Bioinformatic analysis of microRNA expression in Parkinson's disease

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Abstract. Parkinson's disease (PD) is a type of movement disorder caused by loss of dopamine-producing neurons in the midbrain. In order to identify the synergistic microRNA (miRNA) pattern in PD, miRNA and mRNA double expression profiles of PD were downloaded. Differentially expressed miRNA and mRNA were identified [P<0.01, following false discovery rate (FDR) correction]. A cumulative hypergeometric distribution test was then performed to identify synergistic miRNAs (P<0.01, following FDR correction). Gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations were performed to analyze the miRNA regulatory target genes. Subsequently, a synergistic miRNA network was constructed and miRNAs exhibiting a high degree were identified. In total, 200 differentially expressed miRNA and 2,966 differentially expressed mRNA were identified. In addition, 1,502 synergistic miRNA interactions were identified, and miRNAs regulated 304 target genes in total. The GO and KEGG analysis demonstrated that these target genes were enriched in biosynthetic and cellular biosynthetic processes, the assembly of cellular components in morphogenesis, mitogen-activated protein kinase signaling, myometrial relaxation and contraction pathways as well as calcium regulation. The miRNA network demonstrated that miR-627, miR-634, miR-514, miR-563 and miR-613 had a high degree. miRNA with a high degree may be associated with the pathogenesis of PD and, therefore, may assist in the diagnosis and therapy of PD.

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Introduction

Parkinson's disease (PD) is a disease of the nervous system, characterized by the loss of nigrostriatal dopaminergic neurons (NDN) (1). PD, also termed tremors paralysis, is a common age-associated neurodegenerative disease, which is associated with environmental and genetic factors (2,3), with a prevalence of 0.3% in the whole population and an increasing incidence in the elderly (4). The main clinical manifestation of PD includes motionless tremor, which is limited to one side of the body. Clinically, PD features motionless tremor, muscle rigidity, bradykinesia and postural instability (5). At present, the therapeutic methods used for the treatment of PD involve pharmacotherapy, gene therapy, rehabilitation and surgical treatment. In 1967, clinical studies demonstrated that oral levodopa can improve the symptoms of PD, establishing its current status in the treatment of PD. Levodopa remains the most effective drug in PD therapy (6). However, treatment with levodopa is associated with various side-effects, including a decline in efficacy following prolonged usage, switch effect and end-of-dose phenomenon (7). Based on current understanding of the neural circuits of the basal ganglia (8), PD therapy predominantly requires the use of a dopamine D1 receptor-stimulating agent (9).

microRNAs (miRNA) are small, endogenous, RNA-coding molecules, which are important in almost all biological pathways, particularly in the transcriptional regulation of gene expression. As a small molecule regulator of gene transcription and expression, miRNAs are involved in almost all important biological pathways and disease processes in the human body (10). In recent years, there have been an increasing number of studies regarding the association of miRNAs with various diseases and biological functions. miRNAs are important in cancer, inflammation and infection as well as cardiovascular, immune and degenerative diseases acting as molecular markers for screening, but also may be used as new targets for drug development and the prevention and control of severe diseases (11).

Small, gene regulating miRNAs may also be important in PD. It has previously been reported that miRNA regulates leucine repeat kinase-2 (LRRK2) which contributes to the etiology of sporadic PD (12). Jewish individuals of European and North African descent often carry a LRRK2 mutation. These mutations are strongly associated with the occurrence of PD, however, the specific mechanism remains to be elucidated.

Based on data from miRNA and mRNA double expression profiles, the present study identified a synergistic miRNA pattern in PD and constructed an miRNA network. Through gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation, the miRNA-associated pathogenesis of PD was determined. The changes in gene expression observed under pathological conditions assists in improving understanding of the pathogenesis of PD and facilitates the identification of corresponding targets for therapy. Gene expression profile analysis is a fast, high through-put detection method for miRNA expression in tissues or cells. Comparison of the differences in expression between patients and healthy controls using this method, improves knowledge of the pathogenesis and development of PD.

Materials and methods

miRNA and mRNA expression profiles of PD. The miRNA and mRNA expression profiles of PD were downloaded from the Gene Expression Omnibus and were termed GSE16658 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE16658; accessed 9th June 2013) and GSE22491 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE22491; accessed 9th June 2013), respectively (13,14). miRNA expression profiling was performed using the miRCURY LNA microRNA Array platform, which included 32 samples (19 PD samples and 13 normal control samples). The mRNA profiling was performed using the Agilent-014850 Whole Human Genome Microarray 4x44K (G4112F) platform (Agilent Technologies, Inc., Santa Clara, CA, USA), which included 18 samples (10 PD samples and eight normal control samples).

Differential expression analysis for miRNA and mRNA expression profiling. Probes of miRNA and mRNA profiling were mapped to the miRNA name and Entrez Gene ID in the miRBase, respectively. When multiple probes were mapped to one miRNA or gene, the average expression level was calculated. Log2 conversion was then performed on the expression level.

A two-tailed student's t-test was used to analyze the differentially expressed miRNA and mRNA in the PD samples compared with the normal samples when the P-value was <0.01 following false discovery rate (FDR) adjustment.

Predicted target genes of miRNA and function and pathway enrichment analysis. Based on the seven target gene prediction algorithms PicTar, DIANA-microT, miRanda, miRBase, RNAhybrid, RNA22 and TargetScan, a set of predicted target genes of miRNA was determined. In order to reduce false positive predictions, miRNA target genes with high confidence were selected, which were predicted by at least three prediction algorithms. The miRNA target genes set with high confidence were then used for further analysis.

In order to determine which biological functions the synergistic miRNA was involved in, GO (15) was used for the miRNA regulatory target gene enrichment analysis (P<0.001). In addition, GenMAPP software (Gladstone Institute, San Francisco, CA, USA) (16) was used for KEGG pathway analysis.

Identification of synergistic miRNAs. Each miRNA set with high confidence of target genes was obtained and an accumulative hyper-geometric distribution test was performed to locate miRNAs sharing the same target genes. These miRNAs were defined as synergistic miRNAs. P<0.01 was considered to indicate a statistically significant difference following multiple FDR adjustment.

Results

Differential expression analysis. For expression analysis, 200 significantly differentially expressed miRNAs were identified in the PD samples compared with the normal samples. In the mRNA expression profile analysis, 2,966 differentially expressed mRNAs were identified.

Identification of synergistic miRNA and functional annotation for target genes. A total of 3,860 abnormal miRNA interactions were identified, which regulated at least one common target gene. A total of 1,502 miRNA interactions (P≤0.01) were identified based on super geometric distribution algorithm, including 147 miRNAs. These significantly synergistic miRNA pairs were involved in the regulation of 304 abnormally expressed genes.

In order to determine the biological functions that PD-associated disordered miRNAs were involved in, 304 abnormal target genes were used to perform GO function enrichment. A total of 74 GO biological processes were significantly enriched, including the biosynthetic process, the cellular biosynthetic process, the cellular component assembly involved in morphogenesis, mitogen-activated protein kinase (MAPK) signaling, the myometrial relaxation and contraction pathways and calcium regulation in the cardiac cell (Table I). In addition, 304 target genes were significantly enriched in eight KEGG pathways (Table II).

Synergistic miRNA network construction. The synergistic miRNA was used to construct a synergistic miRNA network associated with PD (Fig. 1). The miRNAs in this network comprised all the abnormal molecules in the disease process and are important gene regulatory factors. Therefore, the miRNAs in the network may be beneficial to uncover the mechanism of PD. Through binding to their target genes, miRNAs control post-transcriptional translation or directly degrade the mRNA target genes. Degree represents the number of interaction partners and the node with the highest degree is essential for the stabilization of the network (17). As shown in Table III, a high degree was observed in miR-627, miR-634, miR-514, miR-563 and miR-613, which may be associated with the pathogenesis of PD.

Discussion

PD is a common neurological degenerative disease in elderly individuals. It is well established that miRNA is involved in adjusting the target genes involved in cell proliferation, differentiation, apoptosis and extensive biological processes (18). In addition, miRNA is also important in the differentiation of stem cells (19,20). Dysfunction of miRNA may affect the development of the nervous system, which causes diseases, including Alzheimer's disease and PD (21). In the present

Table I. GO functional annotation of synergistic microRNA regulatory target genes.

GO ID (Biological process)	P-value	Term
GO:0009058	1.63E-04	Biosynthetic process
GO:0044249	1.01E-04	Cellular biosynthetic process
GO:0010927	2.72E-04	Cellular component assembly involved in morphogenesis
GO:0034641	1.27E-04	Cellular nitrogen compound metabolic process
GO:0016265	6.60E-05	Cell death
GO:0006310	8.80E-04	DNA recombination
GO:0033036	9.14E-06	Macromolecule localization
GO:0043412	3.23E-05	Macromolecule modification
GO:0008152	2.26E-04	Metabolic process
GO:0048519	5.61E-05	Negative regulation of biological process
GO:0006807	1.94E-04	Nitrogen compound metabolic process
GO:0008104	4.20E-06	Protein localization
GO:0015031	1.48E-05	Protein transport
GO:0050789	1.00E-04	Regulation of biological process
GO:0010468	2.26E-06	Regulation of gene expression
GO:0019219	1.07E-05	Regulation of nucleobase-containing compound metabolic process
GO:0080090	1.63E-06	Regulation of primary metabolic process
GO:0051252	6.76E-06	Regulation of RNA metabolic process
GO:0044281	3.21E-04	Small molecule metabolic process
GO:0006915	3.52E-04	Apoptosis
GO:0065007	1.47E-04	Biological regulation
GO:0008219	6.39E-05	Cell death
GO:0051641	7.15E-07	Cellular localization
GO:0034645	3.39E-06	Cellular macromolecule biosynthetic process
GO:0044265	2.60E-04	Cellular macromolecule catabolic process
GO:0070727	3.98E-07	Cellular macromolecule localization
GO:0044260	1.69E-08	Cellular macromolecule metabolic process
GO:0044237	5.70E-07	Cellular metabolic process
GO:0009987	2.29E-05	Cellular process
GO:0044257	7.03E-04	Cellular protein catabolic process
GO:0034613	1.06E-06	Cellular protein localization
GO:0044267	6.43E-05	Cellular protein metabolic process
GO:0033554	9.44E-04	Cellular response to stress
GO:0051649	3.09E-05	Establishment of localization in cell
GO:0045184	5.25E-06	Establishment of protein localization
GO:0010467	3.81E-05	Gene expression
GO:0006886	1.58E-05	Intracellular protein transport
GO:0046907	3.68E-08	Intracellular transport
GO:0009059	8.70E-06	Macromolecule biosynthetic process
GO:0043170	6.81E-06	Macromolecule metabolic process
GO:0043632	3.94E-04	Modification-dependent macromolecule catabolic process
GO:0019941	3.58E-04	Modification-dependent protein catabolic process
GO:0016071	1.53E-04	mRNA metabolic process
GO:0006397	1.12E-05	mRNA processing
GO:0048523	2.05E-05	Negative regulation of cellular process
GO:0090304	3.62E-06	Nucleic acid metabolic process
GO:0006139	2.71E-05	Nucleobase-containing compound metabolic process
GO:0006996	3.93E-04	Organelle organization
GO:0006596	6.75E-04	Polyamine biosynthetic process
GO:0051574	3.38E-04	Positive regulation of histone H3-K9 methylation
GO:0044238	2.02E-05	Primary metabolic process

Table I. Table I. Continued.

GO ID (Biological process)	P-value	Term
GO:0012501	2.14E-04	Programmed cell death
GO:0071539	3.38E-04	Protein localization to centrosome
GO:0033365	6.95E-04	Protein localization to organelle
GO:0032446	7.82E-05	Protein modification by small protein conjugation
GO:0070647	1.93E-04	Protein modification by small protein conjugation/removal
GO:0006464	7.95E-06	Protein modification process
GO:0016567	3.19E-04	Protein ubiquitination
GO:0051603	5.37E-04	Proteolysis involved in cellular protein catabolic process
GO:0009889	3.13E-05	Regulation of biosynthetic process
GO:0031326	2.24E-05	Regulation of cellular biosynthetic process
GO:2000112	9.66E-07	Regulation of cellular macromolecule biosynthetic process
GO:0031323	1.34E-06	Regulation of cellular metabolic process
GO:0050794	2.66E-05	Regulation of cellular process
GO:0010556	2.43E-06	Regulation of macromolecule biosynthetic process
GO:0060255	5.68E-06	Regulation of macromolecule metabolic process
GO:0019222	7.90E-06	Regulation of metabolic process
GO:0051171	4.62E-06	Regulation of nitrogen compound metabolic process
GO:0006355	1.54E-05	Regulation of transcription, DNA-dependent
GO:0032774	7.31E-05	RNA biosynthetic process
GO:0016070	8.94E-06	RNA metabolic process
GO:0008380	2.36E-05	RNA splicing
GO:0006351	2.61E-05	Transcription, DNA-dependent
GO:0006511	2.85E-04	Ubiquitin-dependent protein catabolic process

GO, gene ontology.

Table II. Kyoto Encyclopedia of Genes and Genomes pathway annotation of synergistic microRNA regulatory targeted genes.

Pathway	MAPP name	Adjusted P-value
WP382	Mitogen-activated protein kinase signaling	0
WP289	Myometrial relaxation and contraction	0
WP536	Calcium regulation in the cardiac cell	0
WP481	Insulin signaling	0
WP23	B cell receptor signaling	0
WP254	Apoptosis	0
WP707	DNA damage response	0
WP1591	Heart development	0

Table III. Degree of miRNA in the PD-associated network.

miRNA	Degree
miR-627	58
miR-634	50
miR-514	48
miR-563	48
miR-613	46
miR-106a	46
miR-383	44
miR-557	44
miR-505	42
miR-559	42

miRNAs with the 10 highest degrees are presented. miRNA, microRNA.

study, the expression profiles of miRNA and mRNA were used to identify disordered miRNA. A total of 304 abnormal miRNA regulatory target genes were identified. GO function and KEGG pathway analysis demonstrated that miRNA regulatory target genes were enriched in several biosynthetic processes and pathways, among which cell apoptosis (22,23), the MAPK signal pathway (24), calcium ion regulation (25) and insulin signals (26) have been associated with the development or the treatment process and response to DNA damage in PD.

In the present study, a total of 1,502 synergistic miRNA interactions were identified and an miRNA synergistic network was constructed. Within this network, miR-7 has

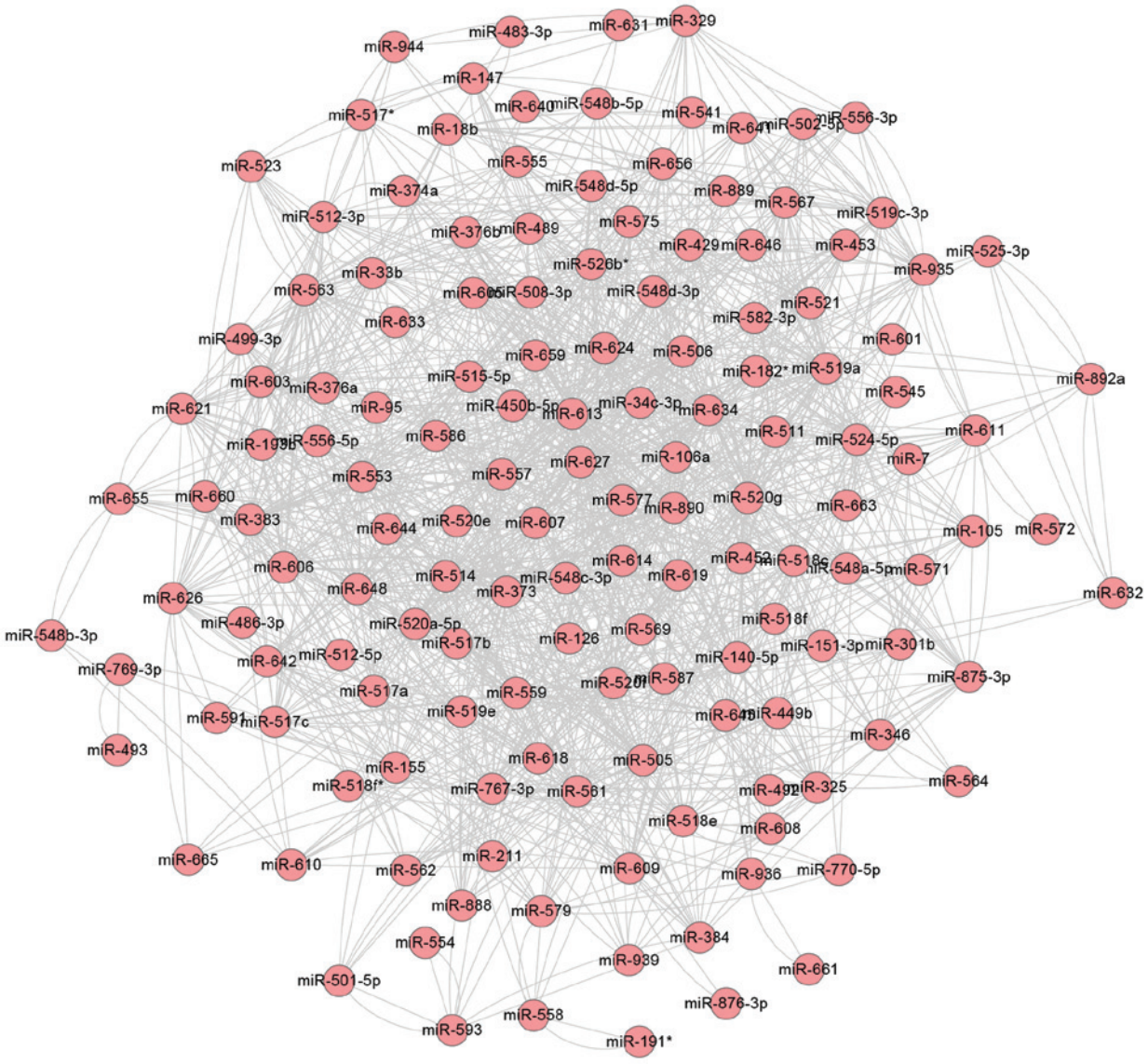

Figure 1. Synergistic miRNA network associated with Parkinson's disease. Nodes represent differentially expressed genes. miRNA, microRNA.

been demonstrated to regulate critical genes in the nervous system and to be involved the processes of PD (14,27). In the synergistic network constructed in the present study, miR-7 and another six miRNAs regulated common target genes. In addition, through binding to the 3'UTR of amyloid precursor protein (APP), miR-147 regulated the level of APP expression and, therefore, affected the risk of developing Alzheimer's disease and PD (28). The miRNA network demonstrated that miR-627, miR-634, miR-514, miR-563 and miR-613 exhibited a high degree. miR-627, miR-634 and miR-514 have been investigated in human colorectal cancer cells (29), acute lymphoblastic leukemia (30) and ovarian cancer (31). miR-563 has been reported to be differentially expressed in Alzheimer's disease samples (32) and targets the nuclear liver X receptor, which is important in the metabolism and homeostasis of cholesterol, bile acids, lipids and steroid hormones (33). However, to the best of our knowledge, no studies have investigated the association between these miRNAs and PD or other nerve-associated diseases. Therefore, the present study demonstrated for the first time, to the best of our knowledge, that miR-627, miR-634, miR-514, miR-563 and miR-613 are associated with PD. Further studies are required in order to confirm these results.

Using the expression profile data, the entire genomic expression situation of PD was examined. This revealed differentially expressed miRNAs and mRNAs. In addition, miRNAs were obtained and a synergistic miRNA network was constructed. From this, miR-627, miR-634, miR-514, miR-563 and miR-613 were obtained, which were newly reported in the present study. However, further studies are required to confirm these results.

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