Therapeutic Advances in Chronic Disease

Original Research

Effect of TO901317 on GF to promote the differentiation of human bone marrow mesenchymal stem cells into dopamine neurons on Parkinson's disease

Ther Adv Chronic Dis

2021, Vol. 12: 1–22

DOI: 10.1177/2040622321998139

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Abstract

Background: Human bone marrow mesenchymal stem cells (hBMSCs) could differentiate into dopamine-producing cells and ameliorate behavioral deficits in Parkinson's disease (PD) models. Liver X receptors (LXRs) are involved in the maintenance of the normal function of central nervous system myelin. Therefore, the previous work of our team has found the induction of cocktail-induced to dopaminergic (DA) phenotypes from adult rat BMSCs by using sonic hedgehog (SHH), fibroblast growth factor 8 (FGF8), basic fibroblast growth factor (bFGF), and TO901317 (an agonist of LXRs) with 87.42% of efficiency in a 6-day induction period. But we did not verify whether the induced cells had the corresponding neural function.
Methods: Expressions of LXRα, LXRβ, and tyrosine hydroxylase (TH) were detected by immunofluorescence and western blot. Adenosine triphosphate-binding cassette transporter A1 (ABCA1) was detected by quantitative real-time PCR. The induced cells were transplanted into PD rats to study whether the induced cells are working.
Results: The induced cells can release the dopamine transmitter; the maximum induction efficiency of differentiation of hBMSCs into DA neurons was 91.67% under conditions of combined use with TO901317 and growth factors (GF). When the induced-cells were transplanted into PD rats, the expression of TH in the striatum increased significantly, and the behavior of PD rats induced by apomorphine was significantly improved.
Conclusion: The induced cells have the function of DA neurons and have the potential to treat PD. TO901317 promoted differentiation of hBMSCs into DA neurons, which may be related to activation of the LXR-ABCA1 signaling pathway.

Keywords: human bone marrow mesenchymal stem cells, Parkinson's disease, liver X receptor agonist, TO901317, differentiation, cell transplantation

Received: 30 December 2020; revised manuscript accepted: 3 February 2021.

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Introduction

Parkinson's disease (PD) is a complex, age-related neurodegenerative disease with early prominent death and loss of dopaminergic (DA) neurons in substantia nigra (SN).[1,2] The main clinical manifestations of PD are static tremors, increased muscle tone, and unstable posture. PD is also related closely to various non-motor symptoms, such as cognitive dysfunction, mood, and psychotic disorder.[3] A considerable number of patients have cognitive impairment in the late stage of PD. Non-motor symptoms might be present up to 20 years before manifestations of the characteristic motor symptoms.[4]

PD is associated with cholinergic and monoaminergic neurons, and oligodendrocytes play an important role in PD.[5] Traumatic brain injury, post-traumatic and stress disorder,[6] history of melanoma, and exposure to pesticides increase risk of PD.[4] In the degenerative diseases of the nervous system, the incidence of PD in the 65-year-old population is more than 1%, second only to Alzheimer's disease, and it escalates the burden in economic terms and effects on quality of life to these patients' families and society.

The main management modes of PD are drug therapy, surgical treatment, and stem cell replacement therapy. Drug therapy mainly improves the symptoms of patients by increasing the concentration of dopamine in the brain. But it is important to consider the side-effects and tolerance levels of the patient.[7] Surgical treatments include thalamicectomy and deep brain stimulation (DBS). Thalamotomy is an intrusive technique. Although it can relieve the tremor of PD patients well, it seems to alter the physiological regulation of PD patients and cause motor dysfunction.[8] When PD patients' symptoms do not respond to medication adjustments, DBS treatment needs to be started. DBS can significantly control the dyskinesia induced by levodopa in the treatment of PD. However, for this invasive treatment, PD patients must experience surgery again and again, which increases the risk of infection.[9] Currently, available drugs or surgery are merely symptomatic treatments and do not slow down or prevent disease progression. There are some data suggesting that supplementing brain-lost DA neurons by cell transplantation may be the most promising therapy for PD.[10] A more immediate and reachable goal of cell transplantation may be neuronal protection.[11] Currently, embryonic stem cells (ESCs), neural stem cells (NSCs), induced pluripotent stem cells (iPSCs), and bone marrow mesenchymal stem cells (BMSCs) are available for stem cell replacement therapy.[12] The use of ESCs and NSCs has inherent ethical problems. So much hope is transferred to iPSCs, which are human fibroblasts induced into a source of patient-specific and disease-specific neurons, especially as, in theory, this approach would avoid many of the ethical issues associated with using ESCs.[13,14] Cell replacement therapy is derived from the patient's own cells without immune rejection. In autologous transplantation, it is necessary to establish iPSCs from each patient, and current technical operations take much time and incur high costs, so it is difficult to spread to general treatment. In addition, since iPSCs possess the patient's own genetic factors, the sensitivity of the disease may be high. There is also a high risk of introducing cancer cells.[15] Specifically, iPSCs often develop chromosomal abnormalities, with gains or losses of whole chromosomes.[16] Unlike these stem cells, BMSCs come from patients themselves without any ethical dispute. Especially, BMSCs have multi-directional differentiation potential,[17] low risk of tumorigenesis,[18] and rich in source. Many *in vitro* and preclinical studies have proved strongly the therapeutic potential of BMSCs when applied as a treatment for different pathological conditions.[19,20]

There have been different methods involved in the differentiation of BMSCs into DA neurons, including cell growth factors,[21,22] chemical inducers,[23] and lentiviral transduction.[24] Currently, *in vitro* induction of stem cells using growth factors with sonic hedgehog (SHH) and fibroblast growth factors (FGFs) have succeeded in inducing adult human BMSCs into DA neurons with 67% of efficiency in 12 days.[25,26] This is the maximum induction efficiency and the shortest induction time for human BMSCs to DA neurons at present.

Liver X receptors (LXRs) include LXRα and LXRβ. LXRs are members of the nuclear receptor supergene family of ligand-activated transcription factors and are major regulators of lipid metabolism.[27] They play a key role in the regulation of cholesterol and fatty acid homeostasis.[28] LXRs are also essential for the central nervous system (CNS).[29,30] The loss of LXRβ in mice affected the formation of progenitor cells and granule cell differentiation, leading to hypoplasia of the dentate gyrus.[31] It has been found that LXRs play crucial roles in the regulation of genes related to *cerebrospinal fluid* (CSF) production and structural integrity of choroid plexus.[32] LXRα and LXRβ are involved in the processes of myelination and remyelination.[33] Activation of LXRα and LXRβ can promote the regeneration and survival of motor neurons.[34] Meanwhile, LXR agonists activate LXR target genes, and play a therapeutic role in different neurodegeneration animal models.[35–37] Therefore, we speculate on whether they can promote the formation of DA neurons and whether LXR agonists have a therapeutic effect on PD.

TO901317 is an LXR agonist that can reduce inflammatory markers and possesses neuroprotective properties.[37,38] TO901317 also significantly increased synaptophysin expression and axonal regeneration in stroke.[39]

Our previous work has found the induction of cocktail-induced to DA phenotypes in adult rat BMSCs by using SHH, fibroblast growth factor 8 (FGF8), basic fibroblast growth factor (bFGF), and TO901317 with 87.42% of efficiency over a 6-day period of induction.[40] LXR agonists significantly shortened induction time and improved induction efficiency compared with reported methods. However, previous studies by our team did not investigate whether induced cells released dopamine. Furthermore, we did not ascertain whether induction can promote differentiation of hBMSCs into DA neurons as well, and if the induced cells have a therapeutic effect on PD.

In this study, we investigated the effect of TO901317 on differentiation of hBMSCs into DA neurons. We also explored whether induced cells had dopamine neuronal function, and the possible mechanism thereof. Finally, we transplanted induced cells into PD rats to observe the therapeutic effect.

Materials and methods

Materials

6-Hydroxydopamine (6-OHDA) was purchased from Selleck (Houston, TX, USA). hBMSCs were purchased from Cyagen Biosciences (Santa Clara, CA, USA). LXR agonist-TO901317 (N-(2, 2, 2-trifluoro-ethyl)-N-[4-(2,2,2-tri-fluoro1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Apomorphine hydrochloride was purchased from the Pharmaceutical Factory of Qinghai, China. Goat serum was purchased from Beijing Dingguo Changsheng Biotechnology, Beijing, China.

Animals

Sprague-Dawley rats were accommodated in the barrier housing facility, in keeping with the national standard of *Laboratory Animal-Requirements of Environment and Housing Facilities*. The care of the laboratory animals and animal experimental operation conforms to the *Chongqing Administration Rule of Laboratory Animal*. The experimental procedures were approved by the animal laboratory administrative center and the institutional ethics committee of Chongqing Medical University (License number: SYXK YU 2018-0003). All animal procedures were performed in strict accordance with the ARRIVE guidelines.

Parkinson's disease rats

All male Sprague-Dawley rats weighed 220–250 g. To establish the rat model of PD, all Sprague-Dawley rats received an intraperitoneal injection of apomorphine (0.5 mg/kg). Rats were selected without rotation behavior to receive a 6-OHDA lesion of the medial forebrain bundle on the right side [MFB, anterior-posterior (AP): 1.8 mm, medial-lateral (ML): 2.0 mm, dorsal-ventral (DV): 8.0/7.8 mm]. A total of 30 male rats received a 6-OHDA lesion of the MFB on the right side and 10 rats were as control group injected with the solvent used to dissolve 6-OHDA only.[41,42] Briefly, rats were anesthetized with chloral hydrate (4% chloral hydrate and 96% saline solution, 1 ml/100 g) by intraperitoneal injection. The MFB was targeted with an injection of 4 µl 0.02% L-ascorbic acid saline solution containing a total amount of 16 µg 6-OHDA. The lesion to stereotaxic coordinates was adjusted to the age and weight of the animals with the help of brain stereotaxic instrument (RWD, Shenzhen, China). The condition of the animals was observed every day; no poor condition was observed. After 2 weeks to 1 month, to determine whether the models were successful, we used apomorphine to induce rotation, and behavior was recorded over a period of 30 min. The model was judged successful if the number of rotations to the healthy side was more than seven rotations per minute.

Differentiation of hBMSCs

Human BMSCs were divided into three groups and plated in 24-well plates where each plate contained 2.0×10^4 cells; cells were cultured at 37°C with 5% CO_2.

Control group. hBMSCs were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12, Gibco, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Biological Industries, Israel) for 24 h. After that, the medium was replaced with neurobasal medium (Invitrogen/Gibco, Carlsbad, CA, USA) and 0.5% B27 supplement (Invitrogen/Gibco).

Growth factors treated group (GF). hBMSCs were cultured in DMEM/F-12 containing 10% FBS for 24h. After that, the medium was replaced with neurobasal medium and 0.5% B27 supplement. The cells were induced only once with a cocktail of 250ng/ml recombinant human SHH (PeproTech, Rocky Hill, NJ, USA), 100ng/ml recombinant human FGF8 (PeproTech), and 50ng/ml recombinant human basic-FGF (bFGF; PeproTech). The medium was not replaced for 12days.

TO901317 and growth factors treated group (LXR+GF). On the basis of induction of GF, the effects of TO901317 on the differentiation of hBMSCs into DA neurons in a time- and concentration-dependent manner were investigated. According to the results of (a), (b), and (c) below, the cells were induced only once with the cocktail, and the medium was not replaced during the induction period.

(a) Explore the concentration of TO901317 added: different concentrations of TO901317 (0.125, 0.25, 0.5, 1 and 2μM) were added with GF (250ng/ml SHH, 100ng/ml FGF8, 50ng/ml bFGF).
(b) Explore the time to add TO901317 during the growth factor induction period: based on GF, TO901317 were only added on the first day, the third day, the sixth day, and the ninth day, respectively, to induce differentiation for 12days.
(c) Explore the induction time of the TO901317 in combination with GF: according to the results of (a) and (b), 0.5μM TO901317 was added to the culture medium, and the cell morphology was observed every 3days.

Cell counting kit-8 assay

A Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) assay was used to test the growth rate by following the manufacturer's instructions. Samples of 3000 cells/well of hBMSCs were cultured in a 96-well plate for each group. At 3-day intervals, the growth rates of cells in each group were measured by application of the CCK-8 kit, and optical density (OD) was determined at 450nm using a microplate reader (Thermo Scientific, Waltham, MA, USA).

Immunofluorescence

In brief, cells of each group were fixed in 4% paraformaldehyde, permeabilized with 0.3% Triton-X100, and blocked in phosphate buffer solution (PBS) containing 5% normal goat serum (Beijing Dingguo Changsheng Biotechnology). Cells were incubated with monoclonal antibodies overnight with 4°C. Antibody dilutions were as follows: β III tubulin, 1:200 (Tuj1; Abcam, Cambridge, UK); tyrosine hydroxylase, 1:200 (TH, Abcam, Cambridge, UK); Nestin, 1:200 (Abcam, Cambridge, UK); Neun, 1:200 (Abcam, Cambridge, UK); LXR α receptor, 1:200 (Abcam, Cambridge, UK); LXR β receptor, 1:200 (Gene Tex, Irvine, CA, USA). After extensive washing three times in PBS, suitable secondary antibodies anti-mouse IgG-Alexa Fluor 488, anti-rabbit IgG-Alexa Fluor 488, anti-mouse IgG-Cy3, and anti-rabbit IgG-Alexa Fluor 594 were diluted at 1:200 in PBS, and then suitable secondary antibodies were added and incubated in darkness for 1h at room temperature. Nuclear stain 4,6-diamidino-2-phenylindole (DAPI; Beyotime Biotechnology, Shanghai, China) was then used for nuclear staining.

Enzyme-linked immunosorbent assay

To investigate whether cells in each group release dopamine, culture supernatant and cells of the control group, GF group, and LXR+GF group were collected. Cells were added to PBS, then ground on ice. The mixture was centrifuged at 3000*g* for 20min at 4°C and the supernatants collected. Dopamine was detected using enzyme-linked immunosorbent assay (ELISA) kits ($n=6$) (Mei biao, Jiangsu, China). The samples and standards were tested according to the manufacturer's instructions.

Western blotting test

Cells were plated on a six-well plate for 24h with 10% FBS, then grown in induction medium for 6days to be used for preparation of whole cell extract. After removing the medium, cells were washed three times with PBS. The cells in each well were then added to 150μl lysis buffer containing 1% phenylmethanesulfonyl fluoride (PMSF) and cracked on ice for 30min. The mixture was centrifuged at 12,000 revolutions per minute for 20min at 4°C and the supernatants

Table 1. List of primers used in qPCR analysis.

Gene	FORWARD sequence (5′->3′)	REVERSE sequence (5′->3′)
ABCA1	GAGGCAATGGCACTGAGGAAGATG	CAACGAGCAGCGGCTTCAGAG
GAPDH	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG

collected. The protein concentration was determined by a bicinchoninic acid (BCA) Protein Assay Kit (Beyotime, Shanghai, China). The protein was separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% bovine serum albumin (BSA) for 2 h at room temperature and then incubated with specific primary antibodies, TH, 1:500 (Abcam); LXR α receptor, 1:500 (Abcam); LXR β receptor, 1:500 (GeneTex) were included and overnight at 4°C. The membranes were rinsed three times in TBST and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for 1 h. After washing three times in TBST, protein signals were visualized by ECL (Bio-Rad, Richmond, CA, USA).

Real-time polymerase chain reaction

Total RNA was isolated from cells in the control group, GF group, and LXR+GF group by Trizol reagent (Vazyme, Nanjing, China) according to the manufacturer's protocol. mRNA was subjected to reverse transcription using HiScript Q Select RT SuperMix (Vazyme). SYBR Green II (Bimake, Houston, TX, USA) incorporation method was used to detect the amount of mRNA. Negative controls were used as no template cDNA reactions and melting curves were used to confirm the results. The results were normalized using the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) concentration of each sample. The primer sequences are reported in Table 1.

Cell transplantation

Four weeks after 6-OHDA infusion, PD rats were divided randomly into two groups: a saline group (6-OHDA group, $n=10$), and a cell transplantation group (6-OHDA+Cells group, $n=20$). The differentiated DA cells were dislodged using an accutase cell dissociation reagent (Invitrogen/Gibco). Cell suspensions of 5 µl (100,000 cells/µl) were transplanted in right SN (AP: 4.6 mm, ML: 2.2 mm, DV: 7 mm).

Histopathological examination

Hematoxylin and eosin (HE) staining was performed to show pathological histological damage in the substantia nigra pars compacta (SNc) and striatum. Rats of the control group, 6-OHDA group, and 6-OHDA+Cells group were anesthetized with sodium chloral hydrate (4%, 1 ml/100 g) and perfused with PBS, and then perfusion with 4% paraformaldehyde. Thereafter, brains were dehydrated in a graded series of alcohols and embedded in paraffin. A series of 5-µm-thick sections were cut from the brain. Finally, the sections were stained with HE reagents for histopathological examination.

Statistical analysis

All results were expressed as the means ± standard deviation (SD) and the statistical significance of differences was analyzed by GraphPad Prism (GraphPad Software, La Jolla, CA, USA). For the comparison of multiple groups, statistical analysis was performed using one-way analysis of variance (ANOVA) with Bonferroni's *post hoc* test. Probability values less than 0.05 ($p<0.05$) were considered to be statistically significant.

Results

Morphological features of hBMSCs

hBMSCs were purchased in Cyagen Biosciences. The cells exhibited a long fusiform and vortex arrangement (Figure 1).

Effect of different concentrations of TO901317 on cell survival

A CCK8 kit was used to detect the survival rate of cells in the control, GF, and LXR+GF groups. In

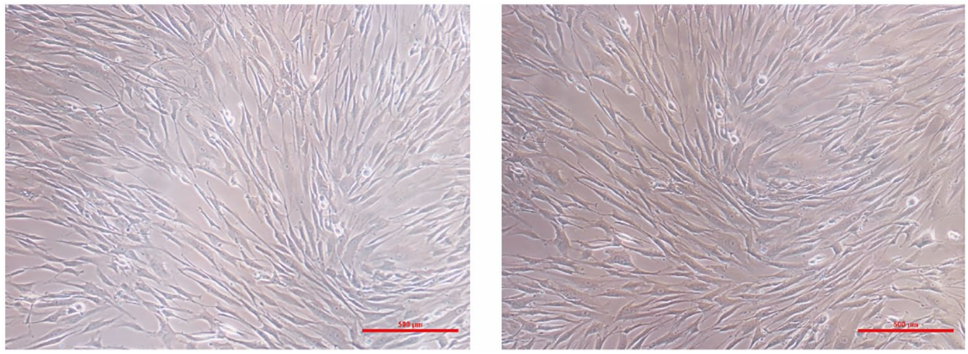

Figure 1. hBMSCs were observed at inverted microscope and showed a fibroblastoid cell profile and produced cell colonies with unique vortex shape.
hBMSCs, human bone marrow mesenchymal stem cells.

addition, the TO901317 was added to the LXR+GF group at concentrations of 0.125 μM, 0.25 μM, 0.5 μM, 1 μM and 2 μM, respectively. After 3 days, the survival rate of cells in each group did not show a significant difference (Figure 2a). The survival rate increased significantly in the GF group and LXR+GF group compared with the control group when cells were cultured for 6 days, 9 days, and 12 days. Interestingly, there was no significant difference in cell survival rate when cells were cultured with different concentrations of TO901317 (Figure 2).

Optimal concentration of TO901317

Immunofluorescence was used to detect the expression of neuronal markers (Neun, Nestin, and Tuj1) and dopamine neuron markers (TH).

In this study, TO901317 was used at five concentrations (0.125 μM, 0.25 μM, 0.5 μM, 1 μM, and 2 μM) combined with GF. When hBMSCs were cultured with 0.5 μM TO901317, the number of TH^+ positive cells reached the maximum (Figure 3).

The time to add TO901317 into GF

In this study, 0.5 μM TO901317 was used in combination with GF to induce hBMSCs to form DA neurons. On the premise of GF addition during 12 days, TO901317 was fully added on the first day, the third day, the sixth day, and the ninth day, respectively. Expression of TH (Cy3, red) and Tuj1 (Alexa Fluor 488, green) were determined by immunofluorescence. The results showed that expression of TH was the highest when TO901317 was added on the first day (Figure 4).

The optimal period induced by TO901317

Immunofluorescence was used to detect expression of Tuj1 and TH after 3 days, 6 days, 9 days, and 12 days of the induction period. Cells in the control group expressed only Tuj1. While cells in the GF group and LXR+GF group expressed Tuj1 and TH simultaneously (Figure 5a). In the LXR+GF group, TH^+ cells reached a maximum after 6 days of induction (Figure 5b). Compared with the control and GF groups, the expression of TH was increased significantly in cells of the LXR+GF group when 0.5 μM TO901317 was added with GF on the first day (Figure 5d). Cells showed typical neuronal morphology with extended long cell processes and enlarged cell bodies in LXR+GF group in 12 days (Figure 5c). In particular, compared with control and GF groups, the morphology of the neurons expressed by the cells was more obvious in LXR+GF group when 0.5 μM TO901317 were added with GF on the first day (Figure 5e).

Nestin, neun expression, and dopamine release

Nestin, a neuroectodermal marker, seems to be a prerequisite for the acquisition of the aptness to progress towards the neural lineage.[43,44] The cells in the control group probed with nestin and neun antibodies were negative and just revealed nuclear staining. Both GF and LXR+GF cells stained with neun and nestin (Figure 6a, b).

A dopamine kit was used to study whether differentiated hBMSCs release dopamine. The results showed that cells in the control and GF groups did not secrete dopamine. Although the GF group cells showed positive staining for neuronal

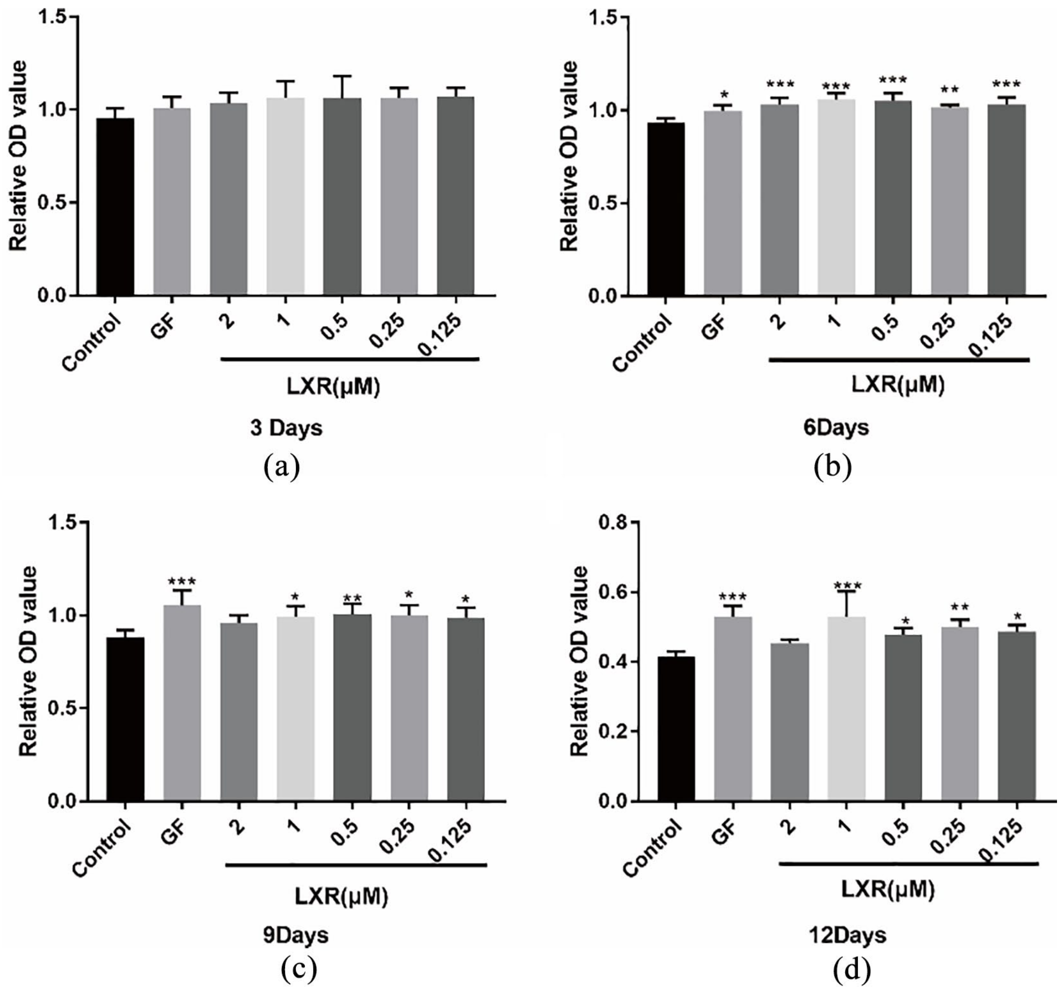

Figure 2. Different concentrations of TO901317 were added to the medium to examine the effect of TO901317 on the viability of hBMSCs. (a) Culture for 3 days. (b) Culture for 6 days. (c) Culture for 9 days. (d) Culture for 12 days. Data were expressed as mean ± SD ($n = 6$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with control group.

GF, growth factor group; hBMSCs, human bone marrow mesenchymal stem cells; LXR, liver X receptors; OD, optical density; SD, standard deviation; TO901317, LXR agonist N-(2,2,2-trifluoro-ethyl)-N-[4-(2,2,2-tri-fluoro1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide.

markers, dopamine levels were extremely low and could not be detected in this experiment. Only cells in the LXR + GF group had the characteristics of DA neurons, and the content of dopamine was high (Figure 6c).

The role of LXRs in cell differentiation and its possible mechanism

The expressions of LXRα and LXRβ did not show significant differences among the control, GF, and LXR+GF groups (Figure 7a, b). The result of western blot showed that expressions of LXRα and LXRβ were decreased significantly in the LXR+GF group compared with the control and GF groups (Figure 7c, d).

Adenosine triphosphate-binding cassette transporter A1 (ABCA1) mRNA in the LXR+GF group was elevated significantly when compared with the control and GF groups (Figure 7e).

Establishment of PD model

Compared with the control group, the number of contralateral rotations per min was scored. Rats

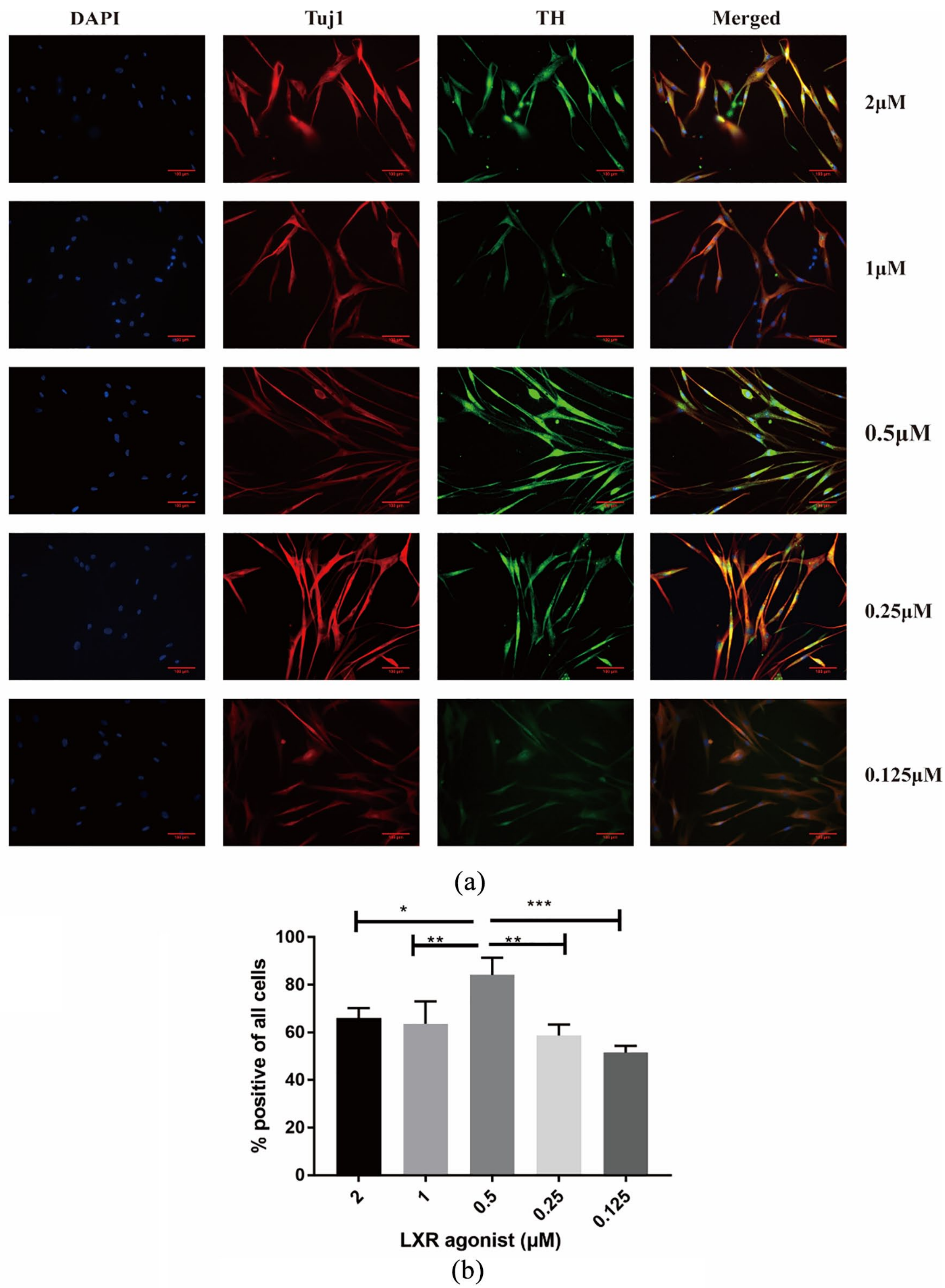

Figure 3. Optimal concentration of TO901317. (200×, Scale bars = 100 μm). (a) The rate of TH^+ positive cells reached the maximum under the induction of 0.5 μM TO901317 combined use with GF. (b) Group data showed change in expression of TH. Data were expressed as mean ± SD ($n=6$).*$p<0.05$, **$p<0.01$, ***$p<0.001$, compared with concentration of 0.5 μM TO901317.

GF, growth factor group; SD, standard deviation; TH, tyrosine hydroxylase; TO901317, LXR agonist N-(2,2,2-trifluoro-ethyl)-N-[4-(2,2,2-tri-fluoro1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide.

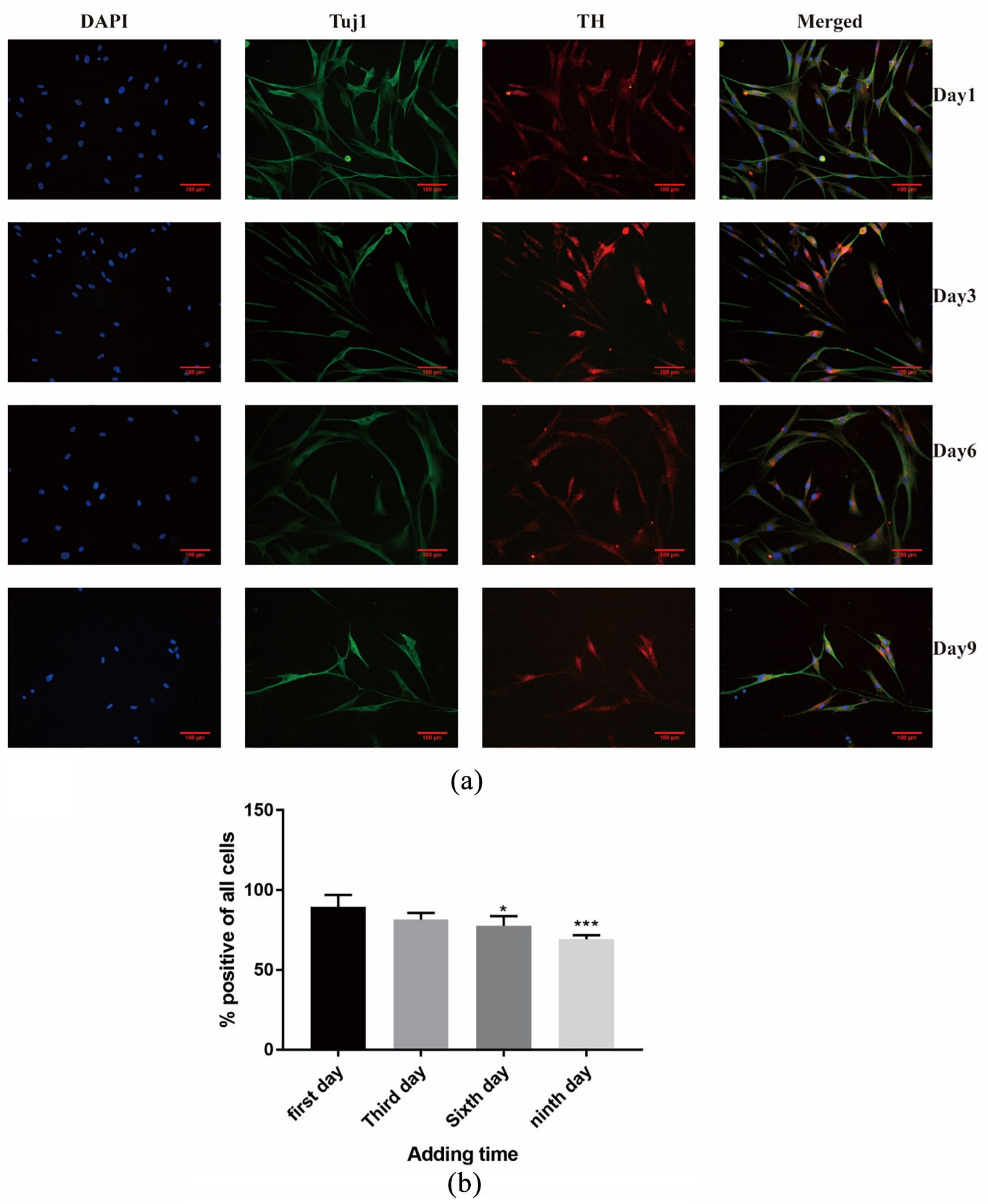

Figure 4. Exploring the best time to add TO901317 into GF (200×, Scale bars = 100 μm). (a) Expressions of Tuj1 and TH between each group. (b) The statistic of TH^+ cells between groups. Data were expressed as mean ± SD ($n = 6$). *$p < 0.5$, ***$p < 0.001$, compared with the first day group.
GF, growth factor group; SD, standard deviation; TH, tyrosine hydroxylase; TO901317, LXR agonist N-(2,2,2-trifluoro-ethyl)-N-[4-(2,2,2-tri-fluoro1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide; Tuj1, β III tubulin.

with stable lesions (>7 rpm/min) were selected as PD models. The expression of TH in the model group decreased significantly in striatum. The results suggested that the PD model was successfully established (Figure 8).

Effect of cell transplantation on PD rats

Two weeks after the injection of the cells, the apomorphine-induced contralateral rotation test was performed. The 6-OHDA+Cells group already exhibited significantly improved behavioral

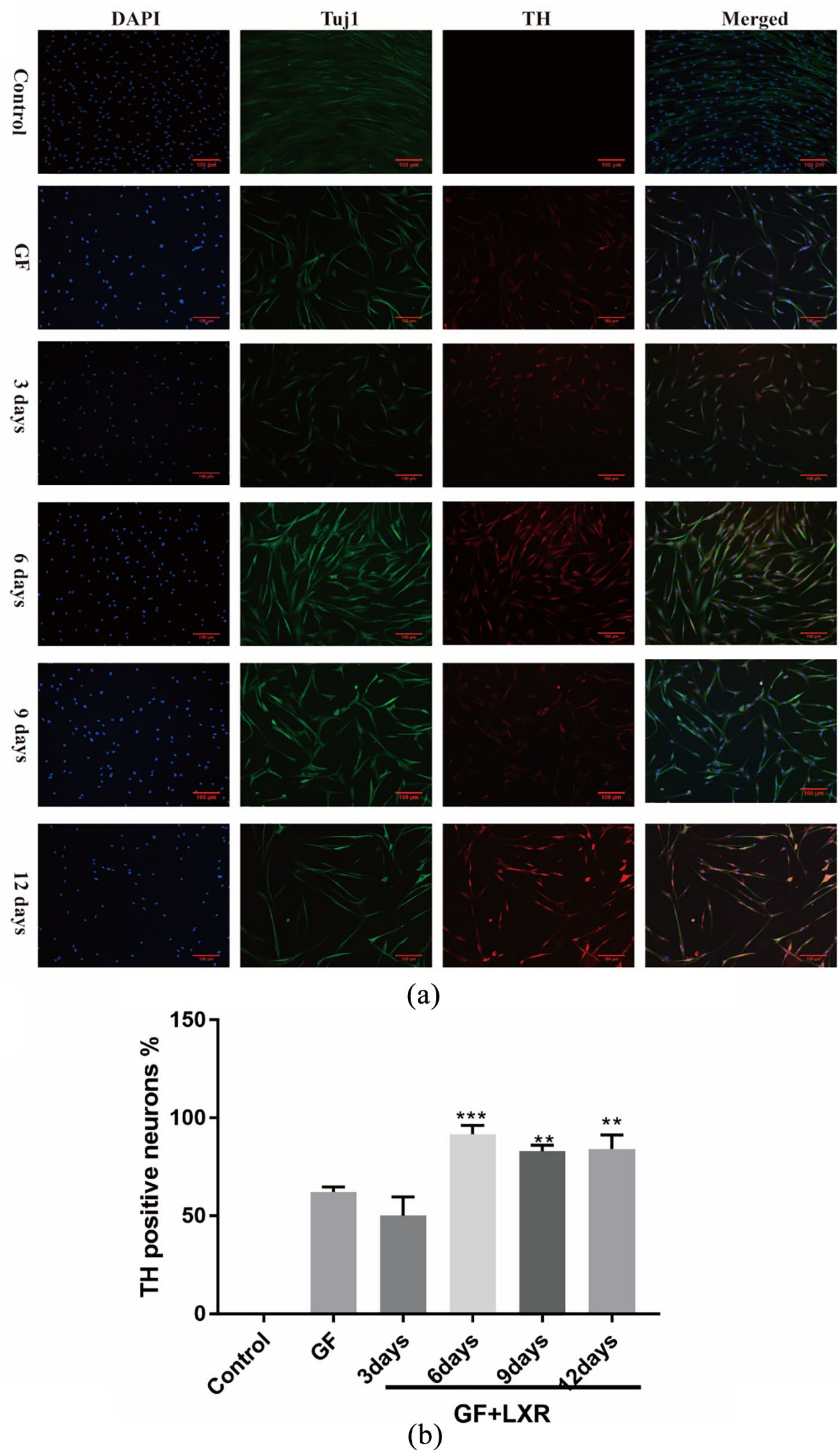

Figure 5. *(Continued)*

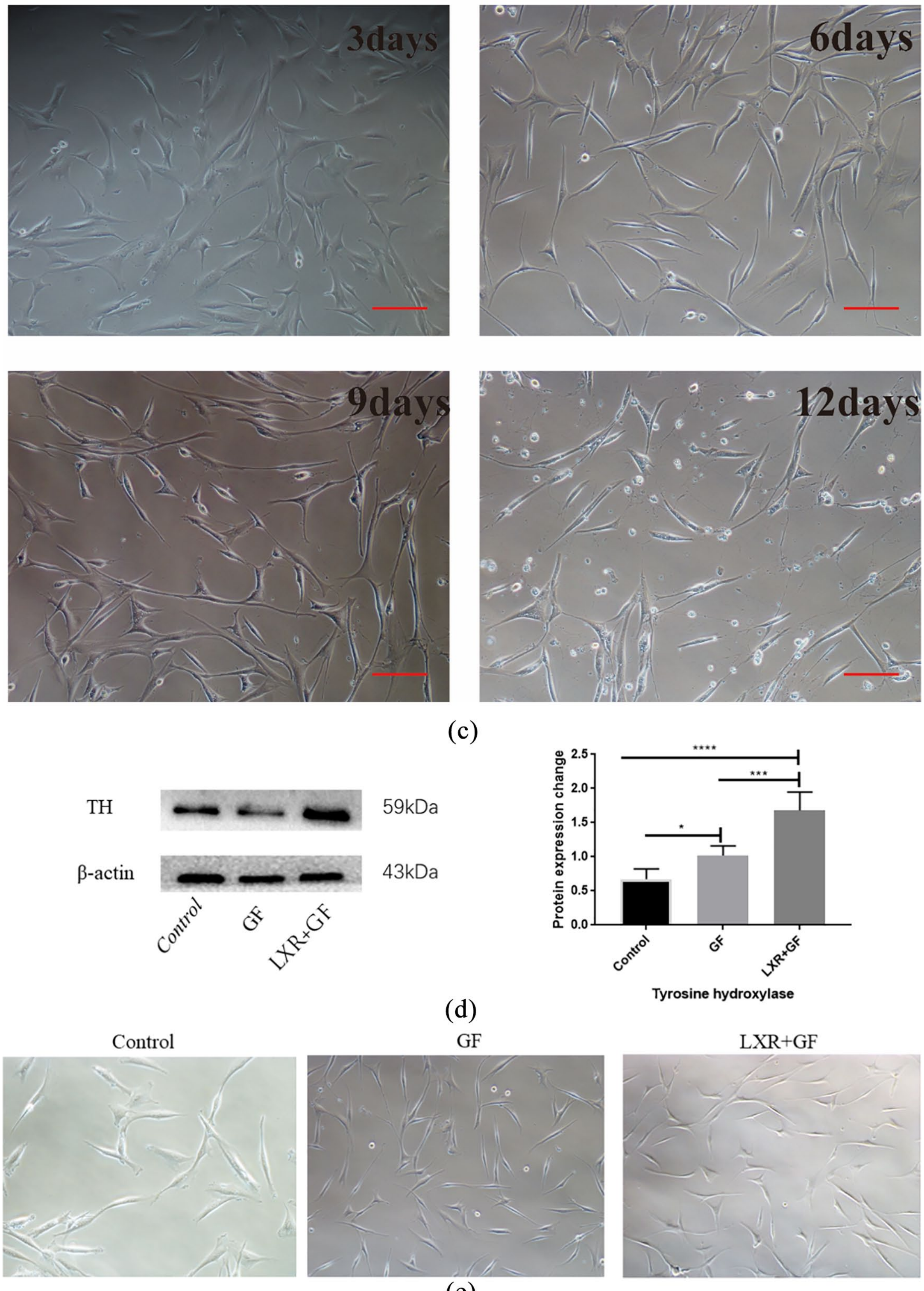

Figure 5. Exploring the optimal period for induction by TO901317. (a) Expression of Tuj1 (Alexa Fluor 488, green) and TH (Cy3, red) in each group (200×). (b) Counting TH+ cells between groups. Data were expressed as mean ± SD (*n* = 6). $^{**}p < 0.01$, $^{***}p < 0.001$, compared with the GF group, respectively. (c) Morphological changes of cells treated with GF and TO901317 under different induction periods (100×). (d) Expression changes of TH when 0.5 μM TO901317 was added with GF on the first day were detected by Western blotting.

Figure 5. *(Continued)*

Figure 5. (Continued)

Data were expressed as mean ± SD ($n=3$). $*p<0.05$, $***p<0.001$, compared with the control group, respectively. $**p<0.01$, compared with the GF group. (e) Bright-field images of hBMSCs in different groups by cultivating for 6 days after 0.5 μM TO901317 were added with GF on the first day (100×).
GF, growth factor group; hBMSCs, human bone marrow mesenchymal stem cells; SD, standard deviation; TH, tyrosine hydroxylase; TO901317, LXR agonist N-(2,2,2-trifluoro-ethyl)-N-[4-(2,2,2-tri-fluoro1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide; Tuj1, β III tubulin.

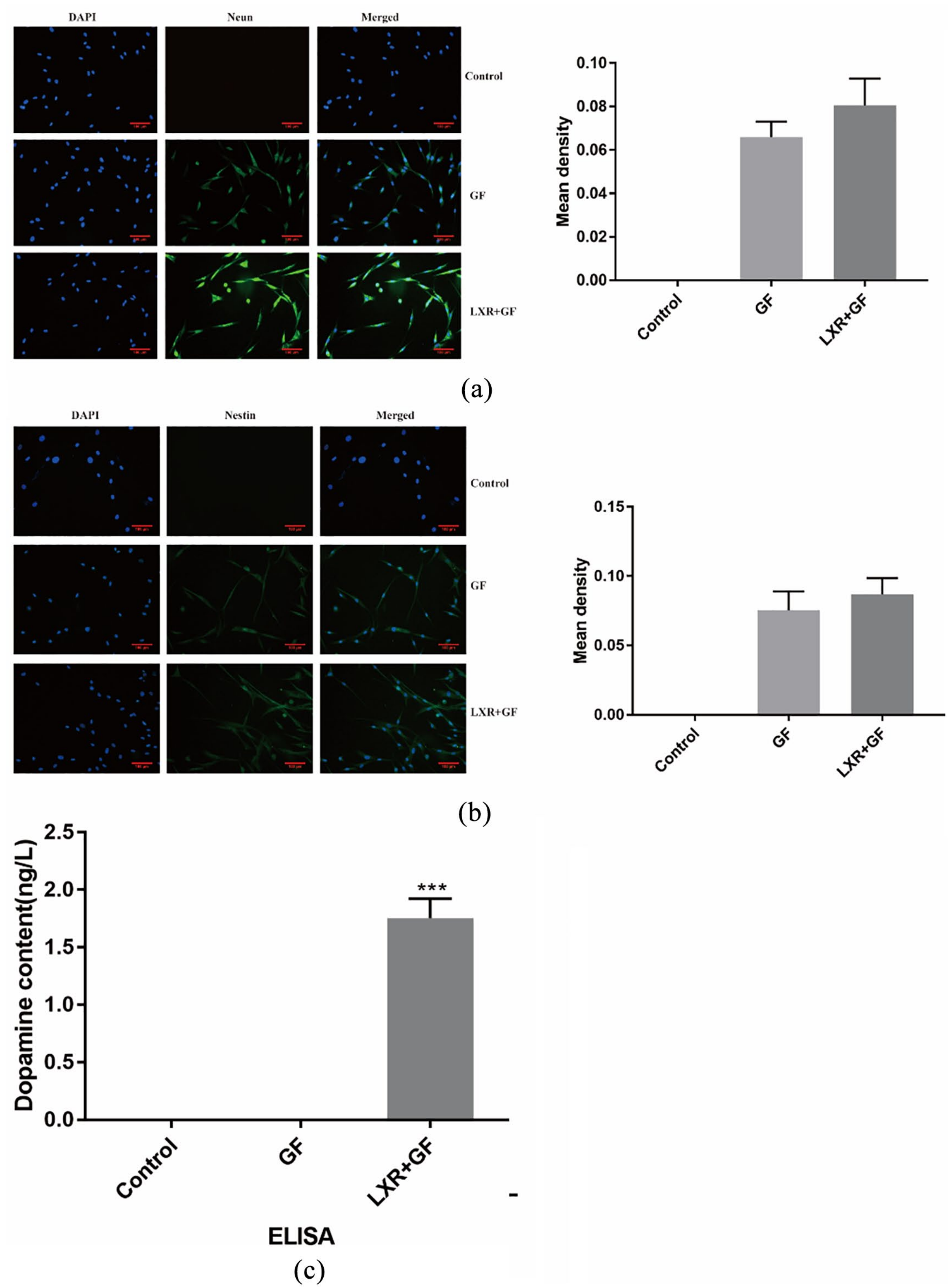

Figure 6. *(Continued)*

Figure 6. Nestin, neun expression, and dopamine release. (200×, scale bars = 100 μm). (a) Expression of neun. Data were expressed as mean ± SD (*n* = 3). (b) Expression of nestin. Data were expressed as mean ± SD (*n* = 3). (c) Secretion of dopamine. Data were expressed as mean ± SD (*n* = 3). ***$p < 0.001$, comparison of the expression of dopamine by LXR+GF group.
GF, growth factor group; LXR, liver X receptors; SD, standard deviation.

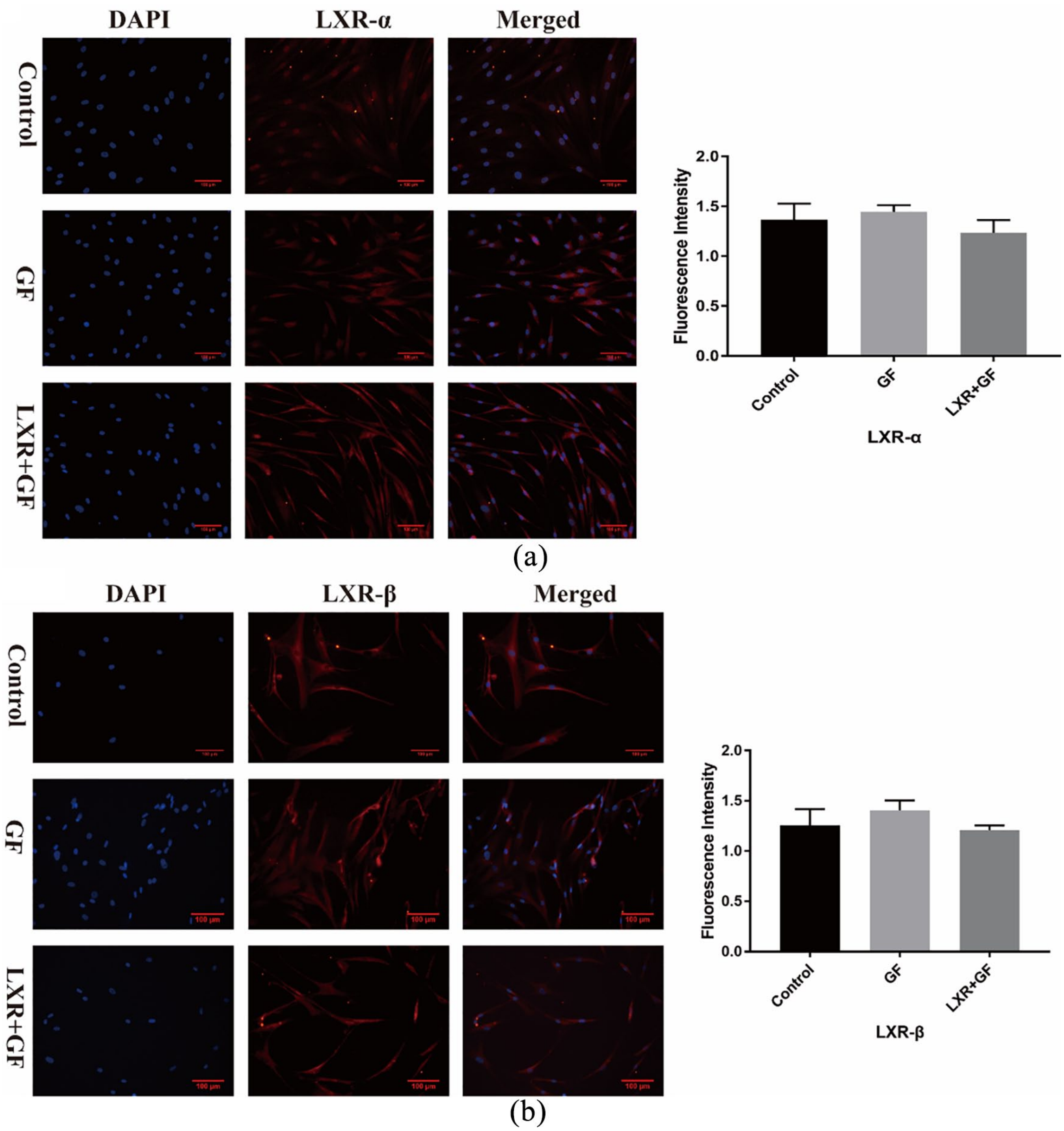

Figure 7. *(Continued)*

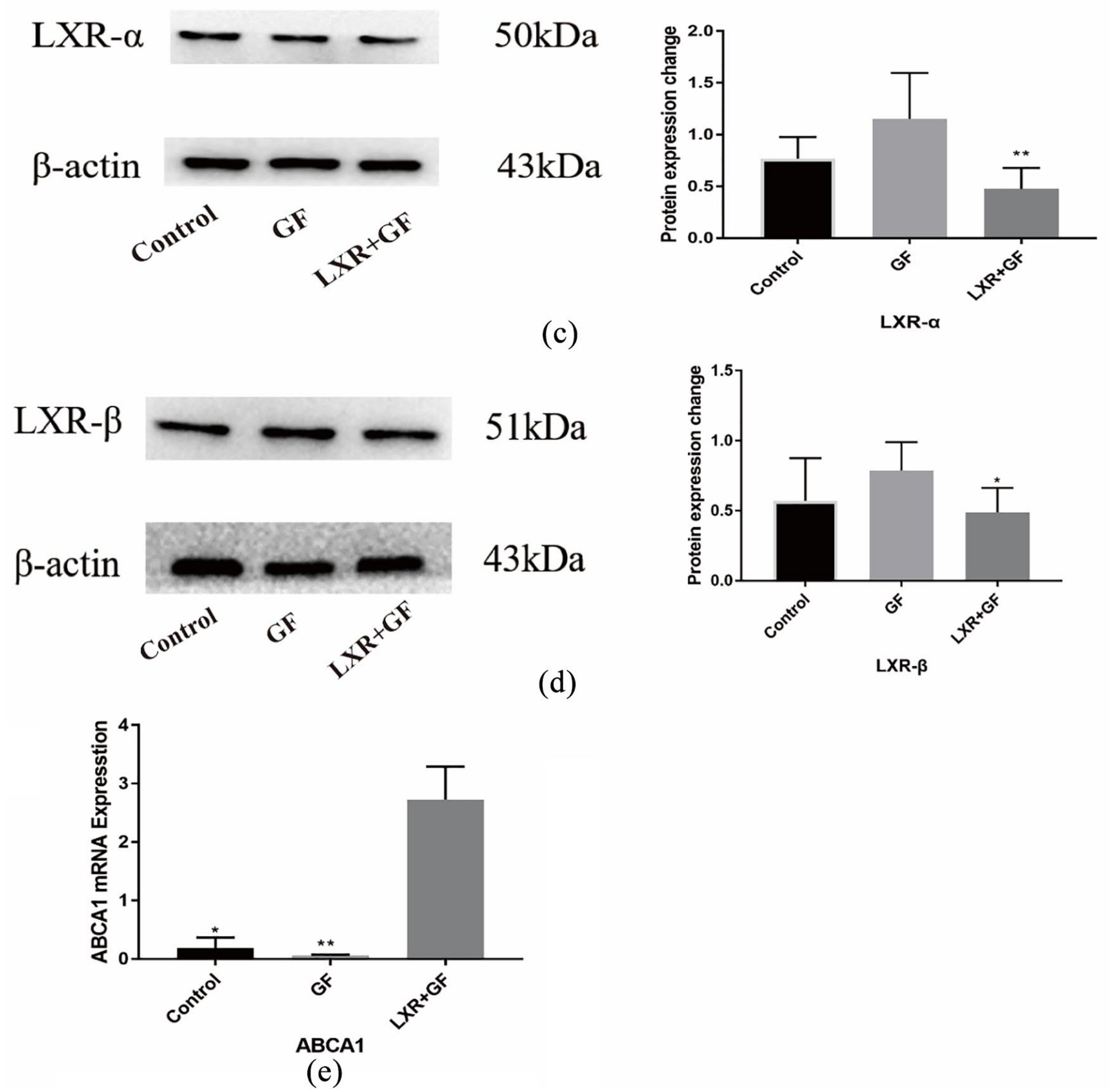

Figure 7. Changes in expressions of LXRs (200×, Scale bars = 100 μm). (a, b) Expressions of LXRα and LXRβ (n = 3). (c, d) The expression of LXRs protein. Data were expressed as mean ± SD (n = 3).$^*p < 0.05$, $^{**}p < 0.01$, compared with GF group. (e) Changes in expression of ABCA1 mRNA. Data were expressed as mean ± SD (n = 3). $^*p < 0.05$, $^{**}p < 0.01$, compared with LXR+GF group.
ABCA1, adenosine triphosphate-binding cassette transporter A1; GF, growth factor group; LXR, liver X receptors; SD, standard deviation.

performance compared with the rats of the 6-OHDA group (Figure 9f). Compared with the control group, cells in the 6-OHDA group presented significant nuclear pyknosis, vacuolization, and nuclear deep staining. After cell transplantation, the nuclear deep staining of the nucleus and the vacuolization of cells in 6-OHDA+Cells were reduced (Figure 9a). Results revealed that TH-positive signals were almost absent at the striatum in the 6-OHDA-lesioned rats that received no grafts. In contrast, in rats in the 6-OHDA+Cells group, TH-positive signals were greatly recovered in the striatum (Figure 9c). TH-positive signals also increased significantly at SNc in the 6-OHDA+Cells group compared with the 6-OHDA group (Figure 9b). Western blot detection also showed the same results in striatum (Figure 9d, e).

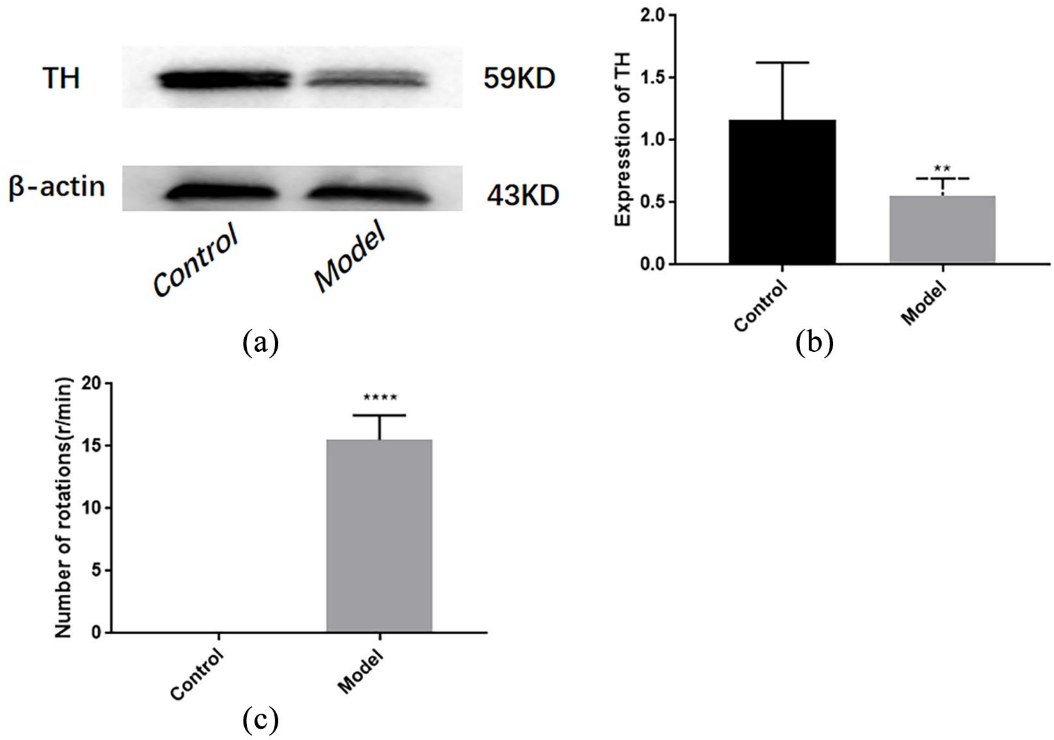

Figure 8. Establishment of Sprague-Dawley rat PD model. (a) The expression of TH in striatum in each group ($n = 3$). (b) Data were expressed as mean ± SD ($n = 3$). $^{**}p < 0.01$, compared with control group. (c) Rotational revolutions per minute. Data were expressed as mean ± SD ($n = 6$). $^{****}p < 0.0001$, compared with control group.
PD, Parkinson's disease; SD, standard deviation; TH, tyrosine hydroxylase.

Discussion

PD is a complex neurodegenerative disease characterized by motor dysfunction, also accompanied by non-motor symptoms. These symptoms are associated tightly with the loss and death of DA neurons. In current PD research, cell transplantation has become an important topic, and has a very important significance in clinical application. NSCs, ESCs, iPSCs, and BMSCs have been used for the treatment of PD.

Some studies have shown that BMSCs express genes and proteins related to the neural lineage, and have been displayed to hold neurogenic differentiation potential under the proper conditions *in vitro*.[45–47] BMSC transplantation restrained multiple parameters of spinal neuroinflammation found in diabetic mice.[48] Furthermore, BMSCs can release neurotrophic factors, including glial cell line-derived neurotrophic factor (*GDNF*) and brain-derived neurotrophic factor (*BDNF*) to protect neurons.[49,50]

In the reported studies, many methods have been adopted to drive BMSCs to differentiate into DA neurons; induction time is generally 12 days or more.[51] The vital method was to induce directional differentiation of cells by using cytokines. Astrocyte-derived bFGF is required for regulation of DA differentiation of stem cells, and promotes growth and survival of midbrain DA cells.[52–55] SHH participates in a broad array of neurodevelopmental processes in the vertebrate embryo, including morphogenesis, cell proliferation and specification, and axon pathfinding.[56,57] SHH exists in the postnatal and adult CNS, modulating neuronal activity in progenitor cells and astrocytes as well as in differentiated neurons.[58–60] FGF8 is essential for the development of multiple brain regions, such as suprachiasmatic nuclei (SCN) and the hypothalamic-pituitary.[61,62] The development and survival of DA neurons are associated with GF-SHH and FGF8.[63,64] ABCA1 affects cognitive function, leading to amyloid-beta (Aβ) production and apoptosis and promoting neurorestoration.[65–67]

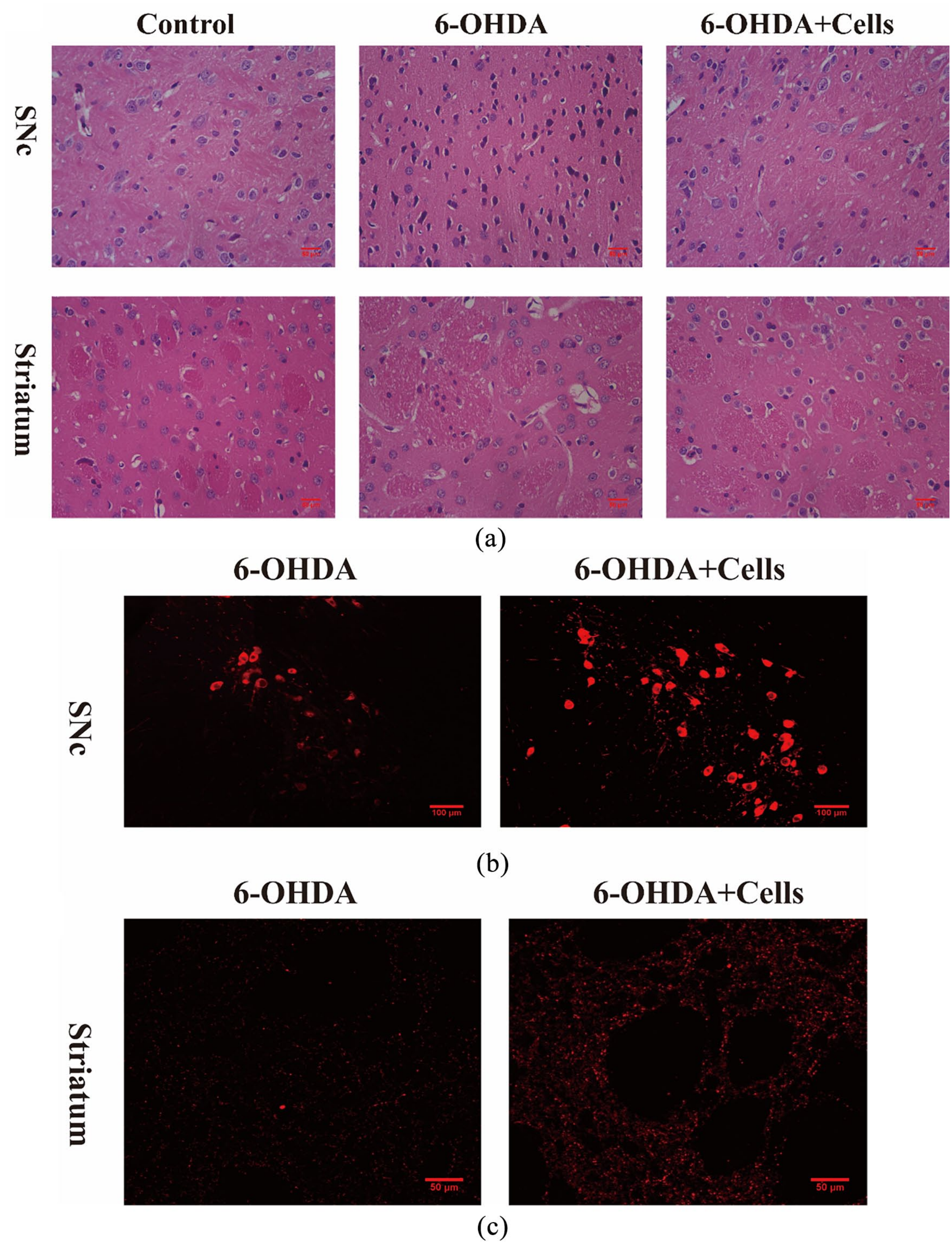

Figure 9. *(Continued)*

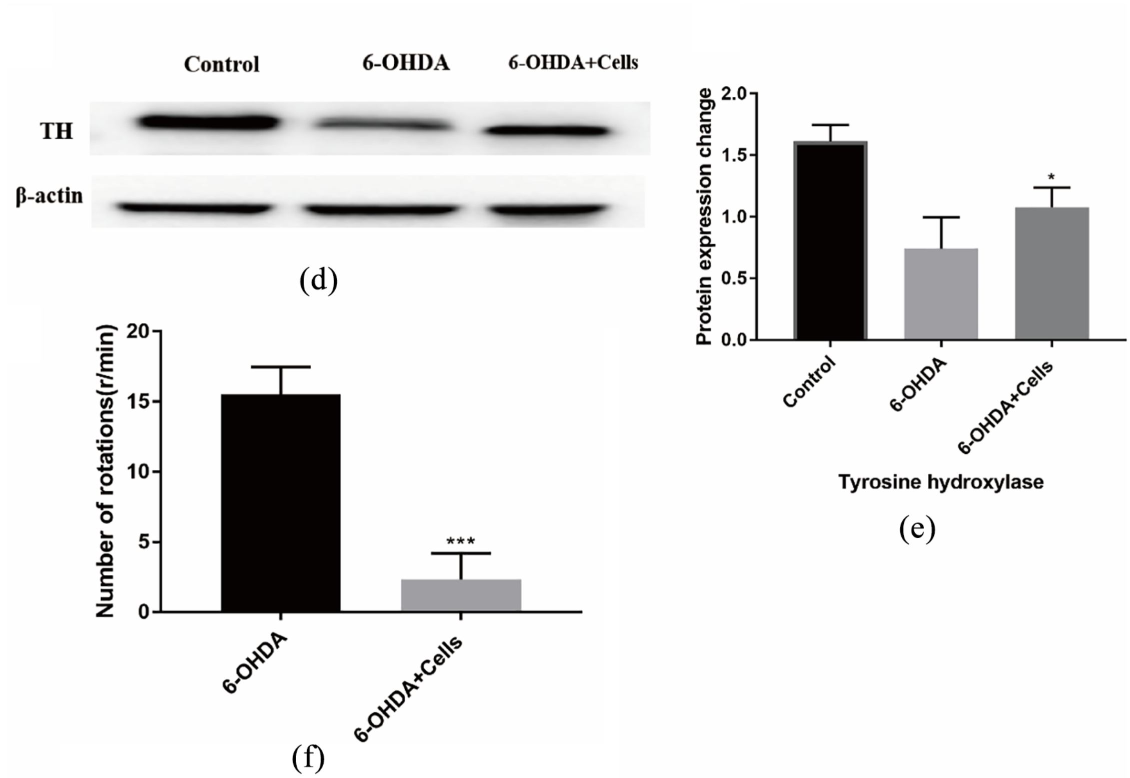

Figure 9. Results of cell transplantation (200×, Scale bars = 100 μm). (a) Results of HE in each group. (b,c) TH immunofluorescent staining for TH in SNc and striatum of each group. (d,e) Expression of TH in striatum. Data were expressed as mean ± SD ($n = 3$). *$p < 0.05$, compared with 6-OHDA group. (f) Results of apomorphine-induced rotations from each group. Data were expressed as mean ± SD ($n = 6$). ***$p < 0.001$, compared with 6-OHDA group.

HE, hematoxylin and eosin; 6-OHDA, 6-hydroxydopamine; SD, standard deviation; SNc, substantia nigra pars compacta; TH, tyrosine hydroxylase.

The aim of this present study was to explore the effect of TO901317 on the differentiation of hBMSCs into DA neurons. We found that the growth rate of cells in the GF and LXR+GF groups were increased initially and decreased latterly with the extension of induction time when compared with a control group. But the growth rate of cells in the LXR+GF group was not different from that of the GF group. The results indicated that TO901317 had little or no effect on the proliferation of cells, and that TO901317 may have a special effect on cell differentiation. Some studies have explored the effect of GF on inducing the differentiation of BMSCs into DA neurons, but the result designated DA neuronal progenitors without neuronal function.[26,68] The results of this study showed that hBMSCs treated with GF alone or TO901317 in combination with GF led to directed neuronal differentiation. But the maximum efficiency of induction was low, and the shortest induction time was 12 days when hBMSCs were treated with GF alone, whereas the maximum efficiency of induction was 91%, and the shortest induction time was 6 days when hBMSCs were treated with 0.5 μM TO901317 in cooperation with GF. The results showed that TO901317 could promote the differentiation of hBMSCs into DA neurons in cooperation with GF. TO901317 improved the efficiency of induction. Cells in the GF and LXR+GF groups expressed neun and nestin. Earlier work on hBMSCs has shown the spontaneous expression of nestin in BM-MSCs and upon DA neuron induction, and the expression of nestin in BMSCs has been shown to be down-regulated. In recent years, some studies have shown that BMSCs have no spontaneous expression of nestin.[40,69] Upon DA neuron induction, the expression of nestin in BM-MSCs has been shown to become down-regulated.[70] Therefore, we speculated that different culture media may cause the different expression of nestin observed. The results revealed that only cells in the LXR+GF group secreted dopamine. The result suggested that TO901317 could promote the maturation of cellular functions. All these results indicated that simultaneous addition of TO901317 and GF could significantly improve the differentiation efficiency of hBMSCs and shorten the induction period of hBMSC differentiation into DA neuron-like cells.

The lack of ABCA1 leads to transport disorders of CNS cholesterol, which, in turn, leads to defects in neuronal structure and function.[71] LXR may be involved in the mechanisms of hBMSC differentiation into DA neuron-like cells. Our study found that the expression of ABCA1 mRNA elevated significantly and LXRs decreased obviously in the LXR+GF group when compared with the GF group. The results indicated that TO901317 can promote the differentiation of hBMSCs into DA neurons by activating the LXRs-ABCA1 signal pathway.

The results of other studies have shown that short-term xenotransplantation has less immune rejection.[72,73] Moreover, BMSCs have immunosuppressive effects *in vivo* and *vitro*.[74,75] The damage caused by 6-OHDA could be reduced after induced-cells transplantation in PD rats. Compared with the 6-OHDA group, the DA neurons in the 6-OHDA+Cells group had a significant increase.

The behavioral symptoms were improved, the pathological damage to morphological structures was reduced, and the expression of TH increased when induced cells were transplanted to PD rats. Together with the results of *in vitro* experiments, these results suggested that the induced cells had corresponding physiological functions after transplantation. This indicated that they could survive after transplantation and DA release. The results showed that induced cells had a potential therapeutic effect on PD. Because of the important position of the current technology for BMSCs to differentiate into DA neurons, and given that the existing technology has serious defects such as low differentiation efficiency, short induction of differentiation time, and low cell survival rate after transplantation, this work is the first to propose LXR as an entry point, to find methods and technologies to significantly improve the differentiation efficiency of BMSCs, shorten the differentiation induction time, and improve the survival rate and function of cells after transplantation, to solve the key problem with the BMSCs that are widely used in cell replacement therapy for PD patients.

Conclusions

Collectively, based on GF to culture cells, 0.5 μM TO901317 could promote the differentiation of hBMSCs into DA neurons. Induced cells had a potential therapeutic effect on PD. The mechanism of TO901317 promoting the differentiation of hBMSCs into DA neurons may be related to the activation of the LXR-ABCA1 signaling pathway.

Author contributions

Junqing Yang and Hong Wang made substantial contribution to conception, design, and performance of the study. Miaomiao Li, Junqing Yang, Oumei Cheng, Zhe Peng, Yin Luo, Dongzhi Ran, Yang Yang, Pu Xiang, Haifeng Huang, Xiaodan Tan took part in all the experiments and carried out the data analysis. Miaomiao li wrote the final manuscript and all authors approved the final manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Chongqing science technology commission of China provided funding for research. This work was supported by the Chongqing science technology commission of China (NO: cstc2017shms-zdyfX0053).

Ethical statement

All animal experiments were approved by the Institutional Animal Care and Use Committee of Chongqing Medical University and in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals.

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Availability of data and materials

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

References

1. Kalia LV and Lang AE. Parkinson's disease. *Lancet* 2015; 386: 896–912.
2. Masliah E, Rockenstein E, Veinbergs I, *et al*. Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. *Science* 2000; 287: 1265–1269.
3. Sahli H, Seddik L and Remy P. Non-motor symptoms of Parkinson disease and their management. *Rev Prat* 2018; 68: 508–512.
4. Ascherio A and Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. *Lancet Neurol* 2016; 15: 1257–1272.
5. Bryois J, Skene NG, Hansen TF, *et al*. Genetic identification of cell types underlying brain complex traits yields insights into the etiology of Parkinson's disease. *Nat Genet* 2020; 52: 482–493.
6. White DL, Kunik ME, Yu H, *et al*. Post-traumatic stress disorder is associated with further increased Parkinson's disease risk in veterans with traumatic brain injury. *Ann Neurol* 2020; 88: 33–41.
7. Langston JW. The promise of stem cells in Parkinson disease. *J Clin Invest* 2005; 115: 23–25.
8. Iacono RP, Henderson JM and Lonser RR. Combined stereotactic thalamotomy and posteroventral pallidotomy for Parkinson's disease. *J Image Guid Surg* 1995; 1: 133–140.
9. Raza C, Anjum R and Shakeel NUA. Parkinson's disease: mechanisms, translational models and management strategies. *Life Sci* 2019; 226: 77–90.
10. Barker RA. Designing stem-cell-based dopamine cell replacement trials for Parkinson's disease. *Nat Med* 2019; 25: 1045–1053.
11. Svendsen CN and Langston JW. Stem cells for Parkinson disease and ALS: replacement or protection? *Nat Med* 2004; 10: 224–225.
12. Barker RA, Drouin-Ouellet J, Parmar M, *et al*. Cell-based therapies for Parkinson disease—past insights and future potential. *Nat Rev Neurol* 2015; 11: 492–503.
13. Barker RA and de Beaufort I. Scientific and ethical issues related to stem cell research and interventions in neurodegenerative disorders of the brain. *Prog Neurobiol* 2013; 110: 63–73.
14. Takahashi K, Okita K, Nakagawa M, *et al*. Induction of pluripotent stem cells from fibroblast cultures. *Nat Protoc* 2007; 2: 3081–3089.
15. Amin N, Tan X, Ren Q, *et al*. Recent advances of induced pluripotent stem cells application in neurodegenerative diseases. *Prog Neuropsychopharmacol Biol Psychiatry* 2019; 95: 109674.
16. Henry MP, Hawkins JR, Boyle J, *et al*. The genomic health of human pluripotent stem cells: genomic instability and the consequences on nuclear organization. *Front Genet* 2018; 9: 623.
17. Pittenger MF, Mackay AM, Beck SC, *et al*. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284: 143–147.
18. Hayashi T, Wakao S, Kitada M, *et al*. Autologous mesenchymal stem cell-derived dopaminergic neurons function in parkinsonian macaques. *J Clin Invest* 2013; 123: 272–284.
19. Uccelli A, Moretta L and Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008; 8: 726–736.
20. Bernardo ME and Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell* 2013; 13: 392–402.
21. Sun Y, Selvaraj S, Pandey S, *et al*. MPP(+) decreases store-operated calcium entry and TRPC1 expression in Mesenchymal Stem Cell derived dopaminergic neurons. *Sci Rep* 2018; 8: 11715.
22. Singh M, Kakkar A, Sharma R, *et al*. Synergistic effect of BDNF and FGF2 in efficient generation of functional dopaminergic neurons from human mesenchymal stem cells. *Sci Rep* 2017; 7: 10378.
23. Black IB and Woodbury D. Adult rat and human bone marrow stromal stem cells differentiate into neurons. *Blood Cells Mol Dis* 2001; 27: 632–636.
24. Barzilay R, Ben-Zur T, Bulvik S, *et al*. Lentiviral delivery ofLMX1a enhances dopaminergic phenotype in differentiated human bone marrow mesenchymal stem cells. *Stem Cells Dev* 2009; 18: 591–602.
25. Trzaska KA, King CC, Li KY, *et al*. Brain-derived neurotrophic factor facilitates maturation of mesenchymal stem cell-derived dopamine progenitors to functional neurons. *J Neurochem* 2009; 110: 1058–1069.

26. Trzaska KA, Kuzhikandathil EV and Rameshwar P. Specification of a dopaminergic phenotype from adult human mesenchymal stem cells. *Stem Cells* 2007; 25: 2797–2808.

27. Jakobsson T, Treuter E, Gustafsson JA, *et al*. Liver X receptor biology and pharmacology: new pathways, challenges and opportunities. *Trends Pharmacol Sci* 2012; 33: 394–404.

28. Hong C and Tontonoz P. Liver X receptors in lipid metabolism: opportunities for drug discovery. *Nat Rev Drug Discov* 2014; 13: 433–444.

29. Gavini CK, Bookout AL, Bonomo R, *et al*. Liver X receptors protect dorsal root ganglia from obesity-induced endoplasmic reticulum stress and mechanical allodynia. *Cell Rep* 2018; 25: 271–277.

30. Theofilopoulos S and Arenas E. Liver X receptors and cholesterol metabolism: role in ventral midbrain development and neurodegeneration. *F1000Prime Rep* 2015; 7: 37.

31. Cai Y, Tang X, Chen X, *et al*. Liver X receptor beta regulates the development of the dentate gyrus and autistic-like behavior in the mouse. *Proc Natl Acad Sci U S A* 2018; 115: E2725–E2733.

32. Dai Y, Wu W, Huang B, *et al*. Liver X receptors regulate cerebrospinal fluid production. *Mol Psychiatr* 2016; 21: 844–856.

33. Meffre D, Shackleford G, Hichor M, *et al*. Liver X receptors alpha and beta promote myelination and remyelination in the cerebellum. *Proc Natl Acad Sci U S A* 2015; 112: 7587–7592.

34. Theofilopoulos S, Griffiths WJ, Crick PJ, *et al*. Cholestenoic acids regulate motor neuron survival via liver X receptors. *J Clin Invest* 2014; 124: 4829–4842.

35. Koldamova RP, Lefterov IM, Staufenbiel M, *et al*. The liver X receptor ligand T0901317 decreases amyloid beta production in vitro and in a mouse model of Alzheimer's disease. *J Biol Chem* 2005; 280: 4079–4088.

36. Riddell DR, Zhou H, Comery TA, *et al*. The LXR agonist TO901317 selectively lowers hippocampal Abeta42 and improves memory in the Tg2576 mouse model of Alzheimer's disease. *Mol Cell Neurosci* 2007; 34: 621–628.

37. Paterniti I, Campolo M, Siracusa R, *et al*. Liver X receptors activation, through TO901317 binding, reduces neuroinflammation in Parkinson's disease. *PLoS One* 2017; 12: e174470.

38. Wu CH, Chen CC, Lai CY, *et al*. Treatment with TO901317, a synthetic liver X receptor agonist, reduces brain damage and attenuates neuroinflammation in experimental intracerebral hemorrhage. *J Neuroinflammation* 2016; 13: 62.

39. Chen J, Zacharek A, Cui X, *et al*. Treatment of stroke with a synthetic liver X receptor agonist, TO901317, promotes synaptic plasticity and axonal regeneration in mice. *J Cereb Blood Flow Metab* 2010; 30: 102–109.

40. Cheng O, Tian X, Luo Y, *et al*. Liver X receptors agonist promotes differentiation of rat bone marrow derived mesenchymal stem cells into dopaminergic neuron-like cells. *Oncotarget* 2018; 9: 576–590.

41. Kirkeby A, Nolbrant S, Tiklova K, *et al*. Predictive markers guide differentiation to improve graft outcome in clinical translation of hESC-based therapy for Parkinson's disease. *Cell Stem Cell* 2017; 20: 135–148.

42. Kirik D, Rosenblad C and Bjorklund A. Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. *Exp Neurol* 1998; 152: 259–277.

43. Tropepe V, Hitoshi S, Sirard C, *et al*. Direct neural fate specification from embryonic stem cells: a primitive mammalian neural stem cell stage acquired through a default mechanism. *Neuron* 2001; 30: 65.

44. Wislet-Gendebien S, Leprince P, Moonen G, *et al*. Regulation of neural markers nestin and GFAP expression by cultivated bone marrow stromal cells. *J Cell Sci* 2003; 116: 3295–3302.

45. Tondreau T, Lagneaux L, Dejeneffe M, *et al*. Bone marrow–derived mesenchymal stem cells already express specific neural proteins before any differentiation. *Differentiation* 2004; 72: 319–326.

46. Blondheim NR, Levy YS, Ben-Zur T, *et al*. Human mesenchymal stem cells express neural genes, suggesting a neural predisposition. *Stem Cells Dev* 2006; 15: 141–164.

47. Deng J, Petersen BE, Steindler DA, *et al*. Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. *Stem Cells* 2006; 24: 1054–1064.

48. Evangelista AF, Vannier-Santos MA, de Assis SG, *et al*. Bone marrow-derived mesenchymal stem/stromal cells reverse the sensorial diabetic neuropathy via modulation of spinal neuroinflammatory cascades. *J Neuroinflammation* 2018; 15: 189.

49. Whone AL, Kemp K, Sun M, *et al*. Human bone marrow mesenchymal stem cells protect catecholaminergic and serotonergic neuronal perikarya and transporter function from oxidative stress by the secretion of glial-derived neurotrophic factor. *Brain Res* 2012; 1431: 86–96.

50. Scheper V, Schwieger J, Hamm A, *et al*. BDNF-overexpressing human mesenchymal stem cells mediate increased neuronal protection in vitro. *J Neurosci Res* 2019; 97: 1414–1429.

51. Xiong N, Yang H, Liu L, *et al*. BFGF promotes the differentiation and effectiveness of human bone marrow mesenchymal stem cells in a rotenone model for Parkinson's disease. *Environ Toxicol Pharmacol* 2013; 36: 411–422.

52. Forget C, Stewart J and Trudeau L. Impact of basic FGF expression in astrocytes on dopamine neuron synaptic function and development. *Eur J Neurosci* 2006; 23: 608–616.

53. Yang F, Liu Y, Tu J, *et al*. Activated astrocytes enhance the dopaminergic differentiation of stem cells and promote brain repair through bFGF. *Nat Commun* 2014; 5.

54. O'Malley EK, Sieber BA, Morrison RS, *et al*. Nigral type I astrocytes release a soluble factor that increases dopaminergic neuron survival through mechanisms distinct from basic fibroblast growth factor. *Brain Res* 1994; 647: 83.

55. Chadi G, Moller A, Rosen L, *et al*. Protective actions of human recombinant basic fibroblast growth factor on MPTP-lesioned nigrostriatal dopamine neurons after intraventricular infusion. *Exp Brain Res* 1993; 97: 145–158.

56. Fuccillo M, Joyner AL and Fishell G. Morphogen to mitogen: the multiple roles of hedgehog signalling in vertebrate neural development. *Nat Rev Neurosci* 2006; 7: 772–783.

57. Ruiz IAA, Palma V and Dahmane N. Hedgehog-Gli signalling and the growth of the brain. *Nat Rev Neurosci* 2002; 3: 24–33.

58. Traiffort E, Angot E and Ruat M. Sonic Hedgehog signaling in the mammalian brain. *J Neurochem* 2010; 113: 576–590.

59. Ahn S and Joyner AL. In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. *Nature* 2005; 437: 894–897.

60. Hill SA, Blaeser AS, Coley AA, *et al*. Sonic hedgehog signaling in astrocytes mediates cell type-specific synaptic organization. *Elife* 2019; 8.

61. Miller AV, Kavanaugh SI and Tsai PS. Disruption of the suprachiasmatic nucleus in fibroblast growth factor signaling-deficient mice. *Front Endocrinol* 2016; 7: 11.

62. McCabe MJ, Gaston-Massuet C, Tziaferi V, *et al*. Novel FGF8 mutations associated with recessive holoprosencephaly, craniofacial defects, and hypothalamo-pituitary dysfunction. *J Clin Endocrinol Metab* 2011; 96: E1709–E1718.

63. Ye W, Shimamura K, Rubenstein JL, *et al*. FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell* 1998; 93: 755–766.

64. Roussa E, Farkas LM and Krieglstein K. TGF-beta promotes survival on mesencephalic dopaminergic neurons in cooperation with Shh and FGF-8. *Neurobiol Dis* 2004; 16: 300–310.

65. Fitz NF, Carter AY, Tapias V, *et al*. ABCA1 deficiency affects basal cognitive deficits and dendritic density in mice. *J Alzheimers Dis* 2017; 56: 1075–1085.

66. Cui X, Chopp M, Zhang Z, *et al*. ABCA1/ApoE/HDL pathway mediates GW3965-induced neurorestoration after stroke. *Stroke* 2017; 48: 459–467.

67. Lee HJ, Ryu JM, Jung YH, *et al*. High glucose upregulates BACE1-mediated Aβ production through ROS-dependent HIF-1α and LXRα/ABCA1-regulated lipid raft reorganization in SK-N-MC cells. *Sci Rep* 2016; 6.

68. Trzaska KA, Reddy BY, Munoz JL, *et al*. Loss of RE-1 silencing factor in mesenchymal stem cell-derived dopamine progenitors induces functional maturity. *Mol Cell Neurosci* 2008; 39: 285–290.

69. Mu T, Qin Y, Liu B, *et al*. In vitro neural differentiation of bone marrow mesenchymal stem cells carrying the FTH1 reporter gene and detection with MRI. *Biomed Res Int* 2018; 2018: 1978602.

70. Mohammad MH, Al-Shammari AM, Al-Juboory AA, *et al*. Characterization of neural stemness status through the neurogenesis process for bone marrow mesenchymal stem cells. *Stem Cells Cloning* 2016; 9: 1–15.

71. Karasinska JM, Rinninger F, Lutjohann D, *et al*. Specific loss of brain ABCA1 increases brain cholesterol uptake and influences neuronal structure and function. *J Neurosci* 2009; 29: 3579–3589.

72. Wang Y, Chen X, Armstrong MA, *et al*. Survival of bone marrow-derived mesenchymal stem cells in a xenotransplantation model. *J Orthop Res* 2007; 25: 926–932.

73. Nakamura Y, Wang X, Xu C, *et al*. Xenotransplantation of long-term-cultured swine bone marrow-derived mesenchymal stem cells. *Stem Cells* 2007; 25: 612–620.

74. Le Blanc K, Tammik C, Rosendahl K, *et al*. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003; 31: 890–896.

75. Tse WT, Pendleton JD, Beyer WM, *et al*. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation* 2003; 75: 389–397.