. Jahng GH, Oh J, Lee DW, Kim HG, Rhee HY, Shin W, Paik JW, Lee KM, Park S, Choe BY and Ryu CW: Glutamine and glutamate complex, as measured by functional magnetic resonance spectroscopy, Alters during Face-name association task in patients with mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis* 53: 745, 2016.
15. Nesteruk T, Nesteruk M, Styczynska M, Barcikowska-Kotowicz M and Walecki J: Radiological evaluation of strategic structures in patients with mild cognitive impairment and Early Alzheimer's disease. *Pol J Radiol* 81: 288-294, 2016.
16. Petit A, Kawarai T, Paitel E, Sanjo N, Maj M, Scheid M, Chen F, Gu Y, Hasegawa H, Salehi-Rad S, *et al*: Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. *J Biol Chem* 280: 34025-34032, 2005.

17. Sun ZK, Ma XR, Jia YJ, Liu YR, Zhang JW and Zhang BA: Effects of resveratrol on apoptosis in a rat model of vascular dementia. *Exp Ther Med* 7: 843-848, 2014.
18. Kaul M and Lipton SA: Signaling pathways to neuronal damage and apoptosis in human immunodeficiency virus type 1-associated dementia: Chemokine receptors, excitotoxicity, and beyond. *J Neurovirol* 10 (Suppl 1): S97-S101, 2004.
19. Huang JL, Fu ST, Jiang YY, Cao YB, Guo ML, Wang Y and Xu Z: Protective effects of Nicotiflorin on reducing memory dysfunction, energy metabolism failure and oxidative stress in multi-infarct dementia model rats. *Pharmacol Biochem Behav* 86: 741-748, 2007.
20. Shi GX, Liu CZ, Wang LP, Guan LP and Li SQ: Biomarkers of oxidative stress in vascular dementia patients. *Can J Neurol Sci* 39: 65-68, 2012.
21. Hernández-Zimbrón LF and Rivas-Arancibia S: Oxidative stress caused by ozone exposure induces β -amyloid 1-42 overproduction and mitochondrial accumulation by activating the amyloidogenic pathway. *Neuroscience* 304: 340-348, 2015.
22. Bayir H, Kapralov AA, Jiang J, Huang Z, Tyurina YY, Tyurin VA, Zhao Q, Belikova NA, Vlasova II, Maeda A, *et al*: Peroxidase mechanism of lipid-dependent cross-linking of synuclein with cytochrome C: Protection against apoptosis versus delayed oxidative stress in Parkinson disease. *J Biol Chem* 284: 15951-15969, 2009.
23. Demirci K, Nazıroğlu M, Övey İS and Balaban H: Selenium attenuates apoptosis, inflammation and oxidative stress in the blood and brain of aged rats with scopolamine-induced dementia. *Metab Brain Dis* 32: 321-329, 2017.
24. Cao Y, Guo N, Lv Y, Shi H and Chen X: Isolation, primary culture and characterization of mouse glomerular mesangial cells. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 29: 1315-1318, 2013 (In Chinese).
25. Xiao S, Wang J and Xiao N: MicroRNAs as noninvasive biomarkers in bladder cancer detection: A diagnostic meta-analysis based on qRT-PCR data. *Int J Biol Markers* 31: e276-e285, 2016.
26. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
27. Naganuma Y, Ichii O, Otsuka S, Hashimoto Y and Kon Y: Analysis of TdT-mediated dUTP nick end labeling (TUNEL)-positive cells associated with cardiac myogenesis in mouse embryo. *J Vet Med Sci* 75: 283-290, 2013.
28. Han M, Choi JW, Rim NJ, Kim SY, Suh HI, Lee KS, Hong JM and Lee JS: Cerebral infarct volume measurements to improve patient selection for endovascular treatment. *Medicine* 95: e4702, 2016.
29. Tanaka Y, Akiyoshi J, Kawahara Y, Ishitobi Y, Hatano K, Hoaki N, Mori A, Goto S, Tsuru J, Matsushita H, *et al*: Infrared radiation has potential antidepressant and anxiolytic effects in animal model of depression and anxiety. *Brain Stimul* 4: 71-76, 2011.
30. Dirani M, Nasreddine W, Abdulla F and Beydoun A: Seizure control and improvement of neurological dysfunction in Lafora disease with perampanel. *Epilepsy Behav Case Rep* 2: 164-166, 2014.
31. Venketasubramanian N, Sahadevan S, Kua EH, Chen CP and Ng TP: Interethnic differences in dementia epidemiology: Global and Asia-Pacific perspectives. *Dement Geriatr Cogn Disord* 30: 492-498, 2010.
32. Battistin L and Cagnin A: Vascular cognitive disorder. A biological and clinical overview. *Neurochem Res* 35: 1933-1938, 2010.
33. Yang HY, Liu Y, Xie JC, Liu NN and Tian X: Effects of repetitive transcranial magnetic stimulation on synaptic plasticity and apoptosis in vascular dementia rats. *Behav Brain Res* 281: 149-155, 2015.
34. Wolozin B, Wang SW, Li NC, Lee A, Lee TA and Kazis LE: Simvastatin is associated with a reduced incidence of dementia and Parkinson's disease. *BMC Med* 5: 20, 2007.
35. Luca M, Luca A and Calandra C: The role of oxidative damage in the pathogenesis and progression of Alzheimer's disease and vascular dementia. *Oxid Med Cell Longev* 2015: 504678, 2015.
36. Schirer Y, Malishkevich A, Ophir Y, Lewis J, Giladi E and Gozes I: Novel marker for the onset of frontotemporal dementia: Early increase in activity-dependent neuroprotective protein (ADNP) in the face of Tau mutation. *PLoS One* 9: e87383, 2014.
37. Kumar A, Sharma N, Gupta A, Kalonia H and Mishra J: Neuroprotective potential of atorvastatin and simvastatin (HMG-CoA reductase inhibitors) against 6-hydroxydopamine (6-OHDA) induced Parkinson-like symptoms. *Brain Res* 1471: 13-22, 2012.
38. Tušková R, Lipták B, Szomolányi P, Vančová O, Uličná O, Sumbalová Z, Kucharská J, Dubovický M, Trattng S, Liptaj T and Kašparová S: Neuronal marker recovery after Simvastatin treatment in dementia in the rat brain: In vivo magnetic resonance study. *Behav Brain Res* 284: 257-264, 2015.
39. El-Dessouki AM, Galal MA, Awad AS and Zaki HF: Neuroprotective effects of simvastatin and cilostazol in L-Methionine-Induced vascular dementia in rats. *Mol Neurobiol* 54: 5074-5084, 2017.
40. Ramos MC, Sierra S, Ramirez C, Velasco J and Burgos JS: Simvastatin modulates the Alzheimer's disease-related gene seladin-1. *J Alzheimers Dis* 28: 297-301, 2012.
41. Urban P, Pavliková M, Sivonová M, Kaplán P, Tatarková Z, Kaminska B and Lehotský J: Molecular analysis of endoplasmic reticulum stress response after global forebrain ischemia/reperfusion in rats: Effect of neuroprotectant simvastatin. *Cell Mol Neurobiol* 29: 181-192, 2009.
42. Bartolomé F, Muñoz U, Esteras N, Alquezar C, Collado A, Bermejo-Pareja F and Martín-Requero A: Simvastatin overcomes the resistance to serum withdrawal-induced apoptosis of lymphocytes from Alzheimer's disease patients. *Cell Mol Life Sci* 67: 4257-4268, 2010.
43. Vitte J, Traver S, Maués De Paula A, Lesage S, Rovelli G, Corti O, Duyckaerts C and Brice A: Leucine-rich repeat kinase 2 is associated with the endoplasmic reticulum in dopaminergic neurons and accumulates in the core of Lewy bodies in Parkinson disease. *J Neuropathol Exp Neurol* 69: 959-972, 2010.
44. Hu YZ, Lu PY and Ling L: The expression of ERK in the hippocampal neurons of mice with vascular dementia. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 25: 466-467, 520, 2009 (In Chinese).
45. Hu M, Liu Z, Lv P, Wang H, Zhu Y, Qi Q and Xu J: Autophagy and Akt/CREB signalling play an important role in the neuroprotective effect of nimodipine in a rat model of vascular dementia. *Behav Brain Res* 325: 79-86, 2017.
46. Yao S, Zong C, Zhang Y, Sang H, Yang M, Jiao P, Fang Y, Yang N, Song G and Qin S: Activating transcription factor 6 mediates oxidized LDL-induced cholesterol accumulation and apoptosis in macrophages by up-regulating CHOP expression. *J Atheroscler Thromb* 20: 94-107, 2013.
47. Chen PY, Sun JS, Tsuang YH, Chen MH, Weng PW and Lin FH: Simvastatin promotes osteoblast viability and differentiation via Ras/Smad/Erk/BMP-2 signaling pathway. *Nutr Res* 30: 191-199, 2010.