Research Square

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Fe_3O_4@Polydopamine Nanoparticle-Loaded Human Umbilical Cord Mesenchymal Stem Cells Improve the Cognitive Function in Alzheimer's Disease Mice by Promoting Hippocampal Neurogenesis

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Abstract

Background

One of the most promising treatments for neurodegenerative diseases is the stem cell therapy; however, there are still some limitations in the treatment of Alzheimer's disease (AD), and the specific molecular mechanism that affects the cognitive function remains unclear. Therefore, it is necessary to develop a strategy to increase the recruitment of stem cells to the lesion site for clinical application. Fe_3O_4 nanoparticles have good physiological stability, biocompatibility, and is conducive to the active uptake of stem cells.

Methods

In this study, superparamagnetic iron oxide nanoparticles composed of magnetic Fe_3O_4 and polydopamine (PDA) shells were used to label human umbilical cord mesenchymal stem cells (hUC-MSCs) in order to increase the targeting of hUC-MSCs. We first detected the effect of Fe_3O_4 nanoparticles on the proliferation and differentiation of hUC-MSCs, and identified the distribution of Fe_3O_4@PDA labeled hUC-MSCs in APP/PS1 transgenic mice. We also determined the effects of hUC-MSCs on OA-induced apoptosis in vitro, and Fe_3O_4@PDA labeled hUC-MSCs on the cognitive function of AD mice by water maze test. The effects of Fe_3O_4@PDA labeled hUC-MSCs on related-proteins in hippocampus of AD mice were determined by WB and immunohistochemistry.

Results

Fe_3O_4@PDA labeling did not affect the biological characteristics of hUC-MSCs, but did increase the efficiency of hUC-MSCs entering the brain. Moreover, the results of the water maze test showed that compared with single hUC-MSCs, Fe_3O_4@PDA-labeled hUC-MSCs improved the cognitive ability of APP/PS1 transgenic mice more significantly. Other experimental data, including WB, immunohistochemistry, showed that the expression of essential proteins in the hippocampus, such as amyloid precursor protein (App), synaptophysin (SYN), brain-derivedneurotrophic factor (BDNF), glial fibrillary acidic protein (GFAP), etc., are affected by Fe_3O_4@PDA coated-hUC-MSCs. The study showed a well-established Aβ deposition by promoting neurogenesis and synaptic plasticity and increased protein levels of BDNF, SYN, and GFAP.

Conclusion

Fe_3O_4@PDA is a promising magnetic nanomaterial, which can increase the targeting of stem cells. The regulation of hUC-MSCs could improve the memory and cognitive ability of AD mice byexcessive generation of neuroprotective factors, which might be considered a viable therapy to treat AD.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease, and its incidence rate and mortality rate are increasing every year as the world's population is aging, which is a global problem [1]. AD is caused due to apoptosis of a large number of neurons leading to cognitive dysfunction and eventually developing into severe dementia [2]. The first case of a patient with AD was recorded in the early 20th century, and since then, researches are going on to investigate the etiology of AD. Although many pathogenesis and targets of AD, such as β-amyloid (Aβ) hypothesis, tau protein hypothesis, etc. have been discovered, the main mechanisms remain unknown. Thus, most of the studies regarding drug research and development in AD have remained unsuccessful.

Currently, there are only a few FDA-approved drugs available for the treatment of AD. However, these drugs can only improve the clinical symptoms of patients, but can neither reduce the pathological changes of AD nor prevent the death of nerve cells or reverse or slow down the disease process [3, 4]. In addition, it is challenging for the drugs to cross the blood-brain barrier (BBB) and reach the focus area due to the contradictory hypothesis and unclear mechanism [5, 6]. Therefore, finding new strategies to prevent or control AD is extremely crucial.

Another promising therapy for the treatment of central nervous system injuries is stem cell transplantation. However, its application in the treatment of AD is limited, and the potential mechanism for cognition improvement remains unclear.

Recently, researches on stem cell therapy are being carried out. Stem cell therapy proves to be a promising strategy for the treatment of AD, as shown by animal experiments. Due to its unknown mechanism and difficult to target due to widespread neuronal cell death, the clinical application of stem cell therapy is limited.

Mesenchymal stem cells (MSCs) differentiate into adipose, bone, cartilage, muscle, nerve, liver, heart muscle, endothelium, and many other tissue cells [7]. MSCs are easily obtained, can be separated, and cultured. MSCs are used in the research and development of stem cell therapy and are effective against AD owing to features such as its potential to differentiate, autotransplantation, damage repair, and homing ability [8].

Few reports suggest that the cognitive ability of AD model mice is restored by BMSCs, ADMSCs, and other MSCs, but there are many challenges during the process [7, 9]. Umbilical cord-derived mesenchymal stem cells (hUC-MSCs)are primitive stem cells and exhibit specific signs of hESC. It is cost-effective, abundant, and do not cause injury to donors. Importantly, it does not involve any ethical implications [10]. On comparing with BMSC and ADMSC, it has a strong amplification ability, no tumorigenic activity, and low immunogenicity. Its proliferation and differentiation ability remain unaffected with time and age when compared with BMSC [11].

Due to the multidirectional aggregation to chronic inflammatory sites, hUC-MSCs lack homing specificity and do not reach the target tissue, and the cognition improvement mechanism remains unclear.

Therefore, different ways to enhance the targeting of stem cells is an important path to improve the limited clinical application.

For the past 15 years, magnetic targeting technique has been used to improve the cell transplantation efficiency [12]. Therefore, nanomaterials with good biocompatibility, high safety, and low toxicity are highly important. Many researches on superparamagnetic nanomaterials are being conducted in recent years. These nanomaterials find a wide range of applications in biological separation, drug-loaded targeted treatment, biological imaging, and magnetic hyperthermia treatment due to the following factors: (i) superparamagnetic properties, (ii) good biocompatibility, (iii) various surface modification properties [13]. The most commonly used superparamagnetic nanoparticles are Fe_3O_4 nanoparticles (Fe_3O_4 NPs) [14]. Moreover, tissue damage repair by Fe_3O_4 NPs-labeled MSC is observed better under the external magnetic field attached to the damaged site, improving the efficiency of MSC cell therapy.

Fe_3O_4 NPs-coated nerve growth factor (NGF) has shown to promote the growth and differentiation of neurons in vitro under the influence of dynamic external magnetic fields. Magnetically targeted cell delivery (MTCD) of Fe_3O_4 NPs-labeled hUC-MSCs can greatly improve the targeting of hUC-MSCs into the lesion area, and achieve efficient direct intracerebroventricular (ICV) injection. This is a noninvasive choice for clinical application [15]. A synthetic process was adopted to improve the biocompatibility and magnetic response of NPs. Fe_3O_4 nanoparticles (Fe_3O_4 NPs) were prepared using a thermal reduction method, and Fe_3O_4 superparticles (Fe_3O_4) were formed through the oil phase to the water phase (SPS). Then, a layer of dopamine was coated on the surface. Finally, Fe_3O_4@PDA SPS was designed and synthesized. This superparticle showed good stability, proper particle size, good magnetic separation, and higher contrast in MRI when compared with ordinary Fe_3O_4 NPs.

In this study, the hUC-MSCs were used for the treatment of AD. hUC-MSCs were combined with polydopamine (PDA)-modified Fe_3O_4 NPs and administered to the AD model mice. A significant improvement in the memory ability of mice was observed, providing us a new method for drug development for AD.

Collectively, the results of our study show that Fe_3O_4@PDA-coated hUC-MSCs improve the cognitive function in APP/PS1transgenic mice model that exhibits well-established Aβ deposition by promoting neurogenesis and synaptic plasticity, increasing protein levels of BDNF, SYN, and GFAP. Our results also suggest that the excessive generation of neuroprotective factors due to the regulation of hUC-MSCs could help in the treatment of AD.

Materials And Methods

Reagents

Human neuroblastoma cells SH-SY5Y was purchased from Jilin Neogene Biotechnology Co. LTD. Okadaic acid (OA) was purchased from cayman chemical company, and CCK-8 kit were purchased from

the Bimake company. DIO was purchased from Meilun Biotechnology. Anti-BDNF, Tau, Synaptophysin, GFAP, Connexin 43, PARP, caspase-3 antibodies were purchased from cell signaling company. Anti-actin antibody was purchased from santa cruz company. Anit-GAPDH and secondary antibodies were purchased from bioss company.

Extraction and culture of human umbilical cord blood-derived mesenchymal stem cells (hUC-MSCs)

The human umbilical cord was sectioned into 2 cm, and the artery and vein blood vessels were removed. The cut sections were washed 3 times using phosphate-buffered saline (PBS) containing penicillin (100 IU/ml) and streptomycin (100 μg/ml). After washing, the umbilical cord was cut into tissue blocks (5 mm × 5 mm × 5 mm) and planted in a 10 cm culture dish with 3 mm interval. Then, 2–3 ml of DMEM medium containing 20% FBS was added into the culture plate, at a 5% CO_2 and 37 °C incubator. The DMEM medium was changed every 3 days. The cells could be passaged when the cells around the tissue grew to 70%–80% (16–20 days).

Cell culture

DMEM high-glucose medium containing 10% fetal bovine serum (100 IU/ml penicillin, 100 μg/ml streptomycin) was used to culture hUC-MSCs and SH-SY5Y cells at 37°c in a thermostatic CO_2 incubator.

SH-SY5Y cells were pretreated with 30 nM OA for 24 hrs and then incubated with hUC-MSCs using transwell plants for another 24 hrs. hUC-MSCs were used for all experiments in this study.

Preparation and Characterization of Fe_3O_4@PDA NPs

Preparationof Fe3O4@PDA NPs

Fe_3O_4@PDA NPs were prepared as described above. Briefly, once Fe_3O_4 NPs are obtained, they (7 mg/ml) are injected into SDS solution, heated to 60 ℃, and toluene is evaporated to obtain SDS-coated Fe_3O_4 superparticles. Then, it was distributed in Tris buffer (pH=8.5). Then, 0.03 M of PDA solution was added and stirred for 3 h to obtain Fe_3O_4@PDA NPs.

Transmission electron microscopy

A total of 10 μl Fe_3O_4 and Fe_3O_4@PDA NPs, respectively, were gently dropped onto the surface of the copper mesh of the complete carbon supporting membrane without crease or scratch. After 40 minutes, excess water is absorbed gently using filter paper, and then deionized water was lightly dropped twice onto the surface of the copper mesh for cleaning. This copper mesh was placed under the voltage of 200 kV for TEM detection.

CCK-8 detection

CCK-8 detection kit was used to measure the activity of the cells. The CCK-8 reagent was added to the cells of each group. This was incubation for 2 h at 37°C, and then the absorbance was measured using a Microplate Reader.

Stem cell preparations and Prussian blue staining analysis

Fe_3O_4 nanomaterials (50 μg/ml) were added into cells and incubated at 37°C for 24 h. The cells were harvested and diluted in PBS (2.0×10^5 cell/0.1 ml) for injection. After 24 h of co-incubation, they were treated according to the Solarbio (Beijing) method. Now the resultant cells were fixed with 4% paraformaldehyde for 10 min and washed with PBS 3 times. Then, the cells were stained with Prussian blue for 10min. The stained cells were observed under the inverted optical microscope.

MSC differentiation potential

Osteogenesis

As per the instruction manual of the kit (StemPro osteogenesis differentiation kit; Gibco), the assay of osteogenic differentiation potential of hUC-MSCs was completed. MSCs (5×10^3 cells/cm^2) were seeded onto a 12-well plate with MSC growth medium for 2–4 days. Then, complete osteogenesis differentiation medium was changed every 3–4 days. The osteogenic cultures were processed for Alizarin Red S staining (2%, pH 4.2) after 28 days using an optical microscope (X51; Olympus Corporation).

Chondrogenesis

StemPro chondrogenesis differentiation kit (Gibco) was used to carry out the assay of chondrogenesis differentiation potential of hUC-MSCs and was completed according to the instruction manual of the kit. Briefly, after trypsinization and centrifugation, MSCs (1.6×10^7 viable cells/ml) solution was generated. A total of 5 ml droplet of cell solution was seeded in the center of 12-well plate for 2 h to generate micromass culture. Then, warmed chondrogenesis media was added to the culture in with 5% CO_2 and incubated at 37°C. Incubation was refed every 2–3 days. After 28 days, the cells were stained using Alcian Blue, and chondrogenic pellets were observed.

Adipogenesis

The assay of adipogenesis differentiation potential of hUC-MSCs was carried out as per manufacturer's protocol (StemPro Adipogenesis differentiation kit; Gibco). Briefly, MSCs (1×10^4 cells/cm^2) were seeded onto a 12-well plate with MSC growth medium for 2–4 days, followed by the addition of adipogenesis differentiation medium and continue the culture. Refeed every 3–4 days. After 21 days, the cells from adipogenic cultures were stained with Oil Red O (Sigma-Aldrich), and the stained cells were observed using an optical microscope (X51; Olympus Corporation, Tokyo, Japan).

hUC-MSCs flow cytometry analysis

Flow cytometry Human MSC Analysis Kit was used to detect hUC-MSCs using multicolor analysis. Briefly, different fluorescence represents different positive markers, and the MSC positive cocktail (FITC CD90, PerCP-Cy™5.5 CD105, and APC CD73) leaves the PE channel open to use in combination with the supplied negative MSC cocktail (PE CD45, PE CD34, PE CD11b, PE CD19, and PE HLA-DR), to analyze multiple samples.

APP/PS1 transgenic mice

APP/PS1 transgenic mice were from Nanjing Biomedical Research Institute of Nanjing University. In this experiment, the Nd-Fe-B permanent magnet cylinder with a diameter of 20 mm was fixed in front of the mouse head for 12 h, and then removed. No animal died in the experiment.

Fluorescence imaging assay

The cells were harvested and the DIO cell membrane fluorescent probe (10 μM) was added and mixed for 30 min. The mice were anesthetized using pentobarbital, and the drug solution was injected via the tail vein. After tail vein injection, the injection efficiency was observed under Fluorescence in vivo Imaging System.

Water maze assay

The memory ability of the mice was evaluated by the water maze assay. This experiment takes 10 days as one cycle, the first 3 days as the training phase, and the last 7 days as the trial phase.

During the training phase, the covert platform was placed 1 cm above the water surface, and the mouse was put into the water to adapt to the environment, and the escape latency (time required for the mouse to board the platform) was recorded. If the mouse did not find the platform after 120 s, it was guided to the platform. Irrespective of whether the mouse found the platform or not, it was allowed to stay on the platform for 30 s. At the end of the experiment, the mice were dried and put back into the cage. In total, four training sessions were conducted.

During the trial period, each mouse was trained 4 times a day. The platform was hidden 1 cm below the water surface, and the mice were randomly put into the water from the surface wall of the four water entry points to record the escape incubation period. If the mouse did not find the platform after 120 s, it was guided to the platform and let it stay for 30 s.

The results of the experiment were evaluated as the average escape latent period and the final average incubation period.

Statistical analysis

Groups of data with mean ± standard deviation (x±s), using SPSS statistic alanalysis software, with analysis of variance for significance test. $P < 0.05$ was used as the criterion for determining statistical difference.

Result

Preparation and characterization of Fe_3O_4@PDA NPs

A synthesis method was used to prepare Fe3O4@PDA NPs. The morphology of the synthesized NPs was observed using TEM (as shown in Fig. 1A). The average size of Fe_3O_4 NP is about 45–50 nm, and the average particle size after PDA coating is 55–60 nm. In this study, PDA and superparamagnetic materials were selected to form uniform shell core nanocomposites to improve the biocompatibility and stability of Fe_3O_4@PDA NPs.

Fe_3O_4@PDA NPs labeling did not affect the differentiation of hUC-MSCs.

Prussian blue staining experiment was done to analyze the labeling efficiency and cell uptake of Fe_3O_4@PDA NPs. The staining results showed no morphological difference between labeled (blue) and unlabeled cells (Fig. 1B). After 24 h incubation with Fe_3O_4@PDA NPs (50 μg/ml), hUC-MSCs showed blue precipitate.

Fluorescence-activated cell sorting of hUC-MSCs loaded with Fe_3O_4@PDA NPs indicated negative and positive surface markers while strongly expressing typical surface antigens, as per the kit protocol (Fig. 1C).

Fe_3O_4@PDA NPs were cultured in osteogenic, adipogenesis, and chondrogenic differentiation induction medium, respectively, and were stained with alizarin red, oil red O and alislan (Fig. 1D), whereas hUC-MSCs was considered as blank control. No difference in osteogenic, adipogenesis, and chondrogenic differentiation of hUC-MSCs with Fe_3O_4@PDA NP was observed when compared with the control group. Also, the osteogenic alkaline phosphatase staining area represented the degree of osteogenic differentiation, with no difference in size and area between the two groups. There was no difference in the number and size of lipid droplets in oil red O positive cells formed by lipogenic differentiation; the number and size of cartilage were same. The results showed that osteogenic, adipogenesis, and chondrogenic potential of stem cells remained unaffected.

Fe_3O_4@PDA NPs have almost no toxic effect on cells and mice

To prove the safety of synthesized Fe_3O_4 NPs, first CCK-8 was used to evaluate the toxicity of Fe_3O_4 NPs, and time dependence and concentration dependence were analyzed. The results showed that Fe_3O_4 NPs had no significant effect on cell activity in either time-course or dose-curve (Fig. 1E and 1F). As per the results obtained in this study, we chose the experimental conditions with 10μg/ml and 24 h for the following experiments.

Subsequently, hematoxylin and eosin staining of wild-type mice's heart, lung, liver, spleen, and kidney showed no significant pathological differences in the tissue sections of the two mice (Fig. 1G). Overall, these data indicated that Fe_3O_4 NPs are not toxic to cells.

hUC-MSCs have a therapeutic effect on OA-induced apoptosis in SH-SY5Y cells

To investigate the effect of hUC-MSCs on neuronal apoptosis, OA-treated SHSY5Y cells were further cocultured with hUC-MSCs in a transwell system (Fig.2A). OA-induced programmed cell death is a common cell model. The results of CCK-8 showed that (Fig.2B) the addition of hUC-MSCs had an antagonistic effect on OA-induced cell death, indicating that some cytokines secreted by hUC-MSCs entered the lower compartment through the transwell compartment. Furthermore, western blot analysis showed no significant difference in the targeting protein expression upon exposure of hUC-MSCs. As shown in Fig.2C, OA-induced PARP cleavage, and the decreasing expression levels of GFAP, pro-BDNF, connexin 43, and synaptophysin were significantly recovered upon the hUC-MSCs treatment, while the expression levels of Tau and CTFα were decreased. These data strongly demonstrated that treatment with hUC-MSCs improves the expression levels of AD-related proteins, thus inhibiting OA-induced neuronal cell death.

Fe_3O_4@PDA NPs enhance the targeting of hUC-MSCs

To explore if Fe_3O_4 nanoparticles could accelerate the ability of hUC-MSCs to target the lesion areas, the cells were stained with a DIO cell membrane fluorescent probe. The injected cells are located in vivo with a fluorescence imaging system after the first tail vein injection. The results showed that MSC labeled with Fe_3O_4 (Fig. 3D left part) gathered more significantly at the disease site than hUC-MSCs alone (Fig. 3D right part), demonstrating that hUC-MSCs labeled with Fe_3O_4 NPs could enhance the ability of hUC-MSCs to target the damaged site in AD model mice.

hUC-MSCs injection improves memory ability in Alzheimer's mice

After the injection cycle was completed (Fig. 3B), a water maze experiment was carried out to investigate the recovery of memory in AD mice. The evasive incubation period of the mice in each group was observed to be gradually shortened with the increase in the training schedule (Fig.4 A, B, C). Mice injected with MSC and mice injected with MSC labeled with Fe3O4 NPs showed better memory recovery than normal AD model mice. At the same time, MSC labeled with Fe_3O_4 NPs had a better recovery effect on the memory ability of mice compared to mice injected with MSC only.

hUC-MSCs injection increased the expression of BDNF and SYN, decreased phosphorylation activity of Tau, and the expression of connexin 43 in the hippocampus of AD mice.

To investigate whether MSC can treat AD in mice, western blot analysis and immunohistochemical experiments were carried out. Western blot analysis showed an increase in expression levels of APP, pro-BDNF, and SYN in mice injected with MSC and MSC labeled with Fe_3O_4 NPs at the age of 7 months (Fig. 4D). While the expression levels of CTFα protein and Tau were decreased, which was consistent with the trend of wild-type mice compared with the control group. Mice aged 10 months showed similar results (Fig. 4E). Mice injected with MSC and MSC labeled with Fe_3O_4 NPs showed significantly increased expression levels of pro-BDNF, SYN, and GFAP, while showed decreased expression levels of CTFα protein

and Tau. This indicates that MSCs affect the key protein expression of AD (Tau, APP, etc.), and have an ideal therapeutic effect on mice in the early and middle stages of AD.

Subsequently, according to the immunohistochemical data (Fig. 5) were statistically analyzed, and it was found that the expression levels of BDNF and SYN in mice injected with MSC and MSC labeled with Fe_3O_4 NPs increased, while the expression levels of Tau and APP-CTFα decreased. This is consistent with the results of western blot analysis.

Discussion

With the increasing aging of the global population, the incidence rate and mortality rate of AD are increasing every year. The 2018 World Alzheimer's disease report indicates that the average world population has 1 people every 3 seconds, the average survival time is 5.9 years, and the need for long-term care, seriously affecting social development, is a global problem threatening the human health [1]. According to the latest statistics of the World Health Organization, about 50 million people suffered from dementia globally in 2018, and the total number is expected to reach 82 million in 2030. By 2050, the number will increase to 152 million. The first recorded AD patients appeared in the early 20th century since then research on the etiology of AD has never been slack in the medical field. Although many pathogeneses and related targets of AD have been found, such as "β-amyloid (Aβ) hypothesis", "tau protein hypothesis", etc., but the complex pathogenesis involving multiple systemdysfunction has not been fully revealed [15, 16].

Even today, traditional drug therapy, especially cholinesterase inhibitors, is considered the first-line treatment for AD; however, the currently available treatment can only improve symptoms in a certain period, but cannot change the course of the disease. Nowadays, researchers are using stem cells and preparations developed by stem cells for the treatment of various neurodegenerative diseases such as AD. Some of them have confirmed that stem cells have broad clinical application prospects for the treatment of AD. Owing to the progressive nature of AD, the key prerequisite for the success of stem cell therapy is to make clear the inclusion criteria of clinical patients to be treated. Due to the involvement of hippocampal circuits in the early stages of the disease, some scholars suggest this region be a potential therapeutic target. An effective treatment strategy is the synaptic neuron loss [3].

The most important step in the development of stem cell therapy is choosing the right cell source. Taking into account the access to cells, ethical relationship, immunogenicity, efficiency, cost-effectiveness and other issues, MSCs were selected in this study. Compared with other mesenchymal stem cells, although hUC-MSCs have great advantages, its poor targeting and homing are still areas to be improved. Therefore, a new strategy to improve its targeting and homing is very important.

Magnetic targeting is a method to improve the efficiency of cell transplantation. At present, reports show that magnetic targeting can enhance the concentration of treatment cells up to 1.5–30 times, and

significantly improve the treatment effect [17]. There are two important factors in the method of repairing magnetic targeted guidance:

(i) Magnetic labeling of therapeutic cells to form magnetized cells.

(ii) Using a magnetic field to target and guide magnetized cells.

At present, the endocytosis of cells is used to transplant nano level magnetic materials into cells. The magnet-labeled NPs will directly affect cell survival and biological function. These NPs will follow the cells into the patient's body, which will also have a certain impact on the body. Fe_3O_4 is the only metal oxide approved by FDA to be used in the biomedical field. It has been widely used in nuclear magnetic resonance, targeted drug carrier, and tissue engineering. In this case, SPIONs have been approved to be safe in clinical applications. SPION, as one of the most widely used MRI drugs, has been widely used in the first-line clinical diagnosis [18, 19]. There have been a lot of reports on the synthesis of SPION shell nanoparticles, in which the shell is composed of inorganic (such as silica) or organic (such as polymer) materials. Due to its unique coating quality and function, polydopamine (PDA) shell structure has attracted much attention. PDA has excellent biocompatibility and biodegradability, and will not produce long-term toxicity during retention in vivo, which improves the stability and biocompatibility of SPION@PDA.

Stem cell therapy has attracted more attention in recent years. However, many researchers still dispute whether stem cells can break through the BBB and enter the brain smoothly. In our study, we use SPION wrapped with dopamine to process MSCs and clarify the effect of MSCs modified with NPs on AD mice. The experimental data show that the NPs can not only help MSCs to pass through the BBB smoothly, but also further enhance the targeting of MSCs to the focus. Moreover, the MSCs modified with NPs improved cognitive function and learning ability. It also changes the important proteins in the hippocampus, such as Aβ deposition, tau protein, and BDNF.

The Morris water mazetest results revealed that APP/PS1 mice exhibit cognitive dysfunction, and this impairment is significantly alleviated after the injection of hUC-MSCs. Moreover, obvious results are seen for hUC-MSCs modified by Fe_3O_4@FDA. The role of hUC-MSCs on Aβ pathology is examined to determine the mechanism of the amelioration of hUC-MSCs on AD. Our results show that by decreasing the generation of CTF fragment, hUC-MSCs modified by Fe_3O_4@FDA and hUC-MSCs could ameliorate the pathology of AD. That's consistent with other researchers [20]. It showed that stem cells can not only improve the memory behavior and learning ability of AD but can also regulate the generation of Aβ in the early and middle stages of AD (7 and 10 months). However, in the late stage of AD (more than 12 months old), it can only affect the memory and other functions of AD and has no effect on the generation of Aβ [21–23].

To further confirm whether hUC-MSCs have an effect on neuronal cell death, OA-treated SHSY5Y cells were further cocultured with hUC-MSCs in a transwell system. The effect of hUC-MSCs on OA-induced apoptosis was detected. The results showed that hUC-MSCs could inhibit OA-induced PARP cleavage,

caspase activation. Furthermore, OA-induced CTFα generation, GFAP, BDNF connexin 43, synaptophysin, and change in Tau protein levels have been influenced by cocultured with hUC-MSCs. These data strongly suggest that treatment with hUC-MSCs can inhibit OA-induced neuronal cell death by improving AD-related proteins.

The protein level of APP, BDNF, SYN, and GFAP, which correlates closely with neurogenesis and synaptic Tau connectivity was examined. The results showed decreasing levels of APP, pro-BDNF, and SYN, increasing in APP/PS1 mice when compared to WT mice. However, these proteins increased or decreased significantly in the hippocampus of APP/PS1 mice after treatment with hUC-MSCs.

Decreasing levels of BDNF are correlated with AD-related cognitive impairment severity, suggesting that reduced BDNF may be an early cofactor involved in the AD development. Furthermore, evidence shows that the neurotrophic factor signaling pathways are also closely related to AD development. A decrease in expression levels of BDNF is reported in the process of AD, which also participates in AD-related cognitive impairment [24, 25].

The early and core clinical manifestation of AD is hypomnesia, while the early pathological damage of AD is caused by the damage of synaptic function and structure. A significant decrease in synaptic connections in the hippocampus is observed in the early stage of AD.

SYN, a membrane protein on synaptic vesicles, is closely related to the release of neurotransmitters, synaptic formation, and ion channels of synaptic vesicles. The content of SYN protein expression reflects the synaptic function [26]. Research shows that loss of SYN is related to the cognitive function of AD patients, and it is also prior to the decrease of acetylcholine transferase activity [27].

As per recent evidence, astrocytes play an important role in the activation of AD, and the characteristic protein of astrocytes is GFAP. Astrocytes transform into reactive astrocytes when the central nervous system is damaged, which results in volume hyperplasia and hypertrophy, increasing the level of GFAP [28, 29].

Thus, our data indicate that Fe_3O_4@PDA-coated hUC-MSCs increase BDNF, SYN, and GFAP, which improves cognitive function in APP/PS1 transgenic mice by promoting hippocampal neurogenesis and enhancing hippocampal synaptic plasticity. The treatment mechanism of Fe_3O_4@PDA-coated hUC-MSCs remains unclear but is effective than treatment with hUC-MSCs. In a further study, we will investigate the mechanism of hUC-MSCs on the activation of endogenous neurogenesis and the reconstruction of synaptic connectivity mediated by BDNF, SYN, and GFAP.

In summary, the results of this study show that Fe_3O_4@PDA-coated hUC-MSCs improve the cognitive function in APP/PS1 mice model that exhibits well-established Aβ deposition by promoting neurogenesis and synaptic plasticity, increasing protein levels of BDNF, SYN, and GFAP. This study also suggests that regulation of hUC-MSCs generates excess neuroprotective factors, which could provide a viable therapy to treat AD.

Abbreviations

AD: Alzheimer's disease;

PDA: polydopamine;

hUC-MSCs: human umbilical cord mesenchymal stem cells;

APP: amyloid precursor protein;

SYN: synaptophysin;

BDNF: brain-derivedneurotrophic factor;

GFAP: glial fibrillary acidic protein;

Aβ: β-amyloid;

BBB: blood-brain barrier;

NPs: nanoparticles;

NGF: nerve growth factor;

MTCD: Magnetically targeted cell delivery;

ICV: intracerebroventricular;

OA: Okadaic acid;

Declarations Section

Ethical Approval and Consent to participate

Informed consent of umbilical cord donation was approved by the medical ethics committees of the China-Japan Union Hospital of Jilin University. All animal experiments comply with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and were approved by the ethics committees of Jilin University.

Consent for publication

Not applicable.

Availability of data and materials

I can confirm that all data and material relevant to the study are included in the research paper.

Competing interests

The authors declare that they have no conflicts of interest with the contents of this article.

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Authors' contributions

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Figures

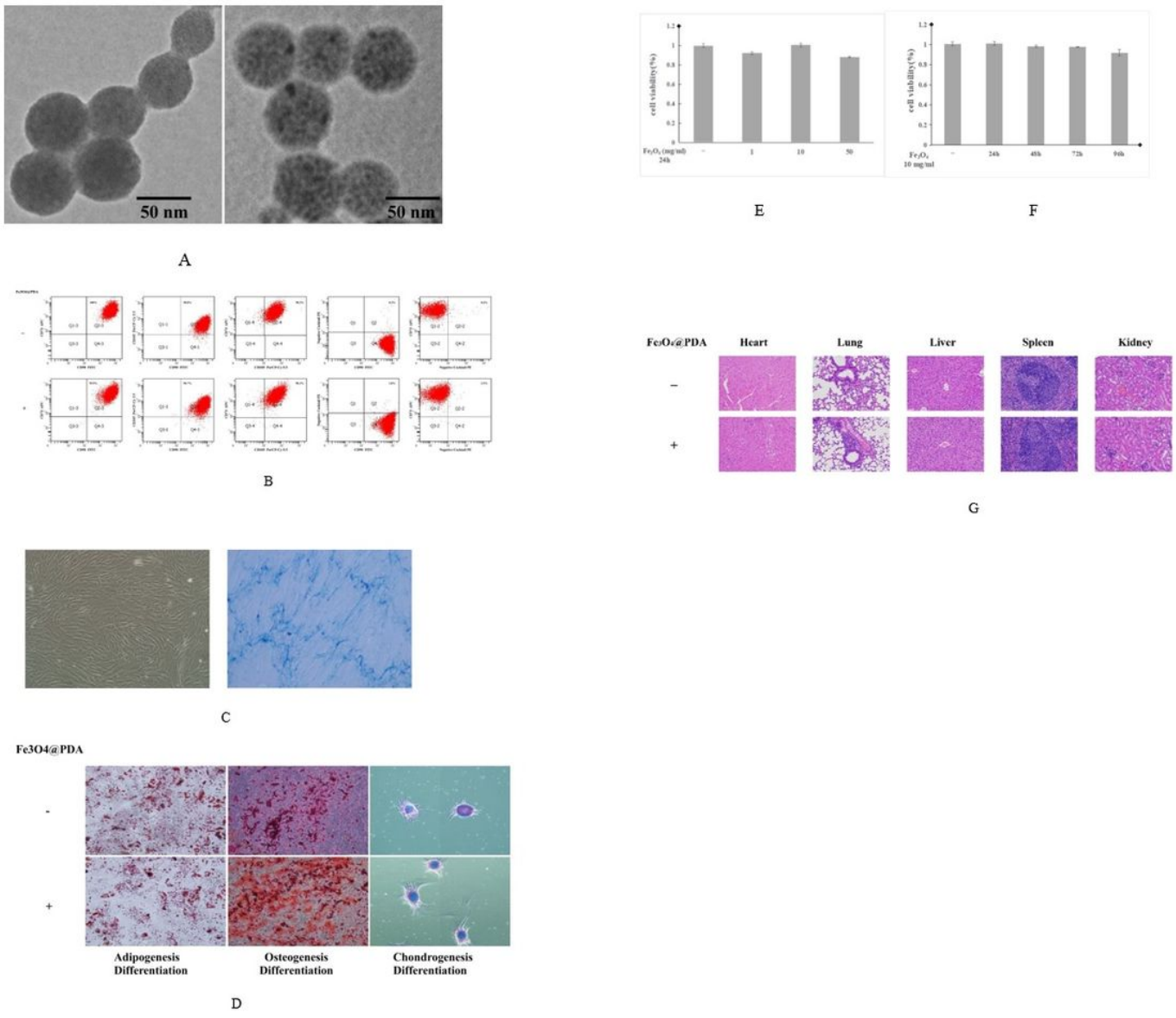

Figure 1

Viability of hUC-MSCs and mice are not affected by Fe3O4@PDA labeling. (A) TEM images of synthesized Fe3O4 (left) and Fe3O4@PDA (right). Scale bar =50 nm. (B) Flow cytometry analysis showed that Fe3O4@PDA did not affect the differentiation of hUC-MSCs. (C) Photomicrographs showing the morphology of the hUC-MSCs labeled with Fe3O4@PDA (50 μg/ml) for 24 hours (right part), then cells were stained with a Prussian blue iron stain kit. (E)& (F) Proliferation of 50 μg/ml Fe3O4@PDA-labeled hUC-MSCs by CCK-8. Cell viability assay was observed in hUC-MSCs labeled with different concentrations (0, 10, 50, or 100 μg/ml) of Fe3O4@PDA for 24 hrs (E), and with Fe3O4@PDA (50 μg/ml) for different hours (0, 24, 48, 72, or 96 hrs) (F). (G) HE staining showed the effects of Fe3O4 on heart, lung, liver, spleen, and kidney toxicity in mice.

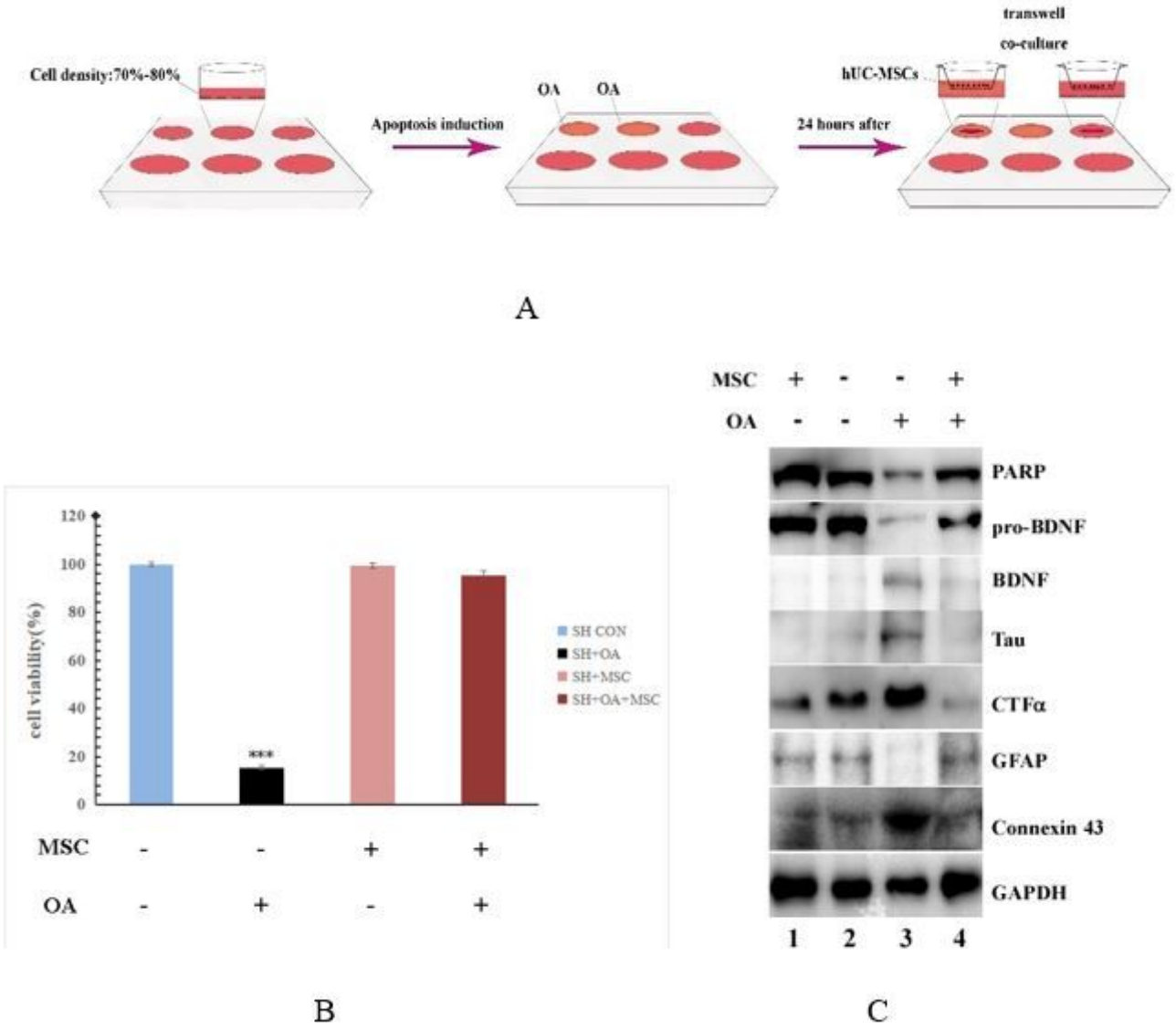

Figure 2

Mesenchymal stem cells can antagonize OA-caused apoptosis in SHSY-5Y cells (A) hUC-MSCs and SHSY-5Y cells were cultured with the same amount of optimized medium in the transwell system. (B) hUC-MSCs protected OA-caused cell death by CCK-8. (C)The expressions of PARP, BDNF, pro-BDNF, Tau, CTFα, GFAP, Connexin 43, and GAPDH were analyzed by western blot.

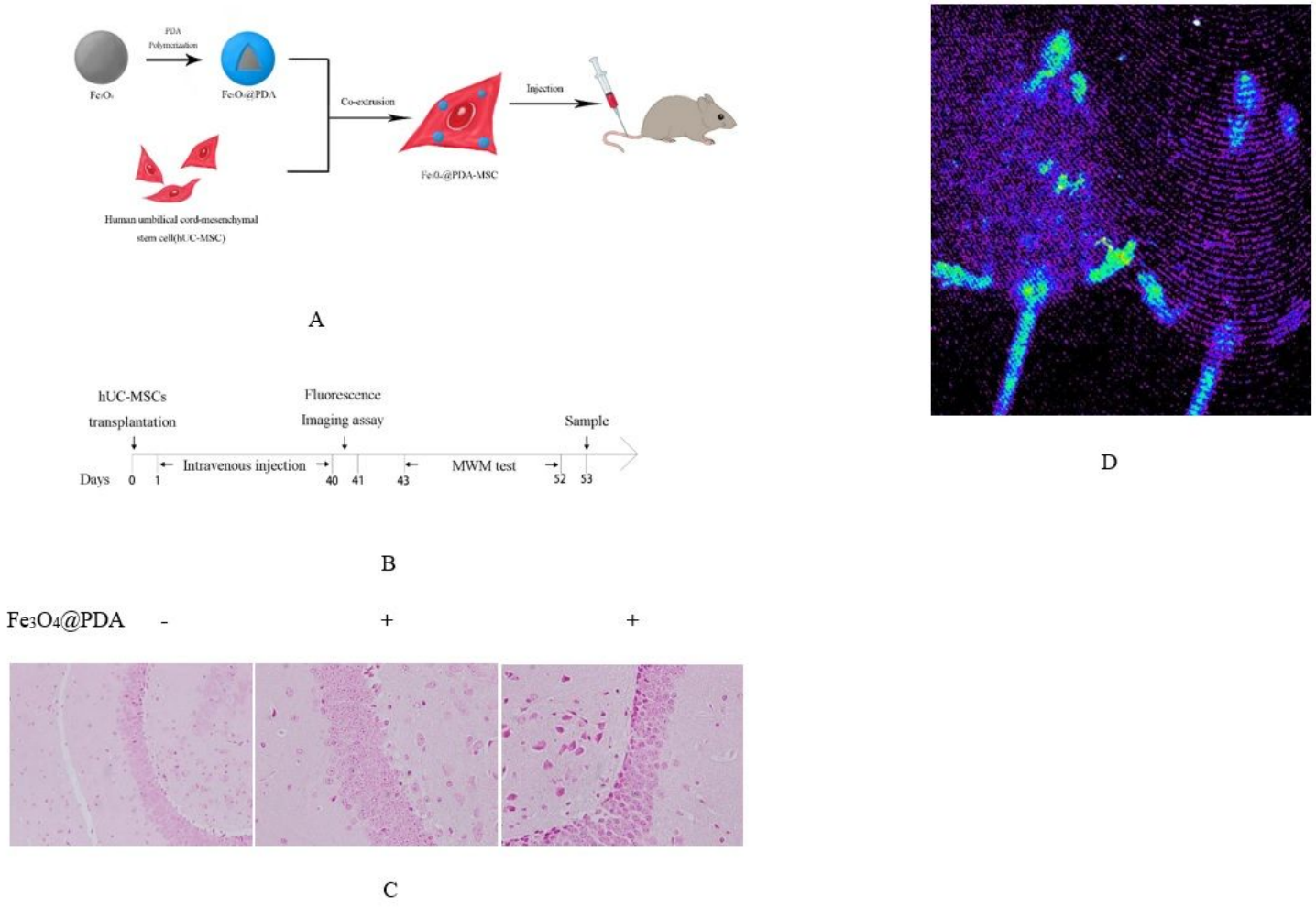

Figure 3

Intra hippocampal hUC-MSCs transplantation improves spatial memory. (A) Schematic of a 3D molecular model of the entire experiment. (B) Experimental design for the hUC-MSCs treatment of an APP/PS1 mouse AD model. (C) The nanoparticles Fe3O4@PDA (50 μg/ml) were injected into mice, and the hippocampal area was sectioned for Prussian blue staining. (D) Fe3O4@PDA injection enhanced the ability of hUC-MSCs to target damage sites in Alzheimer's disease model mice than Fe3O4 nanoparticles.

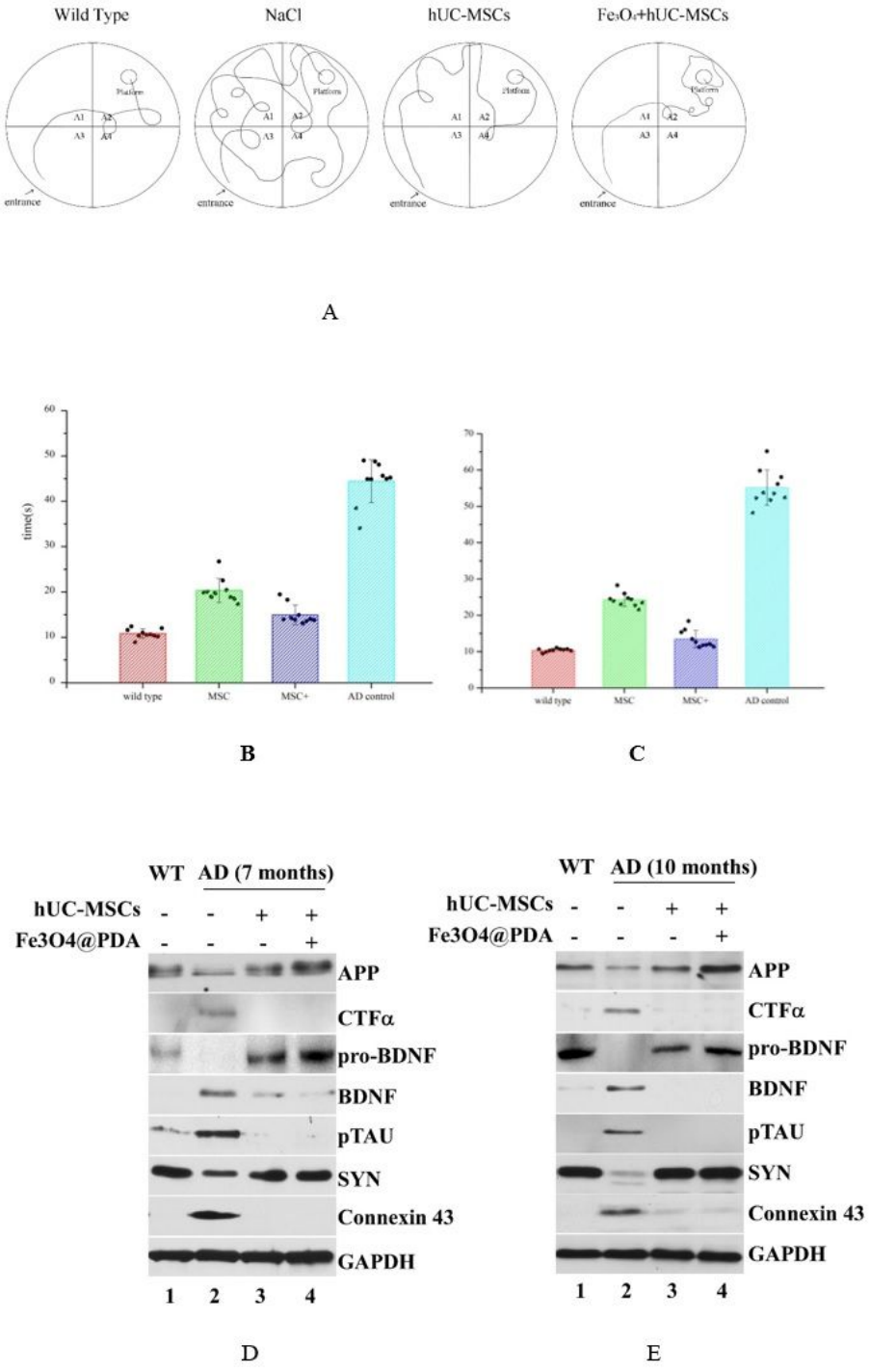

Figure 4

(A) Representative swimming paths on Day 10 of training. (B) & (C) Water maze escape latencies in seconds over a 10-day time period for APP/PS1 mice whose hippocampl were bilaterally injected with PBS or hUC-MSCs and WT controls. For a complete test, a total of 40 sessions over 10 days were given. The graph shows the average escape latencies per day for each condition. Escape latencies in the hUC-MSCs transplanted APP/PS1 group show improved learning over time (n =15 for each of the three

groups, B, 7 months; C, 10 months). (D) & (E) The expressions of PARP, BDNF, pro-BDNF, Tau, CTFα, GFAP, Connexin43, and GAPDH were analyzed by western blot.

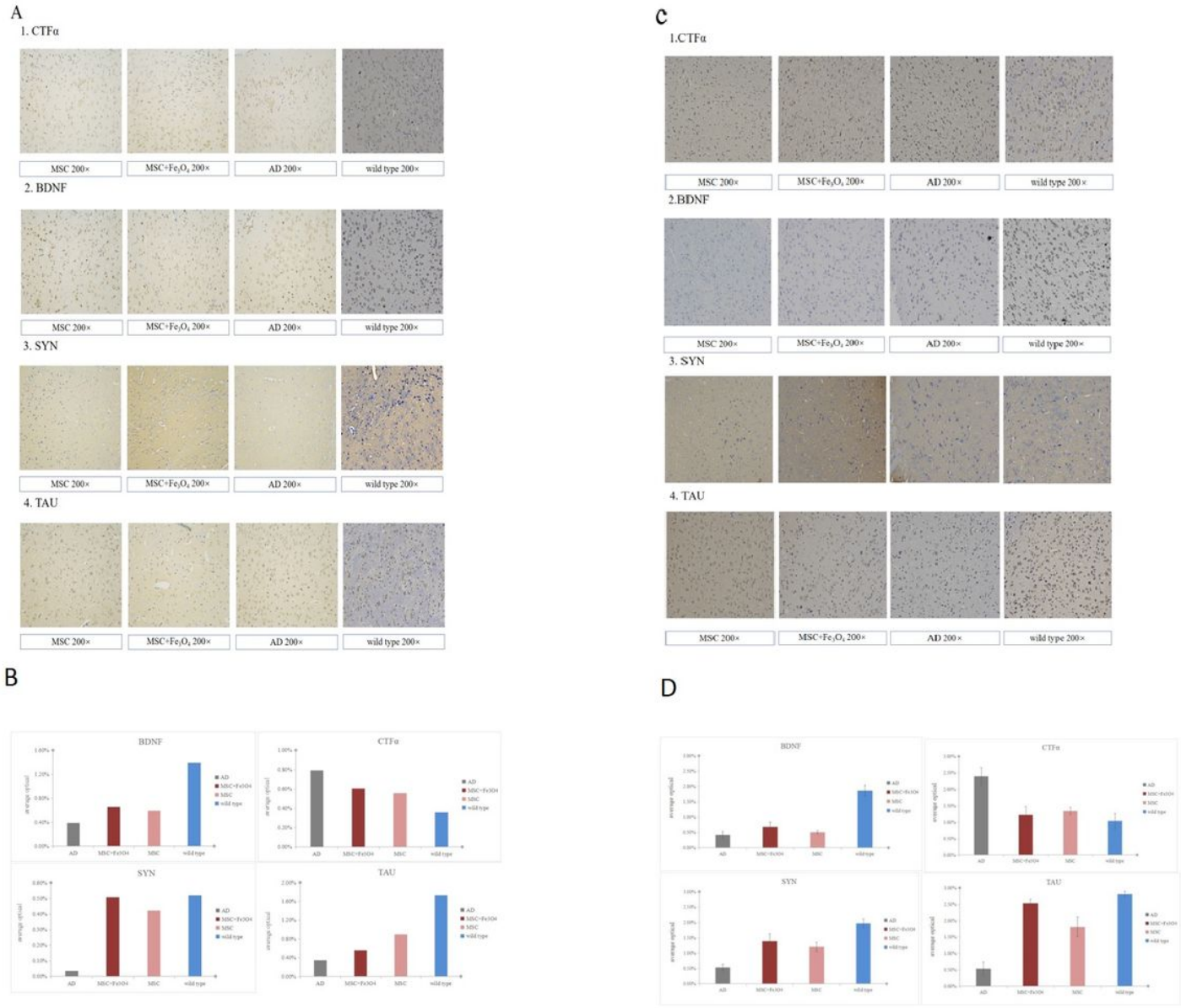

Figure 5

Mesenchymal stem cells regulate Alzheimer's disease-related proteins. (A) Comparison of brain immunohistochemistry in mice aged 7 months. (B) Statistical results of the positive rate of brain immunohistochemistry in mice aged 7 months. (C) Comparison of brain immunohistochemistry in mice aged 10 months. (D) Statistical results of the positive rate of brain immunohistochemistry in mice aged 10 months.