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Article

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Treadmill exercise improves motor function in PD mice by regulating mitochondrial and synaptic functions via miR-7/Drp1/PINK1 axis

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Abstract

Background: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra. Exercise can delay PD progression and improve motor symptoms. However, the underlying molecular mechanisms remain unclear. Whether exercise exerts protective effects by reshaping microRNA (miRNA) expression profiles remains unclear. This study aims to explore differential miRNA expression and their targeted pathways mediated by moderate-intensity exercise, and to reveal novel mechanisms of neuroprotection in PD.

Methods: Eight-week-old male C57BL/6 mice were randomly divided into normal control (NC), PD model (PD), and PD exercise (PE) groups. The PE group received 8 weeks of treadmill exercise. SH-SY5Y cells were treated with MPP^+ to establish a cellular PD model. Motor functions were assessed using pole, hanging, and gait tests.

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Immunohistochemical staining detected the levels of the rate-limiting enzyme of catecholamine biosynthesis Tyrosine hydroxylase (TH) and the pathological marker of PD α-synuclein (α-syn). Immunofluorescence staining detected TH, α-syn, and the neurogenesis markers BrdU and NeuN. Synaptic currents in the substantia nigra were recorded by brain slice patch-clamp. Cell viability was measured by CCK-8 assay, and mitochondrial membrane potential was assessed by JC-1 staining. RNA sequencing was performed to screen for differentially expressed miRNAs. ATP content was determined by biochemical assay. Dual-luciferase assay verified the interaction between miR-7 and its target genes. Western blot detected the expression of TH, α-syn, the synaptic proteins synaptophysin (SYN) and postsynaptic density 95 (PSD95), the mitochondrial fission proteins dynamin-related protein1 (Drp1), phosphorylated Drp1 (p-Drp1), and mitochondria fission protein1 (FIS1), the mitophagy-related proteins PTEN induced putative kinase 1 (PINK1), Parkin, microtubule-associated proteins 1A/1B light chain 3A/B (LC3A/B), ubiquitin-binding protein p62 (p62). qRT-PCR detected the expression of miR-7, Drp1, PINK1, and Parkin.

Results: PD group showed improved motor coordination and gait function after 8 weeks of treadmill exercise. Neurodegeneration was alleviated, and synaptic plasticity and mitochondrial function were enhanced in PD group. In PD mice and MPP^+-treated SH-SY5Y cells, miR-7 expression was significantly decreased. 8 weeks of treadmill exercise increased miR-7 expression in PD mice. Exercise regulated the Drp1/PINK1 pathway via miR-7. Overexpression of miR-7 inhibited MPP^+-induced excessive

mitochondrial fission, and alleviated mitochondrial dynamics imbalance and mitochondrial dysfunction, and improved synaptic function.

Conclusion: 8 weeks of treadmill exercise regulates mitochondrial and synaptic functions via the miR-7/Drp1/PINK1 pathway, thereby improving motor function and delaying PD progression in PD mice.

Key words: Parkinson's disease; Physical activity; mitochondrial dysfunction; miRNA; Drp1

1. Introduction

Parkinson's disease (PD) is a major neurodegenerative disorder, and its global prevalence growth rate now ranks first among all neurological diseases.[1] The core pathological features of PD include progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), with damaged projections to the striatum (ST), the mesolimbic system, and the cerebral cortex. This leads to markedly reduced dopamine levels in these regions, which in turn causes motor symptoms such as resting tremor, rigidity, and bradykinesia. Currently, clinical treatment for PD is predominantly pharmacological, but it cannot prevent the progressive loss of dopaminergic neurons. Moreover, given the long latency, persistent symptoms, and slow progression of PD, early accurate diagnosis, effective disease intervention, and long-term functional rehabilitation remain major challenges. [2]

It is currently believed that progressive degeneration of dopaminergic neurons is

closely associated with mitochondrial dysfunction. Under physiological conditions, moderate fission mediated by dynamin-related protein 1 (Drp1), a key regulator of mitochondrial fission, can activate protective mitophagy, but abnormal activation of Drp1 in PD disrupts mitochondrial network stability, promotes excessive mitochondrial fragmentation, and exacerbates cell death. [3] The E3 ubiquitin ligase Parkin and the ubiquitin kinase PTEN-induced putative kinase 1 (PINK1) are crucial for maintaining mitophagy homeostasis, and mutations in either gene can directly cause early-onset PD. [4] Inhibition of Drp1 activity or overexpression of PINK1/Parkin ameliorates α-synuclein (α-syn)-induced mitochondrial fragmentation, depolarization of mitochondrial membrane potential, and neuronal apoptosis. [5] This suggests that targeting mitochondrial dynamics and mitophagy pathways may be a potential direction for PD therapy.

MicroRNAs (miRNAs) are a class of non-coding small RNAs with a length of 19–24 nucleotides. Dysregulation of miRNAs may promote the onset and progression of PD by modulating various genes and proteins associated with the disease. [6] Among them, aberrant expression of multiple miRNAs is closely linked to mitochondrial dysfunction. [7] miR-7 (also known as miR-7a) is highly enriched in brain tissues, with highly conserved gene structure and sequence. In the genome, its mature form is encoded by multiple gene loci (miR-7-1, miR-7-2, and miR-7-3) located on different chromosomes, each producing a precursor that is ultimately processed into the same mature sequence. Similarly, in mice, miR-7a-1, miR-7a-2, and miR-7b, located on three

different chromosomes, follow this same encoding pattern. miR-7 protects against neuronal damage in MPTP-induced PD models by inhibiting mechanistic target of rapamycin (mTOR) signaling and restoring B-cell lymphoma 2 (BCL-2) levels. [8] However, the specific regulatory network of miR-7 in PD pathogenesis, particularly its direct mechanism of action on neuronal mitochondria, remains to be further elucidated.

Exercise has been proved to exert neuroprotective effects on PD by regulating specific miRNA expression and improving mitochondrial function. However, whether exercise influences mitochondrial function via miR-7, improves motor symptoms, and exerts beneficial effects on PD neurons remains unclear. This study aims to analyze synaptic function and mitochondrial status in PD mice after exercise, investigate whether exercise regulates mitochondrial function, improves synaptic plasticity and motor symptoms through the miR-7/Drp1/PINK1 pathway, and elucidate the potential mechanisms underlying exercise-delayed PD progression, thereby providing a theoretical basis for the prevention and treatment of neurodegenerative diseases.

2. Materials and methods

2.1. Animals and model preparation

Forty-five male C57BL/6 mice (8 weeks, 25-30 g) were obtained from the Hubei Provincial Experimental Animal Research Center and housed under controlled conditions (23~26℃, 40%~70% humidity, 12h light/dark) with free access to food and water. After 1 week acclimation, mice were randomly divided into PD model group (PD group, n=30) and control group (NC group, n=15). The PD group received daily

intraperitoneal MPTP (25 mg/kg) for 7 consecutive days. [9] The NC group received an equal volume of saline. Three mice per group received daily BrdU (50 mg/kg) for 7 consecutive days to track neurogenesis. All animal procedures were approved by the Ethics Committee of Wuhan Sports University.

2.2. Behavioral testing

The wire hanging test (four limbs hanging and fore limbs hanging) measured suspension duration on a grid 50 cm above the floor; each mouse performed three trials with 30-min intervals (max 600 s per trial).

The pole test used a vertical (90°) wooden pole (50 cm × 1 cm diameter, lower end fixed). Mice were placed head-up on top, the times to turn downward and to reach the bottom were recorded in three trials per mouse.

2.3. Treadmill exercise protocol

PD mice were randomly divided into PD group and PD exercise group (PE group). PE group were familiarized with treadmill exericse (8 m/min, 30 min/day, 5days), rested 2days, then treadmill exercise at 12 m/min for 60 min/day (with 5-min warm-up and cool-down at 5 m/min), 5days/week for 8 weeks. The intensity of this exercise protocol was moderate (65-70% VO_{2max}). [10] The timeline is shown in Fig. 1.

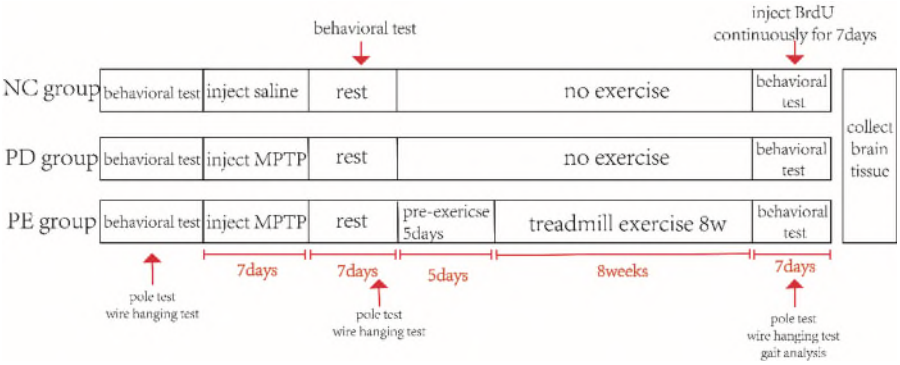


Fig. 1. Exercise intervention flow chart

2.4. Gait analysis

Gait analysis was performed post-exercise via the ink footprint method. The fore limb paws were painted with blue ink and the hind limb paws with red ink for distinction. Mice walked through a transparent corridor to a dark chamber, and the recording paper at the bottom was collected to measure the fore limb and hind limb stride length (the distance between successive placements of the same paw in a complete stride), fore limb and hind limb stance width (the distance between fore limb placements in a complete stride).

2.5. Electrophysiology

After cardiac perfusion, brains were removed and coronal slices (300μm) prepared. Whole-cell patch-clamp recordings from substantia nigra (SN) neurons used standard ACSF with 1 μM Tetrodotoxin and 10 μM Bicuculline (miniature excitatory postsynaptic currents, mEPSCs) or 1 μM Tetrodotoxin, 10 μM CNQX and 50 μM AP-5 (miniature inhibitory postsynaptic currents ,mIPSCs). All current signals were recorded with AxonMultiClamp700B amplifier (voltage-clamp mode, 3kHz filter) and analyzed with Clampfit 11.2.

2.6. Cell cultures and transfection

The human SH-SY5Y cells (CL-0208, Procell, Wuhan, China) were cultured in specialized medium (CM-0208, Procell, Wuhan, China) with 10% fetal bovine serum at 37℃ in a 5%CO_2 incubator. Cells were divided into NC group and 1-methyl-4-phenylpyridinium (MPP^+, M423656, Aladdin, Shanghai, China) at 0, 0.1, 0.3, 0.5, 1, 1.3 mM or with 1mM for 1, 6, 12, 24, and 48 hours before collection. For transfection, cells seeded into 6-well plates to 70% confluence were incubated for 48-hour with mixtures of miR-7 mimic, mimic NC, miR-7 inhibitor, inhibitor NC (Ribobio, Shenzhen, China) plus Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) in Opti-MEM (PWL041, MeilunBio, Dalian, China), then used for subsequent assays.

2.7. CCK8

Treated cells were seeded into 96-well plates. After attachment, CCK8 solution (MA0218, MeilunBio, Dalian, China) was added and incubated for 2 hours, then OD450 nm was measured.

2.8. Immunohistochemistry

Mice were anesthetized with isoflurane (R510-22-10, RWD Life Science, Shenzhen, China) and sacrificed. Brains were fixed in paraformaldehyde, paraffin-embedded, sectioned, dewaxed, and rehydrated. After antigen retrieval and blocking, sections were incubated overnight with primary antibodies against tyrosine hydroxylase (TH, ab152, Sigma-Aldrich, St. Louis, MO,USA) and α-syn (ab51253,

Abcam, Cambridge, MA, USA), then with secondary antibodies and developed with DAB.

2.9. Immunofluorescence

Tissues were fixed, washed, permeabilization, blocked, then incubated with primary antibodies against BrdU (66241-1-lg, proteintech, Wuhan, China), NeuN (Cy5515, Abways, Shanghai, China), TH, and α-syn, followed by secondary antibodies, mounted, and observed under a fluorescence microscope.

2.10. Mitochondrial membrane potential assay

The cells were washed with JC-1 assay buffer, incubated in JC-1 working solution (E-CK-A301, Elabscience, Wuhan, China) for 30min at 37℃, then observed under a fluorescence microscope.

2.11. RNA sequencing

Total RNA was extracted. RNA concentration and purity (Nanodrop instrument), integrity (agarose gel), and RQN value (Agilent 5300) were assessed. Libraries were prepared via adapter ligation, reverse transcription, PCR, enrichment, and purification, then sequenced on NovaSeq X Plus or DNBSEQ platform, as appropriate. After quality control of raw reads, clean reads were mapped (HISAT2 software), assembled (StringTie), quantified (RSEM), and differentially expressed genes ($|\log2F\ C| > 1$, p adjust≤ 0.05) identified (DESeq2). Library preparation and sequencing were performed by Majorbio Biotechnology Co., Ltd (Shanghai, China), and data analyzed on the Majorbio Cloud Platform (www.majorbio.com).

2.12. qRT-PCR

RNA was extracted using TRIzol reagent, and DNA was extracted using FastPure Cell/Tissue DNA Isolation Mini Kit (DC102, Vazyme, Nanjing, China). cDNA was reverse transcribed using Mir-X miRNA First-Strand Synthesis Kit (638313, Takara, Tokyo, Japan) and 5×SweScript All-in-One SuperMix for qPCR (G3337, Servicebio, Wuhan, China). qPCR was run with TB Green Advantage qPCR Premix (639676, Takara, Tokyo, Japan) and 2×Universal Blue SYBR Green qPCR Master Mix (G3336, Servicebio, Wuhan, China) on a Step One Plus Real-time PCR system (Applied Biosystems Inc, Carlsbad, CA, USA), using primers for mmu/hsa-miR-7a-5p, PINK1, Parkin, Drp1 (Ribobio), and nuclear/mitochondrial DNA (Takara).

2.13. Western blot

After tissue lysis, supernatants were collected for protein quantification, mixed with loading buffer, boiled at 100 °C for 10 min, separated by electrophoresis on 12% SDS-PAGE, and transferred to membranes. Membranes were blocked with 5% non-fat milk, incubated overnight with primary antibodies, then with HRP-conjugated secondary antibodies, and detected by chemiluminescent reagents. Primary antibodies: PINK1 (23274-1-AP, proteintech), Parkin (2132, CST, Danvers, MA, USA), microtubule-associated proteins 1A/1B light chain 3A/B (LC3A/B, 14600-1-AP, proteintech), ubiquitin-binding protein p62 (p62, 18420-1-AP, proteintech), Drp1 (81561-1-RR, proteintech), p-Drp1 (AF8470, Affinity Bioscience, Liyang, China), mitochondrial fission protein 1 (FIS1, 10956-1-AP, proteintech), α-syn (10842-1-AP,

proteintech), synaptophysin (SYN, 17785-1-AP, proteintech), TH, postsynaptic density 95 (PSD95, 20665-1-AP, proteintech), and GAPDH (10494-1-AP, proteintech).

2.14. ATP detection

Tissues were lysed and centrifuged, supernatants were incubated with ATP assay working solution (HY-K0314, MCE, Monmouth Junction, NJ, USA) in opaque 96-well plates for 5 min ar RT, then standards/supernatants were added, shaken for 5 s to mix, RLU measured with a multimode microplate reader.

2.15. Dual-luciferase assay

Cells transfected with miR-7-5p mimic or mimicNC were co-transfected with either pmirGLO empty vector or pmirGLO vector containing the full-length 3′UTR of Drp1. Luciferase activity was measured per dual-luciferase assay kit (Genecreate, Wuhan, China).

2.16. Statistical analysis

The statistics and graphing used GraphPad Prism 8.0.2 (GraphPad Software Inc., San Diego, CA, USA). Data are presented as mean±SD. Statistical differences were analyzed by one-way analysis of variance followed by Tukey's test. Differences were considered significant at $P < 0.05$.

3. Results

3.1. PD mice exhibit decreased motor function and neuronal degeneration

As shown in Fig. 2A and 2B, PD group after MPTP injection showed significantly increased turn-down time and total time in the pole test compared with NC group (**p

< 0.01, ***p < 0.001). The fore limbs and four limbs hanging durations were significantly shortened (Fig. 2C and 2D) (*p < 0.05, ***p < 0.001). Compared with PD group before injection, the PD mice after injection showed significantly increased turn-down time and total pole time (****p < 0.0001), and significantly decreased fore limbs and four limbs hanging duration (****p < 0.0001). TH staining density in SN and ST of PD group was significantly lower than that in NC group (*p < 0.05, **p < 0.01), whereas α-syn staining density was significantly higher (*p < 0.05, **p < 0.01) (Fig. 2E-2I). Western blot (Fig. 2J-2M) confirmed higher α-syn expression (***p < 0.001) in SN and lower TH expression in SN and ST (****p < 0.0001) in PD. These results indicated that after MPTP injection, PD mice exhibited motor coordination impariment and decreased muscle strength, reduced expression of the dopamine synthesis rate-limiting enzyme TH, accompanied by abnormal α-syn aggregation and dopaminergic neuron pathology.

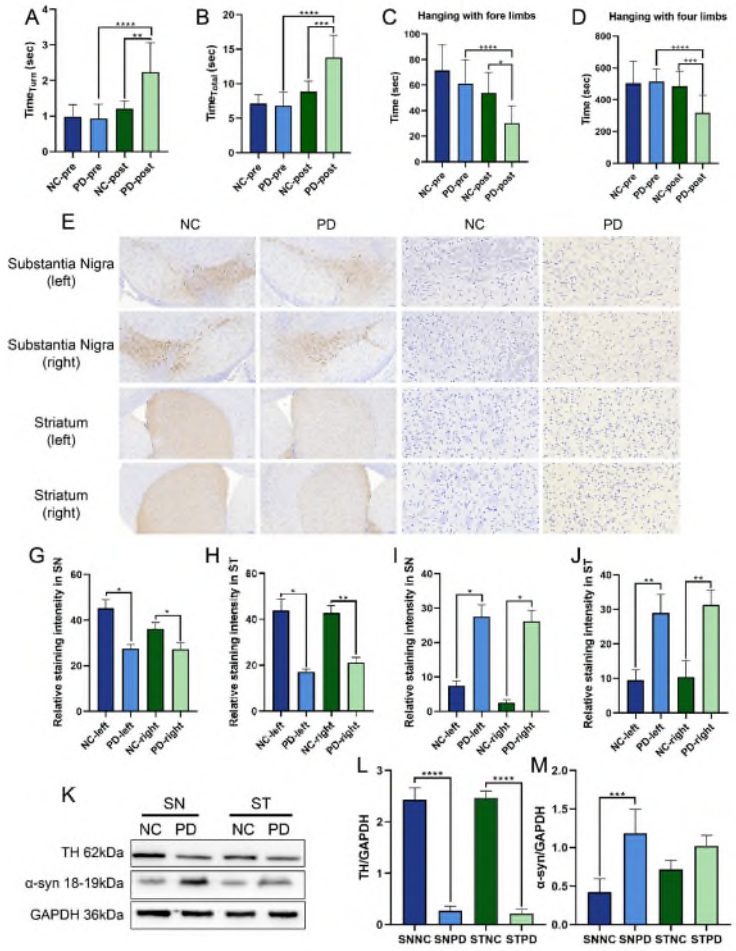


Fig.2. The results of MPTP mouse model. (A-B) Statistical results of turn-down time and total time in the pole test of each group. (C-D) Statistical results of fore limbs and four limbs hanging duration for each group. (E) Immunohistochemical staining of TH and α-syn for each group. (F-I) Statistical results of immunohistochemistry for each group. (J) Representative images of Western blot results. (K-L) Statistical results of TH and α-syn expression in SN and ST. ****p < 0.0001; ***p < 0.001; **p < 0.01; *p < 0.05. NC = normal control group; PD = Parkinson's disease; SN = substantia nigra; ST = striatum; TH = tyrosine hydroxylase; α-syn = α-synuclein.

3.2. Treadmill exercise improves motor function in PD mice

Behavioral test results showed that compared with PD group, PE group exhibited decreased turn-down time and total time in the pole test (**p < 0.01, ***p < 0.001) (Fig.

3A and 3B), increased fore limbs and four limbs hanging durations (*p < 0.05, ****p < 0.0001) (Fig. 3C and 3D),increased fore limbs and hind limbs stride lengths (**p < 0.01) (Fig. 3E and 3G), and decreased fore limbs stance widths (***p < 0.001) (Fig. 3F). These results indicated that PD mice showed impaired motor coordination, significantly reduced muscle strength, and gait deficits, whereas 8-week treadmill exercise improved motor coordination and gait function in PD mice.

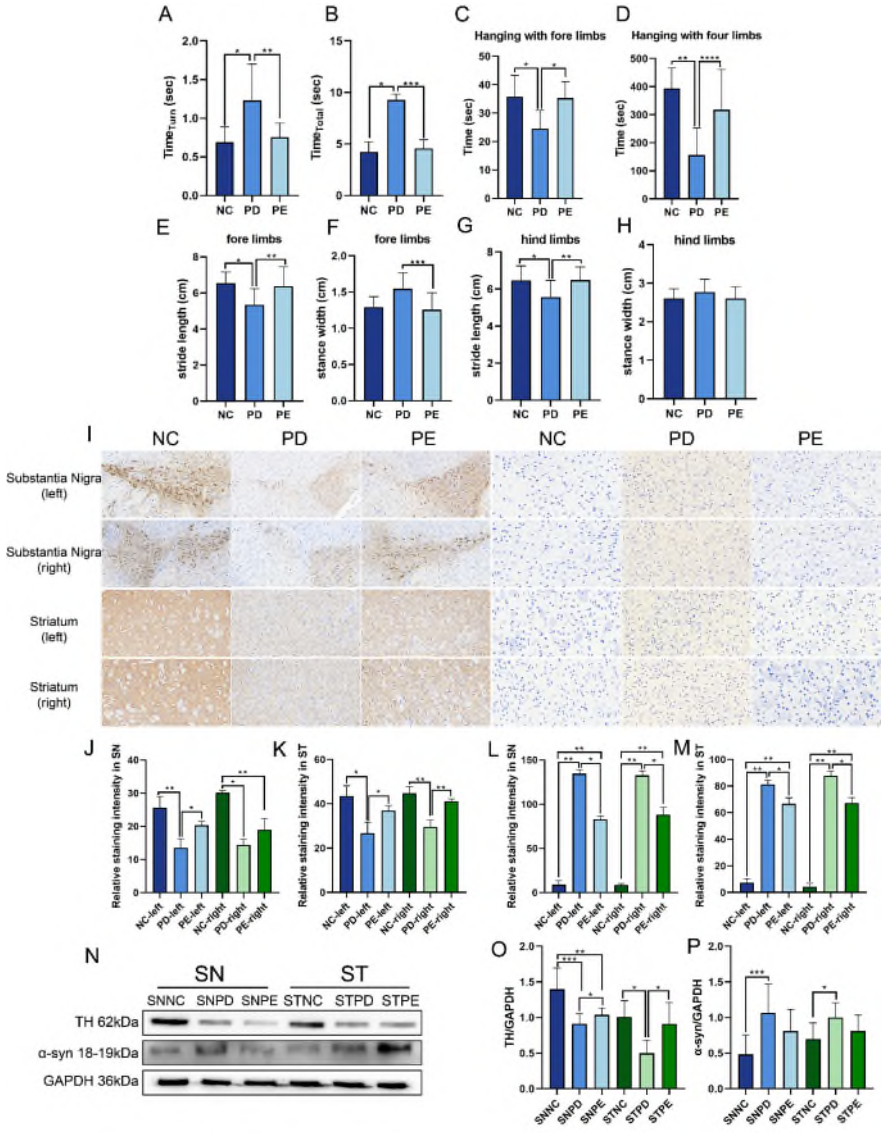


Fig.3. Exercise improves motor symptoms and protects dopaminergic neurons in PD mice. (A-B) Statistical results of turn-down time and total time in the pole test of each group. (C-D)

Statistical results of fore limbs and four limbs hanging duration for each group. (E-F) Statistical results of stride length and stance width in gait test for each group. (I) Immunohistochemical staining of TH and α-syn for each group. (J-M) Statistical results of immunohistochemistry for each group. (N) Representative images of Western blot results. (O-P) Statistical results of TH and α-syn expression. ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. NC = normal control group; PD = Parkinson's disease; PE = PD + exericse; SN = substantia nigra; ST = striatum; TH = tyrosine hydroxylase; α-syn = α-synuclein.

3.3. Treadmill exercise ameliorates neuronal degeneration in PD mice

Immunohistochemical (Fig. 3I-3M) showed that TH staining in SN and ST of PD group was significantly lower than that in NC group (**$p < 0.01$, *$p < 0.05$), and was significantly higher in PE group than in PD group (*$p < 0.05$, **$p < 0.01$). α-syn staining in SN and ST of both the PD and PE groups was significantly higher than that in NC group (**$p < 0.01$), whereas tht in PE group was significantly lower than that in PD group (*$p < 0.05$). Western blot results (Fig. 3N-3P) showed that TH expression in SN and ST of PD group was significantly lower than that in NC group (***$p < 0.001$, *$p < 0.05$), and α-syn expression was significantly increased than NC group (***$p < 0.001$, *$p < 0.05$). After 8-week exercise, TH expression in SN and ST was significantly increased (*$p < 0.05$), and α-syn expression was slightly decreased. These results indicate that 8-week treadmill exercise upregulated TH expression and inhibited abnormal α-syn deposition.

3.4. Treadmill exercise improves synaptic function in PD mice

Synaptic transmission analysis revealed that the mEPSC frequency in SN neurons of PD group was significantly decreased compared with that of NC group (***p < 0.001), whereas exercise increased mEPSC frequency (*p < 0.05). The PD group showed significantly increased mIPSC frequency (*p < 0.05), and exercise reversed this effect (****p < 0.0001). Fig. 4B, 4D, 4F and 4H display cumulative probability of interevent interval and amplitude, showing a significantly shift in the distribution between PD and PE groups. These results indicate that PD mice exhibit synaptic abnormalities in SN neurons, and 8 weeks of treadmill exercise corrects this synaptic dysfunction.

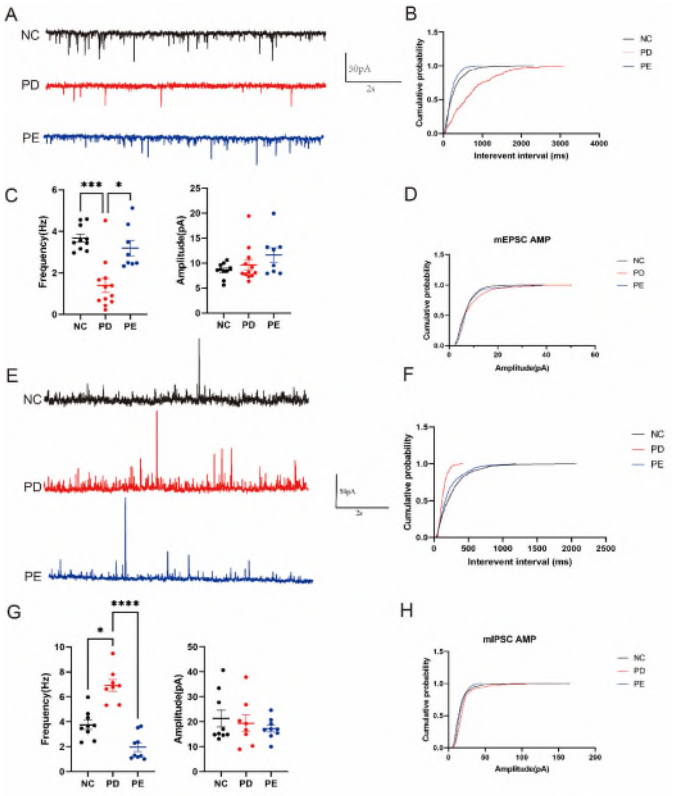


Fig.4. Exercise improves synaptic electrophysiology in PD mice. (A) Representative traces of mEPSCs recorded from SN neurons of each group. (B) Cumulative probability of interevent interval in mEPSC. (C) Quantitation of mEPSCs frequencies and amplitudes. (D) Cumulative

probability of amplitude in mEPSC. (E) Representative traces of mIPSCs recorded from SN neurons of each group. (F) Cumulative probability of interevent interval in mIPSC. (G) Quantitation of mIPSCs frequencies and amplitudes. (H) Cumulative probability of amplitude in mIPSC. ****$p < 0.0001$; ***$p < 0.001$; *$p < 0.05$. NC = normal control group; PD = Parkinson's disease; PE = PD + exericse; mEPSCs = miniature excitatory postsynaptic currents; mIPSCs = miniature inhibitory postsynaptic currents.

Immunofluorescence (Fig. 5A and 5B) showed that BrdU intensity in SN and ST was significantly lower in PD than in NC group (*$p < 0.05$, **$p < 0.01$), but significantly higher in PE compared with PD group (**$p < 0.01$), indicating increased neurogenesis after 8 weeks of exercise. Western blot results (Fig. 5C-5F) showed that PSD95 expression in SN and ST, SYN expression, and dopamine transporter (DAT) expression were significantly decreased in PD compared with NC group (*$p < 0.05$, ***$p < 0.001$, **$p < 0.01$). After 8 weeks of treadmill exercise, PSD95 expression in SN and ST of PE group was significantly upregulated (*$p < 0.05$), SYN expression in ST was significantly upregulated (*$p < 0.05$), and DAT expression showed an increasing trend. These results suggest that 8-week treadmill exercise promoted neurogenesis, upregulated synaptic plasticity-related proteins, and improved synaptic structure and function in PD mice.

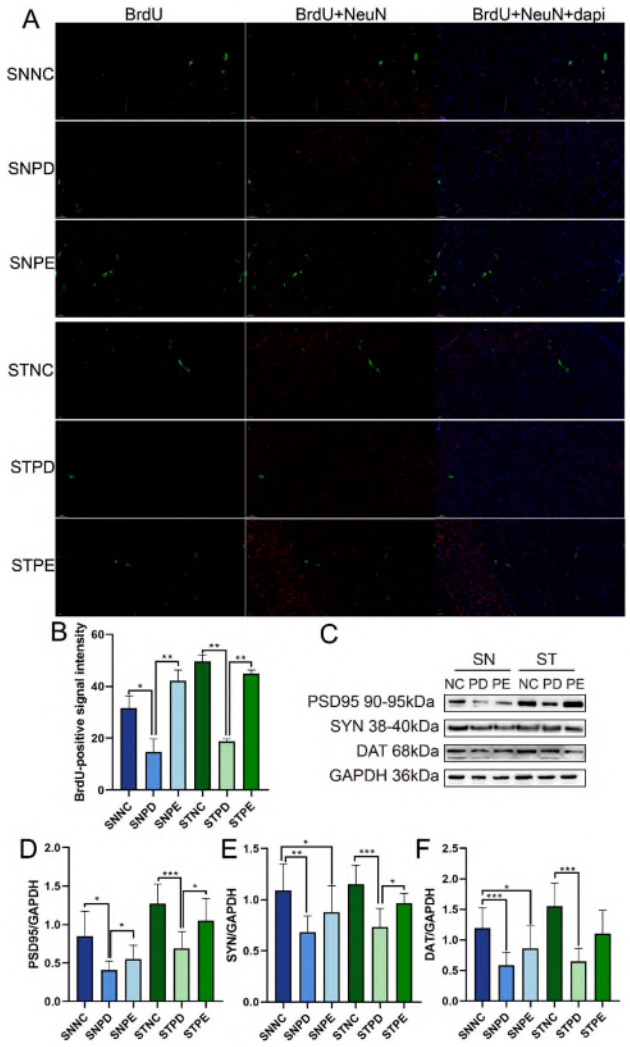


Fig.5. Exercise improves synaptic plasticity in PD mice. (A) Representative images of immunofluorescence for each group. (B) Statistical results of BrdU-positive fluorescence intensity for each group. (C-F) Expression results of synaptic-related protein for each group. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. NC = normal control group; PD = Parkinson's disease; PE = PD + exericse; SN = substantia nigra; ST = striatum; PSD95 = postsynaptic density 95; SYN = synaptophysin; DAT = dopamine transporter.

3.5. Treadmill exercise regulates the mitochondrial respiratory chain in PD mice

Fig. 6A showed ATP content was significantly lower in both PD and PE group than in NC group (***$p < 0.001$, **$p < 0.01$), with PE group slightly higher than PD group.

Western blot results (Fig. 6B-6E) showed that cytochrome c expression in SN and ST of PD group was significantly reduced compared with NC group (*p < 0.05), and cytochrome c oxidase subunit IV (COXIV) and translocase of outer mitochondrial membrane 20 (TOM20) in SN were also reduced (*p < 0.05, **p < 0.01). After 8 weeks of treadmill exercise, cytochrome c expression in SN was significantly upregulated (*p < 0.05), TOM20 expression in ST was significantly upregulated (**p < 0.01), and COXIV slightly increased over PD group. These results suggest 8 weeks of treadmill exercise upregulated key mitochondrial respiratory chain proteins in PD neurons, while restoration of ATP levels may require longer intervention.

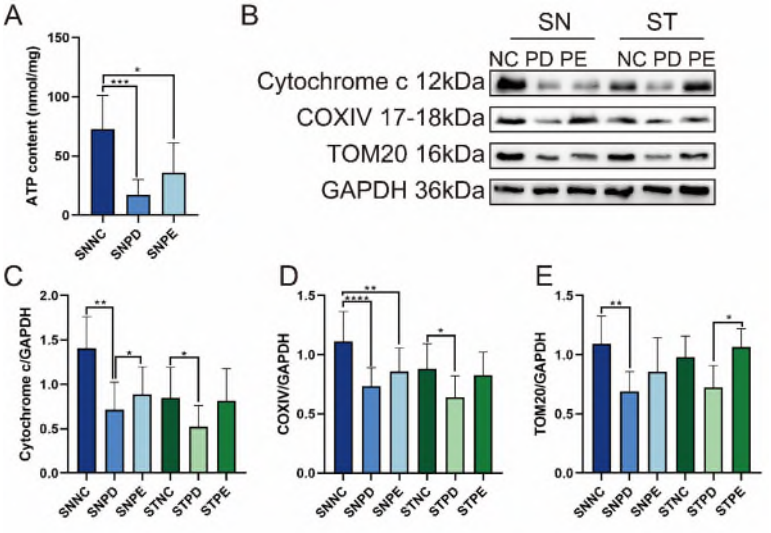


Fig.6. Exercise regulates the mitochondrial respiratory chain in PD mice. (A) Statistical results of ATP content for each group. (B-E) Expression results of the mitochondrial respiratory chain-related protein for each group. ****p < 0.0001; ***p < 0.001; **p < 0.01; *p < 0.05. NC = normal control group; PD = Parkinson's disease; PE = PD + exericse; SN = substantia nigra; ST = striatum; COXIV = cytochrome c oxidase subunit IV; TOM20 = outer mitochondrial membrane 20.

3.6. Treadmill exercise improves mitochondrial function in PD mice

Western blot (Fig. 7A-7H) showed that Drp1 expression in SN and ST of PD group was significantly higher than that in NC group (****$p < 0.0001$, **$p < 0.01$), and p-Drp1 expression was higher than NC group (***$p < 0.001$, *$p < 0.05$). FIS1 expression in the ST was significantly higher than that in NC group (*$p < 0.05$). PINK1 and Parkin expression were significantly decreased compared with NC group (****$p < 0.0001$, *$p < 0.05$, ***$p < 0.001$). LC3A/B and p62 expression were significantly decreased compared with NC group (****$p < 0.0001$, ***$p < 0.001$, *$p < 0.05$). After 8 weeks of treadmill exercise, Drp1 decreased in SN and ST (***$p < 0.001$), p-Drp1 expression was slightly reduced, and FIS1 decreased in ST compared with PD group (**$p < 0.01$). PINK1 and Parkin expression were significantly increased (*$p < 0.05$, **$p < 0.01$), LC3A/B expression was slightly increased, and p62 expression significantly increased in SN (*$p < 0.05$). mRNA analysis showed Drp1 mRNA expression in PD group was significantly higher than that in NC group (*$p < 0.05$), PINK1 and Parkin mRNA expression were significantly lower (***$p < 0.001$, *$p < 0.05$). In PE group, PINK1 mRNA expression was significantly increased compared with PD group (***$p < 0.001$). These results suggest that 8-week treadmill exercise alleviated abnormal mitochondrial fission and improved the mitochondrial fission-mitophagy imbalance in PD mice.

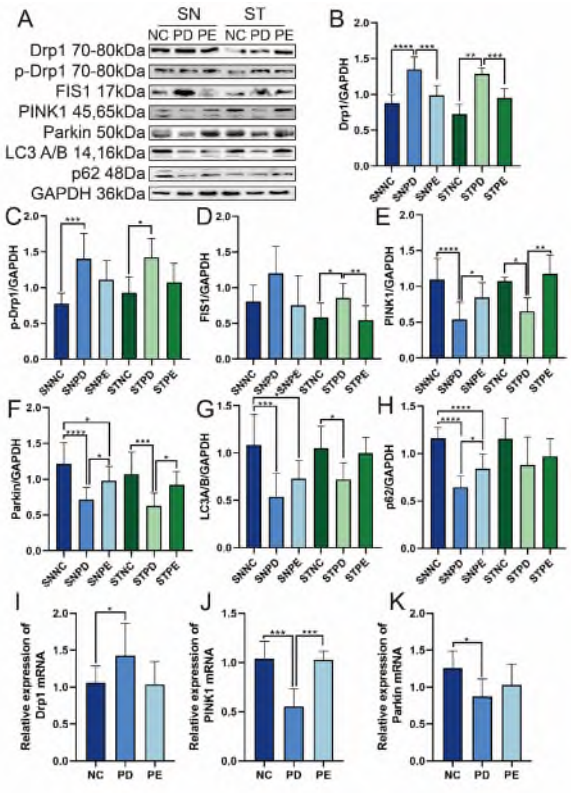


Fig.7. Exercise regulates Drp1/PINK1 pathway in PD mice. (A-H) Expression results of mitochondrial fission-and mitophagy-related protein for each group. (I-K) Expression results of Drp1, PINK1, Parkin mRNA. ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. NC = normal control group; PD = Parkinson's disease; PE = PD + exericse; SN = substantia nigra; ST = striatum; Drp1 = dynamin-related protein1; p-Drp1 = phosphorylated Drp1; FIS1 = mitochondria fission protein1; PINK1 = PTEN induced putative kinase 1; LC3A/B = microtubule-associated proteins 1A/1B light chain 3A/B; p62 = ubiquitin-binding protein p62.

3.7. Treadmill exercise regulates miRNA expression in SN of PD mice

Differential expression analysis (Fig. 8A-8F) revealed 81 significantly upregulated and 72 significantly downregulated miRNAs in PD group compared with NC group, and 7 upregulated and 7 downregulated miRNAs in PE group compared with PD group. Summarized comparison of all differential miRNAs and verified

through PCR revealed that miR-7a-5p was significantly upregulated in PD group and significantly downregulated in PE group (Fig. 8H). GO analysis identified functions including cellular process, biological regulation, metabolic process, cellular component, and binding (Fig. 8G). GO enrichment analysis of differential target genes revealed biological processes including mitophagy and mitochondrial disassembly (Fig. 8I).

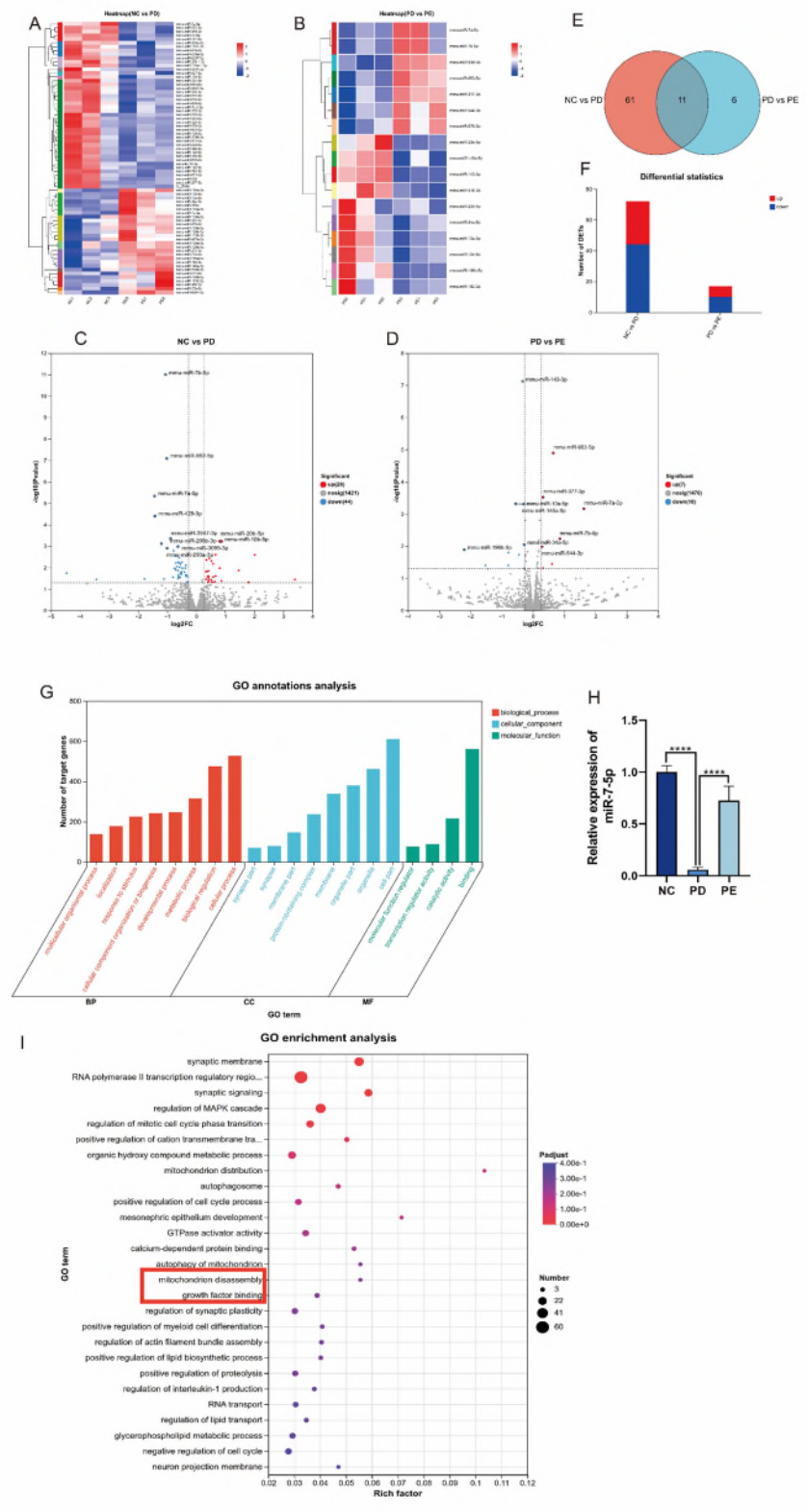


Fig.8. RNA sequencing and miR-7a-5p expression results. (A-B) Clustering heatmap of differential miRNAs between three groups. (C-D) Volcano plot of differential miRNAs between three groups. (E) Venn diagram of differential miRNAs among the three groups. (F) Bar chart

showing the number of differential miRNAs in each group. (G) GO annotation analysis of target genes of differentially expressed miRNAs. (H) miR-7a-5p expression results in each group. (I) GO enrichment analysis of target genes of differentially expressed miRNAs. ****p < 0.0001. NC = normal control group; PD = Parkinson's disease; PE = PD + exericse.

3.8. miR-7 regulates Drp1/PINK1 pathway in MPP+-treated cells

To further explore the effects of miR-7 on neurons, SH-SY5Y cells were exposed to MPP+ at different concentrations. CCK8 assay (Fig .9A) showed that treatment with 1 mM and 1.3 mM MPP+ significantly reduced cell viability compared with the 0 mM (**p < 0.01, ***p < 0.001). miR-7-5p expression decreased at all tested concentrations of MPP+ (****p < 0.0001), and over time it first increased then declined (Fig. 9C). Based on these results, cells treated with 1 mM MPP+ for 24 hours were selected for subsequent experiments.

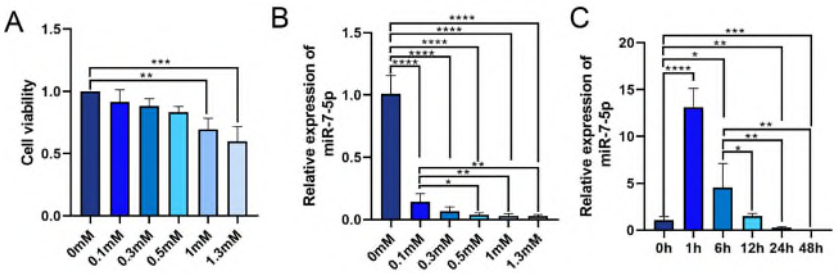


Fig.9. Effects of different concentrations of MPP+ on cells. (A) Cell viability after treatment with different concentrations of MPP+. (B) miR-7-5p expression results after treatment with different concentrations of MPP+. (C) miR-7-5p expression results after treatment with 1 mM MPP+ for different durations. ****p < 0.0001; ***p < 0.001; **p < 0.01; *p < 0.05.

To further verify the direct interaction between miR-7 and Drp1, dual-luciferase reporter assays were performed (Fig.10A and 10B), the results showed that miR-7

overexpression inhibited Drp1 activity (****$p < 0.0001$). In MPP^+-treated cells, miR-7 was significantly lower than NC group (****$p < 0.0001$). Drp1 mRNA expression was significantly higher (***$p < 0.001$), PINK1 and Parkin mRNA expression were significantly lower than NC group (*$p < 0.05$, ****$p < 0.0001$). Overexpressing miR-7 increased miR-7 (****$p < 0.0001$), while Drp1 mRNA expression was slightly decreased compared with the MPP^+ group, PINK1 mRNA expression was slightly increased, and Parkin mRNA expression was significantly increased compared with the MPP^+ group (**$p < 0.01$). And miR-7 inhibition significantly increased Drp1 expression (*$p < 0.05$) and significantly decreased Parkin expression (*$p < 0.05$). PINK1 expression was significantly decreased in both MPP^+ group and miR-7 inhibitor group (****$p < 0.0001$), miR-7 overexpression slightly decreased Drp1, and slightly increased PINK1 and Parkin compared with MPP^+ group. These results suggest that miR-7 may regulate PINK1/Parkin by targeting Drp1.

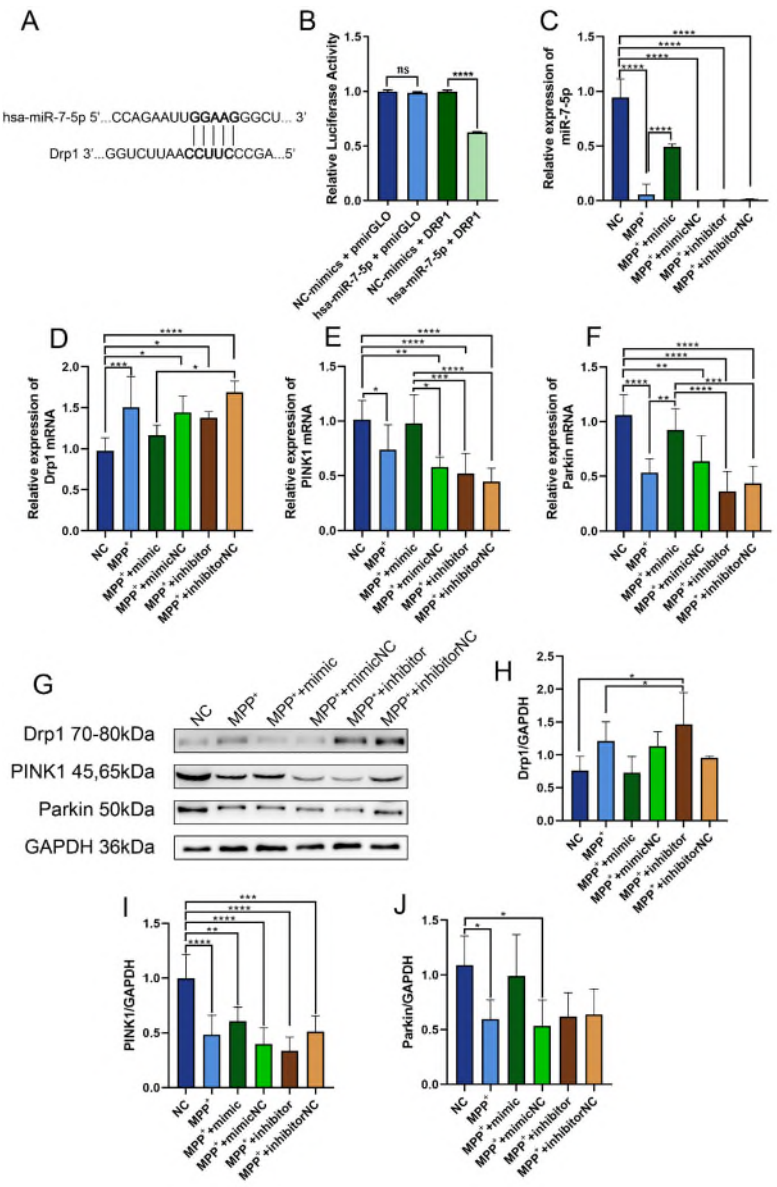


Fig.10. In vitro validation of miR-7/Drp1/PINK1 pathway. (A-B) Dual-luciferase reporter assay results. (C) miR-7-5p expression results in each group of cells. (D-F) Drp1, PINK1, and Parkin mRNA expression results in each group of cells. (G-J) Drp1, PINK1, and Parkin protein expression results in each group of cells. ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. NC = normal control group; Drp1 = dynamin-related protein1; PINK1 = PTEN induced putative kinase 1.

3.9. Overexpression of miR-7 reduces PD-related phenotypes in MPP+-treated

cells

To further verify the role of miR-7 in MPP^+ cells, immunofluorescence staining was performed to detect the fluorescence intensity of TH and α-syn after miR-7 overexpression (Fig. 11A-11C). TH fluorescence intensity was significantly lower in MPP^+ group than NC group (***$p < 0.001$), while α-syn was significantly higher (***$p < 0.001$). Overexpressing miR-7 raised TH intensity (***$p < 0.001$) and lowered α-syn intensity (***$p < 0.001$). Cell viability was significantly reduced in MPP^+ group (*$p < 0.05$), and slightly recovered after miR-7 overexpression. SYN and DAT expression in MPP^+ group were significantly lower than in NC group (***$p < 0.001$), and were slightly increased after miR-7 overexpression. These results indicate that MPP^+ induced PD-like pathological, and miR-7 overexpression effectively reversed the abnormal expression of TH and α-syn, with trends toward improved cell viability and synaptic plasticity.

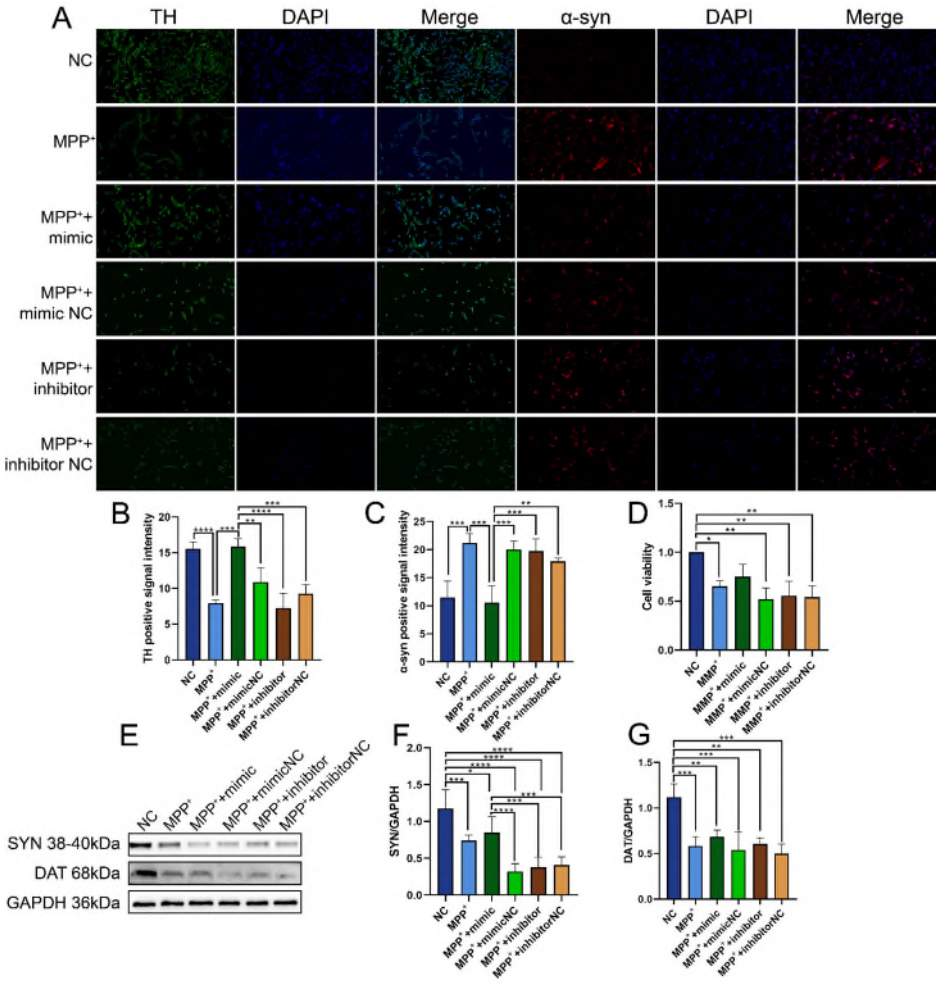


Fig.11. Expression of PD-related and synaptic-related proteins in each group of cells. (A-C) Immunofluorescence results for TH and α-syn in each group of cells. (D) CCK8 assay results in each group of cells. (E-G) Expression results for SYN and DAT in each group of cells. ****p < 0.0001; ***p < 0.001; **p < 0.01; *p < 0.05. TH = tyrosine hydroxylase; α-syn = α-synuclein; SYN = synaptophysin; DAT = dopamine transporter.

3.10. Overexpression of miR-7 improves mitochondrial function in MPP$^+$-treated cells

As shown in Fig. 12A and 12B, mitochondrial membrane potential was assessed by JC-1 staining, the red/green fluorescence intensity ratio in MPP$^+$ group was significantly lower than that in NC group (****p < 0.0001), indicating a marked

decrease in mitochondrial membrane potential. The ratio was significantly increased after miR-7 overexpression (*$p < 0.05$). ATP content was significantly decreased in MPP^+ group than in NC group (****$p < 0.0001$), and was significantly increased after miR-7 overexpression (***$p < 0.001$). mtDNA copy number was significantly lower in MPP^+ group than both NC group and miR-7 overexpression group (*$p < 0.05$). COXIV and TOM20 expression in the MPP^+ group were significantly lower than those in NC group (**$p < 0.01$, ***$p < 0.001$), and were slightly increased after miR-7 overexpression. Collectively, these results indicate that miR-7 overexpression alleviated MPP^+-induced mitochondrial dysfunction, enhanced energy production, and increased mitochondrial membrane potential, mtDNA copy number, and ATP content, with partial restoration of mitochondrial respiratory chain.

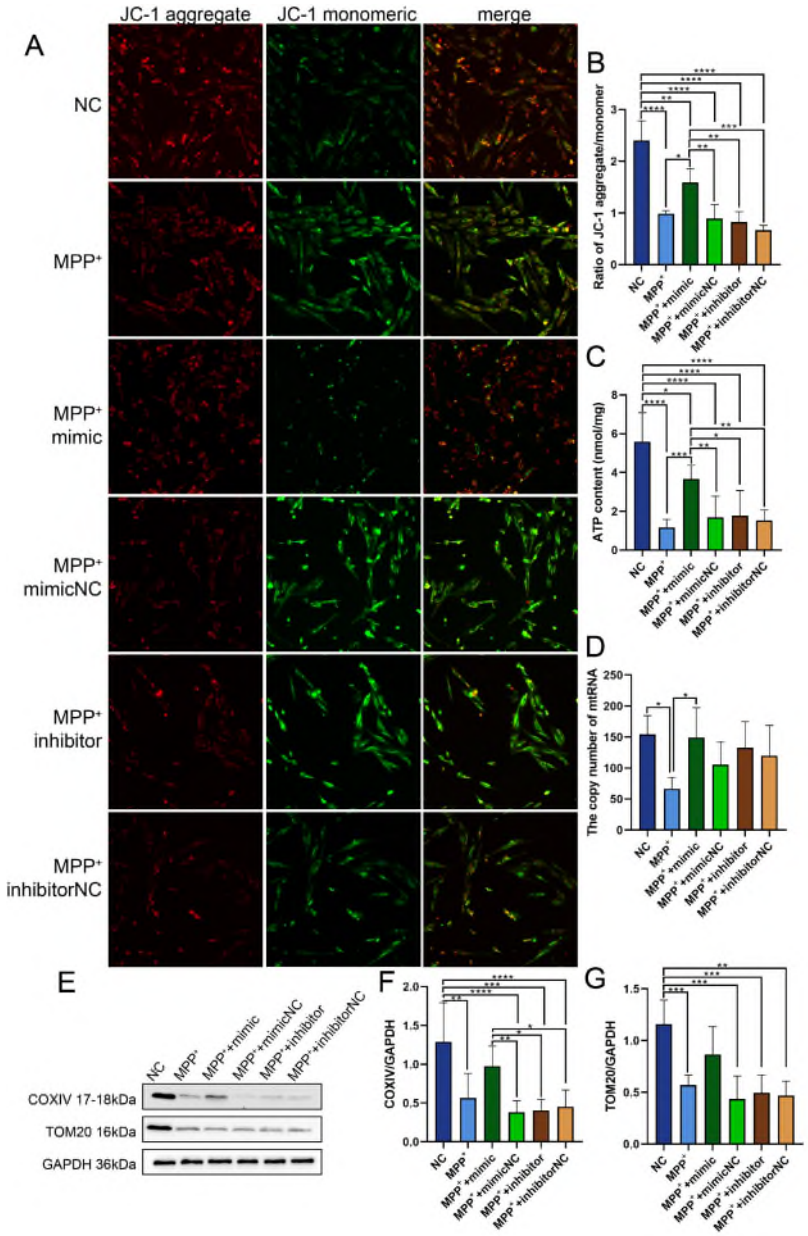


Fig.12. Expression of mitochondrial function-related indicators in each group of cells. (A) Representative images of JC-1 staining in each group. (B) Statistical results of JC-1 fluorescence intensity in each group. (C) ATP content in each group. (D) Statistical results of the copy number of mtDNA in each group. (E-G) Expression results of mitochondria-related proteins in each group. ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. mtDNA = mitochondrial DNA; COXIV = cytochrome c oxidase subunit IV; TOM20 = outer mitochondrial membrane 20.

4.Discussion

4.1 Treadmill exercise regulates motor function and dopaminergic neurons in PD

Exercise is purposeful, planned, and repetitive physical activity for health improvement, with fewer side effects compared with pharmacotherapy. [11] Long-term exercise may delay the onset of PD in at-risk populations and reduce its incidence. [12] Aerobic exercise alleviates motor symptoms, effectively reduces the incidence of falls, improves quality of life, and lessens the burden on families and society in PD patients. [13] Running training notably improves lower-limb mobility in PD patients. [14] Animal studies confirm that gait-impaired rats have shortened stride length and increased stance width, [15] whereas exercise significantly improves gait speed, stride length, and stance width, limits dopaminergic neuron loss, and upregulates TH and BDNF expression. [16] MPTP-treated mice show prolonged pole test times and shortened hanging durations, [17] whereas exercised MPTP mice show reduced turn-down and total pole times. [18] In the present study, intraperitoneal MPTP induced tremor and rigidity in mice. After 7days of injection, mice exhibited slower climbing speed, shorter hanging durations, decreased stride length, and increased stance width, indicating motor coordination impairment and reduced limb strength. TH decreased and α-syn aggregated in SN and ST, confirming successful PD modelling. After 8 weeks of treadmill exercise, PD mice showed improved motor coordination, normalized gait, increased TH expression, and reduced α-syn accumulation. Thus, treadmill exercise limits dopaminergic neuron loss and restores motor function in PD mice.

4.2 Treadmill exercise regulates synaptic plasticity and synaptogenesis

EPSC and IPSC are key electrical signals for neuronal communication. mEPSCs and mIPSCs reflect excitatory and inhibitory postsynaptic currents, respectively. In this study, PD group showed a significantly decreased mEPSC frequency and a significantly increased mIPSC frequency than NC group, indicating reduced excitatory input drive and increased inhibitory neurotransmitter release to dopaminergic neurons. These changes implied impaired synaptic function, reduced neural network activity, and restricted dopaminergic firing, lead to motor initiation difficulties and reduced movement fluency in PD. After 8 weeks of treadmill exercise, these synaptic abnormalities were effectively reversed. PE group showed significantly increased mEPSC frequency and significantly decreased mIPSC frequency compared with PD group, suggesting reversal of excitatory weakening, relief of excessive inhibition of dopaminergic neurons, and enhanced synaptic plasticity.

Study established PD model by injecting α-syn into the mouse striatum and found decreased striatal synaptic currents, reduced dendritic spine density, decreased TH expression, and impaired motor and cognitive function in mice. [19] SYN (a synaptic vesicle phosphoprotein) and PSD95 (a postsynaptic scaffold) are markers of synaptic abundance and structure. Singh et al. reported shortened dendritic length and reduced SYN and PSD95 in PD rats, [20] and exercise restored these in MPTP-treated mice. [21] In the present study, BrdU-positive neurons density in MPTP mice was significantly decreased compared with NC group. 8 weeks of treadmill exercise increased

BrdU-positive neuron numbers and upregulated PSD95 and SYN expression in MPTP mice. These findings suggest that 8 weeks of treadmill exercise promotes neuronal synaptogenesis in PD.

4.3 Treadmill exercise mediates the protective effects of the Drp1/PINK1/Parkin pathway on neuronal mitochondria

Synaptic transmission is a complex and energy-demanding process, and mitochondria, as the energy factories, supply ATP for synaptic function. [22] Mitochondrial dysfunction is a key factor in PD pathogenesis. [23,24] Mitochondrial fission is essential for biogenesis, transport, distribution, and quality control, but excessive fission links to mitophagy. Dysregulation of this fission-mitophagy axis may may underlie PD neuron vulnerability. Drp1 binds FIS1 and mitochondrial fission factor (Mff) to drive mitochondrial membrane constriction and fission, [25] and inhibiting excessive Drp1 activation normalizes fission. [26] PINK1-deficient dopaminergic neurons reduces mitochondrial respiratory capacity, increased expression of Parkin-interacting substrate (PARIS), and reduced numbers of newly generated mitochondria, indicating the key role for PINK1 in regulating mitochondrial biogenesis via PARIS/PGC-1α. [27] Overexpression PINK1/Parkin reduces reactive oxygen species (ROS) and sustains mitophagy. [28] Damaged mitochondria are cleared by macroautophagy and mitophagy through PINK1/Parkin signaling pathway, a process activated by exercise. [29,30] Experimental has shown treadmill exercise upregulates TOM20 expression, protects mitochondrial integrity, and activates mitochondrial

biogenesis, thereby alleviating cerebral ischemia-induced neuronal damage. [31,32] MPTP impairs clearance of dysfunctional SN mitochondria, while exercise restores network balance via mitophagy. [33] However, existing studies report conflicting findings regarding the direction of exercise-induced regulation of the PINK1/Parkin pathway in PD. Hwang et al. found PINK1/Parkin elevated in MPTP-treated mice and lowered by exercise. [33] Chuang et al. reported that PINK1 increased but Drp1/Parkin decreased in PD rats, and exercise reversed this abnormal expression pattern. [34] Additional evidence showed that inhibiting Drp1 upregulates striatal TH, enhances PINK1/Parkin, and improves mitochondrial ultrastructure. [35] Similarly, the present study found Drp1 and p-Drp1 in PD mice were significantly increased, PINK1 and Parkin were significantly decreased, and autophagy-related proteins LC3A/B and p62 were significantly decreased. After 8 weeks of exercise, Drp1 protein expression decreased, p-Drp1 and FIS1 showed a decreasing trend, PINK1 and Parkin protein expression increased, and PINK1 mRNA and p62 protein expression increased. These findings suggest that exercise may promote the clearance of dysfunctional mitochondria, restore mitochondrial homeostasis by inhibiting pathological mitochondrial fission, and supports synaptic regeneration, delaying dopaminergic neuron degeneration in PD.

4.4 Treadmill exercise regulates miR-7 to improve synaptic function in PD

miRNAs can participate in the development and progression of PD by negatively regulating gene expression, and thus targeting PD-related miRNAs holds potential therapeutic value. [36] For example, miR-34b/c downregulates SNCA, causing α-syn

deposition and neuronal death, [37] while miR-29a reduces ROS and inflammation. [38] Furthermore, exercise has been shown to influence neuronal function by modulating various miRNAs. Exercise may inhibit miR-382-3p, upregulate BDNF and improve cognitive impairment in diabetic mice. [39] In this study, RNA sequencing and intersection with exercise-altered miRNAs identified miR-7a as a key miRNA. However, the specific mechanisms remain to be further validated.

Studies have shown that miR-7 levels are significantly decreased in the brain tissues of PD patients and PD animal models, [40] correlating with α-syn deposition and dopaminergic neuron loss. In the present study, miR-7a was significantly decreased in PD group compared with NC group, and similar changes were observed in MPP^+-treated cells. MPP^+ decreased cell viability, reduced TH, SYN and DAT expression, increased α-syn accumulation. miR-7 mimic reversed these changes, raising TH and lowering α-syn fluorescence intensity, with improving trends in cell viability and synaptic proteins. These results suggest that miR-7 protects against MPP^+-induced dopaminergic neuronal injury, and exercise may influence PD by regulating miR-7.

4.5 miR-7/Drp1/PINK1 pathway modulates neuronal mitochondrial function

Balanced mitochondrial fission and fusion are essential for maintaining cell function and viability. Drp1 regulates fission and mitophagy, but its hyperactivity disrupts morphology, elevates oxidative stress, and dysregulates apoptosis and mitophagy, contributing to neurodegeneration. [41] Experimental evidence showed that

Drp1 were significantly increased in PD patients and PD transgenic mice. [42] In recent years, the regulatory role of miRNAs in mitochondrial function has gained increasing attention. In vitro studies showed that miR-128-3p reduces Drp1-mediated fission and restores membrane potential. [43] while miR-455-3p upregulates PINK1, Parkin, PSD95, and SYN and improves cognition in Alzheimer's disease mice. [44] A review summarized that exercise can positively influence mitochondrial function by modulating miRNAs. For example, miR-499, miR-181a, and miR-133a improve mitochondrial biogenesis and affect muscle mass, and exercise such as swimming and running increases miR-181a expression. [45] Studies noted that miR-7 regulates dopaminergic neuron generation, [46] and hippocampal synaptic plasticity by targeting selenoprotein P (SEPP1) and nuclear receptor subfamily 4 group A member 3 (NR4A3). [47,48] To further explore the targeting relationship between miR-7 and Drp1, as well as the role of the miR-7/Drp1 in PD mitochondrial function, dual-luciferase assays were performed in the present study to verify that miR-7 directly targets Drp1. miR-7 mimic in MPP^+-treated SH-SY5Y cells reduced Drp1 activity, increased Parkin mRNA, restored membrane potential, ATP, mtDNA, and trended up COXIV/TOM20. These findings further indicate that miR-7 attenuates abnormal mitochondrial fission and repairs mitochondrial respiratory chain function by targeting and inhibiting Drp1, thereby exerting a protective effect against PD-related mitochondrial damage.

In summary, this study systematically elucidates a complete molecular regulatory axis of "exercise - miR-7/Drp1/PINK1 - mitochondrial function - synaptic plasticity -

PD symptoms" (as shown in Fig .13), providing a new theoretical basis for understanding the mechanisms by which exercise improves PD. However, this study has certain limitations. The upstream regulatory mechanisms by which exercise modulates miR-7 remain unclear and require further investigation. The experiments were performed only in PD mice and cells, lacking evidence from human studies. Future studies may incorporate genetically modified animal models, both sexes, and clinical samples to further deepen the understanding of the neuroprotective mechanisms of exercise.

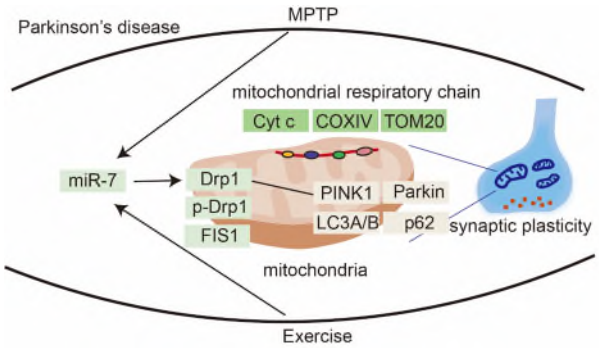


Fig.13. Schematic diagram of the exercise-mediated miR-7/Drp1 pathway.

5.Conclusion

The results of this study indicate that 8 weeks of treadmill exercise may exert neuroprotective effects on PD by regulating miR-7/Drp1/PINK1 pathway, which inhibits excessive mitochondrial fission and activates mitophagy, enhances synaptic plasticity, delays dopaminergic neuron degeneration, and thereby improves motor function.

Authors' contributions

G.X. designed the study and contributed to data collection and analysis ,and writed

the manuscript; C.M. contributed to resource collection and revised the study; Y.Y. participated in the design of the study and reviewed and revised the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Declaration of competing interest

The authors declare that they have no competing interests.

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References

1. Jankovic J, Tan EK. Parkinson's disease: Etiopathogenesis and treatment. *J Neurol Neurosurg Psychiatry* 2020;91(8):795-808.

2. Bloem BR, Okun MS, Klein C. Parkinson's disease. *Lancet* 2021;397(10291):2284-2303.

3. Feng ST, Wang ZZ, Yuan YH, et al. Dynamin-related protein 1: A protein critical for mitochondrial fission, mitophagy, and neuronal death in Parkinson's disease. *Pharmacol Res* 2020;151.

4. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998;392(6676):605-608.

5. Krzystek TJ, Banerjee R, Thurston L, et al. Differential mitochondrial roles for α-synuclein in DRP1-dependent fission and PINK1/Parkin-mediated oxidation. *Cell Death Dis* 2021;12(9).

6. Nies YH, Mohamad Najib NH, Lim WL, Kamaruzzaman MA, Yahaya MF, Teoh SL. MicroRNA dysregulation in Parkinson's disease: A narrative review. *Front Neurosci* 2021;15:660379.

7. Aaluri GR, Choudhary Y, Kumar S. Mitochondria-associated microRNAs and Parkinson's disease. *Neurosci Insights* 2024;19:26331055241254846.

8. Fragkouli A, Doxakis E. miR-7 and miR-153 protect neurons against MPP(+)-induced cell death via upregulation of mTOR pathway. *Front Cell Neurosci* 2014;8:182.

9. Jang Y, Kwon I, Song W, Cosio-Lima LM, Taylor S, Lee Y. Modulation of mitochondrial phenotypes by endurance exercise contributes to neuroprotection against a MPTP-induced animal model of PD. *Life Sci* 2018;209:455-465.

10. Jang Y, Kwon I, Song W, Cosio-Lima LM, Lee Y. Endurance exercise mediates neuroprotection against MPTP-mediated Parkinson's disease via enhanced neurogenesis, antioxidant capacity, and autophagy. *Neuroscience* 2018;379:292-301.

11. Janssen Daalen JM, Schootemeijer S, Richard E, Darweesh SKL, Bloem BR. Lifestyle interventions for the prevention of Parkinson disease: A recipe for action. *Neurology* 2022;99(7):42-51.

12. Alwardat M, Schirinzi T, Di Lazzaro G, et al. Association between physical activity and dementia's risk factors in patients with Parkinson's disease. *J Neural Transm*

2019;126(3):319-325.

13. Langeskov-Christensen M, Franzén E, Grøndahl Hvid L, Dalgas U. Exercise as medicine in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 2024;95(11):1077-1088.

14. Robinson AG, Dennett AM, Snowdon DA. Treadmill training may be an effective form of task-specific training for improving mobility in people with Parkinson's disease and multiple sclerosis: a systematic review and meta-analysis. *Physiotherapy* 2019;105(2):174-186.

15. Baptista PP, De Senna PN, Paim MF, et al. Physical exercise down-regulated locomotor side effects induced by haloperidol treatment in Wistar rats. *Pharmacol Biochem Behav* 2013;104(1):113-118.

16. Hsueh SC, Chen KY, Lai JH, et al. Voluntary physical exercise improves subsequent motor and cognitive impairments in a rat model of Parkinson's disease. *Int J Mol Sci* 2018;19(2):508.

17. Shao Q hang, Chen Y, Li F fang, et al. TLR4 deficiency has a protective effect in the MPTP/probenecid mouse model of Parkinson's disease. *Acta Pharmacol Sin* 2019;40(12):1503-1512.

18. Wang W, Lv Z, Gao J, et al. Treadmill exercise alleviates neuronal damage by suppressing NLRP3 inflammasome and microglial activation in the MPTP mouse model of Parkinson's disease. *Brain Res Bull* 2021;174:349-358.

19. Marino G, Campanelli F, Natale G, et al. Intensive exercise ameliorates motor and

cognitive symptoms in experimental Parkinson's disease restoring striatal synaptic plasticity. *Sci Adv* 2023;9(28):eadh1403.

20. Singh A, Yadawa AK, Chaturvedi S, Wahajuddin M, Mishra A, Singh S. Mechanism for antiParkinsonian effect of resveratrol: Involvement of transporters, synaptic proteins, dendrite arborization, biochemical alterations, ER stress and apoptosis. *Food Chem Toxicol* 2021;155:112433.

21. Toy WA, Petzinger GM, Leyshon BJ, et al. Treadmill exercise reverses dendritic spine loss in direct and indirect striatal medium spiny neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *Neurobiol Dis* 2014;63:201-209.

22. Imbriani P, Martella G, Bonsi P, Pisani A. Oxidative stress and synaptic dysfunction in rodent models of Parkinson's disease. *Neurobiol Dis* 2022;173:105851.

23. Su J, Zhang J, Bao R, et al. Mitochondrial dysfunction and apoptosis are attenuated through activation of AMPK/GSK-3β/PP2A pathway in Parkinson's disease. *Eur J Pharmacol* 2021;907:174202.

24. Chen C, McDonald D, Blain A, et al. Parkinson's disease neurons exhibit alterations in mitochondrial quality control proteins. *NPJ Parkinsons Dis* 2023;9(1):120.

25. Kraus F, Ryan MT. The constriction and scission machineries involved in mitochondrial fission. *J Cell Sci* 2017;130(18):2953-2960.

26. Rahmani S, Roohbakhsh A, Karimi G. Inhibition of Drp1-dependent mitochondrial

fission by natural compounds as a therapeutic strategy for organ injuries. *Pharmacol Res* 2023;188:106672.

27. Wang H, Chen R, Xiao L, et al. Defects in mitochondrial biogenesis drive mitochondrial alterations in PINK1-deficient human dopamine neurons. Preprint posted online June 26, 2023.

28. Shang Y, Xue W, Kong J, et al. Ultrafine black carbon caused mitochondrial oxidative stress, mitochondrial dysfunction and mitophagy in SH-SY5Y cells. *Sci Total Environ* 2022;813:151899.

29. Zhao N, Zhang X, Li B, Wang J, Zhang C, Xu B. Treadmill exercise improves PINK1/Parkin-mediated mitophagy activity against Alzheimer's disease pathologies by upregulated SIRT1-FOXO1/3 axis in APP/PS1 mice. *Mol Neurobiol* 2023;60(1):277-291.

30. Iorio R, Celenza G, Petricca S. Mitophagy: Molecular mechanisms, new concepts on Parkin activation and the emerging role of AMPK/ULK1 axis. *Cells* 2021;11(1):30.

31. Pan G, Zhang H, Zhu A, et al. Treadmill exercise attenuates cerebral ischaemic injury in rats by protecting mitochondrial function via enhancement of caveolin-1. *Life Sci* 2021;264:118634.

32. Zhang Q, Wu Y, Sha H, et al. Early exercise affects mitochondrial transcription factors expression after cerebral ischemia in rats. *Int J Mol Sci* 2012;13(2):1670-1679.

33. Hwang DJ, Koo JH, Kwon KC, et al. Neuroprotective effect of treadmill exercise possibly via regulation of lysosomal degradation molecules in mice with

pharmacologically induced Parkinson's disease. *J Physiol Sci* 2018;68(5):707-716.

34. Chuang CS, Chang JC, Cheng FC, Liu KH, Su HL, Liu CS. Modulation of mitochondrial dynamics by treadmill training to improve gait and mitochondrial deficiency in a rat model of Parkinson's disease. *Life Sci* 2017;191:236-244.

35. Feng ST, Wang ZZ, Yuan YH, et al. Inhibition of dynamin-related protein 1 ameliorates the mitochondrial ultrastructure via PINK1 and Parkin in the mice model of Parkinson's disease. *Eur J Pharmacol* 2021;907:174262.

36. Titze-de-Almeida SS, Soto-Sánchez C, Fernandez E, Koprich JB, Brotchie JM, Titze-de-Almeida R. The promise and challenges of developing miRNA-based therapeutics for Parkinson's disease. *Cells* 2020;9(4):841.

37. Miñones-Moyano E, Porta S, Escaramís G, et al. MicroRNA profiling of Parkinson's disease brains identifies early downregulation of miR-34b/c which modulate mitochondrial function. *Hum Mol Genet* 2011;20(15):3067-3078.

38. Yang YL, Lin TK, Huang YH. MiR-29a inhibits MPP + - Induced cell death and inflammation in Parkinson's disease model in vitro by potential targeting of MAVS. *Eur J Pharmacol* 2022;934:175302.

39. Wang M, Xie K, Zhao S, et al. Aerobic exercise improves cognitive impairment in mice with type 2 diabetes by regulating the MALAT1/miR-382-3p/BDNF signaling pathway in serum-exosomes. *Mol Med* 2023;29(1):130.

40. Horst CH, Schlemmer F, de Aguiar Montenegro N, et al. Signature of aberrantly expressed microRNAs in the striatum of rotenone-induced Parkinsonian rats.

Neurochem Res 2018;43(11):2132-2140.

41. Chan DC. Mitochondrial dynamics and its involvement in disease. *Annu Rev Pathol* 2020;15(1):235-259.

42. Brown HJ, Fan RZ, Bell R, et al. Imbalanced mitochondrial dynamics in human PD and α-synuclein mouse brains. *Neurobiol Dis* 2025;212:106976.

43. Liu X, Cui H, Bai Q, Piao H, Song Y, Yan G. miR-128-3p alleviates airway inflammation in asthma by targeting SIX1 to regulate mitochondrial fission and fusion. *Int Immunopharmacol* 2024;130:111703.

44. Pradeepkiran JA, Bandaru M, Islam MA, et al. Overexpression of miR-455-3p enhances mitophagy, synaptic and mitochondrial proteins in Alzheimer's disease. *Mitochondrion* 2026;89:102151.

45. Long YF, Chow SKH, Cui C, et al. Does exercise influence skeletal muscle by modulating mitochondrial functions via regulating MicroRNAs? A systematic review. *Ageing Res Rev* 2023;91:102048.

46. de Chevigny A, Coré N, Follert P, et al. miR-7a regulation of Pax6 controls spatial origin of forebrain dopaminergic neurons. *Nat Neurosci* 2012;15(8):1120-1126.

47. Pönniö T, Conneely OM. nor-1 regulates hippocampal axon guidance, pyramidal cell survival, and seizure susceptibility. *Mol Cell Biol* 2004;24(20):9070-9078.

48. Stevanato L, Sinden JD. The effects of microRNAs on human neural stem cell differentiation in two- and three-dimensional cultures. *Stem Cell Res Ther* 2014;5(2):49.