Downregulation of miR-384-5p attenuates rotenone-induced neurotoxicity in dopaminergic SH-SY5Y cells through inhibiting endoplasmic reticulum stress

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Jiang M, Yun Q, Shi F, Niu G, Gao Y, Xie S, Yu S. Downregulation of miR-384-5p attenuates rotenone-induced neurotoxicity in dopaminergic SH-SY5Y cells through inhibiting endoplasmic reticulum stress. Am J Physiol Cell Physiol 310: C755–C763, 2016. First published February 10, 2016; doi:10.1152/ajpcell.00226.2015.—Endoplasmic reticulum (ER) stress has been linked to the pathogenesis of Parkinson’s disease (PD). However, the role of microRNAs (miRNAs) in this process involved in PD remains poorly understood. Recent studies indicate that miR-384-5p plays an important role for cell survival in response to different insults, but the role of miR-384-5p in PD-associated neurotoxicity remains unknown. In this study, we investigated the role of miR-384-5p in an in vitro model of PD using dopaminergic SH-SY5Y cells treated with rotenone. We found that miR-384-5p was persistently induced by rotenone in neurons. Also, the inhibition of miR-384-5p significantly suppressed rotenone-induced neurotoxicity, while overexpression of miR-384-5p aggravated rotenone-induced neurotoxicity. Through bioinformatics and dual-luciferase reporter assay, miR-384-5p was found to directly target the 3′-untranslated region of glucose-regulated protein 78 (GRP78), the master regulator of ER stress sensors. Quantitative polymerase chain reaction and Western blotting analysis showed that miR-384-5p negatively regulated the expression of GRP78. Inhibition of miR-384-5p remarkably suppressed rotenone-evoked ER stress, which was evident by a reduction in the phosphorylation of activating transcription factor 4 (ATF4) and inositol-requiring enzyme 1 (IRE1α). The downstream target genes of ER stress including CCAAT/enhancer-binding protein-homologous protein (CHOP) and X box-binding protein-1 (XBP-1) were also decreased by the miR-384-5p inhibitor. In contrast, overexpression of miR-384-5p enhanced ER stress signaling. In addition, knockdown of GRP78 significantly abrogated the inhibitory effect of miR-384-5p inhibitors on cell apoptosis and ER stress signaling. Moreover, we observed a significant increase of miR-384-5p expression in primary neurons induced by rotenone. Taken together, our results suggest that miR-384-5p mediated ER stress by negatively regulating GRP78 and that miR-384-5p inhibition might be a novel and promising approach for the treatment of PD.

endoplasmic reticulum stress; Parkinson’s disease; miR-384-5p; GRP78

PARKINSON’S DISEASE (PD) is one of the most common neurodegenerative diseases worldwide, characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta and a reduction of dopamine production in the striatum (6, 41, 43). The development and progress of the disease mainly depends on age, and approximately 1% of the older population (>60 yr) in industrialized countries are affected by PD (41). An increasing number of studies have suggested that numerous mechanisms, including mitochondrial defects, oxidative stress, dysimmunity, heredity, and cell apoptosis, have been implicated in the pathogenesis of PD (22, 24, 46, 48). However, the precise mechanism underlying the pathogenesis of PD remains poorly understood, hampering the development of efficient treatments for PD. The current therapeutics only temporarily relieve the symptoms, thus improving patients’ quality of life to a certain degree. Recently, increasing evidence has suggested that endoplasmic reticulum (ER) stress plays an important role in the pathogenesis of PD, which indicates a potential therapeutic option for PD treatment (50).

The hallmark of PD is the accumulation of misfolded proteins in neurons (19, 36), which could evoke cellular stress in the ER (25, 30, 53). ER stress, also termed the unfolded protein response, contributes to the protection of cells against toxic stimuli or cellular stress-induced accumulation of misfolded proteins (29, 36). However, the prolonged and excessive ER stress induces cell apoptosis (3). Glucose-regulated protein 78 (GRP78), also named binding protein (BiP), plays an important role in the initiation of ER stress. Under normal circumstances, GRP78 binds to three ER stress receptors, including inositol-requiring enzyme 1 (IRE1), activating transcription factor-6 (ATF6), and pancreatic ER kinase-like ER kinase (PERK), which become dissociated from complexes following the accumulation of misfolded proteins, resulting in the activation of IRE1-, ATF6- and PERK-mediated downstream signaling (38). The phosphorylation of PERK activates eukaryotic initiation factor 2α (eIF2α), thus inhibiting protein synthesis (15, 37). Activated eIF2α subsequently activates ATF4, which could induce cell death proapoptotic genes such as CHOP (CCAAT/enhancer-binding protein-homologous protein) (14, 51, 55). The ATF6 pathway is activated by dissociation from GRP78 followed by trafficking to the Golgi to produce cleaved ATF6, which regulates the expression of ER stress target genes such as CHOP and X box-binding protein-1 (XBP-1) (16, 54). The phosphorylation of IRE1α results in IRE1 pathway activation, which is associated with unconventional splicing of XBP-1 mRNA, thereby activating the transcription of ER stress target genes (17, 26, 54). The ER stress signaling is activated to restore the ER function and enhance the folding capacity and degradation of protein aggregates. However, if the homeostasis is not reestablished, the ER stress target genes

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including CHOP1, XBP-1, and caspase-12 promote cell apoptosis (47). An analysis using post mortem samples from PD patients revealed that phosphorylated PERK and eIF2α were expressed at a higher level in neuronomalin containing dopaminergic neurons in the substantia nigra, implying ER stress was activated in brain tissue (18). Furthermore, ER stress has been found to be involved in cellular models of PD (39, 44). It has been reported that the prolonged ER stress induced by toxic stimuli such as rotenone results in the apoptotic death of neurons (10). In a rotenone rat model of PD, suppression of ER stress provides neuroprotection (49). Therefore, inhibiting the prolonged ER stress-induced cell apoptosis may provide a novel insight into the treatment of PD.

MicroRNAs (miRNAs) are a group of short, noncoding RNAs that negatively regulate gene expression and thus participate in various pathological processes (2). Increasing evidence has suggested that they may be implicated in the pathogenesis of PD (7). However, their molecular mechanism is not fully understood. In recent years, miR-384-5p has been reported to play an important role for cell survival in response to different insults. Ethanol exposure causes expression changes of miR-384-5p in rat brain (20). Spinal cord injury significantly increases the expression of miR-384-5p in the serum (13). Importantly, miR-384-5p has been shown to be an indicator for trimethyltin-induced neurotoxicity (32) and is found to be differentially expressed in dopaminergic neurons following cocaine addiction (42). However, whether miR-384-5p is associated with neurotoxicity in PD remains unknown. In this study, we aimed to investigate the role and the potential underlying mechanism of miR-384-5p in an in vitro model of PD induced by rotenone. Through bioinformatics and expression analysis, we found that miR-384-5p regulated ER stress in dopaminergic SH-SY5Y cells via negatively regulating GRP78 expression, the master regulator of ER stress sensors. Overall, our study for the first time reveals a direct link between miR-384-5p and ER stress in dopaminergic SH-SY5Y cells and suggests that the miR-384-5p–GRP78 axis might serve as a potential molecular target for the prevention of dopaminergic neuronal loss in PD.

MATERIALS AND METHODS

Cell culture. Human dopaminergic neuroblastoma SH-SY5Y cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and were maintained in Dulbecco’s modified Eagle’s medium—Ham’s Nutrient Mixture F-12 (DMEM/F12; Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (FCS) plus penicillin and streptomycin (1%). The cells were cultured in humidified atmosphere containing 5% CO2 at 37°C. Human primary neurons were isolated from the postmortem brain tissues provided by Chinese PLA General Hospital. The cells were cultured in Neuronal Medium (ScienCell Research Laboratories, Carlsbad, CA) supplemented with 1% neuronal growth supplement and 1% penicillin and streptomycin at 37°C and 5% CO2. Cells were used at passage 9. The experimental procedure was approved by the Institutional Human Experiment and Ethics Committee of Chinese PLA General Hospital and carried out in accordance with Declaration of Helsinki.

Cell treatment. Cells were exposed to rotenone (Sigma, St. Louis, MO) at various concentrations (1, 5, 10, 20, and 50 μM) (28) for the indicated times before being harvested for analysis. For miRNA transfection, cells were transfected with miR-384-5p mimics or inhibitors using Lipofectamine 2000 (Invitrogen) at a final concentration of 50 nM for 24 h prior to rotenone (20 μM) exposure. For gene silencing, 50 nM GRP78 siRNA or control siRNA (Santa Cruz Biotechnology, Santa Cruz, CA) was transfected into SH-SY5Y cells in the presence or absence of miR-384-5p inhibitors using Lipofectamine 2000 (Invitrogen). All assays were performed in triplicate.

MTT assay. Cell growth and viability were measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were seeded into 96-well plates at a density of 1 × 104 cells/well and cultured for 24 h. Then, cells were transfected with miR-384-5p mimics or inhibitors for 24 h followed by exposure to 20 μM rotenone for another 24 h. Thereafter, the old medium was replaced by fresh medium plus 20 μl of MTT solution (0.5 mg/ml) and cultured for a further 4 h. Finally, the formazan dye crystals formed in the cell culture were dissolved with 150 μl of dimethylsulfoxide per well and the optical density (OD) value at 490 nm in the solution was measured by a microplate reader (ThermoElectron, Vantaa, Finland). Results are expressed as the percentage of MTT reduction, assuming the absorbance of control cells to be 100%.

Cell apoptosis assay. Cell apoptosis was evaluated by Annexin V/propidium iodide (PI) double staining method. Briefly, cells were harvested and digested with 2.5% trypsin. After being washed with phosphate-buffered saline (PBS), cells were centrifuged, collected, and resuspended in 500 μl of PBS. Then, 5 μl of Annexin V-FITC and 5 μl of PI were added and coincubated in the dark for 15 min at room temperature. Finally, apoptotic cells were quantified by fluorescence-activated cell sorting (BD Biosciences, San Jose, CA) and then analyzed by Cell Quest software (BD Biosciences). The data are represented as cell percentage (%).

Caspase-3 activity assay. Caspase-3 activity was measured using a commercial kit (BioVision, Milpitas, CA). Briefly, cells were lysed in ice-cold cell lysis buffer. The supernatant was collected after centrifugation (10,000 g for 1 min) and the protein concentration was measured. Then, 50 μl of cell lysis buffer containing 100 μg of protein was incubated with 5 μl of 4 mM DEVD-pNA substrate in 50 μl of reaction buffer for 2 h at 37°C. The OD value at 405 nm was determined using microplate reader.

Quantitative PCR analysis. Total RNA was isolated using miR-Neasy Mini Kit (Qiagen, Dusseldorf, Germany), which was used to synthesize cDNA using M-MLV reverse transcriptase (Clontech, Palo Alto, CA) for miRNA analysis, or using the one-step primerscript miRNA cDNA synthesis kit (Takara, Dalian, China) for miRNA analysis according to the manufacturer’s instructions. The PCR assay was performed using SYBR Green qPCR Master Mix (ThermoFisher, Shanghai, China). The primers used for qPCR were as follows: GRP78 forward, 5'-ggaaagagttaccatgc-3' and reverse, 5'-ggagccagtgctagttcgc-3'; miR-384-5p forward, 5'-acactccagctgggattc-3' and reverse, 5'-gcttcggcagcacatatactaaaat-3'; GAPDH forward, 5'-ggagttcactggtctca-3' and reverse, 5'-tggtgtcgtggagtcg-3'; β-actin forward, 5'-caggagttcaactgtccttc-3' and reverse, 5'-tggtgtcgtggagtcg-3'; U6 SnRNA forward, 5'-gcctgcaagcataatataaa-3' and reverse, 5'-gcctgcaagcataatataaa-3'. Relative gene expression was calculated by comparison with the internal reference gene GAPDH or U6 SnRNA using the 2^−ΔΔCt method.

Western blot analysis. Proteins in cell samples were extracted using a protein extraction kit (BCA Protein Assay Kit, Beyotime, Haimen, China) according to the manufacturers’ instructions. Equivalent amounts of protein (50 μg) for each sample were run on 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis. The proteins were then electrothermally transferred to a PVDF membrane which was blocked by 2.5% nonfat milk for 1 h at 37°C. The membrane was blocked with anti-GRP78, anti-CHOP, anti-XBP-1, and anti-GAPDH antibodies purchased from Santa Cruz Biotechnology, and anti-ATF4, anti-phospho-ATF4, anti-IRE1α, and anti-phospho-IRE1α antibodies purchased from Bios (Beijing, China); the membrane was then incubated overnight at 4°C, followed by exposure to horseradish peroxidase-conjugated secondary antibodies.
miR-384-5p REGULATES ROTENONE-INDUCED NEUROTOXICITY

Fig. 1. Detection of the expression of miR-384-5p in rotenone-treated cells. A: miR-384-5p was significantly increased in SH-SY5Y cells treated with different concentrations of rotenone (0, 1, 5, 10, 20, and 50 μM) for 24 h. *P < 0.05. B: miR-384-5p was persistently increased in SH-SY5Y cells treated with 20 μM rotenone for 12, 24, 48, 72, and 96 h. *P < 0.05.

RESULTS

The expression of miR-384-5p is significantly induced by rotenone in SH-SY5Y cells. To investigate whether miR-384-5p played a critical role in rotenone-induced neurotoxicity, we initially detected its expression profile in SH-SY5Y cells treated with different concentrations of rotenone (0, 1, 5, 10, 20, and 50 μM) by the qPCR method. The results showed that miR-384-5p was dose-dependently increased after rotenone treatment.
treatment (Fig. 1A). Furthermore, its expression was persistently increased post-time challenge (Fig. 1B). The results indicate that miR-384-5p plays an important role in regulating rotenone-induced neurotoxicity. Downregulation of miR-384-5p inhibits rotenone-induced neurotoxicity. To explore the precise role of miR-384-5p in regulating rotenone-induced neurotoxicity, we detected the gain or loss of function of miR-384-5p on rotenone-induced neurotoxicity. According to the results of MTT (Fig. 2A), the viability of SH-SY5Y cells was significantly decreased by rotenone (20 μM) treatment relative to the control group. When the cells were pretreated with miR-384-5p inhibitor for 24 h, the cell viability was markedly increased compared with the rotenone-treated group (Fig. 2A). In contrast, cell viability was further decreased by treatment with miR-384-5p mimics (Fig. 2B). The results showed that rotenone treatment significantly increased the activity of caspase-3, which was decreased by miR-384-5p inhibitor or further increased by miR-384-5p mimics (Fig. 2D). Again, the data suggest that downregulation of miR-384-5p exhibited a protective effect against rotenone-induced neurotoxicity.

miR-384-5p directly targets the 3'-UTR of GRP78. To explore the underlying mechanism of miR-384-5p, we sought to investigate its target gene. Using bioinformatics analysis, we found that GRP78 was one of the putative target genes of miR-384-5p (Fig. 3A). To confirm this relationship, we performed a dual-luciferase reporter assay. The results showed that miR-384-5p mimics significantly reduced the luciferase activity in pGL3-3'-UTR of GRP78 (WT) transfected cells, which had no effect on pGL3-mutated 3'-UTR of GRP78 (MT) transfected group (Fig. 3B). In contrast, the miR-384-5p inhib-
itor markedly increased luciferase activity in WT-transfected cells (Fig. 3B). Furthermore, we detected the effect of rotenone treatment on the luciferase activity of WT- or MT-transfected cells. The results showed that rotenone treatment significantly decreased the luciferase activity in WT-transfected cells, whereas it showed no obvious effect on MT-transfected cells (Fig. 3C). Taken together, these results indicate that miR-384-5p directly targets the 3'-UTR of GRP78.

miR-384-5p regulates the expression of GRP78 in SH-SY5Y cells. Owing to the targeting relationship between miR-384-5p and the 3'-UTR of GRP78, the expression of GRP78 could be regulated by miR-384-5p. To test the hypothesis, we detected the expression of GRP78 upon treatment with miR-384-5p inhibitors or mimics. The qPCR results demonstrated that the mRNA expression level of GRP78 was obviously increased by treatment with miR-384-5p inhibitors or decreased by treatment with miR-384-5p mimics (Fig. 4A), which was consistent with the protein levels detected by Western blot analysis (Fig. 4B). These results indicate that miR-384-5p directly regulates GRP78 pathway.

Downregulation of miR-384-5p inhibits rotenone-evoked ER stress signaling. A previous report has suggested that GRP78 is a master regulator for ER stress signaling (53). Thus, ER stress signaling could be affected by modulating miR-384-5p expression. The results showed that the expression levels of phosphorylated ATF4 (pATF4) and phosphorylated IRE1α (pIRE1α) were both significantly increased by rotenone treatment (Fig. 5, A and B), implying that rotenone treatment evoked ER stress signaling, which is consistent with the findings of Goswami et al. (10). As expected, the expression levels of pATF4 and pIRE1α were significantly decreased by miR-384-5p inhibitor treatment or further increased by treatment with miR-384-5p mimics (Fig. 4, A and B). Furthermore, the downstream target genes of ER stress signaling including CHOP and XBP-1 were also decreased by miR-384-5p inhibitor treatment or increased by treatment with miR-384-5p mimics (Fig. 5, C and D). Taken together, our data imply that miR-384-5p regulates ER stress signaling.

Silencing of GRP78 enhanced ER stress and abrogates the protective effect of miR-384-5p on rotenone-induced neurotoxicity. To further confirm that miR-384-5p regulated ER stress via GRP78, we used a knockdown of GRP78 and detected the effect of miR-384-5p downregulation on ER stress and rotenone-induced neurotoxicity. The results exhibited that silencing of GRP78 (Fig. 6A) significantly blocked the inhibitory effect of miR-384-5p inhibitors on ER stress signaling, as indicated by the increased expression of ER stress signaling target genes including CHOP and XBP-1 (Fig. 6, B and C). Furthermore, the protective effect of miR-384-5p inhibitor on rotenone-induced neurotoxicity was also significantly attenuated by the knockdown of GRP78 (Fig. 6D). Overall, these results suggest that miR-384-5p may regulate ER stress signaling via GRP78.

Detection of miR-384-5p in human primary neurons. To further determine the critical role of miR-384-5p involved in PD, we used human primary neurons to validate the role of miR-384-5p.
miR-384-5p in regulating rotenone-induced neurotoxicity. The results showed that miR-384-5p was significantly increased in primary neurons with treatment of rotenone (Fig. 7A). Furthermore, rotenone-induced neurotoxicity was markedly reversed by miR-384-5p inhibition or further aggravated by miR-384-5p overexpression (Fig. 7B). The results indicate that miR-384-5p functions as an important regulator for neuron survival in PD.

**DISCUSSION**

In the present study, we have demonstrated that miR-384-5p is strongly induced in dopaminergic SH-SY5Y cells treated with rotenone. Also, the downregulation of miR-384-5p significantly attenuated rotenone-induced neurotoxicity by inhibiting ER stress. The direct link between miR-384-5p and the 3′-UTR of GRP78 was confirmed by a dual-luciferase activity and miR-384-5p negatively regulated the expression of GRP78. Thus, downregulation of miR-384-5p elevated the expression of GRP78, leading to the inhibition of rotenone-evoked ER stress. Our data are in line with the findings of Gorbatyuk et al. (9), who reported that overexpression of GRP78 relieved α-synuclein neurotoxicity in a rat model of PD by downregulating ER stress.

In this study, we used an in vitro model of PD induced by rotenone in human dopaminergic SH-SY5Y cells. Rotenone can pass through the blood-brain barrier, which induces neuronal dysfunction and neurodegeneration (31, 35). A previous study has demonstrated that rotenone induces ER stress by upregulating the phosphorylation of PERK and eIF2α and that the knockdown of eIF2α significantly blocked rotenone-induced ER stress and cytotoxicity in neuroblastoma cells (4). Wu et al. (52) reported that candesartan cilexetil inhibited rotenone-evoked ER stress by downregulating the expression of ATF4 and CHOP and protected rat dopaminergic neurons. A recent study revealed that ER stress played an important role in rotenone-induced apoptosis of mouse neuroblastoma cells (10). In line with these findings, our data also indicate that rotenone-induced ER stress in human dopaminergic SH-SY5Y cells, which was evident by an increase in phosphorylation of ATF4 and IRE1α. Intriguingly, the miR-384-5p significantly inhibited the phosphorylation of ATF4 and IRE1α, leading to a reduction in ER stress signaling.

In this study, we found that the expression of miR-384-5p was significantly increased in response to rotenone treatment. Several studies have proposed that miR-384-5p serves as a potential biomarker for numerous pathological processes. It has been reported that the serum level of miR-384-5p was significantly increased in mice with acute spinal cord injury and that miR-384-5p might be used as a promising biomarker for predicting the severity of acute spinal cord injury (13). In rats treated with trimethyltin, miR-384-5p was significantly upregulated with the evolution of neuronal cell death that was regarded as potential indicators of neurotoxicity (32).
decreased expression of miR-384-5p is required for spine enlargement associated with long-term potentiation (12). Here, we demonstrated that miR-384-5p was upregulated in response to rotenone treatment, indicating that miR-384-5p might have the potential to be used as a biomarker for PD. Bao et al. (1) reported that miR-384-5p was an important protective factor for cardioprotection in myocardial ischemia by regulating the expression of phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit delta. Here, we reported that GRP78 was a target gene of miR-384-5p, both of which were involved in regulating rotenone-induced neurotoxicity by affecting ER stress signaling.

To uncover the underlying mechanism of miR-384-5p in regulating ER stress, we investigated its target gene. Through bioinformatics analysis, dual-luciferase reporter assay and expression analysis, we identified that miR-384-5p directly targeted the 3′-UTR of GRP78 and negatively regulated the mRNA and protein expression of miR-384-5p. Several reports have suggested that numerous miRNAs regulate the expression of GRP78 and its downstream signaling. It was reported that increased miR-181a aggravated injury in a mouse stroke model by inhibiting GRP78 protein expression levels, whereas reduced levels of miR-181a were associated with increased GRP78 protein levels and reduced injury (33). In several cancer cell lines, GRP78 was reported to be regulated by miR-199a-5p as well as miR-30d and miR-181a and cooperatively suppressed GRP78-mediated chemoresistance (45). Similarly, Dai et al. (5) reported that miR-199a-5p was evaluated during hepatic ER stress and directly regulated the expression of GRP78 in hepatocytes. Downregulation of miR-181b produced neuroprotection against ischemic injury via upregulating GRP78 (34). Iwamune et al. (21) reported that miR-376a mediated the expression of GRP78 in rat granulosa cells (21). In the present study, we identified miR-384-5p as a novel regulator of GRP78 and provided a novel option for modulating GRP78 expression in related pathological processes.

GRP78 functions as the master regulator of ER stress, playing an important role in regulating many diseases. It was reported that silencing of GRP78 increased the expression of CHOP and induced cell apoptosis in retinal ischemia-reperfusion (27). Overexpression of GRP78 suppressed α-synuclein aggregation by alleviating ER stress (23). Consistently, Gorbatyuk et al. (9) found that overexpression of GRP78 attenuated α-synuclein neurotoxicity in a rat model of PD by inhibiting ER stress. GRP78 overexpression reduces cell apoptosis through reprograming ER stress by reducing the protein levels of cleaved pATF6 protein, phosphorylated eIF2α, and the proapoptotic protein CHOP (8). Consistent with these findings, our results demonstrated that overexpression of GRP78 by inhibiting miR-384-5p significantly suppressed neuronal apoptosis induced by rotenone, and reprogrammed ER stress signaling by inhibiting the protein levels of pATF4, pIRE1α, CHOP, and XBP-1. In addition, knockdown of GRP78 significantly abrogated the inhibitory effect of miR-384-5p inhibitor on cell apoptosis and ER stress signaling, further indicating the involvement of miR-384-5p and GRP78 in regulating ER stress.

Previous study has suggested that GRP78 functions not only as the inhibitor for ER stress signaling but also a bona fide target gene of ER stress (50). Therefore, there is a feedback loop for GRP78 in the ER stress signaling pathway. Here, we reported that rotenone treatment activated ER stress but the expression of GRP78 had no obvious change in contrast to other ER stress target genes. The rotenone-induced high ex-
expression of miR-384-5p might contribute to these discrepancies. We speculated that miR-384-5p targeted and inhibited GRP78 expression induced by rotenone treatment, thus leading to uncontrolled ER stress signaling activation. However, expression of GRP78 in response to rotenone remains to be further determined.

In this study, we have demonstrated that miR-384-5p and ER stress were persistently induced in neurons treated with rotenone. We demonstrated that miR-384-5p modulated ER stress by directly targeting and regulating GRP78. Gorbatyuk et al. (9) reported that overexpression of GRP78 diminished α-synuclein neurotoxicity in a rat model of PD by downregulating ER stress. More recently, Salganik et al. (40) found that GRP78 protein overexpression protected aging nigral dopamine neurons in the α-synuclein-induced rat model of PD. Rotenone has been reported to induce ER stress both in cell models and animal models of PD (10, 11, 49). Here, we showed that downregulating miR-384-5p increased the expression and effect of miR-384-5p using an in vitro cell model of PD induced by rotenone. Whether miR-384-5p targets GRP78 to regulate ER stress and neuronal survival were further validated in human primary SH-SY5Y cells. The expression pattern and effect of miR-384-5p on neuron survival were further validated in human primary neurons. However, certain limitations of our study should be noted. Whether miR-384-5p is dysregulated in PD patients remains unknown. In this study, we only investigated the expression and effect of miR-384-5p using an in vitro cell model of PD induced by rotenone. Whether miR-384-5p regulates GRP78 to regulate ER stress and neuronal survival in an in vivo model of PD still needs to be further validated. Therefore, before concluding that miR-384-5p is an important regulator for PD, more in vivo animal and clinical detection studies are needed.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
M.J. and S.Y. conception and design of research; M.J., Q.Y., and S.Y. performed experiments; M.J., Q.Y., F.S., G.N., Y.G., and S.X. analyzed data; M.J., Q.Y., F.S., G.N., and Y.G. prepared figures; M.J. drafted manuscript; M.J. edited and revised manuscript; M.J. and S.Y. approved final version of manuscript.

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