Downregulation of long noncoding RNA SNHG7 protects against inflammation and apoptosis in Parkinson's disease model by targeting the miR-425-5p/TRAF5/NF-κB axis

Haiquan Zhang1,2 | Zhiyong Wang1,2 | Keqi Hu1,2 | Handong Liu1,2

Abstract

Accumulated evidence has manifested that long noncoding RNA (lncRNA) is involved in the progress of Parkinson's disease (PD). SNHG7, a novel lncRNA, has been found to be involved in tumorigenesis. However, SNHG7 expression and its functional effects on PD remain uncharted. Rotenone (Rot) was adopted to construct PD models in Sprague-Dawley (SD) rats and SH-SY5Y cells, respectively. The expression levels of caspase 3, tyrosine hydroxylase (TH), ionized calcium-binding adapter molecule 1 (Iba1) in SD rat striatum were measured via immunohistochemistry and western blot. Additionally, the expressions of inflammatory cytokines (interleukin 1β [IL-1β], IL-6, tumor necrosis factor α) and oxidative stress factors (malondialdehyde, superoxide dismutase, and glutathione peroxidase) in the brain tissues were examined using real-time polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. Moreover, the protein levels of tumor necrosis factor receptor-associated factor (TRAF5), I-κB, nuclear factor-κB (NF-κB), HO-1, Nrf2 were detected via western blot. Bioinformatics was applied to predict the targeting relationship between SNHG7, miR-425-5p, and TRAF5. Dual-luciferase activity assay and RNA immunoprecipitation assays were conducted to verify their interactions. In comparison to healthy donors, SNHG7 was found upregulated while miR-425-5p expression was downregulated in PD patients. Functional experiments confirmed that SNHG7 downregulation or miR-425-5p overexpression attenuated neuronal apoptosis in the Rot-mediated PD model, TH-positive cell loss, and microglial activation by mitigating inflammation and oxidative stress. Mechanistically, SNHG7 served as a competitive endogenous RNA by sponging miR-425-5p and promoted TRAF5 mediated inflammation and oxidative stress. Inhibition of SNHG7 ameliorated neuronal apoptosis in PD through relieving miR-425-5p/TRAF5/NF-κB signaling pathway modulated inflammation and oxidative stress, and similar results were observed in the Rot-mediated rat model of PD.

Keywords

inflammation, miR-425-5p, Parkinson's disease, SNHG7, TRAF5

Haiquan Zhang and Zhiyong Wang contributed equally to this study.
1 | INTRODUCTION

As a typical neurodegenerative disorder disease, Parkinson’s disease (PD) inflicts serious damage to the patients’ quality of life. Clinically, PD patients usually present with symptoms like resting tremors and muscle rigidity. Pathologically, this disease is characterized by the degeneration or progressive loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and striatum of the mid-brain, together with the presence of Lewy bodies (mainly composed of α-synuclein). A series of studies have confirmed that genetic susceptibility, oxidative stress, drug toxicity, and environmental triggers are strongly implicated in PD’s pathogenesis. In recent years, increasing research has focused on the pathogenic mechanism underlying PD. A great many noncoding RNAs (such as long noncoding RNA [lncRNA] and microRNA [miRNA]) that regulate these genes have attracted wider attention. Thus, an improved understanding of lncRNAs in PD helps to provide new insights for finding early diagnostic indicators and effective therapeutic targets of PD.

According to recent studies, lncRNA regulates inflammatory reactions by targeting miRNAs. For example, SNHG16 regulates chemokine ligand 5 (CCL5) expression by competitively binding to miR-146a-5p; SNHG16 overexpression aggravates the apoptosis, as well as inflammation of lipopolysaccharides (LPS)-induced acute pneumonia in WI-38 cells. Moreover, FGD5-antisense 1 (FGD5-AS1) affects the occurrence and development of periodontitis by regulating the miR-142-3p/suppressor of cytokine signaling protein 6 (SOCS6)/nuclear factor-kB (NF-kB) pathways. In PD, similar mechanisms of lncRNA have also been uncovered. For example, lncRNA-p21 is upregulated in a PD model of 1-methyl-4-phenylpyridinium iodide (MPP (+))-induced neuronal damage in SH-SY5Y cells. Besides this, lncRNA-p21 upregulates transient receptor potential melastatin 2 (TRPM2) expression by targeting miR-625. As a group of lncRNAs, the SNHG family is considered a key regulator in PD. For instance, SNHG1 is upregulated in PD and overexpressed SNHG1 promotes MPP+ -induced SH-SY5Y cell apoptosis. In another study, SNHG1 regulates the p27/mTOR pathway by competitively binding to miR-221/222. SNHG1 knockdown mitigates MPP+-induced cytotoxicity of MN9D dopaminergic neurons.

SNHG7 also belongs to one member of lncRNAs, which is located on chromosome 9q34.3 (in the human gene). Besides this, SNHG7 exerts oncogenic effects on multiple cancers, including colorectal cancer, pancreatic cancer, and breast cancer, mainly by modulating the proliferation, migration, invasion, and apoptosis of tumor cells. Additionally, SNHG7 could inhibit high glucose-induced cell proliferation, migration, and angiogenesis, and also alleviate osteoarthritis through modulating the proliferation, apoptosis, and autophagy of chondrocytes. However, the role of SNHG7 in PD progression remains to be further verified.

TRA5 is a member of the tumor necrosis factor receptor-associated factor (TRAF) family, which has been discovered recently. Like other TRAF family members, TRAF5 also has a ring finger domain, and it displays E3 ubiquitin ligase viability. Existing studies have validated that TRAF5, as a cytoplasmic adapter, can activate the NF-κB signaling pathway through its receptors, and then participate in mediating inflammation, neuronal apoptosis, glial cell activation, and other processes in the nervous system. At present, accumulating studies have highlighted that TRAF5 is directly involved in disease progression as a targeted molecule of miRNAs. For example, in gastric cancer cells, miR-135a regulates the NF-κB pathway by targeting TRAF5. MiR-135a overexpression suppresses the NF-κB pathway, thereby inhibiting gastric cancer cell migration.

In the present study, we found that SNHG7 was significantly overexpressed in the serum of PD patients and also positively correlated with the levels of proinflammatory cytokines (interleukin 1β [IL-1β], IL-6, tumor necrosis factor α [TNF-α]). Additionally, we conducted bioinformatics analysis through Starbase and found that miR-424-5p shared the binding sites with SNHG7 and TRAF5. Interestingly, miR-424-5p was markedly downregulated in the serum of PD patients and had negative correlations with IL-1β, IL-6, and TNF-α levels. Therefore, we supposed there is a regulatory network of SNHG7-miR-424-5p-TRA5 in PD development.

2 | MATERIALS

2.1 | Clinical samples

A total of 56 participants were recruited from XiangYang Center Hospital: 36 surgically treated PD patients and 20 healthy donors who had undergone related examinations as required. All the subjects provided written informed consent. The diagnosis of PD was performed by two experienced clinicians, based on the British PD clinical diagnostic criteria for diagnosis. Exclusion criteria: (1) PD induced by chemical drugs, trauma, and cerebrovascular diseases; (2) PD patients with severe heart, kidney, liver, blood system diseases and infectious or inflammatory diseases; (3) patients with other nervous system diseases were excluded. Moreover, this study garnered approval from the Ethics Committee of XiangYang Center Hospital. All the plasma samples (fasting elbow vein blood, 10 ml) were obtained from the recruiters recruited patients and controls in the morning. After that, the samples were subjected to experimental examination after centrifugal separation.

2.2 | Animals and treatment

A total of 45 Sprague-Dawley (SD) rats (male, 240–260 g, clean grade) was obtained from the Experimental Animal Center of Jilin University (license number: SCXK (Ji) 2016-0001). All the rats were kept under standard specific pathogen free conditions. All animal experiments were approved by the Animal Experiment Ethics Review Committee of XiangYang Center Hospital and conducted following the guidelines for laboratory animal care and use of the National Institutes of Health (NIH Publication No.: 8023, 1978 revision). A total of 45 SD rats were randomly divided into three groups,
namely, the Sham group \( (n = 15) \), the Rot + LV-si-NC group \( (n = 15) \), and the Rot + LV-si-SNHG7 group \( (n = 15) \). For the Sham group: sunflower oil was subcutaneously injected into the back of SD rats for 42 days (1 ml/kg/day). For the Rot + LV-si-NC group and Rot + LV-si-SNHG7 group: rotenone (MedChemExpress, HY-B1756) was subcutaneously injected at the rats' back for 42 days consecutively (1.5 mg/kg/day). The small interference RNA (siRNA) lentivirus vector targeting SNHG7 (LV-Si-SNHG7) or LV-si-NC (GeneChem Co. Ltd.) were inserted into the linearized vector GV115 to construct the siRNA recombinant lentiviral vector. Two days before Rot treatment, the rats were anesthetized via isoflurane (2%) in oxygen and nitrous oxide and then positioned on a stereotactic frame (Stoelting). The skull surface was exposed with a hole drilled to locate the needle. Hamilton syringes (5 μl, 33-gauge needle) were applied here to inject the rats with 1 μl/side of recombinant LV-si-SNHG7 or LV-si-NC into the dorsal hippocampus (anterior, −5.5; lateral, +1.6; dorsoventral, −7.5 from bregma) (0.2 μl/min, 10 min). Behavioral evaluation was carried out on days 0, 14, and 42. Rats were sacrificed by decapitation following the behavioral evaluation on the 42nd day. After anesthetization and perfusion, the rat brains were collected, washed, and frozen in liquid nitrogen. They were stored at −80°C for further use.

### 2.3 Behavioral assessment

Using a computer-aided infrared motion detection system (Institute of Materia Medica, Chinese Academy of Medical Sciences, China), spontaneous sports activities were measured. The rats were placed in transparent plexiglass bottles (40 cm across, 13 cm high), making them get used to the environment 5 min before the experiment. Then the number of horizontal and vertical movements was recorded in 10 min.

On the carousel experiment: First, the rats were placed on a fixed rod (3 cm across) for 30 s, during which any fallen animals were placed back on the rod. Next, the conditioned response of the rats was made to formulate in the following 120 s at a constant speed of 15 RP/m. For those who failed to formulate the response, two additional chances were granted. During the fatigue time after the last training, the rats were placed on the rod at a constant speed of 15 RP/m (120 s/time) to evaluate their motor skills. Measurements were recorded three times at 30-min intervals per round.

On the bevel experiment: To begin with, the rats were placed on the bevel rough surface at an angle of 60°. Then, the duration during which the rats of each group stay on the bevel was recorded respectively. When the rat stayed on the slope for over 3 min, it was recorded as 180 s. Three experiments were performed on each animal.

### 2.4 Immunohistochemical staining

On the 42nd day, the rats in different groups were sacrificed, then the brains were collected, fixed in 4% paraformaldehyde, embedded in paraffin, serially sectioned (4 μm). Next, the brain slices were put in a methanol solution (0.3% H2O2) and got incubated (37°C, 15 min) to inactivate the endogenous peroxidase. After that, the sections were rinsed with phosphate-buffered saline (PBS) (0.01 mol/L, pH 7.4) for 5 min (×3), and incubated with 10% normal goat serum (37°C, 15 min). When the goat serum was removed, the slices were added with rabbit antibodies including anti-TH (1: 200; ab75875; Abcam), anti-Caspase 3 (1:200; ab13847; Abcam) and kept in a wet box (4°C) overnight. Then, the samples were rinsed by PBS for 5 min (×3), added with biotin-labeled goat anti-mouse IgG (1:200; Beijing Zhongshan Biotechnology Co., Ltd.), and incubated (37°C, 30 min). Afterward, we washed them with PBS for 5 min (×3), added ABC solution (Beijing Zhongshan Biotechnology Co., Ltd.), and incubated them at 37°C for 30 min. Furthermore, the sections were rinsed with PBS for 5 min (×3) and kept for DAB color development for 10–15 min. Lastly, the sections were dehydrated with gradient alcohol, made transparent via xylene, sealed with neutral gum, and ultimately observed under an optical microscope. Five slices of brain tissue at approximately the same level per rat were taken to observe TH or caspase 3 positive cells around the SN area at five randomly selected fields (×200) and calculated for the number of positive cells.

### 2.5 Immunofluorescence

On the 42nd day, the rats in different groups were sacrificed, then the brains were collected and were frozen quickly in a −20°C refrigerator. The frozen sections were taken, hydrated with 0.01 mol/L phosphate buffer for 20 min, and repaired with citric acid in the microwave for about 5 min. The membranes were ruptured with 0.3% Triton X-100 for 30 min, washed with 0.01 mol/L phosphate buffer for 5 min (×3), blocked with antigen blocking solution (at room temperature for 1 h). Next, the rabbit anti-ionized calcium-binding adapter molecule 1 (Iba1) antibody (1: 200; ab178846; Abcam) was added dropwise for incubation overnight (4°C). Then, the specimens were subjected to 0.01 mol/L phosphate buffer washing for 5 min (×3), added with goat anti-rabbit fluorescent IgGII antibody labeled with AlexaFluor 555, and incubated (at 37°C, 1 h) in the dark. We washed the sections with 0.01 mol/L phosphate buffer for 5 min (for four times), mounted them via the medium with 4′,6-diamidino-2-phenylindole, and ultimately put them under fluorescent microscope observation. Five slices of brain tissue at approximately the same level per rat were taken to observe Iba1-positive microglia around the SN area at five randomly selected fields (×200) and calculated for the number of positive cells.

### 2.6 Cell culture and transfection

Human dopaminergic neuroblastoma SH-SY5Y cells and microglia HMC3 cells were obtained from the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco’s modified Eagle’s medium-Ham’s Nutrient Mixture F-12 (DMEM/F12; Invitrogen)
supplemented with 10% fetal bovine serum (Invitrogen), penicillin and streptomycin (1%; Beyotime) in humidified air (37°C, 5% CO₂). Then, the SH-SY5Y cells were further cultured in neuron medium (ScienCell Research Laboratories) (37°C, 5% CO₂), added with 1% neuron growth agent and 1% penicillin and streptomycin.

Rotenone was dissolved in dimethyl sulfoxide (DMSO). The final DMSO solution concentration was 0.01%. SH-SY5Y cell damage was induced using rotenone (500 nM) for 24 h. HMC3 cell activation was induced by LPS (10 μg/ml; Sigma-Aldrich). Twenty-four hours after transfection, the cells (at logarithmic growth phase) were seeded into a 96-well microplate to achieve a 60%-80% fusion rate. Then the expression vectors of SNHG7 overexpression plasmids, si-SNHG7, miR-425-5p mimics, and their negative controls (GenePharma) were transferred into SH-SY5Y and HMC3 cells using Lipofectamine 2000 Reagent (Thermo Fisher Scientific) in line with the manufacturer’s instructions. The cells were harvested for further experiments after 48-h transfection.

### 2.7 Quantitative real-time polymerase chain reaction (qRT-PCR)

First, total RNA was isolated from human plasma, rat SN, SH-SY5Y cells, and HMC3 cells using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. After the determination of RNA purity, the total RNA was then reversely transcribed into complementary DNA (cDNA) using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Subsequently, SYBR® Premix-ExTaq™ (Takara) was used for qRT-PCR on the ABI7300 system. The total volume of the PCR system was 30 μl containing 300 ng cDNA. Under the amplification procedure, the first denaturation lasted 10 min (95°C), and the following 45 cycles were: 95°C (10 s), 60°C (30 s), and 85°C (20 s). All fluorescence data were converted into relative quantification, using β-actin as the endogenous control for SNHG7. The expression of the miR-425-5p in the human or rat or cellular samples was measured using the All-in-One™ miRNA qRT-PCR Detection Kit (GeneCoepoeia). ABI 7500 Fast Real-Time PCR System (Applied Biosystems) was used to perform qRT-PCR, and U6 was used for the endogenous control to normalize miR-425-5p expression. Additionally, the 2−ΔΔCT method was used for statistics with all experiments repeated in triplicate. The human origin RNA primer sequences were as follows: SNHG7 Forward: 5’-AACAGGAGGAGTGTAAAGACC-3’; Reverse: 5’-GATGGGTTTTCCAGTGTTGTG-3’; miR-425-5p Forward: 5’-GCGGAAAGCAGCAGCAGTTTAA-3’, Reverse: 5’-GCTTCCGAGGTGCAGTTTAA-3’. The expression of the miRNA was calculated using the 2−ΔΔCT method and normalized to the endogenous control (U6). The ΔΔCT of each band was calculated and used to determine the relative expression level of miRNA.

### 2.8 Western blot

When rat brain (SN area) or cells were collected, we removed the medium and added protein lysate (Roche) to lyse the cells or tissues. Then the total proteins were isolated. Next, 50 μg total protein was added to 12% polyacrylamide gel for electrophoresis (100 V, 2 h). Then, the protein samples were electroblotted onto polyvinylidene fluoride membranes. After blocking with 5% skimmed milk powder (1 h, room temperature), the membranes were washed three times with TBST for 10 min each time. Afterward, the membranes were incubated (4°C) with primary antibodies overnight as follows: anti-tirosine hydroxylase (TH) (1:1000; ab57857; Abcam), anti-TRAF5 (1:1000; ab137763; Abcam), anti-I-κB (1:1000; ab32518; Abcam), p-NF-κB (phospho 5536) (1:1000; ab86299; Abcam), anti-NF-κB (1:1000; ab32536; Abcam), anti-Nrf2 (1:1000; ab31163; Abcam), anti-HO-1 (1:1000; ab13248; Abcam), anti-Bax (1:1000; ab32503; Abcam), anti-Bcl2 (1:1000; ab182858; Abcam), and anti-Caspase 3 (1:1000; ab13847; Abcam), anti-Iba1 (1:200; ab178846; Abcam). After being washed with TBST, the membranes were incubated with horseradish peroxidase (HRP) labeled anti-rabbit or anti-mouse secondary antibodies (concentration 1:3000) for 1 h at room temperature. Then, we washed the membranes three times with TBST (10 min each). Finally, a western blot reagent (Invitrogen) was applied for color imaging. Image J was used for analyzing the gray value of each band.

### 2.9 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

A total of 100 μl SH-SY5Y cells (logarithmic growth phase, about 5000 cells) were seeded into a 96-well plate (about 4 × 10⁵/well) and incubated at 37°C, with 5% CO₂ and 100% humidity for 24 h. Afterward, we added an equal volume of PBS to the control group (five repetitive wells in each group) and continued to add 50 μl MTT (5 g/L) after 24 h of incubation. Four hours later, the supernatant was discarded and 150 μl DMSO was added into each well. Then the plate was placed in a platform rocker for shaking for 1 h. When the crystals were dissolved, a microplate reader was utilized to detect the optical density (OD) value in each well (570 nm wavelength).

### 2.10 Measurement of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX)

When the brain tissues (SN area) were weighed, a 9-time volume of cold saline (containing protease inhibitors) was added for homogenization. Then, the samples subjected to centrifugation at 3000g for
20 min (4°C). After that, the supernatant was collected. Regarding Rot-treated SNHG7 cells, the cell supernatant was collected according to the group, and centrifuged at 1000g (10 min, 4°C). Next, the supernatant was collected. SOD and GSH content in brain tissues or cells were determined respectively with the help of SOD (Cat No.: A001-3-2), GSH-PX (Cat No.: A005-1-2), and MDA (Cat No.: A003-1-2), a procedure carried out under the instructions of the relative kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The content of SOD was determined by the WST-1 assay using a microplate reader (the detection wavelength is 450 nm). The content of GSH was determined by the colorimetric method using a spectrophotometer (UV-2000; UNICO) (OD at 412 nm). The content of MDA was determined by the TBA method using a spectrophotometer (UV-2000; UNICO) (OD at 532 nm).

### 2.11 Enzyme-linked immunosorbent assay (ELISA)

To begin with, the test tube was filled with 10 ml of venous blood. Standing for 1 h at room temperature, the sample was subjected to centrifugation (340 g, 5–10 min). Next, the upper serum was collected and put in the refrigerator (−20°C) for overall detection. Following that, the SD rat brains (SN area) were weighed, shredded, and added with 12 volumes of normal saline. The tissues in normal saline were then transported to a homogenizer for thorough mixing and crushing to deliver a tissue homogenate. Furthermore, the brain tissue homogenate was centrifuged (680g, 15 min, 4°C), and then the supernatant was collected for detection. Then, the supernatant of Rot-treated SNHG7 cells was collected based on the group. After a 10 min centrifugation (1000g, 4°C), the supernatant was collected as required.

All experimental steps conformed to the instructions provided by the ELISA kit in determining the content of IL-1β, IL-6, TNF-α, and lactate dehydrogenase (LDH) release. The test kits were obtained from Nanjing JianCheng Bioengineering Institute (Nanjing JianCheng Bioengineering Institute, Nanjing, China).

### 2.12 Luciferase reporter assay

The DNA sequences of IncRNA SNHG7 and TRAF5 were amplified. According to the predicted binding sites from the online database Starbase (http://starbase.sysu.edu.cn), SNHG7 and TRAF5 cDNA containing the predictive binding sites of miR-424-5p were cloned into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega) to form the reporter vectors named pmirGLO-SNHG7-WT or TRAF5-WT. The mutant SNHG7 and TRAF5 containing point mutations of the miR-424-5p seed region binding site were specifically synthesized and inserted into the abovementioned vectors, which were named pmirGLO-SNHG7-MUT and TRAF5-MUT. The cells were seeded into a 24-well plate overnight. Then Lipofectamine 2000 was used to transfect SNHG7-WT, SNHG7-MUT, TRAF5-WT, TRAF5-MUT reporter vectors, and miR-425-5p mimics (Invitrogen) into SH-SY5Y cells. Through the dual-luciferase reporter system (Promega), the dual-luciferase activities were determined after 48 h of transfection. Relative firefly luciferase activity was normalized to Renilla luciferase activity as a control for transfection efficiency.

### 2.13 RNA immunoprecipitation (RIP) assay

To determine the relations between SNHG7 and miR-425-5p, miR-425-5p, and TRAF5, Magna-RNA combined with the binding protein immunoprecipitation kit (Millipore) was used to conduct the RIP test. The SH-SY5Y cells (transfected with miR-424-5p mimics or miR-NC) at 80% confluence were collected and lysed in a complete RIP lysate buffer. Then, the cell lines were co-immunized with RIP buffer containing magnetic beads with anti-argonaute 2 (Ago2) antibody (Millipore) or negative control healthy rat IgG (Millipore). The immunoprecipitated RNA was isolated and the relative enrichment of SNHG7 and TRAF5 in the lysates was examined by qRT-PCR.

### 2.14 Statistical analysis

Data analysis was carried out via SPSS software (version 20.0). The data were displayed as mean ± SD. Student’s t test, along with χ2 test, was employed to access the statistical differences between the two groups. One-way analysis of variance followed by Tukey’s test is used for multiple group comparisons. The correlation relationship was determined by Pearson’s correlation test. p < 0.05 was regarded as statistically significant.

### 3 RESULTS

#### 3.1 SNHG7 and miR-425-5p expression characteristics in PD patients

Aiming at determining the SNHG7 and miR-425-5p expressions in PD patients, qRT-PCR was employed to detect the expressions in 36 sera of PD patients and 20 healthy donors. The results indicated that compared with the healthy donors, SNHG7 was upregulated in PD patients’ serum, while miR-425-5p was downregulated (Figure 1A,B). Pearson’s correlation analysis exhibited that SNHG7 in PD patient serum was negatively correlated with miR-425-5p expression (Figure 1C). Furthermore, the IL-1β, IL-6, and TNF-α expressions in the serum were detected via ELISA. The results indicated that the levels of IL-1β, IL-6, and TNF-α in PD patient serum were significantly increased compared with those in the healthy donors’ serum (Figure 1D). By conducting Pearson’s correlation analysis, we found that SNHG7 was positively correlated with IL-1β, IL-6, TNF-α in PD patients’ serum, while miR-425-5p revealed a negative correlation with IL-1β, IL-6, TNF-α in PD patients’ serum (Figure 1E,F).
Therefore, the results suggested that both SNHG7 and miR-425-5p play a role in PD.

3.2 | SNHG7 inhibition attenuated neuronal apoptosis in PD model in vivo and in vitro

To better understand the biofunctions of SNHG7 on PD, we used Rot to construct a PD model in SD rats. Meanwhile, LV-si-SNHG7 was used to establish a rat model with downregulation of SNHG7. The spontaneous locomotor activity test, carousel experiment, and bevel experiment were conducted to comprehensively assess the motor skills of rats. As Figure 2A shows, the rats in the Rot + LV-si-NC group showed notable behavioral damage in comparison to the rats in the Sham group. However, the Rot + LV-si-SNHG7 group showed markedly attenuated behavioral deficits than those in the Rot + LV-si-NC group (Figure 2A). The TH-positive cells in SN were detected by immunohistochemistry, and it was found that TH-positive cells were prominently reduced in rats of the Rot + LV-si-NC group (compared with that in the Sham group), while knocking down SNHG7 reversed Rot’s effect on TH positive cell number in SD rats (vs. Rot + LV-si-NC group, Figure 2B). Additionally, a western blot was applied to detect TH relative expression in the SN area. The result indicated that TH relative expression was enhanced in the Rot + LV-si-SNHG7 group compared to that in the Rot + LV-si-NC group (Figure 2C). Moreover, caspase 3 staining was used to detect the number of apoptotic neurons. As a result, more caspase 3 positive staining cells were found in the Rot + LV-si-NC group (compared with that in the Sham group). However, SNHG7 knockdown obviously inhibited caspase 3 labeled apoptotic cells (vs. Rot + LV-si-NC group, Figure 2D). To further verify the effect of SNHG7 in modulating rotenone-induced neuronal damage, si-SNHG7 was used to establish an SNHG7-downregulated cell model in
SH-SY5Y cells (Figure 2E). Next, the viability, release of LDH, and apoptosis of SH-SY5Y cells were determined. The results showed that compared with the control group, Rotenone significantly inhibited cell viability (Figure 2F), promoted LDH expression (Figure 2G), and enhanced apoptosis (Figure 2H). However, downregulating SNHG7 increased cell viability, reduced LDH expression, and cell apoptosis (compared with those in the Rot+si-NC group, Figure 2F–H). The above results illustrated that SNHG7 knockdown exerted neuroprotective effects against rotenone-induced PD both in vivo and in vitro.

**FIGURE 2** SNHG7 inhibition attenuated neuronal apoptosis in PD models in vivo and in vitro. Then, the rat PD model was induced by Rotenone and Lv-si-SNHG7 was used for knocking down SNHG7 in the rat brain. (A) The spontaneous locomotor activity test, carousel experiment, and bevel experiment were used to comprehensively evaluate the rat's motor skills (n = 5). (B) Immunohistochemistry was utilized to detect the number of TH positive cells in the SN area of rats (n = 5). (C) Western blot was employed to detect the TH relative expression in the rat SN region (n = 5). (D) Caspase 3 staining was used to detect the apoptotic neuron number in rats (n = 5). SH-SY5Y cells with low SNHG7 expression were constructed and were then treated with rotenone (500 nM) for 24 h. (E) and qRT-PCR was used to detect the SNHG7 relative expression (n = 3). (F) MTT assay was used for detecting neuronal proliferation (n = 3). (G) ELISA assay was used to detect LDH levels of cells in each group (n = 3). (H) The relative expression of apoptotic proteins: Bax, Bcl2, and caspase 3 was detected by western blot (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 (vs Sham); *p < 0.05, **p < 0.01, ***p < 0.001. ELISA, enzyme-linked immunosorbent assay; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; PD, Parkinson's disease; qRT-PCR, quantitative real-time polymerase chain reaction; SN, substantia nigra; TH, tyrosine hydroxylase.
3.3 | SNHG7 inhibition attenuated inflammation and oxidative stress in the PD model

In the PD rat model, we further validated the expressions of inflammation and oxidative stress factors. First, immunofluorescence was performed to test Iba1-labeled microglia. The findings suggested that microglial activation in the PD model was remarkably stimulated, while SNHG7 knockdown notably reduced microglial activation (vs. Rot + Lv-si-NC group, Figure 3A,B). Moreover, the proinflammatory cytokines in brain was measured by ELISA. The results showed that the levels of IL-1β, IL-6, and TNF-α were prominently overexpressed in the Rot+LV-si-NC group (compared with those in the Sham group), while SNHG7 inhibition attenuated the inflammatory responses in the PD model (vs. Rot + Lv-si-NC group, Figure 4A). Next, the expressions of oxidative stress factors including MDA, SOD, and GSH-PX in the brain of rats were also determined. We discovered that the Rot+LV-si-SNHG7 group had attenuated MDA level but enhanced SOD and GSH-PX level in the brain when compared with the Rot+LV-si-NC group, suggesting that SNHG7 downregulation inhibited oxidative stress response (Figure 4B). Then, we examined the profiles of TRAF5, IκB, p-NF-κB, Nrf2, HO-1 using western blot. As the results showed, the expressions of TRAF5 and p-NF-κB in the Rot+LV-si-SNHG7 group were remarkably downregulated compared to those in the Rot+LV-si-NC group, while the expressions of IκB, Nrf2 and HO-1 were significantly elevated in the Rot+LV-si-SNHG7 group (vs. Rot+LV-si-NC group, Figure 4C). Furthermore, we investigated the regulatory role of SNHG7 on the inflammation and oxidative stress reactions on HMC3 cells induced by LPS. ELISA results showed that in the LPS+si-SNHG7 group, the inflammatory cytokines (including IL-1β, IL-6, and TNF-α) were significantly reduced when compared with those in the LPS+si-NC group (Figure 4D). By detecting the oxidative stress factors’ expression, we found that in LPS-treated HMC3 cells, the level of MDA significantly increased, while SOD and GSH-PX were decreased (compared with the control group, Figure 4E). After downregulating SNHG7, the MDA level was inhibited, but the SOD and GSH-PX expressions were enhanced (compared with the LPS+si-NC group, Figure 4E). Additionally, the expressions of TRAF5 and p-NF-κB in the LPS+si-SNHG7 group were significantly downregulated compared with those in the LPS+si-NC group, while the expressions of IκB, Nrf2, and HO-1 were significantly increased (Figure 4F). The above results indicated that SNHG7 inhibition could relieve microglia-mediated inflammation and oxidative stress via regulating the TRAF5/IκB/NF-κB pathway.

3.4 | SNHG7 targeted miR-425-5p

To explore the downstream molecular mechanism of SNHG7, we analyzed the SNHG7 candidate targets through the StarBase database (http://starbase.sysu.edu.cn). Interestingly, miR-425-5p had binding sites with SNHG7 (Figure 5A). The dual-luciferase reporter assay and RIP assay were applied to determine the interactions between SNHG7 and miR-425-5p. As the results show, miR-425-5p mimics reduced the luciferase activity of HMC3 cells transfected with SNHG7 (wild-type) but exerted a minimal effect on the luciferase activity of mutant SNHG7. Moreover, the enrichment of SNHG7 in the anti-Ago2 antibody precipitation complex was remarkably increased in the LPS+si-SNHG7 group (vs. LPS+si-NC group, Figure 4F). The above results indicated that SNHG7 inhibition could relieve microglia-mediated inflammation and oxidative stress via regulating the TRAF5/IκB/NF-κB pathway.

**FIGURE 3** SNHG7 inhibition attenuated Iba1 labeled microglial activation in the rat PD models. (A). Immunofluorescence was used to detect Iba1-labeled microglia in the SN area (n = 5). (B) Western blot was conducted to detect Iba1 expression in the SN area (n = 5). ***p < 0.001. Iba1, ionized calcium-binding adapter molecule 1; PD, Parkinson’s disease; SN, substantia nigra.
increased following miR-425-5p mimics transfection. In addition, the miR-425-5p levels in the PD model (both in vivo and in vitro) were significantly declined compared with that in the Sham or control group, while downregulation of SNHG7 promoted miR-425-5p expression (compared with that in the Rot+si-NC or Rot+Lv-si-NC group, Figure 5D,E). The above results showed that SNHG7 may function as a competitive endogenous RNA (ceRNA) by sponging miR-425-5p.

3.5 SNHG7 overexpression inhibited miR-425-5p mediated neuroprotective effects

To further investigate the SNHG7/miR-425-5p axis in PD, SH-SY5Y cells were transfected with miR-425-5p mimics and/or SNHG7 overexpressing plasmids. The results showed overexpression of miR-425-5p inhibited SNHG7 level (compared with that in the Rot+miR-NC group, Figure 6A). At the same time, SNHG7 overexpression suppressed the miR-425-5p level (compared with that in the Rot+miR-524-5p group, Figure 6B). Next, the cell viability of SH-SY5Y cells was tested by MTT assay, which showed that the cell viability in the Rot+miR-425-5p group was apparently enhanced (compared with that in the Rot+miR-NC group), while supplementation of the SNHG7 overexpressing plasmids resulted in a remarkable decrease in cell viability (compared with that in the Rot+miR-425-5p group, Figure 6C). Additionally, an ELISA assay was performed to ascertain the LDH level. We found that miR-425-5p mimics obviously reduced LDH expression. Followed with the SNHG7 overexpression, the LDH level was significantly promoted (compared with that in the Rot+miR-425-5p group, Figure 6D). Besides this, the expressions of apoptosis-related proteins (including Bax, Bcl2, and caspase 3) were examined via western blot. It was found that miR-425-5p decreased the Rot-induced Bax and caspase 3 overexpression and Bcl2 downregulation (compared with those in the Rot+miR-NC group). Nevertheless, the compensation of SNHG7 reversed those effects (compared with that in the Rot+miR-425-5p group, Figure 6E). Hence, SNHG7 promoted rotenone-mediated neuronal damage via inhibiting miR-425-5p.

3.6 SNHG7 promoted TRAF5/NF-κB pathway activation by competitively binding miR-425-5p

To further verify the underlying mechanism of the SNHG7/miR-425-5p axis in regulating microglial activation, the relative expression of inflammatory factors and oxidative stress factors were detected by ELISA. It turned out that miR-425-5p overexpression inhibited the inflammatory cytokines and oxidative stress induced by LPS in HMC3.
cells, which were inhibited by SNHG7 (compared with that in the Rot + miR-425-5p group, Figure 7A,B). Next, the result of western blot showed that the TRAF5 and p-NF-κB expressions in the LPS + miR-425-5p group declined, while the I-κB, Nrf2, and HO-1 expressions increased in comparison to the LPS+miR-NC group. However, SNHG7 overexpression reversed the effects induced by miR-425-5p mimics (compared with that in the LPS + miR-425-5p group, Figure 7C,D). The above statistics indicated that SNHG7 promoted the activation of the TRAF5/NF-κB pathway in microglia through competitively binding with miR-425-5p.

3.7 | miR-425-5p targeted TRAF5

To reveal the downstream mechanism of miR-425-5p, the bioinformatics database Starbase (http://starbase.sysu.edu.cn/) was employed to find the miR-425-5p downstream genes. In this study, TRAF5 was identified as an important target molecule toward miR-425-5p (Figure 8A). To clarify whether miR-425-5p targeted TRAF5, the dual-luciferase activity assay, and RIP assay were conducted, which showed that miR-425-5p dramatically inhibited the luciferase activity of TRAF5-WT-transfected cells, but exerted a minimal effect on TRAF5-MUT-transfected cells. Moreover, the anti-Ago2 RIP assay demonstrated that miR-425-5p mimics led to a markedly high level of TRAF5 enrichment in the anti-Ago2 group (Figure 8B,C). These findings showed that miR-425-5p could target TRAF5.

4 | DISCUSSION

In the present study, we explored a novel network of the SNHG7-miR-425-5p axis in PD progression. Our data showed that downregulation of SNHG7 markedly repressed the inflammation and oxidative stress through upregulating miR-425-5p, thus inhibiting the TRAF5/NF-κB pathway. With the pathological mechanisms of PD being ceaselessly explored, PD patients are found to manifest reduced TH content, activated microglia, and decreased LDH. In addition, the activation
of inflammation and oxidative stress could be the biomarkers for the early diagnosis of PD.\cite{24,25} Thus, inhibiting the inflammation and oxidative stress of PD might bring out direct therapeutic effects for clinical treatment. For instance, niacin,\cite{26} epalrestat,\cite{27} and bruceine D\cite{28} could ameliorate PD progression via modulating inflammation and oxidative stress. In our study, we also detected the levels of proinflammatory cytokines (including IL-1β, TNF-α, and IL-6) in the serum of PD patients. It was found that the expressions of IL-1β, TNF-α, and IL-6 were overexpressed in PD patients compared with those in healthy donors, and the result was consistent with previous studies.\cite{29,30}

Multiple evidence has indicated that the aberrantly expressed lncRNAs are involved in the development of PD, a positive sign in regulating the neural development, oxidative stress, apoptosis, and neuroinflammation of PD.\cite{31–33} For example, lncRNA-UCA1 is highly expressed in MPP+-induced PD rat brain tissues and SH-SYSY cells. LncRNA-UCA1 leads to neuron damage and apoptosis by upregulating α-synuclein (SNCA).\cite{34} Furthermore, other lncRNAs such as MALAT1,\cite{35} and SNHG14\cite{36} are all overexpressed in PD and promote its progression. In this study, our data suggested that SNHG7 is upregulated in PD patients and PD models (both in vivo and in vitro). Interestingly, the high level of SNHG7 had a positive relationship with TNF-α and IL-6 in the serum of PD patients. Functionally, knockdown of SNHG7 expression not only alleviated rotenone-mediated neuronal damage and microglial activation but also significantly mitigated the levels of inflammatory cytokines and oxidative stress. Therefore, SNHG7 is considered to be a biomarker of PD and also play a role in PD progression via modulating neuronal damage and microglial activation.

MiR-425-5p is a member of miRNAs. Increasing evidence has shown that miR-425-5p is involved in the progression of cancers. For instance, miR-425-5p is overexpressed in gastric cancer (GC) and predicts poor outcomes in GC patients.\cite{37} In addition, several studies have indicated that miR-425-5p has powerful effects against inflammation. For instance, miR-425-5p was found to be downregulated in LPS-induced sepsis, and enhancing miR-425-5p level

**Figure 6** SNHG7 overexpression inhibited the neuroprotective effects of miR-425-5p on neurons. SH-SYSY cells were transfected with miR-425-5p mimics and/or SNHG7 overexpressing plasmids and then subjected to rotenone (500 nM). (A, B) The SNHG7 and miR-425-5p relative expressions in SH-SYSY cells were detected via qRT-PCR (n = 3). (C) MTT was used for the detection of SH-SYSY cells’ viability (n = 3). (D) ELISA was used for detecting LDH level in SH-SYSY cells (n = 3). (E) Western blot was used for detecting the apoptosis-related proteins (Bax, Bcl2, and caspase 3) in SH-SYSY cells (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001. ELISA, enzyme-linked immunosorbent assay; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; LDH, lactate dehydrogenase; qRT-PCR, quantitative real-time polymerase chain reaction.
FIGURE 7  SNHG7 promoted TRAF5/NF-κB activation through competitively binding to miR-425-5p. HMC3 cells were transfected with miR-425-5p mimics and/or SNHG7 overexpressing plasmids and then subjected to LPS (10 μg/ml). (A) ELISA was used to estimate the relative expression of inflammatory factors (including IL-1β, IL-6, and TNF-α) in HMC3 cells (n = 3). (B) ELISA was applied to detect the relative expression of oxidative stress factors (including MDA, SOD, and GSH-PX) in the culture medium of HMC3 cells (n = 3). (C, D) Western blot was used for detecting the expressions of TRAF5, IκB, NF-κB, Nrf2, HO-1 in HMC3 cells (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001. ELISA, enzyme-linked immunosorbent assay; GSH-PX, glutathione peroxidase; IL, interleukin; MDA, malondialdehyde; SOD, superoxide dismutase; TNF-α, tumor necrosis factor α

FIGURE 8  miR-425-5p targeted TRAF5. (A) The online database Starbase (http://starbase.sysu.edu.cn/) was used to predict the binding sites between miR-425-5p and TRAF5. (B) In SH-SY5Y cells, luciferase reporter assay was used to further verify the targeting relationship between miR-425-5p and TRAF5 (n = 3). (C) RIP assay was conducted on SH-SY5Y cells transfected with miR-425-5p mimics or miR-NC, and the expression of TRAF5 in the lysates was determined by RT-PCR. NSp > 0.05, **p < 0.001 (N = 3). RIP, RNA immunoprecipitation; RT-PCR, real-time polymerase chain reaction
markedly relieves the inflammation and septic liver damage via negatively modulating the RIP1-induced necroptosis. Interestingly, miR-425 also exerts a role in central nervous system diseases. For instance, miR-425-5p was significantly downregulated in mild traumatic brain injury (mTBI) at early time points and identified as an ideal candidate for the diagnosis of mTBI. Besides this, miR-425-5p is also considered to be a marker of antidepressant response and regulate MAPK/Wnt-system genes. Interestingly, recent studies showed that a low level of miR-425 is correlated with MPTP induced dopaminergic neuron loss, and miR-425 overexpression obviously attenuated necroptosis activation and dopaminergic neuron loss, and improved locomotor behaviors. Here, our study indicated that miR-425-5p was lowly expressed in PD patients and had negative correlations with the proinflammatory cytokines in the serum. Moreover, upregulation of miR-425-5p showed mighty effects against rotenone-induced neuronal damage and microglial inflammations. Therefore, miR-425-5p serves as a promising mediator and therapeutic target for PD.

The lncRNA-miRNA interaction has attracted accumulated attention, particularly in tumor development and inflammation. Interestingly, by conducting bioinformatics analysis, we found that miR-425-5p contains the binding sites with SNHG7. Our gain- and loss-of-assays showed that downregulating SNHG7 enhanced miR-425-5p level, while overexpressing SNHG7 led to miR-425-5p inhibition as well significantly reduced the neuroprotective and anti-inflammatory effects mediated by miR-425-5p. Thereby, we confirmed that the overexpressed SNHG7 could promote neuron damage and microglial activation at least through sponging miR-425-5p. The TRAF family has been proved to participate in the progress of pathology in PD. For example, triggering receptor expressed on myeloid cells-2 (TREM2), a newly identified receptor expressed on microglia, remarkably reduced MPTP-induced dopaminergic neurodegeneration and neuroinflammation in PD by downregulating the TRAF6/TLR4-mediated activation of the MAPK and NF-κB signaling pathways. In another study, telmisartan has neuroprotective effects in the rotenone rat model of PD through inhibition of endoplasmic reticulum stress-activated IRE1α-TRAF2-caspase-12 apoptotic pathway. TRAF5 is also a vital member of the TRAF family. Interestingly, it has been found that TRAF5 is overexpressed in NeuN- and GFAP-labeled cells in a rat acute spinal cord injury model. Moreover, TRAF5 is a mediator of several CNS diseases such as ischemic brain infarction, Huntington’s disease, and HIV-1 gp120-induced apoptosis in human neurons. In our study, we found that TRAF5 was a target of miR-425-5p by bioinformatics analysis. Downregulating SNHG7 and overexpressing miR-425-5p both inhibited TRAF5 expression in the PD model in vivo and in vitro. Moreover, as an upstream molecule of NF-κB, TRAF5 promotes NF-κB activation, a feature widely recognized by scholars. Accumulated evidence has shown that TRAF5 mediates the activation of the NF-κB signaling pathway, and engages in cell apoptosis, the occurrence of inflammation, and immune regulation. In this study, we found that miR-425-5p inhibited TRAF5, which, in turn, constrained the activation of the IκB/NF-κB pathway, thereby reducing inflammation and oxidative stress, and weakening neuronal apoptosis.

5 | CONCLUSION

In summary, this study indicated that SNHG7 was upregulated in PD patients; SNHG7 functioned as a ceRNA by sponging miR-425-5p and promoted TRAF5 mediated inflammation and oxidative stress. SNHG7 low expression or miR-425-5p overexpression profoundly attenuated neuronal apoptosis, inflammation, and oxidative stress in PD. Taken together, this study established a novel regulatory axis of SNHG7/miR-425-5p/TRA55/NF-κB axis in PD. However, more studies are needed to verify this network in the progression of PD in the future.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS STATEMENT

Our study was approved by the Ethics Review Board of XiangYang Center Hospital.

AUTHOR CONTRIBUTIONS

Operating the experiment and writing—original draft and supervision: Keqi Hu. Formal analysis and visualization: Handong Liu. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

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

ORCID

Handong Liu https://orcid.org/0000-0002-8399-9424

REFERENCES

[1] G. James, J. Marjan, F. Thomas, Brain 2015, 138, 1454.
[2] I. Aviles-Olmos, P. Linoumis, A. Lees, T. Foltynie, Brain 2013, 136, 374.
[3] J. Tianfang, S. Qian, C. Shengdi, Prog. Neurobiol. 2016, 147, 1.
[4] S. H. Tan, V. Karri, N. Tay, K. H. Chang, H. Y. Ah, P. O. Ng, H. S. Ho, H. W. Keh, M. Candasamy, Biomed. Pharmacother. 2019, 111, 765.
[5] W. L. Kuan, E. Poole, M. Fletcher, S. Karneity, P. Tyers, M. Wills, R. A. Barker, J. H. Sinclair, J. Exp. Med. 2012, 209, 1.
[6] J. L. Marques-Rocha, M. Samblas, J. Milagro, J. A. Bressan, A. Martinez, F. I. Marti, FASEB J. 2015, 29, 3595.
[7] Z. Zhou, Y. Zhu, G. Gao, Y. Zhang, Life Sci. 2019, 228, 189.
[8] X. Chen, X. Xi, F. Cui, M. Wen, A. Hong, Z. Hu, J. Ni, Artif Cells Nanomed. Biotechnol. 2019, 47, 2098.
[9] X. M. Ding, L. J. Zhao, H. Y. Qiao, S. L. Wu, X. H. Wang, Chem.-Biol. Interact. 2019, 307, 73.
[10] J. Zhao, L. Geng, Y. Chen, C. Wu, Biol. Res. 2020, 53, 1.
[11] C. Qian, Y. Ye, H. Mao, L. Yao, X. Sun, B. Wang, H. Zhang, L. Xie, H. Zhang, Y. Zhang, S. Zhang, X. He, Exp. Cell Res. 2019, 384, 111614.
[12] Y. Shan, J. Ma, Y. Pan, J. Hu, B. Liu, L. Jia, Cell Death Dis. 2018, 9(7), 722.
[13] D. Cheng, J. Fan, Y. Ma, Y. Zhou, K. Qin, M. Shi, J. Yang, Cell Biosci. 2019, 9, 28.
[14] S. Zucchelli, S. Vilotti, R. Calligaris, Z. S. Lavina, M. Biagioli, R. Foti, L. De Maso, M. Pinto, M. Gorza, E. Speretta, C. Casseler, G. Tell, G. Del Sal, S. Gustincich, Cell Death Differ. 2009, 16, 428.
[15] N. Ke, L. H. Pi, Q. Liu, L. Chen, Biochem. Biophys. Res. Commun. 2019, 514(2), 503.
