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Research article

Alterations of miRNAs reveal a dysregulated molecular regulatory network in Parkinson's disease striatum

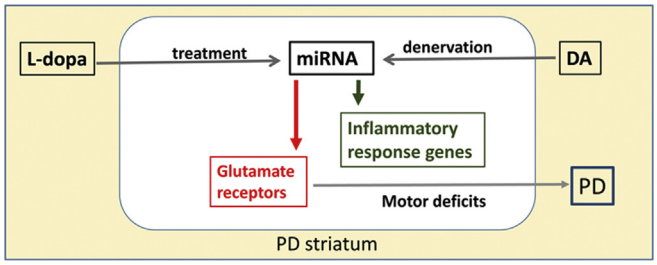
Venugopalan D. Nair*, Yongchao Ge

Department of Neurology and Center for Translational Systems Biology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

HIGHLIGHTS

- 800 miRNAs were screened in the striatal tissues of PD patients and compared with controls.
- 6 significantly upregulated and 7 downregulated miRNAs were identified in postmortem striatal tissues of PD patients.
- Differentially expressed (DE) miRNAs in PD striatum are associated with the inflammatory response.
- Expression of DE miRNAs negatively correlates with transcript levels of genes implicated in inflammatory response.

GRAPHICAL ABSTRACT



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ABSTRACT

Molecular adaptations in the striatum mediated by dopamine (DA) denervation and/or levodopa (L-dopa) treatments have been implicated in the motor deficits found in Parkinson's disease (PD). Alterations in inflammatory response mechanisms and glutamatergic neurotransmission are reported to play important roles in mediating these changes. However, the mechanisms mediating the molecular adaptations in the striatum are not well understood. Small non-coding microRNAs (miRNAs) influence numerous biological processes including the development and maintenance of striatal neurons by regulating gene expression post-transcriptionally. To investigate miRNA function in human PD striatum, we examined the global expression of miRNAs in postmortem putamen (putamen along with caudate forms the striatum) tissues obtained from PD patients and neurologically normal controls using Nanostring miRNA assays. We found that 6 miRNAs were significantly ($p \leq 0.05$) upregulated and 7 miRNAs were downregulated in PD putamen when compared with control. The differential expression (DE) of the 4 highest scoring miRNAs was further confirmed by reverse transcription polymerase chain reaction. Ingenuity pathway analysis demonstrated that these miRNAs are enriched in the processes of inflammatory responses. We found that the expression of DE miRNAs in PD putamen negatively correlates with the expression of gene transcripts implicated in inflammatory

Abbreviations: PD, Parkinson's disease; CNS, central nervous system; DA, dopamine; miRNA, microRNA; mRNA, messenger RNA; RIN, RNA integrity number; L-dopa, levodopa; LID, L-dopa induced dyskinesia; DE, differentially expressed; LTP, long term potentiation; *TNFSF13B*, TNF superfamilymember 13; *ATF4*, activating transcription factor 4; *LTA*l, ymphotoxin alpha; *HMOX1*, heme oxygenase-1; *SLC5A3*, solute carrier family 5 sodium/myo-inositol cotransporter member 3; *OSM*, oncostatin M; *PSMB2*, proteasome subunitbeta type 2; *CCL5*, chemokine C-C motif ligand 5; *GSR*, glutathione reductase; *TXN*, thioredoxin.

* Corresponding author at: Department of Neurology, Annenberg 14-72, Box 1137, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029, USA.
E-mail address: venugopalan.nair@mssm.edu (V.D. Nair).

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response with p53 and NF-kB as central signaling molecules. Taken together, our results suggest that in PD striatum, the DE miRNAs are associated with the oxidative stress pathway. This mechanism may contribute to the molecular adaptations and related motor complications found in PD.

1. Introduction

The striatum is a primary input area of the basal ganglia that receives glutamatergic and dopaminergic inputs from different regions of the brain and controls voluntary movements [1]. The deficiency of the neurotransmitter dopamine (DA) in the striatum resulting from the loss of DA producing neurons in the substantia nigra pars compacta is mainly responsible for the motor symptoms found in PD [2]. The most common parkinsonian medication, the DA precursor levodopa (L-dopa), leads to an increase in DA levels and alleviates the motor symptoms of PD. However, the use of L-dopa is often complicated by the development of abnormal involuntary movements called L-dopa induced dyskinesia (LID) [3]. Several neuronal systems are appeared to be involved in the complex pathophysiology of dyskinesia [3–5]. Glutamatergic transmission and inflammatory response mechanisms have been shown to be altered in striatal neurons following dopaminergic denervation and/or chronic L-dopa treatment [6,7]. It has been suggested that both of these factors are associated with molecular adaptations found in PD striatum and contribute to the development of dyskinesia [4,5]. In spite of the extensive research in this field, the molecular mechanisms mediating these changes in the striatum are still unclear. Therefore, understanding the molecular mediators underlying this process is important for gaining new insights into the pathogenesis of PD.

MicroRNAs (miRNAs) are a class of small non-coding RNAs that control diverse biological functions by regulating gene expression after transcription [8]. miRNAs are known to play important roles in the development [9,10] and motor activities [11] of striatal neurons. Identification of DA D1 receptors as a direct target of miR-382 suggests that miR-382 is an important regulator of dopaminergic neurotransmission [12]. Moreover, miR-126, miR-132, and miR-133b are involved in the differentiation and function of midbrain dopamine neurons [13–15]. miR-133b is specifically expressed in midbrain DA neurons and is shown to be deficient in midbrain tissue from patients with PD. [13]. The ability of miRNAs to influence complex gene networks and pathways in striatal and midbrain neurons suggests that their dysregulation might contribute to the motor complications associated with PD. In the present study, we performed expression analyses of 800 miRNAs in postmortem putamen (putamen along with caudate forms the striatum) tissues originating from individuals with PD and neurologically healthy control subjects. We found modest yet significant ($p < 0.05$) changes in the expression levels of 13 miRNAs in the putamen of individuals with PD when compared to controls. We mapped differentially expressed (DE) miRNAs to the inflammatory response pathway and identified candidate miRNAs for further evaluation of their relationship with gene transcripts and neuronal function in PD striatum.

2. Materials and methods

2.1. Postmortem brain tissue samples

A set of putamen samples with Parkinson's disease (n = 12) and neurologically normal control subjects (n = 12) was obtained from the Human Brain and Spinal Fluid Resource Center (Los Angeles, CA) through NIH NeuroBiobank. For detailed sample information, refer to Table 1 and Supplementary Table S1 in online version at DOI: 10.1016/j.neulet.2016.06.061. This study was reviewed by the Icahn School of Medicine at Mount Sinai's Institutional Review Board and was approved as exempt because the study involves only tissue-collected postmortem and, therefore, not classified as human subjects.

2.2. RNA isolation

Total RNA containing miRNA was isolated from putamen tissues using mirVana RNA isolation kit (Thermo Fisher Scientific) according to the manufacturer's instructions. RNA was quantified using the Nanodrop 1000 (Nanodrop Technologies), and the quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies).

2.3. MicroRNA expression analysis

A total of 225 ng of total RNA was assayed for determining the expression of 800 human miRNAs using the nCounter Human v2 miRNA Expression Assay kit (Nanostring Technologies, USA). The nSolver analysis software (NanoString Technologies) was used for the extraction of raw miRNA data and for checking the quality of the data. Using the control data, a generalized linear model was fitted with 6 negative and 6 positive controls, where the counts for each positive control follow a Poisson's distribution with the mean modeled as a linear function of concentration. Each sample was then normalized to the median slope and intercept of all samples and $\log_2$ transformed. Using the transformed data of all genes, a Bland-Altman plot was generated for each sample to compare the sample to the average of all samples. A loess curve was fitted for each Bland-Altman plot to globally normalize the transformed data as described in our recent publication [16]. A two sample t-statistical was performed on each miRNA, and a p-value ≤ 0.05 and the $\log_2$ fold change between PD and control is greater than 0.263 in absolute value was considered to indicate a statistically significant difference. The significantly differentially expressed miRNAs were displayed in the volcano plot.

2.4. Gene expression analysis

A majority of the DE miRNAs identified were mapped to the inflammatory response, organismal injury, and abnormalities pathways. We queried Pubmed using the terms "inflammation" and "cell death and survival" to assemble the gene set. To identify highly significant genes, the gene set was subsequently uploaded to Ingenuity Pathway Analysis (IPA) database (Qiagen, Redwood City, CA) and analyzed in the context of known biological response and regulatory networks. IPA returns a p-value based on Fisher's exact test for each network and converts it to a score that is $-\log10$ (p-value). A gene panel consisting of 134 genes implicated in inflammatory response (110/134, scores 24–72) and cell death and survival (120/134, scores 24–65) was selected (Supplementary Table S2 in online version at DOI: 10.1016/j.neulet.2016.06.061). Probe sets for each gene in the panel and housekeeping genes were designed and synthesized at Nanostring Technologies. The mRNA expression assay was carried out using 225 ng of total RNA according to the manufacturer's instructions [17], and the data was analyzed as