Journal of Molecular Neuroscience
https://doi.org/10.1007/s12031-020-01660-2

NEAT1 Decreasing Suppresses Parkinson's Disease Progression via Acting as miR-1301-3p Sponge

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Received: 10 May 2020 / Accepted: 6 July 2020

Abstract

Long non-coding RNA (lncRNA) plays a crucial role in multiple disorders, while the role of it in Parkinson's disease (PD) is still unclear. Here, the increased lncRNA NEAT1 was discovered in MPP^+-induced SH-SY5Y cells. Then, we proved that NEAT1 decreasing suppressed MPP^+-induced neuronal apoptosis, upregulation of α-syn and activation of NLRP3 inflammasome. Rescue experiments shown that the inhibition of NEAT1 decreasing to MPP^+-induced activation of NLRP3 inflammasome and subsequent neuronal apoptosis can be reversed by overexpressed α-syn. Subsequently, we indicated the interaction between NEAT1 and miR-1301-3p, as well as between NEAT1 and miR-5047. Interesting, we found that NEAT1 decreasing repressed the expression of GJB1, a downstream target of miR-1301-3p and miR-5047, through promoting miR-1301-3p rather than miR-5047 expression. Finally, we transfected miR-1301-3p inhibitor to MPP^+-induced SH-SY5Y cells following si-NEAT1, and found that downregulation of NEAT1 repressed α-syn-mediated the activation of NLRP3 inflammasome through regulating miR-1301-3p/GJB1 signaling pathway. Overall, our data demonstrated that NEAT1 decreasing effectively suppressed MPP^+-induced neuronal apoptosis. Mechanismly, downregulation of NEAT1 repressed α-syn-induced activation of NLRP3 inflammasome via inhibiting the expression of GJB1 by targeting miR-1301-3p. Our study supported a new and reliable evidence for lncRNA NEAT1 as a potential target for PD treatment.

Keywords Parkinson disease · Nuclear enriched assembly transcript 1 · Endogenous competing RNA · NLRP3 inflammasome · Neuronal apoptosis

Introduction

Parkinson's disease (PD) is a complex and progressive neurodegenerative disorder accompanied by multiple motor symptoms such as postural instability, rigidity, bradykinesia, and tremor, and non-motor symptoms such as depression- and anxiety-like behavior (Schenkman et al. 2018; Seppi and Ray Chaudhuri 2019). Over the past 20 years, more than 20 genes have been recognized as the genetic cause of parkinsonism, for example, *SNCA*, *LRRK2*, *PARK7*, *PARK2*, *VPS35*, *RAB29*, and *LAMP3* (Balestrino and Schapira 2018). Among these genes, *SNCA* encodes α-synuclein (α-syn) protein. α-syn is a highly conserved presynaptic protein with 140-amino acid. It has been indicated that α-syn is a major constituent of Lewy bodies, and the variants in *SNCA* like A53T, E46K, A30P, A53E, and H50Q are the major cause of PD both in sporadic and familial (Nussbaum 2017; Xiong et al. 2016). *SNCA* has been identified as the first PD gene. It has been confirmed that α-syn could induce the activation of nucleotide oligomerization domain-like receptor protein with pyrin domain containing 3 (NLRP3) inflammasome, thus

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Published online: 25 July 2020

contributing to PD neuropathology (Gustot et al. 2015). In recent years, a clinical trial about PRX002/RG7935, an anti-α-syn monoclonal antibody, is going on. PRX002/RG7935 has been designed to target aggregated α-syn (Jankovic et al. 2018). How to reduce aggregated α-syn is the key point for PD improvement.

Long non-coding RNA (lncRNA) is the newest player in gene regulation, and the transcript length of it is more than 200 nucleotides. Several lines of evidence strongly suggested that lncRNA plays an important role in multiple disorders, especially in malignant tumor (Jiang et al. 2019). Furthermore, over the past 10 years, the role of lncRNA in PD development has been gradually expounded, including small nucleolar RNA host gene 1 and HAGLROS (Peng et al. 2019; Qian et al. 2019). Recently, Simchovitz et al. have demonstrated that a new lncRNA, LINC-PINT, is a neuroprotective factor in PD (Simchovitz et al. 2020). Another lncRNA p21 has been upregulated in PD. Knockdown of p21 could significantly suppress the cytotoxicity and apoptosis of human neuroblastoma SH-SY5Y cells induced by 1-methyl-4-phenylpyridinium (MPP^+) (Ding et al. 2019). MPP^+-induced SH-SY5Y cell is a widely accepted cell model for PD study. These data have indicated the potential and key effect of lncRNA on PD progression. However, researchers still have a poor understand about the exact role and action mechanism of lncRNA in PD. Nuclear enriched assembly transcript 1 (NEAT1) is a noted regulator in numerous malignant tumors. It has been revealed that NEAT1 is highly expressed or decreased in tumor tissues, and serves as a competing endogenous RNA (ceRNA) which competes with mRNA for binding to microRNA (miRNA), thus promoting the mRNA expression (Adriaens and Rambow 2019; Yu and Li 2017). Otherwise, NEAT1 also has been reported to be involved in the development of neurodegenerative diseases, but the implications of NEAT1 in PD remain controversial (Wang et al. 2020). Reliable data have demonstrated that NEAT1 is increased both in peripheral blood cells and substantia nigra of patients with PD (Boros et al. 2020, Simchovitz et al. 2019). NEAT1 may act as a crucial regulator in PD development. However, the regulatory effect of NEAT1 on α-syn aggregation and the exact action mechanism of NEAT1 in PD development are still not fully clear.

In this present study, our data suggested that NEAT1 was upregulated in MPP^+-induced SH-SY5Y cells, and knockdown of NEAT1 could notably attenuate the apoptosis of SH-SY5Y cells induced by MPP^+ through inhibiting α-syn-mediated the activation of NLRP3 inflammasome. Mechanismly, downregulation of NEAT1 impeded α-syn-induced NLRP3 inflammasome activation by decreasing connexin32 (Cx32, GJB1) expression via binding with miR-1301-3p rather than miR-5047. Our study identified that NEAT1 played a crucial role in MPP^+-induced SH-SY5Y cells toxicity. Our work demonstrated the value of NEAT1 that acts as a potential target for PD treatment.

Materials and Methods

Materials and Reagents

SH-SY5Y cell line was obtained from American Type Culture Collection (ATCC, Manassas, Va., USA). Dulbecco's Modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were provided by Gibco (Grand Island, NY, USA). MPP^+ was purchased from Sigma (St. Louis, MO, USA). Lipofectamine 2000 reagent, TRIzol regent, the First-Strand Synthesis Kit, and SYBR Green PCR Master Mix Kit were obtained from Invitrogen (Carlsbad, CA). NEAT1 siRNA (si-NEAT1), siRNA negative control (si-NC), miR-1301-3p mimics, miR-5047 mimics, mimics-NC, miR-1301-5p inhibitor, miR-5047 inhibitor, and inhibitor-NC were purchased from company (siPools, siTools Biotech, Planegg, Germany). IL-1β ELISA kit was purchased from R&D Systems. RIPA protein lysis was from Beyotime (Jiangsu, China). Primary antibodies and secondary antibodies were obtained from Abcam (Cambridge, MA). In Situ Cell Death Detection Kit was from (Roche, Mannheim, Germany).

Cell Culture and Treatment

SH-SY5Y cells were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin according to the ATCC guidelines. Si-NEAT1, si-NC, pcDNA-α-syn, pcDNA, miR-1301-3p mimics, miR-5047 mimics, mimics-NC, miR-1301-5p inhibitor, miR-5047 inhibitor, and inhibitor-NC were transfected into cells, respectively, using Lipofectamine 2000 reagent according to the manufacturer's instruction. Meanwhile, SH-SY5Y cells were incubated with 100 μm MPP^+ for 24 h to build PD model in vitro (Xu et al. 2018). Subsequently, the cells were harvested for the following experiments.

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

TRIzol reagent was used to obtain total RNA from SH-SY5Y cells, and subsequent reverse transcribed into cDNA utilizing a First-Strand Synthesis Kit. Next, the transcript levels of NEAT1, miR-1301-3p, miR-5047, GJB1 mRNA, NLRP3 mRNA, caspase-1 mRNA, and IL-1β mRNA were analyzed using a SYBR Green PCR Master Mix Kit on the 7500 FAST Real-Time PCR System. Here, GAPDH was served as the internal reference of NEAT1 and mRNA, and the expression of miRNA was normalized to U6. Finally, the relative expression of genes was calculated in the light of $2^{-\Delta\Delta Ct}$ methods. All experiments were fulfilled according to the specified protocol.

Western Blot Analysis

Total proteins were extracted from SH-SY5Y cells utilizing RIPA protein lysis. Equal amount of protein samples (20 μg) from each group were loaded into 12% SDS-PAGE gel, and then transferred into PVDF membrane. Subsequently, the membrane was successively incubated with 5% non-fat mike for 1 h at room temperature, primary antibodies against c-myc (1:1000), α-syn (1:2000), GJB1 (1:2000), NLRP3 (1:1000), caspase-1 (1:2000), Bax (1:2000), Bcl-2 (1:2000), caspase-3 (1:1000) or cleaved caspase-3 (1:1000) overnight at 4 °C, and secondary antibodies (1:10000) for 1 h at room temperature. At last, ECL kit was used to visualize the protein bands under imaging system.

Cell Apoptosis Analysis

SH-SY5Y cells were washed by 0.01 M PBS solution for two times, and then fixed with 4% paraformaldehyde for 1 h at 4 °C. Subsequently, cells were incubated with 3% H_2O_2 solution in methanol for 15 min, and then 0.1% Triton X-100 for 10 min. Then, TUNEL kit was used to stain the apoptotic cells. DAPI working solution was incubated with cells to stain the nucleus for 5 min in the dark. Cell apoptosis was analyzed under an Olympus DP 74 fluorescence microscope.

IL-1β Production Analysis

A specific IL-1β ELISA kit was used to ensure the concentration of IL-1β in cell culture medium as the introduction protocol.

Dual-Luciferase Activity Assay

The relationships between NEAT1, miR-1301-3p, miR-5047, and GJB1 mRNA were analyzed through the dual-luciferase activity assay. NEAT1 wide type (NEAT1-WT), NEAT1 mutation (NEAT1-MUT), GJB1 mRNA-WT 3'-UTR, and GJB1 mRNA-MUT 3'-UTR luciferase reporter were constructed, respectively, and co-transfected into SH-SY5Y cells with miR-1301-3p mimics or miR-5047 mimics. Forty-eight hours later, the luciferase activity of cells was assessed using Dual-Luciferase Reporter Assay System (Promega, WI, USA).

Statistical Analysis

All data were analyzed using the SPSS 20.0 software (IBM., Armonk, NY) and GraphPad Prism 6.0 (GraphPad, San Diego, CA, USA), and expressed as mean ± standard deviation (SD). Student's *t* test was

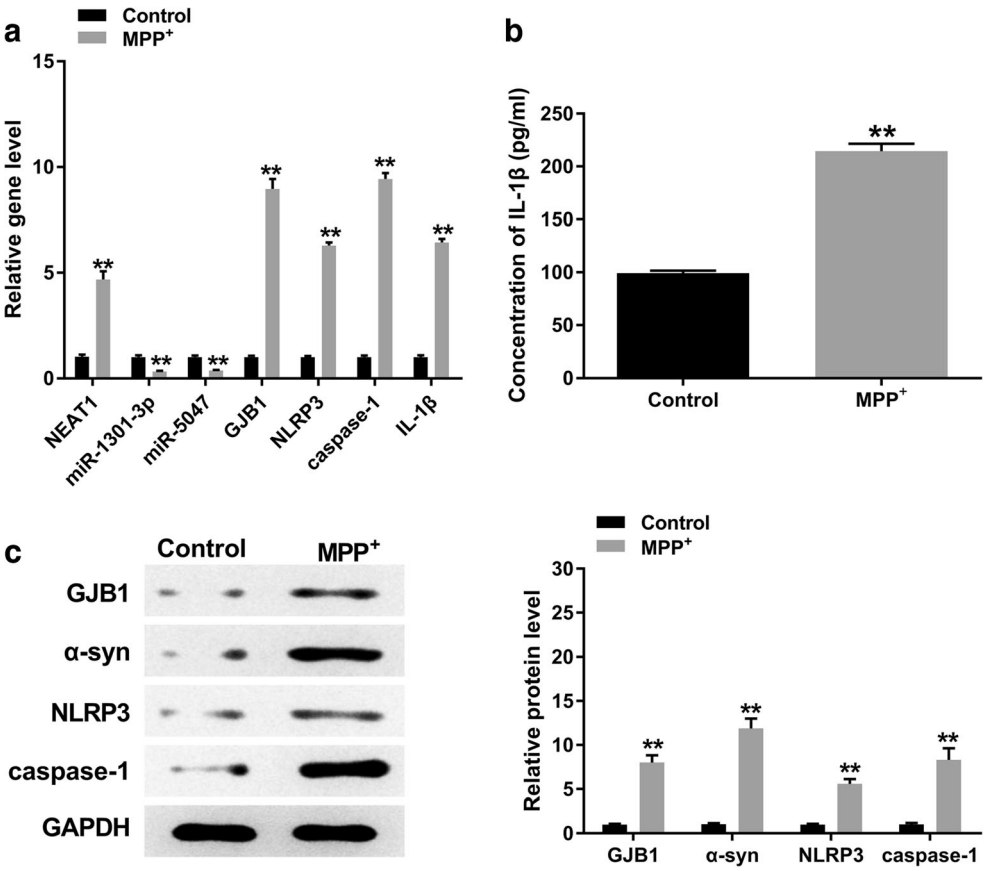


Fig. 1 Expression of the key factors in SH-SY5Y cells treated with or without MPP^+. After 24 h for MPP^+ treatment, (a) qRT-PCR was performed to measure the expression level of NEAT1, miR-1301-5p, miR-5047, GJB1, NLRP3, caspase-1 and IL-1β genes. (b) The concentration of IL-1β in cell culture medium was ensured by ELISA analysis. (c) The expression of GJB1, α-syn, NLRP3, and caspase-1 proteins were detected by western blot. $^{**}P < 0.01$ was contrasted with control group

utilized to analyze the significant difference between two groups, and one-way analysis of variance (ANOVA) was used to analyze the significant difference between multiple groups with normal distribution. $P < 0.05$ was considered statistically significant, and every result was repeated for three times at least.

Results

NEAT1 and GJB1 Were Upregulated in MPP$^+$-Treated SH-SY5Y Cells, while miR-1301-3p and miR-5047 Were Downregulated

To explore the NEAT1, miR-1301-3p, miR-5047, and NLRP3 inflammasome-related proteins expression in PD, we built a PD model in SH-SY5Y cells using MPP$^+$ treatment. Compared with untreated cells (control group), the expressions of NEAT1, GJB1, NLRP3, caspase-1, and IL-1β mRNAs were notably increased in MPP$^+$-treated SH-SY5Y cells (MPP$^+$ groups), while the expressions of miR-1301-3p and miR-5047 were significantly decreased (Fig. 1a). Consistently, the concentration of IL-1β also was upregulated in the culture medium of MPP$^+$-treated SH-SY5Y cells (Fig. 1b). Moreover, the expressions of GJB1, α-syn, NLRP3, and caspase-1 in protein level were upregulated in MPP$^+$-treated SH-SY5Y cells (Fig. 1c). Our data revealed that in MPP$^+$-stimulated SH-SY5Y cells, NEAT1, GJB1, and α-syn expressions were upregulated, NLRP3 inflammasome was activated, while miR-1301-3p and miR-3047 expressed were reduced.

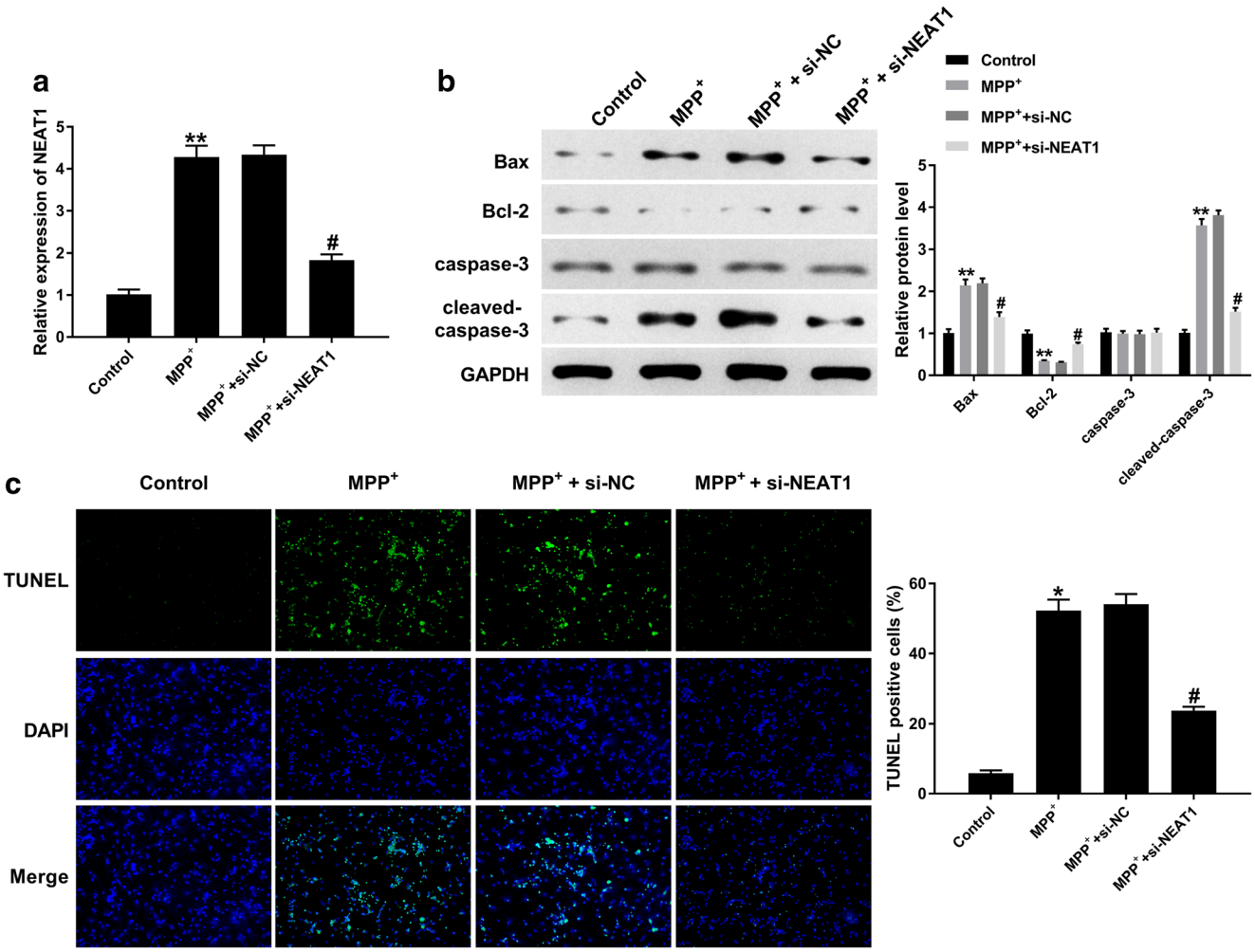


Fig. 2 Effect of NEAT1 knockdown on MPP$^+$-stimulated SH-SY5Y cells apoptosis. MPP$^+$ and si-NEAT1 were co-transfected into SH-SY5Y cells, at 24 h later, (a) qRT-PCR was carried out to detect the expression of NEAT1 in each groups, and ensure the efficiency of RNA interference of NEAT1. (b) The expressions of apoptosis-related proteins were measured by western blot. (c) The number of apoptotic cells was detected using a TUNEL analysis. $^*P < 0.05$ and $^{**}P < 0.01$ were compared with control group, and $^{\#}P < 0.05$ was contrasted with MPP$^+$ groups

Knockdown of NEAT1 Repressed MPP$^+$-Induced Neuronal Apoptosis

Then, to explore the role of NEAT1 in PD development, the specific siRNA of NEAT1 was designed and used to suppress the expression of it. Si-NEAT1 or si-NC was transfected into SH-SY5Y cells following MPP$^+$. Firstly, we verified the transfection efficiency, and the results revealed that si-NEAT1 could effectively reduce the expression of NEAT1 in MPP$^+$-induced SH-SY5Y cells ($^{\#}P < 0.05$, Fig. 2a). Then, we examined the expression of apoptosis-related factors. Our data shown that the expressions of Bax and cleaved-caspase-3 were promoted after MPP$^+$ treatment, while the expression of Bcl-2 was repressed ($^{**}P < 0.01$). However, the effect of MPP$^+$ was partly reversed by NEAT1 decreasing ($^{\#}P < 0.05$, Fig. 2b). Moreover, as shown in Fig. 2c, the number of apoptotic cell in MPP$^+$ group is notably higher than control groups, while NEAT1 knockdown could reduce the number of apoptotic cells. Our data suggested that MPP$^+$ induced neuronal apoptosis that can be prevented by NEAT1 decreasing.

Knockdown of NEAT1 Repressed Neuronal Apoptosis Via Suppressing α-Syn-Induced Activation of NLRP3 Inflammasome

Then, we discovered that downregulation of NEAT1 could significantly suppress the promotion of MPP$^+$ to α-syn, NLRP3, caspase-1, and IL-1β mRNAs expressions

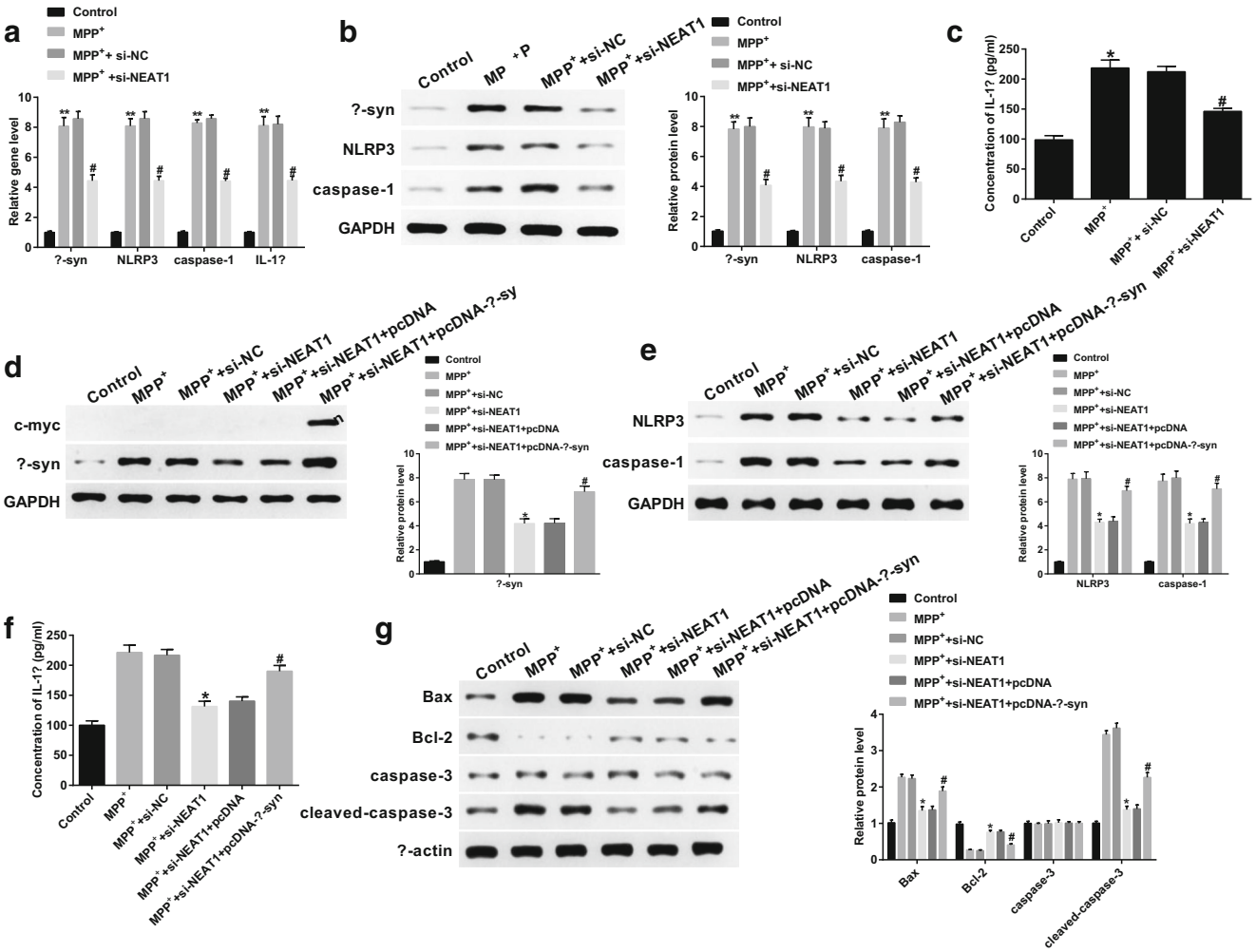


Fig. 3 Role of α-syn-mediated NLRP3 inflammasome activation in the process of NEAT1 downregulation suppressed MPP$^+$-induced neuronal apoptosis. (a–c) MPP$^+$ together with si-NEAT1 or si-NC were transfected into SH-SY5Y cells, at 24 h later, the expression of α-syn, NLRP3, caspase-1, and IL-1β genes were measured by qRT-PCR (a), the expressions of α-syn, NLRP3, and caspase-1 were detected though western blot (b), and ELISA assay was performed to measure the concentration of IL-1β in SH-SY5Y cells culture medium (C). $^{*}P < 0.05$ and $^{**}P < 0.01$ were compared with control group, and $^{\#}P < 0.01$ was contrasted with MPP$^+$ group. Subsequently, (d–g) si-NEAT1 and pcDNA-α-syn tagged with c-myc gene were co-transfected into SH-SY5Y cells treated with MPP$^+$, and 24 h later, the expressions of c-myc and α-syn were detected using western blot to ensure the transfection efficiency of pcDNA-α-syn (d), the expressions of NLRP3 and caspase-1 were measured by western blot (e), the concentration of IL-1β in cell culture medium was surveyed through ELISA assay (f), and the expressions of apoptosis-related proteins like Bax, Bcl-2, caspase-3. and cleaved-caspase-3 were ensured by

(Fig. 3a). Consistently, MPP^+-induced the upregulation of α-syn, NLRP3, and caspase-1 expressions in protein level, and the secretion of IL-1β was effectively repressed by NEAT1 knockdown (Fig. 3 b and c). Our results indicated that NEAT1 knockdown could suppress MPP^+-induced the activation of NLRP3 inflammasome and the increasing of α-syn.

It has been reported that α-syn could activate NLRP3 inflammasome via microglial endocytosis and subsequent lysosomal cathepsin B release (Zhou et al. 2016). Hence, the question we asked is that whether NEAT1 decreasing restricted MPP^+-induced cell apoptosis through targeting α-syn-induced NLRP3 inflammasome activation. To answer this question, we constructed a eukaryotic expressing vector, c-myc-tagged human α-syn (pcDNA-α-syn). Si-NEAT1 and pcDNA-α-syn were co-transfected into MPP^+-induced SH-SY5Y cells. As shown in Fig. 3d, c-myc only was detected in MPP^+ + si-NEAT1 + pcDNA-α-syn group. At the same time, compared with MPP^+ + si-NEAT1 group, the expression of α-syn was significantly upregulated in MPP^+ + si-NEAT1 + pcDNA-α-syn group. Moreover, the inhibitory effect of NEAT1 knockdown on MPP^+-induced upregulation of NLRP3 and caspase-1 was partly reversed by increasing of α-syn (Fig. 3e). Furthermore, the inhibition of NEAT1 decreasing to IL-1β secretion MPP^+-induced SH-SY5Y cells was partly repressed by α-syn overexpression (Fig. 3f). Importantly, the inhibitory effect of NEAT1 decreasing on the expression of Bax and cleaved-caspase-3 and the enhancement of NEAT1 decreasing to expression of Bcl-2 were partly reversed by α-syn overexpression (Fig. 3g). Taken together, in MPP^+-induced PD cell model, downregulation of NEAT1 could effectively repress α-syn-induced activation of NLRP3 inflammasome and neuronal apoptosis.

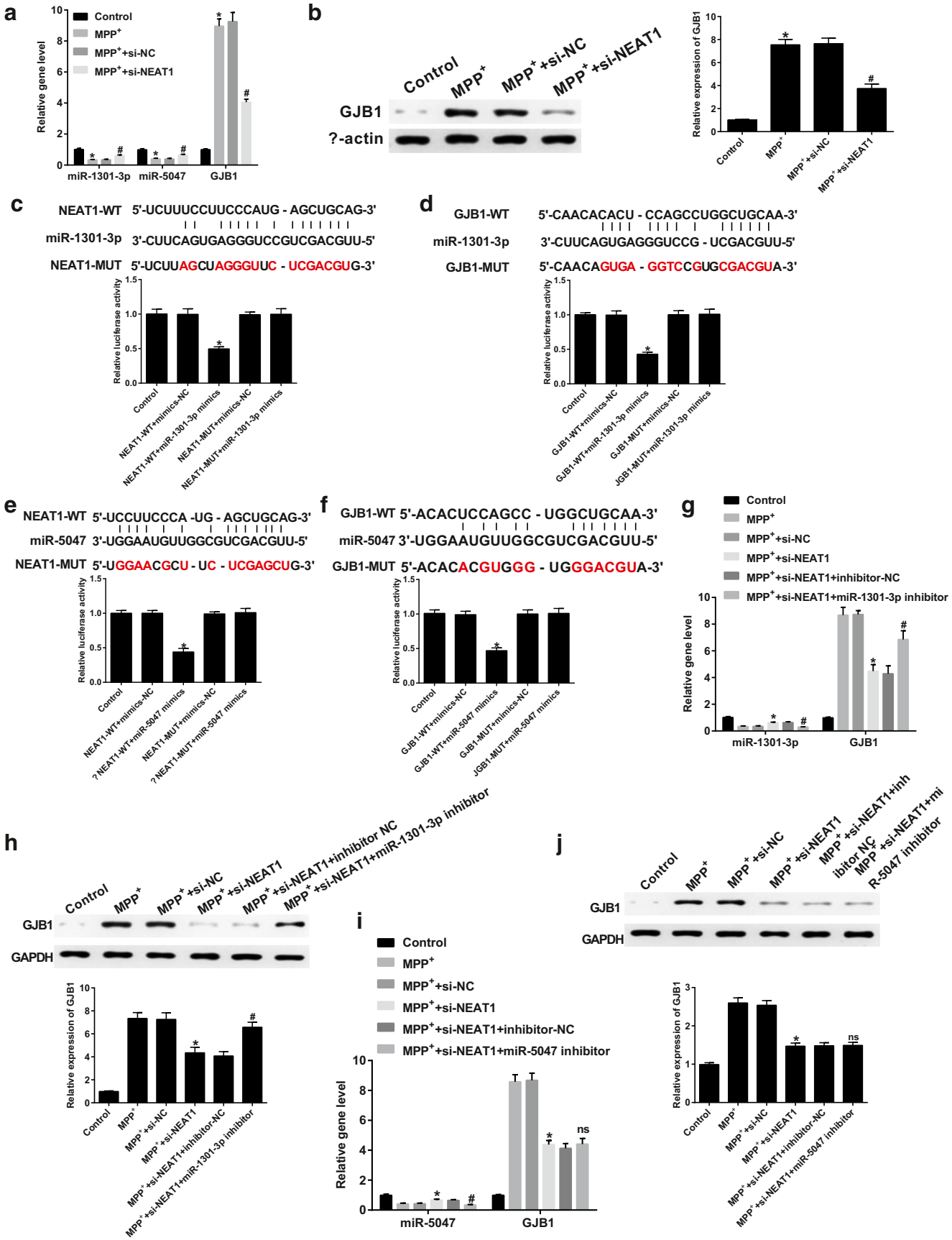
NEAT1 Decreasing Repressed GJB1 Expression Via Targeting miR-1301-3p Rather than miR-5047

Recently, a new research pointed out the upregulation of GJB1 is associated with α-syn accumulation in PD model (Reyes et al. 2019). However, more effects of GJB1 in PD development are unclear. According to the result of bioinformatics prediction using starBase, we found that both NEAT1 and GJB1 may interact with miR-1301-3p and miR-5047. We further investigated the effect of NEAT1 on miR-1301-3p, miR-5047, and GJB1 expression in PD model for SH-SY5Y cells. Our results indicated that compared with MPP^+ group, downregulation of NEAT1 significantly promoted the expression of miR-1301-3p and miR-5047, while inhibited GJB1 mRNA expression (Fig. 4a). MPP^+ induced the increasing of GJB1 protein and also was partly reversed by NEAT1 decreasing (Fig. 4b). To ensure the relationship between NEAT1, miR-1301-3p, miR-5047, and GJB1 3'-UTR, we predicted the binding sites between NEAT1 and miR-1301-3p (Fig. 4c), GJB1 mRNA and miR-1301-3p (Fig. 4d), NEAT1 and miR-5047 (Fig. 4e), and GJB1 mRNA and miR-5047 (Fig. 4f), and then the interactions between NEAT1, miR-1301-3p, miR-5047, and GJB1 were confirmed using luciferase activity assay. Interestingly, our results demonstrated that miR-1301-3p knockdown could significantly reverse NEAT1 knockdown-induced the decreasing of GJB1 both in gene (Fig. 4g) and protein (Fig. 4h) levels in MPP^+-stimulated SH-SY5Y cells. However, inhibition of miR-5047 has no influence on GBJ1 expression both in gene (Fig. 4i) and protein (Fig. 4j) levels. Our data indicated that NEAT1 acted as an endogenous sponge of miR-1301-3p rather than miR-5047 to regulate the expression of GJB1, which is a downstream target of miR-1301-3p and miR-5047. Hence, we thought that NEAT1 decreasing inhibited MPP^+-induced neuronal apoptosis may be target miR-1301-3p/GJB1 signaling.

Fig. 4 Target regulation between NEAT1, miR-1301-3p, miR-5047, and GJB1. (a) The expressions of GJB1 gene in MPP^+-induced SH-SY5Y cells with or without si-NEAT1 were detected by qRT-PCR assay. $^*P < 0.05$ VS. control group, and $^{\#}P < 0.05$ VS. MPP^+ group. (b) The expressions of GJB1 protein in MPP^+-induced SH-SY5Y cells with or without si-NEAT1 were ensured by western blot assay. $^*P < 0.05$ VS. control group, and $^{\#}P < 0.05$ VS. MPP^+ group. (c–f) Dual-luciferase activity assay was performed to discover the target relationships between NEAT1 and miR-1301-3p (c), GJB1 and miR-1301-3p (d), NEAT1 and miR-5047 (e), and GJB1 and miR-5047 (f). (g) The expressions of GBJ1 gene and miR-1301-3p were measured by qRT-PCR after 24 h for SH-SY5Y cells were treated with MPP^+, si-NEAT1, and miR-1301-3p inhibitor. $^*P < 0.05$ was compared with MPP^+ group, and $^{\#}P < 0.01$ was contrasted with MPP^+ + si-NEAT1 group. (h) The expression of GBJ1 protein was measured by western blot after 24 h for SH-SY5Y cells were treated with MPP^+, si-NEAT1 and miR-1301-3p inhibitor. $^*P < 0.05$ was compared with MPP^+ group, and $^{\#}P < 0.01$ was contrasted with MPP^+ + si-NEAT1 group. (i) The expressions of GBJ1 gene and miR-5047 were measured by qRT-PCR after 24 h for SH-SY5Y cells were treated with MPP^+, si-NEAT1, and miR-5047 inhibitor. $^*P < 0.05$ was compared with MPP^+ group, $^{\#}P < 0.01$ was contrasted with MPP^+ + si-NEAT1 group, and ns was presented no significant difference VS. MPP^+ + si-NEAT1 group. (j) The expression of GBJ1 protein was measured by western blot after 24 h for SH-SY5Y cells were treated with MPP^+, si-NEAT1 and miR-5047 inhibitor. $^*P < 0.05$ was compared with MPP^+ group, $^{\#}P < 0.01$ was contrasted with MPP^+ + si-NEAT1 group, and ns was presented no significant difference VS. MPP^+ + si-NEAT1 group

Knockdown of NEAT1 Suppressed MPP^+-Induced Neuronal Apoptosis through Targeting to miR-1301-3p/GJB1 Signaling Pathway

To further investigate the regulatory mechanism of NEAT1 to MPP^+-induced neuronal apoptosis, we accomplished some experiments as following. Si-NEAT1 was transfected into MPP^+-induced SH-SY5Y cells together with or without miR-1301-3p inhibitor. As shown in Fig. 5a, the inhibitory effect of NEAT1 knockdown on α-syn, NLRP3, caspase-1, Bax, and cleaved-caspase-3 expressions and the promotion of NEAT1 knockdown on Bcl-2 expression were partly reversed


a
Control
MPP+
MPP+si-NC
MPP+si-NEAT1
Relative gene level
miR-1301-3p
miR-5047
GJB1
b
Control
MPP+
MPP+si-NC
MPP+si-NEAT1
GJB1
?-actin
Relative expression of GJB1
c
NEAT1-WT 5'-UCUUUCCUUCCCAUG - AGCUGCAG-3'
miR-1301-3p 3'-CUUCAGUGAGGGUCCGUCGACGUU-5'
NEAT1-MUT 5'-UCUUAGCUAGGGUUC - UCGACGUG-3'
Relative luciferase activity
Control
NEAT1-WT+mimics-NC
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NEAT1-MUT+mimics-NC
NEAT1-MUT+miR-1301-3p mimics
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GJB1-WT 5'-CAACACACU - CCAGCCUGGCUGCAA-3'
miR-1301-3p 3'-CUUCAGUGAGGGUCCG - UCGACGUU-5'
GJB1-MUT 5'-CAACAGUGA - GGTCCGUGCGACGUA-3'
Relative luciferase activity
Control
GJB1-WT+mimics-NC
GJB1-WT+miR-1301-3p mimics
GJB1-MUT+mimics-NC
JGB1-MUT+miR-1301-3p mimics
e
NEAT1-WT 5'-UCCUUCCCA -UG - AGCUGCAG-3'
miR-5047 3'-UGGAAUGUUGGCGUCGACGUU-5'
NEAT1-MUT 5'-UGGAACGCU - UC - UCGAGCUG-3'
Relative luciferase activity
Control
NEAT1-WT+mimics-NC
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NEAT1-MUT+mimics-NC
?NEAT1-MUT+miR-5047 mimics
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GJB1-WT 5'-ACACUCCAGCC - UGGCUGCAA-3'
miR-5047 3'-UGGAAUGUUGGCGUCGACGUU-5'
GJB1-MUT 5'-ACACACGUGGG - UGGGACGUA-3'
Relative luciferase activity
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GJB1-WT+miR-5047 mimics
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JGB1-MUT+miR-5047 mimics
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MPP+si-NEAT1
MPP+si-NEAT1+inhibitor-NC
MPP+si-NEAT1+miR-1301-3p inhibitor
Relative gene level
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MPP+si-NEAT1+miR-1301-3p inhibitor
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Relative gene level
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GJB1
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Relative expression of GJB1
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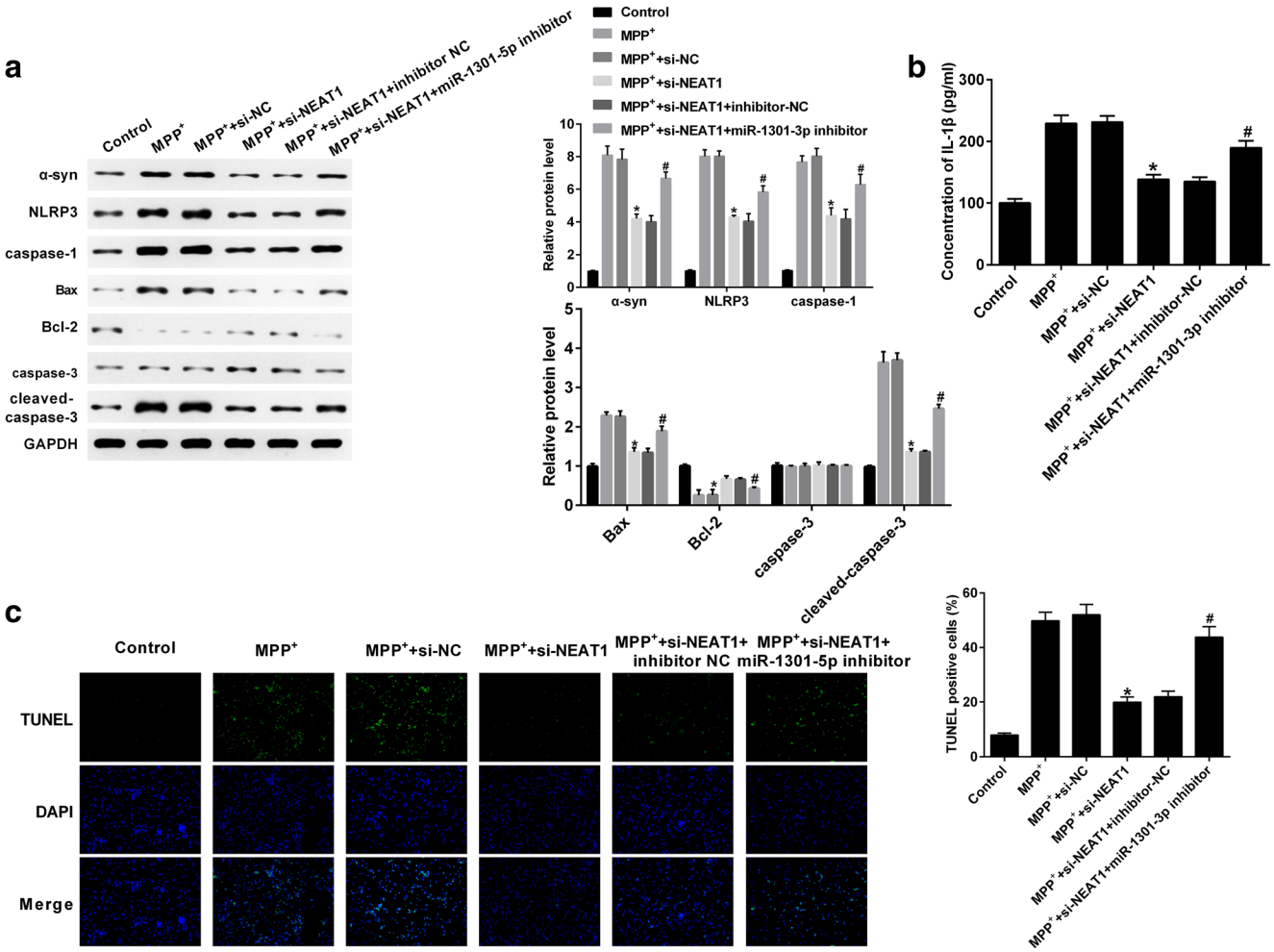

Fig. 5 The regulatory mechanism of NEAT1 knockdown in MPP^+-induced neuronal apoptosis. (a) The expressions of α-syn, NLRP3 inflammasome-related proteins like NLRP3 and caspase-1, and apoptosis-related proteins including Bax, Bcl-2, caspase-3, and cleaved-caspase-3 were detected by western blot. (b) ELISA assay was carried out to ensure the concentration of IL-1β in cell culture medium. (c) TUNEL staining was performed to mark the apoptotic SH-SY5Y cells. $^*P < 0.05$ was compared with MPP^+ group, and $^\#P < 0.05$ was contrasted with MPP^+ + si-NEAT1 group

by miR-1301-3p decreasing. Consistently, silencing of NEAT1 reduced the secretion of IL-1β, while this effect was partly reversed by miR-1301-3p decreasing (Fig. 5b). Moreover, the TUNEL results revealed that compared with MPP^+ group, NEAT1 knockdown could significantly reduce the number of apoptotic cells, while this effect of NEAT1 knockdown was partly reversed by miR-1301-5p decreasing (Fig. 5c). Overall, combined with the above results, we can know that NEAT1 decreasing repressed MPP^+-induced the activation of NLRP3 inflammasome and neuronal apoptosis via promoting miR-1301-3p and subsequent decreasing GJB1.

Discussion

MPP^+-induced PD model in SH-SY5Y cells is widely accepted by researchers for the study about pathogenesis of PD. In this present study, we constructed a PD model in cellular. Our data showed increased α-syn and highly apoptotic rate in MPP^+-induced SH-SY5Y cells. Moreover, we also found that increased NEAT1 and GJB1 activated NLRP3 inflammasome, and decreased miR-1301-3p and miR-5047 in MPP^+-induced SH-SY5Y cells. NEAT1 is a crucial regulator in multiple disorders, especially in malignant tumors. It has been indicated that NEAT1 is increased in breast carcinoma, non-small cell lung carcinoma, hepatocellular carcinoma, ovarian carcinoma, prostate carcinoma, and nasopharyngeal carcinoma. NEAT1 has been recognized as a suitable detection, prognostic, and treatment biomarker of these malignant tumors (Klec et al. 2019). However, the role of NEAT1 in each disorder still needs to be excavated; some reliable researches have suggested that NEAT1 also plays an important role in the development of neurodegenerative disease (Wang et al. 2019). Although some studies already indicated that NEAT1 is increased in MPP^+-induced SH-SY5Y cells, and

promotes the progression of PD via regulating miRNA and PINK1. However, these studies cannot fully reveal the regulatory mechanism of NEAT1 in PD (Xie et al. 2019; Yan et al. 2018). Here, our results proved that downregulation of NEAT1 could effectively improve MPP^+-induced neuronal apoptosis. Then, we explored the regulatory mechanism of NEAT1 in PD progression.

NLRP3 inflammasome is a macromolecular complex composed of NLRP3, ASC, and caspase-1. NRLP3 inflammasome acts as an important composition of innate immunity to involve in multiple disorders progression (Haque et al. 2020). Related reports have indicated that NLRP3 inflammasome-mediated neuroinflammation is associated with the progression of PD. For instance, Gordon et al. have revealed that NLRP3 inflammasome is activated in PD (Gordon and Albornoz 2018). In this present study, we also found that NLRP3 inflammasome was activated in MPP^+-induced SH-SY5Y cells. It has been reported that in PD model, NLRP3 inflammasome can be activated by α-syn via promoting lysosomal cathepsin B release (Zhou et al. 2016). Here, our data indicated that NEAT1 decreasing-induced inactivation of NLRP3 inflammasome was partly reversed following α-syn overexpression. Hence, we thought that NEAT1 decreasing inhibited MPP^+-induced neuronal apoptosis via repressing α-syn-mediated the activation of NLRP3 inflammasome.

GJB1 belongs to the family of connexins. Currently, the crucial role of GJB1 in inflammatory disorders already has been well reported, such as in liver ischemia reperfusion, lung injury, and hepatic injury (Wu et al. 2020; Zhang et al. 2020). Recently, Reyes et al. have demonstrated that GJB1 is involved in the preferential uptake of α-syn oligomeric in neurons, and the upregulation of GJB1 is associated with α-syn accumulation in PD model. This study indicated the potential and crucial role of GJB1 in α-syn-induced neuronal apoptosis (Reyes et al. 2019). Here, we found that GJB1 was increased in MPP^+-induced SH-SY5Y cells. Moreover, we performed the bioinformatics analysis to ensure the potential target of NEAT1, and found that NEAT1 may compete with GJB1 for binding with miR-1301-3p or miR-5047. Actually, decreased miR-1301-3p and miR-5047 were found in MPP^+-induced SH-SY5Y cells. We further ensured the relationship between NEAT1 and miR-1301-3p, miR-1301-3p and GJB1 mRNA, NEAT1 and miR-5047, and miR-5047 and GJB1 mRNA. Interestingly, our data shown that knockdown of NEAT1 could significantly inhibit the expression of GJB1 though promoting miR-1301-3p rather than miR-5047. Subsequently, we revealed that downregulation of NEAT1 effectively suppressed MPP^+-induced activation of NLRP3 inflammasome and subsequent neuronal apoptosis through increasing of miR-1301-3p and decreasing of GJB1.

Conclusion

Overall, our data suggested that knockdown of NEAT1 could effectively suppress neuronal apoptosis in PD. Mechanismly, knockdown of NEAT1 suppressed α-syn-induced activation of NLRP3 inflammasome through promoting the expression of miR-1301-3p and subsequent downregulation of GJB1. Our study provided a new regulatory mechanism of NEAT1 in PD, and a strong evidence for NEAT1 acted as a treatment target in OD.

Funding Information None.

Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

Consent for Publication All of the authors have given their consent for publication.

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