

ORIGINAL RESEARCH ARTICLE

Brain-derived neurotrophic factor-overexpressing adipose-derived stem cells attenuate $A\beta_{1-42}$ -induced hippocampal neurodegeneration by suppressing apoptosis

Fangfang Liu^{1†}, Aiping Wang^{2†}, Xidi Chi¹, Guiyun Ye¹, Haolin Wu^{3*}, and Haofei Fan^{4*}

¹Clinical Laboratory, Nanping First Hospital Affiliated to Fujian Medical University, Nanping, Fujian, China

²Department of Basic Medicine, Faculty of Basic Medical Sciences, Hainan Vocational University of Science and Technology, Haikou, Hainan, China

³International Center for Aging and Cancer, Hainan Medical University, Haikou, Hainan, China

⁴Equipment Section, Logistics Management Office, School of Basic Medicine and Life Sciences, Hainan Medical University, Haikou, Hainan, China

[†]These authors contributed equally to this work.

***Corresponding authors:**

Haolin Wu
(15306156636@163.com)
Haofei Fan
(18876604041@163.com)

Citation: Liu F, Wang A, Chi X, Ye G, Wu H, Fan H. Brain-derived neurotrophic factor-overexpressing adipose-derived stem cells attenuate $A\beta_{1-42}$ -induced hippocampal neurodegeneration by suppressing apoptosis. *Eurasian J Med Oncol*. doi: 10.36922/EJMO026120137

Received: March 22, 2026

Revised: June 5, 2026

Accepted: June 10, 2026

Published online: July 14, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Introduction: Alzheimer's disease (AD) manifests as progressive hippocampal neurodegeneration coupled with deteriorating cognitive performance. Although brain-derived neurotrophic factor (BDNF) exerts neuroprotective effects, its translational potential remains constrained.

Objectives: This study evaluated whether BDNF-overexpressing adipose-derived mesenchymal stem cells (BDNF-ADSCs) exert neuroprotective effects in an AD mouse model.

Methods: AD-like hippocampal injury was induced by stereotaxic injection of $A\beta_{1-42}$ into the CA1 region of mice, followed by local transplantation of BDNF-ADSCs. Behavioral tests assessed anxiety-like behavior and cognitive function. Histological analysis evaluated hippocampal neuronal integrity, and apoptosis-related proteins were examined.

Results: BDNF-ADSC transplantation significantly alleviated anxiety-like behavior and spatial learning and memory impairments induced by $A\beta_{1-42}$. Histological analyses demonstrated reduced hippocampal neuronal loss and partial restoration of hippocampal cytoarchitecture. Moreover, $A\beta_{1-42}$ markedly increased Bax expression and caspase-3 activation while decreasing Bcl-2 levels, whereas BDNF-ADSCs reversed these alterations by suppressing pro-apoptotic signaling and enhancing anti-apoptotic protein expression.

Conclusion: BDNF-ADSCs protect against $A\beta_{1-42}$ -induced hippocampal injury and cognitive impairment, partly via modulation of the Bax/Bcl-2/caspase-3 apoptotic pathway, highlighting their potential as a cell-based therapeutic strategy for AD.

Keywords: Alzheimer's disease; Brain-derived neurotrophic factor; Adipose-derived stem cells; Neuronal apoptosis; Hippocampal neuroprotection

1. Introduction

Alzheimer's disease (AD), the leading cause of dementia, is a progressive neurodegenerative disorder characterized by cognitive decline and neuronal loss.¹ The World Health Organization has identified AD as a major global public health concern and ranks it as the sixth leading cause of death worldwide.² Clinically, AD presents with memory loss, language difficulties, executive dysfunction, visuospatial impairment, and attention deficits, accompanied by behavioral and psychological symptoms that severely reduce patients' quality of life.³ As the disease progresses, patients ultimately develop disability and premature death. By 2015, roughly 46.8 million individuals across the globe had been diagnosed with dementia, and this number is projected to increase dramatically by 2050.⁴ In China, more than 9.8 million individuals aged over 60 years suffer from AD, representing a substantial health burden.⁴

At the histopathological level, AD is distinguished by two signature lesions: extracellular deposits of β -amyloid (A β) plaques and intracellular tangles of paired helical filaments arising from tau hyperphosphorylation. These pathological processes contribute to synaptic function and neuronal loss.⁵ Although the precise mechanisms underlying A β accumulation and tau aggregation remain unclear, increasing evidence indicates that neuronal apoptosis plays a central role in AD progression, especially in memory-related regions, including the hippocampus and cerebral cortex.⁶ A β toxicity and abnormal tau phosphorylation activate multiple apoptotic signaling pathways, among which the Bax/Bcl-2/caspase-3 pathway is a key executor of programmed neuronal death.^{6,7} *In vitro* studies further demonstrate that tau overexpression counteracts A β -potentiated apoptosis through the mitochondrial caspase-3 cascade, whereas A β -induced neuronal death exhibits Bax dependency but operates independently of caspase activation in certain contexts.^{8,9} Reduced brain-derived neurotrophic factor (BDNF) mRNA and protein levels have been reported in hippocampal and cortical tissue and in the peripheral blood of individuals with AD.¹⁰ Gene transfer of BDNF via lentiviral vectors into transgenic AD mouse brains effectively counteracts synaptic injury in the entorhinal cortex and hippocampus, yielding measurable improvements in learning and memory.¹¹ However, BDNF's negligible blood-brain barrier penetrance and rapid systemic turnover severely curtail its therapeutic utility, compelling repeated invasive delivery.^{12,13} Therefore, an effective delivery system is essential to overcome these limitations.

Adipose-derived mesenchymal stem cells constitute multipotent mesenchymal progenitors residing within adipose tissue, exhibiting advantages including

abundant availability, minimal donor injury, stable genetic characteristics, low immunogenicity, and ethical acceptability.¹⁴⁻¹⁸ ADSCs exhibit broad therapeutic potential in tissue repair, regenerative medicine, and therapeutic management of neurological and cardiovascular diseases.¹⁷⁻²⁰ Importantly, ADSCs possess neuroprotective properties and show beneficial effects in models of neurodegenerative diseases, including spinal cord trauma and Parkinson's disease.²¹⁻²⁴ Moreover, ADSCs display strong migratory capacity toward injured tissues and can serve as efficient cellular carriers for exogenous genes or therapeutic molecules, making them suitable vectors for neurotrophic factor delivery.²⁵⁻²⁸ Previous studies have established that ADSC transplantation restores memory function in brain-damaged mice²⁹ and modulates apoptotic protein abundance within the hippocampal CA1 subfield.^{30,31} Given the key role of the Bax/Bcl-2/caspase-3 axis in AD-related neuronal apoptosis⁶, combining the neurotrophic effects of BDNF with the delivery and regenerative capabilities of ADSCs may provide a synergistic therapeutic strategy for AD.

In this work, we assessed the neuroprotective effects of BDNF-overexpressing ADSCs (BDNF-ADSCs) in an AD mouse model. Through behavioral assessments, immunofluorescence analysis, and evaluation of hippocampal protein expression, we aimed to elucidate whether BDNF-ADSCs could attenuate neuronal apoptosis via changes in the Bax/Bcl-2/caspase-3 signaling pathway, reduce A β accumulation, restore hippocampal structure, and improve cognitive function. This study provides mechanistic insight into the neuroprotective effects of BDNF-ADSCs and supports their potential as a novel therapeutic strategy for AD.

2. Materials and methods

2.1. Animals and materials

Male wild-type Kunming mice (7 weeks old, 35–45 g) used in this study were purchased from Changsha Tianqin Biotechnology Co., Ltd (China; License No. 430726230100448425). Animals were housed under standard conditions (22 $\pm$ 2 $^{\circ}$ C, 50 $\pm$ 5% humidity) with a 12-h light/dark cycle and had free access to food and water. Fifty mice were randomly assigned to five groups before surgery (n = 10 per group): control, A β ₁₋₄₂ + PBS, A β ₁₋₄₂ + ADSCs, A β ₁₋₄₂ + Z-DEVD-FMK, and A β ₁₋₄₂ + BDNF-ADSCs groups. A β ₁₋₄₂ oligomer (No. A9810) and dimethyl sulfoxide (DMSO; No. D8418) were purchased from Sigma-Aldrich (United States of America [USA]). Phosphate-buffered saline (PBS; No. C10010500BT) and 0.25% Trypsin-EDTA (No. 25200-072) were obtained from Gibco (USA). Mouse Adipose Stem Cell Complete Medium (No.

CM-M202) was purchased from Pricella (China). Triton X-100 (No. T109027) and bovine serum albumin (No. A116563) were obtained from Aladdin (China). Z-DEVD-FMK, a selective caspase-3 inhibitor (No. HY-12466), and a NeuN antibody (No. HY-P80241) were purchased from MedChemExpress (USA). Antibodies against BDNF (No. ab108319), Bax (No. ab53154), Bcl-2 (No. ab194583), pro-caspase-3 (No. ab32150), and cleaved caspase-3 (No. ab32042) were purchased from Abcam (USA). Enzyme-linked immunosorbent assay (ELISA) kits for BDNF (No. JL12317-48T), Bax (No. JL20134-48T), and Bcl-2 (No. JL13034-48T) were obtained from Shanghai Jianglai Biotechnology Co. (China). RIPA lysis buffer, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) reagents, bicinchoninic acid (BCA) protein assay kit, and related reagents were purchased from Hainan Aoweisi Biotechnology Co. (China).

2.2. Isolation and culture of adipose-derived mesenchymal stem cells

Adipose tissue was harvested from the inguinal and thigh regions of male wild-type mice aged 2–3 weeks. Tissue specimens were dissected into fine pieces, placed into 50-mL conical tubes, and enzymatically dissociated in 0.1% collagenase type I at 37 °C for 60 min under constant gentle agitation. The digestion was terminated by dilution with complete culture medium, followed by gentle pipetting to dissociate cells. The cell suspension was centrifuged at 1,300 rpm for 10 min, the supernatant was removed, and the pellet was washed twice with complete medium. The cell pellet was filtered through a 70- μ m cell strainer and seeded into 75 cm² culture flasks, then incubated at 37 °C in a humidified atmosphere containing 5% CO₂. Adherent cells were examined after 24 h, and the culture medium was replaced after 48 h. Upon reaching 80–90% confluence (approximately 3–4 days), ADSCs were passaged at a ratio of 1:2. Cells at passage 2 were expanded and cryopreserved for subsequent experiments.³²

2.3. Construction and validation of lentivirus vector

The full-length mouse BDNF cDNA was amplified by polymerase chain reaction and cloned into the pHBLV-CMVIE-ZsGreen-Puro lentiviral vector. Recombinant lentiviruses were produced by co-transfecting 293T cells with the transfer plasmid and packaging plasmids psPAX2 and pMD2.G using LipoFiter™ transfection reagent. Forty-eight h after transfection, the viral supernatant was collected and filtered through a 0.22- μ m cellulose acetate membrane. Lentivirus concentration was performed by Shanghai Hanshendong Biotechnology Co., Ltd (China) through ultracentrifugation at 140,000 $\times$ g for 2 h. ADSCs

were transduced with HBLV-BDNF-FLAG-ZsGreen or control HBLV-ZsGreen lentivirus at a multiplicity of infection of 40 in the presence of 5 μ g/mL polybrene. Lentivirus-mediated gene expression was confirmed by fluorescence microscopy, western blotting, and immunofluorescence analysis.³³

2.4. Construction of the hippocampal injury mouse model

Mice were anesthetized and positioned in a stereotaxic apparatus. Following exposure of the skull, a small burr hole was drilled above the left hippocampal CA1 region at the following stereotaxic coordinates: anteroposterior, –2.0 mm from bregma; mediolateral, 1.5 mm left of the midline; and dorsoventral, –2.0 mm from the skull surface. Using a microsyringe connected to a stereotaxic injection pump, A β _{1–42} oligomer solution (10 μ g/ μ L) was administered into the left hippocampal CA1 region at a volume of 1 μ L per mouse. The injection needle was retained in place for 5 min after administration to allow diffusion and prevent reflux. Upon surgical site closure, animals were transferred to a heated recovery platform until emergence from anesthesia was complete. Thereafter, they were randomized into the following treatment groups: control group, A β _{1–42} injury group, ADSC treatment group, caspase-3 inhibitor group, and BDNF-ADSC treatment group. Mice in the control group received a stereotaxic injection of the same volume of vehicle. Mice in the A β _{1–42} group received an A β _{1–42} injection only. In the ADSC treatment group, mice received a stereotaxic injection of ADSCs (1 $\times$ 10⁶ cells in 3 μ L) into the hippocampal CA1 region following A β _{1–42}-induced injury. In the caspase-3 inhibitor group, mice were administered Z-DEVD-FMK (50 mM) at a volume of 0.5 μ L into the hippocampus following A β _{1–42} injection. In the BDNF-ADSC treatment group, mice received a stereotaxic injection of BDNF-modified ADSCs (1 $\times$ 10⁶ cells in 3 μ L) into the hippocampal CA1 region after A β _{1–42}-induced injury. For all stereotaxic injections, the needle was left in place for 5 min following administration to ensure adequate diffusion. BDNF-ADSC or ADSC transplantation was performed four weeks after A β _{1–42}-induced hippocampal injury.³⁴ Twenty-four h after stereotaxic injection of A β _{1–42} oligomers into the hippocampal CA1 region, establishment of the neuronal injury model was assessed by behavioral testing and confirmed histologically in a separate group of mice. BDNF-ADSC transplantation was performed at the same stereotaxic coordinates four weeks after A β _{1–42} injection. Aseptic technique was maintained throughout all operative interventions, with post-procedural observation continuing until animals achieved full recovery from anesthesia.

2.5. Open field test

Behavioral assessment was conducted within a 50 × 50 × 40 cm square open-field arena. Central positioning of visual landmarks provided spatial reference cues, while locomotor activity was captured via a ceiling-mounted video recording system. Each mouse was individually placed in the center of the arena and allowed to freely explore for 30 min. Between successive trials, the apparatus was sanitized with 75% ethanol to remove residual odors. Over the 30-min observation window, Smart 3.0 software (Panlab, Spain) automatically extracted and analyzed spatial metrics, including central dwell time and total locomotor path length.

2.6. Morris water maze

The Morris water maze test was performed over four consecutive days in a circular pool (diameter: 1.2 m; water depth: 0.15 m) maintained at 20 ± 1 °C and rendered opaque with non-toxic black ink. The apparatus comprised a circular pool partitioned into four equal sectors, with an escape platform submerged 1 cm beneath the waterline in the designated target quadrant. Animal trajectories were captured via a ceiling-mounted video system and quantified using Smart 3.0 behavioral tracking software. On day 1, mice were allowed to swim freely for 60 s without a platform for habituation, followed by visible platform training. On days 2 and 3, hidden platform trials were conducted, and escape latency was recorded with a maximum trial duration of 60 s. Mice that failed to locate the platform within 60 s were guided to it and allowed to remain there for 10 s. On the fourth day, the platform was removed for the probe trial, and the number of crossings over the former platform location during the 60-s probe trial was recorded.

2.7. Sample preparation

For cell samples, cells were collected into sterile tubes, resuspended in 200 μ L of PBS, and sonicated for 30 s. Samples were centrifuged at 1,500 × g for 10 min at 4 °C, and the supernatant was collected and stored at –80 °C for subsequent analyses. Following completion of the behavioral tests, mice were deeply anesthetized with isoflurane and transcardially perfused with PBS (pH 7.4), followed by 4% paraformaldehyde. Brains were removed and post-fixed at 4 °C overnight. One portion of the hippocampus was isolated and stored at –80 °C, while the remaining brain tissue was cryoprotected in 30% sucrose solution until equilibration.

2.8. Immunofluorescence staining

For cellular immunofluorescence, untransduced ADSCs, HBLV-ZsGreen-transfected ADSCs, and HBLV-BDNF-

FLAG-ZsGreen-transfected ADSCs were seeded into 24-well plates at a density of 4–5 × 10⁵ cells/well and cultured for 24 h. Cells were incubated with anti-BDNF primary antibody (1:100) at 4 °C overnight, followed by incubation with fluorescent secondary antibody at 37 °C for 1 h in the dark. Images were captured using a fluorescence microscope (BX51+DP72, Olympus Corporation, Japan). For immunofluorescence characterization, hippocampal tissue sections underwent dewaxing, graded ethanol rehydration, and heat-mediated epitope unmasking in citrate buffer. Nonspecific binding was blocked with 10% goat serum prior to overnight probing (4 °C) with primary antibodies directed against caspase-3 (1:2,000), Bax (1:1,000), or Bcl-2 (1:1,000), followed by development with species-matched secondary antibodies. Immunoreactive signals were visualized, and semi-quantitative analysis was performed using ImageJ software (v1.53; NIH, USA). For NeuN staining, fixed brain sections were permeabilized with 0.2% Triton X-100 and incubated with anti-NeuN antibody (1:1,000). Quantitative analysis was performed using ImageJ.

2.9. Cresyl violet staining

Coronal brain slices (35 μ m) were generated using a Cryostat (HM 550, Thermo Fisher Scientific, USA) and maintained in cryoprotective medium at 4 °C. For histological examination, sections were mounted on glass slides and air-dried at room temperature, and subjected to cresyl violet staining for 3 min. Following aqueous washing, sections were dehydrated through graded ethanol solutions, xylene clearing, and final mounting under coverslips with neutral synthetic resin. Bright-field microscopy (BX51+DP72, Olympus Corporation, Japan) was then employed to assess hippocampal cytoarchitectural integrity.

2.10. Hematoxylin and eosin staining

To prepare sections for hematoxylin and eosin (H & E) staining, mouse brain tissues were fixed in 4% paraformaldehyde overnight at 4 °C, cryoprotected in 30% sucrose in PBS until the tissues sank, and embedded in optimal cutting temperature compound. Coronal brain sections (20–40 μ m thick) were prepared using a cryostat (HM 550, Thermo Fisher Scientific, USA) and mounted on gelatin-coated glass slides. Sections were stained using a commercial H & E staining kit (catalog no. C0105S, Beyotime Biotechnology, China) according to the manufacturer's instructions. Briefly, sections were rinsed in distilled water, stained with hematoxylin solution for 3–5 min, differentiated in 1% acid alcohol, and counterstained with eosin Y solution for 30 s to 1 min. After dehydration through graded ethanol solutions (70%, 80%, 95%, and

100%) and clearing in xylene, sections were mounted with neutral balsam and examined under a light microscope at 20 $\times$ magnification.

2.11. Enzyme-linked immunosorbent assay

Protein levels of BDNF, Bax, and Bcl-2 were quantified by sandwich ELISA according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (EPOCH 2, BioTek Instruments, USA), with sample concentrations interpolated from calibration curves.

2.12. Western blot analysis

Six mice from each group were sacrificed, and the left hippocampi were collected. Total protein was extracted using RIPA lysis buffer, and protein concentrations were determined by BCA assay. Equal amounts of protein were separated by SDS-PAGE, transferred onto polyvinylidene fluoride membranes, and blocked with nonfat dry milk. Membranes were incubated overnight at 4 °C with primary antibodies against caspase-3 (1:2,000), Bax (1:1,000), and Bcl-2 (1:1,000), followed by incubation with HRP-conjugated secondary antibodies at room temperature. Protein bands were visualized and quantified using ImageJ software.

2.13. Statistical analysis

Morphometric measurements were quantified using ImageJ. Statistical analyses were performed using GraphPad Prism 8 (GraphPad, USA). Group data are expressed as mean $\pm$ standard deviation (SD). Intergroup differences were evaluated by one-way ANOVA, with pairwise contrasts subjected to Student–Newman–Keuls *q* post hoc test. Statistical significance was set at *p* < 0.05.

3. Results

3.1. Lentiviral transfection induces robust brain-derived neurotrophic factor expression in adipose-derived mesenchymal stem cells

Morphology and ZsGreen fluorescence of ADSCs were observed 72 h after lentiviral transfection. As shown in Figure 1A, primary ADSCs displayed a spindle-shaped, adherent morphology. Strong ZsGreen fluorescence was detected in both the HBLV-ZsGreen and HBLV-BDNF-FLAG-ZsGreen groups, indicating high transfection efficiency. Immunoblotting revealed robust elevation of both BDNF and FLAG signal in ADSCs engineered with the HBLV-BDNF-FLAG-ZsGreen construct, relative to both untransduced controls and empty vector (HBLV-ZsGreen) transfectants (Figure 1B). Densitometric analysis further confirmed a significant increase in BDNF and FLAG protein

levels following BDNF lentiviral transduction (Figure 1C and 1D). Quantitative ELISA revealed a significant increase in secreted BDNF levels in ADSC-conditioned medium of ADSCs transduced with HBLV-BDNF-FLAG-ZsGreen, relative to both naive and empty-vector controls (Figure 1E). Collectively, these results confirm successful lentiviral-mediated overexpression and secretion of BDNF in ADSCs.

3.2. BDNF-ADSC transplantation attenuates behavioral deficits and hippocampal neuronal loss induced by A β ₁₋₄₂

Anxiety-like behavior was first evaluated using the open field test. Compared with the control group, A β ₁₋₄₂-intervention mice (PBS group) spent significantly less time in the central area (Figure 2A) and exhibited a markedly reduced total travel distance (Figure 2B), indicating decreased exploratory activity and increased anxiety-like behavior. Treatment with ADSCs, Z-DEVD-FMK, or BDNF-ADSCs significantly increased the time spent in the center and/or the total distance traveled compared with the A β ₁₋₄₂ (PBS) group, with BDNF-ADSCs showing the most pronounced improvement. Spatial learning and memory were further assessed using the Morris water maze probe test. Representative swimming trajectories are shown in Figure 2C. A β ₁₋₄₂-intervention mice exhibited impaired spatial memory, as evidenced by fewer platform crossings (Figure 2D) and prolonged latency to first entry into the target zone (Figure 2F) compared with controls. Notably, treatment with ADSCs, Z-DEVD-FMK, or BDNF-ADSCs significantly increased platform crossings and shortened the latency to enter the target zone relative to the A β ₁₋₄₂ (PBS) group (Figure 2D and 2E). To further determine whether the behavioral improvements were associated with neuroprotection, cresyl violet staining was performed to assess hippocampal neuronal morphology. As shown in Figure 2F, control mice displayed intact cytoarchitecture and a well-organized pyramidal cell layer in the CA1 region. In contrast, A β ₁₋₄₂ injection caused severe hippocampal damage characterized by disrupted cytoarchitecture and marked loss of pyramidal neurons. Notably, BDNF-ADSC transplantation partially restored the CA1 pyramidal cell layer and improved hippocampal structural integrity, as evidenced by well-defined somatic contours, uniform Nissl body staining, enhanced nucleolar visibility, and increased cellular density surrounding the needle track. The presence of this track directly confirms successful delivery of the cell suspension to the targeted CA1 region, while the surrounding histological alterations collectively indicate that the transplanted cells survived within the local microenvironment and exerted neuroprotective effects. Quantitative analysis confirmed that CA1 neuronal

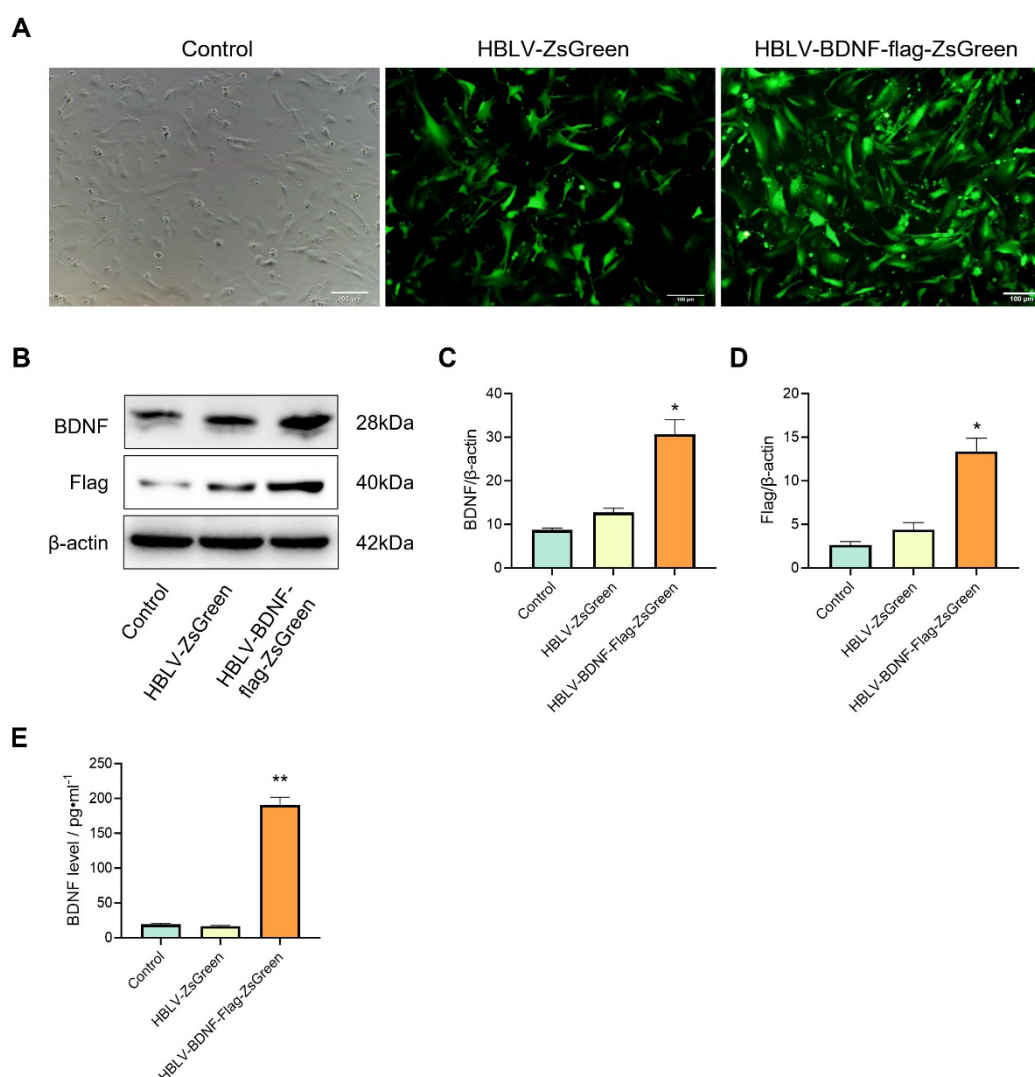


Figure 1. Lentiviral-mediated overexpression of brain-derived neurotrophic factor (BDNF) in adipose-derived mesenchymal stem cells (ADSCs). (A) Representative bright-field and fluorescence images showing ADSC morphology and ZsGreen expression following transfection with HBLV-ZsGreen or HBLV-BDNF-FLAG-ZsGreen. Scale bar: 100 μ m; magnification: 40 $\times$. (B) Representative immunoblots showing protein expression of BDNF and FLAG in ADSCs after lentiviral transfection, normalized to β -actin as the loading control. (C–D) Quantitative densitometric analysis of BDNF and FLAG protein levels normalized to β -actin. (E) ELISA analysis of BDNF concentration in ADSC culture supernatants. Values represent the mean $\pm$ standard deviation from three independent experiments. * $p < 0.05$ and ** $p < 0.01$ versus the control group.

density was significantly reduced following A β_{1-42} injury compared with the control group, whereas BDNF-ADSC transplantation significantly increased neuronal density relative to the A β_{1-42} group, although it did not fully return to control levels (Figure 2G). Together, these findings demonstrate that BDNF-ADSC transplantation alleviates anxiety-like behavior and cognitive deficits and attenuates

hippocampal neuronal loss induced by A β_{1-42} .

3.3. BDNF-ADSC transplantation promotes neuronal and structural recovery in the hippocampus after A β_{1-42} injury

To evaluate hippocampal neuronal integrity following A β_{1-42} -induced injury, NeuN immunofluorescence

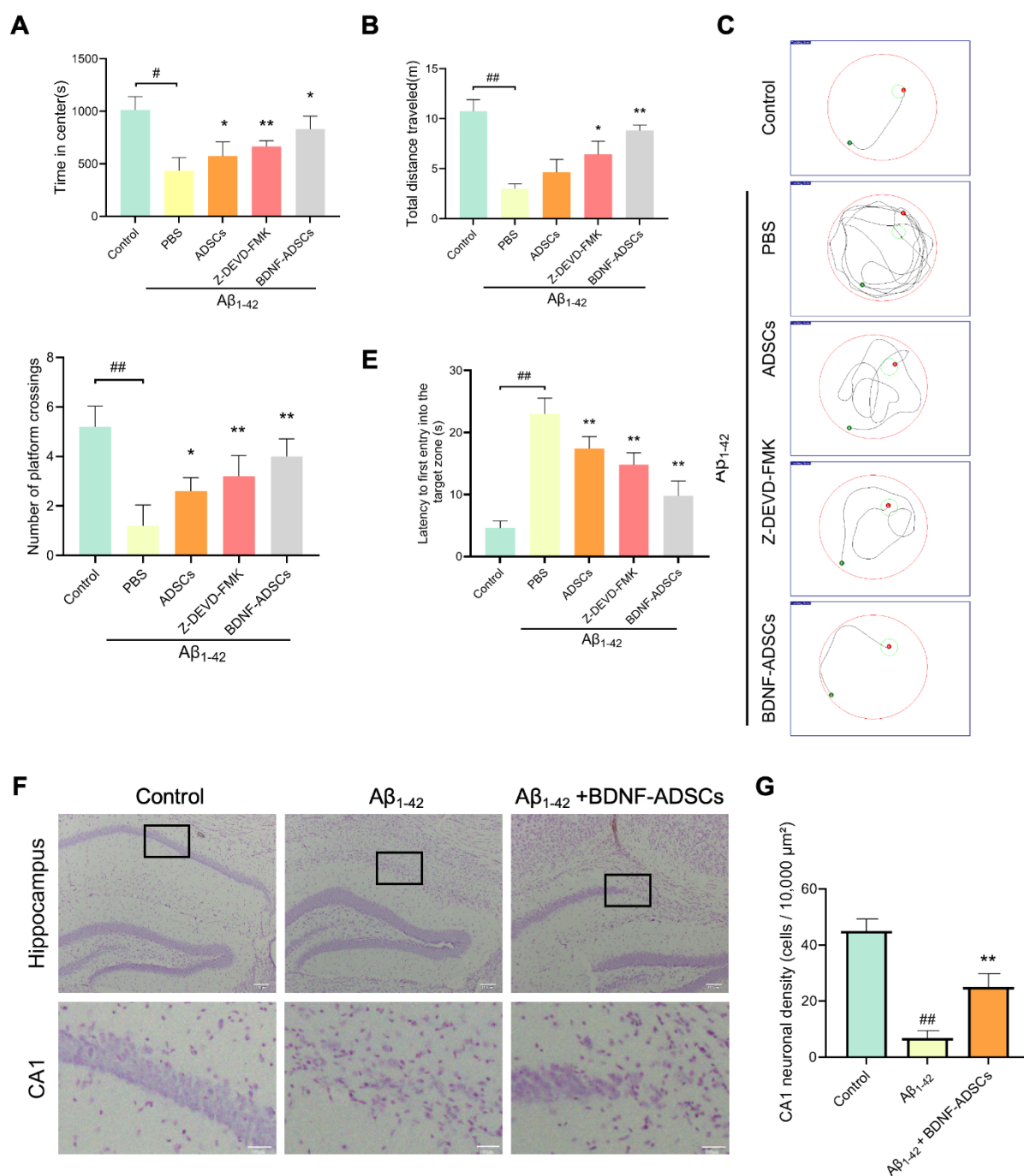


Figure 2. BDNF-ADSC transplantation attenuates A β ₁₋₄₂-induced behavioral deficits and hippocampal neuronal loss. (A) Duration of central zone occupancy during open field assessment. (B) Cumulative ambulatory distance across the open field arena. (C) Representative swimming trajectories during the Morris water maze probe trial. (D) Number of platform crossings during the probe trial. (E) Latency to first entry into the target zone during the probe trial. (F) Representative cresyl violet-stained coronal sections of the hippocampus showing overall hippocampal architecture and CA1 pyramidal neurons in control, A β ₁₋₄₂, and A β ₁₋₄₂ + BDNF-ADSC groups. Boxed areas indicate regions shown at higher magnification. The needle track indicates the transplantation site of BDNF-ADSCs. Scale bars: 100 μ m; magnification: 40 $\times$. (G) Neuronal packing density within hippocampal CA1, quantified as cells per 10,000 μ m². Values denote mean $\pm$ standard deviation ($n = 3$). * $p < 0.05$ and ** $p < 0.01$ versus the control group; * $p < 0.05$ and ** $p < 0.01$ versus the A β ₁₋₄₂-PBS group.

staining was performed. Control animals exhibited densely packed NeuN-immunoreactive neurons throughout the hippocampal formation (Figure 3A). In contrast, A β_{1-42} -intervention mice (PBS group) exhibited a marked reduction in NeuN immunoreactivity in the left hippocampus, indicating substantial neuronal loss. Compared with the A β_{1-42} group, treatment with ADSCs, Z-DEVD-FMK, or BDNF-ADSCs increased NeuN immunoreactivity, with the BDNF-ADSC group showing the most pronounced restoration. Consistent with the immunofluorescence findings, H & E staining revealed clear alterations in hippocampal cytoarchitecture (Figure 3B). The control group displayed intact hippocampal layering with well-organized granule cell and pyramidal cell layers, whereas the A β_{1-42} (PBS) group showed disrupted laminar organization, loosely arranged cells, and abnormal neuronal morphology. Unmodified ADSCs provided only limited improvement in overall structural integrity. In contrast, Z-DEVD-FMK treatment partially normalized hippocampal organization, and BDNF-ADSC transplantation markedly restored hippocampal architecture, as evidenced by improved continuity and reorganization of the granule cell and pyramidal layers. Quantitative analysis showed that the number of hilar interneurons was significantly higher in the BDNF-ADSC group than in the A β_{1-42} + PBS group (Figure 3B). Collectively, these results demonstrate that BDNF-ADSC transplantation effectively attenuates A β_{1-42} -induced hippocampal neuronal loss and promotes structural recovery in the injured hippocampus.

3.4. BDNF-ADSC transplantation suppresses A β_{1-42} -induced hippocampal neuronal apoptosis

To investigate whether BDNF-ADSCs modulate hippocampal apoptosis following A β_{1-42} injury, immunofluorescence double staining for Bax and Bcl-2 was performed. As shown in Figure 4A, Bax immunoreactivity was minimal in the hippocampus of control mice, whereas Bcl-2 exhibited a basal level of expression. In contrast, A β_{1-42} -intervention mice (PBS group) displayed markedly increased Bax immunoreactivity accompanied by a pronounced reduction in Bcl-2 immunoreactivity, indicating activation of pro-apoptotic pathways. Compared with the A β_{1-42} group, ADSC treatment partially reduced Bax expression and modestly restored Bcl-2 immunoreactivity. Notably, treatment with either Z-DEVD-FMK or BDNF-ADSCs resulted in a substantial decrease in Bax immunoreactivity and a marked increase in Bcl-2 expression, with the BDNF-ADSCs group showing the most pronounced shift toward an anti-apoptotic profile. Quantitative analysis further confirmed that BDNF-ADSCs significantly reduced the Bax-positive area,

increased the Bcl-2-positive area, and markedly elevated the Bcl-2/Bax ratio compared with the A β_{1-42} group (Figure 4B). Consistent with the immunofluorescence findings, ELISA analysis revealed that A β_{1-42} exposure significantly increased Bax levels while decreasing Bcl-2 levels compared with the control group. treatment with ADSCs, Z-DEVD-FMK, and BDNF-ADSCs attenuated A β -induced neuronal injury and apoptosis to varying degrees, with BDNF-ADSCs producing the most robust reduction in Bax and elevation in Bcl-2 (Figure 5A and 5B). Western blot analysis further demonstrated that A β_{1-42} injury markedly increased Bax expression and caspase-3 activation, as indicated by elevated cleaved caspase-3 levels and an increased cleaved caspase-3/pro-caspase-3 ratio, while Bcl-2 expression was significantly reduced (Figure 5C). ADSC treatment showed limited effects on these apoptotic markers. In contrast, Z-DEVD-FMK and BDNF-ADSCs significantly increased Bcl-2 expression and suppressed Bax and cleaved caspase-3 levels, resulting in a marked reduction in the Bax/Bcl-2 ratio and caspase-3 activation (Figure 5D and 5E). Collectively, these results indicate that BDNF-ADSCs effectively attenuate A β_{1-42} -induced hippocampal neuronal apoptosis, in association with modulation of Bax/Bcl-2/caspase-3 signaling.

4. Discussion

In this study, we investigated the neuroprotective effects of BDNF-ADSCs in an A β_{1-42} -induced mouse model of AD. Our findings demonstrate that BDNF-ADSC transplantation attenuated hippocampal neuronal loss and ameliorated deficits in spatial learning and memory. Importantly, these functional and structural improvements were accompanied by suppression of hippocampal neuronal apoptosis through modulation of the Bax/Bcl-2/caspase-3 signaling pathway. Together, these results provide evidence that BDNF-ADSCs exert neuroprotective effects against A β -induced neurodegeneration, at least in part, through modulation of apoptotic signaling pathways.

Alzheimer's disease is characterized by progressive cognitive decline and selective vulnerability of hippocampal neurons, yet the precise mechanisms underlying neuronal loss remain incompletely understood.³⁵⁻³⁷ Accumulating evidence suggests that A β , particularly the A β_{1-42} species, initiates a cascade of neurotoxic events, including oxidative stress, calcium dysregulation, mitochondrial dysfunction, and activation of programmed cell death pathways.³⁸⁻⁴¹ These pathological processes converge on apoptotic signaling, making neuronal apoptosis a critical and potentially targetable mechanism in the early stages of AD pathology.^{42,43} Consistent with these observations, intracerebral A β_{1-42} injection in the present study induced pronounced hippocampal neuronal loss, anxiety-like

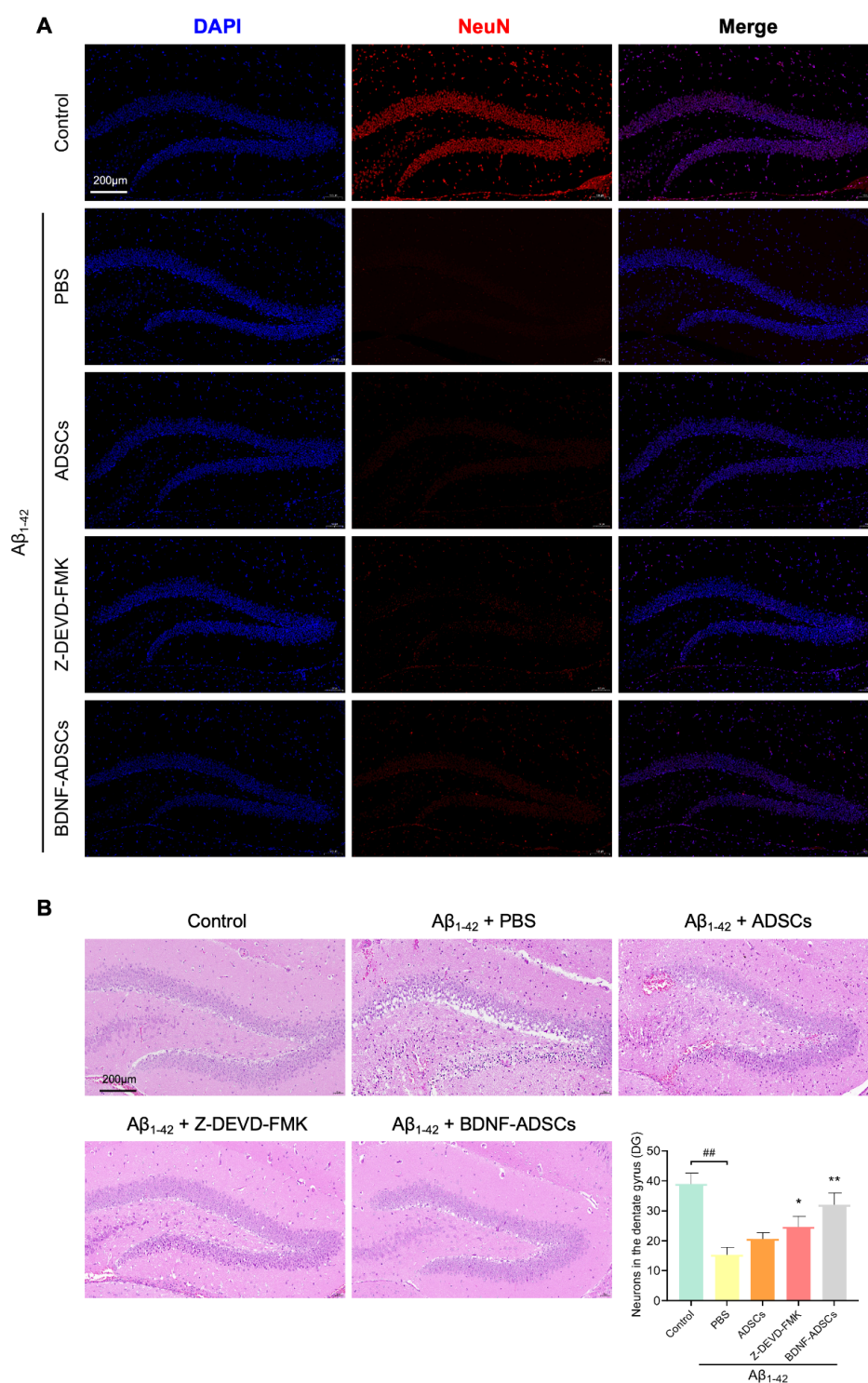


Figure 3. BDNF-ADSC transplantation promotes hippocampal neuronal and structural recovery following A β_{1-42} injury. (A) Representative immunofluorescence images of hippocampal sections stained with NeuN (red) and DAPI (blue) in the control, A β_{1-42} (PBS), ADSC, Z-DEVD-FMK, and BDNF-ADSC groups. Scale bars: 200 μ m; magnification: 20 $\times$. (B) Representative hematoxylin and eosin-stained hippocampal sections showing hippocampal cytoarchitecture, together with quantification of hilar interneurons in the hilus of the dentate gyrus. Data are presented as mean $\pm$ standard deviation ($n = 3$). $^{##}p < 0.01$ vs. control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. A β_{1-42} (PBS) group. Scale bars: 200 μ m; magnification: 20 $\times$.

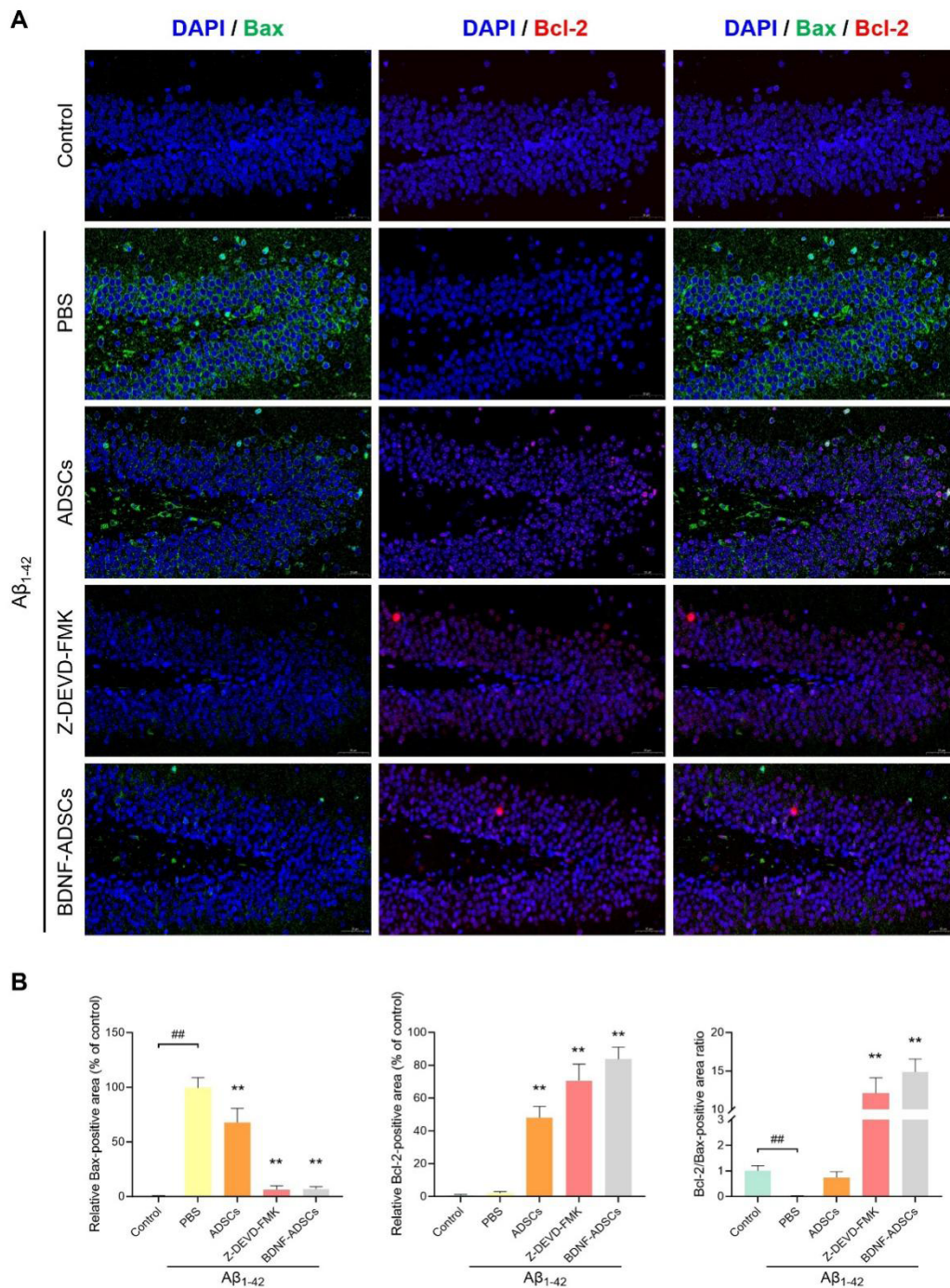


Figure 4. BDNF-ADSC transplantation suppresses A β_{1-42} -induced hippocampal apoptosis by modulating Bax and Bcl-2 expression. (A) Representative immunofluorescence images of hippocampal sections showing Bax (green), Bcl-2 (red), and DAPI (blue) staining in control, A β_{1-42} (PBS), ADSC, Z-DEVD-FMK, and BDNF-ADSC groups. Scale bars: 50 μ m; magnification: 40 $\times$. (B) Quantification of Bax and Bcl-2 immunofluorescence and calculation of the Bcl-2/Bax immunoreactivity ratio. Values denote mean $\pm$ standard deviation ($n = 3$). $^{##}p < 0.01$ versus the control group; $^{**}p < 0.01$ versus the A β_{1-42} -PBS group.

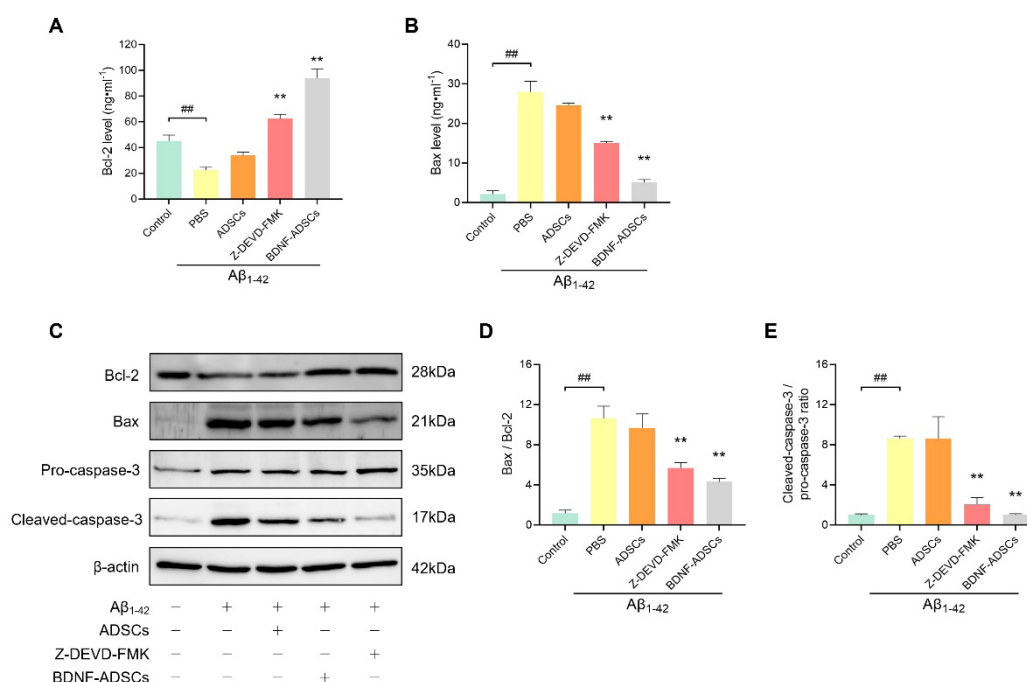


Figure 5. BDNF-ADSCs inhibit A β_{1-42} -induced hippocampal apoptosis via the Bax/Bcl-2/caspase-3 pathway. (A) ELISA quantification of Bcl-2 levels in hippocampal tissue. (B) ELISA quantification of Bax levels in hippocampal tissue. (C) Representative immunoblots showing Bcl-2, Bax, pro-caspase-3, cleaved caspase-3, and β -actin protein bands. (D) Densitometric quantification of the Bax/Bcl-2 expression ratio. (E) Densitometric analysis of the cleaved caspase-3/pro-caspase-3 ratio. Values denote mean $\pm$ standard deviation ($n = 6$). $^{##}p < 0.01$ versus the control group; $^{**}p < 0.01$ versus the A β_{1-42} -PBS group.

behavior, and spatial learning and memory impairment, supporting the validity of this model for mechanistic studies.^{40,44}

Adipose-derived mesenchymal stem cells have attracted increasing attention in regenerative medicine due to their accessibility, low immunogenicity, and potent paracrine activity.^{14,45} Previous studies have shown that ADSCs secrete a variety of neurotrophic and anti-inflammatory factors, including BDNF, nerve growth factor, vascular endothelial growth factor, and insulin-like growth factor, which collectively contribute to neural repair and functional recovery in neurodegenerative disease models.^{46,47} Among these factors, BDNF plays a particularly critical role in promoting neuronal survival, synaptic plasticity, and cognitive function. However, the clinical utility of exogenous BDNF is limited by its poor blood-brain barrier permeability and short biological half-life.^{48,49} By using ADSCs as a cellular vehicle for sustained local BDNF delivery, our approach may overcome these limitations and provide a more physiologically relevant mode of neurotrophic support.

Behavioral analyses in the present study revealed that BDNF-ADSC transplantation significantly alleviated anxiety-like behavior and improved spatial learning and memory deficits induced by A β_{1-42} . These functional improvements were accompanied by enhanced hippocampal neuronal survival and partial restoration of hippocampal cytoarchitecture, as demonstrated by NeuN immunofluorescence and histological analyses. The concordance between behavioral recovery and structural preservation suggests that BDNF-ADSCs exert neuroprotective effects at both functional and cellular levels.

Apoptosis is widely recognized as a major mechanism underlying neuronal loss in AD.⁴² Activation of the intrinsic apoptotic pathway, particularly the Bax/Bcl-2/caspase-3 signaling axis, has been implicated in A β -induced neurotoxicity.^{6,43,50} Further studies have found that A β_{1-42} can directly induce neuronal apoptosis and accelerate AD progression.⁵¹ In this study, A β_{1-42} exposure markedly increased Bax expression and caspase-3 activation while reducing Bcl-2 levels in the hippocampus,

indicating enhanced apoptotic signaling. In contrast, BDNF-ADSC transplantation significantly reversed these molecular alterations by suppressing Bax and cleaved caspase-3 expression while upregulating the anti-apoptotic protein Bcl-2. Notably, these effects were comparable to those observed following pharmacological inhibition of caspase-3, further supporting the involvement of the apoptotic pathway in mediating the neuroprotective effects of BDNF-ADSCs.

Although the anti-apoptotic effects of BDNF-ADSCs and caspase-3 inhibitors appeared comparable in this study, these two strategies differ fundamentally in their mechanistic depth and translational potential, warranting further clarification.

Although caspase-3 inhibitors and BDNF-ADSCs exhibited comparable anti-apoptotic phenotypic effects in this study, they differ fundamentally in mechanistic depth and translational potential. The former merely blocks the terminal execution step of apoptosis, failing to intervene in upstream pathological processes such as A β neurotoxicity, mitochondrial dysfunction, and neuroinflammation; moreover, given the broad non-apoptotic physiological functions of caspases—including regulation of neurogenesis and immune responses—their inhibition carries off-target toxicity risks that have led to the failure of most caspase inhibitors in clinical trials due to safety concerns.^{52,53} In contrast, BDNF-ADSCs achieve disease modification through multi-target synergism: transplanted cells sustainably secrete neurotrophic factors to activate pro-survival pathways, thereby suppressing apoptotic signaling at its origin, while concurrently modulating microglial polarization, inhibiting inflammasome activation, and promoting A β clearance through paracrine effects, thus orchestrating the coordinated regulation of multiple cell death pathways, including apoptosis, autophagy, and pyroptosis.^{53,54} Furthermore, engrafted ADSCs establish a long-acting “biological micropump” of bioactive factors within the host brain, overcoming the limitations of blood-brain barrier permeability and the need for continuous administration that constrain conventional pharmacological approaches. Of particular significance, neuronal apoptosis in AD patients often reaches an irreversible stage where blockade of terminal execution molecules cannot salvage lost neural network function; BDNF-ADSCs not only protect surviving neurons but also promote endogenous neurogenesis, angiogenesis, and synaptic remodeling, providing a structural foundation for functional recovery.^{55,56}

This multi-dimensional integration of neuroprotection, immunomodulation, and microenvironmental remodeling positions BDNF-ADSCs as a disease-modifying strategy

that aligns more closely with the multifactorial, multi-pathway, and chronically progressive pathological nature of AD than single-target pharmacological interventions.

The core innovation of this study does not lie in validating the neuroprotective role of the BDNF-TrkB signaling pathway against A β toxicity, as this mechanism has been extensively confirmed and well established; rather, the contribution resides in the optimization of therapeutic strategy through lentiviral-mediated genetic engineering to transform ADSCs into a “biological pump” capable of sustained and localized BDNF secretion. This approach effectively addresses critical clinical bottlenecks of recombinant BDNF protein, including its short plasma half-life, poor blood-brain barrier permeability, and systemic side effects, while achieving dual functionality of cell replacement and neurotrophic support that is fundamentally distinct from BDNF protein injection or naive ADSC transplantation alone. Notably, the paracrine action of ADSCs represents a core mechanism underlying their neuroprotective effects, as evidenced by the partial functional recovery observed in the empty-vector ADSC group in this study, which corroborates existing findings that ADSCs can modulate microglial polarization toward the M2 phenotype through secretion of transforming growth factor beta, tumor necrosis factor-stimulated gene 6 protein, and interleukin-10.⁵⁷ However, the primary objective of this study was to further enhance this therapeutic potential: BDNF-genetically modified ADSCs not only preserve the inherent paracrine capabilities but also achieve synergistic amplification through BDNF-mediated direct reprogramming of microglial inflammatory states. Consequently, the therapeutic advantage of BDNF-ADSCs lies not in replacing but in amplifying ADSCs’ fundamental functions, with the BDNF-TrkB signaling pathway and the intrinsic paracrine mechanisms forming a multi-layered synergistic effect across anti-inflammatory and antioxidant effects and neuronal survival promotion. This explains the significantly superior efficacy of the BDNF-ADSC group compared to the empty-vector group in this study, providing experimental evidence that genetic engineering modification of ADSCs represents a feasible strategy to overcome pharmacokinetic limitations of conventional neurotrophic factor delivery while maximizing stem cell-based therapeutic outcomes.

Beyond the aforementioned paracrine and anti-apoptotic mechanisms, an idealized yet challenging prospect is whether transplanted ADSCs could directly replace lost neurons through in situ differentiation.

We have also considered the possibility that ADSCs could differentiate into functional neurons *in vivo* and integrate into neural circuits, which would indeed

represent a breakthrough in AD therapeutics and the idealized ultimate goal of stem cell-based treatment for neurological disorders. However, this transdifferentiation process faces multiple biological barriers: inhibitory signals from the adult neural microenvironment, persistent toxic effects of A β pathology, and the functional integration efficiency of transplanted cells with host circuits, all of which render direct *in vivo* differentiation technically unfeasible under current conditions.⁵⁸ Based on the experimental data from this study, we cautiously limit our conclusion to “neuroprotective intervention.” Through immunofluorescence tracking of transplanted cells (ZsGreen-labeled) and colocalization analysis with neuronal markers (NeuN), no definitive evidence was observed that ADSCs directly differentiated into mature neurons to replace dead cells; nevertheless, this does not diminish their therapeutic value—ADSCs effectively delayed A β -induced neurodegeneration through multi-targeted neuroprotection and immunomodulation: constitutively secreted BDNF activated the TrkB-CREB pro-survival pathway, upregulated the Bcl-2/Bax ratio, and suppressed caspase-3 activation.

The balance between Bax and Bcl-2 is a critical determinant of neuronal fate, as elevated Bax promotes apoptosis, whereas increased Bcl-2 confers resistance to apoptotic stimuli.⁵⁹ Our findings indicate that BDNF-ADSCs shift this balance toward cell survival, thereby attenuating hippocampal neuronal apoptosis induced by A β ₁₋₄₂. This neuroprotective effect is likely mediated, at least in part, by the paracrine actions of BDNF released from transplanted ADSCs. Collectively, this study provides mechanistic evidence that BDNF-ADSCs protect hippocampal neurons against A β ₁₋₄₂-induced apoptosis through modulation of the Bax/Bcl-2/caspase-3 signaling pathway, ultimately contributing to improved behavioral and cognitive outcomes in the AD model.

5. Conclusion

The present study demonstrates that transplantation of BDNF-overexpressing ADSCs effectively attenuates A β ₁₋₄₂-induced hippocampal neuronal loss, apoptosis, and cognitive impairment in wild-type mice. The neuroprotective effects of BDNF-ADSCs are closely associated with suppression of the Bax/Bcl-2/caspase-3 apoptotic signaling pathway, likely mediated through sustained paracrine delivery of BDNF. These findings provide novel mechanistic insight into the role of apoptosis in AD pathogenesis and support the therapeutic potential of BDNF-ADSCs as a promising strategy for the treatment of AD.

Acknowledgments

We gratefully acknowledge the Science Laboratory Center

for providing the laboratory equipment.

Funding

This research was funded by the Fujian Provincial Natural Science Foundation of China (Grant number: 2023J011869); Nanping Joint Funding Program for Science and Technology Innovation in Healthcare (Grant number: N2024LH006); Startup Fund for scientific research, Fujian Medical University (Grant number: 2022QH1236); Hainan Province for the Scientific Research Projects of Higher Education Institutions in 2023 (Grant number: Hnky2023-31).

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Fangfang Liu

Formal analysis: Aiping Wang

Investigation: Xidi Chi

Methodology: Guiyun Ye

Writing—original draft: Haofei Fan

Writing—review & editing: Haolin Wu

Ethics approval and consent to participate

The animal study was approved by the Ethical Committee of Hainan Medical University (Ethic Approval Number: HYLL-2023-205). The study was conducted in accordance with the local legislation and institutional requirements.

Consent for publication

Not applicable.

Availability of data

All data generated or analyzed during this study are included in this published article and its supplementary files. Additional raw data are available from the corresponding author upon reasonable request.

References

1. Ashok A, Singh N, Chaudhary S, *et al.* Retinal Degeneration and Alzheimer's Disease: An Evolving Link. *IJMS*. 2020;21(19):7290.
doi: 10.3390/ijms21197290
2. Pritam P, Deka R, Bhardwaj A, *et al.* Antioxidants in Alzheimer's Disease: Current Therapeutic Significance and Future Prospects. *Biology*. 2022;11(2):212.
doi: 10.3390/biology11020212
3. Li P, Quan W, Wang Z, *et al.* Early-stage differentiation between Alzheimer's disease and frontotemporal lobe

- degeneration: Clinical, neuropsychology, and neuroimaging features. *Front Aging Neurosci.* 2022;14.
doi: 10.3389/fnagi.2022.981451
4. Jia L, Du Y, Chu L, *et al.* Prevalence, risk factors, and management of dementia and mild cognitive impairment in adults aged 60 years or older in China: a cross-sectional study. *Lancet Public Health.* 2020;5(12):e661-e671.
doi: 10.1016/S2468-2667(20)30185-7
5. Lin N, Gao XY, Li X, Chu WM. Involvement of ubiquitination in Alzheimer's disease. *Front Neurol.* 2024;15.
doi: 10.3389/fneur.2024.1459678
6. Kumari S, Dhapola R, Reddy DH. Apoptosis in Alzheimer's disease: insight into the signaling pathways and therapeutic avenues. *Apoptosis.* 2023;28(7-8):943-957.
doi: 10.1007/s10495-023-01848-y
7. Park HA, Hayden MM, Bannerman S, Jansen J, Crowe-White KM. Anti-Apoptotic Effects of Carotenoids in Neurodegeneration. *Molecules.* 2020;25(15):3453.
doi: 10.3390/molecules25153453
8. Wang ZF, Yin J, Zhang Y, *et al.* Overexpression of Tau Proteins Antagonizes Amyloid- β -Potentiated Apoptosis Through Mitochondria-Caspase-3 Pathway in N2a Cells. *JAD.* 2010;20(1):145-157.
doi: 10.3233/JAD-2010-1351
9. Selznick LA, Zheng TS, Flavell RA, Rakic P, Roth KA. Amyloid Beta-Induced Neuronal Death is Bax-Dependent but Caspase-Independent. *J Neuropathol Exp Neurol.* 2000;59(4):271-279.
doi: 10.1093/jnen/59.4.271
10. Gao L, Zhang Y, Sterling K, Song W. Brain-derived neurotrophic factor in Alzheimer's disease and its pharmaceutical potential. *Transl Neurodegener.* 2022;11(1).
doi: 10.1186/s40035-022-00279-0
11. Kopec B, Zhao L, Rosa-Molinar E, Siahaan T. Non-invasive Brain Delivery and Efficacy of BDNF in APP/PS1 Transgenic Mice. *MRAJ.* 2020;8(2).
doi: 10.18103/mra.v8i2.2043
12. Nagahara AH, Tuszynski MH. Potential therapeutic uses of BDNF in neurological and psychiatric disorders. *Nat Rev Drug Discov.* 2011;10(3):209-219.
doi: 10.1038/nrd3366
13. Jiao SS, Shen LL, Zhu C, *et al.* Brain-derived neurotrophic factor protects against tau-related neurodegeneration of Alzheimer's disease. *Transl Psychiatry.* 2016;6(10):e907-e907.
doi: 10.1038/tp.2016.186
14. Si Z, Wang X, Sun C, *et al.* Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies. *Biomed Pharmacother.* 2019;114:108765.
doi: 10.1016/j.biopha.2019.108765
15. Cheng KH, Kuo TL, Kuo KK, Hsiao CC. Human adipose-derived stem cells: Isolation, characterization and current application in regeneration medicine. *Genom Med Biomark Health Sci.* 2011;3(2):53-62.
doi: 10.1016/j.gmbhs.2011.08.003
16. Navarro-Perez J, Carobbio S. Adipose tissue-derived stem cells, in vivo and in vitro models for metabolic diseases. *Biochem Pharm.* 2024;222:116108.
doi: 10.1016/j.bcp.2024.116108
17. Zhang J, Liu Y, Chen Y, *et al.* Adipose-Derived Stem Cells: Current Applications and Future Directions in the Regeneration of Multiple Tissues. Li Y, ed. *Stem Cells Int.* 2020;2020:1-26.
doi: 10.1155/2020/8810813
18. Qin Y, Ge G, Yang P, *et al.* An Update on Adipose-Derived Stem Cells for Regenerative Medicine: Where Challenge Meets Opportunity. *Adv Sci.* 2023;10(20).
doi: 10.1002/advs.202207334
19. Mahla RS. Stem Cells Applications in Regenerative Medicine and Disease Therapeutics. *Int J Cell Biol.* 2016;2016:1-24.
doi: 10.1155/2016/6940283
20. Papadopoulos KS, Piperi C, Korkolopoulou P. Clinical Applications of Adipose-Derived Stem Cell (ADSC) Exosomes in Tissue Regeneration. *IJMS.* 2024;25(11):5916.
doi: 10.3390/ijms25115916
21. Kademani A, Avraam C, Montenegro D, *et al.* Exploring the Emerging Role of Stem Cell Therapy in Neurodegenerative Diseases and Spinal Cord Injury: A Narrative Review. *Cureus.* 2025.
doi: 10.7759/cureus.89629
22. Varadarajan SG, Hunyara JL, Hamilton NR, Kolodkin AL, Huberman AD. Central nervous system regeneration. *Cell.* 2022;185(1):77-94.
doi: 10.1016/j.cell.2021.10.029
23. Wang Y, Pan J, Wang D, Liu J. The Use of Stem Cells in Neural Regeneration: A Review of Current Opinion. *CSCR.* 2018;13(7):608-617.
doi: 10.2174/1574888X13666180720100738
24. Sivandzade F, Cucullo L. Regenerative Stem Cell Therapy for Neurodegenerative Diseases: An Overview. *IJMS.* 2021;22(4):2153.
doi: 10.3390/ijms22042153
25. An Y, Zhao J, Nie F, *et al.* Exosomes from Adipose-Derived Stem Cells (ADSCs) Overexpressing miR-21 Promote Vascularization of Endothelial Cells. *Sci Rep.* 2019;9(1).

- doi: 10.1038/s41598-019-49339-y
26. Ying C, Zhang J, Zhang H, *et al.* Stem cells in central nervous system diseases: Promising therapeutic strategies. *Exp Neurol.* 2023;369:114543.
doi: 10.1016/j.expneurol.2023.114543
27. Takata N, Eiraku M. Stem cells and genome editing: approaches to tissue regeneration and regenerative medicine. *J Hum Genet.* 2017;63(2):165-178.
doi: 10.1038/s10038-017-0348-0
28. Bacakova L, Zarubova J, Travnickova M, *et al.* Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells – a review. *Biotechnol Adv.* 2018;36(4):1111-1126.
doi: 10.1016/j.biotechadv.2018.03.011
29. Park D, Yang G, Bae DK, *et al.* Human adipose tissue-derived mesenchymal stem cells improve cognitive function and physical activity in ageing mice. *J Neurosci Res.* 2013;91(5):660-670.
doi: 10.1002/jnr.23182
30. Huang X, Fei G qiang, Liu W juan, *et al.* Adipose-derived mesenchymal stem cells protect against CMS-induced depression-like behaviors in mice via regulating the Nrf2/HO-1 and TLR4/NF- κ B signaling pathways. *Acta Pharmacol Sin.* 2019;41(5):612-619.
doi: 10.1038/s41401-019-0317-6
31. Liu M, Yang Y, Zhao B, *et al.* Exosomes Derived From Adipose-Derived Mesenchymal Stem Cells Ameliorate Radiation-Induced Brain Injury by Activating the SIRT1 Pathway. *Front Cell Dev Biol.* 2021;9.
doi: 10.3389/fcell.2021.693782
32. Lim YC, Jung JI, Hong IK. A Novel Method for Human Adipose-Derived Stem Cell Isolation and Cryopreservation. *Cell Reprogramming* 2023;25(4):171-179.
doi: 10.1089/cell.2023.0017
33. Luo Y, Liu Y, Long H, *et al.* Adipose-Derived Stem Cells Transfected to Express Brain-Derived Neurotrophic Factor Reduce Hippocampal Amyloid Plaque Load and Improve Dendritic Morphology in the APP/PS1dE9 Mouse Model of Alzheimer's Disease. *J Integr Neurosci.* 2026;25(1).
doi: 10.31083/JIN46077
34. Codd LN, Blackmore DG, Vukovic J, Bartlett PF. Exercise reverses learning deficits induced by hippocampal injury by promoting neurogenesis. *Sci Rep.* 2020;10(1).
doi: 10.1038/s41598-020-76176-1
35. Knopman DS, Amieva H, Petersen RC, *et al.* Alzheimer disease. *Nat Rev Dis Primers.* 2021;7(1).
doi: 10.1038/s41572-021-00269-y
36. Nasb M, Tao W, Chen N. Alzheimer's disease puzzle: delving into pathogenesis hypotheses. *Aging Dis.* 2024;15(1):43-73.
doi: 10.14336/AD.2023.0608
37. Coupé P, Manjón JV, Lanuza E, Catheline G. Lifespan Changes of the Human Brain In Alzheimer's Disease. *Sci Rep.* 2019;9(1).
doi: 10.1038/s41598-019-39809-8
38. Breijyeh Z, Karaman R. Comprehensive Review on Alzheimer's Disease: Causes and Treatment. *Molecules.* 2020;25(24):5789.
doi: 10.3390/molecules25245789
39. Ge M, Zhang J, Chen S, *et al.* Role of Calcium Homeostasis in Alzheimer's Disease. *NDT.* 2022;Volume 18:487-498.
doi: 10.2147/NDT.S350939
40. Karapetyan G, Fereshetyan K, Harutyunyan H, Yenkovyan K. The synergy of β amyloid 1-42 and oxidative stress in the development of Alzheimer's disease-like neurodegeneration of hippocampal cells. *Sci Rep.* 2022;12(1).
doi: 10.1038/s41598-022-22761-5
41. Kraeuter AK, Guest PC, Sarnyai Z. The Open Field Test for Measuring Locomotor Activity and Anxiety-Like Behavior. In: *Methods in Molecular Biology.* New York: Springer; 2018:99-103.
doi: 10.1007/978-1-4939-8994-2_9
42. Moujalled D, Strasser A, Liddell JR. Molecular mechanisms of cell death in neurological diseases. *Cell Death Differ.* 2021;28(7):2029-2044.
doi: 10.1038/s41418-021-00814-y
43. Louneva N, Cohen JW, Han LY, *et al.* Caspase-3 Is Enriched in Postsynaptic Densities and Increased in Alzheimer's Disease. *Am J Pathol.* 2008;173(5):1488-1495.
doi: 10.2353/ajpath.2008.080434
44. Barnhart CD, Yang D, Lein PJ. Using the Morris Water Maze to Assess Spatial Learning and Memory in Weanling Mice. Chapouthier G, ed. *PLoS ONE.* 2015;10(4):e0124521.
doi: 10.1371/journal.pone.0124521
45. Yang S, Sun Y, Yan C. Recent advances in the use of extracellular vesicles from adipose-derived stem cells for regenerative medical therapeutics. *J Nanobiotechnol.* 2024;22(1).
doi: 10.1186/s12951-024-02603-4
46. Liu H, Jin M, Ji M, Zhang W, Liu A, Wang T. Hypoxic pretreatment of adipose-derived stem cell exosomes improved cognition by delivery of circ-Epc1 and shifting microglial M1/M2 polarization in an Alzheimer's disease mice model. *Aging.* 2022;14(7):3070-3083.
doi: 10.18632/aging.203989
47. Chang C, Yan J, Yao Z, Zhang C, Li X, Mao H. Effects of

- Mesenchymal Stem Cell-Derived Paracrine Signals and Their Delivery Strategies. *Adv Healthc Mater.* 2021;10(7).
doi: 10.1002/adhm.202001689
48. Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ. Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology.* 1998;37(12):1553-1561.
doi: 10.1016/s0028-3908(98)00141-5
 49. Arévalo JC, Deogracias R. Mechanisms Controlling the Expression and Secretion of BDNF. *Biomolecules.* 2023;13(5):789.
doi: 10.3390/biom13050789
 50. Wójcik P, Jastrzębski MK, Zięba A, Matosiuk D, Kaczor AA. Caspases in Alzheimer's Disease: Mechanism of Activation, Role, and Potential Treatment. *Mol Neurobiol.* 2023;61(7):4834-4853.
doi: 10.1007/s12035-023-03847-1
 51. Kienlen-Campard P, Miolet S, Tasiaux B, Octave JN. Intracellular Amyloid- β 1-42, but Not Extracellular Soluble Amyloid- β Peptides, Induces Neuronal Apoptosis. *J Biol Chem.* 2002;277(18):15666-15670.
doi: 10.1074/jbc.M200887200
 52. D'Amelio M, Sheng M, Cecconi F. Caspase-3 in the central nervous system: beyond apoptosis. *Trends Neurosci.* 2012;35(11):700-709.
doi: 10.1016/j.tins.2012.06.004
 53. Dhani S, Zhao Y, Zhivotovsky B. A long way to go: caspase inhibitors in clinical use. *Cell Death Dis.* 2021;12(10).
doi: 10.1038/s41419-021-04240-3
 54. Nan D, Dou X, Qi Y, Zhang W, He G, Zhang X. *In Vitro* Study of Adipose-Derived Mesenchymal Stem Cells Transduced with Lentiviral Vector Carrying the Brain-Derived Neurotrophic Factor Gene. *IJSC.* 2020;13(3):386-393.
doi: 10.15283/ijsc20038
 55. Joyce N, Annett G, Wirthlin L, Olson S, Bauer G, Nolta JA. Mesenchymal stem cells for the treatment of neurodegenerative disease. *Regen Med.* 2010;5(6):933-946.
doi: 10.2217/rme.10.72
 56. Lin TC, Kawabori M, Yoshie E, Nakahara Y, Tsai LK, Fujimura M. Intraventricular continuous BDNF administration ameliorates neuroinflammation and enhances neurogenesis against rodent intracerebral hemorrhage model. *Front Neurol.* 2025;16.
doi: 10.3389/fneur.2025.1606606
 57. Hay AJD, Murphy TJ, Popichak KA, Zabel MD, Moreno JA. Adipose-derived mesenchymal stromal cells decrease prion-induced glial inflammation in vitro. *Sci Rep.* 2022;12(1).
doi: 10.1038/s41598-022-26628-7
 58. Zavan B, Vindigni V, Gardin C, *et al.* Neural potential of adipose stem cells. *Discovery Medicine.* 2010;10(50):37-43.
 59. Luo X, O'Neill KL, Huang K. The third model of Bax/Bak activation: a Bcl-2 family feud finally resolved? *F1000Res.* 2020;9:935.
doi: 10.12688/f1000research.25607.1