European Review for Medical and Pharmacological Sciences 2018; 22: 7908-7915

LncRNA-UCA1 promotes PD development by upregulating SNCA

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Abstract. – OBJECTIVE: The study aims to investigate whether Long non-coding RNA (LncRNA)-UCA1 can regulate the progression of Parkinson's disease (PD) by mediating a-synuclein (SNCA) expression.

MATERIALS AND METHODS: PD mouse model was first constructed by intraperitoneal injection of MPTP. SH-SY5Y cells were treated with MPP+ for inducing in vitro PD model. Expression levels of lncRNA-UCA1 and SNCA in brain tissues extracted from PD mice and MPP+-induced SH-SY5Y cells were detected by quantitative real-time polymerase chain reaction (qRT-PCR). Protein expression of SNCA was accessed by Western blot. After transfection of pcDNA-NC+DMSO, pcDNA-UCA1+DMSO, pcDNA-NC+α-amantin or pcDNA-UCA1+α-amanitin in SH-SY5Y cells, SNCA expression was detected. Cell viability and SNCA expression were determined after UCA1 overexpression or knockdown in SH-SY5Y cells. Neuronal apoptosis in MPP+-induced SH-SY5Y cells was detected by flow cytometry after the UCA1 knockdown.

RESULTS: UCA1 and SNCA were highly expressed in brain tissues extracted from PD mice and MPP+-induced SH-SY5Y cells. UCA1 overexpression remarkably upregulated mRNA and protein expressions of SNCA in SH-SY5Y cells. Higher viability was seen after the UCA1 knockdown in MPP+-induced SH-SY5Y cells. UCA1 knockdown remarkably inhibited caspase-3 activity and decreased MPP+-induced neuronal apoptosis in SH-SY5Y cells.

CONCLUSIONS: LncRNA-UCA1 promotes the occurrence and progression of PD by upregulating SNCA expression.

Key Words:
LncRNA-UCA1, SNCA, SH-SY5Y, PD.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disease, and its incidence is second only to Alzheimer's disease (AD) in neurodegenerative diseases. It is reported that the prevalence of PD in all populations is about 0.3%, which sharply increases to 1% in people over 60 years old[1, 2]. More seriously, the prevalence of PD is as high as 4% in people over 80 years old[3]. It is concluded that the prevalence of PD increases with age. The main pathological lesions of PD are the deletion of dopaminergic neurons in substantia nigra condense and the deposition of Lewy bodies. It is currently believed that the pathogenesis of PD is mainly related to oxidative stress, mitochondrial dysfunction and protein clearance[4-6]. Oxidative stress and mitochondrial dysfunction mainly cause neuronal apoptosis in the substantia nigra of the midbrain[7-9]. In addition, gene mutations are the main cause of familial PD. So far, LRRK2, SNCA, VPS35, EIF4G1, PARK2, PINK1 and DJ-1 have been confirmed as pathogenic factors in PD[10]. Among them, SNCA is the second most common gene leading to PD, which is located on chromosome 4q21-23 with certain neurospecificity. SNCA is abundantly expressed in the hippocampus, amygdala, corpus callosum, and caudate nucleus[11]. SNCA encodes alpha-synuclein, and therefore, SNCA mutation leads to Lewy body deposition[12-14].

Long non-coding RNA (LncRNA) is a kind of non-coding RNA with over 200 nucleotides in length. LncRNA exerts important biological functions by acting as a signaling regulator, protein complex scaffold or gene transcription enhancer[15]. LncRNA is crucial in development and differentiation, showing stronger cell and tissue specificities than the coding RNA[16]. A large number of reports have confirmed that lncRNA is closely related to the occurrence and progression of diseases, such as tumors[17], endocrine and metabolic diseases[18], immune diseases[19], cardiovascular diseases[20], and neurological diseases[21]. Many ln-

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cRNAs are dysregulated in neurodegenerative diseases, which may serve as potential therapeutic targets for neurodegenerative diseases. Liu et al[22] pointed out that lncRNA-HOTAIR induces PD development by regulating LRRK2 expression. Kraus et al[23] found upregulated lncRNA-H19, as well as downregulated lncRNA-p21, Malat1, SNHG1 and TncRNA in PD, and their expression levels are related to PD progression. These lncRNAs may be utilized as potential targets for monitoring and treating PD.

LncRNA UCA1 was found to be abnormally expressed in a variety of tumors. It could promote tumor development and progression, including bladder cancer, breast cancer, colorectal cancer, esophageal squamous cell carcinoma, gastric cancer, hepatocellular carcinoma, osteosarcoma, ovarian cancer and squamous cell carcinoma of tongue[24-30]. However, the biological function of UCA1 in PD remains to be further explored.

Materials and Methods

Construction of PD Mouse Model

Male C57BL/6 with 8 to 10 weeks old were maintained in an environment with 19±2°C under a 12 h/12 h dark/light cycle. Mice were given free access to water and food. After habituation for one week, mice were intraperitoneally injected with 0.5 mL/100 g MPTP for four times with an interval of 2 h. This study was approved by the Animal Ethics Committee of Nanjing Medical University Animal Center.

Cell Culture and Transfection

Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (fetal bovine serum), 100 U/mL penicillin and 100 μg/mL streptomycin (Hyclone, South Logan, UT, USA). 24 hours later, cells were incubated with 500 μM MPP^+. Cell passage was performed every other day. SH-SY5Y cells were transfected with pcDNA-NC+DMSO, pcDNA-UCA1+DMSO, pcDNA-NC+α-amantin or pcDNA-UCA1+α-amanitin, respectively using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Quantitative Real Time-Polymerase Chain Reaction (RT-qPCR)

The TRIzol kit (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA, which was then reversely transcribed into complementary Deoxyribose Nucleic Acid (cDNA). After the cDNA was amplified, qRT-PCR was performed to detect the expressions of related genes. Primer sequences of UCA1 were: forward, 5'-CTCTCCTATCTCCCTTCACTGA-3'; reverse, 5'-CTTTGGGTTGAGGTTCGTGT-3'.

Western Blot

Cells were lysed for protein extraction. The concentration of each protein sample was determined by a BCA (bicinchoninic acid) kit (Abcam, Cambridge, MA, USA). The protein sample was separated by gel electrophoresis and transferred to PVDF (polyvinylidene difluoride) membranes (Millipore, Billerica, MA, USA). After incubation with primary and secondary antibody, immunoreactive bands were exposed by enhanced chemiluminescence method.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide) Assay

Culture medium was replaced with 20 μL of MTT solution (5 g/L) (Sigma-Aldrich, St. Louis, MO, USA) and incubated at 37°C for 4 h. The supernatant was discarded and 160 μL of DMSO (dimethyl sulfoxide; Sigma-Aldrich, St. Louis, MO, USA) was added into each well. The absorbance value was recorded at the wavelength of 490 nm with a microplate reader (Bio-Rad, Hercules, CA, USA).

Flow Cytometry

Cell density was first adjusted to 5×10^5/mL, followed by centrifugation at 4°C, 1000 $r\cdot min^{-1}$ for 15 min. Cells were resuspended in 200 μL of binding buffer, incubated with 10 μL of Annexin V-FITC in the dark for 15 min. Finally, 300 μL of binding buffer and 5 μL of Propidium Iodide (PI) were added, followed by apoptosis detection using flow cytometry within 1 h.

Determination of Caspase-3 Activity

Transfected cells were lysed in an ice bath for 15 min. 10 μL of Ac-DEVD-pNA was added in cells and incubated at 37°C for 60-120 min. The absorbance value was recorded at the wavelength of 405 nm with a microplate reader. Protein expression of each sample was quantified using the Bradford method.

Statistical Analysis

We used Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) for statistical analysis. The quantitative data were represented as mean ± standard

deviation ($\bar{x}\pm s$). Differences among groups were analyzed by one-way ANOVA, followed by the post-hoc test. $p<0.05$ was considered statistically significant.

Results

Expressions of UCA1 and SNCA in PD Mouse

PD mouse model was established by intraperitoneal injection of MPTP. We found that the number of ipsilateral TH^+ cells in PD mice was significantly higher than that of the controls, suggesting the successful establishment of a PD mouse model ($p<0.001$, Figure 1A). The qRT-PCR assay showed that the mRNA levels of UCA1 and SNCA are significantly higher in PD mice than that of the controls (Figure 1B and 1C). Moreover, the PD mice presented higher protein expression of SNCA (Figure 1D). The above results indicated that UCA1 and SNCA are highly expressed in the PD mouse model.

Expressions of UCA1 and SNCA in MPP^+-Induced SH-SY5Y Cells

SH-SY5Y cells were treated with MPP^+ for inducing *in vitro* PD model. Expression levels of UCA1 and SNCA in MPP^+-induced SH-SY5Y cells were detected by qRT-PCR. The data showed that UCA1 and SNCA are highly expressed in MPP^+-induced SH-SY5Y cells (Figure 2A and 2B). The protein expression of SNCA increased in SH-SY5Y cells treated with MPP^+ (Figure 2C). It is suggested that MPP^+ induction could upregulate expressions of UCA1 and SNCA, indicating their potential roles in PD development.

UCA1 Overexpression in SH-SY5Y Cells Regulated SNCA Expression

SH-SY5Y cells were transfected with pcDNA-UCA1, followed by the determination of its transfection efficacy. The qRT-PCR results showed that the transfection of pcDNA-UCA1 remarkably increases the mRNA level of UCA1 (Figure 3A). Both mRNA and protein levels of SNCA were upregulated by pcDNA-UCA1 transfection (Figure 3B and 3C). To examine whether UCA1

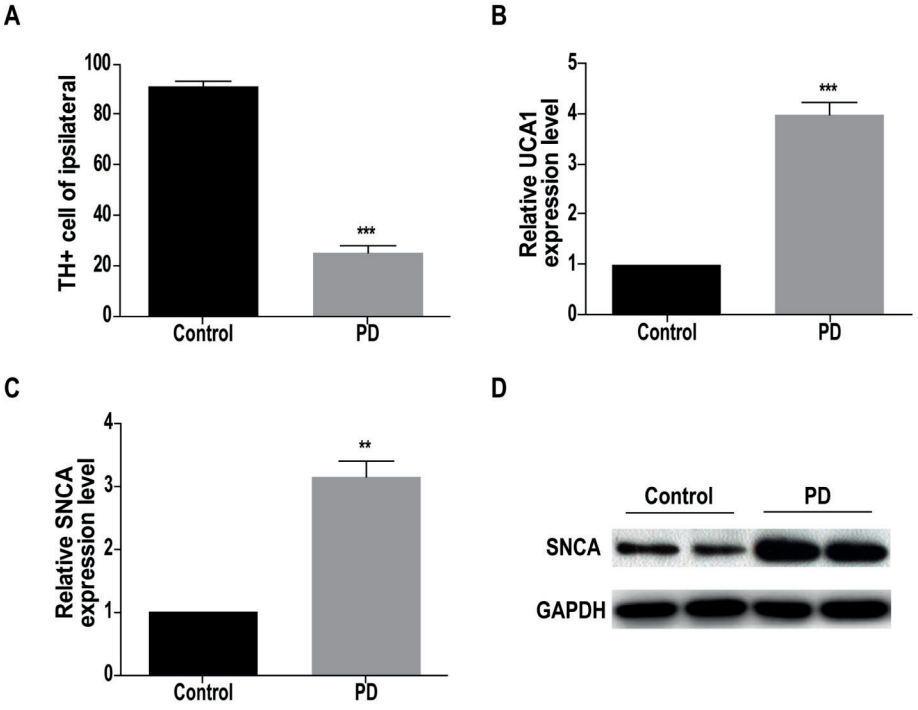

Figure 1. Expressions of UCA1 and SNCA in PD mouse. ***A,*** The number of ipsilateral TH^+ cells in PD mice was significantly higher than that of the controls. ***B, C,*** QRT-PCR assay showed that the mRNA levels of UCA1 and SNCA were significantly higher in PD mice than that of the controls. ***D,*** PD mice presented higher protein expression of SNCA.

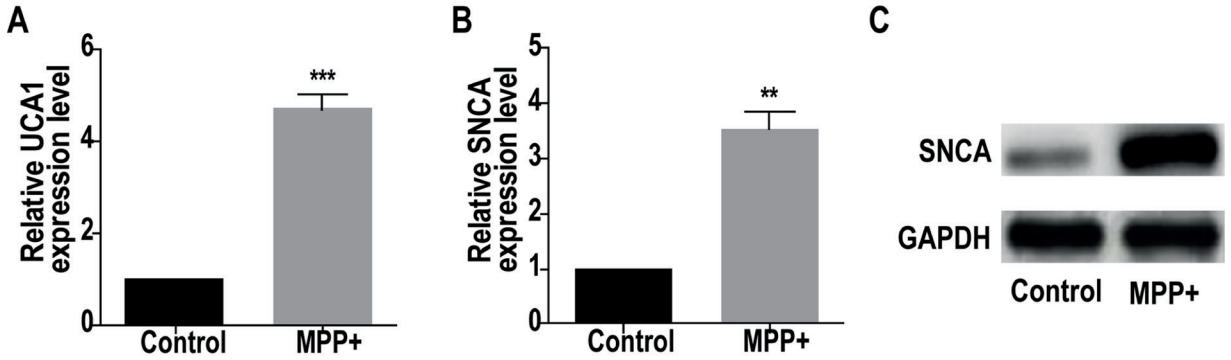

Figure 2. Expressions of UCA1 and SNCA in MPP^+-induced SH-SY5Y cells. ***A, B,*** UCA1 and SNCA were highly expressed in MPP^+-induced SH-SY5Y cells. ***C,*** Protein expression of SNCA increased in SH-SY5Y cells treated with MPP^+

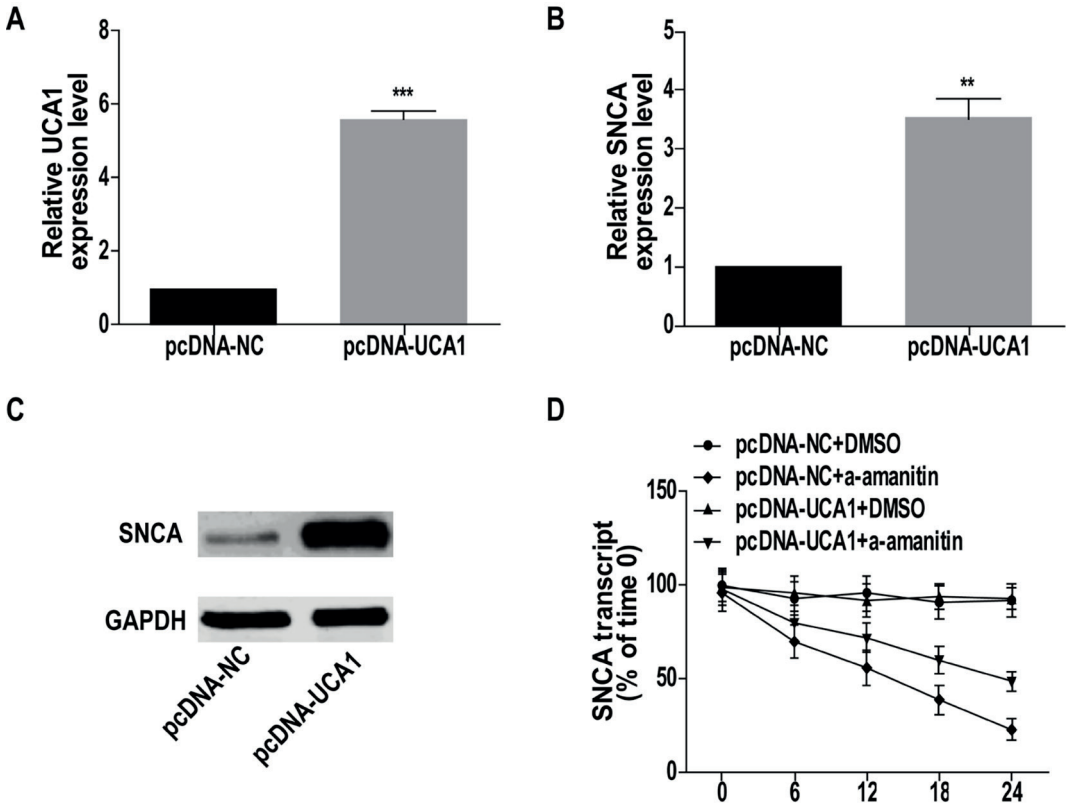

Figure 3. UCA1 overexpression in SH-SY5Y cells regulated SNCA expression. ***A,*** QRT-PCR results showed that transfection of pcDNA-UCA1 remarkably increases mRNA level of UCA1. ***B, C,*** Both mRNA and protein levels of SNCA were upregulated by pcDNA-UCA1 transfection. ***D,*** UCA1 overexpression markedly inhibited mRNA degradation of SNCA induced by α-amanitin.

could regulate SNCA expression, SH-SY5Y cells were transfected with pcDNA-NC+DMSO, pcDNA-UCA1+DMSO, pcDNA-NC+α-amantin or pcDNA-UCA1+α-amanitin, respectively. The UCA1 overexpression markedly inhibited mRNA degradation of SNCA induced by α-amanitin (Figure 3D). We concluded that UCA1 could enhance the stability of SNCA mRNA and upregulate its expression level.

UCA1 Inhibited SNCA Expression and Viability of SH-SY5Y Cells

To further explore the regulatory role of UCA1 in SNCA expression and cell viability, SH-SY5Y cells

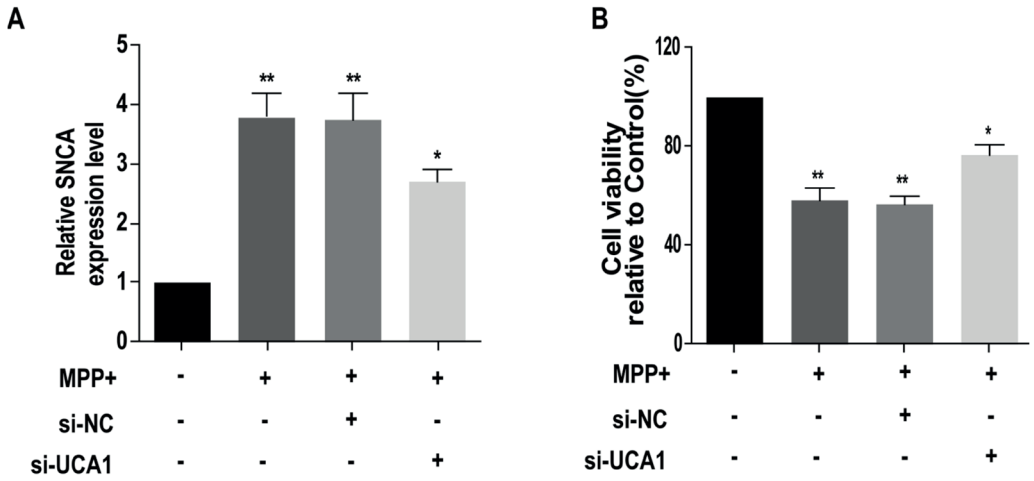

Figure 4. UCA1 inhibited SNCA expression and viability of SH-SY5Y cells. ***A,*** UCA1 knockdown in MPP^+-induced SH-SY5Y cells partially reversed the increased SNCA expression. ***B,*** UCA1 knockdown in MPP^+-induced SH-SY5Y cells partially reversed the decreased cell viability.

were induced with MPP^+, followed by transfection of si-NC or si-UCA1. SNCA expression in SH-SY5Y cells was remarkably upregulated by MPP^+ induction, whereas cell viability greatly decreased. UCA1 knockdown in MPP^+-induced SH-SY5Y cells partially reversed the increased SNCA expression and decreased cell viability (Figure 4A and 4B).

UCA1 Knockdown Decreased MPP^+-Induced Neuronal Apoptosis

We further investigated whether UCA1 can regulate neuronal survival by inducing cell apoptosis. The flow cytometry results showed that the knockdown of UCA1 reduces apoptosis of MPP^+-induced SH-SY5Y cells (Figure 5A). Also, the knockdown of UCA1 reduced the increase in caspase-3 activity caused by MPP^+ treatment (Figure 5B). The above results indicated that interference with UCA1 expression can reduce neuronal apoptosis induced by MPP^+.

Discussion

In recent years, there is increasing evidence suggested that lncRNA exerts an important role in the development of PD. LncRNA shows advantages of easy synthesis, high bioavailability and long half-life, which is expected to be potential target for treating neurodegenerative diseases. However, in-depth researches on the underlying mechanism of lncRNAs in neurodegenerative diseases are still lacked.

UCA1 was first reported by Wang et al[31,32]. It is located on human chromosome 19p13.12, with three exons and two introns. In recent years, there have been many reports on the specific role of UCA1 in tumors. Wu et al[33] elucidated that UCA1 can regulate the proliferation of bladder cancer cells through PI3K-AKT-mTOR signaling pathway. Fang et al[34] reported that UCA1 is highly expressed in tongue cancer and may be associated with metastasis of tongue cancer cells. Han et al[35] demonstrated that UCA1 is highly expressed in colon cancer, affecting the proliferation, apoptosis, and cell cycle of colon cancer cells. The role of UCA1 in the occurrence and development of PD has not been reported yet.

MPTP itself is not toxic, but it can pass through the blood-brain barrier and metabolize to 1-methylpyridine-l-ium iodide (MPP^+) by monoamine oxidase B, which is toxic. MPP^+ is highly specific for dopaminergic neuron damage. It transits to the nerve endings by competing with dopamine transporters, resulting in large production of oxygen free radicals through directly inhibiting mitochondrial complex I function. Subsequently, changes in the expressions of Bcl family proteins and release of cytochrome C eventually initiate apoptotic signaling pathways[36].

This study first constructed PD mouse model by MPTP injection. The number of TH^+ cells in brain tissues was remarkably lower than those of controls. UCA1 was highly expressed in brain tissues extracted from PD mice and MPP^+-induced SH-SY5Y cells, suggesting the significant role of

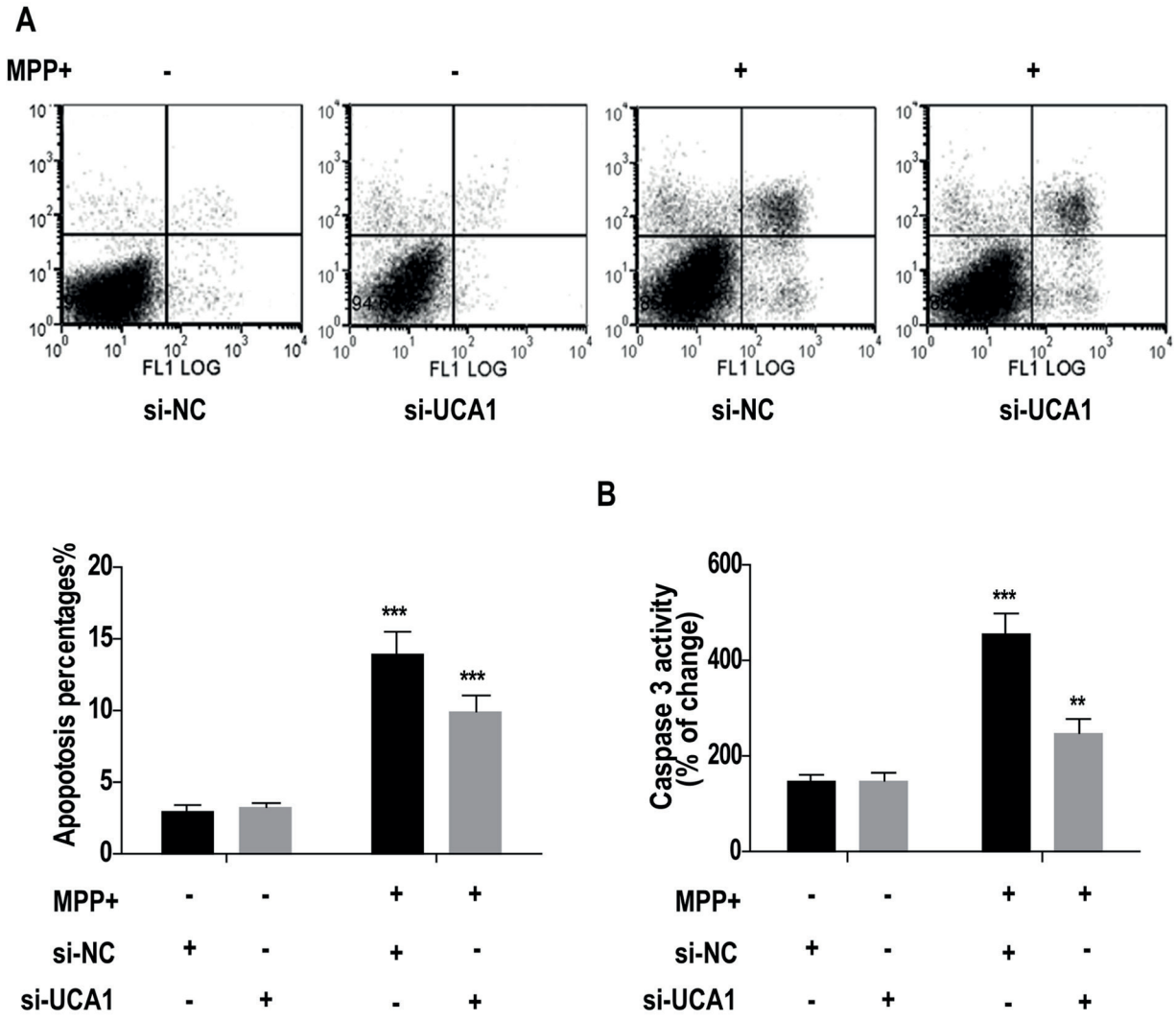

Figure 5. UCA1 knockdown decreased MPP$^+$-induced neuronal apoptosis. ***A,*** Flow cytometry results showed that knockdown of UCA1 reduces apoptosis of SH-SY5Y cells treated with MPP$^+$. ***B,*** Knockdown of UCA1 reduced the increase in caspase-3 activity caused by MPP$^+$ treatment.

UCA1 in PD. Furthermore, the UCA1 overexpression remarkably upregulated SNCA expression. On the contrary, the UCA1 knockdown decreased SNCA expression and viability of SH-SY5Y cells. Our results also showed that UCA1 knockdown could decrease caspase-3 activity. These results indicated that UCA1 promotes the PD development by upregulating the SNCA expression.

Conclusions

Our study found that UCA1 and SNCA are highly expressed in brain tissue of PD mouse. UCA1 promotes the occurrence and progression of PD by upregulating SNCA expression. Inhibition of UCA1 may be a potential strategy for treating PD.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Nussbaum RL, Ellis CE. Alzheimer's disease and Parkinson's disease. N Engl J Med 2003; 348: 1356-1364.
2) de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol 2006; 5: 525-535.

3) de Rijk MC, Breteler MM, Graveland GA, Ott A, Grobbee DE, van der Meche FG, Hofman A. Prevalence of Parkinson's disease in the elderly: the Rotterdam Study. Neurology 1995; 45: 2143-2146.

4) Jangamreddy JR, Los MJ. Mitoptosis, a novel mitochondrial death mechanism leading predominantly to activation of autophagy. Hepat Mon 2012; 12: e6159.

5) Jenner P. Oxidative stress in Parkinson's disease. Ann Neurol 2003; 53 Suppl 3: S26-S36, S36-S38.

6) McNaught KS, Olanow CW. Protein aggregation in the pathogenesis of familial and sporadic Parkinson's disease. Neurobiol Aging 2006; 27: 530-545.

7) Keeney PM, Xie J, Capaldi RA, Bennett JJ. Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. J Neurosci 2006; 26: 5256-5264.

8) Gatt AP, Duncan OF, Attems J, Francis PT, Ballard CG, Bateman JM. Dementia in Parkinson's disease is associated with enhanced mitochondrial complex I deficiency. Mov Disord 2016; 31: 352-359.

9) Schapira AH. Mitochondrial complex I deficiency in Parkinson's disease. Adv Neurol 1993; 60: 288-291.

10) Spatola M, Wider C. Genetics of Parkinson's disease: the yield. Parkinsonism Relat Disord 2014; 20 Suppl 1: S35-S38.

11) Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A, Mucke L. Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. Science 2000; 287: 1265-1269.

12) Berg D, Niwar M, Maass S, Zimprich A, Moller JC, Wuellner U, Schmitz-Hubsch T, Klein C, Tan EK, Schols L, Marsh L, Dawson TM, Janetzky B, Muller T, Woitalla D, Kostic V, Pramstaller PP, Oertel WH, Bauer P, Krueger R, Gasser T, Riess O. Alpha-synuclein and Parkinson's disease: implications from the screening of more than 1,900 patients. Mov Disord 2005; 20: 1191-1194.

13) Hattori N, Machida Y, Noda K. [Pathogenesis of Parkinson's disease: a common pathway between alpha-synuclein and parkin and the mechanism of Lewy bodies formation]. Rinsho Shinkeigaku 2005; 45: 905-907.

14) Venda LL, Cragg SJ, Buchman VL, Wade-Martins R. alpha-Synuclein and dopamine at the crossroads of Parkinson's disease. Trends Neurosci 2010; 33: 559-568.

15) Yang L, Froberg JE, Lee JT. Long noncoding RNAs: fresh perspectives into the RNA world. Trends Biochem Sci 2014; 39: 35-43.

16) Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem 2012; 81: 145-166.

17) Tang JY, Lee JC, Chang YT, Hou MF, Huang HW, Liaw CC, Chang HW. Long noncoding RNAs-related diseases, cancers, and drugs. ScientificWorldJournal 2013; 2013: 943539.

18) Pullen TJ, Rutter GA. Could lncRNAs contribute to beta-cell identity and its loss in Type 2 diabetes? Biochem Soc Trans 2013; 41: 797-801.

19) Wu GC, Pan HF, Leng RX, Wang DG, Li XP, Li XM, Ye DQ. Emerging role of long noncoding RNAs in autoimmune diseases. Autoimmun Rev 2015; 14: 798-805.

20) Uchida S, Dimmeler S. Long noncoding RNAs in cardiovascular diseases. Circ Res 2015; 116: 737-750.

21) Ng SY, Lin L, Soh BS, Stanton LW. Long noncoding RNAs in development and disease of the central nervous system. Trends Genet 2013; 29: 461-468.

22) Wang S, Zhang X, Guo Y, Rong H, Liu T. The long noncoding RNA HOTAIR promotes Parkinson's disease by upregulating LRRK2 expression. Oncotarget 2017; 8: 24449-24456.

23) Kraus T, Haider M, Spanner J, Steinmaurer M, Dietinger V, Kretzschmar HA. Altered long noncoding RNA expression precedes the course of Parkinson's disease--a preliminary report. Mol Neurobiol 2017; 54: 2869-2877.

24) Wang F, Ying HQ, He BS, Pan YQ, Deng QW, Sun HL, Chen J, Liu X, Wang SK. Upregulated lncRNA-UCA1 contributes to progression of hepatocellular carcinoma through inhibition of miR-216b and activation of FGFR1/ERK signaling pathway. Oncotarget 2015; 6: 7899-7917.

25) Wang X, Gong Y, Jin B, Wu C, Yang J, Wang L, Zhang Z, Mao Z. Long non-coding RNA urothelial carcinoma associated 1 induces cell replication by inhibiting BRG1 in 5637 cells. Oncol Rep 2014; 32: 1281-1290.

26) Kumar PP, Emechebe U, Smith R, Franklin S, Moore B, Yandell M, Lessnick SL, Moon AM. Coordinated control of senescence by lncRNA and a novel T-box3 co-repressor complex. eLife 2014; 3:

27) Wang Y, Chen W, Yang C, Wu W, Wu S, Qin X, Li X. Long non-coding RNA UCA1a(CUDR) promotes proliferation and tumorigenesis of bladder cancer. Int J Oncol 2012; 41: 276-284.

28) Wen JJ, Ma YD, Yang GS, Wang GM. Analysis of circulating long non-coding RNA UCA1 as potential biomarkers for diagnosis and prognosis of osteosarcoma. Eur Rev Med Pharmacol Sci 2017; 21: 498-503.

29) Maddika S, Ande SR, Panigrahi S, Paranjothy T, Weglarczyk K, Zuse A, Eshraghi M, Manda KD, Wiechec E, Los M. Cell survival, cell death and cell cycle pathways are interconnected: implications for cancer therapy. Drug Resist Updat 2007; 10: 13-29.

30) Xue M, Li X, Li Z, Chen W. Urothelial carcinoma associated 1 is a hypoxia-inducible factor-1alpha-targeted long noncoding RNA that enhances hypoxic bladder cancer cell proliferation, migration, and invasion. Tumour Biol 2014; 35: 6901-6912.

31) Wang XS, Zhang Z, Wang HC, Cai JL, Xu QW, Li MQ, Chen YC, Qian XP, Lu TJ, Yu LZ, Zhang Y, Xin DQ, Na YQ, Chen WF. Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. Clin Cancer Res 2006; 12: 4851-4858.

32) Wang F, Li X, Xie X, Zhao L, Chen W. UCA1, a non-protein-coding RNA up-regulated in bladder carcinoma and embryo, influencing cell growth and promoting invasion. FEBS Lett 2008; 582: 1919-1927.

33) Wu W, Zhang S, Li X, Xue M, Cao S, Chen W. Ets-2 regulates cell apoptosis via the Akt pathway, through the regulation of urothelial cancer associated 1, a long non-coding RNA, in bladder cancer cells. PLoS One 2013; 8: e73920.

34) Fang Z, Wu L, Wang L, Yang Y, Meng Y, Yang H. Increased expression of the long non-coding RNA UCA1 in tongue squamous cell carcinomas: a possible correlation with cancer metastasis. Oral Surg Oral Med Oral Pathol Oral Radiol 2014; 117: 89-95.

35) Han Y, Yang YN, Yuan HH, Zhang TT, Sui H, Wei XL, Liu L, Huang P, Zhang WJ, Bai YX. UCA1, a long non-coding RNA up-regulated in colorectal cancer influences cell proliferation, apoptosis and cell cycle distribution. Pathology 2014; 46: 396-401.

36) Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003; 39: 889-909.