

miRNA-384-5p regulates the progression of Parkinson's disease by targeting SIRT1 in mice and SH-SY5Y cells

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Abstract. Parkinson's disease (PD) is the second most common neurodegenerative disorder. miR-384-5p expression has been shown to be increased in an *in vitro* model of PD; however, it remains unknown whether there are other molecules that can be regulated by miR-384-5p in *in vivo* and *in vitro* models of PD; thus, the present study aimed to elucidate this matter. Rotenone was applied for the establishment of *in vitro* and *in vivo* models of PD in the present study. Motor disability and equilibrium were determined by a swimming test and traction test, respectively. mRNA and protein levels were detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analysis, respectively. The association between miR-384-5p and Sirtuin 1 (SIRT1) expression was verified by dual luciferase reporter assay. The α -synuclein aggregation was evaluated by immunofluorescence. The results from the *in vitro* model of PD demonstrated that, the mice in the PD group exhibited decreased scores in the swimming test and traction test, which were accompanied by increased α -synuclein aggregation. In addition, the expression of miR-384-5p, which targeted the 3'untranslated region (3'UTR) of SIRT1, was verified to be increased in mice and SH-SY5Y cells in the PD group, whereas SIRT1 exhibited the opposite changes. Moreover, increased mRNA and protein levels of p53 and FOXO1 were observed in mice and SH-SY5Y cells in the PD group. In addition, the SH-SY5Y cells in the PD group exhibited a higher cell apoptotic rate. On the whole, the findings of this study demonstrate that miRNA-384-5p promotes the progression of PD by targeting SIRT1.

Introduction

Parkinson's disease (PD), which was first documented in 1817 by Dr James Parkinson, is the second most common neurodegenerative disorder with a prevalence of approximately 1-2% among individuals >65 years of age (1) and approximately 4-5% among individuals >85 years of age (2). The neuropathological hallmarks of PD are the loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and the accumulation of Lewy bodies (LBs), which mainly consist of α -synuclein (3), thus generating symptoms, including bradykinesia, resting tremor, muscle rigidity and postural instability (4). Previous studies have demonstrated that various environmental factors, such as nutrition, and exposure to metals and pesticides can affect the progression of PD (5-7). Currently, however, the identification of an efficient method with which to attenuate the development of PD is warranted.

MicroRNAs (miRNAs or miRs) are a group of small and non-coding RNAs comprising of 20-22 nucleotides in length, which regulate genes associated with signaling pathways through either mRNA cleavage or post-transcriptional silencing by targeting the 3'untranslated region (3'UTR) of their targets (8,9). miRNAs are expressed throughout the developmental stages spatially and temporally (10). miRNAs are dysregulated in numerous diseases, including PD (11). In addition, numerous miRNAs have been discovered to be associated with PD. For instance, miRNA-155-5p expression has been shown to be increased, whereas the expression of miRNA-146a-5p has been shown to be decreased in patients with PD compared with healthy individuals (12). Furthermore, the decreased miR-433 and miR-133b expression levels are potential biomarkers for PD (13). A previous study also demonstrated that miR-410 exerts neuroprotective effects in an *in vitro* model of 6-hydroxydopamine-induced PD (14). Recently, in another previous study, in an *in vitro* model of rotenone-induced PD, an increased miR-384-5p level was observed, and the downregulation of this miRNA exerted a neuroprotective effect against rotenone by targeting GRP78 (15). However, the role of miR-384-5p in an *in vivo* model of PD remains unknown. In addition, it remains unknown as to whether there are other molecules that may be regulated by miR-384-5p. Therefore, the present study aimed to investigate the role of miR-384-5p in an

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in vitro and *in vivo* model of PD in order to elucidate this matter. The findings of this study may prove to be of paramount importance, as numerous miRNAs are being applied in various clinical trial phases for the purpose of being used as therapeutic drugs.

Materials and methods

Animals. Experiments were performed in 30 adult male C57B/6J mice (weighing 20-30 g, 8 weeks old), which were obtained from the Model Animal Research Center of Nanjing University. The mice were kept in a controlled environment with a temperature of 22-25°C, a 12:12 h light/dark cycle, with free access to water and food. Manipulations were conducted during the light phase of the day. Efforts were made to minimize animal suffering and to reduce the number of animals used. The present study was performed in accordance with the guidelines of the Committee on Care and Use of Experimental Animals Resources and with the approval of Ethical Committee for The First Hospital of Yulin.

Experimental groups. The mice were randomly divided into 3 different groups with 10 mice in each group, including the control group (n=10), the dimethyl sulfoxide (DMSO) control group (n=10) and the rotenone-induced PD group (n=10). The duration of the experiment was 1 month.

Rotenone (Sigma Chemical Co.) was first dissolved in DMSO, which was completed with sunflower oil. Rotenone was administered orally once daily (0.1 ml/10 g, 30 mg/kg) for 30 days to establish an *in vivo* model of PD, as previously described (16). The mice in the DMSO control group were orally administered once daily with DMSO for 30 days. The mice in the control group received no treatments. Afterwards, the mice in each group were submitted to a swimming test and traction test.

Swimming test. The swimming test was conducted to determine the motor disability of the mice in each group using a round glass swimming tank (length, 40 cm; width, 25 cm; height, 16 cm), which was filled with water (at a temperature of 22-25°C) to a depth of 12 cm. The mice were scored using the following scale: 0, No swimming with the head above the water; 1, occasional swim with mice floating using the hind paws; 2, alternations between swim-floating and passively floating; and 3, continuous swimming, as previously described (17).

Traction test. The traction test was performed to determine the muscle strength and equilibrium of the mice. The forepaws of the mice were placed on a rope (diameter, 5 mm) which was approximately horizontally 70 cm at a distance from the ground. The hind limb placements of the mice were scored on a scale of 1 to 3, with the lowest score indicating the most severe deficits. The score was determined using the following criteria: 1 or 2, No or one hind limb seizing the rope; and 3, both hind limbs seizing the rope. Mice were allowed to hang upside down and stay on the rope for 30 sec, as previously described (18).

Tissue preparation. Animal health and behavior was monitored once every 2 days with the following principles: Soft

and smooth hair, even breath and no scabs, etc. During the process, no mouse had died. After the swimming test and traction test, the animals were euthanized by barbiturate overdose (pentobarbital sodium, 150 mg/kg; i.p.) and were immediately decapitated. Death was verified by the absence of breathing, heartbeat or neurofeedback, etc. SN tissues were then extracted from the mice and fresh-frozen at -80°C which were then subjected to the following experiments.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the SH-SY5Y cells (please see below) and SN tissues using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Reverse transcription and TaqMan microRNA assay were carried out using the Hairpin-it™ miRNA qPCR Quantitation kit (GenePharma Co.). Thereafter, qPCR was performed using the SYBR-Green kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) on the ABI 7500 Real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions were as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 15 sec, 58°C for 30 sec and 72°C for 30 sec. The primer sequences were as follows: Sirtuin 1 (SIRT1) forward, 5'-CTG GGGTGTCTGTTTCATGTGG-3' and reverse, 5'-GCTTGA GGATCTGGAAGATCTGG-3'; GAPDH forward, 5'-CCA CTCCTCCACCTTTGACG and reverse, 5'-CCACCACCC TGTTCGTGTAG-3'; forkhead box protein O1 (FOXO1) forward, 5'-TCCCAGTGAGCAGTAAATC-3' and reverse, 5'-CCAGCAGTTGAACAAGTC-3'; p53 forward, 5'-GCT GGTAGGTAGAGGGAGTTG-3' and reverse, 5'-GTGTGG GATGGGGTGAGATTTC-3'; miR-384-5p, 5'-CGCGTATGA ACAATTTCTAGGAAT-3'; and U6, 5'-TTGGTGAAGCGT TCCATATTTT-3'. The relative expression levels of miRNA and mRNAs were determined using the $2^{-\Delta\Delta C_q}$ method (19). The expression of miR-384-5p was normalized to U6, while the expression of SIRT1, p53 and FOXO1 was normalized to GAPDH.

Prediction of targeting mRNAs for miR-384-5p. The binding sites between miR-384-5p and SIRT1 were analyzed by TargetScan release 7.1 (http://www.targetscan.org/vert_71/).

Western blot analysis. Proteins were isolated from the SH-SY5Y cells (please see below) or SN tissues by radio-immunoprecipitation assay (RIPA) (Beyotime Institute of Biotechnology). The protein concentration in each sample was detected by BCA assay (Beyotime Institute of Biotechnology). The same amount of proteins from each sample (15 µg) were separated by 8-10% SDS-PAGE and transferred onto PVDF membranes (Millipore). After blocking with 5% non-fat milk at room temperature for 1 h, the PVDF membranes were incubated with respective primary antibodies for α -synuclein rabbit mAb (dilution, 1:1,000, cat. no. 4179), SIRT1 rabbit mAb (dilution, 1:1,000, cat. no. 9475), p53 rabbit mAb (dilution, 1:1,000, cat. no. 2527), FOXO1 rabbit mAb (dilution, 1:1,000, cat. no. 2880), and GAPDH rabbit mAb (dilution, 1:1,000, cat. no. 5174) overnight at 4°C and anti-rabbit IgG, HRP-linked secondary antibodies (dilution, 1:2,000, cat. no. 7074) at room temperature for 2 h, successively. The above-mentioned antibodies were obtained from Cell Signaling Technology. ECL system (Bio-Rad Laboratories Inc.) was used to detect antibody

bound proteins. Densitometric analysis was performed using Image J 1.8.0 software (National Institutes of Health).

Cells and cell culture. SH-SY5Y cells human dopaminergic neuroblastoma were obtained from the American Type Culture Collection (cat. no. ATCC® CRL-2266™; ATCC). STR profiling was used for the authentication of the SH-SY5Y cells. The SH-SY5Y cells were cultured in Dulbecco's modified Eagle's medium-Ham's Nutrient Mixture F-12 (DMEM/F12; Invitrogen; Thermo Fisher Scientific) which was supplemented with 10% fetal calf serum (FCS), and 1% penicillin/streptomycin in a humidified atmosphere containing 5% CO₂ at 37°C. The cells were randomly divided into 3 groups, including the control group, DMSO group and rotenone-induced PD group. The SH-SY5Y cells were exposed to rotenone (20 μM) for 24 h, as previously described (20) before being harvested for use in subsequent experiments.

Cell transfection. The SH-SY5Y cells (2×10⁵ cells/well) were seeded into 6-well plates and cultured till 90% confluence prior to cell transfection. miR-NC inhibitor and miR-384-5p inhibitor were obtained from GenePharma. Briefly, miR-384-5p inhibitor (2.5 μl) or miR-NC inhibitor (2.5 μl) and Lipofectamine 2000 (5 μl, Invitrogen; Thermo Fisher Scientific, Inc.) were added into 250 μl DMEM. Subsequently, the mixture was mixed thoroughly and added into the 6-well plates to a final work concentration of 20 nmol/l. The above mixture was incubated for 48 h prior to use in the subsequent experiments.

Dual luciferase reporter assay. The 293 cells (ATCC) and SH-SY5Y cells transfected with miR-384-5p inhibitor or miR-NC inhibitor were seeded into 96-well plates (1×10³ cells/well) and cultured for 24 h. The SIRT1 3'UTR was amplified from the cDNA of 293 cells and inserted into pLight Switch Prom (Switchgear Genomics). Subsequently, pLightSIRT1-WT-3'UTR reporter construct or pLight-SIRT1-MUT-3'UTR was transfected into the 293 cells and SH-SY5Y cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). At 24 h after cell transfection, Firefly luciferase activity was determined by Light Switch Luciferase assay (Switchgear Genomics) through normalization to *Renilla* luciferase activity.

Cell apoptosis assay. The Annexin V-FITC/PI Staining Apoptosis Detection kit was used for the detection of cell apoptosis. SH-SY5Y cells were collected and washed with PBS. Thereafter, the SH-SY5Y cells (3×10⁵) were resuspended and incubated with Annexin V-FITC (5 μl) and PI (10 μl) at 37°C for 15 min. The cell apoptotic rate was analyzed using a flow cytometer (BD Biosciences).

Evaluation of α-synuclein aggregation. Following incubation at 37°C for 48 h, the SH-SY5Y cells were fixed with 4% formaldehyde for 15 min at 4°C, followed by permeabilization with 0.2% Triton X-100 for 10 min at room temperature. The primary antibody for α-synuclein rabbit mAb (dilution, 1:200, cat. no. 4179) was incubated at 4°C overnight. Anti-rabbit IgG (Alexa Fluor 488 conjugate, cat. no. 4412; dilution, 1:500) acted as a secondary antibody to detect fluorescence and

was incubated at room temperature for 2 h. Subsequently, the cells were incubated with DAPI (cat. no. 4083; 0.5 μg/ml) for 10 min at room temperature for cell nuclei staining. The above-mentioned antibodies were obtained from Cell Signaling Technology. Images were captured under a confocal microscope (TCS SP 5 II, Leica). Fluorescence intensity was analyzed using MicroBrightField Stereo-Investigator (MBF Bioscience).

Statistical analysis. GraphPad Prism 7 software (GraphPad Software, Inc.) was applied for the analysis of the data. The results are expressed as the means ± SEM. Comparisons between 2 groups were analyzed using a Student's t-test, while comparisons among 3 groups were analyzed by one-way analysis of variance followed by Newman-Keuls post-hoc analysis. The correlation between miR-384-5p and SIRT1 was analyzed by Pearson's correlation analysis. A value of P<0.05 was considered to indicate a statistically significant difference.

Results

Increased α-synuclein and decreased swimming/traction scores in mice with rotenone-induced PD. In the *in vivo* model of PD in this study, the severity of parkinsonian signs was first evaluated through swimming scores and traction scores at day 1 following rotenone induction, as displayed in Fig. 1A and B. The results demonstrated that there were no significant differences in swimming scores between the control group and DMSO group, whereas in the group of rotenone-exposed PD mice, the swimming scores became significantly lower than those of the DMSO group (Fig. 1A).

Furthermore, the data presented in Fig. 1B demonstrated that there were no significant differences in traction scores between the control group and DMSO group, whereas the induction of PD with rotenone led to a significant decrease in the score of the traction test below the DMSO values.

In addition, the protein expression levels of α-synuclein were examined in each group. As shown in Fig. 1C and D, no significant were observed between the control group and DMSO group; however, in the group of rotenone-exposed PD mice, the protein expression levels of α-synuclein became significantly higher than those of the DMSO group. Taken together, these aforementioned results verify that the constructed PD model was successful.

Increased miR-384-5p expression in mice with rotenone-induced PD and targeting of SIRT1 by miR-384-5p in 293 cells. Subsequently, the differences in the expression levels of miR-384-5p in the mice among the 3 groups were examined by RT-qPCR. The results revealed that compared with the control group, there were no obvious differences in miR-384-5p levels in the DMSO group; however, in the group of mice with rotenone-induced PD, the expression levels of miR-384-5p became significantly higher than those of the mice in the DMSO group (Fig. 2A).

The following experiment was performed in an aim to predict the potential targets for miR-384-5p. As exhibited in Fig. 2B, using the online software TargetScan, it was discovered that the 3'UTR of SIRT1 had the complementary site for

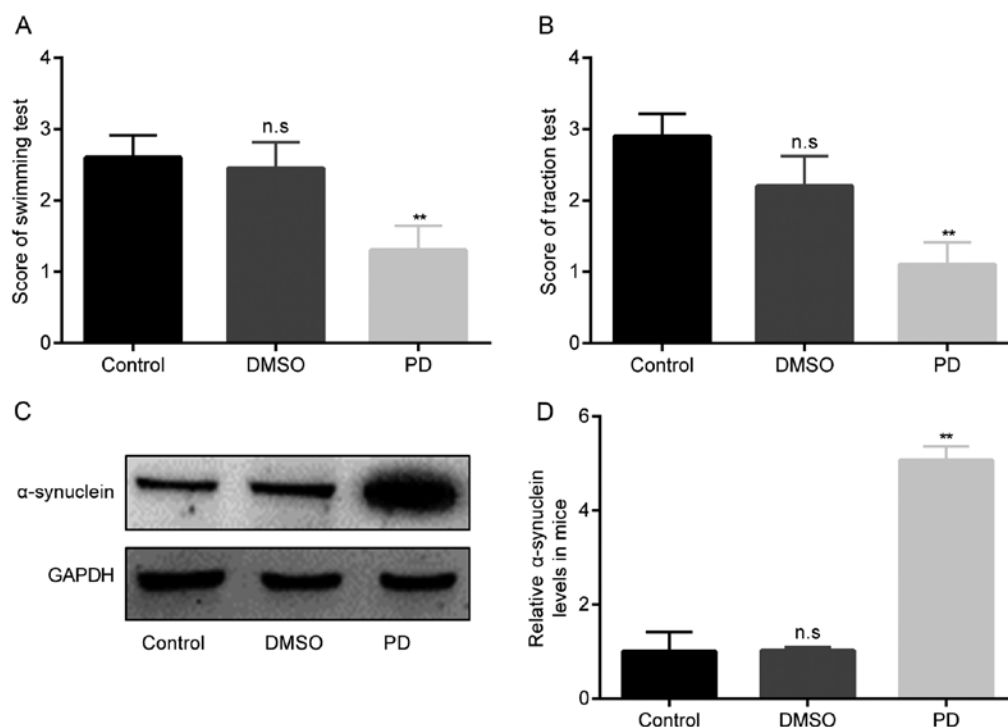


Figure 1. PD increases α -synuclein levels and decreases swimming/traction scores. (A and B) No significant differences were found in swimming scores or traction scores between the control group and DMSO group; however, these were lower in the PD group than in the DMSO group. (C and D) In addition, no significant differences were found in the α -synuclein levels between the control group and DMSO group; the levels were significantly higher in the PD group than the DMSO group. PD, Parkinson's disease; n.s, not significant. ** $P < 0.01$ compared with the controls.

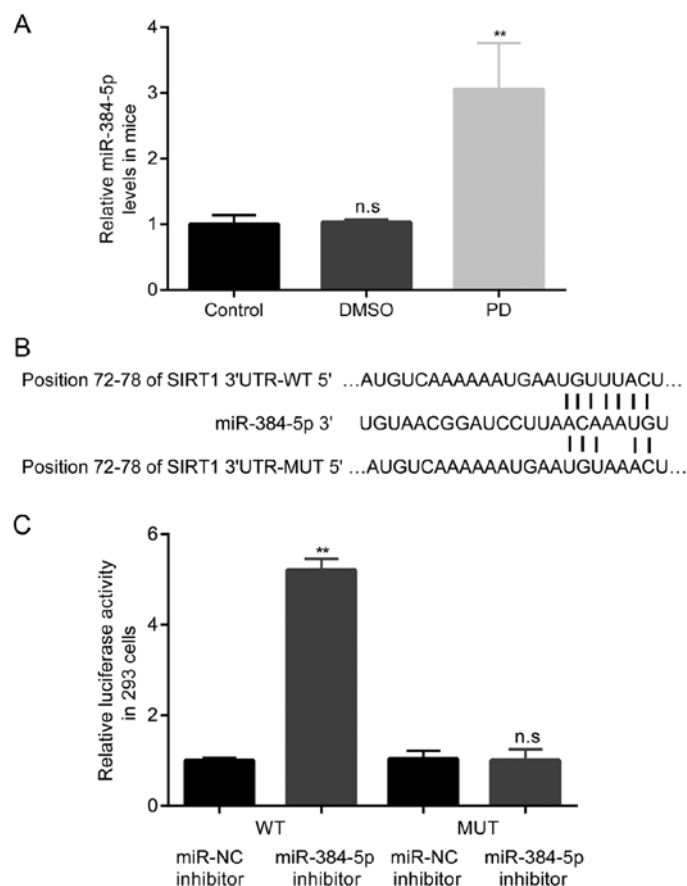


Figure 2. PD increases miR-384-5p expression and miR-384-5p target SIRT1 in 293 cells. (A) No obvious differences were found in the miR-384-5p levels between the DMSO group and control group; however, the levels were higher in the PD group than in the DMSO group. (B) The 3'UTR of SIRT1 had the complementary site for miR-384-5p. (C) miR-384-5p targeted the 3'UTR of SIRT1 in 293 cells. PD, Parkinson's disease; SIRT1, Sirtuin 1; n.s, not significant. ** $P < 0.01$ compared with the controls.

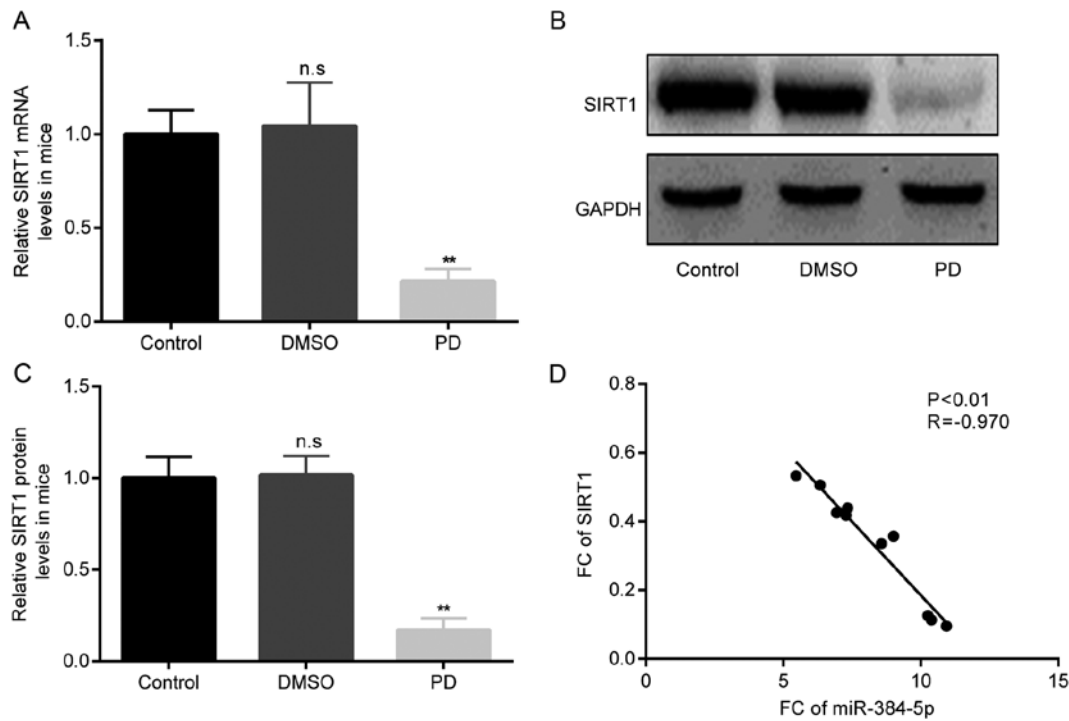


Figure 3. miR-384-5p negatively correlates with SIRT1 in mice with PD. (A-C) No obvious differences were found in the SIRT1 mRNA or protein levels between the control group and DMSO group; the levels were lower in the PD group than in the DMSO group. (D) miR-384-5p negatively correlated with SIRT1 in mice with PD. PD, Parkinson's disease; SIRT1, Sirtuin 1; n.s., not significant. ** $P < 0.01$ compared with the controls.

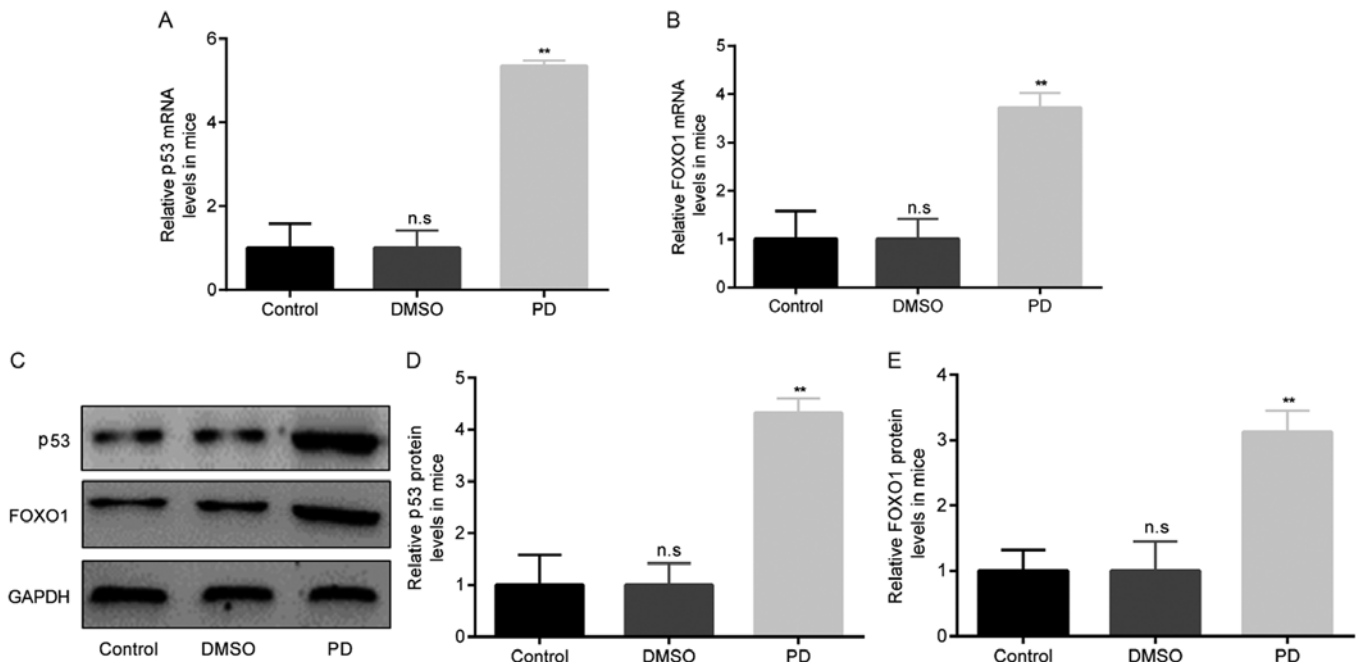


Figure 4. PD increases p53 and FOXO1 expression levels. (A and B) No obvious differences were found in the p53/FOXO1 mRNA levels between the control group and DMSO group; however, the levels were higher in the PD group than in the DMSO group. (C-E) The protein levels of p53 and FOXO1 exhibited similar trends with the mRNA levels. PD, Parkinson's disease; SIRT1, Sirtuin 1; n.s., not significant. ** $P < 0.01$ compared with the controls.

miR-384-5p; moreover, the mutant sequence of the 3'UTR of SIRT1 was obtained.

Subsequently, the interaction between miR-384-5p and SIRT1 was confirmed by dual luciferase reporter assay. The results demonstrated that transfection with miR-384-5p inhibitor significantly induced the relative luciferase activity of 293

cells transfected with luciferase activity plasmid containing SIRT 3'UTR-WT, but not SIRT 3'UTR-MUT (Fig. 2C).

Negative correlation between miR-384-5p and SIRT1 expression in mice with rotenone-induced PD. Thereafter, the differences in the mRNA and protein expression levels of

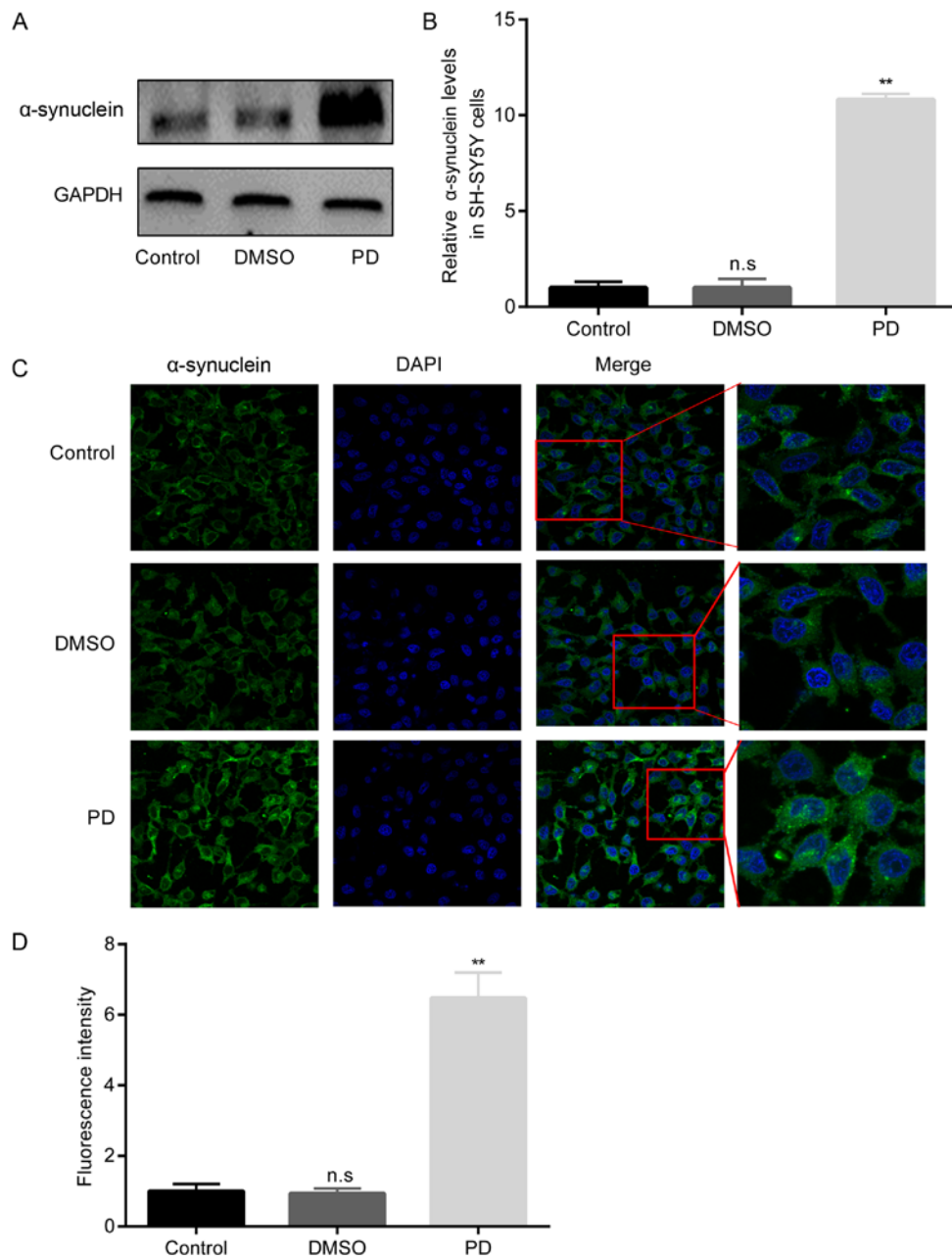


Figure 5. PD increases α -synuclein expression in SH-SY5Y cells. (A-D) No significant differences were found in α -synuclein protein levels or fluorescence intensity between the control group and DMSO group; however, the levels were higher in the PD group than in the DMSO group. PD, Parkinson's disease; n.s, not significant. ** $P < 0.01$ compared with the controls.

SIRT1 in mice with rotenone-induced PD mice among the 3 groups were examined by RT-qPCR and western blot analysis, respectively. The results of RT-qPCR revealed that, compared with the control group, there were no obvious differences in the SIRT1 mRNA levels between the control and the DMSO group; however, in the group of mice with rotenone-induced PD, the mRNA levels of SIRT1 became significantly lower than those of the mice in the DMSO group (Fig. 3A). Moreover, the results of western blot analysis revealed similar results to those obtained with RT-qPCR (Fig. 3B and C).

Of note, the results of Pearson's correlation analysis demonstrated that there was a significant negative correlation between miR-384-5p and SIRT1 expression in the mice with rotenone-induced PD (Fig. 3D).

Increased p53 and FOXO1 expression in mice with rotenone-induced PD. Based on the above-mentioned findings, the molecules that are associated with SIRT1 in mice with rotenone-induced PD were then investigated. A previous study found that p53 and FOXO1 were associated with SIRT1 in MPP⁺-induced SH-SY5Y cells (21). Therefore, this study also examined the association between SIRT1 and P53/FOXO1 in mice with rotenone-induced PD.

The differences in the mRNA and protein expression levels of p53/FOXO1 were determined among the 3 groups by RT-qPCR and western blot analysis, respectively. The results of RT-qPCR revealed that compared with the control group, there were no obvious differences in the p53/FOXO1 mRNA levels in the DMSO group. However, in the group of mice

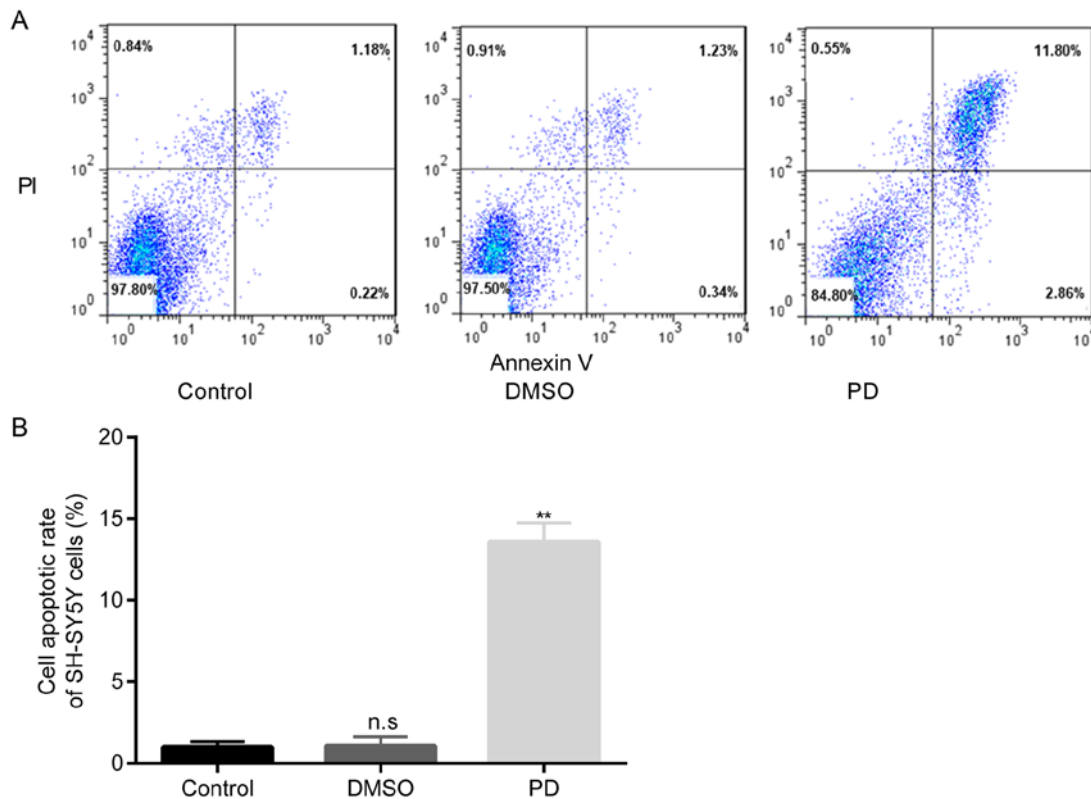


Figure 6. PD increases the apoptosis of SH-SY5Y cells. (A and B) No significant differences were found in SH-SY5Y cell apoptosis between the control group and DMSO group; however, apoptosis was increased in the PD group compared with the DMSO group. PD, Parkinson's disease; n.s, not significant. ** $P < 0.01$ compared with the controls.

with rotenone-induced PD, the mRNA levels of p53/FOXO1 became significantly higher than those of the mice in the DMSO group (Fig. 4A and B). Moreover, the results of western blot analysis revealed similar results to those obtained with RT-qPCR (Fig. 4C-E).

Increased α -synuclein in rotenone-treated SH-SY5Y cells. In the *in vitro* model of PD in this study, the protein expression level of α -synuclein was first evaluated in the SH-SY5Y cells in the 3 groups. The results presented in Fig. 5 demonstrated that there were no significant differences in α -synuclein protein levels between the control group and DMSO group. However, in the PD group of rotenone-exposed SH-SY5Y cells, the α -synuclein protein levels became significantly higher than those in the DMSO group.

Increased apoptosis of rotenone-exposed SH-SY5Y cells. The data presented in Fig. 6 demonstrated that there were no significant differences in the apoptotic rate of SH-SY5Y cells between the control group and DMSO group; however, the exposure of the cells in the PD group to rotenone led to a significant increase in the cell apoptotic rate compared with the DMSO group.

miR-384-5p targets SIRT1 in rotenone-exposed SH-SY5Y cells. Subsequently, the differences in the expression levels of miR-384-5p in the SH-SY5Y cells among the 3 groups were examined by RT-qPCR. The results revealed that compared with the control group, there were no obvious differences in miR-384-5p levels in the DMSO group. However, in the

PD group of rotenone-exposed SH-SY5Y cells, the expression levels of miR-384-5p became significantly higher than those in the DMSO group (Fig. 7A).

Subsequently, the interaction between miR-384-5p and SIRT1 was confirmed by dual luciferase reporter assay, and the results demonstrated that transfection with miR-384-5p inhibitor significantly induced the relative luciferase activity of SH-SY5Y cells transfected with luciferase activity plasmid containing the SIRT 3'UTR-WT, but not SIRT 3'UTR-MUT (Fig. 7B).

Negative correlation between miR-384-5p and SIRT1 in rotenone-exposed SH-SY5Y cells. Thereafter, the differences in the mRNA and protein expression levels of SIRT1 in the rotenone-exposed SH-SY5Y cells in the PD group in the 3 groups were examined by RT-qPCR and western blot analysis, respectively. The results of RT-qPCR revealed that compared with the control group, there were no obvious differences in SIRT1 mRNA levels in the DMSO group; however, in the PD group of rotenone-exposed SH-SY5Y cells, the mRNA levels of SIRT1 became significantly lower than those in the DMSO group (Fig. 8A). Moreover, the results of western blot analysis revealed similar results to those obtained with RT-qPCR (Fig. 8B and C).

Of note, the results of Pearson's correlation analysis demonstrated that there was a significant negative correlation between miR-384-5p and SIRT1 expression in the rotenone-exposed SH-SY5Y cells in the PD group (Fig. 8D).

Increased p53 and FOXO1 expression in rotenone-exposed SH-SY5Y cells. The association between SIRT1 and

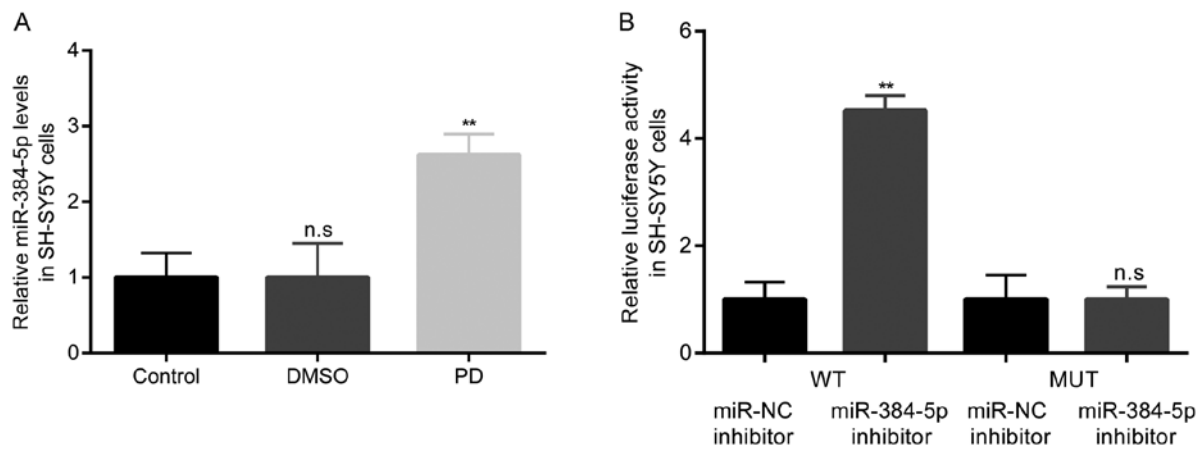


Figure 7. miR-384-5p targets SIRT1 in rotenone-exposed SH-SY5Y cells. (A) No obvious differences were found in the miR-384-5p levels between the control and DMSO group; the levels were higher in the PD group than in the DMSO group. (B) miR-384-5p targeted the 3'UTR of SIRT1 in rotenone-exposed SH-SY5Y cells. PD, Parkinson's disease; SIRT1, Sirtuin 1; n.s., not significant. ** $P < 0.01$ compared with the controls.

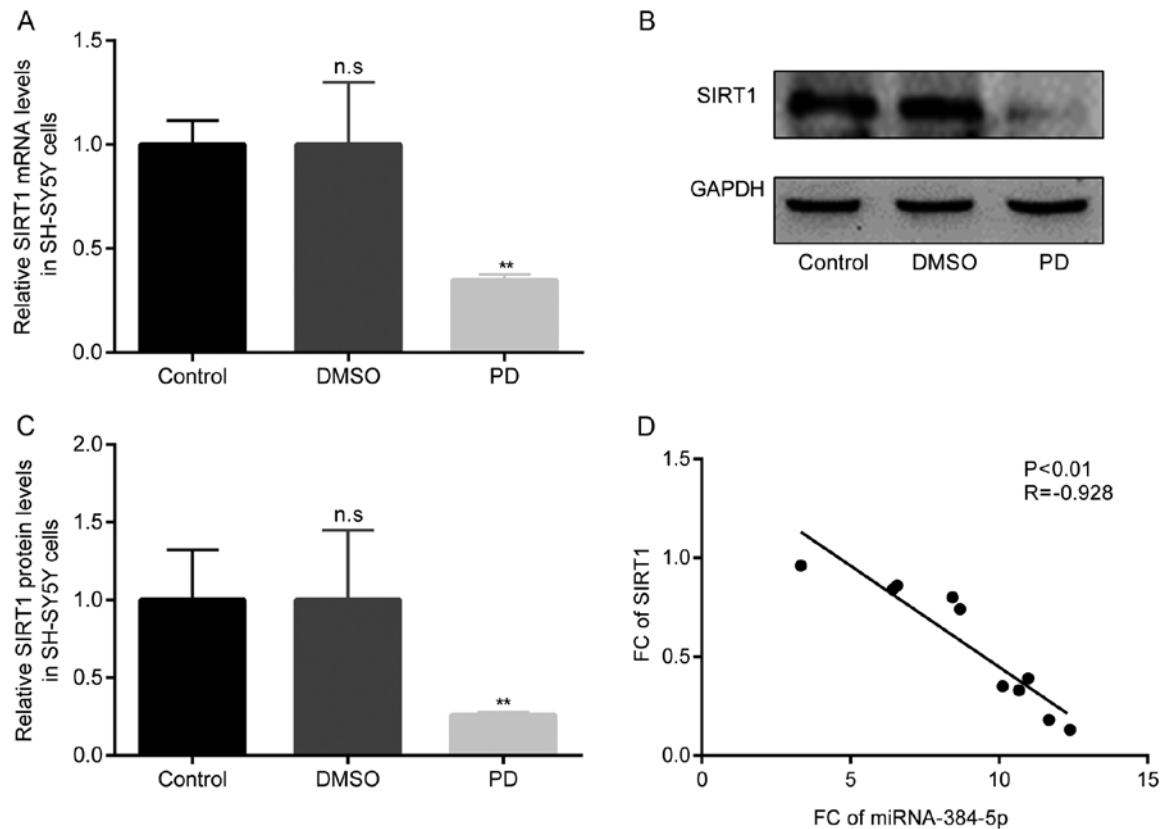


Figure 8. miR-384-5p negatively correlates with SIRT1 in rotenone-exposed SH-SY5Y cells in PD group. (A) No obvious differences were found in SIRT1 mRNA levels between the control and DMSO group; the levels were lower in the PD group than in the DMSO group. (B and C) The protein levels exhibited similar trends with those of the mRNA levels. (D) miR-384-5p negatively correlated with SIRT1 in rotenone-exposed SH-SY5Y cells. PD, Parkinson's disease; SIRT1, Sirtuin 1; n.s., not significant. ** $P < 0.01$ compared with the controls.

p53/FOXO1 expression in rotenone-exposed SH-SY5Y cells was then examined. The differences in the mRNA and protein expression levels of p53/FOXO1 among the 3 groups were examined by RT-qPCR and western blot analysis, respectively. The results of RT-qPCR revealed that compared with the control group, there were no obvious differences in p53/FOXO1 mRNA levels in the DMSO group. However, in the PD group of rotenone-exposed SH-SY5Y cells, the mRNA levels of p53/FOXO1 became significantly higher than those

in the DMSO group (Fig. 9A and B). Moreover, the results of western blot analysis revealed similar results to those of RT-qPCR (Fig. 9C-E).

Discussion

The prevalence of PD is 1-2% for patients >65 years of age (1) and 4-5% for patients >85 years of age (2). Numerous miRNAs have been discovered to be associated with PD. For

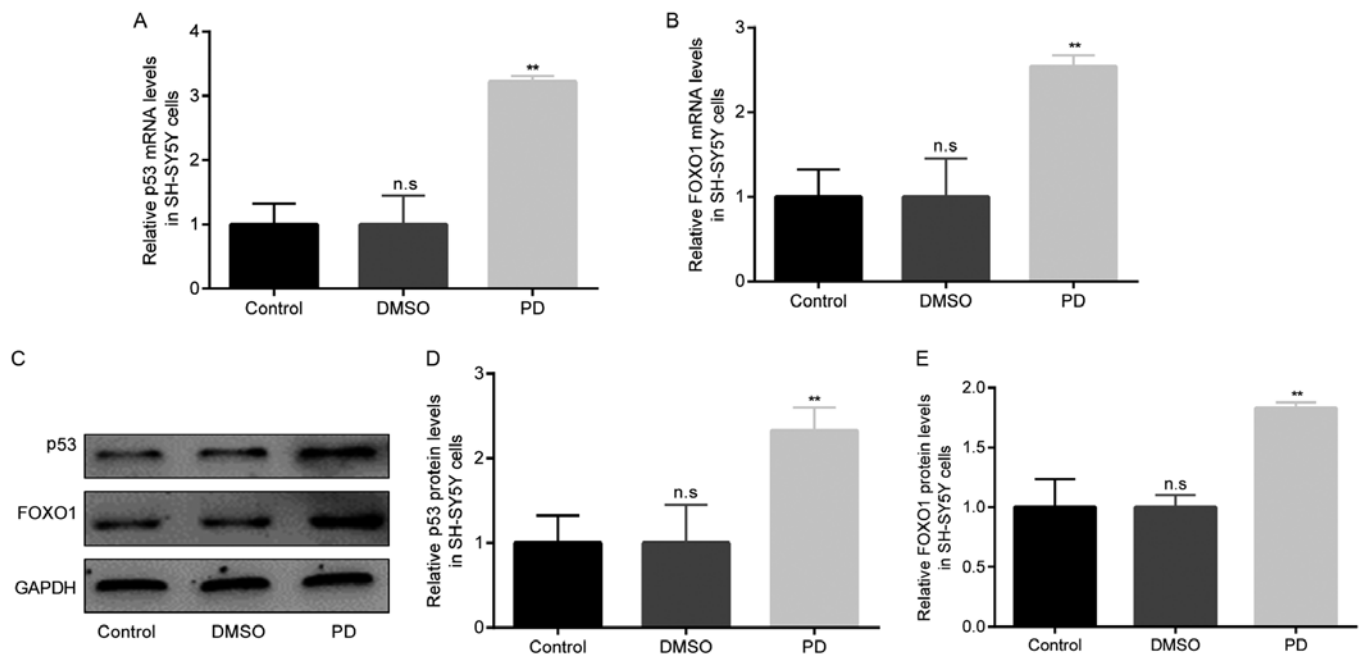


Figure 9. PD increases p53 and FOXO1 expression in SH-SY5Y cells. (A and B) No obvious differences were found in p53/FOXO1 mRNA levels between the control and DMSO group; the levels were higher in the PD group than in the DMSO group. (C-E) The protein levels exhibited similar trends with those of the mRNA levels. **P<0.01 compared with the controls.

instance, miRNA-155-5p and miRNA-146a-5p (12), miR-433 and miR-133b (13) and miR-410 (14). In a recent study, in an *in vitro* model of PD induced by rotenone, miR-384-5p expression was increased, and the downregulation of this miRNA exerted a neuroprotective effect against rotenone by targeting GRP78 (15). However, the role of miR-384-5p in the *in vivo* model of PD and other molecules that may be regulated by miR-384-5p have yet to be investigated.

This study found that the swimming and traction scores of the mice with rotenone-induced PD were reduced, while the α -synuclein levels were increased by rotenone in the mice with PD compared with the DMSO group. Moreover, the α -synuclein levels were increased by rotenone in the SH-SY5Y cells in the PD group compared with the DMSO group, indicating the successful establishment of the *in vivo* and *in vitro* models of PD, respectively.

Thereafter, increased miR-384-5p levels were found in the *in vivo* and *in vitro* PD groups compared with the DMSO group, which was in line with the findings of a previous study (15). miR-384-5p was found to target SIRT1 in 293 cells and SH-SY5Y cells in the present study.

SIRT1 has been reported to be downregulated in PD (22). Consistently, this study found that the SIRT1 levels were decreased in the *in vivo* and *in vitro* PD groups compared with the DMSO group; moreover, a negative correlation was found between miR-384-5p and SIRT1 in mice with rotenone-induced PD and in SH-SY5Y cells exposed to rotenone.

SIRT1 affects mitochondrial-related apoptotic signaling pathways (23); and reduces oxidative stress-induced neuronal cell death in PD (22). SIRT1 also mediates salidroside-elicited protective effects against the MPP⁺-induced apoptosis of SH-SY5Y cells (24), regulates the development of PD and is targeted by miR-9-5p (25). This study found that rotenone

induced a significant increase in the apoptosis of SH-SY5Y cells compared with the DMSO group, and these findings are in agreement with those of previous studies showing the role of SIRT1 in apoptosis in PD (22,24,25).

Subsequently, this study aimed to explore the molecules that are associated with SIRT1 in mice with rotenone-induced PD and in SH-SY5Y cells exposed to rotenone. SIRT1 induces the function of p53 (26); SIRT1 has also been shown to inhibit the H₂O₂-induced apoptosis of osteoblasts via the FOXO1/ β -catenin pathway (27). FOXOs and p53 are regulated by SIRT1 under conditions of oxidative stress (28); p53 and FOXO1 have been shown to be associated with SIRT1 in MPP⁺-induced SH-SY5Y cells (21). Consistently, this study found that in the *in vivo* and *in vitro* models of PD, the mRNA and protein levels of p53/FOXO1 became significantly higher than those in the DMSO group.

In conclusion, this study demonstrates that miRNA-384-5p promotes the progression of PD by targeting SIRT1, providing a potential therapeutic target for the treatment of PD. However, there is a limitation to the present study: The detailed mechanisms of SIRT1 as regards cell apoptosis were not elucidated. Thus, further studies are required to investigate this in the future.

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Availability of materials and data

All data generated or analyzed during this study are included in this published article or are available from the corresponding author on reasonable request.

Authors' contributions

HT and YL carried out the experiments and analyzed the data. YH designed the study, analyzed the data, and prepared the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed in accordance with the guidelines of the Committee on Care and Use of Experimental Animals Resources and with the approval of Ethical Committee for The First Hospital of Yulin.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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