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Identification of serum microRNA alterations associated with long-term exercise-induced motor improvements in patients with Parkinson disease

Ziyi Zhang, BS[a], Ziwei Wu, MS[a], Shenglan Hu, MS[a], Miao He, MD[a,*]

Abstract

Background: Long-term physical exercise has been shown to benefit patients with Parkinson disease (PD), but there is a lack of evidence regarding the underlying mechanism. A better understanding of how such benefits are induced by exercise might contribute to the development of therapeutic targets for improving the motor function in individuals with PD. The purpose of this study was therefore to investigate the possible association between exercise-induced motor improvements and the changes in serum microRNA (miRNA) levels of PD patients through small RNA sequencing for the first time.

Methods: Thirteen PD patients completed our 3-month home-and-community-based exercise program, while 6 patients were assigned to the control group. Motor functions were measured, and small RNA sequencing with data analysis was performed on serum miRNAs both before and after the program. The results were further validated by quantitative real-time polymerase chain reaction. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were then conducted to determine the role of differentially expressed miRNAs.

Results: The 3-month home-and-community-based exercise program induced significant motor improvements in PD patients in terms of Unified Parkinson's Disease Rating Scale activities of daily living and Motor Subscale ($P < .05$), comfortable walking speed ($P = .003$), fast walking speed ($P = .028$), Six-Minute Walk Test ($P = .004$), Berg Balance Scale ($P = .039$), and Timed Up and Go ($P = .002$). A total of 11 miRNAs (10 upregulated and one downregulated) were identified to be remarkably differentially expressed after intervention in the exercise group, but not in the control group. The results of miRNA sequencing were further validated by quantitative real-time polymerase chain reaction. It was found that the targets of altered miRNAs were mostly enriched in the mitogen-activated protein kinase, Wnt, and Hippo signaling pathways and the GO annotations mainly included binding, catalytic activity, and transcription regulator activity.

Conclusion: The exercise-induced motor improvements were possibly associated with changes in circulating miRNA levels in PD patients. These miRNAs, as well as the most enriched pathways and GO terms, may play a critical role in the mechanism of exercise-induced benefits in PD and serve as novel treatment targets for the disease, although further investigations are needed.

Abbreviations: ADL = activities of daily living, ALS = amyotrophic lateral sclerosis, CG = control group, EG = exercise group, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MAPK = mitogen-activated protein kinase, miRNA = microRNA, MMSE = mini-mental state examination, NDs = neurodegenerative disorders, PD = Parkinson disease, qRT-PCR = quantitative real-time polymerase chain reaction, SD = standard deviation, TUG = Timed Up and Go, UPDRS = Unified Parkinson's Disease Rating Scale.

Keywords: long-term exercise, motor improvement, Parkinson disease, serum microRNAs

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Informed consent was obtained in writing from all individual participants included in the study. Participation was voluntary and anonymous. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are publicly available.

This study was approved by the Ethics Committee of the Second Xiangya Hospital, Central South University (LYG2021001).

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1. Introduction

Parkinson disease (PD) is a common progressive neurodegenerative disorder (ND). MicroRNAs (miRNAs) are endogenous, non-coding, single-stranded, stable small RNAs that play a critical role in regulating gene expression.[1] Circulating miRNAs have been shown to act as effective diagnostic and prognostic biomarkers of PD, which can be accessed and measured easily with high reliability and consistency.[2,3] Changes in their expression levels have been associated with the pathophysiological processes of PD.[4] For instance, the miR-29 family was found significantly downregulated in the serum of PD patients; the expression levels of hsa-miR-29a and hsa-miR-29c were negatively associated with PD severity[5]; and the plasma hsa-miR-132-3p level was found significantly upregulated in PD, and a higher level was closely associated with an increased risk of PD in males, as well as higher disease severity and later stage.[6,7] The beneficial effects of exercise for PD in terms of muscle strength, gait, functional capacity, balance and quality of life have been supported by multiple high-quality meta-analyses and systematic reviews.[8–10] It was reported that proper aerobic exercise training could potentially delay PD progression, and long-term community-and-home-based exercise interventions were demonstrated effective and safe for PD patients.[10–14] In addition, Flynn et al[12] reviewed and analyzed 16 clinical trials and concluded that home-based prescribed exercise achieved similar improvements to equivalent center-based exercise in terms of gait speed and balance-related activities in individuals with PD. Nonetheless, the specific mechanism underlying the exercise-induced positive effects in PD still warrants further exploration.

Altered levels of circulating miRNAs were shown to associate with changes triggered by physical exercise and can be used as biomarkers of adaptive responses.[15,16] However, there is a lack of evidence regarding the levels of differentially expressed circulating miRNAs after exercise in PD. Although Da Silva et al[17] conducted some relevant research, but they only measured 3 specific miRNAs, which limited the application of their findings.

The purpose of the current study was therefore to investigate the possible association between exercise-induced motor improvements and the changes in serum miRNA levels in PD patients through small RNA sequencing for the first time. This technique allows unbiased analysis of all miRNAs without target preselection.

2. Materials and methods

2.1. Participants

This study was approved by the Ethics Committee of the Second Xiangya Hospital, Central South University (LYG2021001). Informed consent was obtained in writing from all participants. The inclusion criteria for participant recruitment were age ≤70, Hoehn-Yahr stage 1–3 (Hoehn-Yahr scale is a scoring system commonly used to describe PD stages), absence of cognitive impairment (the mini-mental state examination score ≥26), being independently ambulatory with or without the use of an assistive device, undergoing stable treatment regimen for PD during the exercise program. Subjects were excluded if they had secondary Parkinsonism or Parkinson plus; neurological conditions other than idiopathic PD; any current cardiovascular, respiratory, orthopedic, psychiatric, visual, or vestibular dysfunctions that limited their participation in the program. Eventually, a total of 20 patients diagnosed with idiopathic PD between March and August 2021 were recruited. Based on the participants' own intentions, 14 patients were put onto the exercise training program, while the other 6 patients were assigned to the control group without exercise intervention. Each participant's information was collected through a case report form.

2.2. Experiment procedures and measures

The locomotor performance was measured by the Unified Parkinson's Disease Rating Scale III (UPDRS), comfortable and fast walking speed, Six-Minute Walk Test, Timed Up and Go test, and Berg Balance Scale. Each participant was tested in the "on phase" twice, that is, once at baseline (T0, baseline) and once at the end of the program (T1, 12 weeks after T0), by the same neurologist who was blinded to the grouping. At T0 and T1, venous blood samples were taken from all subjects at rest in the morning (8–10 AM) before the testing session while after intake of medication but not food. The participants were then required to have breakfast before testing. Blood samples were drawn into coagulation-promoting tubes and left to stand for about 30 minutes at 4°C. The serum was isolated from each sample at 2000×*g* for 15 minutes, and then stored immediately at −80°C for further analysis. The anti-PD medication dose remained consistent for each participant throughout the entire training period.

2.3. Exercise intervention

Sixty home-and-community-based exercise sessions were given to each participant in the exercise group (one session per day, 5 days a week for 12 weeks). Each session included 30 minutes of fast walking or jogging and 30 minutes of aerobics, which was specially designed by our research team for PD patients. The aerobics program was composed of stretching, stepping, strengthening, high knees marching, repetitive body weight shifting, and big movements of the body and limbs. All participants in the exercise group were instructed by our trainers face to face in a pre-training practice session and were provided with demonstration videos and booklets to help them grasp correct movements. To ensure adherence, they were also enrolled in a WeChat group, which was managed by the person in charge of their training. In the WeChat group, the participants were asked to report their compliance to the exercise program in a daily manner, and they could also communicate with other participants and the trainers about the problems they met or encourage each other. Feedback was given to each participant via video call on a weekly basis to improve their exercise quality and to progress the intensity of fast walking or jogging (the goal was to remain within 70%–80% of the maximal heart rate throughout fast walking or jogging).

2.4. MiRNAs extraction

The serum samples were directly lysed with TRIzol LS Reagent (Thermo Fisher Scientific, Cleveland, OH), and miRNAs were extracted using the miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The RNA concentration and purity were determined by NanoDrop 2000 (Thermo Fisher Scientific).

Table 1

Baseline characteristics of PD subjects (n = 19).

	Exercise group	Control group	*P* value
Gender (male/female)	6/7	4/2	-
Age (year, mean [SD])	53.231 (6.735)	52.667 (7.685)	0.831
BMI (kg/m^2, mean [SD])	22.739 (2.315)	23.568 (2.639)	0.765
Hoehn and Yahr (points, mean [SD])	1.500 (0.612)	1.833 (0.516)	0.179
Years from onset (mean [SD])	2.231 (1.641)	3.167 (2.137)	0.368
MMSE (points, mean [SD])	28.308 (1.437)	28.167 (1.169)	0.765

BMI = body mass index, MMSE = mini-mental state examination, PD = Parkinson disease, SD = standard deviation.

2.5. Small RNA sequencing and data analysis

Sequencing was performed on the DNBSEQ platform (BGI, Shenzhen, China). Before data analysis, impurities in the raw data, including reads of low quality and reads with adaptor sequences or with high levels of N base, were filtered out first. The clean reads were then aligned to the reference genome (Reference Genome Version: GCF_000001405.39_GRCh38.p13, NCBI) using Bowtie2. The miRNAs and other non-coding RNAs were predicted based on the position of the sequencing data on the genome. The reads were also aligned to the non-coding RNA and Rfam databases, and non-coding RNA annotation was performed on the sequencing data.

2.6. Quantitative real-time polymerase chain reaction

To validate the initial results of small RNA sequencing, quantitative real-time polymerase chain reaction (qRT-PCR) analysis was conducted on 3 miRNAs (miR-320c, miR-181a-2-3p, and miR-619-5p). The RNA was reversely transcribed with the miRNA First Strand cDNA Synthesis (Tailing Reaction) Kit (B532451; Sangon Biotech Co, Ltd., Shanghai, China) according to the manufacturer's instructions. The cDNA was quantitated using the SYBR miRNA RT-PCR kit (B532461; Sangon Biotech Co, Ltd.) on a Roche LightCycler 96 instrument (Roche, Basel, Switzerland) under the following conditions: 30 seconds at 95°C, followed by 5 seconds at 95°C plus 30 seconds at 60°C for 40 cycles, then 10 seconds at 95°C, 60 seconds at 65°C, 1 seconds at 97°C, and 30 seconds at 37°C. The relative expression levels of miRNAs were normalized to an endogenous control (miR-423–5p).[18,19] All primers were synthesized by Sangon Biotech Co, Ltd.

2.7. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis

The Gene Ontology (GO) enrichment analysis was conducted using the GO database (http://www.geneontology.org/). The *P* value was calculated using the basis function phyper of R and was then corrected to *Q* value. The GO terms with *Q* value ≤0.05 were considered as significantly enriched in the candidate genes. Besides, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was also conducted (http://www.genome.jp/kegg/), and pathways with a final *Q* value ≤0.05 were defined as significantly enriched.

2.8. Statistical analysis

Statistical analysis was performed using SPSS Statistics (version 22.0; IBM Corp., Armonk, NY). The descriptive data were expressed as mean, standard deviation, and median. The differences between 2 groups were compared by Mann–Whitney *U* test, and then final versus baseline outcomes were compared by Wilcoxon signed-rank test. *P* values <0.05 were considered statistically significant.

Table 2

Comparison of symptoms and functional performance (pre and post) of the exercise intervention (e.g., n = 13; CG: n = 6).

Indicator (score range)	Pre, mean (SD)/median		*P* value between groups (pre)	Post, mean (SD)/median		*P* value between groups (post)	*P* value within group (pre-post)	
	EG	CG		EG	CG		EG	CG
UPDRS Mentation, Behavior, and Mood subscale (0–16)	1.31 (1.11)/1	1.67 (1.21)/1.5	0.579	1.08 (0.95)/1	1.50 (1.05)/1.5	0.416	0.083	0.317
UPDRS ADL subscale (0–52)	6.08 (3.68)/6	9.33 (2.80)/10	0.054	5.38 (3.28)/6	9.67 (3.27)/10.5	0.022	0.039	0.414
UPDRS Motor subscale (0–108)	14.92 (7.11)/15	19.67 (6.77)/21	0.179	12.15 (5.80)/11	19.67 (6.92)/20	0.029	0.005	1.000
UPDRS motor and other complications of advanced disease subscale (0–23)	0.77 (0.93)/1	1.67 (1.37)/1.5	0.152	0.69 (0.85)/0	1.50 (1.05)/1.5	0.127	0.564	0.317
Comfortable walking speed, m/s	1.26 (0.22)/1.21	0.99 (0.11)/0.95	0.007	1.41 (0.20)/1.42	0.99 (0.11)/0.96	0.000	0.003	0.674
Fast walking speed, m/s	1.62 (0.25)/1.62	1.27 (0.15)/1.24	0.003	1.73 (0.16)/1.73	1.31 (0.10)/1.27	0.000	0.028	0.833
Six-Minute Walk Test, m	454.52 (75.81)/483.2	356.00 (53.01)/350.4	0.012	531.45 (61.54)/537.6	361.60 (53.17)/346.8	0.000	0.004	0.173
Berg Balance Scale score (0–56)	54.92 (1.66)/56	55.33 (0.82)/55.5	1.000	55.62 (0.87)/56	55.50 (0.55)/55.5	0.467	0.039	0.317
Timed Up and Go, s	6.84 (1.03)/6.88	8.62 (0.79)/8.39	0.005	5.73 (0.92)/5.47	8.69 (0.98)/8.42	0.000	0.002	0.462

ADL = activities of daily living, CG = control group, e.g. = exercise group, SD = standard deviation, UPDRS = Unified Parkinson's Disease Rating Scale III.

Table 3

Differentially expressed miRNAs after exercise training.

miRNA ID	Regulation	Average read count (before training)	Average read count (after training)	Average expression (before training)	Average expression (after training)	Log2 fold change	*P* value	*Q* value
hsa-miR-1268a	Up	0.667	33.917	0.057	1.474	4.693	2.19e-05	0.00775
hsa-miR-181a-2-3p	Up	118.25	248.667	8.22	16.189	0.978	0.000225	0.03343
hsa-miR-320c	Up	183.417	478.917	13.073	26.368	1.0122	3.26e-05	0.00775
hsa-miR-320d	Up	36.25	111.417	2.67	5.343	1.0008	5.91e-05	0.01171
hsa-miR-619-5p	Up	90.583	285.083	6.207	12.102	0.9633	9.52e-06	0.00565
hsa-miR-877-5p	Up	54.167	203.417	4.021	12.95	1.6873	0.000355	0.04222
novel-hsa-miR115-5p	Up	205.417	473.25	14.876	23.479	0.6584	3.01e-05	0.00774
novel-hsa-miR116-5p	Up	154	517	10.25	24.439	1.2536	3.51e-07	0.00042
novel-hsa-miR209-3p	Up	14.333	69	1.082	3.42	1.6603	0.000302	0.03993
novel-hsa-miR255-5p	Up	48.25	166.75	3.576	8.186	1.1948	0.000456	0.04920
novel-hsa-miR181-3p	Down	80.167	94.333	6.131	5.144	−0.2532	0.000218	0.03343

miRNA = microRNA.

DEGseq2 was used to calculate the differential expression of miRNAs. The *P* value of each gene was corrected for multiple hypothesis tests using *Q* value. The miRNAs with a |log2 Fold Changel ≥1 and a *Q* value ≤0.05 were considered significantly differentially expressed.

3. Results

3.1. Effect of exercise

Of the 20 patients recruited, 19 completed our program (including controls), and 1 from the exercise group dropped out due to personal reason (moved to another city) (drop-out rate: 5%). The baseline characteristics of the subjects are summarized in Table 1. No significant differences were detected at baseline (T0) between the exercise group and control group in terms of age ($P = .831$), body mass index ($P = .765$), year of onset ($P = .368$), Hoehn and Yahr stage ($P = .179$), mini-mental state examination scores ($P = .765$), or UPDRS total score ($P = .152$). Improvements after exercise intervention were observed in UPDRS activities of daily living subscale ($P = .039$), UPDRS motor subscale ($P = .005$), comfortable walking speed ($P = .003$), fast walking speed ($P = .028$), aerobic capacity as measured by Six-Minute Walk Test ($P = .004$), balance (Berg Balance Scale) ($P = .039$), and functional mobility (Timed Up and Go) ($P = .002$). No significant improvement was observed in the control group (Table 2). The between-group differences were found to be more significant at T1 compared to T0 in all the items evaluated, indicating that the exercise program induced significant improvements (Table 2). No adverse events were reported in both groups.

3.2. Differential expression of miRNAs

A total of 38 samples were sequenced on the DNBSEQ platform, with an average yield of 32.87M reads per sample. The average alignment ratio of the sample comparison genome was 85.86%, and 1291 miRNAs were detected. In the present study, increased volume of exercise was shown to associate with obvious differences in the levels of circulating miRNAs in PD patients. Regarding the effect of exercise training, 11 miRNAs (10 upregulated and 1 downregulated) were identified to be remarkably differentially expressed between T0 and T1 in the exercise group, but not in the control group (Table 3 and Fig. 1). Then, 3 of them (hsa-miR-320c, hsa-miR-181a-2-3p, and hsa-miR-619-5p) were selected for further validation by qRT-PCR, and their expression levels were found to be significantly elevated in the exercise group after intervention ($P < .01$), but not in the control group (Fig. 2).

3.3. GO and KEGG pathway analysis

GO and KEGG pathway analyses were performed on the target gene candidates of differentially expressed miRNAs. Multiple GO terms were found to be significantly enriched, including binding, catalytic activity, and transcription regulator activity (Fig. 3). According to the results of KEGG analysis, the most significantly enriched pathways included the mitogen-activated protein kinase (MAPK) signaling pathway, the Wnt signaling pathway, the Hippo signaling pathway, pathways and miRNAs in cancer, axon guidance, regulation of actin cytoskeleton and focal adhesion, and the Rap1 signaling pathway. The top 20 enriched pathways are listed in Figure 4.

4. Discussion

Our long-term community-and-home-based exercise interventions were found to be well-tolerated and effective for PD patients (Table 2). Certain miRNAs in the blood were identified to be capable of serving diagnostic and prognostic biomarkers for PD.[2] Unfortunately, very few studies have focused

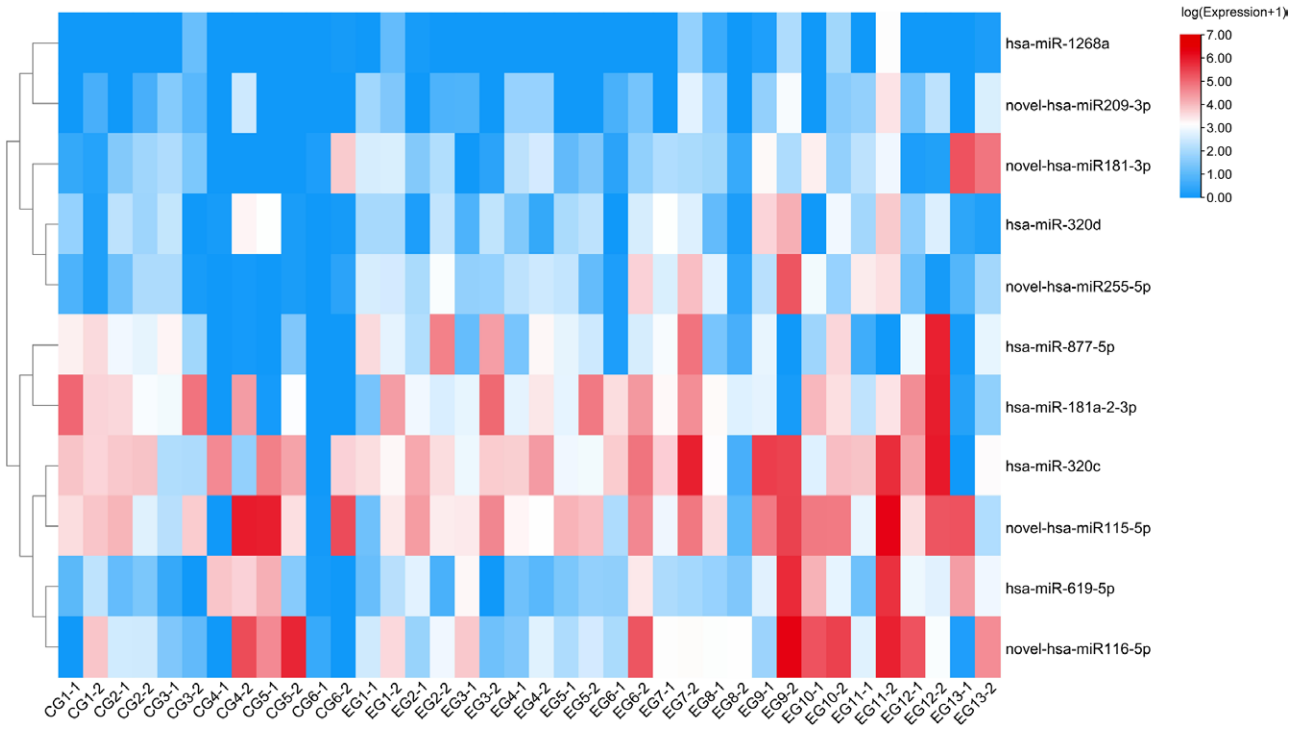

Figure 1. Heatmap summarizing the expression of differentially regulated miRNAs before and after exercise training. Colors range from blue (low expression) to red (high expression). "-1" refers to the expression level at T0 (pretest) (e.g., EG1-1), and "-2" refers to the expression level at T1 (posttest) (e.g., EG1-2). CG = control group, e.g. = exercise group, miRNAs = microRNA.

on the impact of exercise on circulating miRNAs in PD and other NDs. Da Silva et al[17] investigated the effects of a cycle ergometer-based exercise training program on the expression levels of serum miR-106a-5p, miR-103a-3p, and miR-29a-3p in male patients with PD, and reported that the levels of all these miRNAs were elevated in the exercise group (n = 8). However, the authors only measured 3 specific miRNAs in a small group of PD patients, which limited the application of their research findings. In a study focusing on 18 patients with amyotrophic lateral sclerosis (ALS), a decrease in the levels of serum muscle-specific miRNAs (including miR-1, miR-133a, miR-133b, and miR-206) was detected after exercise rehabilitation compared to baseline.[20] Our study was the first of its kind that aimed to clarify the association between the changes in serum miRNA levels and exercise intervention in PD patients through small RNA sequencing, which is a technique allowing for unbiased analysis of all miRNAs in a specimen without target preselection.

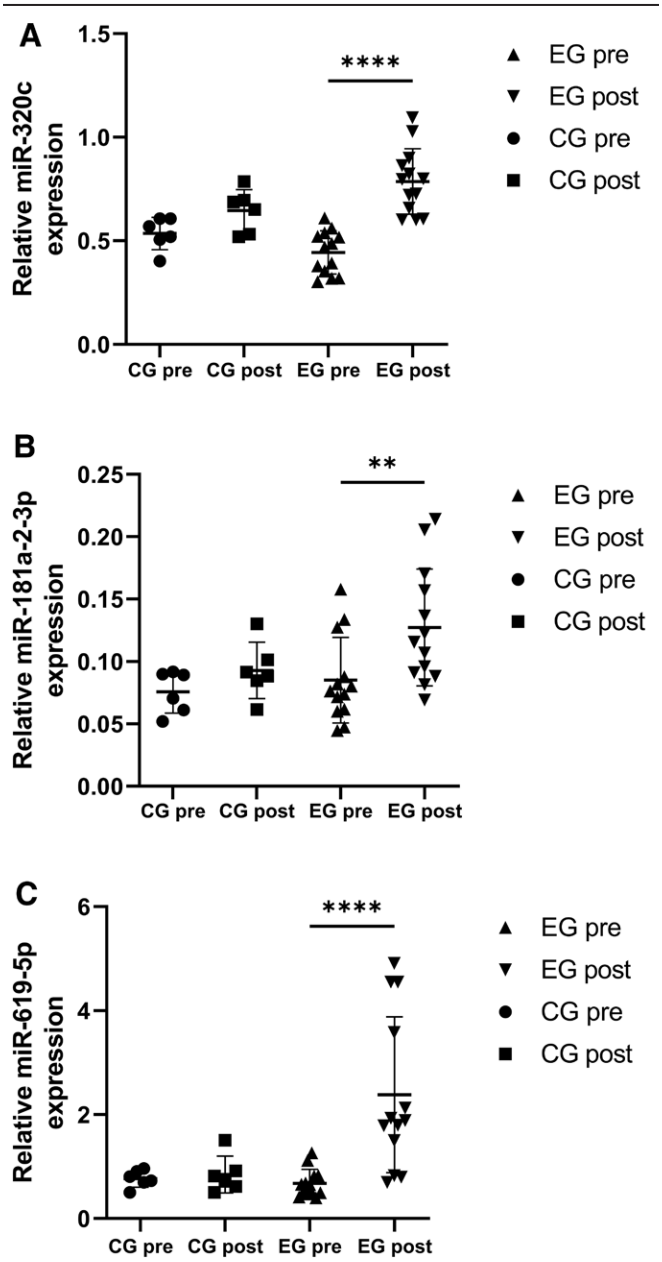

Figure 2. Relative expression levels of (A) miR-320c, (B) miR-181a-2-3p, and (C) miR-619-5p in the control group and exercise group at pretest (T0) and posttest (T1). $^{**}P < .01$, $^{****}P < .0001$. CG = control group, e.g. = exercise group.

Besides, the functional biological processes and pathways that the differentially expressed miRNA targets were most significantly enriched in were detected by GO and KEGG Pathway analyses.

In our study, a series of miRNAs were identified to be associated with the exercise-induced beneficial effects in PD (Table 3). Some of these miRNAs have already been reported to be closely related to NDs, especially PD. For example, circulating miR-320c was found to be downregulated in PD and ALS patients compared to healthy controls, and miR-1268a was found to be significantly downregulated in neuron-derived extracellular vesicles, as well as in motor cortex samples, from ALS patients.[21–23] Recently, it was reported that miR-181a-2-3p transferred by mesenchymal stem cell extracellular vesicles could inhibit oxidative stress in PD by regulating EGR1 via inhibition of NOX4/p38 MAPK axis.[24] Therefore, downregulation of miR-320c, miR-1268a, and miR-181a-2-3p has been potentially involved in the progression of NDs and vice versa. In addition, the miR-181 family plays an important role in regulating mitochondrial dynamics, redox homeostasis, and energy balance and has been associated with PD pathogenesis.[25,26] Overexpression of miR-181 was shown to exacerbate αSyn-induced dopaminergic neuronal loss, whereas miR-181 inhibition could exert a neuroprotective effect compared to controls.[26] Moreover, miR-320d was also identified to be differentially expressed in PD blood samples and significantly related to PD.[27] The levels of some differentially expressed miRNAs were reported to vary in response to exercise. For example, circulating miR-320d was found upregulated after a marathon in runners, indicating miR-320d as a biomarker in response to endurance exercise with high volume and relatively low intensity.[28] The miR-181 family is a kind of muscle-related miRNAs that are highly related to exercise response. Specifically, miR-181-3p can potentially target calpain.[29,30] It was reported that aerobic exercise training downregulated the cardiac levels of miR-181a, whereas circulating miR-181 was upregulated immediately after the exercise.[31–33]

Some most significantly enriched pathways detected by KEGG Pathway analysis, such as the MAPK signaling pathway, the Wnt signaling pathway, and the Hippo pathway (Fig. 4), were reported to be closely related to the pathology of PD and/or the differentially expressed miRNAs found in our study.[34–37] Specifically, inhibition of the MAPK signaling pathway has been associated with the repression of neuroinflammation, oxidative stress, autophagy, and death of dopaminergic neurons in PD.[34,36] Inhibition of the MAPK pathway was shown to associate with the upregulation of miR-181a-2-3p, playing a role in repressing oxidative stress in PD.[24] The Wnt pathway exerts a neuroprotective effect in both cell and animal PD models and restoring the balance between active and non-active Wnt signaling is essential for the development of dopaminergic midbrain neurons and cell biological functions disrupted in PD.[35,38–40] Meanwhile, miR-320 was reported to act as a modulator of the Wnt signaling pathway.[41,42]

Besides, upregulation of the Hippo pathway was identified to be involved in promoting dopaminergic neuronal loss in PD via the regulation of Netrin1.[37] The actin cytoskeleton pathways are important for neurite outgrowth in PD, especially in LRRK2 models.[43] The axon guidance pathways also play a role in PD, whereas the Rap1 signaling pathway is involved in dopamine pathway regulation.[44,45] Our study indicates that aforementioned pathways may also be associated with in the exercise-induced beneficial effects in PD. Clarifying the relationship between exercise-induced improvements and changes in circulating miRNAs in PD may not only aid us in understanding the etiology of this disease but also provide insights into molecular targets for the development of therapeutic drugs. These differentially expressed miRNAs can partially explain the exercise-induced benefits in PD and may be

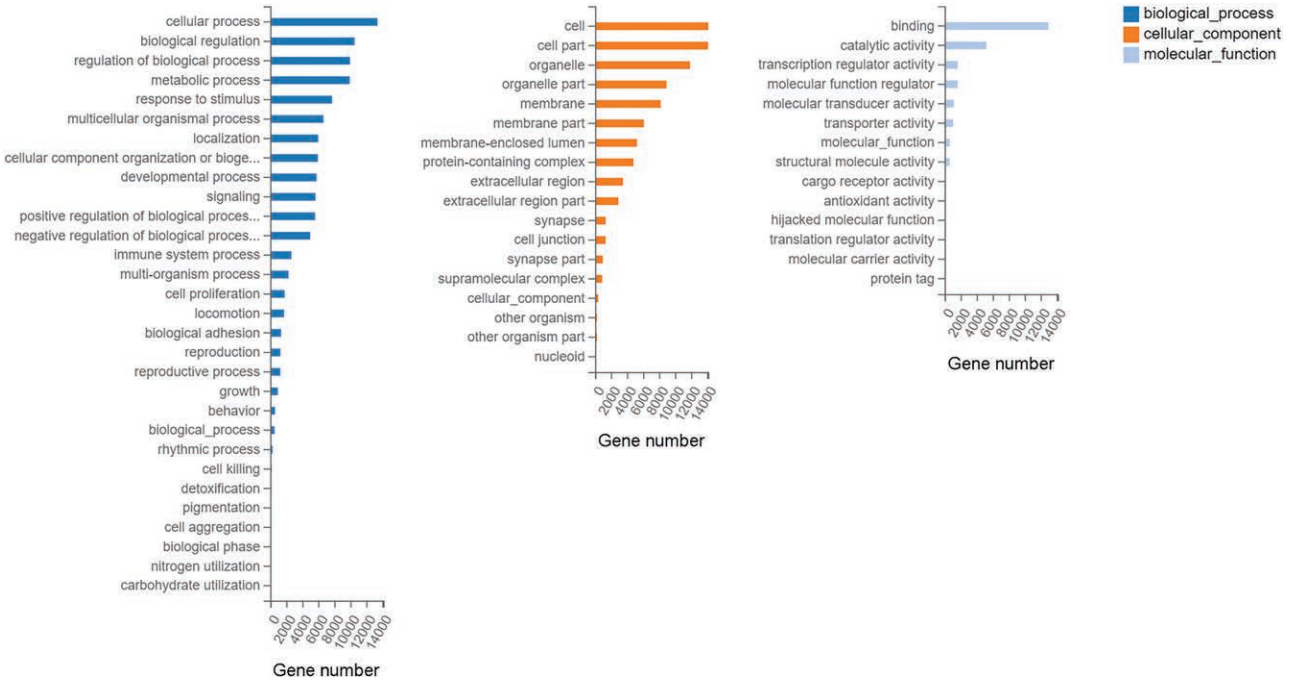

Figure 3. GO enrichment analysis of differentially expressed miRNA-targeted genes. GO = Gene Ontology, miRNA = microRNA.

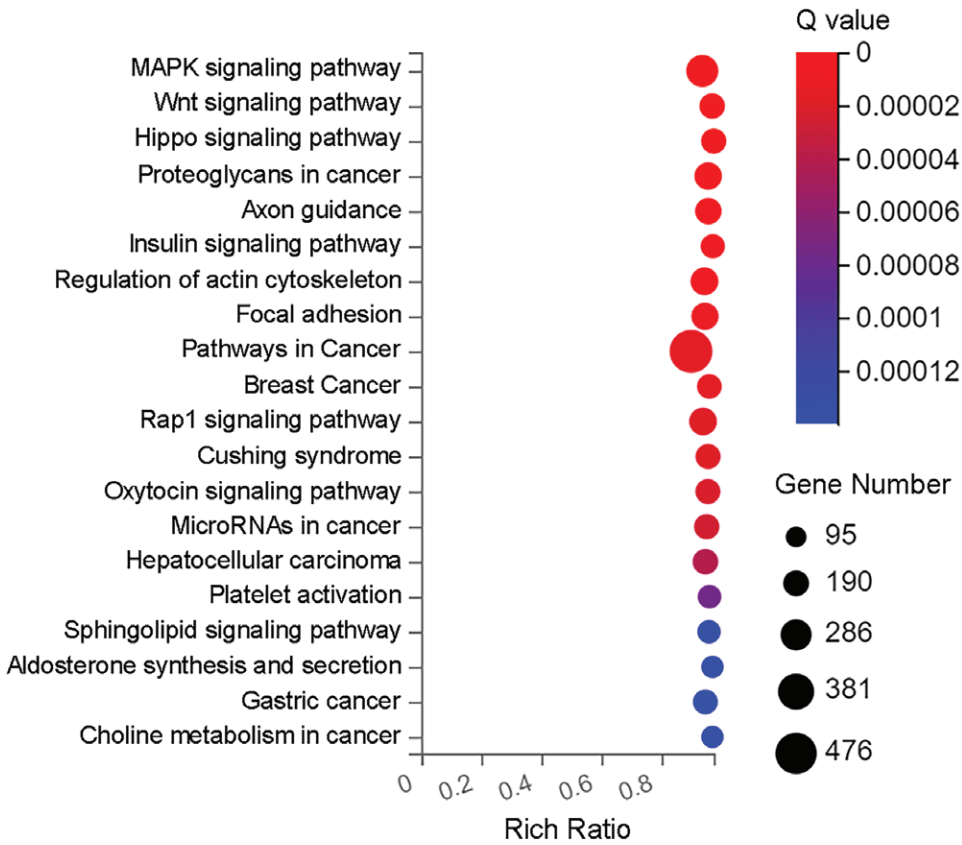

Figure 4. KEGG pathway enrichment analysis of differentially expressed miRNA-targeted genes. The larger the bubble, the higher the degree of enrichment. *Q* value, the closer to zero, the more significant the enrichment is. KEGG = Kyoto Encyclopedia of Genes and Genomes, MAPK = mitogen-activated protein kinase, miRNA = microRNA.

used as biomarkers of PD patients' adaptive capacity to exercise training, though clinical trials with larger sample sizes were needed to confirm the results. In addition, the potential neuroprotective effect induced by changing the expression levels of these miRNAs is also worth to be further investigated in PD models.

Limitations of this study included due to requests of the patients, randomization was not feasible in the grouping; a relatively small sample size; lack of a follow-up period to determine whether the effects of exercise and its impact on circulating miRNAs can last over time; the grouping was based on the patients' won intentions which might introduce bias; sample size imbalance between groups. These limitations may affect the generalizability of our results, which warrant randomized controlled trails with larger sample sizes to confirm.

5. Conclusion

According to evidence from small RNA sequencing, the motor improvements induced by our 3-month home-and-community-based exercise program in PD patients were possibly associated with changes in circulating miRNA levels. A better understanding of these miRNAs, as well as the pathways and GO terms they are mostly enriched in, may provide more concrete insights into the mechanism of exercise-induced adaptation in PD. Besides, these miRNAs can be used as possible novel treatment targets for PD, although further investigations are needed.

Author contributions

Investigation: Ziyi Zhang, Ziwei Wu, Shenglan Hu, Miao He.
Methodology: Ziyi Zhang, Shenglan Hu, Miao He.
Writing—original draft: Ziyi Zhang, Ziwei Wu, Shenglan Hu.
Writing—review & editing: Miao He.

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