H2S alleviates Parkinson-like phenotypes by modulating lncRNA-CasC7/miR-30c/BNIP3L signaling pathway

Type

Research paper

Keywords

Parkinson's disease, hydrogen sulfide, lncRNA-CasC7, miR-30c, BNIP3L

Abstract

Introduction

The pathogenesis of Parkinson's disease (PD) is closely related to mitophagy, a process regulated by miRNAs and long non-coding RNAs (lncRNAs). In this study, we investigated the role of the lncRNA-CasC7/miR-30c/BNIP3L (BCL2 Interacting Protein 3 Like) signaling pathway in PD.

Material and methods

A 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD was generated and treated by hydrogen sulfide (H₂S) inhalation. The SH-SY5Y cell model of PD was generated by MPP⁺ and the treatment was NaHS. The open field, rotarod and tail suspension tests were performed to assess motor deficits. TUNEL and immunofluorescence assays were used to evaluate neuronal apoptosis in mice and in SH-SY5Y cell culture. Real-time PCR was performed to measure the expression level of the lncRNA-CasC7, miR-30c and BNIP3L, and western blotting was used to assess the protein levels of CBS, BNIP3L and PINK1. Luciferase assays were conducted to examine the regulatory relationship between miR-30c and lncRNA-CasC7/BNIP3L.

Results

H₂S inhalation alleviated the motor disorder and neuronal apoptosis in PD mice, and NaHS treatment inhibited apoptosis in the SH-SY5Y cell culture model of PD. The sulfide compounds also ameliorated the dysregulated expression of CasC7, miR-30c, BNIP3L, and PINK1 in the PD models. Furthermore, miR-30c significantly inhibited the expression of lncRNA-CasC7 and BNIP3L, as assessed with the luciferase assays.

Conclusions

Our findings suggest that the lncRNA-CasC7/miR-30c/BNIP3L mitophagy signaling pathway is involved in the pathogenesis of PD.

H₂S alleviates Parkinson-like phenotypes by modulating lncRNA-CasC7/miR-30c/BNIP3L signaling pathway

* Ling Long and Xiaodong Cai contributed equally to this study.

Running title: H₂S affects PD via lncRNA-CasC7/miR-30c/BNIP3L

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Key words: Parkinson's disease, hydrogen sulfide, lncRNA-CasC7, miR-30c, BNIP3L

Preprint

Introduction

Parkinson's disease (PD) is characterized by progressive and chronic neuronal degeneration in the substantia nigra [1, 2]. While the pathogenesis of PD remains unclear, many studies have shown a protective role of hydrogen sulfide (H_2S) in neurons, and inhaled H_2S has been reported to prevent the neurodegeneration and progression of PD in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD [3]. Moreover, chronic H_2S inhalation has been demonstrated to have a neuroprotective effect on dopaminergic neurons in animal models of PD [4].

Mitophagy is a critical quality control process that selectively eliminates damaged or superfluous mitochondria in eukaryotic cells [5]. Mitochondria are the hub of cellular metabolism and the primary site of energy generation; therefore, dysregulation of mitochondrial functions can impair programmed cell death. Indeed, a wide range of pathological conditions, including many neurodegenerative diseases, have been linked to mitochondrial dysfunction. In particular, PD is a neurological disease caused by the degeneration of neurons in the midbrain. Different types of familial PD and several sporadic PD cases are associated with defective mitophagy functions caused by insufficient degradation of RHOT1/Miro1, both of which are mitochondrial kinesin adaptor proteins, and the inability to remove damaged mitochondria [6, 7].

H_2S has been reported to exert a neuroprotective effect by increasing the expression of cancer susceptibility candidate 7 (CasC7, a ~9.3 kb lncRNA) in a rat model of spinal cord ischemic injury [8]. Furthermore, lncRNA-CasC7 can function as a decoy of miR-30c, and inhibitors of miR-30c can diminish the effects of si-CasC7 [8]. Moreover, as competing endogenous RNAs (ceRNAs) of miRNAs, lncRNAs can bind to miRNAs and

affect their regulation of the expression of their target genes. For example, CasC7 knockdown was shown to increase the expression of miR-30c, and thereby downregulate the expression levels of its target genes, such as Bcl-1, in SH-5YSY cells exposed to oxygen-glucose deprivation/reoxygenation (OGD/R) injury. Furthermore, H₂S upregulates the expression of lncRNA-CasC7, and overexpression of lncRNA-CasC7 downregulates miR-30c expression [8, 9].

Gao et al. reported that the mitochondrial protein BNIP3L, as a substrate of PARK2, participates in the pathogenesis of PD by promoting mitophagy and by thereby regulating neuronal apoptosis [10]. PTEN-induced kinase 1 (PINK1), which is mitochondrially localized and plays a role in cytoprotection against cellular stress, was also reported to be involved in the pathogenesis of PD [11]. Furthermore, cystathionine β -synthase (CBS) overexpression increases sulfide generation and perturbation of the CBS–H₂S pathway was reported to contribute to the pathogenesis of PD [12]. In this study, we investigate the neuroprotective effect of H₂S in cell culture and animal models of PD, and we examine its impact on the expression of BNIP3L, PINK1 and CBS to clarify the underlying mechanisms of action.

Materials and Methods

Animals and PD model production

All experimental procedures complied with the procedures approved by the Animal Care Committee of our hospital. Weight-matched 10-week-old male C57BL/6J mice were purchased from the Animal Center of Sun Yat-sen University (Guangzhou, China) and subjected to a 7-day acclimation period with unlimited access to water and food. The

animals were housed in a room maintained at 22 ± 2 °C, under a 12-h/12-h light/dark cycle. The mice were divided into four groups, with 12 mice in each group, i.e., a control group, a control + H₂S group, an MPTP group, and an MPTP + H₂S group. The mice in the control group were given four intraperitoneal injections of saline with 2 h between each injection. The mice in the MPTP group were given four intraperitoneal injections of 25 mg/kg MPTP (Sigma-Aldrich, St. Louis, MO, USA) dissolved in saline with a 2-h interval between each injection. The mice in the control + H₂S group were given intraperitoneal injections of saline in the same way as the mice in the control group plus inhalation of 40 ppm H₂S for 8 h per day. Finally, the mice in the MPTP + H₂S group were given four intraperitoneal injections of 25 mg/kg MPTP, with 2 h between each injection, plus inhalation of 40 ppm H₂S for 8 h per day. The inhalation of H₂S was continued for 7 days. During the treatment period, the rectal temperature of mice in all groups was monitored daily. Finally, all mice were euthanized on day 7, and their brains were collected.

Behavioral testing

All behavioral tests were carried out by two independent investigators who were blind to the treatment groups. In the open field test, the mice were individually housed in rectangular plastic cages and were allowed to move freely for 5 min to adapt to the experimental environment. The floor of each cage had a 10-cm² black grid to enable the distance travelled by each mouse to be recorded. The number of rearings in the open field, a measure of exploratory activity, was counted over a period of 1 h. For the rotarod test, mice were trained for five sessions. Mice were then placed on the rotating rod, and the speed was increased from 0 rpm to 60 rpm over a 5-min period. The time that each mouse

remained on the rotating rod, i.e., the fall latency, was used as a measure of motor function. In the tail suspension test, mice were suspended in the air for 5 min by their tails, and the time spent immobile was recorded.

RNA isolation and real-time PCR

Total RNA was extracted using TRI reagents (Sigma-Aldrich) from tissue and cell samples according to the manufacturer's instructions. Equal amounts of RNA from the samples were reverse transcribed into cDNA using the Quanti Tect Reverse Transcription Assay Kit (Qiagen, Valencia, CA, USA). cDNA templates were subjected to real-time PCR using an LC480 real-time PCR machine and SYBR Green Master Mix (Roche, Basel, Switzerland) following the standard protocol recommended by the manufacturer. The expression levels of lncRNA-CasC7 (forward: 5'-TGTTTGATTTCCTCGTCGCT-3'; reverse: 5'-GGCACCGTAATGGCACTG-3'), miR-30c (forward: 5'-TGTAACATCCTACACTCTCAGC-3'; reverse: 5'-CAGTGCGTGTCTGGAGT 3'), and BNIP3L mRNA (forward: 5'-TCAGGAACTTCTTCTGCTCACA-3'; reverse: 5'-AGGTTCCCAGGTACT-3') were calculated using the $2^{-\Delta\Delta C_t}$ methods and normalized to the expression of the housekeeping gene, GAPDH (forward: 5'-CTGCCAACGTGTCAGTGGTG-3'; reverse: 5'-TCAGTGTAGCCCAGGATGCC-3').

Cell culture and treatment

SH-SY5Y cells were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences and cultured in RPMI-1640 medium containing penicillin, streptomycin and 10% heat-inactivated fetal bovine serum. The culture conditions were 37 °C, 95% air/5% CO₂, and saturated humidity. When the cells reached logarithmic growth, they were divided into the following four groups: (1) Control group; (2) Control + 200μM NaHS

group; (3) 2 mM active toxic cation 1-methyl-4-phenylpyridinium (MPP⁺, the active metabolite of MPTP) group; and (4) 2 mM MPP⁺ + 200 μM NaHS group. The cells were treated for a period of 7 days prior to subsequent assays.

Vector construction, mutagenesis and luciferase assays

To examine the interaction between lncRNA-CasC7 and miR-30c and between miR-30c and BNIP3L, the 3'-UTR of BNIP3L and the promoter of lncRNA-CasC7 containing the miR-30c binding sites were cloned into pmiR-RB luciferase vectors (Ribobio, Guangzhou, China) to generate wild-type BNIP3L and lncRNA-CasC7 plasmids, respectively. Mutagenesis was performed on the miR-30c binding sites in the BNIP3L 3'-UTR and lncRNA-CasC7 promoter, and the resulting sequences were also cloned into pmiR-RB to generate mutant BNIP3L and lncRNA-CasC7 plasmids. SH-SY5Y cells in the logarithmic growth phase were transfected with wild-type or mutant BNIP3L and lncRNA-CasC7 vectors in conjunction with miR-30c using Lipofectamine 3000 (Invitrogen, Carlsbad, CA) following the standard protocol of the manufacturer. Then, 48 h after transfection, the luciferase activity of transfected cells was measured using a Bright-Glo luciferase assay kit (Promega, Madison, WI) according to the manufacturer's instructions.

Cell proliferation assay

SH-SY5Y cells were plated into 96-well tissue culture plates at 2×10^4 cells/well and divided into the following treatment groups: (1) Control group; (2) Control + NaHS group; (3) MPP⁺ group; and (4) MPP⁺ + NaHS group. A CCK8 kit (Beyotime, Beijing, China) was used to assess cell viability according to the manufacturer's instructions.

Western blot analysis

Cell and tissue samples were lysed in RIPA lysis buffer (Beyotime), and total protein was collected by centrifugation. Equal quantities of protein from the various samples were resolved by 10% SDS-PAGE and blotted onto polyvinylidene fluoride membranes. Membranes were then blocked with Tris-buffered saline containing 5% skimmed milk and incubated at 4 °C overnight with anti-CBS, anti-BNIP3L, and anti-PINK1 primary antibodies (1:1000 dilution; all purchased from Abcam, Cambridge, MA). After several washes with TBS containing 0.1% Tween-20, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 2 h at room temperature, and developed with enhanced chemiluminescence reagents (Beyotime) and visualized on an E-Gel Imager (Thermo Fisher Scientific, Waltham, MA, USA). The intensities of the target protein bands were quantified relative to the internal control housekeeping gene, β -actin.

Immunofluorescence microscopy

Immunofluorescence assays were performed to evaluate the apoptosis of SH-SY5Y cells exposed to MPP⁺. SH-SY5Y cells, cultured in 96-well tissue culture plates, were fixed in 4% paraformaldehyde, permeabilized for 10 min with PBS containing 0.3% Triton X-100, blocked with 10% FBS, and then stained at room temperature for 1 h with the corresponding FITC-labeled secondary antibody (1:100 dilution; Sigma-Aldrich). The SH-SY5Y cells were then counter-stained with DAPI (Thermo Fisher Scientific) and examined by fluorescence microscopy.

ELISA assay

H₂S content in cell supernatants was determined using an ELISA assay (Thermo Fisher Scientific) following the manufacturer's instructions.

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay

The apoptotic status of neurons and cultured cells was assessed using a TUNEL assay (Thermo Fisher Scientific) according to the manufacturer's instructions.

Statistical analysis

Open source R software was used for statistical analysis. Each experiment was repeated in triplicate, and all results were expressed as the mean $\pm$ standard deviation. Inter-group comparisons were made using one-way analysis of variance (ANOVA). A *P*-value of < 0.05 was chosen to represent statistical significance.

Results

H₂S inhalation alleviates the movement disorder in MPTP-treated mice.

MPTP was used to establish a mouse model of PD, which was treated with H₂S before behavioral testing. The open field test showed that MPTP significantly decreased the distance travelled (Fig. 1A) and the number of rearings (Fig. 1B). Inhalation of H₂S markedly diminished the reductions in locomotor activity and the number of rearings in the PD model mice. MPTP substantially reduced the latency to fall in the rotarod test. H₂S inhalation partially restored the latency towards normal levels (Fig. 1C). MPTP increased the immobility time in the tail suspension test, and inhalation of H₂S reduced this increase in immobility time in these PD mice (Fig. 1D).

H₂S inhalation reduces MPTP-induced apoptosis of neurons in mice.

TUNEL assays were performed to evaluate the apoptotic status of neurons in the MPTP-induced PD model mice. As shown in Fig. 2, administration of MPTP robustly increased

neuronal apoptosis. H₂S inhalation effectively reduced the apoptosis of neurons in these PD mice.

H₂S inhalation restores the levels of lncRNA-CasC7, miR-30c and BNIP3L mRNA towards normal levels in PD model mice.

Quantitative real-time PCR was performed to analyze the levels of lncRNA-CasC7, miR-30c and BNIP3L mRNA in PD model mice given different treatments. Levels of the lncRNA-CasC7 were markedly decreased in mice administered MPTP. H₂S inhalation partially restored the levels of CasC7 in these animals (Fig. 3A). In contrast, miR-30c was notably upregulated by MPTP treatment, and this increase in expression was suppressed by H₂S inhalation (Fig. 3B). MPTP downregulated BNIP3L, and inhalation of H₂S effectively upregulated BNIP3L in this mouse model of PD (Fig. 3C).

H₂S inhalation restores the levels of CBS, BNIP3L and PINK1 proteins towards normal levels in PD mice.

Western blotting was used to evaluate the levels of CBS, BNIP3L and PINK1 proteins, which are closely correlated with the progression of PD, in PD model mice given the various treatments. Levels of CBS (Fig. 4A, 4B), BNIP3L (Fig. 4A, 4C) and PINK1 (Fig. 4A, 4D) proteins were drastically reduced by MPTP treatment. Inhalation of H₂S effectively reversed this effect of MPTP on the levels of BNIP3L and PINK1, but not CBS.

NaHS protects SH-SY5Y cells against MPP⁺-induced apoptosis.

Immunofluorescence assays were performed to evaluate apoptosis in MPP⁺-treated SH-SY5Y cells. Apoptosis was markedly increased by MPP⁺ treatment, and this effect of the toxin was diminished by NaHS (Fig. 5A). Furthermore, the viability of SH-SY5Y cells

was notably reduced by MPP^+ treatment, and NaHS treatment partially restored viability towards normal levels (Fig. 5B). ELISA was performed to analyze the H_2S content of cell supernatants. H_2S production by SH-SY5Y cells was significantly inhibited by MPP^+ treatment, while treatment with NaHS reversed this effect of MPP^+ (Fig. 5C).

NaHS protects SH-SY5Y cells against MPP^+ -induced dysregulation of lncRNA-CasC7, miR-30c and BNIP3L mRNA.

The levels of lncRNA-CasC7, miR-30c and BNIP3L mRNA were analyzed in SH-SY5Y cells given the various treatments. Expression of lncRNA-CasC7 was markedly decreased by MPP^+ . NaHS treatment partially restored the expression of lncRNA-CasC7 in these MPP^+ -treated cells (Fig. 6A). In contrast, the expression of miR-30c was upregulated by MPP^+ in SH-SY5Y cells. NaHS treatment partially reduced this effect of MPP^+ (Fig. 6B). BNIP3L was downregulated by MPP^+ , and NaHS treatment partially restored the expression of BNIP3L in the MPP^+ -exposed cells (Fig. 6C).

NaHS protects SH-SY5Y cells against MPP^+ -induced perturbation of CBS, BNIP3L and PINK1 protein levels.

The levels of CBS, BNIP3L and PINK1 proteins in the PD cell culture model were analyzed by western blotting. The levels of CBS (Fig. 7A, 7B), BNIP3L (Fig. 7A, 7C) and PINK1 (Fig. 7A, 7D) were substantially reduced by MPP^+ . Treatment with NaHS significantly suppressed this effect of MPP^+ on the levels of BNIP3L and PINK1, but not CBS.

miR-30c inhibits the luciferase activities of lncRNA-CasC7 and BNIP3L.

Because lncRNA-CasC7 (Fig. 8A) and BNIP3L (Fig. 8C) are candidate targets of miR-30c, we generated luciferase vectors containing lncRNA-CasC7 and BNIP3L sequences

and transfected them together with miR-30c into SH-SY5Y cells. The luciferase activities of the wild-type *lncRNA-CasC7* (Fig. 8B) and BNIP3L (Fig. 8D) constructs were effectively inhibited by miR-30c, suggesting that H₂S upregulates *lncRNA-CasC7* by downregulating miR-30c. This downregulation of miR-30c also increased the expression of BNIP3L, resulting in the upregulation of PINK1 and PARK as well. Thus, H₂S activates the PINK1 signaling pathway, thereby suppressing neuronal apoptosis and reducing the risk of PD (Fig. 9).

Discussion

PD is one of the most frequently diagnosed diseases of the human nervous system, particularly affecting those ≥ 65 years of age [13]. PD is characterized by the loss and degeneration of dopaminergic neurons in the substantia nigra [14-17]. Although the exact etiology of PD remains unclear, levodopa is widely used to treat PD. However, levodopa only ameliorates PD symptoms, and cannot cure the disease or reverse the progressive degeneration of dopaminergic neurons [18-21]. Thus, novel therapeutic agents are urgently needed to effectively treat PD. In this study, we generated a PD mouse model using MPTP and evaluated motor behavior. We found that inhalation of H₂S effectively alleviated the movement disorder and decreased the rate of neuronal apoptosis in the PD model mice.

Expression of *lncRNA-CasC7* is reduced in SH-SY5Y cells exposed to OGD/R as well as in the rat model of spinal cord ischemia/reperfusion injury, and NaSH treatment upregulates *lncRNA-CasC7* and decreases the size of spinal cord infarction [8]. Therefore, NaSH might exert its neuroprotective actions by regulating miR-30c and *lncRNA-CasC7*

expression. Additionally, silencing **lncRNA-CasC7** **increases** apoptosis of SH-SY5Y cells exposed to OGD/R and **treated with NaHS**, suggesting that **NaHS exerts its anti-apoptotic effect by modulating the expression of lncRNA-CasC7** [8, 9]. In this study, we **evaluated** the expression of **lncRNA-CasC7**, **miR-30c** and **BNIP3L mRNA** in mouse and cell **culture** models of PD, and we examined the effects of **H₂S inhalation and NaHS treatment, respectively, on the levels of these RNAs**. We found that inhaled H₂S and NaHS treatment **restored expression of BNIP3L and PINK1**. Furthermore, NaHS protected SH-SY5Y cells against **MPP⁺**-induced apoptosis.

In cisplatin-induced apoptosis of renal tubular cells, **miR-30a-e** expression decreases, **and** increased expression of **miR-30c** **reduces** the expression **levels** of **Hspa5** and **BNIP3L**, **which** can be increased by cisplatin. Therefore, it **has been suggested** that **Hspa5** and **BNIP3L** are the targets of **miR-30c** [22]. In this study, we performed luciferase assays to explore the regulatory relationship between **CasC7** and **miR-30c** as well as between **BNIP3L** and **miR-30c**. The **assays revealed** that **miR-30c** **inhibits** the **expression of lncRNA-CasC7** and **BNIP3L**.

BNIP3L **is localized** to the outer membrane of mitochondria and is essential for autophagic mitochondrial degradation. Its expression is increased during the maturation of reticulocytes [23-25]. Increased **BNIP3L** expression can also induce mitophagy in cells expressing wild-type **PARK2**, but not **in** cells deficient in **PARK2**. **BNIP3L** is required to complete the selective elimination of damaged mitochondria. **Also**, as a **PARK2** substrate, **BNIP3L** can drive the mitophagy mediated by **PARK2** [10]. Furthermore, **PARK2** can ubiquitinate **BNIP3L** and recruit **NBR1** into mitochondria to induce mitochondrial degradation. As a result, **as a substrate of PARK2**, **BNIP3L** enhances the mitophagy

involved in the pathogenesis of PD [23, 26-32]. The BNIP3/BNIP3L complex can also disrupt the binding between BCL2 and Beclin1 to release Beclin1, which in turn initiates mitophagy [33]. BNIP3L also plays a key role in recognizing damaged mitochondria targeted for autophagy by interacting with GABARAP and LC3 [31, 32, 34]. Mitophagy induced by AMBRA1 plays a neuroprotective role in rotenone-induced cytotoxicity to SH-SY5Y cells. In addition, transfection of plasmids carrying the AMBRA1^{ActA} fusion protein construct into SH-SY5Y cells significantly alleviates apoptosis induced by reactive oxygen species [35]. The AMBRA1^{ActA} fusion protein can reduce oxidative stress and inhibit the apoptosis by inducing mitophagy in cells exposed to rotenone or 6-OHDA [35]. Oxidative stress plays an essential role in the pathogenesis of PD [36], and therefore, the mitophagy mediated by PARKIN and PINK1 may help to maintain cellular homeostasis. Consequently, mutations in these genes may increase the risk of PD by inducing the accumulation of impaired mitochondria [37].

Conclusion

In this study, we used cell culture and animal models of PD to investigate the impact of H₂S on the lncRNA-CASC7/miR-30c/BNIP3L mitophagy signaling pathway. Our findings show that H₂S upregulates lncRNA-CasC7, which in turn downregulates miR-30c. BNIP3L, a predicted target of miR-30c, is implicated in mitophagy, an important process that regulates the apoptosis of neurons, and which plays a critical role in the pathogenesis of PD.

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Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1

H₂S inhalation alleviates the movement disorder in MPTP-exposed mice (**P* < 0.05, vs. control group; #*P* < 0.05, vs. MPTP group).

A: MPTP decreased the distance traveled, and H₂S treatment suppressed this change.

B: MPTP decreased the number of rearings, and H₂S treatment restored the number to control values.

C: MPTP decreased the latency to fall from the rotarod, and H₂S treatment restored the latency to control values.

D: MPTP increased the immobility time in the tail suspension test, and H₂S treatment restored the immobility time to control values.

Figure 2

MPTP induces neuronal apoptosis, which can be inhibited by H₂S treatment.

A: TUNEL assay for neuronal apoptosis in the various groups.

B: Relative apoptosis index for the various groups. The index was increased by MPTP, and H₂S treatment reduced this index (**P* < 0.05, vs. control group; #*P* < 0.05, vs. MPTP group).

Figure 3

H₂S inhalation restores expression of lncRNA-CasC7, miR-30c and BNIP3L mRNA towards control levels in PD model mice (**P* < 0.05, vs. control group; #*P* < 0.05, vs. MPTP group).

A: MPTP inhibited the expression of lncRNA-CasC7 in PD model mice, and H₂S treatment partially restored the expression of lncRNA-CasC7.

B: MPTP enhanced the expression of miR-30c in PD model mice, and H₂S treatment partially suppressed this change in expression.

C: MPTP inhibited the expression of BNIP3L in PD model mice, and H₂S treatment partially restored the expression of BNIP3L.

Figure 4

H₂S inhalation restores the protein levels of BNIP3L and PINK1 in PD model mice ($*P < 0.05$, vs. control group; $^{\#}P < 0.05$, vs. MPTP group).

A: Graphical representation of the changes in the expression levels of CBS, BNIP3L and PINK1 in PD model mice given the various treatments.

B: CBS was downregulated in MPTP-induced PD mice.

C: BNIP3L was downregulated in MPTP-induced PD mice, and H₂S treatment partially restored expression of BNIP3L.

D: PINK1 was downregulated in MPTP-induced PD mice, and H₂S treatment partially restored the levels of PINK1.

Figure 5

NaHS protects SH-SY5Y cells against MPP⁺-induced apoptosis ($*P < 0.05$, vs. control group; $^{\#}P < 0.05$, vs. MPP⁺ group).

A: Immunofluorescence analysis showed an elevated rate of apoptosis in MPP⁺-treated SH-SY5Y cells. NaHS treatment reduced this increase.

B: Cell viability was decreased by MPP⁺. NaHS treatment increased the viability of these MPP⁺-exposed SH-SY5Y cells.

C: H₂S content in the cell supernatant was decreased by MPP⁺. NaHS treatment increased the H₂S content in the cell supernatant.

Figure 6

NaHS treatment restores the levels of lncRNA-CasC7, miR-30c and BNIP3L mRNA towards normal values in the SH-SY5Y cell culture model of PD ($*P < 0.05$, vs. control group; $^{\#}P < 0.05$, vs. MPP⁺ group).

A: MPP⁺ inhibited the expression of lncRNA-CasC7 in the PD cell culture model. NaHS treatment partially restored the expression of lncRNA-CasC7.

B: MPP⁺ upregulated miR-30c in the PD cell model. NaHS treatment partially diminished this change in expression of miR-30c.

C: MPP⁺ inhibited the expression of BNIP3L in the PD cell culture model. NaHS treatment partially restored the expression of BNIP3L.

Figure 7

NaHS treatment restores CBS, BNIP3L and PINK1 protein expression levels in the PD cell culture model ($*P < 0.05$, vs. control group; $^{\#}P < 0.05$, vs. MPP⁺ group).

A: Graphical representation of the changes in the expression levels of CBS, BNIP3L and PINK1 in the PD cell culture model under the various treatment conditions.

B: CBS was downregulated in the PD cell culture model induced by MPP⁺.

C: BNIP3L was downregulated in the PD cell culture model induced by MPP⁺. NaHS treatment partially restored expression of BNIP3L.

D: PINK1 was downregulated in the PD cell culture model induced by MPP⁺. NaHS treatment partially restored the expression of PINK1.

Figure 8

miR-30c inhibits expression of lncRNA-CasC7 and BNIP3L, as assessed by luciferase assay ($*P < 0.05$, vs. control group).

A: Sequence analysis **showing** a potential miR-30c target site in **lncRNA-CasC7**.

B: Luciferase activity of wild-type **lncRNA-CasC7 fusion protein** was inhibited by miR-30c.

C: Sequence analysis **showing** a potential miR-30c target site in BNIP3L.

D: Luciferase activity of wild-type BNIP3L **fusion protein** was inhibited by miR-30c.

Figure 9

The effect of the **lncRNA-CasC7/miR-30c/BNIP3L** mitophagy signaling pathway on the risk of PD.

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Preprint

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