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Downregulation of lncRNA UCA1 ameliorates the damage of dopaminergic neurons, reduces oxidative stress and inflammation in Parkinson's disease through the inhibition of the PI3K/Akt signaling pathway

Lijun Cai[a], Li Tu[b], Tian Li[c,d], Xiulin Yang[d], Yipin Ren[d], Ran Gu[e], Qian Zhang[d], Huan Yao[d], Xiang Qu[d], Qian Wang[e], Jinyong Tian[d,*]

[a] Department of Neurology, the Affiliated Hospital of Guizhou Medical University; Guiyang 550000, PR China
[b] Department of General Medical, the Affiliated Hospital of Guizhou Medical University, Guiyang 550000, PR China
[c] Zunyi Medical University, Zunyi 563006, PR China
[d] Department of Emergency, Guizhou Provincial People's Hospital, Guiyang 550004, PR China
[e] Department of Neurology, Guizhou Provincial People's Hospital, Guiyang 550004, PR China

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ABSTRACT

This study is conducted to investigate the role of lncRNA urothelial carcinoma-associated 1 (UCA1) in the protection of dopaminergic neurons in Parkinson's disease (PD) through regulating the PI3K/Akt signaling pathway. PD rat model was induced by injection of 6-hydroxydopamine (6-OHDA) to damage the substantia nigra striatum. The successfully modeled PD rats were introduced with siRNA-negative control (NC) or UCA1-siRNA. The expression of UCA1 in neurobehavioral change, neuroinflammatory response and oxidative stress of PD rats were explored. The effect of UCA1 on the PI3K/Akt signaling pathway and downstream proteins IκBα and ERK was also investigated. The rats with PD exhibited aggregated neurobehavioral change, increased neuroinflammatory response and oxidative stress. Down-regulation of UCA1 up-regulated the expression of TH positive cells and DA content, reduced the apoptosis of substantia nigra neurons, the apoptosis of substantia nigra neurons and oxidative stress and improved the neuroinflammatory response in PD rats. Down-regulation of UCA1 inhibited the activation of the PI3K/AKT signaling pathway in substantia nigra of PD rats. Our study suggests that the downregulated lncRNA UCA1 ameliorates the damage of dopaminergic neurons, reduces oxidative stress and inflammation in PD rats through the inhibition of the PI3K/Akt signaling pathway.

1. Introduction

Parkinson's disease (PD) is defined as a neuropathological disorder which is characterized by the substantia nigra dopaminergic neurons denatured and then lost their striatal terminals [1]. The core symptoms of PD are clearly related to the degeneration and death of dopamine (DA) neurons in the substantia nigra pars compacta [2]. Although the definite pathogenesis of PD remains unknown, accumulating studies have indicated that excessive generation of oxidative stress, reactive oxygen species (ROS) as well as mitochondrial dysfunction lead to the pathogenesis of PD [3,4]. An in-depth understanding of the mechanisms of action of toxins, such as 6-hydroxydopamine (6-OHDA), rotenone or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have achieved significant progress in parkinsonian neurodegeneration [5]. 6-OHDA is known as a neurotoxin that causes the death of DA neurons, which is commonly applied to establish experimental models of PD in rodents [6]. Therefore, finding a new target against PD which is able to alleviate the symptoms of PD, prevent neurodegeneration as well as restore the degenerated dopaminergic neurons is essential for the treatment of PD.

Recently, long noncoding RNAs (lncRNAs) have gained much attention owing to its abnormal expression in a great deal of carcinomas and they plat diversiform roles in tumorigenesis, and metastasis [7–9]. The human nervous system is reported to be the most highly evolved and also, sophisticated biological system. LncRNAs are different from other RNAs, they are transcribed from a location of the genome, which are overexpressed in the nervous system [10,11], implying that their networks are highly subjected to complicated neurobiological functions. The lncRNA urothelial carcinoma-associated 1 (UCA1) is an lncRNA initially detected from bladder carcinoma, which was dysregulated in many types of tumors, including melanoma, breast cancer,

* Corresponding author at: Department of Emergency, Guizhou Provincial People's Hospital, Zhongshan East Road No. 83, Guiyang 550004, Guizhou Province, PR China.
E-mail address: jinyong_Tian@126.com (J. Tian).

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colorectal cancer; esophageal squamous cell carcinoma, and gastric cancer [12–16]. Besides, UCA1 act as an oncogene to promote proliferation, migration, invasion while inhibiting apoptosis of the tumor cell [17–20]. Sun Y et al. have shown that UCA1 is up-regulated in glioma tissues, and lncRNA UCA1 acts as an endogenous sponge of miR-122, thereby promoting proliferation, migration and invasion of glioma cells [21]. Zheng J et al. have demonstrated that knocking out UCA1 gene can inhibit the differentiation of neural stem cells into astrocytes and promote the differentiation of neural stem cells into neurons [22]. Furthermore, UCA1 is reported to be highly expressed in brain tissue and peripheral blood of epilepsy model rat [23]. The above studies indicate that lncRNA UCA1 is associated with neurological diseases. Therefore, it is speculated that lncRNA UCA1 plays a vital role in tumorigenesis, even though the mechanism of action is still unclear. Furthermore, the effective modulation of the PI3K/Akt signaling pathway has been associated with several diseases, such as cancer, atherosclerosis as well as neurodegenerative diseases [24,25]. A study has suggested that the activation of PI3K/AKT signaling prominently affected PD in terms of regrowth of axons within the adult nigrostriatal projection [26]. Based on these aforementioned, we could assume that lncRNA UCA1 and the PI3K/Akt signaling pathway might participate in the progression of PD.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Medical Ethics Committee of Guizhou Provincial People's Hospital. (No. 2015013) The animal experiment was strictly complied with the principle to minimize the pain and suffering to experimental animals.

2.2. Experimental animals

Healthy male Wistar rats (weighing 220–250 g), were provided Hunan SJA Laboratory Animal Co., Ltd. (Hunan, China). The rats were kept in a room at 22–25 °C with free access to drinking and food, with 12 h day/night cycle. The cage and pad were replaced every 2 days, and the rats were adapted to the laboratory environment for one week before experiment.

2.3. Establishment of rat model of PD

The randomly-assigned experimental rats were anesthetized with 0.5 mL/100 g chloral hydrate (Shanghai Chemical Reagent Co. Ltd. (Shanghai, China)). The rats were fixed in the prone position in a stereotaxic apparatus (RWD Life Science Co.; Shenzhen, China) after holding the hind foot without reaction. The rats' head skin was disinfected with 75% ethanol, the skin was cut by a sharp knife to expose the skull, and the anterior fontanelle was chosen as the origin point. Two points of the medial forebrain bundle was selected for drilling according to the Paxinos & Watson pattern: (1) 4.4 mm behind the anterior fontanelle, 1.2 mm right of the lineae mediana, and 7.8 mm below the endocranium; (2) 4.0 mm behind the anterior fontanelle, 0.8 mm right of the lineae mediana, and 8.0 mm below the endocranium. The dosage of 6-OHDA (Sigma-Aldrich, St Louis, MO, USA) was 2.25 μL and 2.7 μL, and the speed was 1 μL/min for 5 min. The needle was produced slowly, the skin was disinfected and the scalp was sutured. Penicillin was used to prevent infection for 3 days, with 200,000 U per day. The same method was used to inject 2.25 μL and 2.7 μL saline into the medial forebrain bundle of rats in the sham group.

2.4. Animal treatment

A total of 60 healthy male Wistar rats were randomly assigned into five groups (n = 12) by random digital table: normal group (without any treatment), sham group (two points injection of medial forebrain bundle of normal saline), PD group (two-point injection of the medial forebrain bundle of 6-OHDA), siRNA-negative control (NC) (the NC was used to pretreat the substantia nigra of rats, and then the two points injection of the medial forebrain bundle of 6-OHDA was performed), and UCA1-siRNA group (UCA1-siRNA was used to pretreat the substantia nigra of rats, and then two points injection into the medial forebrain bundle of 6-OHDA). The nigra injection steps were the same with the model establishment procedures. The injection dose of siRNA-NC and UCA1-siRNA was 1 μL and the speed was 0.1 μL/min for 10 min. The needle was produced slowly, the skin was disinfected and the scalp was sutured. Penicillin was used to prevent infection for 3 days, with 200,000 U per day. siRNA-NC and UCA1-siRNA were provided by Shanghai Genechem Co., Ltd. (Shanghai, China).

2.5. Animal behavior determination

Rotation test: After 4 weeks of 6-OHDA injection, the rats in each group were put into the behavioral detector in advance for 5 min. The rats were injected subcutaneously with apomorphine according to the dose of 0.05 mg/kg, and the rotation times for half an hour were counted.

Open field test: According to the methods proposed by Kawai [27], the rats in each group were placed in the dark box of the automatic motility apparatus for 5 min. The number of autonomous activities in 2 min was recorded for 3 consecutive times, and the average value was taken.

2.6. Hematoxylin-eosin (HE) staining and Nissl staining

After the behavioral measurement, the rats in each group were euthanized, and the brain tissues of detection sites in each group were obtained and fixed with paraformaldehyde. The brain tissues were hydrated with 70%, 80% and 95% gradient alcohol for 12 h each, and n-butyl alcohol for 6 h. The tissues were then waxed at 60 °C overnight. Subsequently, the tissues were embedded and sliced continuously with the slice thickness of 6–8 um. One slice was taken in every 3 pieces.

HE staining: Paraffin sections were routinely washed with distilled water, stained with Mayer hematoxylin at room temperature for 5 min, flushed under tap water for l min to make the nucleus return to blue. Next, the sections were re-stained with 1% eosin solution for l min, followed by alcohol dehydration, xylene clearance and sealing. Results observation: The nucleus was blue and the cytoplasm was purplish red.

Nissl staining: The brain coronal sections were attached to the slides, dried naturally, and then soaked in xylene for 10 min, 100% ethanol for 10 min, 95% ethanol for 5 min and 70% ethanol for 5 min. The sections were then stained in 0.5% coke violet solution (pH = 3.9) for 30 min, and rinsed with distilled water for 3 min. Subsequently, the sections were decolorized in 70% ethanol, 95% ethanol (pH = 4.1) and 100% ethanol, followed by xylene clearance and sealing with neutral balsam. The positive neurons by Nissl staining were counted under a microscope.

2.7. Transmission electron microscope observation

After cardiac perfusion fixation, about 1 mm^3 substantia nigra striatum tissue was obtained. The tissues were fixed at 4 °C for 3 h in 2.5% glutaraldehyde (prepared with 0.01 MPBS, pH = 7.4) and fixed at 4 °C for 2 h in 1% Osmium acid solution. Lastly, the specimens were dehydrated by alcohol (20–100%) and acetone gradient, embedded with epoxy resin, sliced by ultra-thin slicer, double stained with uranium acetate and lead citrate. The ultrastructure of neurons was observed by a transmission electron microscope.

2.8. Determination of dopamine (DA) in striatum

After the behavioral measurement, the rats in each group were euthanized. The bilateral striatum tissue was separated and preserved on an ice box, and the tissue sample was accurately weighed and transferred into the prepared centrifuge tube. Next, the tissues were added with cold 0.1 mol/L perchloric acid ($HClO_4$) and 100 ng/mL 2,6-dihydroxy-benzoic acid (2,6-DHBA). The supernatant was centrifuged for 15 min at 4 °C for 13,000 r/min. The content of DA was determined by high performance liquid chromatography (HPLC) assay.

2.9. Immunohistochemical staining

After paraffin section dewaxing and hydration, the sections were treated with 3% methanol-hydrogen peroxide solution for 15 min at 37 °C, antigen repaired with sodium citrate buffer and added with normal goat serum sealant for 30 min at 37 °C. Afterwards, the primary antibody to TH (1: 1000) was supplemented and washed for several times, and then the secondary antibody horseradish peroxidase-labeled IgG (1: 200) was supplemented and washed for several times. Lastly, the sections were developed with diaminobenzidine (DAB), followed by hematoxylin counterstaining, dehydration, clearance, neutral balsam sealing. THH immunoreactive neurons were observed and counted under a light microscope.

2.10. TUNEL assay

Paraffin sections were routinely dewaxed to water, added with fresh 3% hydrogen peroxide for 8–10 min to inactivate endogenous peroxidase, and rinsed with distilled water for 3 times. The prepared protease K solution was added dropwise to the section and incubated at room temperature for 30 min. Next, the sections were supplemented with 50 μL TUNEL reaction mixture, incubated in a wet box at 37 °C for 60 min and then added with peroxidase (POD) for incubation at 37 °C for 30 min. Lastly, the sections were developed with diaminobenzidine (DAB), followed by hematoxylin counterstaining 1% hydrochloric acid alcohol differentiation, anhydrous ethanol dehydration dehydration, xylene clearance, and neutral balsam sealing. The expression of apoptotic cells was observed under an optical microscope.

2.11. Detection of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) in striatum

The rats were euthanized to obtain the striatum of the substantia nigra. The ultrasonic homogenate was obtained after weighing the brain tissue. The brain tissue homogenate was centrifuged at 4 °C, with the supernatant collected. The protein concentration was determined by Coomassie brilliant blue method. The absorbance of MDA, SOD and GSH-Px in homogenate was measured by a spectrophotometer at 532 nm, 420 nm, 550 nm and 412 nm respectively based on the instructions of MDA, SOD and GSH-Px kits (NanJing JianCheng Bioengineering Institute, Nanjing, China).

2.12. Enzyme-linked immunosorbent assay (ELISA)

The nigral tissues were maintained at the low temperature condition. After weighing, the samples in each group were diluted based upon a fixed proportion of normal saline. After homogenization, the samples were transferred to a centrifuge tube and centrifuged at 4 °C for 20 min (3000 rpm). The expression of tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6) in the supernatant of substantia nigra was detected by ELISA kit. The optical density (OD) value was measured at 450 nm. All OD values should be calculated after zero-well value was deducted. The standard curve was drawn by computer Curve Expert 1. 3 analysis software to calculate the sample content.

Table 1
Primer sequence.

Gene	Primer sequence (5′ - 3′)
UCA1	Forward: 5′-CATGCTTGACACTTGGTGCC-3′ Reserve: 5′-GGTCGCAGGTGGATCTCTTC-3′
TNF-α	Forward: 5′-TCAGCCGATTTGCCATTTCAT-3′ Reverse: 5′-ACACGCCAGTCGCTTCACAGA-3′
IL-1β	Forward: 5′-GTCCTTTCACTTGCCCTCAT-3′ Reverse: 5′-CAAACTGGTCACAGCTTTCGA-3′
IL-6	Forward: 5′-AAATGCCTCGTGCTGTCTGACC-3′ Reverse: 5′-GGTGGGTGTGCCGTCTTTCATC-3′
BDNF	Forward: 5′-TCGCTTCATCTTAGGAGT-3′ Reverse: 5′-TCAACATAAACCACCAAC-3′
NGF	Forward: 5′-CTGCTGAACCAATAGCTGCCCG-3′ Reverse: 5′-CGCCTTGACAAAGGTGTGAGTCG-3′
GAPDH	Forward: 5′-AACGGATTTGGTCGTATTGGG-3′ Reverse: 5′-TCGCTCCTGGAAGATGGTGAT-3′

Note: TNF-α, tumor necrosis factor-α; IL-1β, interleukin 1β; IL-6, interleukin 6; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor, GAPDH, glyceraldehyde phosphate dehydrogenase.

2.13. Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The Trizol method (Takara, Dalian, China) was used for extracting the total RNA. Nanodrop2000 (Thermo Fisher Scientific, San Jose, CA, USA) was adopted to determine the concentration and quality of RNA. The complementary DNA (cDNA) was obtained based on the instructions of the reverse transcription kit (DRR047S, Takara, Dalian, China). The obtained cDNA was added with 65 μL diethyl pyrocarbonate (DEPC) and mixed. PCR primer was designed and synthesized by Shenzhen Huada Gene Research Institute (Shenzhen, China) (Table 1). Glyceraldehyde phosphate dehydrogenase (GAPDH) was selected as internal control of UCA1, TNF-α, IL-6, IL-1β, brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). There were six parallel repeated in each gene of each sample. The $2^{-\Delta\Delta Ct}$ method [19] was used to analyze the ratio relation of target gene expression between the experimental group and the control group: $\Delta\Delta Ct = [Ct_{(target\ gene)} - Ct_{(internal\ control\ gene)}]_{experimental\ group} - [Ct_{(target\ gene)} - Ct_{(internal\ control\ gene)}]_{control\ group}$.

2.14. Western blot analysis

The extracted proteins from tissues were were determined referring to the instructions of the bicinchoninic acid (BCA) assay (Beyotime Institute of Biotechnology, Shanghai, China). Tthe proteins were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a nitrocellulose membrane. Subsequently, the protein samples were transferred to polyvinylidene fluoride (PVDF) membrane and blocked with 5% bovine serum albumin (BSA) for at 4 °C. Afterwards, the membranes were supplemented with the primary antibodies to BDNF (1:1000), NGF (1 μg/mL), Cleaved-Caspase-3 (1:500), Bax (1:1000), Bcl-2 (1:500), IκBα (1:1000) (all from Abcam, Cambridge, MA, USA), PI3K (1:100), p-PI3K (1:100), Akt (1:100), p-Akt (1:100), p-IκBα (1:1000), ERK (1:1000) (all from Cell Signaling Technology, Danvers, MA, USA), p-ERK (1:1000) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and GAPDH (1, 1000) (Millipore Corporation, Bedford, CA, USA) and incubated at 4 °C overnight. The membranes were then rinsed with Tris-buffered saline and Tween 20 (TBST) for 3 times. The corresponding secondary antibodies-labeled by horseradish peroxidase (Abcam, Cambridge, MA, USA) were incubated for 1 h at 37 °C. An electrogenerated chemiluminescence (ECL) solution was used for developing. The gray value of the target band was analyzed by Image J software.

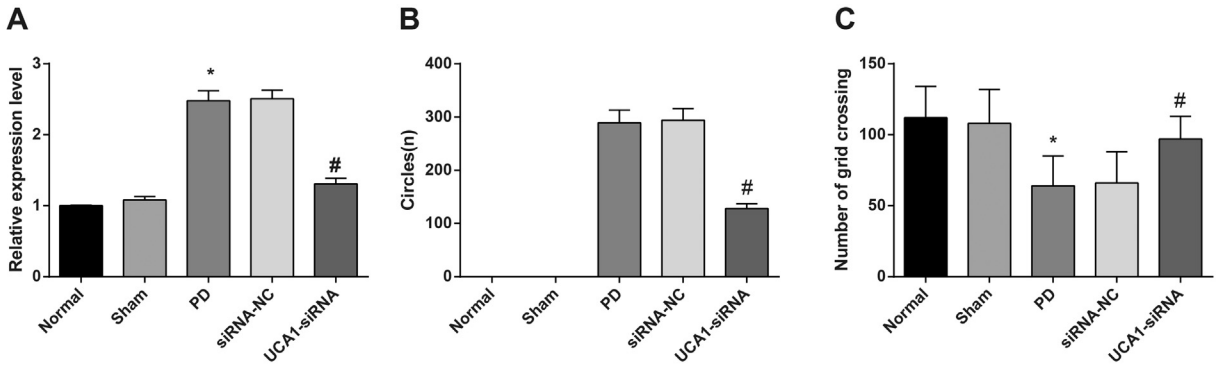

Fig. 1. The expression of UCA1 in the substantia nigra of rats in each group and the results of behavioral examination (n = 12). A. The expression of UCA1 in the substantia nigra of rats in each group. B. The number of rotation circles in each group of PD rats induced by apomorphine; C. The number of grid crossing of rats in each group; * $P < 0.05$ vs the normal group; # $P < 0.05$ vs the siRNA-NC group. The measurement data were expressed as mean ± standard deviation and ANOVA was used for the comparison among multiple groups. After ANOVA analysis, the LSD-*t*-test was used for pairwise comparison.

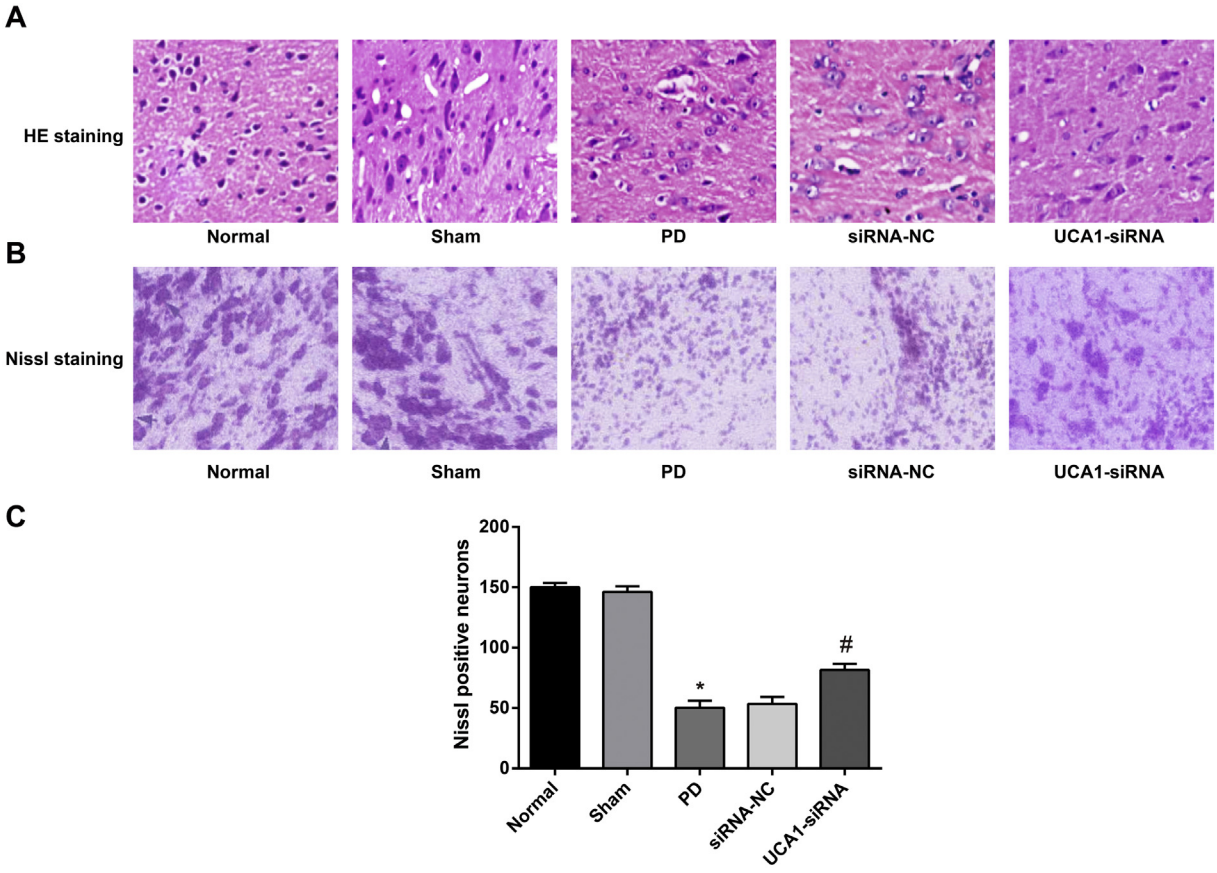

Fig. 2. Morphological observation of rats with PD in each group (n = 12). A. HE staining of substantia nigra in each group (× 400); B. Nissl staining of substantia nigra in each group (× 200); C. Comparison of the number of positive cells in the substantia nigra of rats in each group; * $P < 0.05$ vs the normal group; # $P < 0.05$ vs siRNA-NC group. The measurement data were expressed as mean ± standard deviation and ANOVA was used for the comparison among multiple groups. After ANOVA analysis, the LSD-*t*-test was used for pairwise comparison.

2.15. Statistical analysis

All statistical analyses were performed using the SPSS 21.0 software (IBM SPSS, Inc., Chicago, IL, USA). The data were normally distributed by Kolmogorov-Smirnov test. The measurement data were expressed as mean ± standard deviation. The *t*-test was used for the comparison between the two groups, and one-way analysis of variance (ANOVA) was used for the comparison among multiple groups. After ANOVA analysis, the Fisher's least significant difference *t*-test (LSD-t) was used for pairwise comparisons. P values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Expression of UCA1 is upregulated in the substantia nigra of PD rats

Initially, the expression of UCA1 in the substantia nigra of rats in each group was detected by RT-qPCR. As shown in Fig. 1A, no significant difference was found in the expression of UCA1 in the substantia nigra of rats between the normal and the sham groups as well as between the siRNA-NC and the PD groups (all $P > 0.05$). Compared with the normal group, the expression of UCA1 in the substantia nigra of rats in the PD group was increased ($P < 0.05$), suggesting that the

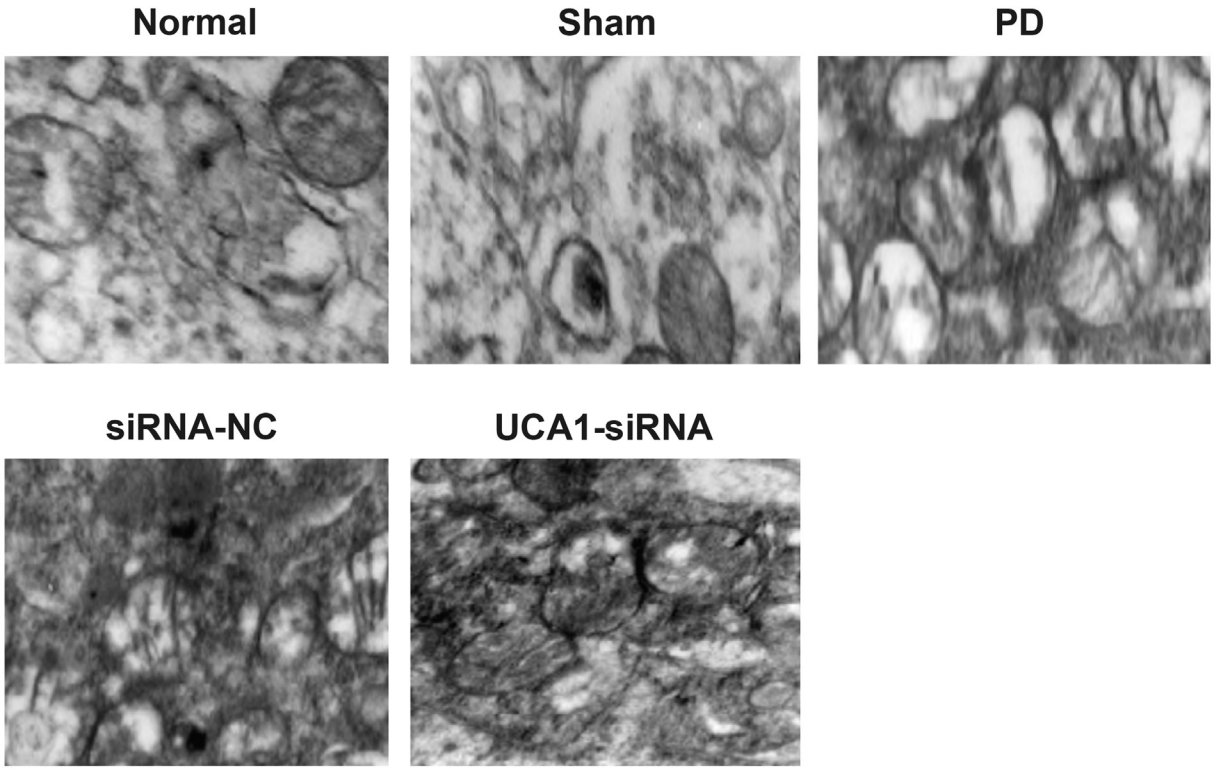

Fig. 3. Ultrastructure of substantia nigra striatum by a transmission electron microscope scanning (× 30,000) (n = 12).

expression of UCA in the substantia nigra of PD rats was up-regulated. Relative to the siRNA-NC group, the expression of UCA1 was decreased in the substantia nigra of rats in the UCA1-siRNA group ($P < 0.05$).

3.2. Behavioral examination of rats with PD in each group

Four weeks post operation, none of the rats in the normal group and the sham group showed rotational behavior after induced by apomorphine, while the other three groups showed varying degrees of left rotation behavior. There was no significant difference was found in the number of rotation circles among the PD, siRNA-NC and the UCA1-siRNA groups (all $P > 0.05$). Compared with the siRNA-NC group, the number of rotation circles of the UCA1-siRNA group was significantly decreased ($P < 0.05$; Fig. 1B).

As presented in Fig. 1C, there was no significant difference in the number of grid crossing between the normal group and the sham group as well as between the siRNA-NC and the PD groups (all $P > 0.05$). In comparison to the normal group, the number of grid crossing was decreased significantly in the PD group ($P < 0.05$). In contrast to the siRNA-NC group, the number of grid crossing was increased significantly in the UCA1-siRNA group ($P < 0.05$). It is suggested that down-regulation of UCA1 can improve neurobehavioral changes in PD rats induced by 6-OHDA.

3.3. Morphological observation of rats with PD in each group

According to the results of HE staining (Fig. 2A), we found that the morphology of substantia nigra was normal in the normal and the sham groups. In the PD and the siRNA-NC groups, substantia nigra cells decreased significantly, large numbers of neuroastrocytes proliferated, neuronal cell bodies constricted, edema vacuoles and swollen stellate processes were seen around the capillaries and pyknotic nerve cells. The damage in the UCA1-siRNA group was less than that in the PD group, which showed that the number of neurons in substantia nigra was more, inflammatory cell and glial cell proliferation were alleviated.

Nissl staining (Fig. 2B-C) showed that there was no significant difference in the number of positive cells in substantia nigra between the normal group and sham group as well as between the siRNA-NC and the PD groups (all $P > 0.05$). The number of positive cells in substantia nigra in the PD group was significantly less than that in the normal group ($P < 0.05$), and the number of positive cells in substantia nigra in the UCA1-siRNA group was significantly more than that in the siRNA-NC group ($P < 0.05$).

3.4. Ultrastructural observation of substantia nigra striatum neurons in rats of each group

In order to observe the ultrastructural changes of substantia nigra-striatum neurons, the structure of substantia nigra-striatum in rats was scanned by a transmission electron microscope. The results in Fig. 3 suggested that in the substantia nigra striatum of rats in the normal group and the sham group, the neuronal cell structure was clear, the cytoplasmic organelle was abundant, the nucleus was round or irregular, the nuclear membrane was intact, the nuclear chromatin was dispersed and the nucleolus was obvious. In the sham group, mitochondria were slightly swollen, but the structure was intact, and the double layer mitochondrial membrane and mitochondrial ridge could be clearly distinguished. In the PD group and the siRNA-NC group, the structure of striatum organelle was unclear, the nucleus shrank, the heterochromatin in the nucleus increased, gathered into small lumps and distributed around the nuclear membrane, showing the early morphology of apoptosis. The volume of mitochondria increased, vacuolated, and the mitochondria were dissolved in different degrees. However, the pre-treatment of UCA1-siRNA injection in the substantia nigra significantly reduced the shrinkage of the nucleus and the abnormal aggregation of the chromatin, and significantly improved the swelling of the mitochondria and the change of the vacuolation.

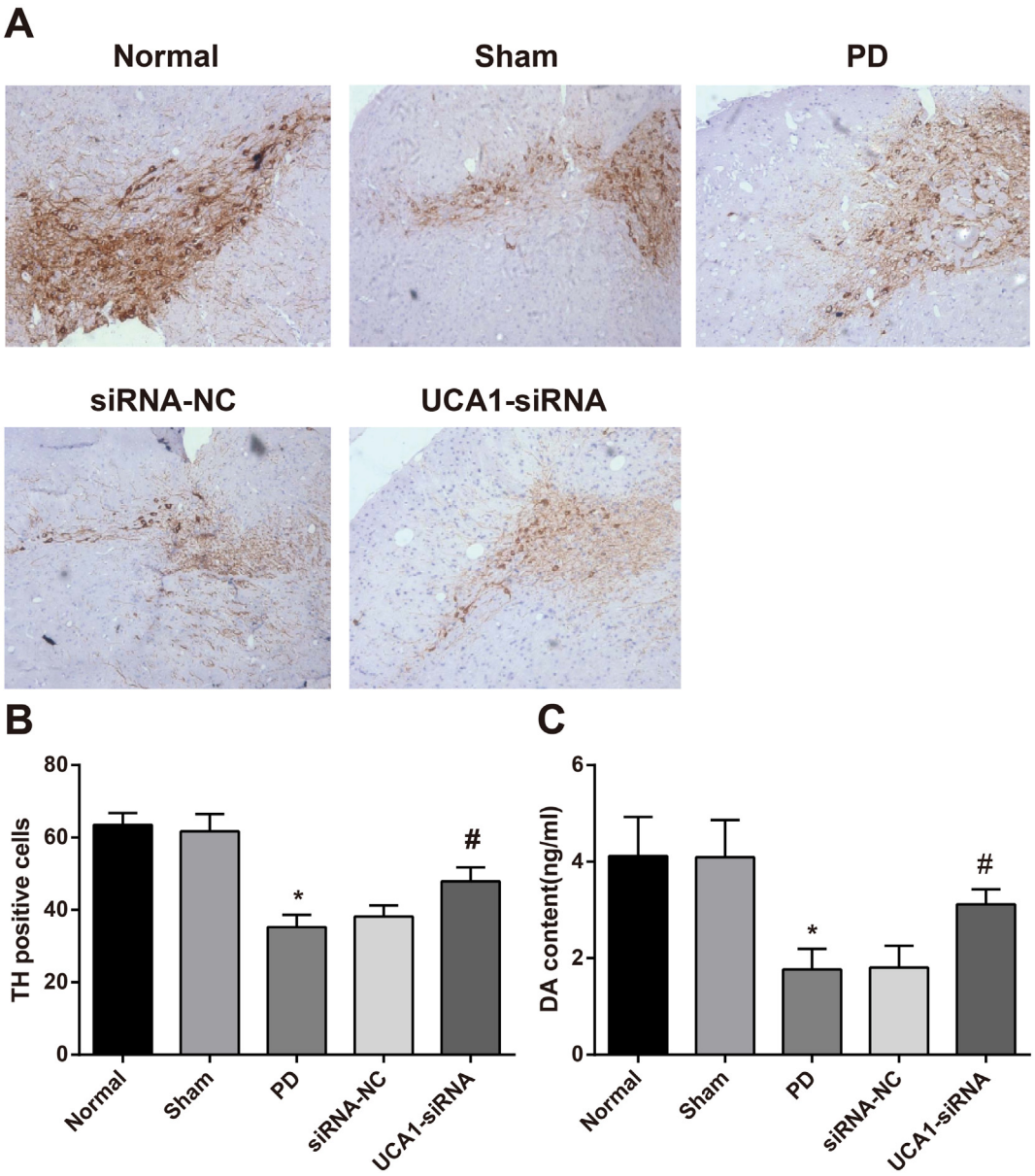

Fig. 4. Down-regulation of UCA1 up-regulates the expression of TH positive cells and DA content in substantia nigra of PD rats (n = 12). A. Immunohistochemical staining of TH in substantia nigra of rats in each group (× 100); B. Number of TH positive cells in substantia nigra of rats in each group; C, The DA content in the striatum of rats in each group. * $P < 0.05$ vs the normal group; # $P < 0.05$ vs siRNA-NC group. The measurement data were expressed as mean ± standard deviation and ANOVA was used for the comparison among multiple groups. After ANOVA analysis, the LSD-*t*-test was used for pairwise comparison.

3.5. Down-regulation of UCA1 up-regulates the expression of TH positive cells and DA content in substantia nigra of PD rats

TH positive cells were found in the substantia nigra of each group (Fig. 4A), and the positive expression of TH protein was located in the cytoplasm and was brown. The number of TH positive cells showed no significant difference between the normal and the sham groups as well as the siRNA-NC and the PD groups (all $P > 0.05$). The number of TH positive cells in the PD group was significantly less than that in the normal group ($P < 0.05$), and the number of TH positive cells in the UCA1-siRNA group was significantly more than that in the siRNA-NC group ($P < 0.05$; Fig. 4B). It is suggested that down-regulation of UCA1 can up-regulate the expression of TH positive cells in substantia nigra of PD rats.

HPLC was utilized to measure the DA content in the striatum, and the results showed (Fig. 4C) that there was no significant difference in DA content in striatum of rats between the normal group and the sham group as well as the siRNA-NC and the PD groups (all $P > 0.05$). Compared with the normal group, DA content in striatum of rats was significantly decreased in the PD group ($P < 0.05$). The content of DA in striatum of rats in the UCA1-siRNA group was significantly higher than that in the siRNA-NC group ($P < 0.05$), suggesting that down-regulation of UCA1 upregulated DA content in striatum of PD rats.

3.6. Down-regulation of UCA1 could reduce the apoptosis of substantia nigra neurons in PD rats

The apoptosis of dopaminergic neurons in substantia nigra was detected by TUNEL assay. As shown in Fig. 5A, there were several apoptotic cells in the normal group and the sham group. It was found that most of the apoptotic cells were stained brown, which were located in the nucleus, and the cytoplasm was not stained, the nucleus became smaller or pyknotic, and some of them dissolved into vacuolation in the PD, siRNA-NC and the UCA1-siRNA groups. Additionally, the number of

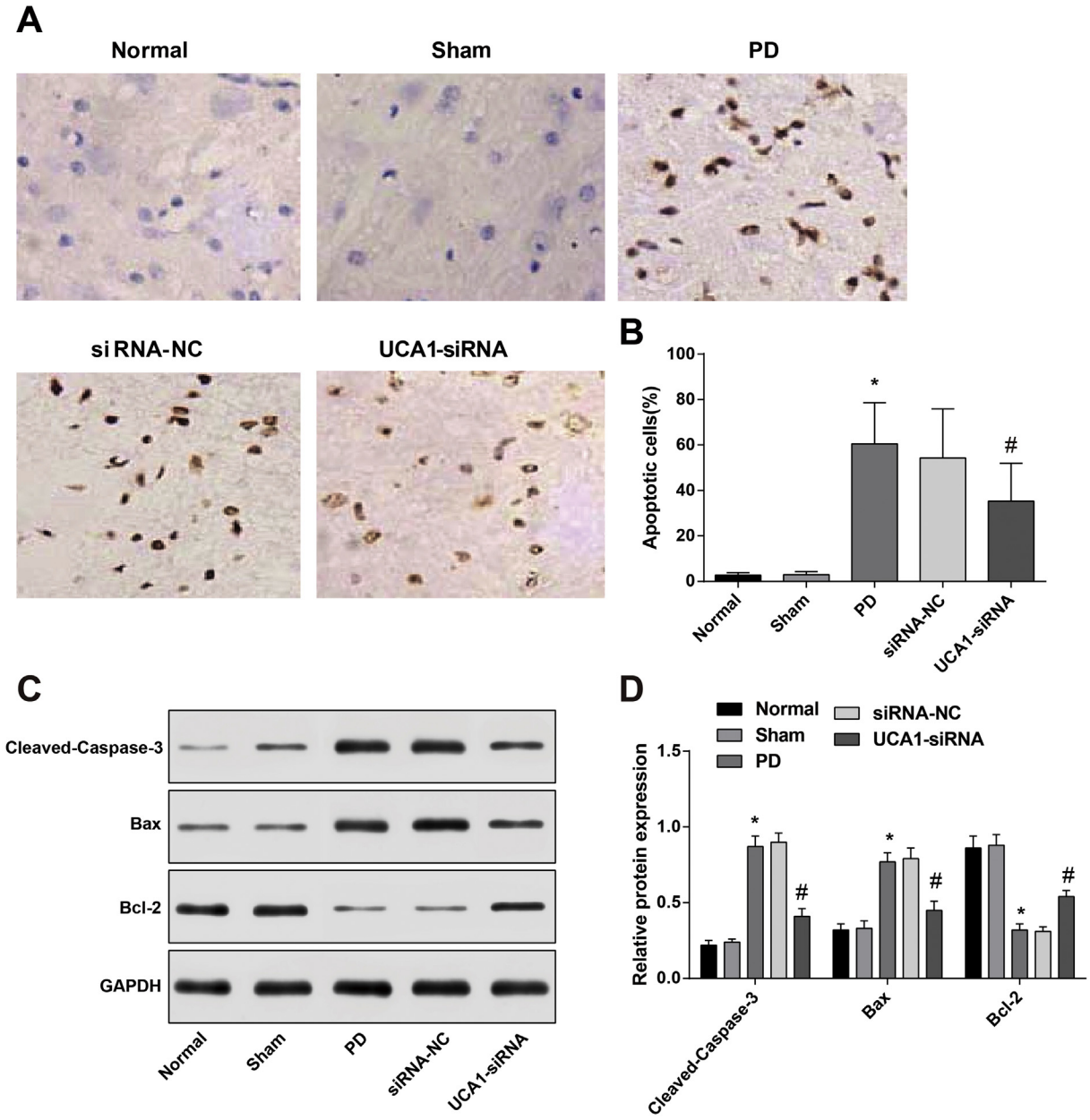

Fig. 5. Down-regulation of UCA1 could reduce the apoptosis of substantia nigra neurons in PD rats ($n = 12$). A. TUNEL staining of dopaminergic neurons in the substantia nigra of rats in each group. B. Comparison of the number of apoptotic dopamine neurons in the substantia nigra of rats in each group. C. Protein bands of apoptosis-related proteins Cleaved-Caspase-3, Bax and Bcl-2 in substantia nigra of rats in each group. D. Expression of apoptosis-related proteins Cleaved-Caspase-3, Bax and Bcl-2 in substantia nigra of rats in each group; * $P < 0.05$ vs the normal group; # $P < 0.05$ vs the siRNA-NC group. The measurement data were expressed as mean ± standard deviation and ANOVA was used for the comparison among multiple groups. After ANOVA analysis, the LSD-*t*-test was used for pairwise comparison.

apoptotic cells was statistically analyzed (Fig. 5B). There was no significant difference in the number of apoptotic dopaminergic neurons between the normal group and the sham group as well as between the PD and the siRNA-NC groups ($P > 0.05$). Compared with the normal group, the number of apoptotic dopaminergic neurons in rats was significantly increased in the PD group ($P < 0.05$). The number of apoptotic dopaminergic neurons in rats in the UCA1-siRNA group was significantly less than that in the siRNA-NC group ($P < 0.05$).

The expression of apoptosis-related proteins (Cleaved-Caspase-3, Bax and Bcl-2) in substantia nigra was detected by western blot analysis (Fig. 5C-D). The results showed that the expression of apoptosis-related protein Cleaved-Caspase-3, Bax and Bcl-2 in substantia nigra of rats between the normal and the sham groups as well as between the PD and the siRNA-NC groups were not significantly different (all $P > 0.05$). In the PD group, the protein expression of pro-apoptotic factor Cleaved-Caspase-3 and Bax was up-regulated, and the expression of anti-apoptotic factor Bcl-2 was down-regulated in the substantia nigra of rats relative to the normal group (all $P < 0.05$). In contrast to the siRNA-NC group, the protein expression of pro-apoptotic factor Cleaved-Caspase-3 and Bax was down-regulated, and the expression of anti-apoptotic factor Bcl-2 was up-regulated in the substantia nigra of rats in the UCA1-siRNA group (all $P < 0.05$). It came to a conclusion that down-regulation of UCA1 could reduce the apoptosis of substantia nigra neurons in PD rats.

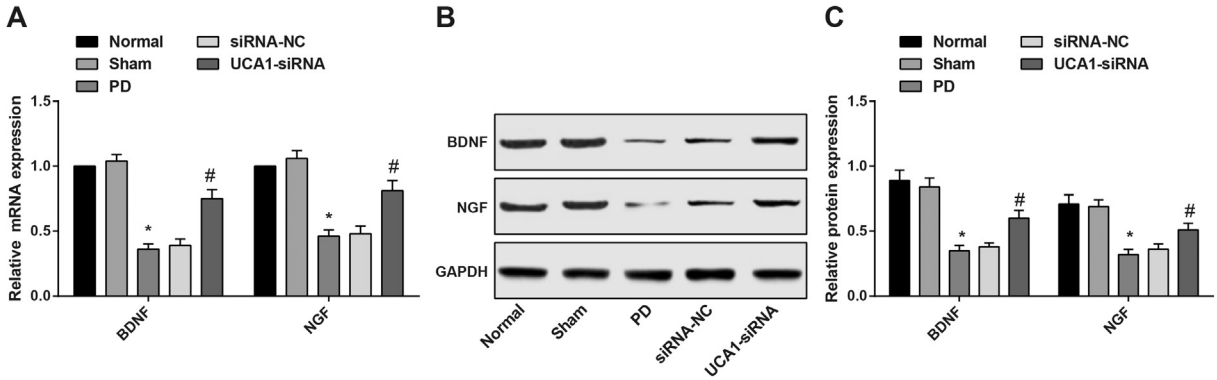

Fig. 6. Down-regulation of UCA1 could up-regulate the expression of nerve growth factor (BDNF and NGF) in substantia nigra of PD rats (n = 12). A. The expression of BDNF and NGF mRNA in substantia nigra of rats in each group. B. Protein bands of BDNF and NGF in substantia nigra of rats in each group. C. The expression of BDNF and NGF protein in substantia nigra of rats in each group. * $P < 0.05$ vs the normal group; # $P < 0.05$ vs the siRNA-NC group. The measurement data were expressed as mean ± standard deviation and ANOVA was used for the comparison among multiple groups. After ANOVA analysis, the LSD-t-test was used for pairwise comparison.

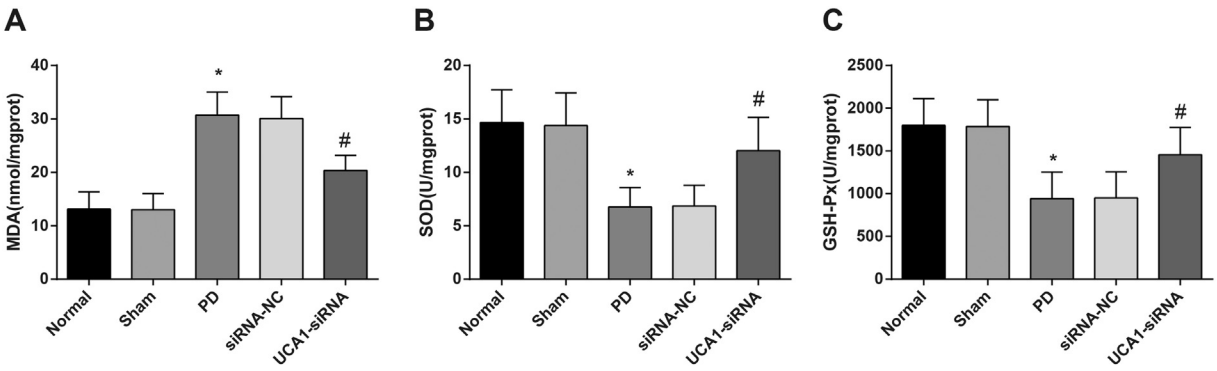

Fig. 7. Down-regulation of UCA1 reduces oxidative stress in substantia nigra of PD rats (n = 12). A. MDA content in substantia nigra of rats in each group; B. SOD activity in substantia nigra of rats in each group; C. GSH-Px activity in substantia nigra of rats in each group; * $P < 0.05$ vs the normal group; # $P < 0.05$ vs the siRNA-NC group. The measurement data were expressed as mean ± standard deviation and ANOVA was used for the comparison among multiple groups. After ANOVA analysis, the LSD-t-test was used for pairwise comparison.

3.7. Down-regulation of UCA1 up-regulates the expression of nerve growth factor (BDNF and NGF) in substantia nigra of PD rats

RT-qPCR and western blot analysis were used to detect the expression of BDNF and NGF mRNA and protein in substantia nigra of rats. The results in Fig. 6 suggested that there was no significant difference was found in the expression of BDNF and NGF mRNA and protein in substantia nigra of rats between the normal and the sham groups as well as between the PD and the siRNA-NC groups were not significantly different (all $P > 0.05$). Compared with the normal group, the expression of BDNF and NGF mRNA and protein in substantia nigra of rats in the PD group was significantly decreased (all $P < 0.05$). The expression of BDNF and NGF mRNA and protein in substantia nigra of rats was significantly increased in the UCA1-siRNA group compared with the siRNA-NC group (all $P < 0.05$). It suggested that down-regulation of UCA1 could up-regulate the expression of nerve growth factor (BDNF and NGF) in substantia nigra of PD rats.

3.8. Down-regulation of UCA1 reduces oxidative stress in substantia nigra of PD rats

The contents of SOD, GSH-Px and MDA in the substantia nigra of rats in each group were determined (Fig. 7). The results indicated that there was no significant difference in the contents of SOD, GSH-Px and MDA in the substantia nigra of rats between the normal and the sham groups as well as between the PD and the siRNA-NC groups (all $P > 0.05$). Compared with the normal group, the content of MDA increased significantly while the activities of SOD and GSH-Px decreased significantly in the substantia nigra of rats in the PD group (all $P < 0.05$). The content of MDA decreased significantly while the activities of SOD and GSH-Px increased significantly in the substantia nigra of rats in the UCA1-siRNA group relative to the siRNA-NC group (all $P < 0.05$), indicating that down-regulation of UCA1 could reduce oxidative stress in substantia nigra of PD rats.

3.9. Down-regulation of UCA1 improves the neuroinflammatory response of PD rats

The expression of TNF-α, IL-6 and IL-1β in substantia nigra of rats in each group was detected (Fig. 8). The results suggested that there was no significant difference in the expression of TNF-α, IL-6 and IL-1β in the substantia nigra of rats between the normal and the sham groups as well as between the PD and the siRNA-NC groups (all $P > 0.05$). Relative to the normal group, the expression of TNF-α, IL-6 and IL-1β was increased significantly in the substantia nigra of rats in the PD group (all $P < 0.05$). The expression of TNF-α, IL-6 and IL-1β was decreased significantly in the substantia nigra of rats in the UCA1-siRNA group in contrast to the siRNA-NC group (all $P < 0.05$). It is suggested that down-regulation of UCA1 can improve the neuroinflammatory response of PD rats induced by 6-OHDA.

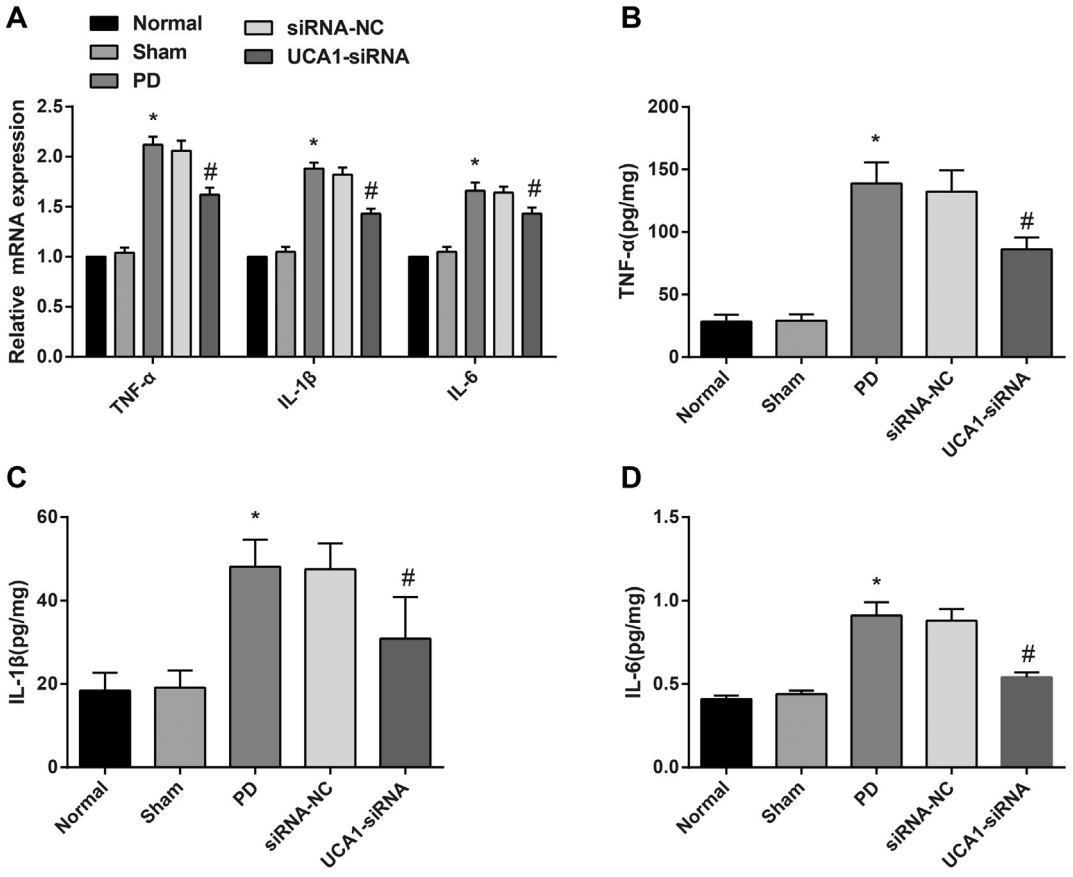

Fig. 8. Down-regulation of UCA1 improves the neuroinflammatory response of PD rats (n = 12). A. mRNA expression of inflammatory factors TNF-α, IL-1 β and IL-6 in substantia nigra of rats in each group; B-D. Protein expression of inflammatory factors TNF-α, IL-1 β and IL-6 in substantia nigra of rats in each group; * $P < 0.05$ vs the normal group; # $P < 0.05$ vs the siRNA-NC group. The measurement data were expressed as mean ± standard deviation and ANOVA was used for the comparison among multiple groups. After ANOVA analysis, the LSD-t-test was used for pairwise comparison.

3.10. Down-regulation of UCA1 inhibits the activation of the PI3K/AKT signaling pathway in substantia nigra of PD rats

Detection of PI3K, p-PI3K, Akt, p-Akt, IκBα, p-IκBα, ERK and p-ERK protein expression in substantia nigra of rats was carried out by western blot analysis. The results in Fig. 9 indicated that there was no significant difference in p-PI3K/t-PI3K, p-Akt/t-Akt, p-IκBα/t-IκBα and p-ERK/t-ERK expression in the substantia nigra of rats between the normal and the sham groups as well as between the PD and the siRNA-NC groups (all $P > 0.05$). Relative to the normal group, the p-PI3K/t-PI3K, p-Akt/t-Akt, p-IκBα/t-IκBα and p-ERK/t-ERK expression was increased significantly in the substantia nigra of rats in the PD group (all $P < 0.05$). The p-PI3K/t-PI3K, p-Akt/t-Akt, p-IκBα/t-IκBα and p-ERK/t-ERK was decreased significantly in the substantia nigra of rats in the UCA1-siRNA group in contrast to the siRNA-NC group (all $P < 0.05$). It suggested that down-regulation of UCA1 could inhibit the activation of the PI3K/AKT signaling pathway and phosphorylation of downstream proteins IκBα and ERK in substantia nigra of PD rats.

4. Discussion

PD commonly arises due to a complex net of pathological changes, such as oxidative stress, neuroinflammation and mitochondrial and proteasomal dysfunction [28]. The exposure of DA neurons to 6-OHDA has been suggested to contribute to the neurotoxin uptake by the DA transporter together with its subsequent oxidation to both reactive oxygen species and free radicals [6]. Certain studies have demonstrated that several lncRNAs, such as naPINK1 and ASUchl1, exhibited significant potential in the pathogenesis of PD [29,30]. Nevertheless, the genome-wide expression together with the functional significance of lncRNAs is still unclear in PD. Furthermore, upregulated UCA1 is reported to promote cancer progression through regulating either mTOR or Wnt signaling pathway [31,32]. Although UCA1 has been suggested to have imporatnt functions in a growing number of cancers, little is known on the expression pattern and specific role of UCA1 in PD. In this present study, we aim to investigate the role of UCA1 in the progression of PD through the regulation of the PI3K/AKT signaling pathway.

One of the most significant findings in our study was that expression of UCA1 is upregulated in the substantia nigra of PD rats, and UCA1 expression was implicated in the development of PD. Similarly, UCA1 was identified to be upregulated in non-small cell lung cancer (NSCLC) patient tissues and cell lines, suggesting the pathological and clinical implication of UCA1 expression in NSCLC [17]. In recent years, different lncRNA profiles enables to serve as phenotypic signatures for different types of cancers for their application in cancer prognostics and therapeutics [17]. Neural stem cell is served as therapeutic regimen for neurological disorders, such as spinal cord injuries, Huntington's disease, Alzheimer's disease and PD, and UCA1 modulated the cell proliferation and differentiation of neural stem cells [22]. It has been suggested that it's the decoy function of UCA1 through sponging microRNAs (miRNAs) to control the latter's downstream oncogenic targets is the most common molecular mechanism of UCA1 in promoting its role in cell survival and carcinogenesis [33,34]. Meanwhile, a current study has expanded the knowledge that UCA1 plays a role in promoting cell survival in both the physiological status and under stress conditions [35].

In addition, our study also suggested that down-regulation of UCA1 inhibits the activation of the PI3K/AKT signaling pathway in substantia

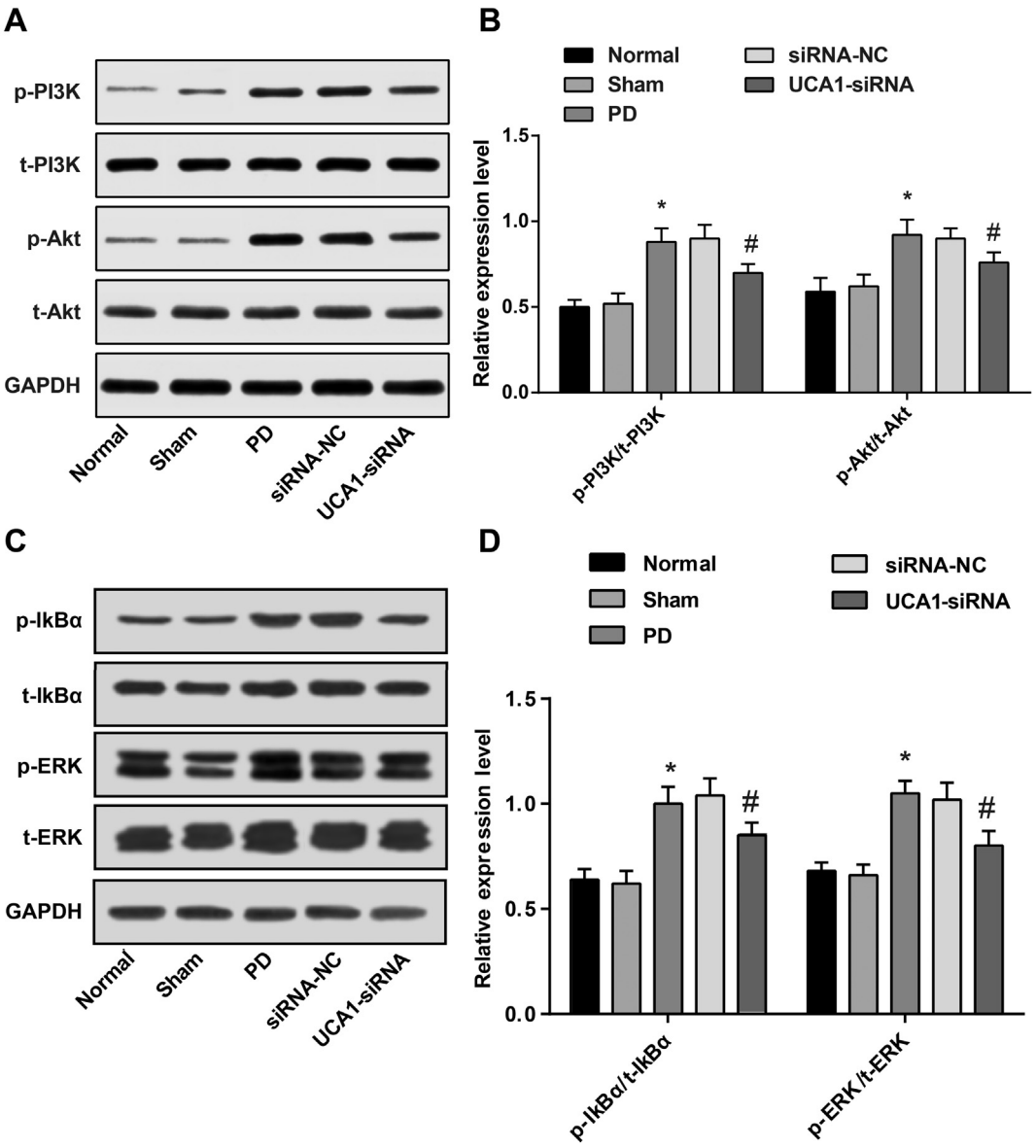

Fig. 9. Down-regulation of UCA1 inhibits the activation of the PI3K/AKT signaling pathway in substantia nigra of PD rats (n = 12). A. Protein bands of PI3K, p-PI3K, Akt and p-Akt. B. p-PI3K/t-PI3K and p-Akt/t-Akt expression in substantia nigra of PD rats. C. Protein bands of IκBα, p-IκBα, ERK and p-ERK. D. p-IκBα/t-IκBα and p-ERK/t-ERK expression in substantia nigra of PD rats. * $P < 0.05$ vs the normal group; # $P < 0.05$ vs the siRNA-NC group. The measurement data were expressed as mean ± standard deviation and ANOVA was used for the comparison among multiple groups. After ANOVA analysis, the LSD-t-test was used for pairwise comparison.

nigra of PD rats. Numerous pathological processes such as inflammation, mitochondrial dysfunction, mitochondrial dysfunction, oxidative stress, and apoptosis together with the genetic factors may result in neuronal degeneration [36]. Many dysfunctional lncRNAs may be implicated in biological pathways associated with PD, including axon guidance pathway via cis- or trans-regulating target genes [37]. Functioning of the PI3K/Akt signaling pathway reveals that neuro-defense is active for the purpose of rendering neuroprotection through preventing apoptosis and neuro-inflammation [36]. A previous study has elucidated the neuroprotective functions of pramipexole-induced hypothermia through regulating the PI3K/Akt signaling pathway [38]. Scholars have proposed that UCA1 is able to affect the cell cycle and promote proliferation of tumor cells via the regulation of the PI3K/Akt signaling pathway [39,40]. Additionally, UCA1 shows an unfavorable prognosis and induces tumorigenesis in cholangiocarcinoma (CCA) via regulating the Akt/GSK-3β signaling pathway [41]. Furthermore, the molecular mechanism of UCA1 indicates that UCA1 alters gastric cancer progression both in vitro and in vivo by regulating the PI3K-Akt-mTOR signaling-related proteins and their downstream mediators [42]. In this present study, we also found out that with the treatment of down-regulation of UCA1, the PD rats displayed improved neurobehavioral change, increased neuroinflammatory response and oxidative stress through inhibiting the activation of the PI3K/Akt signaling pathway. In accordance with the results in our study, a study has revealed that Vitexin inhibited the ratio of Bax/Bcl-2 and caspase-3 activity by enhancing the activation of PI3K and Akt in mitochondrial permeability transition pore (MPTP)-treated PD mice [43]. Another study has demonstrated that UCA1 could suppress CCA cell apoptosis, and knockdown of UCA1 increased apoptosis-associated factors (caspase-3, -9) [41].

In conclusion, this present study helped to define the role of UCA1 in PD tumorigenesis. The study of the molecular mechanism of UCA1 suggested that downregulation of lncRNA UCA1 ameliorates the damage of dopaminergic neurons, reduces oxidative stress and

inflammation in PD through the inactivation of the PI3K/Akt signaling pathway. Based upon our study, we suggest that UCA1 might be a novel target for therapeutic intervention of PD. In future research, we would use transcriptomics to find differentially expressed lncRNA in order to achieve better results. Additionally, we did not conducted relevant experiments on the inhibitors of PI3K/AKT signaling pathway, but we will focus on it in the future work in order to provide a theoretical basis for the treatment of PD.

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Ethical statement

This study was approved by the Medical Ethics Committee of Guizhou Provincial People's Hospital. (No. 2015013) The animal experiment was strictly complied with the principle to minimize the pain and suffering to experimental animals.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Authors' contributions

Guarantor of integrity of the entire study: Lijun Cai, Jinyong Tian
Study design: Li Tu, Tian Li
Literature research: Xiulin Yang, Yipin Ren
Experimental studies: Ran Gu, Qian Zhang
Statistical analysis: Huan Yao, Xiang Qu
Manuscript editing: Qian Wang, Jinyong Tian
Manuscript review: Lijun Cai, Jinyong Tian

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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