Identification of Potential miRNA-mRNA Regulatory Network Contributing to Parkinson's Disease

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Research Article

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Abstract

Parkinson's disease (PD) is a common neurodegenerative disease and the mechanism underlying PD pathogenesis is incompletely understood. Increasing evidence indicates that microRNA (miRNA) plays critical regulatory role in the pathogenesis of PD. This study aimed to determine the miRNA-mRNA regulatory network for PD. The differentially expressed miRNAs (DEmis) and genes (DEGs) between PD patients and healthy donors were screened from miRNA dataset GSE16658 and mRNA dataset GSE100054 downloaded from the Gene Expression Omnibus (GEO) database. Target genes of the DEmis were selected when predicted by 3 or 4 online databases and overlapped with DEGs from GSE100054. Next, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted by Database for Annotation, Visualization and Integrated Discovery (DAVID) and Metascape analytic tool. The correlation between the screened genes and PD was evaluated by the online tool Comparative Toxicogenomics Database (CTD). The protein-protein interactions (PPI) network was built by STRING platform. Finally, we testify the expression of members of the miRNA-mRNA regulatory network in the blood samples collected from PD patients and healthy donors by using qRT-PCR. 1505 upregulated and 1302 downregulated DEGs, 77 upregulated DEmis and 112 downregulated DEmis were preliminarily screened from GEO database. Through further functional enrichment analysis, 10 PD-related hub genes were selected, including RAC1, IRS2, LEPR, PPARGC1A, CAMKK2, RAB10, RAB13, RAB27B, RAB11A and JAK2, which were mainly involved in Rab protein signaling transduction, AMPK signaling pathway and signaling by Leptin. The miRNA-mRNA regulatory network was constructed with 10 hub genes and their interacting miRNAs overlapped with DEmis, including miR-30e-5p, miR-142-3p, miR-101-3p, miR-32-3p, miR-508-5p, miR-642a-5p, miR-19a-3p and miR-21-5p. Analysis on clinical samples verified significant upregulation of LEPR and downregulation of miR-101-3p in PD patients compared with healthy donors. In the study, the potential miRNA-mRNA regulatory network was constructed in PD, which may provide novel insight into pathogenesis and treatment of PD.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide characterized by progressive degeneration of dopaminergic (DA) neurons in the pars compacta region of substantia nigra (SN) [1, 2]. It is estimated that about 2 out of 1000 people of all ages would suffer from this disease [3, 4]. The pathogenesis of PD involves complicated malfunction of multiple systems, manifested by motor dysfunctions as well as other non-motor symptoms [3]. The characteristic motor symptoms of PD include tremor (in hands, arms, legs, jaw, or head), rigidity (of the limbs and trunk), bradykinesia (of movement), and postural instability [3, 5, 6]. PD is a chronic and progressive disease, suggesting that it persists and gets worse over time [4, 7]. The heredity seems to be involved in the pathogenesis of PD since some of the genetic factors occur in as many as 5–10% of PD patients [8]. Among these, the heterozygous GBA mutations, α -synuclein variants and tau variants are representative genetic factors [3, 9, 10]. On the other hand, environmental factors are also involved in the pathogenesis of PD, such as smoking and alcohol [3]. Nevertheless, the precise mechanism underlying PD is not fully understood. While recently the epigenetic regulation such as microRNA (miRNA) emerges as a promising pathological factor for PD [11, 12].

MiRNA belongs to the non-coding RNA family that do not translate to any protein, featuring short single-strand RNA consists of 20–25 nucleotide [13]. The common function of miRNA is interacting with the 3' untranslated region (3'-UTR) of the target mRNAs, disrupting the translation or accelerating mRNA degradation [14]. Over the past decades, it has been indicated that miRNAs participate in pathogenesis of various diseases through regulating multiple biological processes such as cell proliferation, differentiation and apoptosis [15]. Recent studies also gradually disclosed the involvement of miRNAs in PD pathogenesis [12]. For example, miR-124 was reported to be a potential diagnostic and therapeutic biomarker of PD, through regulating NF- κ B, STAT3, AMPK and ERK signaling and subsequent cell apoptosis, autophagy, oxidative damage and neuroinflammation [16]. Goh et al. implicated several miRNAs including miR-30, let-7 and miR-485 to be associated with PD pathogenesis [12]. This evidence suggested the promising therapeutic role of miRNA for PD.

In the past decade, the development of bioinformatics analysis has provided a proficient manner to decipher genetic and epigenetic regulations related to diseases, and presented numerous evidence for diagnosis and treatment strategies [17, 18]. In

the current study, we adopted integrated bioinformatics methods to analyze miRNA and mRNA dataset collected from Gene Expression Omnibus (GEO) database, constructing a potential miRNA-mRNA regulatory network associated with PD pathogenesis. And blood samples collected from PD patients and healthy donors were tested the expression of mRNA and miRNA by quantitative real-time PCR to verify the findings of bioinformatics analysis. Our work may promise new insight into pathogenic mechanisms and treatment of PD.

Material And Methods

Clinical samples

The peripheral blood samples were collected from 8 PD patients and 9 healthy donors, following strict protocol and precautions, and placed in heparin-covered tubes. All PD patients were diagnosed according to the MDS diagnostic criteria proposed in 2015 [19], with the Hoehn and Yahr stage 2 to 4. While all the healthy donors were age- and gender-matched persons, and were free of diseases such as dementia, rheumatism, tumors, etc. All experimental procedures were conducted following the guidelines and approved by the Ethics Boards of Chinese PLA General Hospital. All PD patients and healthy donors have signed informed consent. The information of donors was listed in Supplementary Table 1.

Quantitative real-time PCR (qRT-PCR)

The total RNA was extracted from the isolated lymphocytes by TRIzol Reagent (Invitrogen, CA, USA) following the manufacture's protocol. QRT-PCR was conducted via Fast SYBR® Green Master Mix Bulk Pack (Invitrogen, USA) according to the manufacturer's instructions. Small endogenous nucleolar U6 snRNA and GAPDH were used as an internal control for normalization of miRNAs and mRNAs, respectively. Gene expression levels were calculated by the ($2^{-\Delta\Delta Ct}$) method. The sense and antisense primers were used in this study:

GAPDH: sense, 5'-GGAGCGAGATCCCTCCAAAAT-3', antisense, 5'-GGCTGTTGTCATACTTCTCATGG-3';

U6: sense, 5'-CTCGCTTCGGCAGCAC-3', antisense, 5'-AACGCTTCACGAATTTGCGT-3';

LEPR: sense, 5'-TGGGATTAGGTGGGATTT-3', antisense, 5'-CCGCTCCTACCAATCTAA-3';

IRS2: sense, 5'-TTGAC TTCTTGTCACCACCTTG-3', 5'-GCTGAG CGTCTTCTTTTAATGATACT-3';

JAK2: sense, 5'-TCTGGGGAGTATGTTGCAGAA-3', antisense, 5'-AGACATGGTTGGGTGGATACC-3';

miR-30e-5p: sense, 5'-GGGTGTAAACATCCTTGAC-3', antisense, 5'-TGCGTGTCGTGGAGTC-3'

miR-101-3p: sense, 5'-GCCGCCACCATGGTGAGCAAGG-3', antisense, 5'-AATTGAAAAAAGTGATTTAATTT-3';

Microarray Data

A miRNA expressing dataset GSE16658 and an mRNA expressing dataset GSE100054 were downloaded from the online Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) database. All samples were peripheral blood mononuclear cells (PBMCs) collected from PD patients and healthy donors. GSE16658 dataset consisted of 32 samples, among which 19 were from PD patients and 13 were from healthy donors. The GSE 100054 contained 19 samples including 10 from PD patients and 9 from healthy donors. The GSE16658 and GSE100054 dataset were detected by Illumina Human v2 MicroRNA expression beadchip platform and Illumina HumanHT-12 V3.0 expression beadchip platform, respectively.

Identification of Differentially Expressed Genes (DEGs) and miRNAs (DEmis)

To obtain differentially expressed genes (DEGs) and miRNAs (DEmis) between PD patients and healthy control, the raw data from GSE16658 and GSE100054 datasets were analyzed by GEO2R, a GEO-provided analytic tool. The $|\log_2FC| \geq 1$ and $p <$

0.05 were introduced as cut-off criteria. The DEGs and DEmis were visualized by volcano plots.

Prediction of miRNA targets

The targets of the selected upregulated and downregulated DEmis from GSE16658 were predicted by four online databases, including miRDB (<http://mirdb.org/miRDB/index.html>), TargetScan (http://targetscan.org/vert_72/), miRWalk (<http://mirwalk.umm.uni-heidelberg.de/>) and miRTarBase database (<http://mirtarbase.mbc.nctu.edu.tw>). The targets which were simultaneously predicted by at least 3 databases and overlapped with the DEGs from GSE100054 would be selected for further study.

Construction of miRNA-mRNA regulatory network

The miRNA-mRNA regulatory network was established and visualized by Cytoscape version 3.7.2 software [20] using the selected miRNAs and overlapped target genes.

Functional enrichment analysis

We conducted Gene Ontology (GO) analysis (biological process, molecular function and cellular component), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis to identify biological functions and pathways enriched from the predicted target genes of the DEmis. The functional enrichment analysis was conducted by using the online bioinformatics tool, the Database for Annotation, Visualization and Integrated Discovery (DAVID, <https://david.ncifcrf.gov/>). The terms with adjusted p value < 0.05 were selected.

The enriched functional terms were also determined by Metascape online analytic tool (<https://metascape.org/gp/index.html#/main/step1>) based on several databases such as GO, KEGG, Reactome and CORUM. The top 20 enriched terms were visualized as enrichment bar graph.

Comparative Toxicogenomics Database

The correlation between the screened genes and PD was evaluated by the online tool Comparative Toxicogenomics Database (CTD, <https://ctdbase.org/>), scored and showed as bar graphs.

Protein-protein Interaction network

The protein-protein interactions (PPI) network was built by on line platform Search Tool for the Retrieval of Interacting Genes (STRING, <https://string-db.org/>) (version 11.0) [21] to analyze the connection of target genes of the DEmis. The interacted pairs with a confidence score ≥ 0.4 were preserved, and visualized by Cytoscape software [20].

Statistical analysis

The data were presented as the means $\pm$ standard deviation (SD). Student's t-test and one-way ANOVA were used to assess the difference between two groups and multiple groups, respectively. $p < 0.05$ was considered as statistical threshold.

Results

Identification of differentially expressed mRNAs (DEGs) and miRNAs (DEmis) in PD

To determine critical genes and miRNAs involved in the progression of PD, we analyzed the peripheral blood mononuclear cell samples of mRNA dataset GSE100054 and miRNA dataset GSE16658. We obtained 1505 significantly upregulated DEGs and 1302 downregulated DEGs in PD patients comparing with the healthy donors (Fig. 1A). Besides, 77 notably upregulated DEmis and 112 downregulated DEmis were identified in PD patients (Fig. 1B), among which the top ten were selected for further analysis (Table 1).

Table 1
10 up-regulated miRNAs and 10 down-regulated miRNAs
were screened in GSE16658 dataset.

ID	P.Value	logFC	miRNA ID
10 up-regulated miRNAs			
42812	0.03403022	1.948885	hsa-miR-508-5p
42762	0.00241734	0.55537	hsa-miR-665
17836	0.04180705	0.481978	hsa-miR-30b-3p
42785	0.00280149	0.473074	hsa-miR-921
42701	0.02314422	0.463982	hsa-miR-30c-2-3p
28150	0.01187665	0.412631	hsa-miR-765
29575	0.0063856	0.412492	hsa-miR-32-3p
42727	0.01131039	0.386259	hsa-miR-668
42679	0.00076206	0.382502	hsa-miR-642a-5p
13131	0.00741607	0.362588	hsa-miR-518c-5p
10 down-regulated miRNAs			
17660	0.00219334	-2.754213	hsa-miR-550a-3p
33596	0.00002234	-1.592887	hsa-miR-126-5p
28191	0.00029506	-1.497504	hsa-miR-30e-5p
31026	0.00023161	-1.398265	hsa-miR-101-3p
10997	0.00015777	-1.383207	hsa-miR-19a-3p
10947	0.00052467	-1.353895	hsa-miR-142-3p
11053	0.00090354	-1.313697	hsa-miR-32-5p
5740	0.00147611	-1.306943	hsa-miR-21-5p
13143	0.00011819	-1.306042	hsa-miR-301a-3p
10998	0.000103	-1.30473	hsa-miR-19b-3p

Target prediction of DEmis

After identification of the top 10 DEmis, we tried to determine their target genes. Four online tools, namely the miRDB, TargetScan, miRWalk and miRTarBase database, were introduced to predict the potential targets. The target genes of the upregulated DEmis predicted by at least 3 databases were overlapped with the downregulated DEGs from GSE100054 dataset, and those overlaps were selected to build a potential regulatory network (Fig. 2A). A similar screening was performed on the downregulated DEmis and upregulated DEGs (Fig. 2B). This analysis provided a preliminary screening for prognostic miRNA-mRNA regulatory network for PD.

Functional enrichment analysis of the DEGs in PD

To optimize the critical genes and miRNAs associated with the development of PD, we conducted functional enrichment analysis to screen the significant biological processes and signaling pathways, and selected the genes involved in these terms. We first introduced two bioinformatics tools, that is DAVID and Metascape database, to conduct the functional enrichment analysis on the predicted targets of DEmis mentioned above in Fig. 2. As shown in Fig. 3, The results from DAVID database

revealed the representative GO terms, which include the biologic process (Fig. 3A), cellular component (Fig. 3B) and KEGG signaling pathways (Fig. 3C). Another enrichment analysis performed through Metascape website revealed the significant functional processes, basing on the GO, KEGG, Reactome and CORUM database, and the top 20 terms were listed in the bar graph (Fig. 3D).

Subsequently, we optimized the PD-related terms from the enrichment outcome of DAVID and Metascape analysis, by searching each term together with “Parkinson” term on Pubmed website. As listed in Table 2, four terms were screened out for further analysis, including the Rab GTPase binding, AMPK signaling pathway, Rab protein signaling transduction, and signaling by Leptin.

Table 2
Four terms related to PD.

Term	Literature number	Count	P.Value	Genes
GO:0017137 ~ Rab GTPase binding	109	6	0.026593331	DMXL2, EVI5, ACAP2, RAC1, RABGAP1L, SYTL3
hsa04152: AMPK signaling pathway	53	6	0.009607244	IRS2, LEPR, ACACB, RAB10, PPARGC1A, CAMKK2
Rab protein signal transduction	27	5	0.000709612	RAB13, RAB27B, RAB11A, RAB10, RAB34
Signaling by Leptin	11	3	0.000127852	JAK2, LEPR, IRS2

Selection of hub genes related to PD pathogenesis

Among the genes involved in the selected 4 functional terms, 10 were previously reported in PD studies, that is RAC1, IRS2, LEPR, PPARGC1A, CAMKK2, RAB10, RAB13, RAB27B, RAB11A and JAK2. To confirm their functional roles in PD, we introduced the CTD database to testify their correlation with Nervous System Diseases via evaluating the disease score. The genes with PD score > 20 were identified as significantly correlated genes and were marked as orange bars in Fig. 4. Among these 10 genes, JAK2 (Fig. 4A), CAMKK2 (Fig. 4B), RAB27B (Fig. 4C), LEPR (Fig. 4D), RAC1 (Fig. 4E), IRS2 (Fig. 4F) and PPARGC1A (Fig. 4G) scored 22.71, 24.17, 27.91, 32.18, 34.51, 42.85 and 81.08, respectively, herein were considered to be crucial PD-related genes. Whereas the RAB10, RAB13 and RAB11A were excluded, due to their low scores (Supplementary Fig. 1).

To confirm the hub genes related to PD, we also established a PPI network (Fig. 5) basing on the target genes of the DEmis. Intriguingly, the PPI network showed that four of the seven CTD-selected genes, the IRS2, LEPR, JAK2 and PPARGC1A, were closely correlated, suggesting their potential regulatory functions in PD development (Fig. 5).

Determination and validation of critical miRNA-mRNA regulatory network in clinical PD samples

Basing on these screening and selection, we finally constructed a simplified miRNA-mRNA regulatory network consisting of 10 PD-related genes and their interacting miRNAs selected from the DEmis (Fig. 6), including miR-30e-5p, miR-142-3p, miR-101-3p, miR-32-3p, miR-508-5p, miR-642a-5p, miR-19a-3p and miR-21-5p. We next collected blood samples from PD patients and healthy donors to examine the expression of the hub genes and their interacting miRNAs. Among the hub genes and their interacting miRNAs, the level of IRS2, LEPR and JAK2 was upregulated, the level of miR-101-3p and miR-30e-5p was downregulated in PD patients from qRT-PCR results. The changes of LEPR and miR-101-3p are statistically different (Fig. 7).

Discussion

Parkinson's disease (PD) is a common age-related neurodegenerative disorder characterized by progressive degeneration of dopaminergic neurons in the substantia nigra region [3, 22]. While the mechanism underlying PD pathogenesis is still poorly understood [1, 4]. So far, it is believed that PD results from a complicated interplay of genetic and environmental factors affecting numerous fundamental cellular processes [8]. Moreover, epigenetic factors such as miRNAs have been shown to be

important biological molecules involved in the pathogenesis of PD [11, 12]. In this study, we adopted miRNA and mRNA datasets from GEO database, conducted multiple bioinformatics methods to construct a potential miRNA-mRNA regulatory network, and identified several signaling pathways and hub genes that associate with PD development, which were verified by qRT-PCR on blood samples of PD patients and healthy donors. And an upregulation of LEPR and downregulation of miR-101-3p in PD patients compared to healthy donors were discovered.

MiRNAs have been emerging as pivotal molecules involved in PD pathogenesis. A previous study suggests that miR-425 deficiency triggers necroptosis of dopaminergic neurons, and targeting miR-425 in PD mouse model restored dysfunctional dopaminergic neurodegeneration and ameliorated behavioral deficits[23]. And miR-27a and miR-27b were thought to regulate autophagic clearance of damaged mitochondria and PINK1 gene expression which is the most common cause of autosomal recessive PD[24]. Moreover, miRNAs analyzed in our study, including miR-30e, miR-21, miR-101, miR-19a and miR-142 which were downregulated, were previously presented as regulators of PD [25, 26]. Among which, the miR-30 family was studied in multiple studies and seemed to play critical roles during PD [12, 27, 28]. Bioinformatics analysis on various patient samples of PD identified miR-30 family as the potential upstream regulators of progression rates-related biomarkers of PD [29]. Moreover, miR-30e could attenuate the levels of inflammatory cytokines, such as TNF- α , COX-2, iNOS, and ameliorates neuroinflammation in a MPTP model of PD through directly targeting Nlrp3 [30]. MiR-101-3p was previously reported to mediate lncRNA Mirt2 suppressed inflammation [31]. Our study determined the notably reduced level of miR-101-3p in PBMCs of PD patients, supported its negative regulatory role during PD. Noteworthy, the level of miR-30e-5p was decreased but not statistically significant in PD patients, which may be restricted to the small patient pool adopted in this study. A larger cohort of patients with different Hoehn and Yahr stages would be adopted in further study. Based on previous and our research, it is believed that the existence of miRNA-mRNA regulatory network in PD pathogenesis might be promising target for treatment of PD.

Traditionally, miRNAs function by regulating target genes in a post-transcriptional way and play critical roles in various biological processes. We further listed the members of these enriched functional terms, predicted their potential interacting mRNAs, obtained the overlaps with the DEGs, and then constructed a miRNA-mRNA regulatory network. It's worth noting that 4 of the genes among the regulatory network, the IRS2, LEPR, JAK2 and PPARGC1A, were identified as the hub genes, suggesting their critical roles during PD progression. The three upregulated genes IRS2, LEPR, JAK2 participate in leptin-mediated metabolism and inflammatory regulation in PD, which is consistent with their roles in fatty acid transport and gluconeogenesis, further highlighted the important role of leptin signaling in PD progression [32, 33]. Moreover, we identified LEPR as a significantly elevated gene in PD patients, comparing with the healthy control. Protein leptin encoded by gene LEPR serves as a regulator of energy homeostasis and feeding behavior[34], having neurotrophic actions in the central nervous system development during perinatal development and into the adult[35]. Ho et al showed that leptin preserved cell survival in neuronal SH-SY5Y cells against MPP + toxicity (Parkinsonian models) by maintaining ATP levels and mitochondrial membrane potential. The upregulation of gene LEPR might indicate activated feedback protection by neurons which will be investigated in future studies with a larger PD cohort. As for downregulated gene PPARGC1A, it encodes a transcriptional co-activator, PGC-1 α , which has been implicated in the pathogenesis of neurodegenerative disorders and found repressed in PD [36]. Targeting PGC-1 α was proposed as a potential therapeutic method for PD [37]. Interestingly, PGC-1 α was also reported to regulate the IRS2 level in hepatic metabolism [38]. In our study, the gene PPARGC1A interacted with three different MiRNAs, whose role in PD pathogenesis might be more complicated.

By functional enrichment analysis, we screened out biological signaling pathways to be closely related with PD, namely the Rab protein signaling transduction, AMPK signaling pathway and signaling by Leptin. The four hub genes IRS2, LEPR, JAK2 and PPARGC1A were mainly involved in AMPK and Leptin signaling pathways. Numerous evidence from PD models has supported the participation of AMPK in PD, via regulating cellular metabolism, enhancing autophagy, promoting mitochondrial quality control, suppressing oxidation, and alleviating inflammation [39–41]. AMPK activation was therefore regarded as a therapeutic target for PD treatment [39]. Moreover, it is proved that AMPK activation may facilitate clearance of α -synuclein and thereby promote neuronal survival to ameliorate PD [42]. Abnormal leptin signaling is also frequently observed in neurodegeneration diseases [43]. And increasing evidence has presented the role of leptin in regulating metabolic homeostasis

during PD [43, 44]. Candia et al. also suggested that leptin played a key role in linking metabolic imbalance and the damage of nervous system [44]. Besides, leptin was also involved in blood pressure changes during orthostatic stress in PD patients [45]. Our research and previous evidence have indicated the interaction of miRNAs and mRNAs which build a regulatory network and function in various signaling pathways, participating in the pathogenesis of PD.

In conclusion, our research gave a comprehensive study on the role of miRNAs and mRNAs in PD, identifying the potential miRNA-mRNA regulatory network correlated with PD pathogenesis by using bioinformatics tools, and testified the finding in blood samples collected from PD patients. This work may provide novel insight into the pathogenesis and potential therapeutic target for PD.

Declarations

- Ethics approval and consent to participate

All experimental procedures in the manuscript were conducted following the guidelines and approved by the Ethics Boards of Chinese PLA General Hospital. All PD patients and healthy donors have signed informed consent.

- Consent for publication

Not applicable.

- Availability of data and material

The data used to support the findings of this study are available from the corresponding author upon request.

- Competing interests

The authors declare that there are no competing interests.

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- Authors' contributions

XY designed the study and wrote the initial draft of the article; ZBG and ZFW supervised the study; MW, GS and YXN collected clinical samples and developed major methodologies, databases, and experiments; ZYJ contributed to the literature search; WW and TC reviewed and edited the article. All authors read and approved the article.

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Figures

Figure 1

Identification of DEGs and DEmis between PD patients and healthy controls. Volcano plots of 1505 upregulated DEGs and 1302 downregulated DEGs in PD patients (A), and 77 upregulated DEmis and 112 downregulated DEmis were identified in PD patients (B) comparing with the control groups. $p < 0.05$ as a threshold. Blue dots represent upregulated genes or miRNAs, red dots indicate upregulated ones and grey dots represents no significance. Abscissa axis represents $\log_2$ |Fold Change|, vertical axis represents $-\log_{10}$ |p value|.

Figure 2

miRNA target prediction and selection. The target prediction of each miRNA was conducted using miRDB, TargetScan, miRWalk and miRTarBase database. (A) The target genes of upregulated DEmis commonly predicted by 3 and 4 databases, and simultaneously overlapped with the downregulated DEGs from GSE100054 dataset were selected to construct the regulatory network. (B) The regulatory network of downregulated DEmis and their target genes selected from overlaps between upregulated DEGs from GSE100054 dataset and predicted targets by 3 and 4 databases.

Figure 3

Functional enrichment analysis via DAVID & Metascape database. The GO analysis was performed on the target genes of the DEmis, and the most significantly enriched biological process terms (A) and the molecular function terms (B) were presented as bar graphs. KEGG signaling pathways enriched from the target genes of the DEmis(C) . The enriched functional terms enriched from target genes of the DEmis were determined by Metascape online analytic tool basing on several databases such as GO, KEGG, Reactome and CORUM. The top 20 enriched terms were visualized as enrichment bar graph (D).

Figure 4

Parkinson Disease score determined by CTD. The correlation between the genesis of PD and JAK2 (A), CAMKK2 (B), RAB27B (C), LEPR (D), RAC1 (E), IRS2 (F) and PPARGC1A (G) was evaluated by the online tool Comparative Toxicogenomics Database (CTD), scored and showed as bar graphs.

Figure 5

Construction of PPI network. The protein-protein interactions (PPI) network was constructed via Search Tool for the Retrieval of Interacting Genes (STRING), and visualized by Cytoscape software. Confidence score ≥ 0.4 were regarded as criterion.

Figure 6

Optimized miRNA-mRNA regulatory network. The regulatory network consists of 10 optimized genes, JAK2, CAMKK2, RAB27B, LEPR, RAC1, IRS2 and PPARGC1A, and their corresponding miRNAs, miR-30e-5p, miR-142-3p, miR-101-3p, miR-32-3p, miR-508-5p, miR-642a-5p, miR-19a-3p and miR-21-5p.

Figure 7

Evaluation of critical miRNA-mRNA regulatory network in clinical PD samples. Real-time PCR was performed to examine the RNA levels of miR-30e-5p, miR-101-3p, JAK2, LEPR, and IRS2 in the peripheral blood samples of 8 patients with PD and 9 healthy donors. * $p < 0.05$, vs control (CON).

Supplementary Files

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