Ginsenoside Rg1 reduces β-amyloid levels by inhibiting CDK5-induced PPARγ phosphorylation in a neuron model of Alzheimer's disease

QIANKUN QUAN[1], XI LI[1], JIANJUN FENG[1], JIXING HOU[2], MING LI[1] and BINGWEI ZHANG[1]

[1]Department of Geriatrics, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710004; [2]Department of Psychiatry, Xi'an Mental Health Center, Xi'an, Shaanxi 710061, P.R. China

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Abstract. The accumulation of β-amyloid peptides (Aβ) in the brain is a hallmark of Alzheimer's disease (AD). Studies have indicated that ginsenoside Rg1, a primary component of ginseng (*Panax ginseng*), reduces brain Aβ levels in an AD model through peroxisome proliferator-activated receptor γ (PPARγ), thereby regulating the expression of insulin-degrading enzyme (*Ide*) and β-amyloid cleavage enzyme 1 (*Bace1*), which are PPARγ target genes. However, the effects of ginsenoside Rg1 on PPARγ remain unclear. Since cyclin-dependent kinase 5 (CDK5) mediates PPARγ phosphorylation in adipose tissue, this study aimed to investigate whether ginsenoside Rg1 regulates PPARγ target genes and reduces Aβ levels by inhibiting PPARγ phosphorylation through the CDK5 pathway. In the present study, a model of AD was established by treating primary cultured rat hippocampal neurons with $A\beta_{1-42}$. The cells were pretreatment with ginsenoside Rg1 and roscovitine, a CDK5-inhibitor, prior to the treatment with $A\beta_{1-42}$. Neuronal apoptosis was detected using TUNEL staining. PPARγ phosphorylation and protein expression levels of PPARγ, CDK5, IDE, BACE1, amyloid precursor protein (APP) and $A\beta_{1-42}$ were measured by western blotting. The mRNA expression levels of PPARγ, CDK5, IDE, BACE1 and APP were assessed using reverse transcription-quantitative PCR. The results of the present study demonstrated that in an AD model induced by $A\beta_{1-42}$, ginsenoside Rg1 significantly decreased CDK5 expression, inhibited PPARγ phosphorylation at serine 273, elevated IDE expression, downregulated BACE1 and APP expression, decreased $A\beta_{1-42}$ levels and attenuated neuronal apoptosis. The CDK5 inhibitor, roscovitine, demonstrated similar effects. These results suggest that ginsenoside Rg1 has neuroprotective properties and has potential for use in the treatment of AD.

Correspondence to: Professor Xi Li, Department of Geriatrics, The Second Affiliated Hospital of Xi'an Jiaotong University, 157 Xiwu Road, Xi'an, Shaanxi 710004, P.R. China
E-mail: lixi2100@163.com

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Introduction

Ginseng (*Panax ginseng*), which is documented in Shennong's Classic of Materia Medica as having intellect-enhancing effects, has been widely used in China for millennia (1). Ginsenoside Rg1, a primary ginseng components, has multiple neuroprotective effects against Alzheimer's disease (AD), including the improvement of memory impairment (2), inhibition of neuronal apoptosis (3), amelioration of oxidative stress (4) and attenuation of mitochondrial dysfunction (5). The accumulation of β-amyloid peptides (Aβ) is the most prominent pathological feature of AD and significantly influences the pathogenesis of the disease (6). The aggregation and accumulation of Aβ in the brain may lead to neuroinflammatory responses (7), neurofibrillary tangle formation (8), synaptic loss (9), oxidative stress (10), neuronal apoptosis (11), cholinergic dysfunction (12) and tau phosphorylation (13). It has been reported that ginsenoside Rg1 has Aβ-scavenging effects, for example, ginsenoside Rg1 inhibits the transcription and translation of β-amyloid cleavage enzyme 1 (*Bace1*), a target gene of peroxisome proliferator-activated receptor γ (PPARγ), by enhancing the binding of PPARγ to the *Bace1* promoter, thereby decreasing BACE1 activity, which ultimately attenuates Aβ production (14). Additionally, ginsenoside Rg1 upregulates PPARγ expression in the rat hippocampus AD model, thereby increasing the expression of insulin-degrading enzyme (*Ide*) (another PPARγ target gene) and promoting the clearance of $A\beta_{1-42}$ from the hippocampus (15). These studies suggest that PPARγ is involved in the reduction of Aβ levels caused by ginsenoside Rg1; however, the mechanisms by which ginsenoside Rg1 affects PPARγ remain unclear.

Cyclin-dependent kinase 5 (CDK5), a cyclin-dependent kinase, is a proline-directed serine/threonine kinase predominantly activated in post-mitotic cells and has various activities including cytoskeletal dynamics, signaling cascades, gene expression, cell survival, neurodevelopment and brain function (16-19). Phosphorylation is an important post-translational modification of PPARγ. It has been demonstrated that in the adipose tissue, CDK5 induces PPARγ phosphorylation

at serine 273 (Ser273), which is located in the hinge region between the DNA- and the ligand-binding domains *in vivo* and *in vitro* (20). Furthermore, our previous study demonstrated that in rat primary hippocampal neurons CDK5 regulates the expression of IDE and BACE1 by mediating the phosphorylation of PPARγ, resulting in decreased Aβ clearance and increased Aβ production (21). The present study aimed to investigate whether ginsenoside Rg1 inhibits the phosphorylation of PPARγ through the downregulation of the CDK5 pathway. The findings of this study will deepen the understanding of the neuroprotective properties of ginsenoside Rg1 and its potential use in the treatment of AD.

Materials and methods

Reagents. Ginsenoside Rg1 (molecular formula: C42H72O14; molecular weight: 801.01; HPLC purity: 98%) was purchased from Baoji Herbest Bio-Tech Co., Ltd. $A\beta_{1-42}$ and roscovitine were purchased from Sigma Aldrich; Merck KGaA. Rabbit anti-rat IDE (cat. no. ab133561), BACE1 (cat. no. ab10716) and amyloid precursor protein (APP; cat. no. ab15272) polyclonal antibodies were purchased from Abcam. Rabbit anti-rat p-PPARγ-Ser273 polyclonal antibody (cat. no. bs-4888R) was purchased from BIOSS Antibodies. Rabbit anti-rat CDK5 (cat. no. WL01673), PPARγ (cat. no. WL0269) and $A\beta_{1-42}$ (cat. no. WL01427) polyclonal antibodies, anti-β-actin antibody (cat. no. WL01845), goat anti-rabbit secondary horseradish peroxidase-conjugated antibody (cat. no. WLA023), TUNEL assay kit, total protein extraction kit, bicinchoninic acid (BCA) protein assay kit and enhanced chemiluminescence (ECL) reagent were purchased from Wanleibio Co., Ltd. Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco; Thermo Fisher Scientific, Inc. Fetal bovine serum (FBS) was purchased from Biological Industries. Trypsin and 4',6-diamidino-2-phenylindole (DAPI) were purchased from Beyotime Institute of Biotechnology. TRIzol® and 2X Power Taq PCR MasterMix were purchased from BioTeke Corporation. SYBR Green master mix was purchased from Beijing Solarbio Science & Technology Co., Ltd.

Isolation and culture of rat hippocampal neurons. Rat hippocampal neurons were isolated and cultured using the methods previously described (22). In total, 150 2-day-old Sprague Dawley rats (weight, 8±2 g; males, 75; females, 75) were used in the study. These rats were obtained from the Experimental Animal Center of Xi'an Jiaotong University Health Science Center [License no. SCXK (Shaan) 2018-001], which were housed in a specific-pathogen free facility maintained at 23˚C with a 12-h light-dark cycle and 24-h atmosphere purification, and were allowed free access to breastmilk from their mother. In brief, brain tissues were isolated from these rats under aseptic conditions. The hippocampal tissues were then dissected on an ultra-clean bench, cut into pieces, and digested by trypsin. Subsequently, DMEM containing 10% FBS and penicillin/streptomycin was added for trypsin neutralization. Cell suspension was repeatedly digested five-six times after centrifugation (168 x g, 7 min, 25˚C). After filtration and centrifugation, the hippocampal neurons were placed in a poly-lysine-coated 6-well plate at a density of $5x10^5$ cells/ml and cultured for 8 h at 37˚C with 5% CO_2 and saturated humidity. Subsequently, the culture medium was changed to neurobasal medium containing 2% B27, 0.5 mM glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin and the cells were cultured for 48 h. Cytarabine was then added to the medium (final concentration, 10 μM) to inhibit the growth of glial cells. The media were changed once every 3 days until maturation and network formation of the hippocampal neurons in ~15 days. All experimental procedures in this study were approved by the Ethics Committee of The Second Affiliated Hospital of Xi'an Jiaotong University (Shaanxi, China).

Drug treatment. The cultured neurons were divided into the following three groups: Control, model and ginsenoside Rg1 (Rg1) groups. The control group was used as the vehicle treated group, in which no drugs were added to the culture medium; in the model group, cultured neurons were treated with 8 μM $A\beta_{1-42}$ (23) for 24 h at 37˚C; in the Rg1 group, the cultured neurons were exposed to 60 μM ginsenoside Rg1 (24) for 1 h at 37˚C and then to 8 μM $A\beta_{1-42}$ for 24 h at 37˚C. To confirm whether ginsenoside Rg1 regulates PPARγ phosphorylation by acting on CDK5, the effects of ginsenoside Rg1 on cultured neurons that were treated with $A\beta_{1-42}$ after CDK5 expression was inhibited using the CDK5 inhibitor roscovitine were investigated. Neurons were divided into the three following groups: Model, roscovitine and roscovitine+Rg1 groups. In the model group, the cultured neurons were treated with 8 μM $A\beta_{1-42}$ for 24 h at 37˚C; in the roscovitine group, the cultured neurons were first exposed to 25 μM roscovitine (25) for 1 h at 37˚C and then to 8 μM $A\beta_{1-42}$ for 24 h at 37˚C; in the roscovitine+Rg1 group, cultured neurons were first treated with 25 μM roscovitine for 0.5 h at 37˚C followed by 60 μM ginsenoside Rg1 for 1 h at 37˚C and subsequently 8 μM $A\beta_{1-42}$ for 24 h at 37˚C.

TUNEL staining. TUNEL staining was performed using an assay kit according to the manufacturer's instructions. After the slides were fixed with 4% paraformaldehyde at 25˚C for 10 min, the cells were permeabilized with 50 μl of 0.1% Triton X-100 for 15 min at 25˚C. After being washed with phosphate-buffered saline (PBS), the cells were incubated with the TUNEL reaction mixture (formulated by mixing the enzyme and label solutions at a ratio of 1:9) in a wet chamber in the dark at 37˚C for 60 min. Subsequently, the cells were washed with PBS and counterstained with DAPI in the dark at 25˚C for 5 min. After washing with PBS again, the slides were mounted using mounting medium with anti-fluorescent quenchers. The neurons were counted in a blind manner by two pathologists under a BX 53 fluorescence microscope (Olympus Corporation) at x400 magnification.

The average number of neurons from four random fields of view was used as the final result for each pathologist. The results from the two pathologists were averaged and used to calculate the percentage of TUNEL-positive neurons.

Western blotting. Total proteins of the neurons were extracted using the total protein extraction kit according to the manufacturer's instructions and the protein concentration was measured using the BCA protein assay method. After denaturation at 95˚C for 5 min, 20 μl protein sample (including 40 μg protein) was added into each electrophoretic lane,

Table I. Primers used for reverse transcription-quantitative PCR.

Gene	Primer sequence (5'→3')	Primer length	Temperature, ˚C	PCR product length, bp
Cyclin-dependent kinase 5	F: GGACACCGACTGAGGAAC	18	52.0	103
	R: TTGGGCACGACATTCAC	17	52.5	
Peroxisome proliferator-activated receptor γ	F: TACCACGGTTGATTTCTC	18	47.7	155
	R: AATAATAAGGCGGGGACG	18	55.3	
Insulin-degrading enzyme	F: TCCCGTGAAGCGACTGT	17	54.3	180
	R: GACTTGTCCGTGGTGGG	17	53.6	
β-amyloid cleavage enzyme 1	F: TCCGCATCACCATCCTT	17	54.0	123
	R: TGACCGCTCCCATAACG	17	55.1	
Amyloid precursor protein	F: ACTCTGTGCCAGCCAATA	18	51.2	158
	R: TGAATCATGTCCGAACTCC	19	53.0	
β-actin	F: GGAGATTACTGCCCTGGCTCCTAGC	25	60.1	155
	R: GGCCGGACTCATCGTACTCCTGCTT	25	62.0	

F, Forward; R, Reverse; bp, base pairs.

separated in an 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose (NC) membrane. The membrane was blocked with 5% non-fat powdered milk at 37˚C for 1 h and was then incubated with rabbit anti-rat CDK5 (1:500), p-PPARγ-Ser273 (1:500), PPARγ (1:400), IDE (1:900), BACE1 (1:600), APP (1:800), and $A\beta_{1-42}$ (1:500) polyclonal antibodies overnight at 4˚C. After being washed with Tris-buffered saline buffer with 0.05% Tween 20, the NC membrane was incubated with the secondary horseradish peroxidase-conjugated antibody (goat anti-rabbit, 1:1,000) at 37˚C for 45 min. Subsequently, the NC membrane was developed using ECL reagent and the blots were scanned. The optical densities of the target bands were analyzed using Gel-Pro Analyzer software (version 4.0, Media Cybernetics, Inc.).

Reverse transcription-quantitative (RT-q)PCR. Total RNA from the neurons of the three groups was extracted using TRIzol and the concentrations were measured using a UV spectrophotometer. Reverse transcription was performed according to the manufacturer's instructions. In brief, each RNA sample was added into a nuclease-free centrifuge tube in an ice bath based on the concentration of the extracted RNA sample (consistent RNA concentrations during sample loading), followed by the addition of 1 μl of oligo $(dT)_{15}$, 1 μl of random primers, and a sufficient volume of double-distilled water to reach a total volume of 12.5 μl. The mixture was incubated at 70˚C for 5 min and was then rapidly cooled on ice for 2 min. After centrifugation (671 x g, 1 min, 4˚C), the reaction mixture was mixed with 2 μl of deoxynucleoside triphosphate (2.5 mM each), 4 μl of 5X buffer, 0.5 μl of RNase inhibitor and 1 μl of Moloney-murine leukemia virus (200 U), and was then sequentially subjected to the following conditions: 25˚C for 10 min, 42˚C for 50 min and 80˚C for 10 min to terminate the reaction. The resultant cDNA was stored at -20˚C for further use. Primers (Table I) of rat *Cdk5, Pparγ, Ide, Bace1* and *App* genes were designed using Primer Premier 5.0 (Premier Biosoft International). All primers were synthesized by Sangon Biotech Co., Ltd. Fluorescence-based RT-qPCR was performed using a 2X Power Taq PCR Master Mix kit with a Exicycler™ 96 real-time PCR instrument (Bioneer Corp.). The PCR reaction was performed according to the manufacturer's instructions in a 20 μl mixture including 1 μl of cDNA, 0.5 μl of the forward primer (10 μM), 0.5 μl of the reverse primer (10 μM), 10 μl of the SYBR Green Master Mix and sufficient double-distilled water. Reaction conditions were as follows: Initial denaturation at 94˚C for 5 min, followed by denaturation at 94˚C for 10 sec, annealing at 60˚C for 20 sec and extension at 72˚C for 30 sec for 40 cycles. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (26). β-actin was used as the internal control.

Data analysis. All data are expressed as the mean ± SEM. One-way analysis of variance (ANOVA) followed by a Least Significant Difference post hoc test was performed for multiple comparisons. Statistical analyses were conducted using SPSS (version 16.0, SPSS, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Ginsenoside Rg1 inhibits PPARγ phosphorylation in the AD model. In primary cultured rat hippocampal neurons $A\beta_{1-42}$ treatment significantly enhanced PPARγ phosphorylation at Ser273, increased the p-PPARγ/PPARγ ratio and decreased PPARγ protein and mRNA expression levels compared with those in the control group ($P<0.05$; Fig. 1). These results suggested the presence of PPARγ phosphorylation in the AD neuron model induced by $A\beta_{1-42}$. In addition, pretreatment with ginsenoside Rg1 significantly attenuated the aforementioned $A\beta_{1-42}$-induced effects in these neurons ($P<0.05$; Fig. 1). Notably, after inhibiting CDK5 expression using roscovitine, the results demonstrated that compared with those in the model group PPARγ phosphorylation was

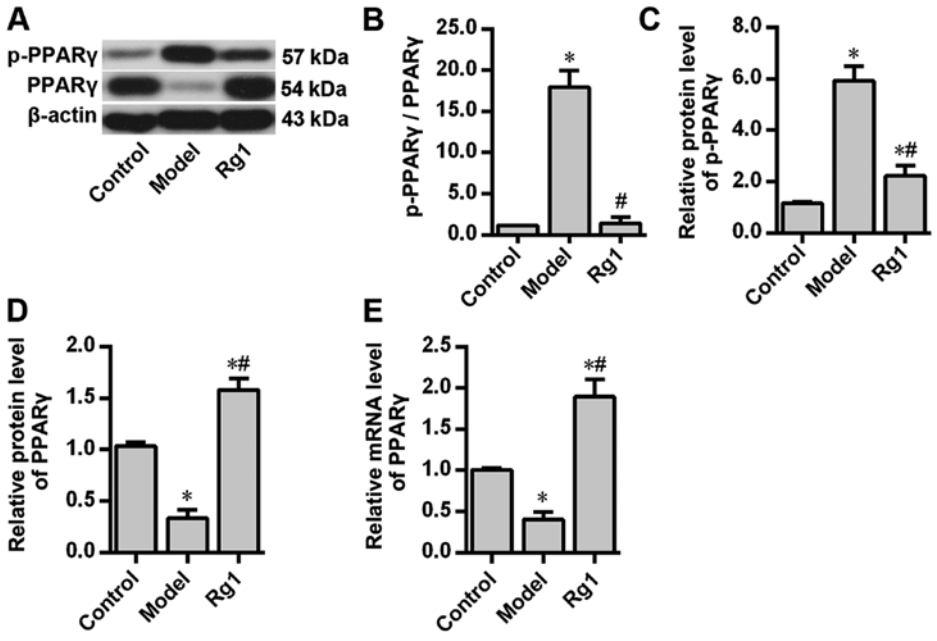

Figure 1. Effects of ginsenoside Rg1 on PPARγ phosphorylation in the Alzheimer's disease neuron model. (A) Western blots for p-PPARγ-Ser273 and PPARγ. (B) Comparison of the p-PPARγ-Ser273/PPARγ ratios among the three groups. (C) Comparison of the p-PPARγ-Ser273 levels among the three groups. Comparison of the PPARγ (D) protein and (E) mRNA expression levels among the three groups. n=6. *P<0.05 vs. control group; #P<0.05 vs. model group. PPARγ, peroxisome proliferator-activated receptor γ; Ser, serine.

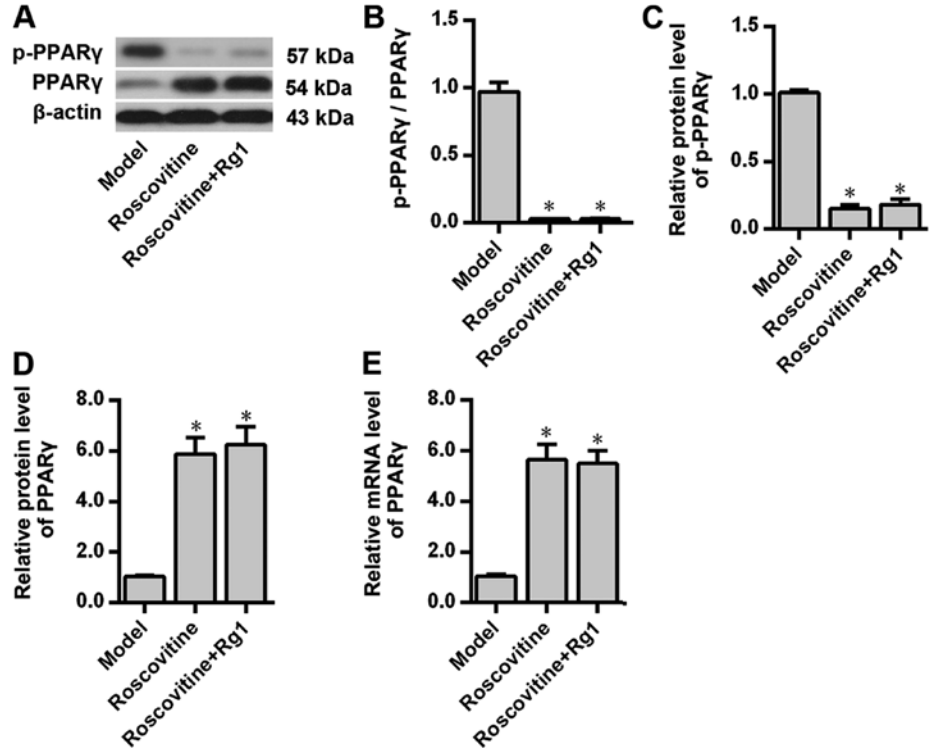

Figure 2. Effects of ginsenoside Rg1 on PPARγ phosphorylation in the Alzheimer's disease neuron model after cyclin-dependent kinase 5 inhibition. (A) Western blots for p-PPARγ-Ser273 and PPARγ. (B) Comparison of the p-PPARγ-Ser273/PPARγ ratios among the three groups. (C) Comparison of the p-PPARγ-Ser273 protein expression levels among the three groups. Comparison of the PPARγ (D) protein and (E) mRNA expression levels among the three groups. n=6. *P<0.05 vs. model group. PPARγ, peroxisome proliferator-activated receptor γ; Ser, serine.

significantly inhibited and PPARγ expression levels were significantly increased in the $A\beta_{1-42}$-treated neurons (P<0.05; Fig. 2). Additionally, PPARγ phosphorylation and the expression levels of PPARγ protein in the $A\beta_{1-42}$-treated neurons were not further affected by ginsenoside Rg1 treatment after CDK5 inhibition (P>0.05; Fig. 2), suggesting that CDK5 may be involved in the inhibition of PPARγ phosphorylation induced by ginsenoside Rg1.

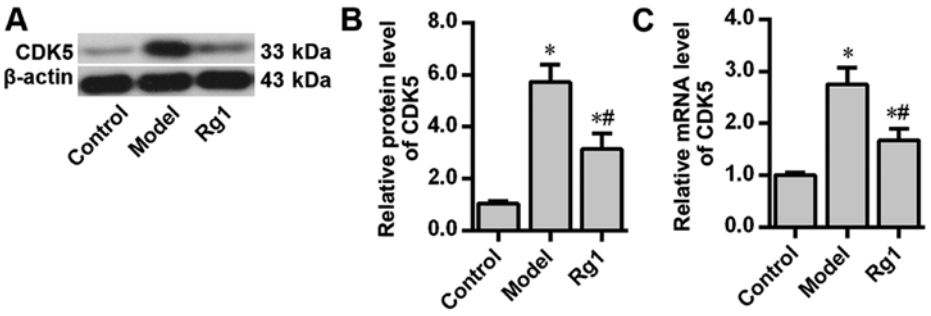

Figure 3. Effects of ginsenoside Rg1 on CDK5 expression levels in the Alzheimer's disease neuron model. (A) Western blots for CDK5. Comparison of the CDK5 (B) protein and (C) mRNA expression levels among the three groups. n=6. *P<0.05 vs. control group; #P<0.05 vs. model group. CDK5, cyclin-dependent kinase 5.

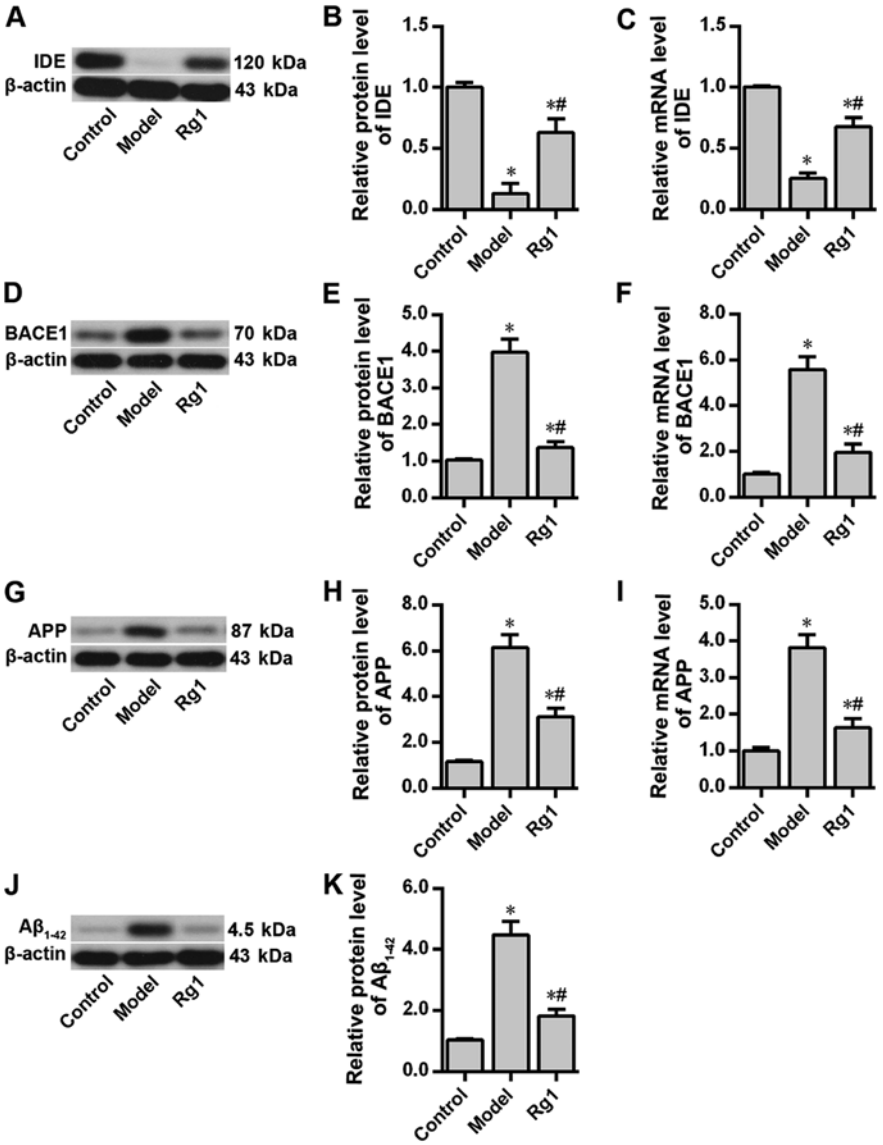

Figure 4. Effects of ginsenoside Rg1 on the expression levels of PPARγ target genes and intracellular $A\beta_{1-42}$ levels in the Alzheimer's disease neuron model. Protein expression levels of (A) IDE, (D) BACE1, (G) APP and (J) $A\beta_{1-42}$ were assessed by western blotting analysis. IDE (B) protein and (C) mRNA expression levels, BACE1 (E) protein and (F) mRNA expression levels, APP (H) protein and (I) mRNA expression levels, and (K) $A\beta_{1-42}$ protein expression levels were compared among the three groups. n=6. *P<0.05 vs. control group; #P<0.05 vs. model group. PPARγ, peroxisome proliferator-activated receptor γ; IDE, insulin-degrading enzyme; BACE1, β-amyloid cleavage enzyme 1; APP, amyloid precursor protein; Aβ, β-amyloid peptides.

Ginsenoside Rg1 decreases CDK5 expression levels in the AD model. The effects of ginsenoside Rg1 on CDK5 expression in the neuron model of AD were investigated. Western blotting and RT-qPCR analyses demonstrated that in the primary

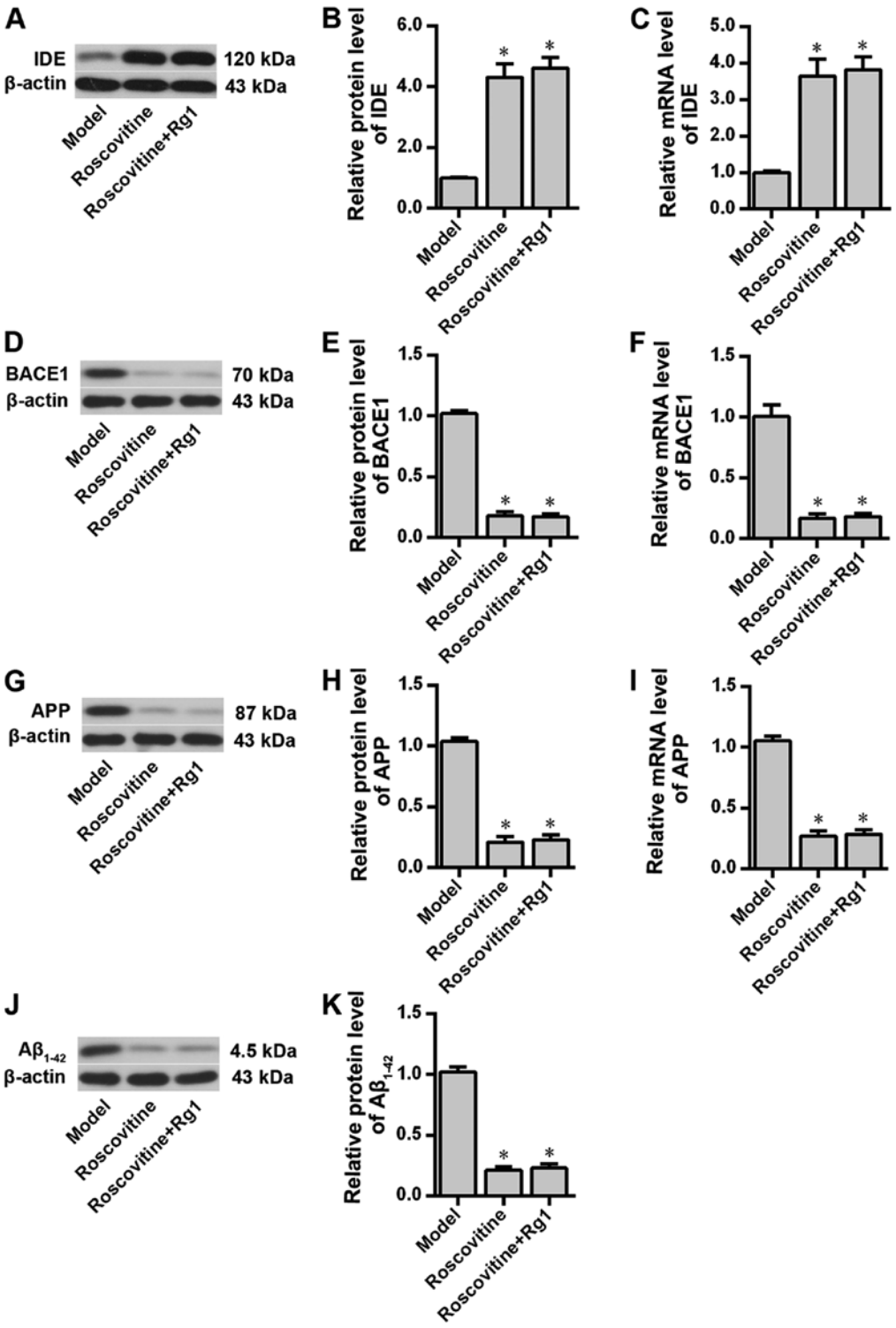

Figure 5. Effects of ginsenoside Rg1 on the expression levels of PPARγ target genes and intracellular $A\beta_{1-42}$ levels in the Alzheimer's disease neuron model after CDK5 inhibition. Protein expression levels of (A) IDE, (D) BACE1, (G) APP and (J) $A\beta_{1-42}$ were assessed by western blotting analysis. IDE (B) protein and (C) mRNA expression levels, BACE1 (E) protein and (F) mRNA expression levels, APP (H) protein and (I) mRNA expression levels and (K) $A\beta_{1-42}$ protein expression levels were compared among the three groups. n=6. *P<0.05 vs. model group. PPARγ, peroxisome proliferator-activated receptor γ; IDE, insulin-degrading enzyme; BACE1, β-amyloid cleavage enzyme 1; APP, amyloid precursor protein; Aβ, β-amyloid peptides.

cultured rat hippocampal neurons $A\beta_{1-42}$ treatment significantly increased the protein and mRNA expression levels of CDK5 compared with those in the control group (P<0.05); but, ginsenoside Rg1 pretreatment significantly attenuated the $A\beta_{1-42}$-induced increase in the protein and mRNA expression levels of CDK5 (P<0.05; Fig. 3).

Ginsenoside Rg1 regulates the expression of PPARγ target genes and decreases intracellular $A\beta_{1-42}$ levels in the AD model. The effects of ginsenoside Rg1 on the expression of PPARγ target genes and intracellular $A\beta_{1-42}$ level when CDK5 was inhibited were examined. The results demonstrated that compared with the rat hippocampal neurons in the control

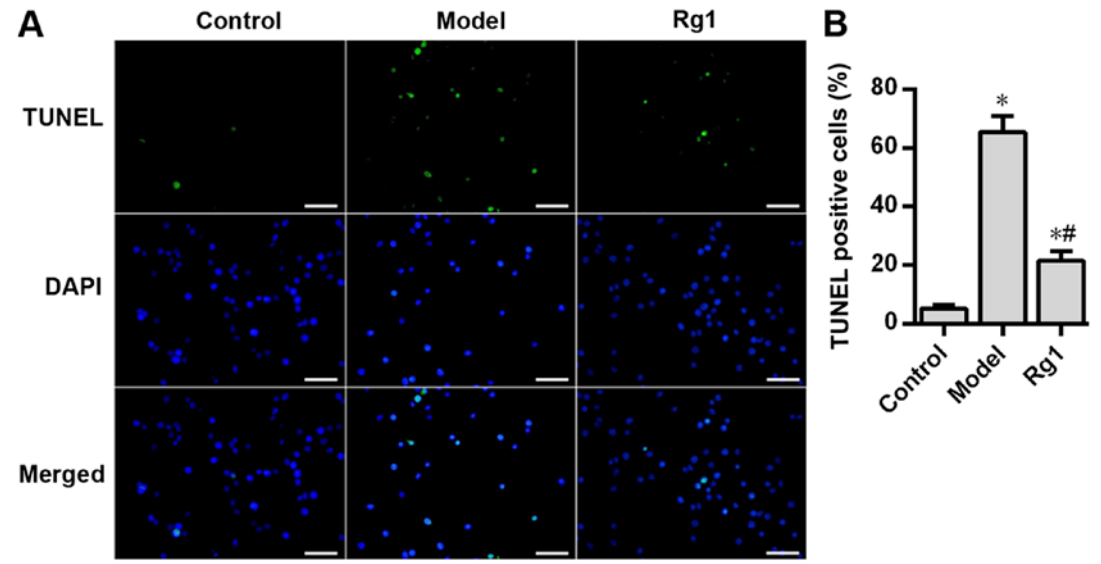

Figure 6. Effects of ginsenoside Rg1 on β-amyloid peptides1-42-induced apoptosis in rat hippocampal neurons. (A) TUNEL staining. Green spots represent TUNEL-positive nuclei and blue spots represent DAPI-counterstained nuclei. Scale bar, 50 μm. (B) Comparison of the percentages of TUNEL-positive neurons among the three groups. n=6. *P<0.05 vs. control group; #P<0.05 vs. model group. TUNEL, transferase dUTP nick end-labeling.

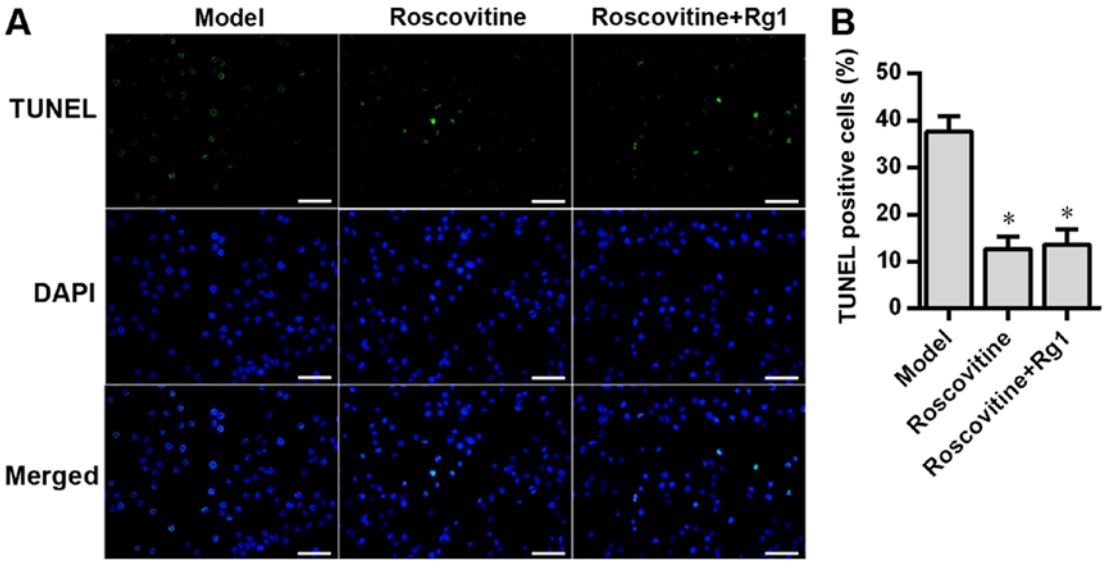

Figure 7. Effects of ginsenoside Rg1 on β-amyloid peptides1-42-induced apoptosis in rat hippocampal neurons after cyclin-dependent kinase 5 inhibition. (A) TUNEL staining. Green spots represent TUNEL-positive nuclei and blue spots represent DAPI-counterstained nuclei. Scale bar, 50 μm. (B) Comparison of the percentages of TUNEL-positive neurons among the three groups. n=6. *P<0.05 vs. model group. TUNEL, transferase dUTP nick end-labeling.

group, those in the model group exhibited significantly decreased IDE protein and mRNA expression levels (P<0.05), but significantly increased BACE1, APP and enhanced intracellular $A\beta_{1-42}$ levels (P<0.05; Fig. 4). Additionally, pretreatment with ginsenoside Rg1 significantly attenuated $A\beta_{1-42}$-induced effects in these neurons (P<0.05; Fig. 4). Additionally, compared with those in the model group inhibition of CDK5 expression using roscovitine significantly increased the expression levels of IDE and reduced the expression levels of BACE1, APP and $A\beta_{1-42}$ in rat hippocampal neurons treated with $A\beta_{1-42}$ (P<0.05; Fig. 5). In addition, no significant differences were observed in the expression levels of IDE, BACE1, APP and $A\beta_{1-42}$ after ginsenoside Rg1 treatment following CDK5 inhibition with roscovitine (P>0.05; Fig. 5).

Ginsenoside Rg1 attenuates $A\beta_{1-42}$-induced apoptosis in rat hippocampal neurons. In the present study, TUNEL staining was performed to determine the effects of $A\beta_{1-42}$ and ginsenoside Rg1 on the apoptosis of rat hippocampal neurons. Compared with that in the control group, $A\beta_{1-42}$ treatment significantly increased neuronal apoptosis in the model group (P<0.05; Fig. 6), but pretreatment with ginsenoside Rg1 significantly decreased $A\beta_{1-42}$-induced neuronal apoptosis (P<0.05; Fig. 6). In addition, the neuronal apoptosis rate in the roscovitine group was significantly lower compared with that in the model group (P<0.05; Fig. 7). Additionally, no significant difference was observed in the in the neuronal apoptosis rate between the roscovitine group and the roscovitine+Rg1 group (P>0.05; Fig. 7).

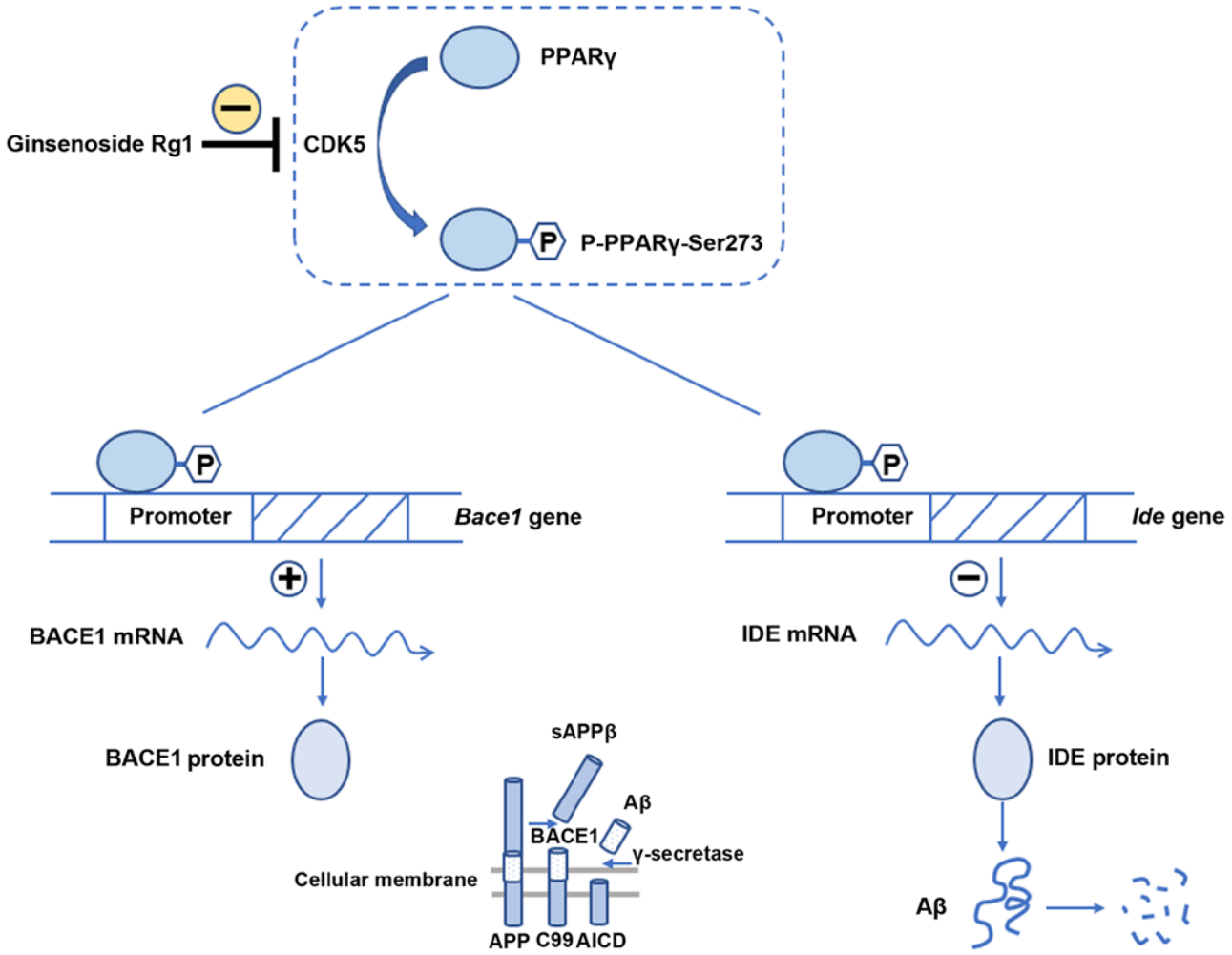

Figure 8. The possible mechanisms by which ginsenoside Rg1 reduces Aβ level by affecting PPARγ. *Ide* and *Bace1* are PPARγ target genes. IDE degrades Aβ directly, whereas BACE1 proteolyzes APP to produce soluble β-fragment of the amyloid precursor protein and C-terminal fragment containing 99 amino acids, which is then enzymatically cleaved by γ-secretase to generate Aβ. CDK5 downregulate the expression of IDE and upregulate the expression of BACE1 by mediating PPARγ phosphorylation at Ser273. The results of the present study demonstrated that ginsenoside Rg1 inhibited PPARγ phosphorylation at Ser273 by downregulating the expression of CDK5, thereby affecting the expression of IDE and BACE1 and decreasing Aβ levels. Aβ, β-amyloid peptides; PPARγ, peroxisome proliferator-activated receptor γ; IDE, insulin-degrading enzyme; BACE1, β-amyloid cleavage enzyme 1; CDK5, cyclin-dependent kinase 5; Ser, serine.

Discussion

This study investigated whether ginsenoside Rg1 inhibits the phosphorylation of PPARγ through the downregulation of the CDK5 pathway, thereby affecting the expression of PPARγ target genes *Bace1* and *Ide* and reducing Aβ levels (Fig. 8). PPARs are ligand-activated nuclear transcription factors, which regulate the transcription of target genes by binding to the peroxisome proliferator response element (PPRE) located on the promoters of these genes (27). Different types of fatty acids (FAs), including docosahexaenoic acid, activate PPARs in adipose tissues (28,29). FA binding to PPARs control the transcription of specific genes including those encoding for various metabolic and cellular processes such as FA β-oxidation and adipogenesis, making them key mediators of lipid homeostasis (29). PPARγ is one of PPARs superfamily members (along with PPARα, PPARβ/δ). A previous study has reported that PPARγ inhibits BACE1 expression by binding to the PPRE on the *Bace1* gene promoter in N2a/APP695 cells (30), which is an essential enzyme in the generation of Aβ as it hydrolyzes APP to form Aβ (31). Additionally, PPARγ also bind to the PPRE in the *Ide* promoter, thereby regulating Ide gene transcription and promoting IDE protein expression (32), which has been demonstrated to degrade Aβ (33). These findings indicate that PPARγ serves a key role in the inhibition of Aβ generation and the promotion of Aβ degradation. Moreover, PPARγ has anti-inflammatory effects through inhibiting the generation of certain proinflammatory cytokines (such as tumor necrosis factor, interleukin-1β and interleukin-6), the production of nitric oxide, and the expression of matrix metalloproteinase 9 and macrophage scavenger receptor 1 (34). Studies have reported that these molecules are closely associated with the onset of AD (35-40). Furthermore, oxidative stress usually occurs during early stages of AD and elevates with increased AD severity (41); PPARγ inhibits Aβ-induced oxidative stress (42). Therefore, PPARγ agonists may provide neuroprotective effects against AD.

Several clinical and experimental studies have investigated the role of thiazolidinediones (TZDs), PPARγ agonists, in AD, for example, pioglitazone reduces brain Aβ levels (43)

and improves learning and memory in APP/PS1 mice (44), and decreases tau phosphorylation (45) and neuronal apoptosis (46) in an AD cell model; rosiglitazone decreases Aβ1-40 and $A\beta_{1-42}$ levels, reduces tau phosphorylation, alleviates memory impairment (47), and inhibits Aβ-induced oxidative stress (48), inflammatory responses (49) and mitochondrial dysfunction (50) in both *in vitro* and *in vivo* systems. Oral administration of rosiglitazone improves cognitive function in patients with mild-to-moderate AD (51). Troglitazone and ciglitazone prevent Aβ-induced microglial- and monocyte-mediated neurotoxicity and inhibit Aβ-induced increased expression of interleukin 6, tumor necrosis factor α, and cyclooxygenase-2 (52). Recently, a study demonstrated that pioglitazone inhibits the phosphorylation of PPARγ at Ser273 *in vitro* by inhibiting CDK5 expression, which in turn affected the expression of PPARγ target genes *Ide* and *Bace1*, thereby promoting Aβ degradation and reducing Aβ production. This reduced Aβ levels in the brain, thereby exerting neuroprotective effects in an AD model (53). These findings indicate that TZDs have neuroprotective effects against AD. However, as a long-term medication is required for AD, which is a chronic disease with complex pathogenic mechanisms and a long disease course, the side effects of TZDs will substantially limit their application in AD treatment. Studies have demonstrated that rosiglitazone may lead to an increased risk of fractures (54), heart failure (55,56) and increased incidence of stroke (56); pioglitazone may lead to fractures and bladder cancer (54,57); and troglitazone may induce severe hepatotoxicity (58). Therefore, there is a need for TZD replacement with safer and effective drugs presenting mild side effects and PPARγ-agonistic effects in the study of AD therapies.

Ginseng is a natural herbal remedy that has been used in China over several millennia (1). Ginseng has multi-target therapeutic and pharmacological effects in central nervous system, which have been well-demonstrated in the clinical practice (59). Ginsenosides, which mainly include Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1 and Rg2, are the main components of ginseng (60). In particular, ginsenoside Rg1 is one of the most studied and representative ginsenoside components in the field of AD treatment (61). Previous studies have observed that ginsenoside Rg1 may potentially activate PPARγ and facilitate Aβ removal by enhancing the binding of PPARγ to target genes or by upregulating PPARγ expression (14,15). Accordingly, the present study further elucidated the mechanisms by which ginsenoside Rg1 affects PPARγ. The results of the present study suggested that ginsenoside Rg1 inhibits PPARγ phosphorylation by downregulating the expression of CDK5, thereby affecting the expression of PPARγ target genes.

Extracellular and intracellular Aβ serve essential roles in the onset of AD (62). BACE1-mediated APP proteolysis produces a soluble β-fragment of the amyloid precursor protein and a C-terminal fragment containing 99 amino acids (C99). Subsequently, C99 is enzymatically cleaved by γ-secretase to generate APP intracellular domain and Aβ; the latter is released into the extracellular matrix (63,64) (Fig. 8). APP proteolysis also occurs in the endoplasmic reticulum and golgi apparatus (64,65), producing intracellular Aβ. Gouras *et al* (66) reported that intraneuronal Aβ immunoreactivity appeared to precede the deposition of both neurofibrillary tangles and senile plaques, indicating that intraneuronal accumulation of Aβ is an early event in the onset of AD. The neuron model of AD in this study was established with the addition of exogenous Aβ, so exogenous and neuro-secreted Aβ could not be distinguished, and this study only observed the effect of ginsenoside Rg1 on the intracellular Aβ and did not investigate the changes in extracellular Aβ levels.

This study demonstrated that Rg1 inhibited CDK5 expression. However, the mechanism remains unclear. The results of the present study suggested that ginsenoside Rg1 may affect CDK5 directly, or through other mechanisms; this needs to be investigated. The results of the present also demonstrated that exogenously added Aβ increased CDK5 expression levels, aggravated PPARγ phosphorylation, decreased PPARγ expression levels, and affected the expression levels of the downstream PPARγ target genes *Ide* and *Bace1*. The exact mechanisms of these phenomena remain unclear and are subjects for future studies. Previous studies have observed a negative correlation between IDE expression and Aβ levels in AD brains (67), and a decrease in IDE expression levels were also observed in an AD rat model established through the injection of $A\beta_{1-42}$ in the hippocampus (15). These findings are consistent with those of the present study, which demonstrated decreased IDE expression levels in Aβ-treated primary cultured hippocampal neurons. After treatment with ginsenoside Rg1, IDE expression levels increased and Aβ levels decreased. Several possible reasons may account for these findings: i) The consumption of IDE through its effects on degradation of Aβ exceeds the compensatory generation of IDE; ii) decreased IDE expression may consequently promote Aβ generation; and iii) mutual interactions may exist between IDE and Aβ, i.e., generated Aβ affects IDE expression, whereas decreased IDE expression promotes an increase in Aβ levels. Accordingly, further studies are required to verify these explanations. This study demonstrated that CDK5 mediates the ginsenoside Rg1 reduction of Aβ levels by phosphorylating PPARγ. In addition to CDK5, other CDKs such as CDK7 and CDK9 also take part in PPARγ phosphorylation. A previous study has reported that CDK7 phosphorylates PPARγ at Ser112 to inhibit the activity of PPARγ (68). However, CDK9 increases PPARγ activity after phosphorylating PPARγ at Ser112 (69). Thus, further investigation is necessary into whether the phosphorylation of PPARγ by CDK7 and CDK9 is involved in the ginsenoside Rg1 reduction of Aβ levels. Additionally, the results of the present study demonstrated that the protein and mRNA expression levels of PPARγ in the Rg1 group were higher compared with those in the control group. It was speculated this is due to the high concentrations of ginsenoside Rg1 used in the study, which were able to stimulate a significant response compared with the control.

There are several limitations to this study. In the study, no experiments such as chromatin immunoprecipitation RT-qPCR, were performed to confirm whether CDK5 directly regulates PPARγ. However, our previous study reported that CDK5 regulates PPARγ (21), and performed co-immunoprecipitation experiments to confirm that Aβ promotes the binding of CDK5 to PPARγ (53). In addition, our previous study demonstrated that the PPARγ agonist pioglitazone inhibits PPARγ phosphorylation by inhibiting CDK5 expression, thereby promoting Aβ degradation and reducing Aβ production (53). Therefore, no PPARγ agonist

was used for comparison with ginsenoside Rg1 in this study. Since a CDK5 inhibitor was used in the study, no *in vivo* studies were conducted and only *in vitro* experiments were performed; therefore, the effects of ginsenoside Rg1 *in vivo* are unknown. Furthermore, since there are no available drugs that target CDK5, no positive-control drug was used in the study. A set of CDK5 overexpressing cells would allow for testing of the results of this study; unfortunately, CDK5 overexpression experiments were not be performed due to limitations on time and funding.

In conclusion, the results of the present study suggested that ginsenoside Rg1 inhibits PPARγ phosphorylation possibly through the downregulation of CDK5 expression, thereby affecting the expression of PPARγ target genes (*Ide* and *Bace1*) and decreasing Aβ levels through the promotion of Aβ degradation and reduction of Aβ synthesis, which ultimately provides neuroprotective effects against AD.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

QQ, XL and JF designed the study and wrote the manuscript. QQ, JH, ML and BZ performed the experiments. QQ and JH collected and analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experimental procedures in the present study were approved by the Ethics Committee of The Second Affiliated Hospital of Xi'an Jiaotong University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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