RETRACTED ARTICLE: Long noncoding RNA HAGLROS regulates apoptosis and autophagy in Parkinson’s disease via regulating miR-100/ATG10 axis and PI3K/Akt/mTOR pathway activation

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ABSTRACT
Parkinson’s disease (PD) is a common age-related neurodegenerative disease resulting from the progressive degeneration of dopaminergic neurons in the pars compacta region of substantia nigra. The goal of this study was to investigate the effects and mechanisms of long noncoding RNA (lncRNA) HAGLROS on the apoptosis and autophagy in PD. The MPTP-induced PD mouse model and MPP+-intoxicated SH-SYSY cell model were established, and the expression levels of HAGLROS and miR-100 were determined. Subsequently, the effects of suppression of HAGLROS on apoptosis and autophagy in MPTP-induced PD mouse model and in MPP+-intoxicated SH-SYSY cells were investigated. In addition, the association between HAGLROS and miR-100 as well as HAGLROS and activation of phosphoinositide-3 kinase/protein kinase-B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway in MPP+-intoxicated SH-SYSY cells was explored. HAGLROS was increasingly expressed in MPTP-induced PD mouse model and MPP+-intoxicated SH-SYSY cells and suppression of HAGLROS decreased apoptosis and autophagy in both in vivo and in vitro PD models. Further in vitro studies showed that HAGLROS negatively regulated miR-100 expression, and HAGLROS regulated apoptosis and autophagy of MPP+-intoxicated SH-SYSY cells through sponging miR-100. Moreover, ATG10 was identified as a target of miR-100. Besides, suppression of HAGLROS alleviated MPP+-intoxicated SH-SYSY cell injury by activating PI3K/Akt/mTOR pathway. Our findings reveal that upregulation of HAGLROS may contribute to the development of PD via inhibiting apoptosis and autophagy, which may be achieved by regulating miR-100/ATG10 axis and PI3K/Akt/mTOR pathway activation.

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Introduction
Parkinson’s disease (PD) is a common age-related neurodegenerative disease resulting from the progressive degeneration of dopaminergic neurons in the pars compacta region of substantia nigra [1–4]. The age at PD onset is approximately 55 years, and always occurs among people with the incidence rising from 1% in the population aged >60 years to 4% in those aged >80 years [5,6]. Despite great advance in the understanding of PD pathophysiology, current therapy is unavailable for halting this neurodegenerative process [7]. PD is a complex disease that is caused by the various risk factors, including genetic and environmental risk factors or the conjunction effects of those factors [8,9]. However, the pathogenesis of PD remains unknown. Further elucidation of the key mechanism underlying PD still has great significances for designing effective therapies for halting this disease.

Long noncoding RNAs (lncRNAs), non-protein coding transcripts longer than 200 nucleotides, are confirmed to play extensive roles in a wide range of biological and pathological processes [10–13]. LncRNAs have been considered as novel culprits or bodyguards in the development of neurodegenerative diseases [14,15]. LncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is reported to be upregulated in PD, and can regulate MPP+-intoxicated cell apoptosis in MN9D cells by sponging miR-205-5p to target LRRK2 (leucine-rich repeat kinase 2) [16]. LncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) is increased expressed in MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-treated SH-SY5Y cells as well as in MPTP mouse model and can promote the MPTP-induced autophagy in PD via inhibition of PINK1 (PTEN induced putative kinase 1) protein degradation [17]. LncRNA small nucleolar RNA host gene 1 (SNHG1) is found upregulated in MPP+-induced SH-SY5Y cells as well as in MPTP-induced mouse model of PD, and can promote α-synuclein aggregation and toxicity in SH-SY5Y cells [17]. Moreover, a preliminary report has suggested that the downregulated H19 upstream conserved 1 and 2 as well as the upregulated...
MALAT1, lncRNA-p21, TncRNA and SNHG1 may precede the course of PD [18]. These findings all confirm the potential role of key IncRNAs in PD development. Despite these, the key IncRNAs involved in PD development have not been fully disclosed. Therefore, exploration of the role of key IncRNAs in PD will further deepen our understanding of the mechanisms underlying this disease.

In a previous study, overexpression of IncRNA HAGLROS is shown to promote the progression of gastric cancer through mTOR-mediated autophagy inhibition [19]. Nevertheless, the role of HAGLROS and its underlying mechanism in PD are largely unknown. To verify these, we determined the expression of HAGLROS in the midbrain of MPTP-induced PD mouse model and in MPP⁺-intoxicated SH-SY5Y cells. Subsequently, the effects of the suppression of HAGLROS on apoptosis and autophagy in MPTP-induced PD mouse model and in MPP⁺-intoxicated SH-SY5Y cells were investigated. Notably, HAGLROS is found to regulate apoptosis and autophagy of lipopolysaccharides-induced WI-38 cells via sponging miR-100 [20]. We thus explored whether the effects of HAGLROS on apoptosis and autophagy of MPP⁺-intoxicated SH-SY5Y cells were through sponging miR-100. Moreover, the potential target of miR-100 was identified to further explore the downstream regulatory mechanism of miR-100. Besides, the phosphoinositide-3 kinase/protein kinase-B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway may contribute to PD development via regulating autophagy and subsequent inflammasome triggering [21]. We thus explored the association between HAGLROS and activation of PI3K/Akt/mTOR pathway. All efforts of our study were to provide a new insight for the discovery of new strategies for the treatment of PD.

Materials and methods

Animals and treatment

The animal experiments were performed and approved by the research committee of our hospital. Ten-week-old male C57BL/6 mice weighing 20–25 g were purchased from Chinese Academy of Medical Sciences Laboratory Animal Center (Beijing, China). The mice were housed in individual cages and had free access to standard pellet diet and water under a 12 h light/dark cycle.

To establish PD mouse model, the mice were intraperitoneal injected with 30 mg/kg/day of MPTP-HCl (Sigma, St. Louis, MO) for 4 consecutive days. An equivalent volume of 0.9% sterile saline used as the negative control (NC) was also injected into mice. Mice were randomly sacrificed at 0, 1, 3, 5 and 7 days after the last MPTP-HCl injection (N=6 in each treatment). The ventral midbrain containing the SNpc of mice was then resected and stored at −80 °C.

For exogenous delivery of HAGLROS in PD mice, short hairpin RNA (shRNA) recombinant lentivirus vectors were established by inserting shRNA-targeting HAGLROS (sh-HAGLROS) or sh-control (GeneChem Co. Ltd, Shanghai, China) into linearized vector GV115 carrying green fluorescent protein (GFP) gene, named recombinant lenti-sh-control or lenti-sh-HAGLROS. Subsequently, mice were deep anaesthesia with isoflurane in oxygen and nitrous oxide and then placed into a stereotaxic frame (Stoelting, Wood Dale, IL). The skull surface was exposed and a hole was drilled to achieve needle positioning. Using a 5-μl Hamilton syringe with a 33-gauge tip needle, mice were then bilateral injected with 1 μl side of recombinant lenti-sh-control or lenti-sh-HAGLROS into the dorsal hippocampus (anterior −5.5; lateral +1.6; dorsoventral −7.5 from bregma) at a rate of 0.2 μl/min for 10 min. Two days after vector injection, MPTP-HCl or 0.9% sterile saline was administrated as above description. Taken together, 48 C57BL/6 mice were randomly divided into four groups, including NC, MPTP, MPTP+sh-HAGLROS and MPTP+sh-control groups (N=6 in each group). The ventral midbrain was also resected and stored at −80 °C.

Terminal deoxynucleotidyltransferase-biotin nick end-labelling (TUNEL) assay

The isolated midbrain from different treatments was fixed with 4% paraformaldehyde, dehydrated, paraffin-embedded and sectioned. Subsequently, the sections were permeabilized with 20 μg/ml proteinase K (Solarbio Science & Technology Co., Ltd., Beijing, China) for 10 min, followed by detection of the apoptosis-specific nuclear DNA fragmentation using the In Situ Cell Death Detection Kit (Roche Molecular Bioscience, Mannheim, Germany). The TUNEL-positive cell nuclei that were stained brown were visualized under an Axiovert 200 fluorescence microscope (Olympus, Tokyo, Japan) and captured with a Photometrics SenSys cooled CCD camera (Roper Scientific, Tucson, AZ). The percentage of apoptotic cells was then analysed by counting the number of TUNEL-positive cells and total cells in five randomly selected high fields.

Cell culture and transfection

Human neuroblastoma cell line SH-SY5Y (American Type Culture Collection, Manassas, VA) was cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum (FBS; Gibco, Grand Island, NY) in a 37 °C incubator with 5% CO₂. To establish cellular PD model in vitro, SH-SY5Y cells were treated with 0.25, 0.5 or 1 mM MPP⁺ (Sigma, St. Louis, MO) for 24 h.

For detecting the role of HAGLROS and miR-100 in PD, SH-SY5Y cells were then transfected with shRNA against HAGLROS (sh-HAGLROS), shRNA control (si-NC), miR-100 mimic, mimic NC, miR-100 inhibitor and inhibitor NC (Ambion, Foster City, CA) using Lipofectamine 2000 (Invitrogen, San Diego, CA), followed by the treatment of 1 mM MPP⁺ solution for 24 h.

Real-time quantitative PCR (qPCR)

Total RNA was extracted from the resected midbrain and cultured SH-SY5Y cells using TRIzol Reagent (Invitrogen, San Diego, CA), followed by detection of the concentration and purity of the isolated RNA using SMA 400 UV-VIS (Merinton, Shanghai, China). Reverse transcription into complementary DNA (cDNA) from the isolated RNA was conducted using the
reverse transcription system kit (Invitrogen, San Diego, CA). Real-time qPCR for detecting gene expression was conducted using SYBR Green PCR kit on a CFX96 real-time PCR System (Bio-Rad, Hercules, CA). The reaction conditions were as below: 95 °C for 10 min, 45 cycles of 20 s at 95 °C, 30 s at 60 °C and 20 s at 72 °C. GAPDH was used as the internal control and the relative gene expression levels were calculated by the 2^ΔΔCt method.

**Western blot**

Total protein samples were extracted from mouse midbrain or SH-SY5Y cells using NP-40 lysis buffer (Beyotime, Haimen, China) and then quantified with the Bradford Protein Assay Kit (Beyotime, Haimen, China). Equal amounts of protein samples (30–50 μg) were loaded on 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). Subsequently, the membranes were blocked with 5% non-fat milk for 1 h at room temperature, incubated with appropriate primary antibodies (1:1500; Abcam, Cambridge, MA) overnight at 4 °C and following horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000; Cell Signaling Technology, Danvers, MA) for 1 h at 37 °C. Primary antibodies against Bcl-2, Bax, pro-caspase-3, cleaved-caspase-3, Cyc, LC3-II, LC3-I, Beclin-1, p62, ATG10, p-PI3K, PI3K, p-AKT, AKT, p-mTOR, mTOR and β-actin (1:1500; Cell Signaling Technology, Danvers, MA) were all purchased from Abcam (Cambridge, MA). β-Actin was used as the internal control. Subsequently, the protein blots were visualized with ECL chemiluminescent reagents (Pierce, Rockford, IL) and normalized to the internal control β-actin.

**Flow cytometry analysis**

Following different treatments, SH-SY5Y cells were collected and resuspended in the annexin V binding buffer. Subsequently, SH-SY5Y cells were stained with 5 μL Annexin V-FITC and 5 μL propidium iodide (PI) at room temperature for 15 min according to the recommend protocols of Annexin V-FITC Apoptosis Detection Kit (Beyotime Institute of Biotechnology, Shanghai, China). The percentage of apoptotic cells was then analysed by flow cytometry using BD FACSDiva software V6.1.3 (BD Biosciences, San Jose, CA).

**Luciferase reporter assay**

The sequence of ATG10 carrying the predicted binding site of miR-100 was synthesized from Sangon (Shanghai, China) and cloned into the downstream of the Renilla luciferase gene of pGL3 vectors (Promega, Madison, WI) to form the reporter vector pGL3-ATG10-wild-type (ATG10-wt). To mutate the potential miR-100 binding sites in ATG10 gene, nucleotide-substitution mutation analysis was conducted using a QuikChange Site-Directed Mutagenesis kit (Agilent Technologies, Palo Alto, CA). The mutant fragment of ATG10 was also inserted into pGL3 vectors to form reporter vector pGL3-ATG10-mutant (ATG3-mut). pRL-TK vector was used as an internal control. SH-SY5Y cells were then co-transfected with 50 ng luciferase vectors (ATG10-wt or ATG3-mut), 10 ng pRL-TK vectors and 50 nM miR-100 or miR-control by Lipofectamine 2000 (Invitrogen, San Diego, CA). After incubating the cells for 48 h, the luciferase activity was then measured using the Dual-Glo Luciferase assay system (Promega, Madison, WI).

**Statistical analysis**

All experiments were repeated three times. Quantitative results were expressed as mean ± standard deviation (SD). Statistical differences between groups were analysed using one-way ANOVA by means of GraphPad Prism 7.0 software (GraphPad Prism, San Diego, CA). p Value < .05 was considered to be statistically significant.

**Results**

**MPTP induced PD mouse model and MPP⁺ intoxicated SH-SYSY cells**

To investigate whether MPTP successfully induced PD mouse model, apoptosis and autophagy were detected. The results of the TUNEL assay showed that MPTP injection significantly induced apoptosis of midbrain in a time-dependent manner (p < .05, Figure 1(A)). Consistent changes in the expression of apoptosis-related proteins were also obtained that MPTP injection dramatically decreased Bcl-2 expression and increased the expression of Bax, pro/cleaved-caspase-3 and Cyc in a time-dependent manner (p < .05, Figure 1(B)). In addition, we found that MPTP injection significantly increased the expression of LC3-II/I and Beclin-1 and obviously decreased p62 expression in midbrain in a time-dependent manner (p < .05, Figure 1(C)), indicating that MPTP injection significantly promoted autophagy of midbrain. On the other hand, whether MPP⁺ intoxicated SH-SYSY cells was also investigated by detection of cell apoptosis and autophagy. The results of flow cytometry showed that MPP⁺ treatment significantly promoted apoptosis of SH-SYSY cells in a dose-dependent manner (p < .05, Figure 1(D)). Also, MPP⁺ treatment resulted in a remarkable downregulation of Bcl-2 expression and obvious upregulation of the expression of Bax, pro/cleaved-caspase-3 and Cyc in SH-SYSY cells in a dose-dependent manner (p < .05, Figure 1(E)). Furthermore, MPP⁺ treatment promoted autophagy of SH-SYSY cells by significantly increasing the expression of LC3-II/I and Beclin-1 and decreasing p62 expression (p < .05, Figure 1(F)). These data indicated the PD mouse model and cellular model were all successfully established.

**HAGLROS was upregulated and miR-100 was downregulated in MPTP-induced PD mouse model and in MPP⁺-intoxicated SH-SYSY cells**

To explore the role of HAGLROS and miR-100 in PD, the expressions of HAGLROS and miR-100 were determined in the
midbrain of MPTP-induced PD mouse model and MPP⁺-intoxicated SH-SYSY cells. The results showed that HAGLROS expression in mouse midbrain was significantly increased after MPTP injection (p < .05, Figure 1(G)), whereas miR-100 expression was markedly decreased after MPTP injection (p < .05, Figure 1(H)). Similarly, inverse expression between HAGLROS (upregulated) and miR-100 (downregulated) was also obtained in MPP⁺-intoxicated SH-SYSY cells (p < .05, Figure 1(J)).
Suppression of HAGLROS attenuated apoptosis and autophagy in MPTP-induced PD mouse model and in MPP⁺-intoxicated SH-SY5Y cells

Subsequently, the effects of the suppression of HAGLROS on apoptosis and autophagy of midbrain in MPTP-induced PD mouse model were first investigated. As shown in Figure 2(A), HAGLROS expression in the sh-HAGLROS group was significantly lower than that in the sh-NC group (p < .01), indicating that HAGLROS expression was successfully suppressed in the midbrain of MPTP-induced PD mouse models. TUNEL assay showed that MPTP could significantly promote apoptosis of midbrain compared with the control group, whereas lentivirus-mediated HAGLROS suppression strikingly inhibited this effect (p < .001, Figure 2(B)). Likely, lentivirus-mediated HAGLROS suppression conspicuously reversed the effect of MPTP on the expression of apoptosis-related proteins, in other words, Bcl-2 expression was significantly upregulated and the expression of Bax, pro-cleaved-caspase-3 and Cyc was downregulated in MPTP + sh-HAGLROS group with respect to those in MPTP + sh-NC group (p < .001, Figure 2(C)). Moreover, lentivirus-mediated HAGLROS suppression also attenuated autophagy of midbrain in MPTP-induced PD mouse model by conspicuously decreasing the expression of LC3-II/I and Beclin-1 and increasing p62 expression (p < .05, Figure 2(D)). On the other hand, the effects of the suppression of HAGLROS on apoptosis and autophagy of MPP⁺-intoxicated SH-SY5Y cells were also investigated. HAGLROS was successfully suppressed in SH-SY5Y cells by transfection with sh-HAGLROS#1 and sh-HAGLROS#2 compared with sh-NC (p < .05, Figure 2(E)). As the inhibition efficiency of sh-HAGLROS#2 was stronger than sh-HAGLROS#1, sh-HAGLROS#2 was thus used for subsequently knockdown experiments. The results of flow cytometry showed that the suppression of HAGLROS significantly inhibited apoptosis of MPP⁺-intoxicated SH-SY5Y cells (p < .01, Figure 2(F)). Moreover, suppression of HAGLROS exhibited the consistent effects on the expression changes of apoptosis-related proteins in MPP⁺-intoxicated SH-SY5Y cells (p < .01, Figure 2(G)). Besides, suppression of HAGLROS significantly inhibited autophagy of MPP⁺-intoxicated SH-SY5Y cells via remarkably decreasing the expression of LC3-II/I and Beclin-1 and increasing p62 expression (p < .05, Figure 2(H)). These data indicated that the suppression of HAGLROS attenuated apoptosis and autophagy in MPTP-induced PD mouse model and in MPP⁺-intoxicated SH-SY5Y cells.

HAGLROS negative regulated miR-100 expression and the effects of HAGLROS on apoptosis and autophagy of MPP⁺-intoxicated SH-SY5Y cells through sponging miR-100

It has been reported that IncRNAs could serve as a competing endogenous RNA (ceRNA) to modulate mRNA expression

Figure 2. Suppression of HAGLROS attenuated apoptosis and autophagy in MPTP-induced PD mouse model and in MPP⁺-intoxicated SH-SY5Y cells. (A) HAGLROS expression in the midbrain of MPTP-induced PD mouse models transfected with sh-HAGLROS and sh-NC. (B) TUNEL assay showed apoptosis of mouse midbrain after different treatments. (C) Western blot showed the expression changes of apoptosis-related proteins in mouse midbrain after different treatments. (D) Western blot showed the expression changes of autophagy-related proteins in mouse midbrain after different treatments. (E) HAGLROS expression in SH-SY5Y cells transfected with sh-HAGLROS#1, sh-HAGLROS#2 and sh-NC. (F) Flow cytometry showed apoptosis of SH-SY5Y cells after different treatments. (G) Western blot showed the expression changes of apoptosis-related proteins in SH-SY5Y cells after different treatments. (H) Western blot showed the expression changes of autophagy-related proteins in SH-SY5Y cells after different treatments. Data were expressed as mean ± standard deviation (SD) (n = 3). *p < .05, **p < .01 and ***p < .001 compared with control.
by sponging miRNA [22]. HAGLROS has been shown to regulate apoptosis and autophagy of lipopolysaccharides-induced Wi-38 cells through sponging miR-100 [20]. In this study, we found that inverse expression of HAGLROS (upregulated) and miR-100 (downregulated) was obtained in MPTP-induced PD mouse model and in MPP⁺-intoxicated SH-SYSY cells (Figure 3), we thus hypothesized that HAGLROS might play a key role in AD via regulating miR-100. We further investigated the expression of miR-100 in SH-SYSY cells transfected with sh-HAGLROS#1 and sh-HAGLROS#2. The results showed that miR-100 expression was remarkably increased in SH-SYSY cells after transfection with sh-HAGLROS#1 and sh-HAGLROS#2 compared with sh-NC (p < .05, Figure 3(A)), indicating that HAGLROS negative regulated miR-100 expression. We further overexpressed and suppressed the expression of miR-100 by transfection with miR-100 mimic and miR-100 inhibitor and the high transfection efficiency was confirmed by qPCR (p < .05, Figure 3(B)). Subsequently, the combined effects of the suppression of HAGLROS and inhibition of miR-100 concurrently on apoptosis and autophagy of MPP⁺- intoxicated SH-SYSY cells were explored. The results showed that the effects of suppression of HAGLROS alone on apoptosis and autophagy of MPP⁺-intoxicated SH-SYSY cells were strikingly reversed after the suppression of HAGLROS and inhibition of miR-100 concurrently (p < .05, Figure 3(C–E)).

**ATG10 was a target of miR-100**

To further explore the downstream regulatory mechanism of miR-100, the potential target of miR-100 was predicted using Targetscan software. As shown in Figure 4(A), ATG10 was predicted as a potential target of miR-100 (http://www.targetscan.org/cgi-bin/targetscan/vert_71/view_gene.cgi?rs=ENST00000282185.3&taxid=9606&members=miR-100-3p&showcnc=0&shownc=0&showncf1=1&showncf2=1&subset=1). To further verify the interaction between miR-100 and ATG10, luciferase reporter assay was performed. The luciferase activities of ATG-wt were significantly inhibited in miR-100-overexpressing cells (p < .05), but the luciferase activities of ATG-mut did not exhibit obvious change (Figure 4(B)). Furthermore, qRT-PCR was carried out to detect ATG10 expression in SH-SYSY cells after transfection with miR-100 mimic and miR-100 inhibitor. The results showed that overexpression of miR-100 markedly suppressed ATG10 expression, while inhibition of miR-100 distinctly enhanced ATG10 expression (p < .01, Figure 4(C)). Likely, western blot showed that the protein expression of ATG10 exhibited consistent changes in SH-SYSY cells after transfection with miR-100 mimic and miR-100 inhibitor (Figure 4(D)). These data indicated that ATG10 was a target of miR-100 and was negative regulated by miR-100.

**Inhibition of miR-100 aggravated apoptosis and autophagy of MPP⁺-intoxicated SH-SYSY cells by targeting ATG10**

To further detect whether miR-100 regulated apoptosis and autophagy of MPP⁺-intoxicated SH-SYSY cells by targeting ATG10, ATG10 was knocked down by transfection of si-ATG10, followed by detection of the combined effects of miR-100 inhibitor and si-ATG10 on apoptosis and autophagy of MPP⁺-intoxicated SH-SYSY cells. As shown in Figure 5(A), the mRNA and protein expression levels of ATG10 in si-ATG10 group were significantly lower than that in si-NC group (p < .01), indicating that ATG10 was successfully knocked down by transfection of si-ATG10. In addition, our results showed that inhibition of miR-100 significantly promoted apoptosis (Figure 5(B,C)) and autophagy (Figure 5(B,C)) of MPP⁺-intoxicated SH-SYSY cells, which were reversed after inhibition of miR-100 and knockdown of ATG10 concurrently (all p < .05, Figure 5(B–D)). These data indicated that inhibition of miR-100 aggravated apoptosis and autophagy of MPP⁺-intoxicated SH-SYSY cells by targeting ATG10.

**Effects of HAGLROS on apoptosis and autophagy of MPP⁺-intoxicated SH-SYSY cells were possible by through PI3K/AKT/mTOR pathway**

It is reported that dysregulation of the PI3K/Akt/mTOR pathway is found to be associated with the loss of dopaminergic neurons in PD [23]. PI3K/AKT/mTOR pathway plays a key role in the modulation of the autophagy in neuronal protection [24]. Therefore, whether HAGLROS contributed to PD via regulating the activation of PI3K/AKT/mTOR pathway was investigated. As shown in Figure 5(E), MPP⁺ treatment resulted in remarkable decreases in the expression levels of p-PI3K, p-AKT and p-mTOR, indicating that MPP⁺-intoxicated SH-SYSY cells, which were further reversed by the suppression of HAGLROS and inhibition of miR-100 concurrently. These data indicated that the effects of HAGLROS on apoptosis and autophagy of MPP⁺-intoxicated SH-SYSY cells were possible by PI3K/AKT/mTOR pathway.

**Discussion**

The effects and mechanisms of HAGLROS on PD development were investigated using the MPTP-induced PD mouse model and MPP⁺-intoxicated SH-SYSY cells. Our results showed that HAGLROS was increasingly expressed in in vivo and in vitro PD model. Animal and cellular studies demonstrated that the suppression of HAGLROS decreased cell apoptosis and autophagy. Further in vitro studies showed that HAGLROS positively regulated miR-100 expression, and HAGLROS regulated apoptosis and autophagy of MPP⁺-intoxicated SH-SYSY cells through sponging miR-100. Moreover, ATG10 was identified as a target of miR-100. Besides, our results showed that the suppression of HAGLROS alleviated MPP⁺-intoxicated SH-SYSY cell injury by activating PI3K/AKT/mTOR pathway. These findings suggest the key role of HAGLROS in PD.

Apoptosis and autophagy are important physiologic processes mediating brain homeostasis. The dysfunction of apoptosis and autophagy contributes to the development of
Figure 3. HAGLROS negatively regulated miR-100 expression and the effects of HAGLROS on apoptosis and autophagy of MPP⁺-intoxicated SH-SY5Y cells through sponging miR-100. (A) miR-100 expression in SH-SY5Y cells after transfection with sh-HAGLROS#1, sh-HAGLROS#2 and sh-NC. (B) miR-100 expression in SH-SY5Y cells after transfection with miR-100 mimic, miR-100 inhibitor and their controls. (C) Flow cytometry showed apoptosis of SH-SY5Y cells after different treatments. (D) Western blot showed the expression changes of apoptosis-related proteins in SH-SY5Y cells after different treatments. (E) Western blot showed the expression changes of autophagy-related proteins in SH-SY5Y cells after different treatments. Data were expressed as mean ± standard deviation (SD) (n = 3). *p < .05, **p < .01 and ***p < .001 compared with control.
neurodegenerative disorders, including PD [25–27]. Apoptosis is identified as an essential signal for neuronal degradation in PD [28]. Growing evidences obtained from animal models of PD and PD patients have confirmed that autophagy plays a pivotal role in the pathogenesis of PD [29,30]. In this study, HAGLROS was increased in MPTP-induced PD mouse model and MPP⁺-intoxicated SH-SY5Y cells, and suppression of HAGLROS decreased apoptosis and autophagy in in vivo and in vitro PD model. Consistent with the previous findings that HAGLROS could regulate apoptosis and autophagy of lipopolysaccharides-induced WI-38 cells [20], we thus speculate that HAGLROS may contribute to the development of PD through regulating apoptosis and autophagy.

Furthermore, it is revealed that IncRNAs function as competitive endogenous RNA (ceRNA) to competetively regulate the expressions of specific miRNAs [31]. miRNAs, small, endogenous, non-protein coding RNAs, have been shown to participate to the diagnosis, pathogenesis and therapy of PD [32–34]. Several miRNAs are also identified as promising biomarkers or therapeutic targets for PD [35,36]. In addition, miR-7 is shown to play neuroprotective effects in a cellular PD model through inhibiting neuronal apoptosis [37]; upregulation of miR-124 can reduce the loss of dopaminergic neurons in the model of PD by regulating autophagy and apoptosis [38]. These findings suggest that miRNAs may involve in PD by regulating cell autophagy and apoptosis. In this study, we found that HAGLROS regulated apoptosis and autophagy of MPP⁺-intoxicated SH-SY5Y cells through sponging miR-100 [20]. Based on our results, we speculate that HAGLROS may regulate cell apoptosis and autophagy in PD through modulating miR-100.

Furthermore, miRNAs are shown to regulate a variety of biological processes through targeting the 3'UTR of mRNAs [41]. In this study, ATG10 was identified as a target of miR-100 and its expression was negatively regulated by miR-100. ATG10 is shown to participate in autophagosome formation in the reaction of ATG5–ATG12 conjugation [42]. Moreover, ATG10 expression is found necessary for autophagy initiation, and may play a role in PD via modulating autophagy [43]. Our results showed that the inhibition of miR-100 aggravated apoptosis and autophagy of MPP⁺-intoxicated SH-SY5Y cells by targeting ATG10, confirming that ATG10 was a downstream regulatory mechanism of HAGLROS/miR-100 in regulating apoptosis and autophagy in PD.

Besides, several cellular signalling pathways, including the PI3K/AKT/mTOR pathway, have been shown to be involved in autophagy process and consequently clearance of protein aggregates in neurodegeneration [44]. PI3K/AKT/mTOR pathway plays a key role in the modulation of the autophagy in neuronal protection [24]. Moreover, dysregulation of the PI3K/Akt/mTOR pathway could result in loss of dopaminergic neurons in PD [23]. Activation of the PI3K/AKT pathway could mediate the neuroprotective effect of caffeine in a cellular model of PD [45]. Furthermore, mTOR is proved to be a key regulator to drive apoptosis and autophagy, and AMPK/mTOR-dependent autophagy may regulate the induction of oxidative stress and subsequent apoptotic death in SH-SY5Y cells [46]. In this study, we found a clear downregulation of the PI3K/AKT/mTOR pathway in MPP⁺-intoxicated SH-SY5Y cells as proven by lower phosphorylation levels of PI3K, Akt and mTOR. Suppression of HAGLROS significantly increased...
Figure 5. Inhibition of miR-100 aggravated apoptosis and autophagy of MPP⁺-intoxicated SH-SY5Y cells by targeting ATG10. (A) ATG10 expression in SH-SY5Y cells after transfection with si-ATG10 and si-NC. (B) Flow cytometry showed apoptosis of SH-SY5Y cells after different treatments. (C) Western blot showed the expression changes of apoptosis-related proteins in SH-SY5Y cells after different treatments. (D) Western blot showed the expression changes of autophagy-related proteins in SH-SY5Y cells after different treatments. (E) The expression of PI3K/AKT/mTOR pathway-related proteins in MPP⁺-intoxicated SH-SY5Y cells after suppression of HAGLROS and/or inhibition of miR-100. Data were expressed as mean ± standard deviation (SD) (n = 3). *p < .05, **p < .01 and ***p < .001 compared with control.
the expression levels of p-PI3K, p-AKT and p-mTOR in MPP\(^+\)-intoxicated SH-SY5Y cells, which were further reversed by suppression of HAGLROS and inhibition of miR-100 concurrently. These results suggest that the suppression of HAGLROS alleviated MPP\(^+\)-intoxicated SH-SY5Y cell injury by activating PI3K/AKT/mTOR pathway.

In conclusion, our findings reveal that upregulation of HAGLROS may contribute to the development of PD via inhibiting apoptosis and autophagy. The miR-100/ATG10 axis inhibiting apoptosis and autophagy. The miR-100/ATG10 axis may be key downstream mechanisms to mediate the role of HAGLROS in PD development. Our study provided a new strategy for the treatment of PD. Further studies are still required to verify our findings.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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