Blockage of Drp1 phosphorylation at Ser579 protects neurons against Aβ1-42-induced degeneration

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Abstract. Alzheimer's disease (AD), one of the most common types of chronic neurodegenerative diseases, is pathologically characterized by the formation of amyloid β (Aβ) peptide-containing plaques and neurofibrillary tangles. Among Aβ peptides, Aβ1-42 induces neuronal toxicity and neurodegeneration. In our previous studies, Cdk5 was found to regulate Aβ1-42-induced mitochondrial fission via the phosphorylation of dynamin-related protein 1 (Drp1) at Ser579. However, whether blockage of Drp1 phosphorylation at Ser579 protects neurons against Aβ1-42-induced degeneration remains to be elucidated. Thus, the aim of the present study was to examine the effect of mutant Drp1-S579A on neurodegeneration and its underlying mechanism. First, the phosphorylation-defect (phospho-defect) mutant, Lenti-Drp1-S579A was constructed. Phospho-defect Drp1-S579A expression was detected in primary cultures of mouse cortical neurons infected with Lenti-Drp1-S579A using western blotting and it was found to successfully attenuate the phosphorylation of endogenous Drp1 at Ser579. In primary neuronal cultures, the neuronal processes were evaluated under microscopy. Treatment with 10 µM Aβ1-42 significantly decreased dendritic density and length, spine outgrowth and synapse number. As expected, infection of neurons with Lenti-Drp1-S579A efficiently alleviated the inhibitory effect of Aβ1-42 on neurite outgrowth and synapse density. In addition, infection with Lenti-Drp1-S579A abolished the cleavage of caspase-3 and apoptosis in neurons exposed to Aβ1-42. Thus, the current data demonstrated that blockage of Drp1 phosphorylation at Ser579 may be an effective strategy to protect neurons against Aβ1-42-induced degeneration and apoptosis. These findings underline the therapeutic potential of targeting Drp1 in the treatment of AD.

Introduction

Alzheimer's disease (AD) is an age-related, progressive neurodegenerative disease, with clinical symptoms of cognitive decline and changes in behavior and personality (1,2). AD is the commonest type of dementia in people aged >65 (3). With the increase in human lifespan AD is becoming, at present, a major health concern for the elderly. Worldwide >50 million people are suffering from AD (3). However, there is no effective treatment for delaying the disease progression of patients with AD (4). The disease is characterized by two hallmark lesions: Senile plaques (SP) and neurofibrillary tangles (NFTs). SP and NFTs mainly result from the deposition of amyloid β (Aβ) peptides and hyperphosphorylated tau, respectively (3). Aβ fibrils have been reported to compose SP and to be the main cause of the massive neurodegeneration observed in AD brains (5). Along with NFTs and SP, additional neuropathological characteristics of this disease include synapse loss and neuronal death (6). Synapses are a unique architecture formed by nerve cells and are considered to be the critical sites for pathogenesis in neurodegenerative diseases associated with aging (7,8). The cognitive impairment in AD mainly results from synaptic loss in neurons and the deterioration of synapses usually begins at the level of dendritic spines (9,10). Dendritic spines are tiny, bulbous structures protruding from the dendrites of neurons, which receive fast excitatory synaptic input in the brain. These structures compartmentalize the postsynaptic machinery and biochemical signaling molecules needed to respond to input from single presynaptic
terminals (11). Recently, it has been reported that Aβ1-42 can decrease dendritic spine density in rat primary hippocampal neuron cultures (12). Moreover, some studies conducted in AD mouse models have shown that the trajectories of axons and dendrites were altered in the proximity of amyloid plaques, which affected synaptic integration of signals (13,14).

Among Aβ peptides, Aβ1-42 is known to be the most neurotoxic (15). Its soluble oligomers may disrupt intracellular calcium homeostasis, leading to the activation of Cdk5. This proline-directed serine/threonine kinase regulates neuronal migration during development and maintains the survival and synaptic functions of mature neurons (16). It has been shown that the aberrant activity of Cdk5 induces the hyperphosphorylation of the neurofilament and microtubule-associated protein tau and serves an important role in neurodegeneration in AD (17). In addition, Cdk5 has been reported to act as an upstream regulator of mitochondrial fission during neuronal apoptosis. Inhibition of dynamin‑related protein 1 (Drp1)‑dependent mitochondrial fission alleviates neuronal apoptosis induced by aberrant Cdk5 expression (18). However, the underlying mechanism remains unknown. In our recent study, the mitochondrial fission protein Drp1 was identified as a direct substrate for Cdk5 (19). Aβ1-42 stimulates Cdk5-mediated phosphorylation of Drp1 at Ser579 in cortical neurons, thereby regulating mitochondrial fission-mediated neuronal apoptosis. However, whether Cdk5-mediated Drp1 phosphorylation is also involved in Aβ1-42-induced neurodegeneration is yet to be fully elucidated.

In the present study, we hypothesized that phosphorylation of Drp1 at Ser579 may be involved in the pathogenesis of neurodegeneration. To this end, a phosphorylation-defective (phosphor-defect) mutant lentiviral vector (Lenti-Drp1-S579A) was constructed to block Drp1 phosphorylation at Ser579. After infection, the expression level of Drp1-S579A was first confirmed in primary cultures of cortical neurons. The neurite outgrowth and synapse density of cortical neurons were observed under microscope. Consistent with our previous findings (19), blockage of Drp1 phosphorylation also prevented Aβ1-42-induced cleavage of caspase-3 and neuronal apoptosis. Taken together, the present findings demonstrated that phosphorylation of Drp1 at Ser579 served an important role in Aβ1-42-induced neurodegeneration, suggesting that this may be an effective strategy for the protection of neurons in this context.

Materials and methods

Experimental animals. A total of 26 C57BL/6 mice (6–8 weeks, 90-110 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd. Mice had free access to water and food at 22–25°C with a 12-h light/dark cycle. The humidity was ~60%. All animal handling was performed in accordance with the guidelines of Animal Research Committee of Nanchang University (20). All protocols described in this article were approved by the Ethics Committee for Animal Experimentation of Nanchang University (approval no. 2018-035). All surgical procedures involving experimental animals were performed under anesthesia with 1.0% pentobarbital sodium (50 mg/kg body weight) by intraperitoneal injection and the suffering of animals was minimized to the best of our ability.

Primary cortical neuronal cultures. As previously described (19), primary cortical neuronal cultures were derived from embryonic day 14-15 fetal C57BL/6 mouse brains. In brief, the cortex isolated from embryonic mouse brains was placed in DMEM (HyClone; Cytiva) and treated with 0.125% trypsin (Beijing Solarbio Science & Technology Co., Ltd.) and 0.004% DNase-I (Sigma-Aldrich; Merck KGaA) at 37°C for 15 and 10 min, respectively. Neurons were mechanically dissociated by pipetting and were seeded on poly-L-lysine (Sigma-Aldrich; Merck KGaA)-coated glass- or plastic-bottom 35-mm culture dishes (cell density was ~25,000–30,000/35-mm dish for microscopic observation, or ~45,000–50,000/35-mm dish for western blotting). Cells were first cultured in neurobasal plating medium (neurobasal medium (Thermo Fisher Scientific, Inc.), 2% B27 supplement, 0.5 mM L-glutamine, 25 µM L-glutamic acid, 1% penicillin-streptomycin (P/S), 10 mM HEPES, 10% FBS (Biological Industries)) and incubated at 37°C in a humidified incubator with 95% air and 5% CO2. On the second day, neuronal cells were cultured in neurobasal feeding medium (neurobasal medium, 2% B27 supplement, 0.5 mM L-glutamine, 1% P/S, 10 mM HEPES). Half the volume of media was replaced with the same volume of fresh neurobasal feeding media every 4 days.

Construction of Lenti-Drp1-S579A and infection procedure. Lenti-Drp1-S579A was constructed by Cyagen Biosciences, Inc. Briefly, Drp1-S579A site-directed mutagenesis was performed using a QuickChange kit (Agilent Technologies, Inc.) according to the manufacturer's instructions. Drp1-S579A was further subcloned into the pLV [Exp]-Puro-EF1A vector (Cyagen Biosciences, Inc.) and fused with a 6X His tag. The expression vector and package vectors (2 µg of each vector) were co-transfected into 293T cells (the American Type Culture Collection) using Lipopectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C for 6 h. After 48 h of culture, the supernatants containing the lentivirus were harvested. Purification was then performed using ultracentrifugation at 80,000 x g for 2 h at 4°C and the lentiviral titer was determined.

To evaluate the infection efficiency, neurons at 3 days in vitro (DIV) were infected with the empty lentiviral vector pLV [Exp]-Puro-EF1A-mCherry at a multiplicity of infection (MOI) of 1-5. Then, 3 days after infection, the fluorescence of mCherry was used to monitor and visualize the lentiviral infection under fluorescence microscope (IX71; Olympus Corporation). The formula for calculating the infection efficiency was as follows: (The number of infected cells/total cells in the field) x100%. To examine the expression level of the exogenous mutant Drp1-S579A, neurons at 3 DIV were infected with Lenti-Drp1-S579A or empty lentiviral vector at MOI of 1, 2 and 5 at 37°C for 8 h. At day 5 after infection, western blot analysis probed with anti-6HIs was carried out to examine the expression of phospho-defect Drp1-S579A in neurons.

Western blot analysis. Cultured neurons for western blot analysis were washed twice with PBS and lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology) for 30 min on ice. The whole cell lysate was harvested via sonication (20 KHz; 20 sec; 4°C) in 4X sample buffer and the protein concentration...
was measured using a BCA protein assay reagent kit (Beyotime Institute of Biotechnology). Proteins (20 μg per lane) were separated by 10% SDS-PAGE gel and further transferred onto PVDF membranes (MilliporeSigma). After blocking with 5% skim milk in TBS-0.1% Tween 20 (TBST) buffer for 30 min at room temperature, the membranes were probed overnight at 4°C with the following primary antibodies: Rabbit anti-Drp1 (cat. no. 8570, 1:1,000; Cell Signaling Technology, Inc.), phospho-Drp1-Ser616 (cat. no. 3455, 1:1,000; Cell Signaling Technology, Inc.), anti-6His (cat. no. CW0083S; CWBIO), cleaved caspase-3 (cat. no. 9644, 1:1,000; Cell Signaling Technology, Inc.), rabbit anti-microtubule associated protein 2 (MAP2; cat. no. 8707, 1:1,000, Cell Signaling Technology, Inc.) and β-actin (cat. no. 4967, 1:5,000; Cell Signaling Technology, Inc.). After three washes in TBST, the membranes were incubated with HRP-conjugated secondary antibodies (1:1,000; CWBIO) for 1 h at room temperature. Protein bands were detected using an ECL solution (CWBIO). Densitometric analysis was performed using ImageJ software (version 1.48; National Institutes of Health).

**Immunofluorescence staining.** The cortical neurons were fixed in 4% ice-cold paraformaldehyde (PFA; Beijing Solarbio Science & Technology Co., Ltd.) at 4°C for 10 min. After fixation, cells were washed three times with 1X PBS and permeabilized with 0.5% Triton-X 100 and 0.5% BSA (cat. no. A2058; Sigma-Aldrich; Merck KGaA) in PBS at room temperature for 30 min. Then, the cells were incubated with monoclonal rabbit anti-MAP2 (cat. no. 8707, 1:200; Cell Signaling Technology, Inc.) or monoclonal rabbit anti-synapsin-1 (cat. no. 6710, 1:200; Cell Signaling Technology, Inc.) antibodies at room temperature for 2 h and washed three times with 0.5% Triton-X 100 in 1X PBS. For immunofluorescence staining, cells were further incubated with Alexa Fluor 594-conjugated goat-rabbit IgG (1:200; Abcam) or Alexa Fluor 488-conjugated AffiniPure goat anti-rabbit IgG (1:200; ProteinTech Group, Inc.) antibodies in the dark at room temperature for 2 h. The primary and secondary antibodies were diluted in 1X PBS with 0.5% Triton-X 100 and 0.5% BSA. Immunofluorescence signals were observed using an inverted fluorescence microscope (IX71; Olympus Corporation; magnification, x40). To measure neurite length, ~30 neurons were randomly selected and captured using fluorescence or DIC images. Neurites longer than the diameter of the soma were defined as neurites. The fluorescent signals from MAP2 and synapsin-1 immunofluorescence staining were measured to evaluate neurite length and synapse density using ImageJ software. The neurites originating from the soma were calculated as the number of primary dendrites per neuron.

**Neuronal apoptosis assays.** As described previously (19), mouse cortical neuronal cultures were treated with or without 10 μM Aβ1-42 at 37°C for 24 h. To examine the effect of Cdk5-mediated Drp1 phosphorylation on Aβ1-42-induced neuronal apoptosis, neurons were infected with Lenti-Drp1-S579A 3 days prior to Aβ1-42 treatment. Then, 24 h after Aβ1-42 incubation, the cells were fixed with 4% ice-cold PFA at 4°C for 10 min. Immunofluorescence staining of MAP2 was performed to label cortical neurons. Hoechst 33258 (cat. no. 94403, Sigma-Aldrich; Merck KGaA) was used for nuclear DNA staining to evaluate chromosomal condensation and its morphological changes in neurons. After MAP2 immunofluorescence staining, the cells were further stained with Hoechst 33258 at room temperature for 5 min, according to the manufacturer's protocol. Then, the fluorescence of MAP2 and Hoechst 33258 was detected under a fluorescence microscope (IX71; Olympus Corporation; magnification, x40). Normal neuronal nuclei were stained blue, whereas apoptotic nuclei with decreased volume and condensed chromatin were stained shiny white. Neurons with condensed and fragmented Hoechst 33258 staining were counted as dead cells.

**Statistical analysis.** Statistical analysis was performed using SPSS software (version 17.0; SPSS, Inc.). Data are presented as the mean ± SEM and multiple comparisons between groups were performed using one-way ANOVA followed by post hoc Tukey's test. The comparisons between two groups were analyzed via unpaired Student's t-test. All experiments were repeated at least three times. P<0.05 was considered to indicate a statistically significant difference.

**Results.**

**Expression level of phospho-defective Drp1-S579A in primary cortical neurons.** To examine the role of Drp1 phosphorylation at Ser579 in Aβ1-42-induced neurodegeneration and apoptosis, a lentiviral vector carrying the phospho-defective mutant Drp1-S579A was constructed. As shown in Fig. 1A, cells were infected with a lentiviral vector carrying mCherry at different multiplicity of infections (MOIs; varying from 0-5) to evaluate infection efficiency. The percentage of mCherry...
fluorescence-positive cells reached 50-70% at a MOI of 2. Thus, in subsequent experiments, neuronal cultures were infected with Lenti-Drp1-S579A at an MOI of 2. As the phospho-defective Drp1-S579A is fused with a 6His tag in the lentiviral vector, the expression of Drp1-S579A in neurons after infection could be immunoblotted using 6His antibody. As presented in Fig. 1B, the expression of phospho-defective Drp1-S579A was detected in neurons infected with Lenti-Drp1-S579A. The exogenous mutant Drp1-S579A effectively downregulated the phosphorylation of Drp1 at Ser579 in neurons.

**Lenti-Drp1-S579A protects neurons against Aβ<sub>1-42</sub>-induced reduction in neurite length and dendritic loss.** Our previous study reported that Aβ<sub>1-42</sub> exerted an inhibitory effect on neurite outgrowth (19). The present results confirmed that primary cortical neurons at 9 DIV had long axons and well-developed dendrites. By contrast, neurons treated with 10 µM Aβ<sub>1-42</sub> had shortened atrophic axons and dendrites (Fig. 2A). When neurons were infected with Lenti-Drp1-S579A, the deleterious effect of Aβ<sub>1-42</sub> on neurites was markedly weakened (Fig. 2B and C). Moreover, immunofluorescence staining of MAP2 was used to label the neuronal processes (Fig. 3A). It was found that Lenti-Drp1-S579A prevented Aβ<sub>1-42</sub>-induced axonal and dendritic atrophy in cortical neurons. Aβ<sub>1-42</sub> significantly decreased neurite lengths and primary dendrite numbers in neurons, which was effectively alleviated by blockage of Drp1 phosphorylation via Lenti-Drp1-S579A (Fig. 3B and C). In addition, Lenti-Drp1-S579A prevented the downregulation of MAP2 in neurons following Aβ<sub>1-42</sub> stimulation (Fig. 3D).

**Lenti-Drp1-S579A attenuates the inhibitory effect of Aβ<sub>1-42</sub> on dendritic spine growth.** It has been reported that Aβ<sub>1-42</sub> has a deleterious effect on neurite outgrowth, including dendritic spines (21). For visualization of spines, primary cortical neurons were transfected with the pEGFP plasmid at 5 DIV. GFP allowed for the identification of neurons and quantification of spines under an inverted fluorescence microscope. As shown in Fig. 4A, mature dendritic spines were observed in control neurons. Compared with control cells, neurons subjected to 10 µM Aβ<sub>1-42</sub> exhibited a significant decrease in mature dendritic spines (4.44±0.21 vs. 1.45±0.26 spines/10 µm for control vs. Aβ<sub>1-42</sub>, respectively; Fig. 4B). To investigate the role of Drp1 phosphorylation in Aβ<sub>1-42</sub>-induced dendritic spine shrinkage, cortical neurons were infected with Lenti-Drp1-S579A at 3 DIV. Lenti-Drp1-S579A significantly restored dendritic spines in neurons after Aβ<sub>1-42</sub> treatment (1.45±0.26 vs. 3.44±0.43 spines/10 µm for Aβ<sub>1-42</sub> vs. Lenti-Drp1-S579A, respectively; Fig. 4B).

**Lenti-Drp1-S579A suppresses Aβ<sub>1-42</sub>-induced synaptic loss.** It is well recognized that synapses serve an important role in interneuronal communication and memory formation (3). Progressive synaptic loss is one of the major hallmarks of AD and is the main cause of memory impairment in patients with AD (14). Several studies have shown that incubation of cortical neurons with Aβ<sub>1-42</sub> decreases synapse number (22,23). Immunofluorescence staining of synapsin was conducted to detect synapses in neurons. As shown in Fig. 5A, synapsin-1-positive puncta were prevalent in neuronal processes in the control group. By contrast, synapsin-1 staining displayed a localization in the soma and decreased along neuronal processes after Aβ<sub>1-42</sub> incubation, indicating reduced synapse density (1.00 vs. 30.11±5.80% for Aβ<sub>1-42</sub> vs. Lenti-Drp1-S579A, respectively; Fig. 5B). Moreover, infection with Lenti-Drp1-S579A restored synapsin-1 staining along neuronal processes (30.11±5.80 vs. 100.90±13.66% for Aβ<sub>1-42</sub> vs. Lenti-Drp1-S579A, respectively; Fig. 5B).

**Lenti-Drp1-S579A protects synapses against Aβ<sub>1-42</sub>-induced apoptosis.** Exposure of cultured neurons to submicromolar concentrations of Aβ<sub>1-42</sub> may induce direct neurotoxicity (24).
Here, the effect of Lenti-Drp1-S579A on Aβ<sub>1-42</sub>-induced apoptosis was examined. To detect neuronal apoptosis, the cells were stained with Hoechst 33258 and MAP2 antibody. As presented in Fig. 6A, MAP2-positive cells were stained with Hoechst 33258 and MAP2 under fluorescence microscope. Fluorescence immunostaining of MAP2 was conducted in neurons after the indicated treatments. Neurite morphology was detected under a fluorescence microscope (magnification, x20). Scale bar, 50 µm. Effect of Lenti-Drp1-S579A on Aβ<sub>1-42</sub>-induced neurodegeneration and ATP levels in neurons (5.00±1.08 vs. 85.75±6.14% for control vs. Aβ<sub>1-42</sub>, respectively). Furthermore, infection with Lenti-Drp1-S579A efficiently alleviated Aβ<sub>1-42</sub>-induced apoptosis (85.75±6.14 vs. 39.25±2.25% for Aβ<sub>1-42</sub> vs. Lenti-Drp1-S579A, respectively; Fig. 6B).

Next, the cleavage of caspase-3 was examined using western blot analysis in neurons after Aβ<sub>1-42</sub> exposure. The results demonstrated that Aβ<sub>1-42</sub> significantly stimulated the cleavage of caspase-3 in neurons (1.00 vs. 1.73±0.08 for control vs. Aβ<sub>1-42</sub>, respectively), which was efficiently prevented by Lenti-Drp1-S579A (1.73±0.08 vs. 1.14±0.15 for Aβ<sub>1-42</sub> vs. Lenti-Drp1-S579A, respectively) (Fig. 7).

**Discussion**

AD is the most common neurodegenerative disease, with Aβ plaques as one of the major pathological hallmarks (25). In several AD mouse models, Aβ peptide deposition in the brain has been reported to be associated with various neuronal abnormalities, including the dystrophic neurites (26), dendritic spine loss (27), development of synaptic dysfunction (28) and abnormal neuronal firing (29). The variety of neuronal deficits associated with the deposition of Aβ peptides likely contributes to cognitive decline and memory loss in patients with AD (8, 30). It is considered that the neurotoxicity of Aβ<sub>1-42</sub> is responsible for neurodegeneration in the AD brain (31). In addition, aberrant activity of Cdk5 is also involved in the Aβ-evoked neurotoxic cascade (32). Cdk5 serves a vital role in the development of the central nervous system, maintenance of synaptic plasticity and neuronal apoptosis in response to stress (33). Moreover, it acts as an upstream regulator of Drp1-dependent mitochondrial fission during neuronal apoptosis (18). However, the underlying mechanism remains to be elucidated.

In our previous study, the mitochondrial fission protein Drp1 was identified as the direct substrate of Cdk5 and Aβ<sub>1-42</sub> effectively induced Cdk5-mediated Drp1 phosphorylation at Ser579. Furthermore, it was observed that Cdk5-mediated Drp1 phosphorylation at Ser579 was involved in Aβ<sub>1-42</sub>-induced mitochondrial fission and neuronal apoptosis (19), indicating that blockage of this process may be a possible strategy to prevent Aβ<sub>1-42</sub>-induced neurodegeneration. To prove this hypothesis, the current study constructed a lentiviral vector carrying phospho-defective Drp1-S579A. The expression level of total Drp1 and exogenous mutant Drp1-S579A in neurons were detected using western blotting with Drp1 antibody and anti-6His antibody, respectively. Notably, the mutant Drp1-S579A effectively decreased the level of Drp1 phosphorylation at Ser579 in neurons. This result was consistent with that of a previous study (34), in which the phosphorylation site at Ser579 remains to be elucidated.

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examined in neurons after the indicated treatments. β-actin was used as an endogenous control. (A) The expression level of cleaved caspase-3 was determined as synapse density. The graph indicates the relative synapse density along neuronal processes in each group. The data are presented as the mean ± SEM of at least three independent experiments. P<0.01. Cont, control; Aβ, amyloid β; Drp1, dynamin-related protein 1.

Next, the effect of Lenti-Drp1-S579A on Aβ1-42-induced neurite atrophy, synapse loss was examined. The results demonstrated that the Aβ1-42-mediated decrease in neurite length, synapse number and density of dendrites and dendritic spines was mostly prevented by the blockage of Drp1 phosphorylation at Ser579, but it does not suggest that Lenti-Drp1-S579A completely blocked all of the Aβ1-42-induced neuronal injury. Moreover, the Aβ1-42-induced neuronal apoptosis was still detected in Drp1-S579A + Aβ1-42 group. Neuronal apoptosis might be a later event in the Aβ1-42-induced degeneration of dendritic growth and loss of spines and synapses and the inhibitory effect of Lenti-Drp1-S579A on Aβ1-42-induced neurodegeneration is greater than neuronal apoptosis. Taken together, the current data support the important role of Cdk5-mediated Drp1 phosphorylation at Ser579 in Aβ1-42-induced neurodegeneration. In addition, the decrease in neurite length, synapse number and density of dendrite and dendrite spine is the common characteristic of neurodegeneration. Therefore, the present study indicates that blockage of Drp1 phosphorylation at Ser579 efficiently protects neurons against Aβ1-42-induced neurodegeneration.

On the other hand, it has been well documented that mitochondrial dysfunction serves a crucial role in various neurodegenerative diseases, including AD, Parkinson's disease and Huntington disease (35-37). Mitochondria are highly dynamic organelles, with their morphology changing frequently via fission and fusion events. Some large GTPases have been identified as regulators of mitochondrial fission and fusion. Mitochondrial outer membrane fission is mediated by Drp1 and mitochondrial fission protein (Fis1) (38). Optic atrophy and mitochondrial fission proteins regulate mitochondrial inner or outer membrane fusion, respectively (39). Several studies have reported abnormalities in mitochondrial function and dynamics in neurodegenerative diseases (14,40,41). Impaired balance of mitochondrial fusion and fission has been observed in the hippocampal tissue of patients with AD (41). Furthermore, inhibition of Drp1-mediated mitochondrial fission protects dopaminergic neurons against neurite loss and apoptosis following mitochondrial stress (42). Consistent with previous studies, the mutant Drp1-S579A alleviated Aβ1-42-induced cleavage of caspase-3 and neuronal apoptosis. In addition, the post translational modifications of Drp1 are closely associated with its activity and mitochondrial fission, including phosphorylation, SUMOylation and nitrosylation (43-48). Calmodulin-dependent protein kinase Ia phosphorylates Drp1 at Ser616 and triggers mitochondrial fission by promoting the interaction between Drp1 and Fis1 (43). GSK-3-mediated phosphorylation of Drp1 at Ser40 and Ser44 enhances the GTPase activity of Drp1 and induces mitochondrial fragmentation (45). In addition, S-nitrosylation of Drp1 also bridges excessive mitochondrial fission with neuronal injury during neurodegeneration (48). Therefore, it would be useful to further examine whether there is a crosstalk between Cdk5-mediated Drp1 phosphorylation and S-nitrosylation of the same protein in neurodegeneration.

The present study indicated that blockage of Drp1 phosphorylation at Ser579 protected cortical neurons against Aβ1-42-induced degeneration and apoptosis. However, there are some limitations in the present study. First, the effect of lenti-Drp1-S579A on neurodegeneration was only examined in primary cultured cortical neurons. It is still unclear whether...
blockage of Drp1 phosphorylation at Ser579 prevents neurodegeneration in vivo. Therefore, it is necessary to examine the effect of lenti-Drp1-S579A on neurodegeneration and neuronal apoptosis in AD animal model. Second, the functional consequences of Cdk5-mediated Drp1 phosphorylation remain controversial. It has been reported that Cdk5-mediated phosphorylation of Drp1 at Ser616 inhibits mitochondrial fission during neuronal maturation (47). By contrast, Jahani-Asl et al (47) and our previous study (19) revealed that Cdk5-mediated phosphorylation of Drp1 at Ser616 or Ser579 (the same conserved serine residue as Ser585 in different Drp1 isoforms) stimulates mitochondrial fission in neurons after exposure to N-methyl-d-aspartate or Aβ1-42, respectively. Although it is possible that the opposite effect of Cdk5-mediated Drp1 phosphorylation at the same conserved serine residue may be explained by the level of maturity of neurons, it would be useful to further investigate the underlying mechanism.

In conclusion, it was suggested that the atrophy of neuronal processes, synapse loss and neuronal apoptosis are the characteristic alterations in AD, a common neurodegenerative disease and that mitochondrial dysfunction is involved in the pathogenesis of AD. In our previous study, the Cdk5-mediated phosphorylation of Drp1 at Ser579 was found to regulate Aβ1-42-induced mitochondrial fission and neuronal apoptosis (19). Thus, it was necessary to further investigate the role of Drp1 phosphorylation at Ser579 in neurodegeneration. The current study constructed a lentiviral vector carrying phospho-defect Drp1-S579A. It was found that inhibition of Drp1 phosphorylation at Ser579 by Lenti-Drp1-S579A efficiently attenuated the Aβ1-42-mediated decrease in neurite length, synapse number, density of dendrites and dendritic spines and neuronal apoptosis. This suggests the involvement of Drp1 phosphorylation at Ser579 in neurodegeneration in AD and corroborates the potential of blocking this process to prevent neurodegeneration.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XJH and XHQ conceived and designed the research. DX and ZJY conducted the cellular and molecular biological experiments. PY, LPJ and YTO raised C57BL/6 mice and conducted neuronal primary culture. TY, JHS, QGL and YYW performed fluorescence microscopy. DX, PY and QGL analysed and interpreted the data. XJH and XHQ confirm the authenticity of all the raw data. XJH and LPJ wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The animal experiments in the present study were approved by the Animal Research Ethics Committee of Affiliated People's Hospital of Nanchang University (approval no. 2018-035).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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