



MiR-410 exerts neuroprotective effects in a cellular model of Parkinson's disease induced by 6-hydroxydopamine via inhibiting the PTEN/AKT/mTOR signaling pathway



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ABSTRACT

Parkinson's disease (PD) is a chronic neurodegenerative disease characterized by loss of dopaminergic neurons in the substantia nigra. Recently, microRNAs (miRNAs) were emerging as important mediators in dopaminergic neuron biology. This study determined miR-410 expression in the 6-hydroxydopamine (6-OHDA)-induced in vitro cellular model of PD and explored the mechanistic role of miR-410 in the modulation of neuronal cell viability and apoptosis. Our data showed that 6-OHDA concentration-dependently suppressed neuronal cell viability and apoptosis. Overexpression of miR-410 partially restored the effects of 6-OHDA on neuronal cell viability, apoptosis, caspase-3 activity as well as reactive oxygen species (ROS) production. On the other hand, inhibition of miR-410 decreased neuronal cell viability and increased apoptotic rates, caspase-3 activity as well as ROS production. Furthermore, the potential targets of miR-410 were predicted by TargetScan tool, and we verified that phosphatase and tensin homolog (PTEN) was a target of miR-410 as confirmed by the dual-luciferase reporter assay. MiR-410 overexpression attenuated PTEN expression and mediated the effects in the 6-OHDA-treated cells via targeting PTEN in SH-SY5Y and PC12 cells. Furthermore, 6-OHDA treatment suppressed the protein expression of phosphorylated AKT and phosphorylated mTOR, which was partially attenuated by miR-410 overexpression in SH-SY5Y and PC12 cells. MiR-410 overexpression increased phosphorylated AKT and phosphorylated mTOR protein expression, and this effect was attenuated by PTEN overexpression in both SH-SY5Y and PC12 cells. Collectively, this is the first study to demonstrate the neuroprotective effects of miR-410 in a 6-OHDA-induced cellular model of PD, and our data implied that miR-410 exerted its neuroprotective effects via regulating PTEN/AKT/mTOR signaling axis. The present study may suggest new paradigm to study the pathology of PD.

1. Introduction

Parkinson's disease (PD) is a movement disorder with high prevalence and is a chronic neurodegenerative disorder (Parashos, 2018; Rocca, 2018). PD is mainly characterized by the loss of dopaminergic neurons in the substantia nigra, which results in dysregulation of neuronal signaling that eventually causes movement disorder (Castrìoto et al., 2014; Obeso et al., 2014; Stoessl et al., 2014). To our best knowledge, the pathophysiological mechanisms underlying the loss of dopaminergic neurons remain elusive. Several studies indicated that loss of dopaminergic neurons was related to oxidative stress, mitochondrial dysfunction and protein aggregation, which could induce

apoptosis and impair the autophagy process of dopaminergic neurons (Guo et al., 2018; Hu and Wang, 2016; Michel et al., 2016). In this regard, finding novel molecular mechanisms regarding the loss of dopaminergic neurons can equip us with new strategy to attenuate the symptoms of PD.

MicroRNAs (miRNAs) are highly conserved small non-coding RNAs with 21–23 nucleotides in length (Singh and Sen, 2017). MiRNAs modulate the expression of relevant genes via binding to 3' untranslated region (3'UTR) (Leggio et al., 2017). Up to date, several lines of evidence indicated that miRNAs played essential roles in cellular functions of dopaminergic neurons and in the pathophysiology of neurodegenerative diseases. McMillan et al., showed that down-regulation of miR-7

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was detected in the substantia nigra of patients with PD and depletion of miR-7 caused dopaminergic neuron loss and the accumulation of α -synuclein in vivo, suggesting the importance of miR-7 in α -synuclein and dopaminergic physiology (McMillan et al., 2017). Let-7d alleviates dopaminergic neuron injury induced by 6-OHDA via modulating caspase-3 expression (Li et al., 2017b). Mao et al., showed that upregulation of astrocytic miR-34a in shedding vesicles promoted dopaminergic neuron loss in response to lipopolysaccharide stimulation by modulating Bcl-2 expression (Mao et al., 2015). Recently, studies by using miRNA profiling identified the dysregulation of multiple miRNAs in the midbrain dopaminergic neurons in PD, and among these miRNAs, miR-410 was found to be down-regulated in midbrain dopaminergic neurons (Briggs et al., 2015). However, the mechanistic actions of miR-410 in the biology of dopaminergic neurons has not been studied yet.

In this study, we explored molecular mechanisms of miR-410 in the modulation of neuronal cell viability and apoptosis of the 6-hydroxydopamine (6-OHDA)-treated neuronal cells. The current investigations identified the downregulation of miR-410 in SH-SY5Y and PC12 cells upon 6-OHDA treatment, and miR-410 overexpression partially restored the effects of 6-OHDA treatment on neuronal viability, apoptosis and reactive oxygen species (ROS) production via targeting phosphatase and tensin homolog (PTEN). These data shed the importance of miR-410 in modulating neuronal cell viability and apoptosis and may suggest new paradigm to study the pathology of PD.

2. Materials and methods

2.1. Cell lines and cell culture

SH-SY5Y and PC12 cell lines were from ATCC (Manassas, USA). Cells were cultured in the DMEM/F-12 medium (Thermo Fisher Scientific, Waltham, USA) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific), and cells were maintained in a humidified incubator with 5% CO₂ at 37 °C.

2.2. Chemicals, RNA oligoribonucleotides and cell transfections

The 6-OHDA chemical was from Sigma-Aldrich (St. Louis, USA), and neuronal cell lines were treated with different concentrations of 6-OHDA (10–200 μ M) for a duration of 48 h. MiRNAs including miR-410 mimics (sequence: 5'-AAUAUACACAGAUGGCCUGU-3') and inhibitors (5'-ACAGGCCAUCUGUGUUUAUU-3') and the corresponding scrambled negative controls (mimics NC: 5'-GAACCAUGAGUCCGAAUUUAU-3' and inhibitors NC: 5'-GCAUUGACGUUAGCACUAUU-3') were from Ribobio (Guangzhou, China). The plasmids including pcDNA3.1 and PTEN-overexpressing plasmid (pcDNA3.1-PTEN without 3'UTR of PTEN) were from General Biosystems Ltd. (Hefei, China). Cell transfections were done using Lipofectamine 2000 (Invitrogen, Carlsbad, USA). In vitro assays were performed at 24 h after cell transfections.

2.3. MMT assay for cell viability

The neuronal cell line viability was detected using MTT assay. Briefly, the treated neuronal cell lines were incubated with the MTT reagent (Sigma-Aldrich) for a duration of 4 h at room temperature. After discarding the supernatant, dimethyl sulfoxide was used to dissolve the precipitations. The cell viability index was determined by the measurement of optical density at 570 nm.

2.4. Quantitative real-time PCR (qRT-PCR)

Isolation of RNA from cells was performed using the TRIzol reagent (Sigma-Aldrich), and the qRT-PCR determination of miR-410 and PTEN mRNA was done by using the ABI7900 system (Applied Biosystems, Foster City, China). For miR-410 detection, qRT-PCR was performed by

using the Hairpin miRNAs qPCR Quantification kit (GenePharma, Shanghai, China). MiR-410 expression was normalized by U6. For PTEN mRNA detection, qRT-PCR was performed using the SYBR Premix Ex Taq kit (Takara, Dalian, China). PTEN mRNA expression was normalized by glyceraldehyde 3-phosphate dehydrogenase. Calculation of relative miR-410 and PTEN mRNA expression was performed using the 2^{- $\Delta\Delta$ Ct} method.

2.5. Determination of cell apoptosis by flow cytometry

The cell apoptotic rates were measured by using the Annexin V-FITC/propidium iodide (PI) Detection kit (Thermo Fisher Scientific). Briefly, the cells after receiving different treatments were collected followed by washing with ice-cold phosphate buffered saline for 3 times. Cell staining was performed by incubating with Annexin V-FITC and PI in binding buffer at room temperature for 10 min. The apoptotic rates of stained cells were then analyzed on a BD FACSCanto II flow cytometer (BD Biosciences, San Jose, USA).

2.6. Caspase-3 activity assessment

The caspase-3 activity of the neuronal cell lines was detected by the Caspase-3 activity assay kit (Abcam, Cambridge, USA). Briefly, the treated cells (5 \times 10⁶) were incubated with ice-cold 50 μ l cellular lysis buffer for 30 min followed by 5 min centrifugation at 4 °C. After that, the supernatant was incubated with 5 μ l caspase-3 substrate and 50 μ l 2 \times Reaction buffer at 37 °C in the dark for 4 h. Measurement of caspase-3 activity was detected as the optical density at a wavelength of 405 nm.

2.7. Reactive oxygen species (ROS) production analysis

The ROS production analysis was performed by following previous methods (Dong et al., 2018). Briefly, the treated cells were incubated with fluorescent probe CM-H₂DCFDA (5 μ M; Thermo Fisher Scientific) at 37 °C for 20 min in the dark. The ROS production was detected by measuring fluorescence intensity with a fluorimeter (Spectra Max M5; Molecular Device, San Jose, USA) with 492 nm excitation and 522 nm emission wavelengths, respectively.

2.8. Luciferase reporter assay

Reporter constructs containing PTEN 3'UTR was generated by PCR-amplifying the PTEN 3'UTR from genomic DNA and inserted into the psiCHECK-2 plasmid (Promega, Madison, USA). QuikChange II XL Site-Directed Mutagenesis Kit was used to mutate the predicted miR-410-binding sites (Stratagene, San Diego, USA). Neuronal cell lines were co-transfected with the luciferase reporter constructs and miRNAs, and 48 h later, cells were processed for luciferase assay using the Dual-Luciferase Reporter Assay System (Promega, Madison, USA).

2.9. Western blot assay

Proteins from neuronal cell lines was extracted using the RIPA lysis buffer (Sigma-Aldrich) and protein concentrations were detected by using the BCA method (Beyotime, Beijing, China). Equal amounts of proteins were separated on a 10% SDS-PAGE gel and transferred to the nitrocellulose membranes (Beyotime). The membrane was incubated with Tris-buffered saline Tween-20 containing 5% skimmed milk for 1 h at room temperature followed by incubating with corresponding primary antibodies against PTEN, total AKT (t-AKT), phosphorylated AKT (p-AKT), total mTOR (t-mTOR), phosphorylated mTOR (p-mTOR), and β -actin (Cell Signaling Technology, Beverly, USA) at 4 °C overnight. The membranes were further incubated with corresponding secondary antibodies conjugated with horseradish peroxidase at room temperature for 2 h. The blotting signal was detected by using the enhanced

chemiluminescence detection kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol.

2.10. Statistical analysis

Graph plotting and statistical analyses were performed by using the GraphPad Prism software (Version 6.0; GraphPad Software, La Jolla, USA). All the data were expressed as mean \pm standard deviation. Differences between groups were analyzed by unpaired Student's *t*-test or one-way ANOVA followed by Bonferroni's multiple comparison test. Statistical significance was defined when $P < .05$.

3. Results

3.1. 6-OHDA inhibited cell viability and suppressed miR-410 expression in SH-SY5Y and PC12 cells

The present study first determined the outcomes of 6-OHDA treatments on the viability of SH-SY5Y (a neuroblastoma cell line being widely used as in vitro model in PD research (Xicoy et al., 2017)) and PC12 cells (a pheochromocytoma cell line widely used in PD studies (Rostamian Delavar et al., 2018; Talepoor Ardakani et al., 2019)) and found that 6-OHDA at different concentrations with 48 h treatment significantly inhibited the viability of SH-SY5Y and PC12 cells, and this inhibitory effect was concentration-dependent (Fig. 1A and B). Further qRT-PCR showed that treatment with 6-OHDA at concentrations from 20 to 200 μ M for 48 h concentration-dependently suppressed the expression level of miR-410 in both SH-SY5Y and PC12 cells (Fig. 1C and D). As 6-OHDA at 100 μ M had the sub-maximal effects, this concentration was chosen in the subsequent study.

3.2. MiR-410 overexpression partially restored the actions of 6-OHDA treatments on the viability, apoptotic rates and ROS production of SH-SY5Y and PC12 cells

To determine the direct involvement of miR-410 in the 6-OHDA-mediated in vitro effects in the neuronal cell lines, we performed transfection experiments to transiently overexpress or inhibit miR-410 expression by using miR-410 mimics (chemically synthesized miRNAs which mimic naturally occurring miR-410) or miR-410 inhibitors (modified miRNAs that specifically inhibitor miR-410 expression). As shown in Fig. 2A–2D, transfection with miR-410 mimics (50 nM) caused an upregulation of miR-410 in SH-SY5Y and PC12 cells when compared to mimics NC transfection (Fig. 2A and 2B; see Supplemental Fig. S1A and B for the concentration-dependent effects of miR-410 mimics transfection on miR-410 expression); while miR-410 inhibitors (50 nM) treatment downregulated miR-410 expression in these cells when compared to inhibitors NC group (Fig. 2C and D; see Supplemental Fig. S1C and D for the concentration-dependent effects of miR-410 inhibitors transfection on miR-410 expression). Furthermore, the in vitro functional assays were performed to determine the treatment effects on the viability, cell apoptotic rates, caspase-3 activity and ROS production. SH-SY5Y and PC12 cells were treated with different miRNA oligonucleotides, and at 24 h post-transfection, cells were continued with the treatment of 100 μ M 6-OHDA for 48 h. 6-OHDA (100 μ M) significantly suppressed the cell viability (Fig. 2E and F), induced cell apoptosis (Fig. 2G and H), increased caspase-3 activity (Fig. 2I and J) and ROS production (Fig. 2K and L) in SH-SY5Y and PC12 cells when compared to NC group. Treatment with miR-410 partially impaired the actions of 6-OHDA on the viability, cell apoptotic rates, caspase-3 activity and ROS production in SH-SY5Y and PC12 cells (Fig. 2E–2L).

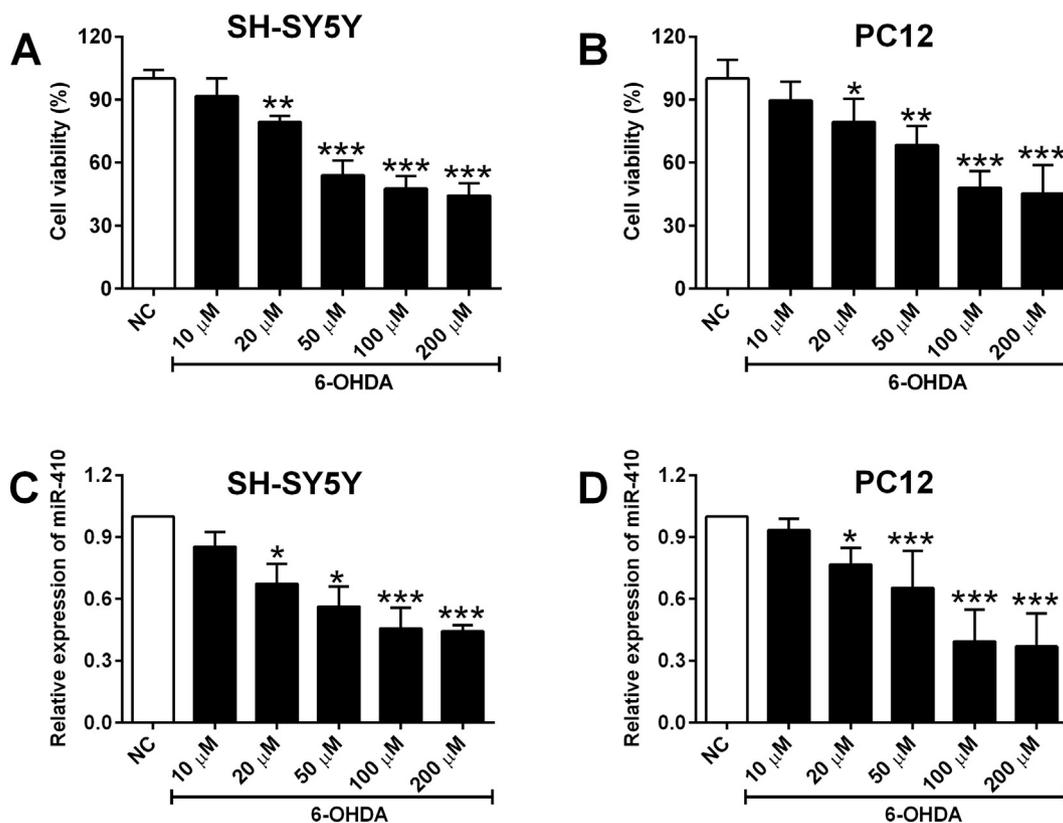


Fig. 1. 6-OHDA inhibited cell viability and suppressed miR-410 expression in SH-SY5Y and PC12 cells. SH-SY5Y and PC12 cells were treated with different concentrations of 6-OHDA (10, 20, 50, 100 and 200 μ M) for 48 h, and then cell viability of SH-SY5Y (A) and PC12 cells (B) was assessed by MTT assay, and the expression of miR-410 in SH-SY5Y (A) and PC12 cells (B) were analyzed by qRT-PCR assay. NC = negative control; $n = 3$; * $P < .05$, ** $P < .01$ and *** $P < .001$ compared to NC group.

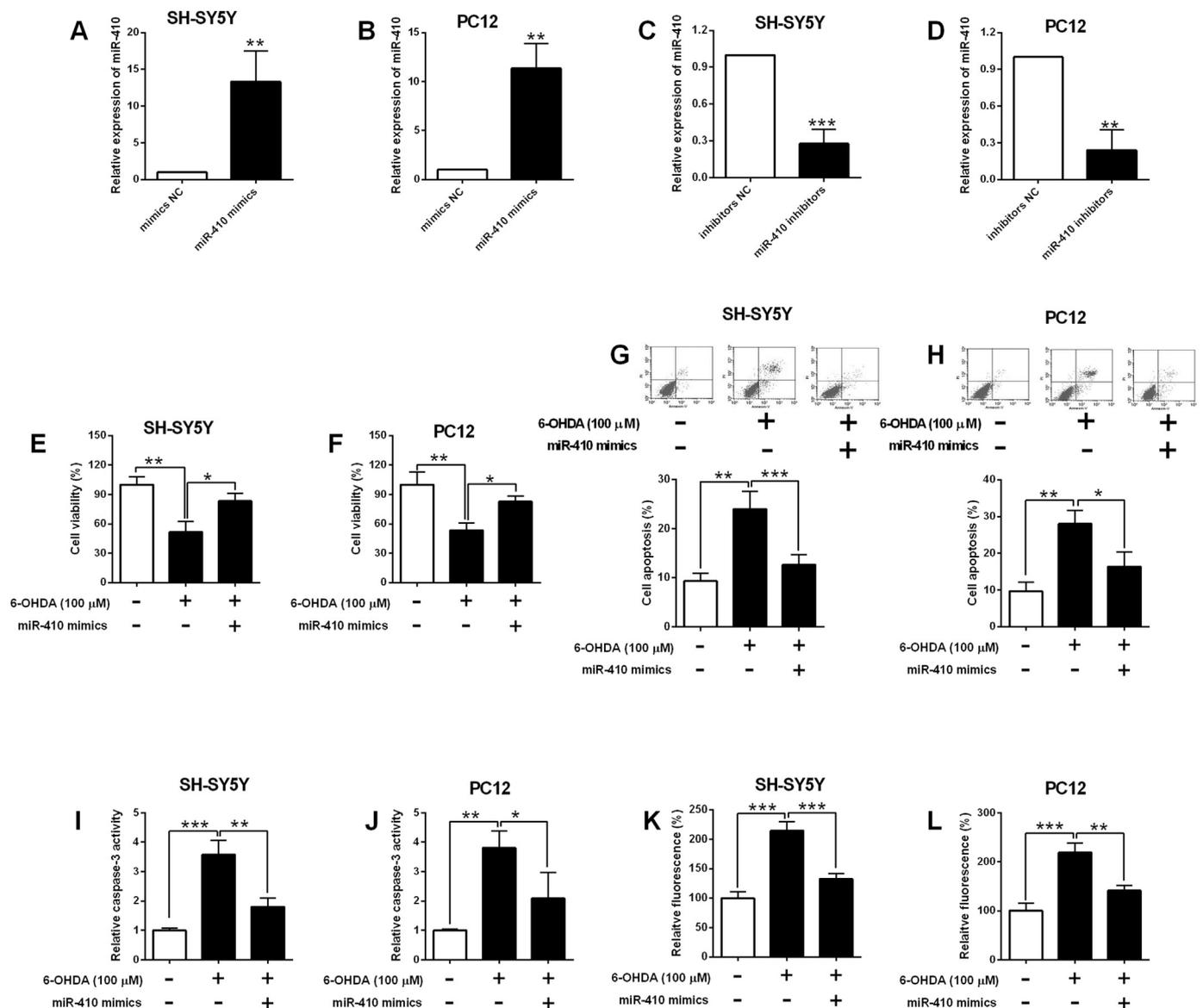


Fig. 2. MiR-410 overexpression attenuated the effects of 6-OHDA treatments on the cell viability, cell apoptosis and ROS production in SH-SY5Y and PC12 cells. (A) SH-SY5Y and (B) PC12 cells were transfected with miR-410 mimics or mimics NC, at 24 h after transfection, the expression of miR-410 in these cells was detected by qRT-PCR assay. (C) SH-SY5Y and (D) PC12 cells were transfected with miR-410 inhibitors or inhibitor NC, at 24 h after transfection, miR-410 expression in these cells was determined by qRT-PCR assay. For the in vitro functional assays, SH-SY5Y and PC12 cells were transfected with miR-410 mimics or mimics NC, at 24 h after transfection, cells were again treated with 6-OHDA (100 μM) or NC for 48 h, and then cell viability of SH-SY5Y (E) and PC12 cells (F) was measured by MTT assay; cell apoptosis of SH-SY5Y (G) and PC12 cells (H) was determined by flow cytometry; caspase-3 activity of SH-SY5Y (I) and PC12 cells (J) was detected by Caspase-3 activity kit; ROS production of SH-SY5Y (K) and PC12 cells (L) was evaluated by ROS production assay. $N = 3$; significant differences between treatment groups were indicated as * $P < .05$, ** $P < .01$ and *** $P < .001$.

3.3. MiR-410 inhibition caused a suppression in cell viability, an enhancement in cell apoptosis and ROS production in SH-SY5Y and PC12 cells

Cells were treated with miRNA oligonucleotides, at 24 h post-transfection, treatment with miR-410 inhibitors markedly impaired cell viability (Fig. 3A and B), caused an increase in cell apoptotic rates (Fig. 3C and D) and caspase-3 activity (Fig. 3E and F), and enhanced ROS production (Fig. 3G and H) in SH-SY5Y and PC12 cells when compared to treatment with inhibitors NC.

3.4. MiR-410 directly targets PTEN 3'UTR and down-regulated PTEN expression in SH-SY5Y and PC12 cells

The downstream targets of miR-410 were analyzed using the online

tool (TargetsCan), and PTEN was selected for further investigation for its well-known role in regulating cell proliferative potential as well as apoptosis. As shown in Fig. 4A, the 3'UTR of PTEN that harbors the complementary sequences with miR-410 was cloned into the psi-CHECK-2 vector to construct the wild type luciferase reporter vector; and the segments of 3'UTR of PTEN with three-point mutations were used to construct the mutated luciferase reporter vector. Treatment with miR-410 mimics resulted in a decreased luciferase activity of the wild type luciferase reporter vector when compared to mimics NC group (Fig. 4B and C); while overexpression of miR-410 did not affect the luciferase activity of the mutated luciferase reporter vector (Fig. 4D and E). Moreover, treatment with miR-410 mimics resulted in PTEN down-regulation in the neuronal cell lines when compared to mimics NC group (Fig. 4F-4I; see Supplemental Fig. S2 for the concentration-dependent effects of miR-410 mimics transfection on PTEN mRNA

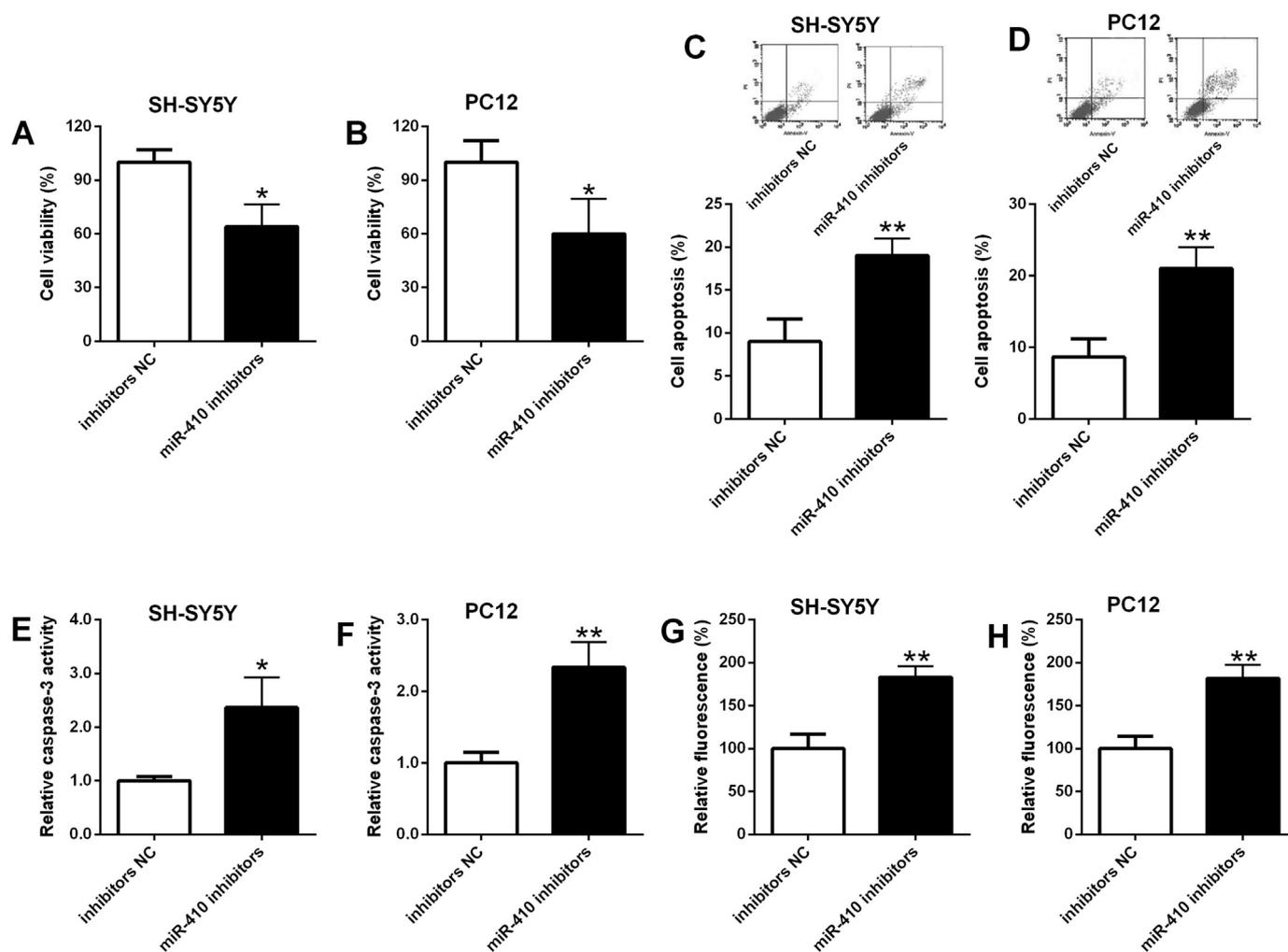


Fig. 3. MiR-410 inhibition suppressed cell viability, induced cell apoptosis and ROS production in SH-SY5Y and PC12 cells. SH-SY5Y and PC12 cells were transfected with miR-410 inhibitors or inhibitors NC, at 24 h after transfection, cell viability of SH-SY5Y (A) and PC12 cells (B) was measured by MTT assay; cell apoptosis of SH-SY5Y (C) and PC12 cells (D) was determined by flow cytometry; caspase-3 activity of SH-SY5Y (E) and PC12 cells (F) was detected by Caspase-3 activity kit; ROS production of SH-SY5Y (G) and PC12 cells (H) was evaluated by ROS production assay. $N = 3$; significant differences between treatment groups were indicated as * $P < .05$ and ** $P < .01$.

expression).

3.5. MiR-410 mediated the actions of 6-OHDA treatments on the viability, apoptosis and ROS production of SH-SY5Y and PC12 cells via targeting PTEN

The involvement of PTEN in the miR-410 mediated-effects on the cell viability, cell apoptosis and ROS production was further determined by in vitro assays in SH-SY5Y and PC12 cells. PTEN mRNA and protein overexpression was detected in both SH-SY5Y and PC12 cells after being transfected with pcDNA3.1-PTEN (Fig. 5A-5D). Transfection with pcDNA3.1-PTEN impaired the actions of miR-410 overexpression in 6-OHDA-treated SH-SY5Y and PC12 cells (Fig. 5E-5L).

3.6. MiR-410 regulated AKT/mTOR signaling pathway via targeting PTEN in SH-SY5Y and PC12 cells

The miR-410-mediated effects on the AKT/mTOR signaling pathway were determined by western blotting analysis in SH-SY5Y and PC12 cells. As shown in Fig. 6A and B, 6-OHDA treatment suppressed the protein expression of p-AKT and p-mTOR, which was partially attenuated by miR-410 overexpression in SH-SY5Y and PC12 cells (Fig. 6A

and 6B). Furthermore, miR-410 overexpression induce an increase in the p-AKT and p-mTOR protein levels but not the t-AKT and t-mTOR protein levels, and the effects of miR-410 overexpression on the protein levels were partially reversed by transfection with pcDNA3.1-PTEN (Fig. 6C and D).

4. Discussion

To look into the exact actions of miR-410 on dopaminergic neuronal cell viability and apoptosis, we employed two neuronal cell lines for establishment of a 6 cellular model of PD induced by 6-OHDA. Our data revealed that 6-OHDA concentration-dependently suppressed cell viability and miR-410 expression in these cell lines. MiR-410 overexpression partially restored the effects of 6-OHDA treatments on viability, cell apoptotic rates, caspase-3 activity as well as ROS production of these cell lines. On the other hand, inhibition of miR-410 led to a decrease in cell viability and an increase in cell apoptotic rates, caspase-3 activity as well as ROS production. Furthermore, the potential targets of miR-410 were predicted by TargetScan tool, and we verified that PTEN was a target of miR-410. In vitro assays further revealed that miR-410 repressed PTEN expression and mediated the effects in the 6-OHDA-treated cells via targeting PTEN in both SH-SY5Y and PC12 cells. Finally, 6-OHDA treatment suppressed the protein expression of p-AKT

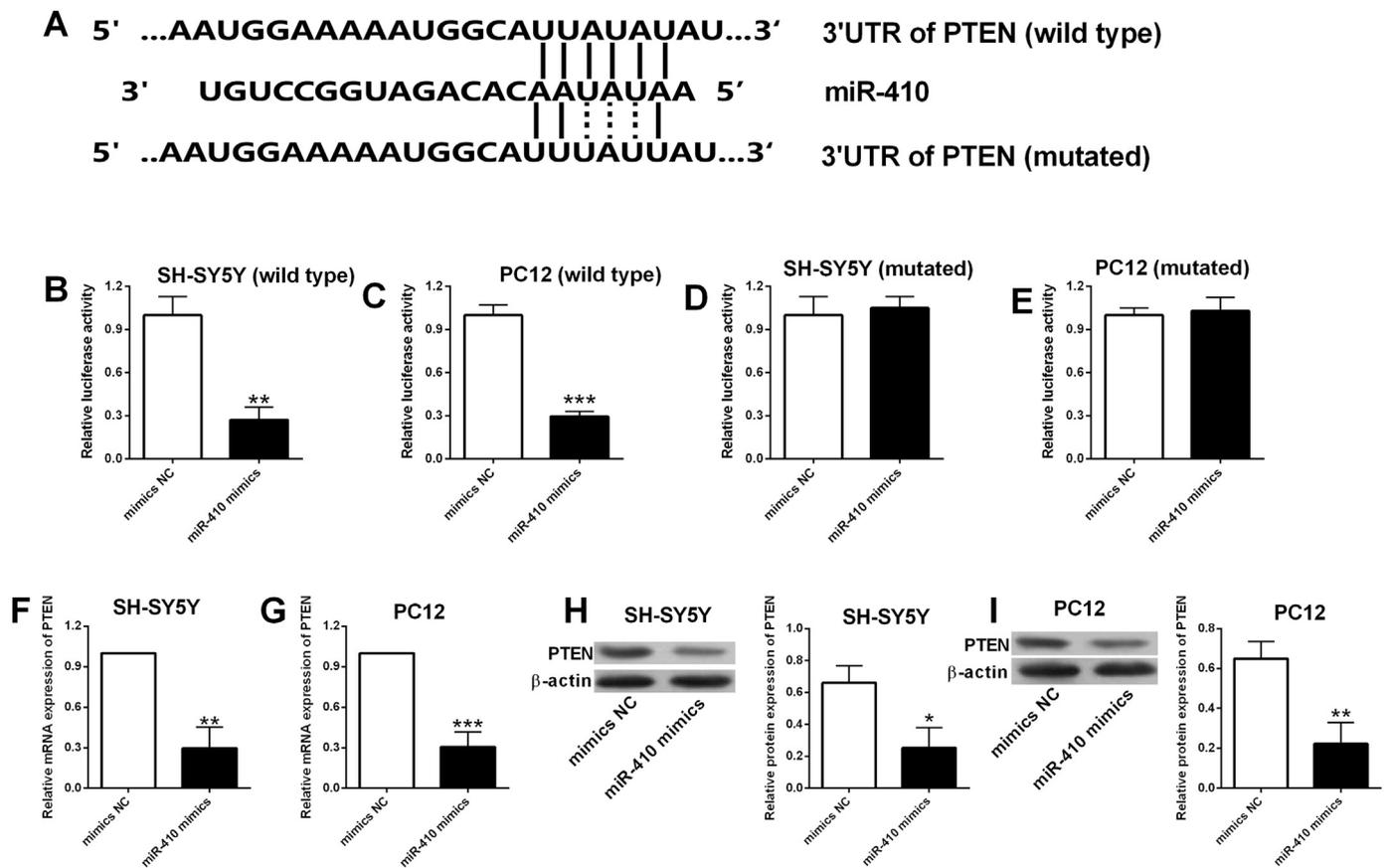


Fig. 4. MiR-410 directly targets the 3'UTR of PTEN and suppressed the expression of PTEN in SH-SY5Y and PC12 cells. (A) The complementary sequences between miR-410 and 3'UTR of PTEN (wild type) as predicted by Targetscan tool, the mutated sites were indicated as dashed line. (B&C) SH-SY5Y and PC12 cells were co-transfected with reporter vectors containing wild type 3'UTR of PTEN and miR-410 mimics (or mimics NC), at 48 h after transfection, luciferase activity was determined by Dual-luciferase reporter system. (D&E) SH-SY5Y and PC12 cells were co-transfected with reporter vectors containing mutated 3'UTR of PTEN and miR-410 mimics (or mimics NC), at 48 h after transfection, luciferase activity was determined by Dual-luciferase reporter system. The mRNA expression of PTEN in SH-SY5Y (F) and PC12 cells (G) at 24 h after being transfected with miR-410 mimics or mimics NC was determined by qRT-PCR assay. The protein expression of PTEN in SH-SY5Y (H) and PC12 cells (I) at 24 h after being transfected with miR-410 mimics or mimics NC was detected by western blot assay. N = 3; significant differences between treatment groups were indicated as *P < .05, **P < .01 and ***P < .001.

and p-mTOR, which was partially attenuated by miR-410 over-expression in SH-SY5Y and PC12 cells. MiR-410 overexpression induced p-AKT and p-mTOR protein expression, and this effect was attenuated by PTEN overexpression in both SH-SY5Y and PC12 cells. Collectively, these data suggest that PTEN involves in mediating the neuroprotective effects of miR-410 on 6-OHDA-induced neuronal cell injury.

The biological actions of miR-410 were mainly elucidated in cancer studies. MiR-410 was shown to promote cell proliferation in the lung cancer by modulation of bromodomain-containing protein 7 expression (Li et al., 2015). Wang et al., also revealed that the up-regulation of miR-410 was detected in the hepatocellular carcinoma and colorectal cancer and increased cell growth via suppressing four-and-a-half IM domains protein 1 (Wang et al., 2014). MiR-410 was also up-regulated in the prostate cancer tissues and cell lines and inhibition of miR-410 caused an inhibition of prostate cancer progression (Zhang et al., 2018). Recently, the neuroprotective effects of miR-410 were also identified, where miR-410 was demonstrated to confer neuroprotective functions against oxidative stress-induced neuronal cell death in the mice model of ischemic stroke (Tian et al., 2018); miR410 was found to control the neurogenesis in the adult subventricular zone by targeting neurogenic genes (Tsan et al., 2016). MiR-410 at the imprinted Dlk-1Dio3 locus domain controls the neonatal metabolic adaption (Labialle et al., 2014), and lack of miR-410 at the imprinted Dlk-1Dio3 locus increased anxiety-related behavior (Marty et al., 2016). In the present study, 6-OHDA treatment suppressed the viability, increased cell apoptotic rates,

capase-3 activity and ROS production in the SH-SY5Y and PC12 cells, which were consistent with 6-OHDA-induced cellular model of PD (Watanabe et al., 2018; Xie et al., 2018; Zhou et al., 2019). Over-production of ROS was suggested as a main contributor to the neuronal cell death in the midbrain from PD patients (Choi et al., 2018). Furthermore, the suppressive effects of 6-OHDA treatment on miR-410 expression were also found in the SH-SY5Y and PC12 cells. Gain-of-function and loss-of-function studies revealed that miR-410 inhibition inhibited cell viability, increased cell apoptosis and ROS production, while overexpression of miR-410 significantly attenuated the 6-OHDA-induced neuronal injury in SH-SY5Y and PC12 cells, suggesting the neuroprotective role of miR-410 in 6-OHDA-conditioned neuronal cells.

To reveal the neuroprotective effects of miR-410, we performed bioinformatics analysis, and we focused PTEN in our study. PTEN is a dual-specificity phosphatase and is well-known for its role as a tumor suppressor (Li et al., 2017a). In PD studies, elevated nuclear PTEN was found in the substantia nigra of the patients with PD (Sekar and Taghibiglou, 2018). Depletion of PTEN in adult dopaminergic neurons was found to be neuroprotective in the disease models of PD (Domanskyi et al., 2011). Studies also showed that neuronal cell death was attenuated upon transnitrosylation from DJ-1 to PTEN in the cellular model of PD (Choi et al., 2014). Our data revealed that PTEN was inversely regulated by miR-410. Effects of miR-410 overexpression on the viability, apoptotic rates and ROS production of 6-OHDA-treated neuronal cells were partially reversed upon PTEN overexpression.

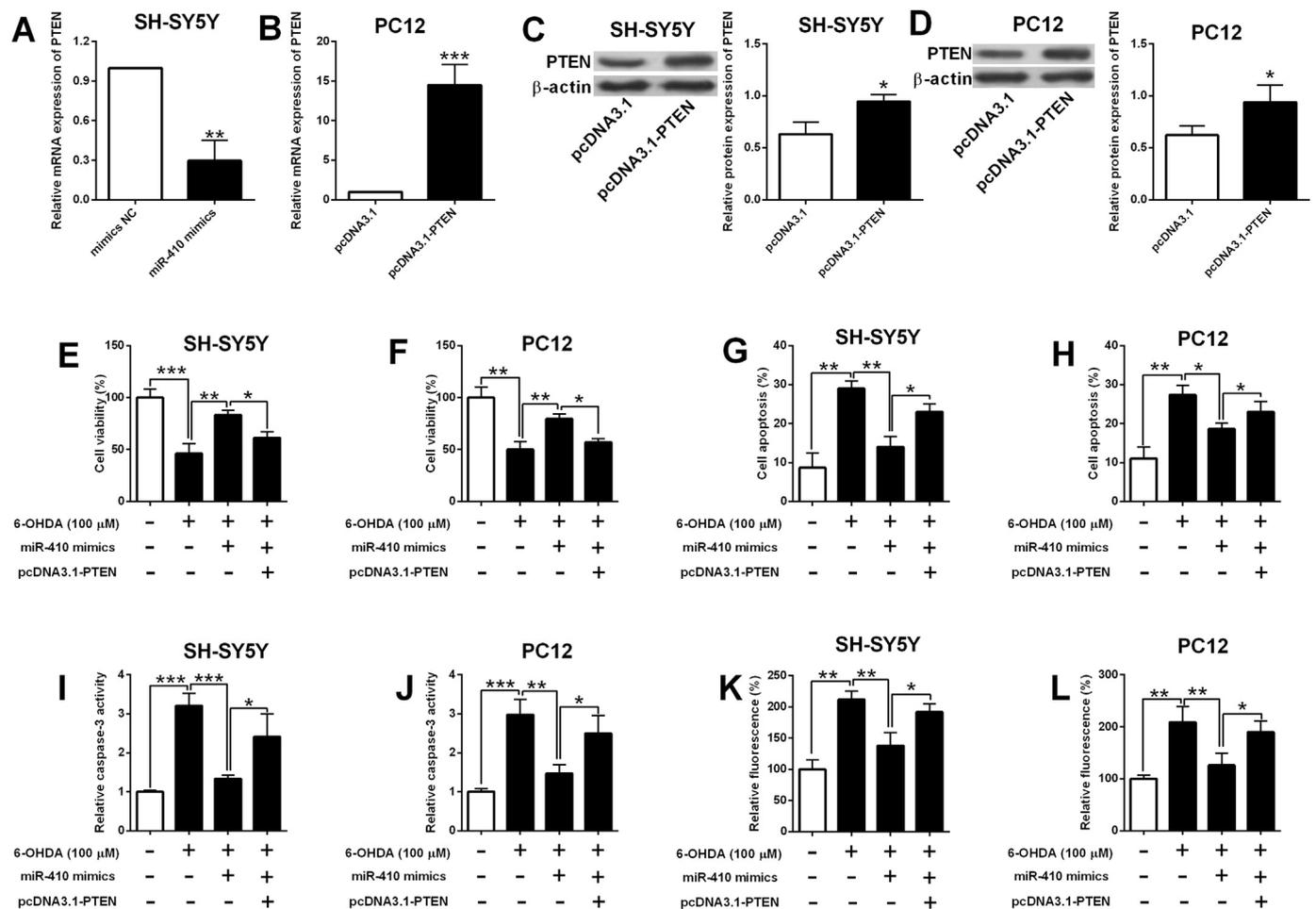


Fig. 5. MiR-410 mediated the effects of 6-OHDA treatments on the cell viability, cell apoptosis and ROS production via targeting PTEN in SH-SY5Y and PC12 cells. The mRNA expression of PTEN in SH-SY5Y (A) and PC12 cells (B) at 24 h after being transfected with pcDNA3.1-PTEN or pcDNA3.1 was determined by qRT-PCR assay. The protein expression of PTEN in SH-SY5Y (C) and PC12 cells (D) at 24 h after being transfected with pcDNA3.1 or pcDNA3.1-PTEN was detected by western blot assay. For the in vitro functional assays, cells were co-transfected with different plasmids and miRNAs, at 24 h after transfection, cells were then treated with 6-OHDA (100 μM) or NC for 48 h, and then cell viability of SH-SY5Y (E) and PC12 cells (F) was measured by MTT assay; cell apoptosis of SH-SY5Y (G) and PC12 cells (H) was determined by flow cytometry; caspase-3 activity of SH-SY5Y (I) and PC12 cells (J) was detected by Caspase-3 activity kit; ROS production of SH-SY5Y (K) and PC12 cells (L) was evaluated by ROS production assay. N = 3; significant differences between treatment groups were indicated as *P < .05, **P < .01 and ***P < .001.

These results suggested that miR-410 exerted its neuroprotective effects via suppressing PTEN. More importantly, suppression of AKT/mTOR signaling was also implicated for its contribution to dopaminergic neuronal loss (Rieker et al., 2011). Studies found that PTEN exerted suppressive effects on AKT/mTOR signaling axis (Cully et al., 2006). Our data demonstrated that 6-OHDA treatment suppressed the protein expression of p-AKT and p-mTOR, which was attenuated by miR-410 overexpression in SH-SY5Y and PC12 cells. Furthermore, miR-410 overexpression increased p-AKT and p-mTOR protein expression, which was attenuated by PTEN overexpression in SH-SY5Y and PC12 cells, suggesting that miR-410 activates the AKT/mTOR signaling via suppressing PTEN.

The present study limited to the in vitro studies using the commonly used cells lines in PD research. In the future studies, the expression profile of miR-410 should be further investigated in the animal model of 6-OHDA-induced PD; and whether restoration of miR-410 level would alleviate the PD symptoms in the animal model is also necessary for investigation. Furthermore, clinical samples from PD patients should be collected to further validate the clinical significance of miR-410 in the pathophysiology of PD. As miR-410 may have other potential targets that have not been studied in the current study, future studies are warranted to fully elucidate the regulatory network of miR-410 in

the neuronal cells. The underlying mechanisms for the dysregulation of miR-410 in the cellular model of PD may be related to methylation status in the promoter region of miR-410 (Wu et al., 2018), which also requires further investigations.

5. Conclusions

In summary, this study is for the first study to demonstrate the neuroprotective actions of miR-410 in a 6-OHDA-induced cellular model of PD, and our results indicated that miR-410 exerted its neuroprotective effects via regulating PTEN/AKT/mTOR signaling axis (see Fig. 7 for the schematic illustration). The current findings may suggest novel paradigm to study the pathology of PD.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yexmp.2019.05.002>.

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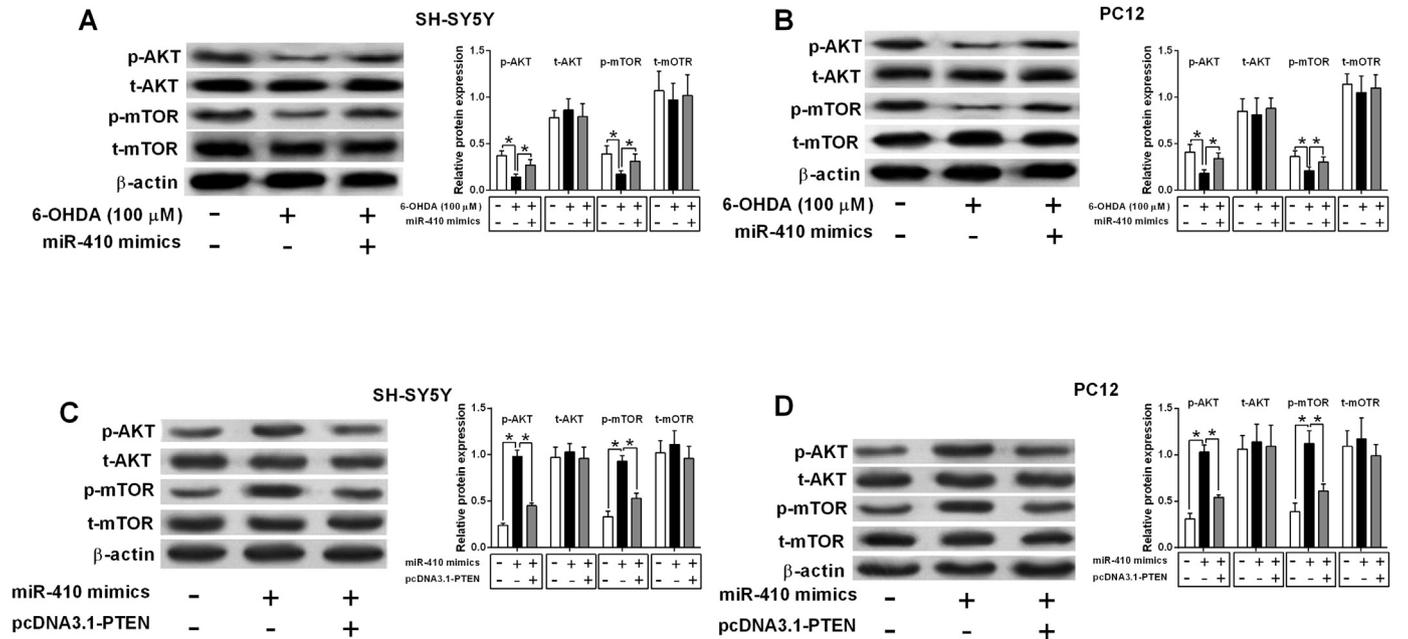


Fig. 6. MiR-410 regulated the AKT/mTOR signaling pathway via targeting PTEN in SH-SY5Y and PC12 cells. Cells were co-transfected with different plasmids and miRNAs, at 24 h after transfection, cells were then treated with 6-OHDA (100 μM) or NC for 48 h, the protein expression of p-AKT, t-AKT, p-mTOR and t-mTOR in SH-SY5Y (A) and PC12 cells (B) was detected by western blot assay. Cells were co-transfected with mimics NC + pcDNA3.1, miR-410 mimics + pcDNA3.1, or miR-410 mimics + pcDNA3.1-PTEN, at 24 h after transfection, the protein expression of p-AKT, t-AKT, p-mTOR and t-mTOR in SH-SY5Y (C) and PC12 cells (D) was detected by western blot assay. N = 3; significant differences between treatment groups were indicated as *P < .05.

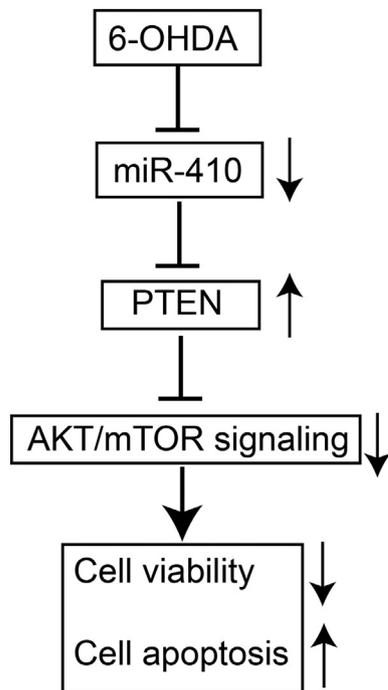


Fig. 7. Schematic illustration of the 6-OHDA-mediated molecular signaling pathways in neuronal cells.

Conflict of interest

None.

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