PTX3 secreted by human adipose-derived stem cells promotes dopaminergic neuron repair in Parkinson's disease via inhibiting apoptosis

Changlin Lian
Zhujiang Hospital
Qiongzhen Huang
Zhujiang Hospital
Xiangyang Zhong
Zhujiang Hospital
Zhenyan He
Zhujiang Hospital
Boyang Liu
Zhujiang Hospital
Huijun Zeng
Zhujiang Hospital
Ningbo Xu
Zhujiang Hospital
Zhao Yang
Zhujiang Hospital
Chenxin Liao
Zhujiang Hospital
Zhao Fu
Zhujiang Hospital
Hongbo Guo (guohongbo911@126.com)
Zhujiang Hospital https://orcid.org/0000-0003-3671-0445

Research

Keywords: Pentraxin 3, Human Adipose-Derived Mesenchymal Stem Cells, Parkinson's Disease, Dopaminergic Neuron, Apoptosis

Posted Date: January 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-151668/v1

Abstract

Background

Adipose-derived human mesenchymal stem cells (hADSCs) transplantation has recently emerged as a promising method in the treatment of Parkinson's disease (PD), however, the mechanism underlying has not been fully illustrated.

Methods

In this study, the therapeutic effects of the striatum stereotaxic injected hADSCs in 6-OHDA-induced mouse model were evaluated. Furthermore, an in vitro model of PD was constructed using tissue-organized brain slices. And the therapeutic effect was evaluated by co-culture of hADSCs and 6-OHDA-constructed brain slice. Within the analysis of hADSCs' exocrine proteins through RNA-seq, Human protein cytokine arrays and label-free quantitative proteomics, key extracellular factors were identified in hADSCs secretion environment.The degeneration of DA neurons and apoptosis were measured in PD samples in vivo and vitro models, and the beneficial effects were evaluated through quantitative reverse transcription polymerase chain reaction (qRT-PCR), western blot,Fluoro-Jade C, Tunel assay and immunofluorescence analysis.

Results

In this study, we discovered that hADSCs protected the dopaminergic (DA) neurons in vivo and vitro models.we identified Pentraxin3 (PTX3) as a key extracellular factor in hADSCs secretion environment. Moreover, we found that human recombinant Pentraxin3 (rhPTX3) treatment could rescue the physiological behaviour of the PD mice in-vivo, as well as prevent DA neurons from death and increase the neuronal terminals in the Ventral tegmental area (VTA) + substantia nigra pars compacta (SNc) and striatum (STR) on the PD brain slices in-vitro. Furthermore, within testing on the pro-apoptotic markers of PD mice brain following the treatment of rhPTX3, we found that rhPTX3 can prevent the apoptosis and the degeneration of DA neurons.

Conclusions

Overall, the current study investigated that PTX3, a hADSCs secreted protein, played a potential role in protecting the DA neurons from apoptosis and degeneration in PD progression as well as improving the motor performances in PD mice to give a possible mechanism of how hADSCs works in the cell replacement therapy in PD. Importantly, our study also provided potential translational implications for the development of PTX3-based therapeutics in PD.

Introduction

PD is the second most frequent neurodegenerative disorder that occurs in approximately 1% of the population over the age of 60 [1]. It's primarily characterized by a massive loss of DA neurons in the VTA + SNc and the consequent deficit in DA release in the STR[2]. However, treatment with L-dopa or DA agonists could just recover the symptoms slightly, even worse these are symptomatic treatments with considerable side effects and their effectiveness diminishes with time [2–4].

In recent years, mesenchymal stem cells (MSCs) transplantation have become a potential therapy for PD [5, 6]. Studies show that hADSCs had become hotspots for regeneration research due to their convenient materials and abundant sources [7–9]. It also had immune properties and the ability to induce differentiation into fat, osteoblasts, and neural-like cells [10, 11]. It is reported that MSCs could induce local repair mechanisms through the release of paracrine factors and inducing changes in the microenvironment [12]. Some previous studies had found that transplanting undifferentiated mesenchymal stem cells into a 6-OHDA induced PD mouse model could restore DA pathway [13–15]. It's recently reported that hADSCs-conditioned medium (hADSCs-CM) could restore H_2O_2-induced toxic SH-SY5Yd cells to normal axon morphology [16]. However, whether hADSCs could protect DA neurons by secreting cytokines still needs to be further explored.

PTX3 belongs to the pentraxin family and is a typical acute phase protein [17]. Studies had reported that PTX3 played an important role in acute inflammation and could also inhibit cell apoptosis [18, 19]. In recent years, it's reported that PTX3 secreted by bone marrow mesenchymal stem cells (MSCs) could promote wound healing through fibrin remodeling [20]. At the same time, some studies showed that PTX3 secreted by human umbilical cord blood mesenchymal stem cells (hUCB-MSCs) promotes functional recovery, vascular remodeling and nerve regeneration in stroke rat models [21, 22]. Both clinical and laboratory studies have found that in some chronic central nervous system diseases, such as Parkinson's disease and Alzheimer's disease, there was increased expression of PTX3 [23, 24]. For now, PTX3 was involved in a variety of pathological processes such as central nervous system diseases, especially its role in clinical practice is getting more and more attention, but its mechanism of action is not yet fully understood.

Apoptosis as one of the pathogenesis of PD [25]. A large number of *in-vivo* and *in-vitro* experiments have confirmed the activation of caspase during the apoptosis process induced by 6-OHDA [26]. *In-vitro* experiments confirmed that 6-OHDA could be selectively absorbed by DA neurons, and activated caspase to cause a cascade reaction and promote cell apoptosis [27, 28]. Regarding the signal transduction system in the process of apoptosis, it is currently believed that the main pathways are membrane receptor pathways and mitochondrial pathways [29]. Both pathways activate the caspase cascade and ultimately lead to cell death. Studies have shown that FADD is involved in the pathogenesis of DA neuron apoptosis in Parkinson's disease, and it is speculated that the death receptor signal transduction pathway mediated by FADD is one of the pathogenesis of Parkinson's disease [30].

In current study, we not only confirmed that hADSCs had a protective effect on PD mice models, but also verified that hADSCs do have neuroprotective effects through the *in-vitro* model which co-cultureing the hADSCs and PD tissue organotypic brain slices. Subsequently, through RNA-seq, Human protein cytokine arrays and label-free quantitative proteomics, we found that PTX3 secreted by hADSCs plays an important role in the protection of dopaminergic neurons. Moreover, we established that topical application of PTX3 protected DA neuronal via inhibiting apoptosis pathway.

Materials And Methods

Animals

All experimental protocols were performed in accordance with guidelines issued by the committee on animal research of Zhujiang Hospital of Southern Medical University and were approved by the institutional ethics committee. C57BL/6 male mice were maintained in 12hour light/dark cycles in cages and acclimated to the experimental environment for 1 week before modeling. All procedures were reviewed and approved by the institutions animal care committee. We have made all efforts to minimize the animal suffering.

6-Hydroxydopamine hydrobromide Lesioning

The PD model was induced by 6-OHDA lesion [31]. Following adequate anesthesia, animals were secured onto a stereotactic frame(RWD,68001,China). A solution of 6-OHDA (Sigma-Aldrich, 3ul, 5 mg/ml in sterilized saline containing 0.02% ascorbic acid) was injected into the right SNc via microliter syringe at an infusion rate of 0.5 ul/minute by Hamilton syringe & back pump (RWD) for a total dose of 15ug at coordinates anteroposterior (AP), -3mm; mediolateral (ML), + 1.3 mm; dorsoventral (DV), -4.7mm relative to bregma. Following a wait of 5 minutes, the needle was withdrawn slowly.

Transplantation of hADSCs

hADSCs in suspension (phosphate-buffered saline,PBS) were implanted at the striatumstereotaxic coordinates(AP, + 0.9mm, ML, + 2.2mm, DV, -2.8mm). A total of 1 X 10^5 cells were injected per point in a volume of 5uL with a 5uL Hamilton syringe (1.5 ul/min).

Labeling hADSCs with CM-DiI

hADSCs(1×10^5/well) were incubated into 6-well plates 24 hours, the cell membrane were stained with DiI (MedChemExpress, USA) for 10 min. Then, hADSCs were changed the medium.

Organotypic brain slices cultures

Organotypic slice cultures were prepared according to the membrane interface method [32]. Brains were removed from (3–4 weeks) mice. Sagittal nigrostriatal brain slices [33], 350um thick, were cut using a McIlwain tissue chopper (Microslicer™ DTK-1000N, Japan, Dosaka Company) Subsequently, four slices were plated onto each 0.4um porous polytetrafluoroethylene (PTFE) membrane insert (TCS000012, JET

Biofil) placed in a 6-well plates filled with 1 ml of slice culture media containing 50% HEPES-buffered MEM (Gibco), 25% heat-inactivated horse serum (Gibco), 25% HBSS (Gibco), and 1 mM L-glutamine (Gibco) at pH 7.4 and maintained in a cell-culture incubator at 37°C, 5% CO_2. Media were exchanged 1 day after preparation and subsequently every 3–4 days.

PD models-organotypic brain slices co-culture with hADSCs

Organotypic brain slices were cultured with 6-OHDA (600nM; Fig.S3) for 1 hours, 1 x 10^5 hADSCs were tiled in the lower ventricle of the small ventricle, and the 6-OHDA-induced brain slices were located in the upper ventricle. They were cultured for 4 days with Serum-free medium (75% HEPES-buffered MEM (Gibco), 25% HBSS (Gibco), and 1 mM L-glutamine (Gibco), and the medium was changed every 2 days.

These experiments were performed as described in Supplementary Materials and Methods.

RNA-seq analysis

Oligo(dT)-attached magnetic beads were used to purified mRNA. Purified mRNA was fragmented into small pieces with fragment buffer at appropriate temperature. Then First-strand cDNA was generated using random hexamer-primed reverse transcription, followed by a second-strand cDNA synthesis. Afterwards, A-Tailing Mix and RNA Index Adapters were added by incubating to end repair. The cDNA fragments obtained from previous step were amplified by PCR, and products were purified by Ampure XP Beads, then dissolved in EB solution. The product was validated on the Agilent Technologies 2100 bioanalyzer for quality control. The double stranded PCR products from previous step were heated denatured and circularized by the splint oligo sequence to get the final library. The single strand circle DNA (ssCir DNA) was formatted as the final library. The final library was amplified with phi29 to make DNA nanoball (DNB) which had more than 300 copies of one molecular, DNBs were loaded into the patterned nanoarray and single end 50 bases reads were generated on BGIseq500 platform.

RNA-seq data were deposited in the GEO database as GSE163176. For reviewer access, the following link can be used to view the raw data: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE163176.

Proteome Profiler TM Array

The cytokine profiles of protein (200ug) from medium of single hADSCs group, 6-OHDA group and co-hADSCs group were analyzed using Human XL Cytokine Array Kit and Cytokine Array Kit (R&D, ARY022B) according to the manufacturer's instructions. The detected signals were quantified by pixel density analysis.

LC-MS/MS Analysis

LC-MS/MS analysis was performed on a Q Exactive mass spectrometer (Thermo Scientific) that was coupled to Easy nLC (Proxeon Biosystems, now Thermo Fisher Scientific) for 120 min. The mass spectrometer was operated in positive ion mode. MS data was acquired using a data-dependent top 10 method dynamically choosing the most abundant precursor ions from the survey scan (300–1800 m/z) for HCD fragmentation. Automatic gain control (AGC) target was set to 3e6, and maximum inject time to

10 ms. Dynamic exclusion duration was 40.0s. Survey scans were acquired at a resolution of 70,000 at m/z 200 and resolution for HCD spectra was set to 17,500 at m/z 200, and isolation width was 2 m/z. Normalized collision energy was 30 eV and the underfill ratio, which specifies the minimum percentage of the target value likely to be reached at maximum fill time, was defined as 0.1%. The instrument was run with peptide recognition mode enabled. The MS data were analyzed using MaxQuant software version 1.3.0.5 (Max Planck Institute of Biochemistry in Martinsried, Germany). The following parameters were set (see **Table 4S**).

Brain slices Immunofluorescence

Organotypic brain slices were washed in PBS and then fixed on their inserts in 4% paraformaldehyde for 1 h and stained [34]. Individual slice cultures were cut out from their membranes after fixation and then treated as freefloating sections for the following steps. Slice cultures were permeabilized for 18h in 0.5% Triton X-100 at 4°C and then blocked in 20% BSA (Sigma-Aldrich) for 4 h at room temperature (RT). Slice cultures were then incubated with appropriate primary antibodies overnight at 4°C in 5% BSA, washed and then incubated with fluorophore-coupled secondary antibodies for 4h at RT. Slice cultures were washed a final time before mounting on slides with Fluoromount-G with DAPI (Thermo Fisher Scientific, P36941) and then imaged by Multiphoton Laser Scanning Microscope (Olympus, FV1200MPE).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8.0. Statistical significance between means ± SEM was assessed using a one-way analysis of variance (ANOVA), two-way ANOVA and Student's t test for paired or unpaired data.Values of $p \leq 0.05$ were considered statistically significances.

See further details in the Supporting Information Materials and Methods.

Results

hADSCs transplantation improved motor performance and TH expression in the 6-OHDA-induced PD mice

Using the same method as previous described [31], 6-OHDA or vehicle (saline) was injected into the SNc in situ of mice brain to get the 6-OHDA-induced PD mice model, as well as control mice cohort. APO rotation experiment [35] was conducted after 1, 2, 3 weeks injection to select the PD mice. Mice with a rotation frequency above 7r/min were selected as PD mice and were used in the following experiments (video S1-S2). To evaluate the potential effects of hADSCs (Fig. 1S) on PD, hADSCs were transplanted in situ into the striatum of PD mice brain and the behavioral tests were performed at the 4th, 5th, and 6th weeks (Fig. 1a). We found that hADSCs grafted PD mice displayed faster moving velocity ($F(2,12) = 28.11$, $p < 0.0001$) and further distance ($F(2,13) = 19.50$, $P = 0.0001$), while less sedentary resting time ($F(2,12) = 21.73$, $p = 0.0001$) than the vehicle treated PD mice (Fig. 1b). Moreover, significant reduction of numbers in APO rotation experiments were observed in hADSCs grafted mice compared with the vehicle treated PD mice ($F(2,12) = 130.3$, $P < 0.0001$, Fig. 1c & video S3). These data indicated that hADSCs could improve the motor performances in the 6-OHDA-induced PD mice model.

To further investigate the effective treatment of hADSCs transplantation on 6-OHDA-induced PD mice, brain tissues were harvested at the end of the 6th week when the behavioral tests were completed. Then, western blot and immunohistochemistry were conducted on brain tissues and brain slices respectively. Western blot showed that hADSCs transplantation increased the expression of TH protein, a major marker of dopaminergic (DA) neurons, in the STR nucleus and the VTA + SNc (for STR: $F(2,17) = 20.46$, $P < 0.0001$; for VTA + SNc:$F(2,6) = 16.09$, $P = 0.0039$; Fig. 1d,e). Consistent with the observation in western blot, histological analyses showed that hADSCs treatment increased the percentage of TH-positive densitometry in STR on 6-OHDA-induced PD mice (6-OHDA-inuced PD mice without hADSCs transplantation: 8.7% ± 9.2%, with hADSCs transplantation: 48.15% ± 9.57%, $F(2, 9) = 109.6$, $p < 0.0001$, Fig. 1f). Subsequently, using densitometry analysis, we found that hADSCs transplantation could also increase the TH-positive fibers in VTA and SNc nucleus on 6-OHDA-induced PD mice ($F(2, 6) = 73.98$, $p < 0.0001$, Fig. 1g).

hADSCs protected the dopaminergic systems of 6-OHDA-damaged brain slices with an indirect manner

To further explore the origin of rejuvenated DA fibers around the grafts, we used Celltracker CM-DiI (located in the cell membrane and cytoplasm) to label hADSCs followed by the previous transplantation procedures. At 3rd week, brain tissues were harvested for sagittal immunofluorescence staining. We found that CM-DiI-labeled hADSCs were distributed in STR region of brain tissue, where these hADSCs were transplanted in situ of mice brain, while were not observed in the SNc region (Fig. 2a). These observations showing that grafted hADSCs didn't transfer from STR to SNc region. Moreover, TH staining neurons showed no co-localization with CM-DiI-labeled hADSCs and revealed that hADSCs didn't differentiate into DA neurons. These data showing that hADSCs protect DA neurons through an indirect manner, while not by transferring to SNc or differentiate into DA neurons.

Next, we evaluated the protective effects of hADSCs on DA neurons via hADSCs co-cultured with PD models-organic brain slices in vitro. Organotypic slice cultures were prepared according to the membrane interface method as previous described [32]. In current study, we harvested the brains from young mice, and cut 350um thick sagittal black striatal brain slices with Tissue Culture Plate Insert and placed four slices on the membrane insert. Brain slices were allowed to recover for 7 days from cutting trauma (Fig.S2). On day 8, brain slices were exposed to combined 6-OHDA (600nM; Fig.S3) for 1h, followed by 1 x 10^5 hADSCs co-cultureing procedure with serum-free medium for 4 days (Fig. 2b). Subsequently, western blot was conducted to analysis the TH expression on brain slices of hADSCs co-cultured 6-OHDA damaged brain slices and we found that hADSCs effectively prevent the 6-OHDA induced TH annihilate ($F(2, 9) = 69.38$, $p < 0.0001$, Fig. 2c). Moreover, 6-OHDA damaged brain slices secrected extracelluar Lactate dehydrogenase (LDH) in culture medium was also tested with LDH assay, and we observed that hADSCs could markedly decrease the LDH release ($F(6, 28) = 23.58$, $p < 0.0001$, Fig. 2d). These data indicated that hADSCs could protect the dopaminergic neurons from damage which caused by 6-OHDA in an indirect manner.

Pentraxin 3 was a potential hADSCs secrected protective molecule on DA neurons in PD models.

As hADSCs showed protective effects on PD models both *in-vitro* and *in-vivo* through an indirect way, we hypothesized that hADSCs may protect the 6-OHDA damaged brain slices through secrecting extracullar cytokines. To further explore the extracellular mechanism of how hADSCs protected the DA neurons on 6-OHDA-treated brain slices, ater 4 days co-cultivation of hADSCs and 6-OHDA-treated brain slice (Fig. 3a), we collected the hADSCs for RNA-seq high-throughput sequencing and RT-qPCR. Hierarchical clustering heat map showed RNA-seq high-throughput sequencing results of distinct gene expression patterns in hADSCs and hADSCs which co-cultured with brain slices ($p < 0.05$, Fig. 3b). Co-cultureing with 6-OHDA-treated brain slices lead to 1577 mRNAs appeared differential expression including 276 upregulated and 1301 downregulated (Table. S1). Meanwhile, we collected the conditioned media from hADSCs single culturing group and hADSCs cultured with 6-OHDA-treated brain slice group, as well as 6-OHDA-treated brain slice single culturing group. These conditioned media were then applied to human cytokine array and label-free quantitative proteomics. Human protein cytokine arrays showed that cytokines appeared to a difference between hADSCs cultured conditioned media, 6-OHDA-treated brain slices cultured conditional media and the conditioned media of hADSCs co-cultured with 6-OHDA-treated brain slices (Fig. 3c, Table. S2). In label-free quantitative proteomics, a total of 153 proteins with significant differences were identified (Table. S3). Screening of differentially expressed proteins according to the standard of expression fold change of more than 2.0 times (up-regulation greater than 2.0-fold or down-regulation less than 0.5-fold), a total of 153 differential proteins were selected as target cytokines (Fig. 3d). The results of RNA-seq high-throughput sequencing and Human protein cytokine arrays as well as label-free quantitative proteomics were subsequently subjected to Venn intersection and we found that PTX3 was the exclusive protein which showed remarkable difference in all three tests and analysis (Fig. 3e). The following RT-qPCR showed that the mRNA level of PTX3 in the hADSCs co-cultured 6-OHDA-treated brain slices group was higher than the hADSCs single culturing group ($p < 0.05$, Fig. 3f). Therefore, these data suggested that PTX3 which secreted by hADSCs may play an important role in the hADSCs transplantation therapy on 6-OHDA-induced PD mice model.

PTX3 treatment mimicked the effect of hADSCs transplantation to improve the motor performances on 6-OHDA-induced PD mice

Successful PD mice model were selected as previous descried and were used in following experiments (Fig. 4a). To evaluate the potential effects of PTX3 on Parkinson's disease, 1×10^5 hADSCs ($n = 7$), 1×10^5 si-PTX3 ($n = 7$, hADSCs with PTX3 knockdown), equal volumes of rhPTX3 (4ul, 0.50 mg/ml, $n = 7$, Fig. S4) or vehicle (saline) (4ul, $n = 7$) were injected in situ into the right striatum of PD mice, open field experiment (Fig. 4b) showed that hADSCs, si-PTX3 and rhPTX3(2000ng/ml, Fig. S5) treatment improved the motor performances on 6-OHDA-induced PD mice. Both hADSCs, si-PTX3 and rhPTX3 treatment groups showed further moving distance ($F(4, 13) = 12.71$, $p = 0.0002$, Fig. 4c), faster moving velocity ($F(4, 13) = 12.70$, $P = 0.0002$, Fig. 4d) and less resting time ($F(4, 14) = 25.25$, $p < 0.0001$, Fig. 4e) when compared with vehicle treatment group on 6-OHDA-induced PD mice. Consisted with what were observed in open field experiments, APO rotation test showed that both si-PTX3 and rhPTX3 could decrease the rotation frequency on PD mice. Importantly, APO rotation test also showed that si-PTX3 blunted the

behavioral improvement effects of hADSCs on PD mice. Moreover, rhPTX3 administration decreased rotations showing a effect similar to those in the hADSCs grafted mice ($F(4, 16) = 37.37$, $p < 0.0001$, Fig. 4f, vedio 4S-5S). These data indicated that the transplantation of hADSCs had a neuroprotective effect in the 6-OHDA-induced mouse model, part of the effect was attributed to PTX3 secreted by hADSCs.

PTX3 treatment mimics the effects of hADSCs transplantation to improve TH expression in vivo **and** in vitro

By the end of behavioral test, mice were anesthetized and fixed with formaldehyde perfusion, and then the brain tissues of VTA + SNc and STR regions were harvested to immunofluorescence staining for TH. Mice with 6-OHDA administration in the right side showed that there was a remarkable loss of dopamine-containing STR and VTA + SNc neurons at the right side, while 3 weeks after hADSCs were injected into 6-OHDA-induced mice, TH-positive neurons increased significantly. Moreover, rhPTX3 (2000ng/ml, Fig. S5) administration increased TH-positive cells showing a effect similar to those in the hADSCs grafted mice. What's more, si-PTX3 were injected into 6-OHDA-induced mice, the density of TH positive cells wwas less pronounced than that in the hADSCs group (Fig. 5a-d). These results indicated that PTX3 could mimic the effects of hADSCs on protecting dopaminergic neurons from 6-OHDA neurotoxicity in mice.

PD brain slices are prepared as described above,1×10^5 hADSCs ($n = 7$), 1×10^5 si-PTX3 ($n = 7$), equal volumes of rhPTX3 (1000ng, $n = 7$, Fig. S6) or vehicle(saline) ($n = 7$) were co-culturing procedure with serum-free medium for 4 days. Then, we performed brain slice immunofluorescence, western blot and LDH test. The data indicated that the same tendency wwas observed in immunofluorescent staining, western blot and LDH test in vitro ($F(4, 20) = 46.81$, $P < 0.0001$, Fig. 5e-h, Immunofluorescence video-S). The above results indicated that PTX3 secreted by hADSCs has a protective effect on DA neurons.

PTX3 protected DA neuron through inhibition of apoptotic death-inducing signal complex

Next, we sought to investigate the underlying mechanism of PTX3-mediated neuroprotection effects in parkinson's disease. As apoptosis is one of the most important pathogenesis of PD, we hypothesised that PTX3 may protect DA neurons by inhibiting the apoptotic pathway. To further confirm this, another individual experiments were conducted and the VTA + SNc regions of brains from saline treated with vehicle mice, saline treated in 6-OHDA-induced PD mice, hADSCs transplanted in 6-OHDA-induced PD mice, siPTX3 transplanted in 6-OHDA-induced PD mice and rhPTX3 treated in 6-OHDA-induced PD mice, were harvested for Fluoro-Jade C staining(for the severity of neuronal degeneration), Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and TH dual immunofluorescence staining(for apoptosis). We found that the number of Fluoro-Jade C positive cells in VTA + SNc regions of 6-OHDA-induced PD mice injected saline was higher than 6-OHDA-induced PD mice treated with hADSCs, but its' effect was in part reversed by si-PTX3. More interestingly, there was a similar effect in rhPTX3 injected PD mice to hADSCs-treated PD mice (Fig. 6a). Finally, the same trend could be also obtained in the results of TH and TUNEL co-staining (Fig. 6b).

Since PTX3 is also called tumor necrosis factor (TNF)-inducible gene 14 protein (TSG-14). The TNF family is closely related to exogenous apoptosis. Therefore, we speculated whether PTX3 exerted DA neuron protection by inhibiting the exogenous apoptosis pathway. RT-qPCR and western blot were used to test the changes of exogenous apoptosis related genes and proteins with saline transplanted vehicle mice, saline transplanted 6-OHDA-induced PD mice, hADSCs transplanted 6-OHDA-induced PD mice, rhPTX3 treated 6-OHDA-induced PD mice. RT-qPCR analysis showed that 6-OHDA treatment increased the mRNA level of caspase3 ($F (4, 17) = 13.47$, $P < 0.0001$), caspase8 ($F (4, 18) = 17.52$, $P < 0.0001$), FADD ($F (4, 18) = 20.51$, $P = 0.0001$) and TRADD ($F (4, 16) = 32.71$, $P < 0.0001$), where were decreased in hADSCs-treated mice, siPTX3-treated mice, rhPTX3-treated mice. Howerver, si-PTX3-treated could decreased the effect partly, while rhPTX3-injected group having similar inhibitory apoptosic effect as hADSCs-treated group (Fig. 6c). Western blots showed lower expression of apoptotic genes levels in hADSCs-injected mice than in 6-OHDA-injected mice, but these genes were not lower in the si-PTX3 group than in the hADSCs group, which had the lowest among the 3 groups. Although rhPTX3 group was also lower than 6-OHDA group, while rhPTX3 group, while the decrease in rhPTX3 group was not as obvious as that in hADSCs group (Fig. 6d).

Finally, after 4 days co-culturvation, collecting brain slices, q-PCR and western blot were also conduceted, and we could find the trend was the same as in vivo model (Fig. 6e,f). Since DISC (death-inducing signal complex) composed of TRADD/FADD/caspase8 induced a downstream cascade reaction. Thus, the above data indicated that PTX3 could protect dopaminergic neurons by inhibiting exogenous apoptosis similar to hADSCs.

Discussion

Parkinson’s disease (PD) is a progressive disease characterized by a massive loss of DA neurons in the substantia nigra pars compacta (SNc) and the consequent deficit in DA release in the striatum (STR) [2]. The symptoms of Parkinson's patients are mildly restored after treatment with levodopa or DA agonists. These are symptomatic treatments with considerable side effects, and their effectiveness will decrease over time [3, 4]. Cell-based therapies for PD have been studied for more than 30 years using various MSCs types [5]. In previous studies, hADSCs transplantation therapies may effectively restore and replace DA neurons in PD mice [14]. However, the molecular mechanism of hADSCs transplantation to treat Parkinson's disease is still unclear. Our study deepens the understanding of the paracrine mechanisms of hADSCs in the treatement of parkinsons' disease.

hADSCs transplantation has a protective effect on DA neurons which damaged by 6-OHDA

Many studies in PD animal models have verified that MSCs have the capacity to protect damaged DA neurons [36]. In our study, hADSCs transplantation were shown to improve behavioral symptoms and exert neuroprotective effects against nigrostriatal degeneration in 6-OHDA lesioned mice. Thus, the above data in current study clarified that hADSCs had neuroprotective effects on DA neurons and improve the motor performances in PD mice. What’s more, we transplanted hADSCs with CM-Dil according to the

previous procedure. The data of immunofluorescence staining for TH showed that hADSCs with CM-Dil were basically located in the STR at the 5th week, and the TH positive staining in the SNc and STR regions increased significantly, but we did not observe direct differentiation of hADSCs into functional neurons. In support of our findings, previous studies suggested that ADSCs had neuroprotective effects on DA neurons damaged by 6-OHDA, and they speculated that this was achieved by secreting nutritional factors [37]. Therefore, we hypothesized that hADSCs may protect dopaminergic neurons via secreting paracrine pathway.

hADSCs may protect DA neurons through paracrine PTX3

Organotypic cultures have great potential for disease modeling providing an ideal platform between in vitro and in vivo [32, 34]. They were easy to prepare and culture, while importantly, when sectioned appropriately, they retain the architecture and microenvironment of the original organ. Using this model, we not only evaluated the therapeutic effect of the treatment but also revealed stem cell responses and the role in the microenvironment. In this study, 6-OHDA-induced brain slices were used as *vitro* PD model, then co-cultured PD brain slices with hADSCs in a serum-free medium for 4 days. And we found that hADSCs increased TH expression of 6-OHDA-induced brain slices, and decreased the release of LDH. These results further suggested that hADSCs may protect DAergic neurons via paracrine.

It is reported that proteomics and genomics could comprehensively describe the state and dynamic changes of specific tissues or cell lines, thereby being able to identify key molecules and pathways involved in a variety of physiological and pathological processes [38]. The development of protein spectrum label free had greatly improved the sensitivity and fidelity of proteomics analysis, and promoted the application of proteomics methods in neuroscience [39]. Thus, we performed RNA-seq sequencing on the co-cultured hADSCs to screen out differentially expressed genes to further explore the mechanism of hADSCs protecting DA neurons damaged by 6-OHDA. Subsequently, we collected the supernatant after 4 days of co-cultivation, perform protein profile label-free quantitative proteomics and human cytokine array to screen out the different cytokines. Finally, the above high-throughput sequencing results after Venn intersection,we could found that hADSCs could significantly increase the secretion of PTX3 in a co-culture environment.

The protective effect of rhPTX3 on PD was similar to hADSCs

PTX3 is also called tumor necrosis factor (TNF)-inducible gene 14 protein (TSG-14) and belonged to the acute-phase protein superfamily which included C-reactive protein (CRP) and serum amyloid P-component (SAP) [18]. Thus, we speculated that PTX3 secreted by hADSCs can protect DA neurons. In this study, we showed that rhPTX3 restored DA neurons in a *vivo* model and in a *vitro* model, and improved the behavior symptoms of 6-OHDA-induced mice. Supporting this finding, it was reported that PTX3 could target to promote long-term neurovascular repair, and protect neurons after ischemic stroke and other possible cerebral ischemic damage [21]. In the MCAO model,compared with wt mice, PTX3 KO mice have significantly increased neuronal damage and reduced blood vessel diameters [22]. This was

consistent with what we had observed. Therefore, the data suggested that rhPTX3 was similar to hADSCs in protecting DA neurons and improving the behavioral symptoms of PD mice.

The effect of hADSCs transplantation in the treatment of PD is partly attributed to PTX3

In addition, PTX3 was proven to have a repairing effect on skin wounds Claudia and colleagues found that compared with wt MSC, PTX3(-/-) MSC delayed wound closure and reduced granulation tissue formation [20]. Our data suggested hADSCs transfected with siPTX3 could ameliorate behavioral symptoms and improve the number of TH possitive cells. However, the effects in the siPTX3 group were not as obvious as in the hADSCs group. Furthermore, we also got a consistent trend in 6-OHDA-induced brain slice model. In this study, we could find siPTX3 still had a therapeutic effect, suggesting that PTX3 was not the only factor or pathway of hADSCs' action. Thus, these data indicated that the therapeutic benefits of hADSCs in PD could be attributed, at least in part, to the induction of PTX3 secreted by hADSCs.

Anti-apoptotic effects of PTX3

Growing evidences suggested the role of apoptosis in the pathogenesis of PD [25]. 6-OHDA neurotoxicity caused DA neurons death and activated apoptosis in both cultured brain slices and the PD mice at both gene and protein levels [26]. As is known to all, PTX3 not only played an important role in acute inflammation, but also inhibitted cell apoptosis. Our data suggested that hADSCs significantly decreased tunel apoptosis-positive DA neurons and FJC-positive neurons, and interestingly, rhPTX3 could also have a similar effect to hADSCs. Furthermore, we found that hADSCs downstrated the expression of FADD, TRADD, caspase-8 and caspase-3 at gene and protein levels. In our study, we found that rhPTX3 could also reduce the expressions the above apoptisic genes. The activation of caspase proteases is known to constitute a central step in the apoptotic process. Upon activation of exogenous apoptosis, TRADD/FADD activated caspase-8, which in turn induced a cascade reaction to trigger caspase3, and finally induced cell apoptosis.

Although siPTX3 group also had anti-apoptotic effects which is similar to hADSCs, the effect was intact and not as valid as hADSCs. Accordingly, the remaining therapeutic effect on siPTX3 group suggested that PTX3 was not the only factor or pathway of hADSCs' function. Moreover, the function of rhPTX3 to inhibit the expression of exogenous apoptosis genes was similar to hADSCs.

In this study, we demonstrated the neuroprotective function of hADSCs secreted PTX3 on PD mice, indicating that the protective effect of hADSCs on DA neurons was partly due to the secretion of PTX3 (Fig. 6g. In addition, rhPTX3 alone is sufficent to inhibit the apoptosis of DA neurons, and protect the nerves as well as improve the behavioral symptoms of PD mice. In this process, our work showed the potential of PTX3 in the treatment of Parkinson's disease and provid a new idea for cell-based treatments.

Abbreviations

hADSCs, human adipose mesenchymal stem cells;

6-OHDA: 6-hydroxidopamine;

W: weeks;

APO rotation: Apomorphine rotation;

VTA: Ventral tegmental area;

SNc: substantia nigra pars compacta;

SNr: substantia nigra pars reticulata;

STR: striatum;

TH(+): Tyrosine Hydroxylase (positive);

DA: dopamine;

DiI: Celltracker CM-DiI;

LDH: Lactate dehydrogenase;

hADSCs group: single hADSCs;

co-hADSCs group: hADSCs coculture with 6-OHDA-induced brain slices;

6-OHDA group: 6-OHDA-induced brain slices.

si-PTX3, knocking down PTX3

Declarations

Acknowledgements

This study was supported by Guangdong Provincial Clinical Medical Centre for Neurosurgery (no. 2013B020400005).

Authors' contributions

CL, QH conception and design, experiments conduction, data analysis and manuscript writing; ZF, XZ, CL and ZY: experiments conduction and data analysis; ZH, BL, HZ and NX: final approval of manuscript; HG: conception and design, final approval of manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by the Natural Science Foundation of Guangdong Province-Major Basic Cultivation Project, Grant/Award Numbers: 2017A030308001, the National Natural Science Foundation of China, Grant/Award Numbers: 81874079, 81672477, Guangdong Province Science and Technology Innovation Strategy Special Fund (2018A030310422) Guangdong Medical Science and Technology Research Fund(A2018542).

Availability of data and materials

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

Ethics approval and consent to participate

All animal experiments were conducted in accordance with the Animal Ethics Committee of Zhujiang Hospital of Southern Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Neurosurgery Center, The National Key Clinical Specialty, The Engineering Technology Research Center of Education Ministry of China on Diagnosis and Treatment of Cerebrovascular Disease, Guangdong Provincial Key Laboratory on Brain Function Repair and Regeneration, The Neurosurgery Institute of Guangdong Province, Zhujiang Hospital, Southern Medical University, 253 Gongye Middle Avenue, Haizhu District, Guangzhou, Guangdong, China, 510280

[2] Department of Neurosurgery, the affiliated Tumor Hospital of Zhengzhou University, Zhengzhou, Henan, China, 450008

References

1. Tysnes OB, Storstein A. Epidemiology of parkinson's disease. *Journal of neural transmission (Vienna, Austria : 1996)*. 2017;124:901-905
2. Balestrino R, Schapira AHV. Parkinson disease. *European journal of neurology*. 2020;27:27-42
3. Armstrong MJ, Okun MS. Diagnosis and treatment of parkinson disease: A review. *Jama*. 2020;323:548-560

4. Pires AO, Teixeira FG, Mendes-Pinheiro B, Serra SC, Sousa N, Salgado AJ. Old and new challenges in parkinson's disease therapeutics. *Prog Neurobiol*. 2017;156:69-89
5. Glavaski-Joksimovic A, Bohn MC. Mesenchymal stem cells and neuroregeneration in parkinson's disease. *Exp Neurol*. 2013;247:25-38
6. Xue J, Liu Y, Darabi MA, Tu G, Huang L, Ying L, et al. An injectable conductive gelatin-pani hydrogel system serves as a promising carrier to deliver bmscs for parkinson's disease treatment. *Mater Sci Eng C Mater Biol Appl*. 2019;100:584-597
7. Bacakova L, Zarubova J, Travnickova M, Musilkova J, Pajorova J, Slepicka P, et al. Stem cells: Their source, potency and use in regenerative therapies with focus on adipose-derived stem cells - a review. *Biotechnology advances*. 2018;36:1111-1126
8. Cai Y, Li J, Jia C, He Y, Deng C. Therapeutic applications of adipose cell-free derivatives: A review. *Stem Cell Res Ther*. 2020;11:312
9. Liu G, Sun X, Bian J, Wu R, Guan X, Ouyang B, et al. Correction of diabetic erectile dysfunction with adipose derived stem cells modified with the vascular endothelial growth factor gene in a rodent diabetic model. *PLoS One*. 2013;8:e72790
10. Rafat A, Mohammadi Roushandeh A, Alizadeh A, Hashemi-Firouzi N, Golipoor Z. Comparison of the melatonin preconditioning efficacy between bone marrow and adipose-derived mesenchymal stem cells. *Cell J*. 2019;20:450-458
11. Fang B, Liu Y, Zheng D, Shan S, Wang C, Gao Y, et al. The effects of mechanical stretch on the biological characteristics of human adipose-derived stem cells. *Journal of cellular and molecular medicine*. 2019;23:4244-4255
12. Tran C, Damaser MS. Stem cells as drug delivery methods: Application of stem cell secretome for regeneration. *Advanced drug delivery reviews*. 2015;82-83:1-11
13. Park HJ, Oh SH, Kim HN, Jung YJ, Lee PH. Mesenchymal stem cells enhance α-synuclein clearance via m2 microglia polarization in experimental and human parkinsonian disorder. *Acta neuropathologica*. 2016;132:685-701
14. Park HJ, Shin JY, Kim HN, Oh SH, Lee PH. Neuroprotective effects of mesenchymal stem cells through autophagy modulation in a parkinsonian model. *Neurobiology of aging*. 2014;35:1920-1928
15. Schwerk A, Altschüler J, Roch M, Gossen M, Winter C, Berg J, et al. Human adipose-derived mesenchymal stromal cells increase endogenous neurogenesis in the rat subventricular zone acutely after 6-hydroxydopamine lesioning. *Cytotherapy*. 2015;17:199-214
16. Palomares T, Cordero M, Bruzos-Cidon C, Torrecilla M, Ugedo L, Alonso-Varona A. The neuroprotective effect of conditioned medium from human adipose-derived mesenchymal stem cells is impaired by n-acetyl cysteine supplementation. *Mol Neurobiol*. 2018;55:13-25
17. Bonavita E, Gentile S, Rubino M, Maina V, Papait R, Kunderfranco P, et al. Ptx3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. *Cell*. 2015;160:700-714
18. Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. Ptx3, a humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. *Physiological reviews*. 2018;98:623-639

19. Lee HH, Kim SY, Na JC, Yoon YE, Han WK. Exogenous pentraxin-3 inhibits the reactive oxygen species-mitochondrial and apoptosis pathway in acute kidney injury. *PLoS One*. 2018;13:e0195758
20. Cappuzzello C, Doni A, Dander E, Pasqualini F, Nebuloni M, Bottazzi B, et al. Mesenchymal stromal cell-derived ptx3 promotes wound healing via fibrin remodeling. *The Journal of investigative dermatology*. 2016;136:293-300
21. Park HW, Moon HE, Kim HS, Paek SL, Kim Y, Chang JW, et al. Human umbilical cord blood-derived mesenchymal stem cells improve functional recovery through thrombospondin1, pantraxin3, and vascular endothelial growth factor in the ischemic rat brain. *J Neurosci Res*. 2015;93:1814-1825
22. Rajkovic I, Wong R, Lemarchand E, Rivers-Auty J, Rajkovic O, Garlanda C, et al. Pentraxin 3 promotes long-term cerebral blood flow recovery, angiogenesis, and neuronal survival after stroke. *J Mol Med (Berl)*. 2018;96:1319-1332
23. Lee HW, Choi J, Suk K. Increases of pentraxin 3 plasma levels in patients with parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2011;26:2364-2370
24. Webster SJ, Van Eldik LJ, Watterson DM, Bachstetter AD. Closed head injury in an age-related alzheimer mouse model leads to an altered neuroinflammatory response and persistent cognitive impairment. *J Neurosci*. 2015;35:6554-6569
25. Venderova K, Park DS. Programmed cell death in parkinson's disease. *Cold Spring Harbor perspectives in medicine*. 2012;2
26. Singh S, Dikshit M. Apoptotic neuronal death in parkinson's disease: Involvement of nitric oxide. *Brain research reviews*. 2007;54:233-250
27. Hernandez-Baltazar D, Zavala-Flores LM, Villanueva-Olivo A. The 6-hydroxydopamine model and parkinsonian pathophysiology: Novel findings in an older model. *Neurologia (Barcelona, Spain)*. 2017;32:533-539
28. Zhang C, Li C, Chen S, Li Z, Jia X, Wang K, et al. Berberine protects against 6-ohda-induced neurotoxicity in pc12 cells and zebrafish through hormetic mechanisms involving pi3k/akt/bcl-2 and nrf2/ho-1 pathways. *Redox biology*. 2017;11:1-11
29. Schneider-Brachert W, Heigl U, Ehrenschwender M. Membrane trafficking of death receptors: Implications on signalling. *International journal of molecular sciences*. 2013;14:14475-14503
30. Hartmann A, Mouatt-Prigent A, Faucheux BA, Agid Y, Hirsch EC. Fadd: A link between tnf family receptors and caspases in parkinson's disease. *Neurology*. 2002;58:308-310
31. Blandini F, Armentero MT. Animal models of parkinson's disease. *FEBS J*. 2012;279:1156-1166
32. Humpel C. Organotypic brain slice cultures. *Curr Protoc Immunol*. 2018;123:e59
33. Cavaliere F, Vicente ES, Matute C. An organotypic culture model to study nigro-striatal degeneration. *J Neurosci Methods*. 2010;188:205-212
34. Croft CL, Cruz PE, Ryu DH, Ceballos-Diaz C, Strang KH, Woody BM, et al. Raav-based brain slice culture models of alzheimer's and parkinson's disease inclusion pathologies. *J Exp Med*. 2019;216:539-555

35. Pan Q, Zhang W, Wang J, Luo F, Chang J, Xu R. Impaired voluntary wheel running behavior in the unilateral 6-hydroxydopamine rat model of parkinson's disease. *J Korean Neurosurg Soc*. 2015;57:82-87
36. Volkman R, Offen D. Concise review: Mesenchymal stem cells in neurodegenerative diseases. *Stem Cells*. 2017;35:1867-1880
37. Nakhaeifard M, Haji Ghasem Kashani M, Goudarzi I, Rezaei A. Conditioned medium protects dopaminergic neurons in parkinsonian rats. *Cell J*. 2018;20:348-354
38. Aebersold R, Mann M. Mass-spectrometric exploration of proteome structure and function. *Nature*. 2016;537:347-355
39. Schulze WX, Usadel B. Quantitation in mass-spectrometry-based proteomics. *Annual review of plant biology*. 2010;61:491-516

Figures

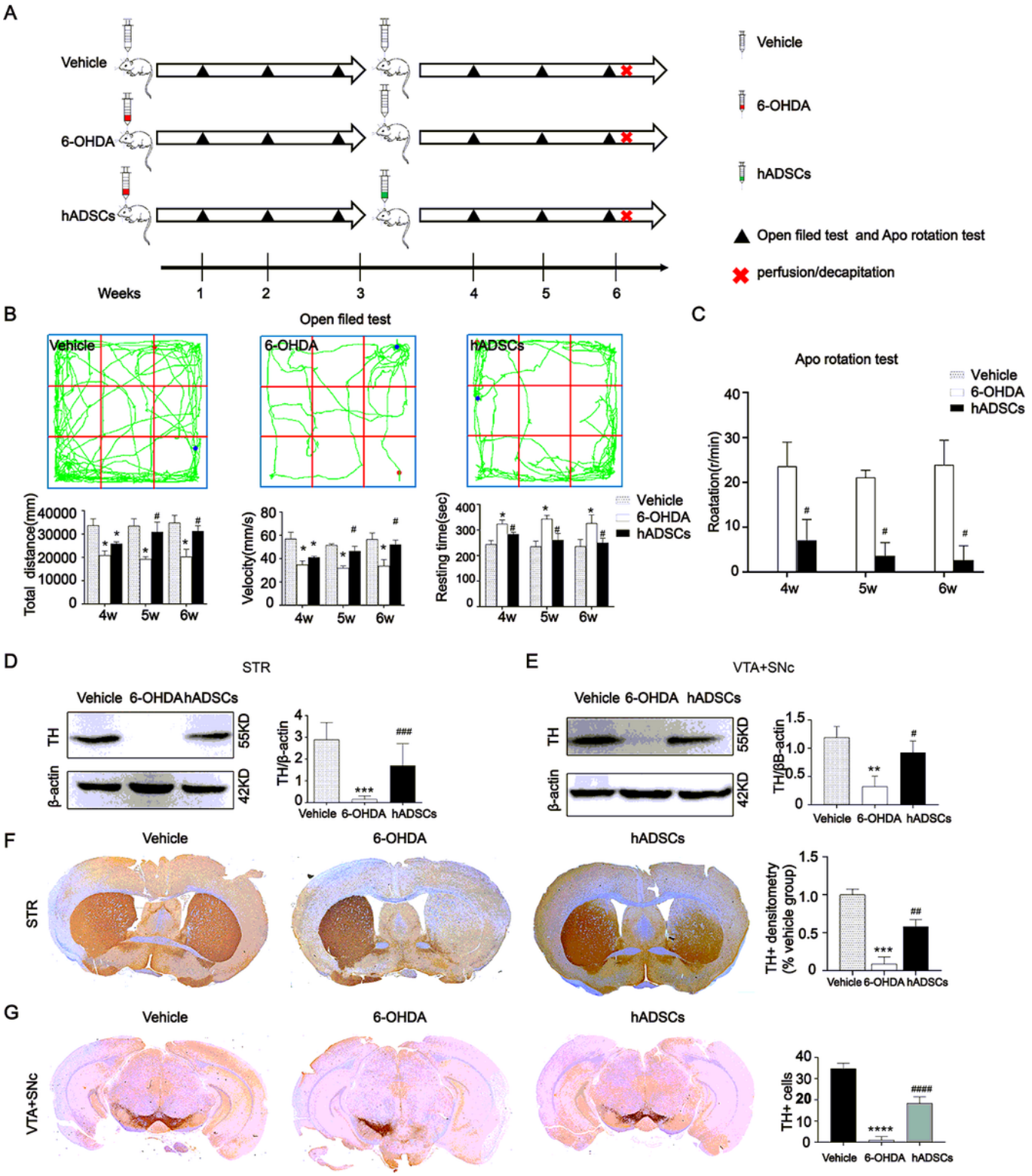

Figure 1

hADSCs transplantation improved motor performance and TH expression in the 6-OHDA-induced PD mice. a.Scheme of experimental design and workflow; b.Open field experiment: total distance moved moving velocity and resting times in 10 min at 4th, 5th, and 6th weeks; c.Side-biased rotational behavior in the APO-induced rotation test was observed at 4th, 5th, and 6th weeks; d,e.Representative Western blots of TH were presented in SNc and STR, with results after quantification, all the ratios were then

normalized with the ratio of vehicle group; f,g. Immunohistochemistry staining was performed to the protein-expression of TH(mice number in each group is equal or above 7, *p <0.05, **p<0.001, ***p<0.0005,****p<0.0001, versus vehicle group, #p<0.05, ##p<0.001, ###p<0.0005,####p<0.0001, versus 6-OHDA group).

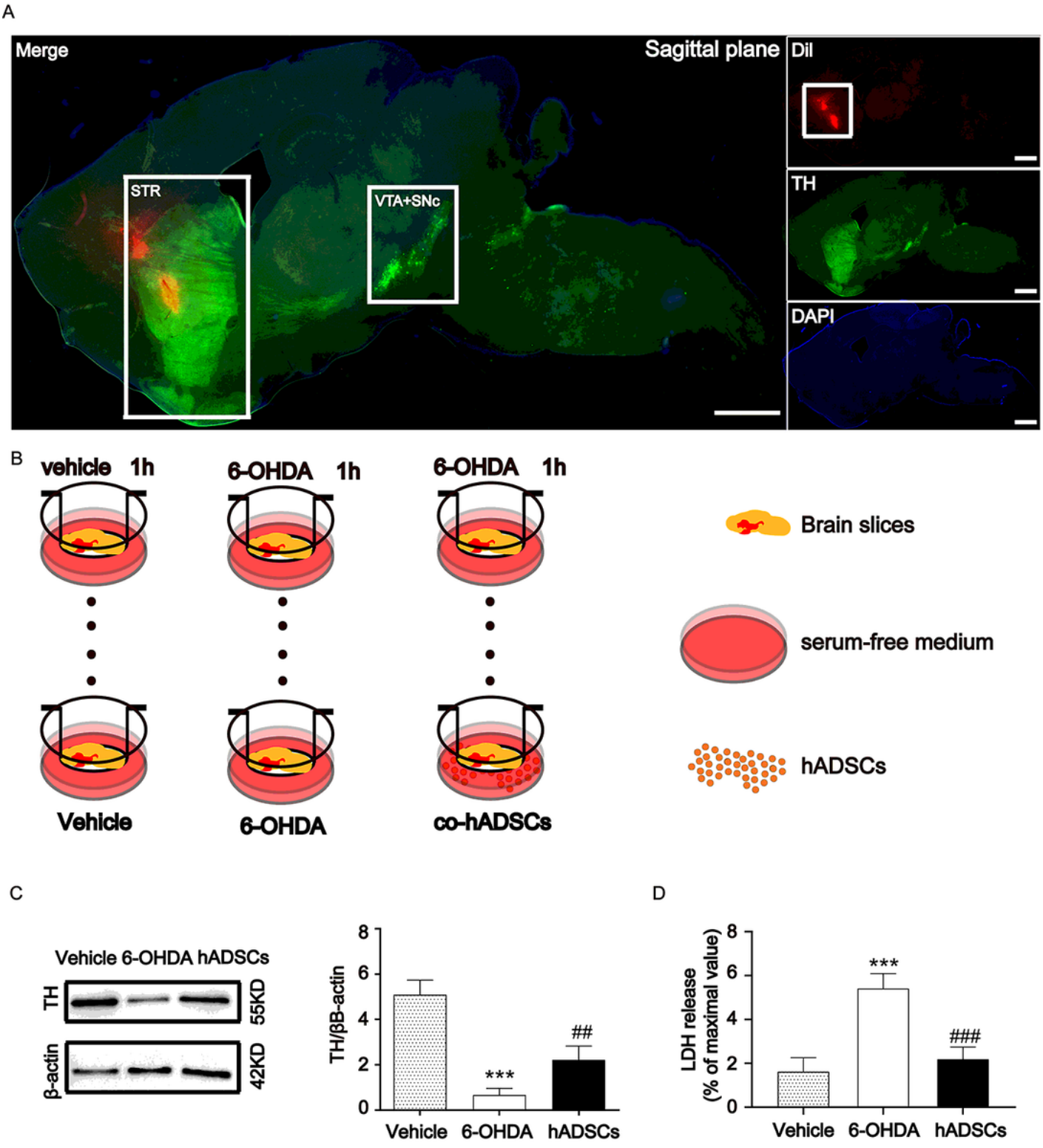

Figure 2

hADSCs protected the dopaminergic systems of 6-OHDA-damaged brain slices with an indirect manner a. hADSCs were located in STR□while not in SNc after 3 weeks transplantation (Green: TH, Blue: DAPI, Red: Dil-positive-hADSCs); b. 6-OHDA-induced brain slices co-culturing with hADSCs procedure; c. The expression of TH was analysised by Western blot in vitro; d. The cell LDH activity in the culture medium was analysed in vitro (scale bars=100um, mice number in each group is equal or above 7, ***p<0.0005, versus vehicle group, ##p<0.001, ###p<0.0005, versus 6-OHDA group).

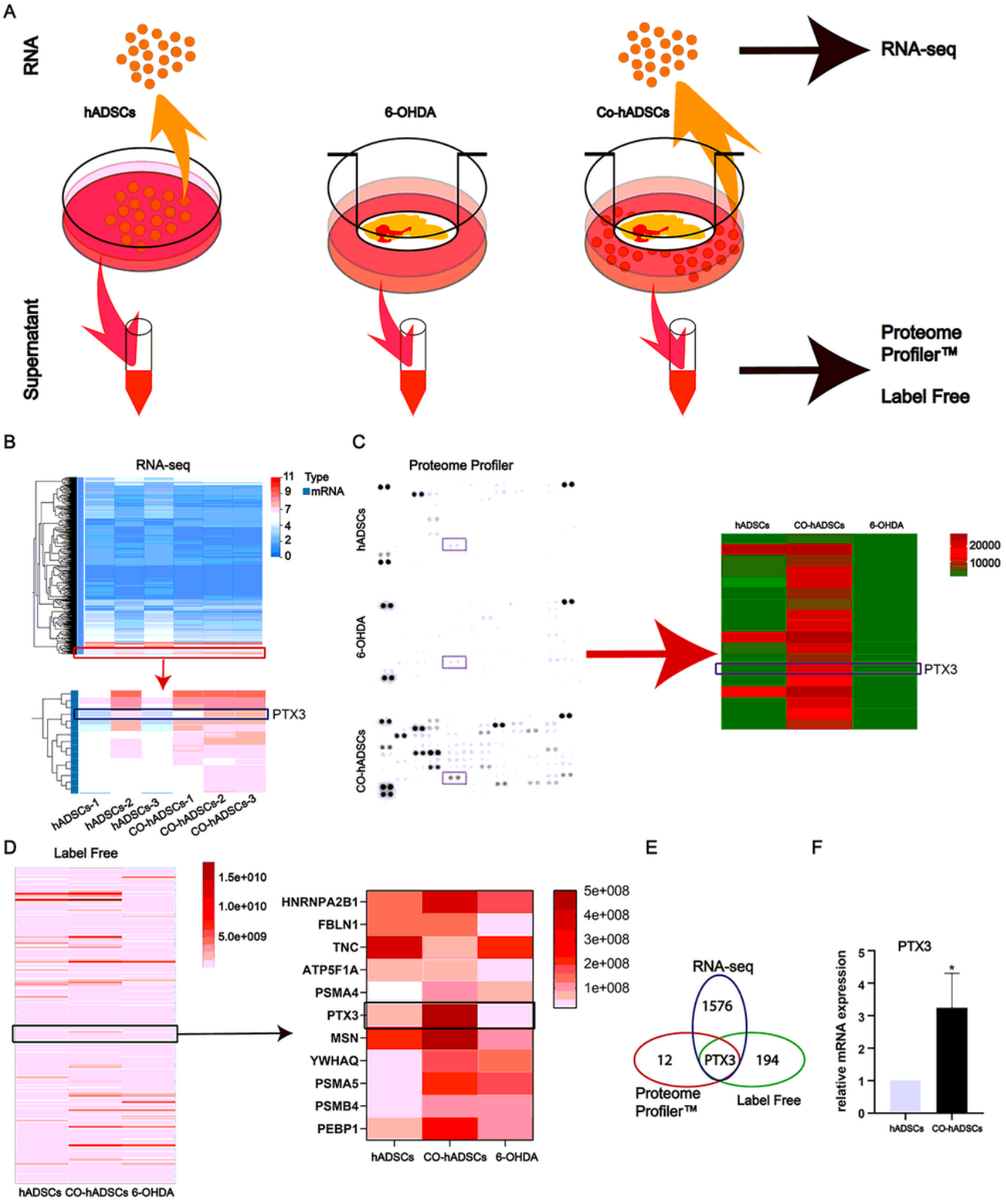

Figure 3

Pentraxin 3 is a potential hADSCs secrected protective molecule on in PD DA neuron models repair. a. High-throughput sequencing sample collection procedure; b. Heatmap showing the genes upregulated and downregulated (|logFC|>1) in hADSCs with or without co-culturing with 6-OHDA-treated brain slices (n=3, p<0.05); c. Conditioned media from hADSCs single culturing group, 6-OHDA-treated brain slices single culturing group and hADSCs co-cultured with 6-OHDA-treated brain slices group were measured by human cytokine array; d. Conditioned media from different groups were measured by label-free quantitative proteomics; e. RNA-seq high-throughput sequencing, Human protein cytokine arrays as well as label-free quantitative proteomics were appled to Venn intersection; f. PTX3 mRNA expression levels in hADSCs from different groups were analyzed by RT-qPCR (*p<0.05).

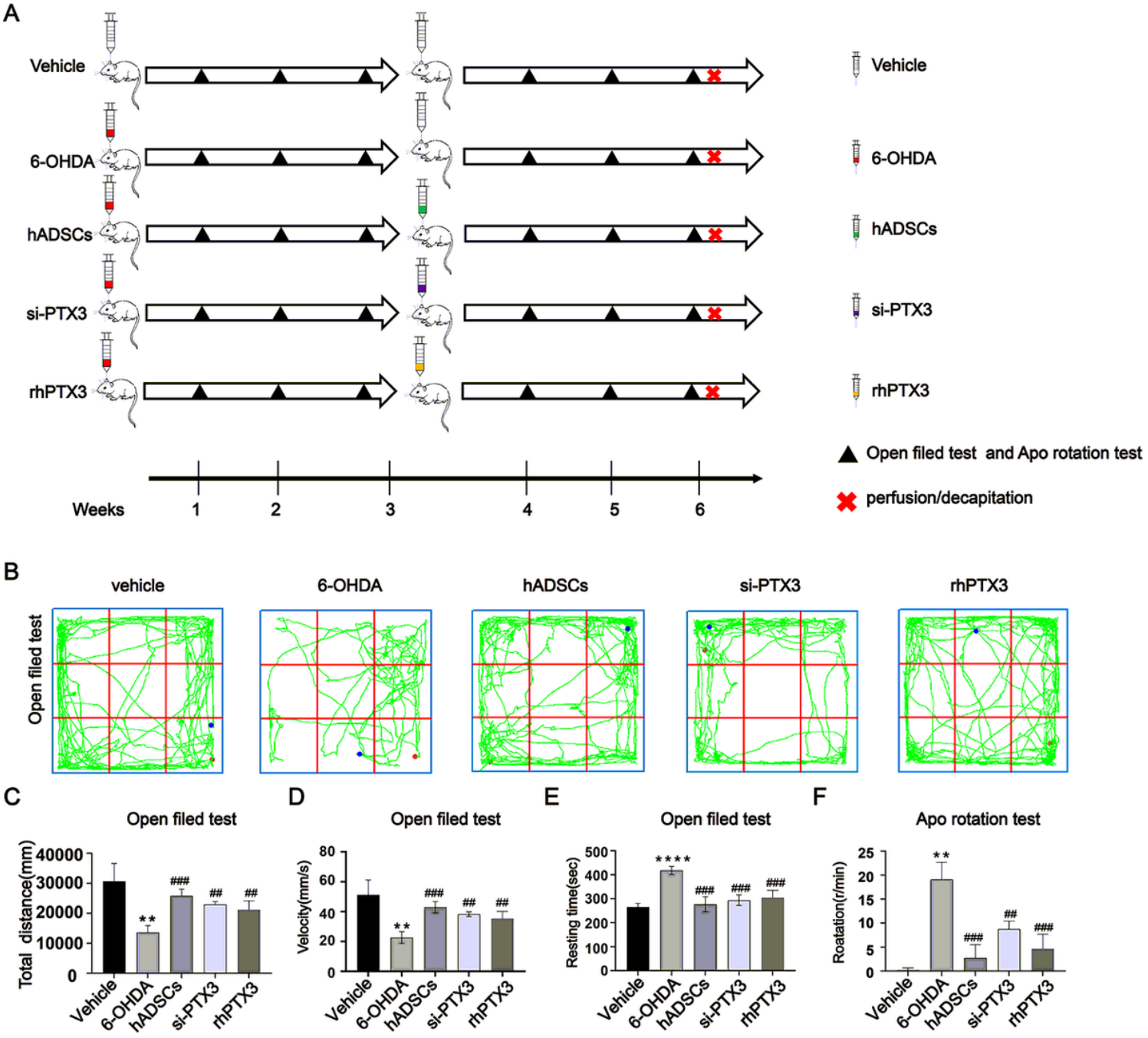

Figure 4

PTX3 treatment mimics the effects of hADSCs transplantation to improve the motor performances in the 6-OHDA-induced PD mice. a. Scheme of experimental design and workflow; b-e. At 5 weeks, open field experiment(b), total distance moved (c), velocity (d) and resting times (e) in 10 min; F. Side-biased rotational behavior in the APO-induced rotation test was observed from 5 weeks. (mice number in each group is equal or above 7, **p<0.001, ***p<0.0005, versus vehicle group, ##p<0.001, ###p<0.0005, versus 6-OHDA group)

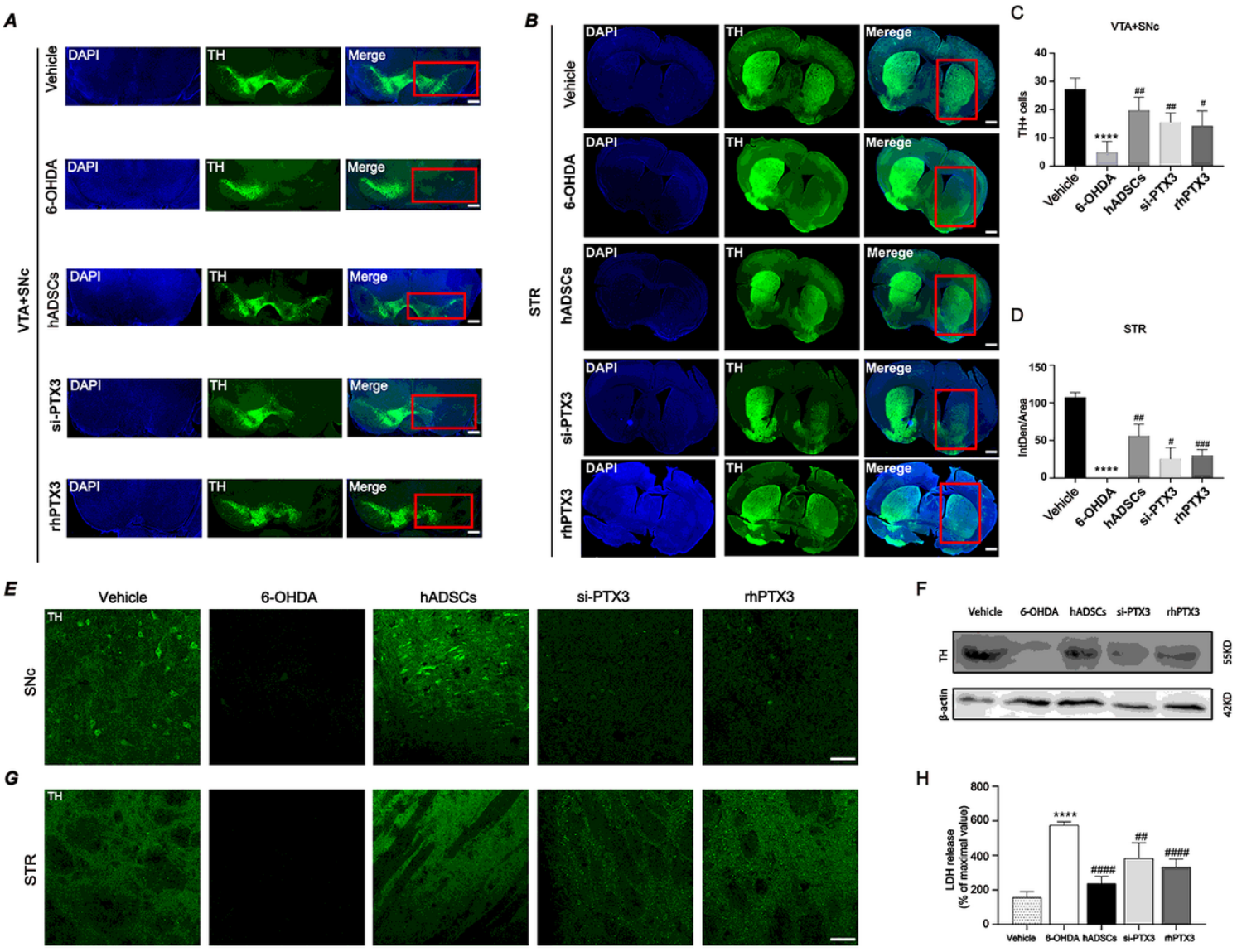

Figure 5

PTX3 treatment mimics the effects of hADSCs transplantation to improved TH expression in vivo and in vitro. At 3 weeks after saline transplanted vehicle mice, saline transplanted in 6-OHDA-induced PD mice, hADSCs transplanted in 6-OHDA-induced PD mice, siPTX3 transplanted in 6-OHDA-induced PD mice and rhPTX3 to treat 6-OHDA-induced PD mice, a,b. Immunofluorescent staining for TH in the VTA+SNc and STR;c. Quantification of TH-positive cells on VTA+SNc regions(scale bars=100um); d. At the STR level, the quantification of TH staining; After 4 days of co-cultivation, 6-OHDA-induced brain slices co-cultured with hADSCs, 6-OHDA-induced brain slices co-cultured with si-PTX3, 6-OHDA-induced brain slices treated

with rhPTX3 and treated with saline Of 6-OHDA-induced brain slices; e, g. brain slice immunofluorescence detection to observe the VTA+SNc and STR regions (video-S6-15, z = 350um, scale bars=50um, mice number in each group is equal or above 7); f. We performed western blot to assess TH protein expression level; h. The cell LDH activity in the culture medium was analysed, performing LDH test to evaluate the activity of brain slice cell (mice number in each group is equal or above 7, ****p<0.0001, versus vehicle group,#p<0.05, ##p<0.001, ###p<0.0005, ####p<0.0001, versus 6-OHDA group).

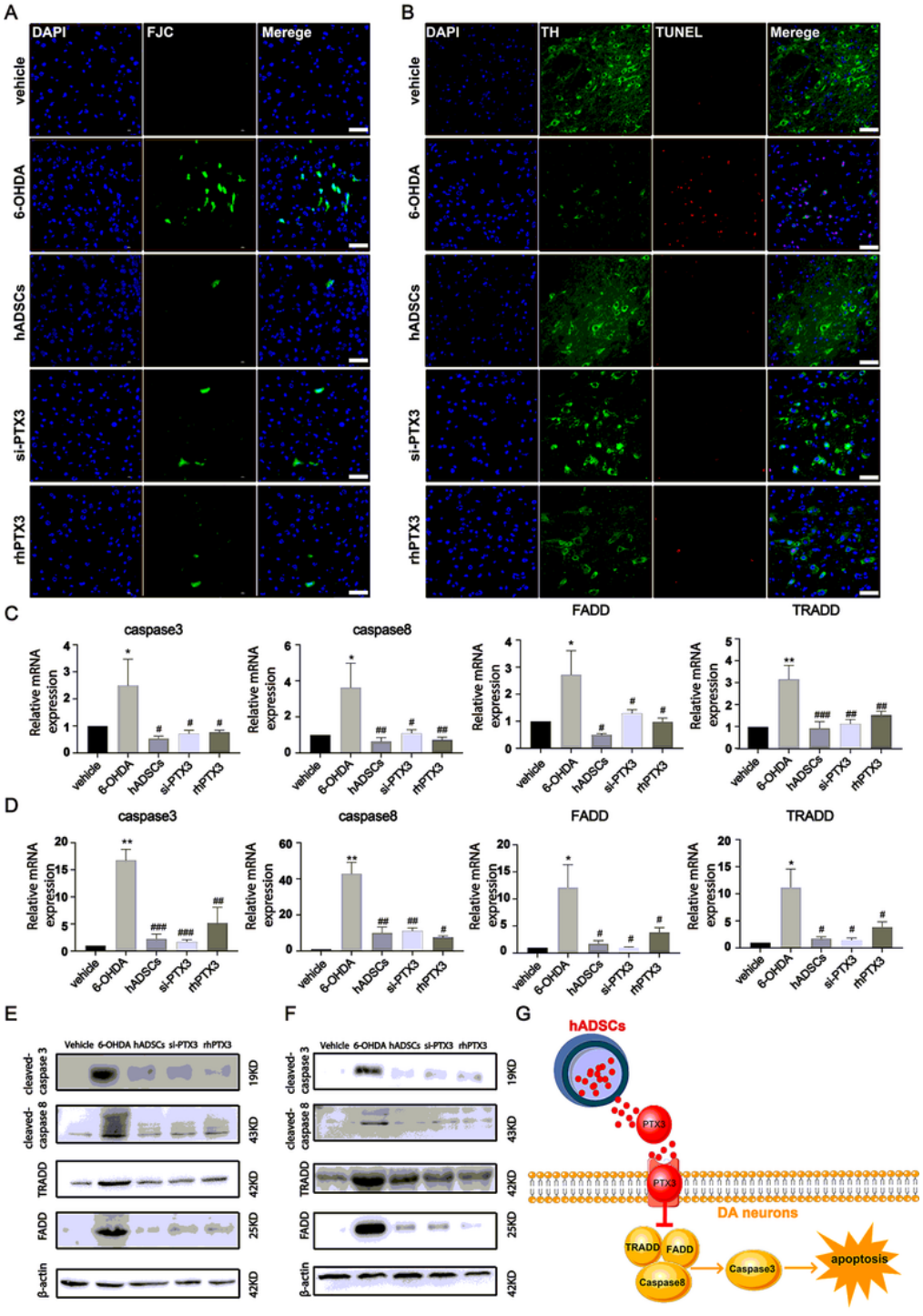

Figure 6

Pentraxin 3 protected DA neuron through inhibition of apoptotic death-inducing signal complex. At 3 weeks after saline transplanted vehicle mice, saline transplanted in 6-OHDA-induced PD mice, hADSCs transplanted in 6-OHDA-induced PD mice, siPTX3 transplanted in 6-OHDA-induced PD mice and rhPTX3 to treat 6-OHDA-induced PD mice, a. Representative images of Fluoro-Jade-C (green)staining in the VTA+SNc (scale bars=50um, mice number in each group is equal or above 7); b. Representative images of terminal deoxynucleotidyl transferase dUTP nick end labeling in the VTA+SN (TUNEL, red; TH, green)-staining (scale bars=50um, mice number in each group is equal or above 7); c. Representative picture of qPCR showing the expression of caspase 3, caspase 8, FADD, and TRADD in the VTA+SN (mice number in each group is equal or above 7, *p<0.05,**p<0.001, versus vehicle group, #p<0.05, ##p<0.001, ###p<0.0005, versus 6-OHDA group); d. After 4 days co-culturvation, subsequently, collecting brain slices, representative picture of qPCR showing the expression of caspase 3, caspase 8, FADD, and TRADD (mice number in each group is equal or above 7, *p<0.05,**p<0.001, versus vehicle group, #p<0.05, ##p<0.001, ###p<0.0005, versus 6-OHDA group); e. The protein expressions of cleaved-caspase 3, cleaved-caspase 8, FADD and TRADD analysised by western blot in the VTA+SN (mice number in each group is equal or above 7);f. After 4 days co-culturvation, subsequently, collecting brain slices, cleaved-caspase 3, cleaved-caspase 8, FADD and TRADD were analyzed by Western blot(mice number in each group is equal or above 7). g. Schematic diagram of the mechanism of hADSCs in promoting DA neurons repaired.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table.S1.xls
- SupplementaryMaterials.docx
- Table.S3.xlsx
- Table.S2.xlsx
- videoS1.mpg
- videoS10.mpg
- videoS11.mpg
- videoS12.mpg
- videoS13.mpg
- Figure.S1.tif
- videoS14.mpg
- Figure.S3.tif
- Figure.S4.tif
- Figure.S5.tif
- Figure.S6.tif
- Figure.S2.tif

- videoS15.mpg
- Table.S4.docx
- videoS2.mpg
- videoS3.mpg
- videoS4.mpg
- videoS5.mpg
- videoS6.mpg
- videoS7.mpg
- videoS8.mpg
- videoS9.mpg