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Differential expression of miRNA 155 and miRNA 146a in Parkinson's disease patients

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ABSTRACT

Parkinson's disease is a neurodegenerative disorder and its etiology is unknown, numerous studies show how different environmental factors can influence the development of disease. miRNAs are involved in several pathologies and their dysregulation contribute to different pathologies, also in neurodegenerative such as Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotrophic lateral sclerosis. In this study, we profiled the expression of different candidate miRNAs: miR-155, miR-26a, miR-146a, and miR132, in PBMCs of L-dopa treated Parkinson patients and unaffected controls (HCs).We investigated the expression of miRNAs by RT-real time PCR, the results were subjected to statistical analysis. miRNA-155-5p was generally up-regulated in PD patients compared to HCs whereas miRNA-146a-5p was down-regulated in PD patients in comparison to HCs. It is interesting to point out that the expression of miR-155-5p was modified by levodopa treatment, in fact a down-regulation of miR-155-5p in PD patients with the highest dosage was observed.

In conclusion, miRNA 155 could not only be a promising target for the anti-inflammatory therapy in PD but also a good candidate as a disease progression biomarker. The role of levodopa in modulating the levels of miRNA 155 requires further studies.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons, causing symptoms such as muscle rigidity, resting tremor, bradykinesia, and postural instability [1]. The etiology is unknown, but the interaction between genetic and environmental factors seems to be crucial in causing the disease. Several genes including α-synuclein, Parkin, PINK and others are involved in pathogenesis of PD [2]. Numerous studies show how different environmental factors such as nutrition, exposure to metals and pesticides can influence the development of the disease [3–8]. Alpha-synuclein (α-syn), one of the most abundant proteins in Lewy bodies and Lewy neuritis, plays a leading role in initiation and progression of Parkinson-like neurodegeneration [9]. Neuroinflammation has been increasingly studied as a chief mediator in the pathogenesis and progression of PD [10]. miRNAs, small non coding RNA, are involved in several pathologies since their activity consists in controlling the genetic expression and their dysregulation contribute to different pathologies, including PD [11]. miRNA could be perfect candidates as biomarkers for diseases in which they are altered. Furthermore, they could be potentially used in order to monitor the progression of the disease. Peripheral blood mononuclear cells (PBMCs) share more than the 80% of the transcriptome with other tissues, including the SNC, so peripheral blood could be considered a great source of biomarkers being also widely available [12]. Several studies show how a dysregulation of miRNA is involved in the pathogenesis of different neurodegenerative diseases like: Alzheimer's disease, Parkinson's disease, Huntington's disease and Amyotrophic lateral sclerosis. The molecular mechanisms underlying the pathological implications of misregulated miRNA expression and the regulation of the key genes involved in neurodegenerative disorders remain largely unknown [13–16]. Since most PD symptoms are caused by a lack of dopamine in the striatum, many Parkinson's drugs are aimed at either temporarily replenishing or mimicking the action of dopamine, Levodopa is most commonly used drug [17].

In this study, we profiled the expression of different candidate PD miRNAs in PBMCs of L-dopa-treated PD patients and unaffected controls. We tested different miRNA such as miR-155, miR-26a, miR-146a and miR-132. We have selected these miRNAs because they are

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Table 1
Demographic and clinical characteristics of study participants.

Group	Age, Mean ± SD	Sex, females/males	Disease duration, Mean ± SD	H&Y score, Median ± SD	Levodopa mg, Mean ± SD
PD(#37)	71,3 ± 9,6	0.8	8.3 ± 4.8	3.3 ± 1.2	458,3 ± 227,71
HC(#43)	60 ± 13.14	1.7			
p value	0.0031	0.8036			

commonly studied in neurodegenerative diseases, but to date only few studies have been conductedin PD patients except formiR-155 that hasonly been studied in a mouse model of Parkinson disease [18-23]. Our primary aim is to investigate the potential of circulating miRNAs as non invasive diagnostic candidate biomarkers of PD patients.

2. Materials and methods

2.1. Samples

The peripheral blood of Sardinian PD patients, enrolled at the Neurology Clinic of the University Hospital of Sassari, Italy, and Healthy Controls (HCs) provided by a family physician of Li Punti district, Sassari, Italy, were collected. The diagnosis of PD was based on the established criteria [24]. The cohort included 37 PD patients (M/F = 0.8, mean age 71.3 ± 9.6, mean disease duration 8.3 ± 4.8 years, mean Hoehn-Yahr scale 3.3 ± 1.2) and 43 HCs (M/F = 1.7, mean age 60 ± 13.14), Table 1. Immediately after collection, PBMCs were isolated from 10 ml of blood by density gradient centrifugation on Ficoll-Paque Plus, (GE Healthcare Bioscience, Sweden), washed twice in phosphate-buffered saline (PBS), counted and stored at −80 °C with RNA later (Sigma) until further use.

The study was approved by ethics committee of the Azienda Sanitaria Locale 1, Sassari, Italy (Prot. N 22, 2015). The patients and the volunteers gave written informed consent.

2.2. miRNAs cDNA synthesis and real-time PCR

Purification of total RNA containing miRNA from PBMCs was performed using miRNeasy Mini kit (Qiagen, USA) according to the manufacturer's recommendations. Quality of extracted RNA was determined according to 260/280 absorbance ratio, measured by Nano Drop spectrometer (Thermo Scientific, USA). 500 ng/RNA were used in reverse-transcription reaction.

cDNA synthesis for miR-155, miR-132, miR-146a and miR-26a was fulfilled using a miSCript II RT Kit (Qiagen) according to the manufacturer's leaflet. MiRNAs quantification was performed with Custom miScript miRNA PCR Array.

2.3. Heat maps

We performed heat maps using GeneGlobe Data Analysis Center (Qiagen). The heat map provides a visualization of the fold changes in expression between the selected groups for every gene in the array in the context of the array layout. The table provides the fold regulation data used for the map as well as the Comments associated with each one. The color of the square denotes the relative up- or down-regulation of the miRNA in that sample. In addition, it produces dendrograms for the rows and columns, which are computed using hierarchical clustering. The ordering of the rows and columns is the most compatible with the dendrograms.

2.4. Statistical analysis

miRNAs data analysis was performed using the ΔΔCT method by Qiagen miRNA detection software and final data were normalized for small nuclear RNA, miRTC (median Ct = 24.86 ± 0.614) PPC (median Ct = 21.27 ± 0.302), RNU6-6P (median Ct = 23.34 ± 0.116), SNORD68 (median Ct = 22.54 ± 0.211) expression levels as endogenous controls.

3. Results

3.1. miRNA expression in patients with PD and their matched controls

microRNAs derived from PBMC samples of PD patients and HCs were extracted and the total miRNA isolation was analyzed for the expression of miRNA 155-5p, 146a-5p, 132-3p and 26a-5p.

The analysis of different miRNA expression showed that miRNA-155-5p (fold change = 27.18; $p > .000001$) were generally up-regulated in PD patients compared to HCs whereas miRNA-146a-5p (fold change = −1.76; $p = .0015$) were down-regulated in PD patients in comparison to HCs. Other miRNA (miRNA-132-5p and miRNA-26a-5p) did not show a different expression between PD patients and HCs (Fig.1).

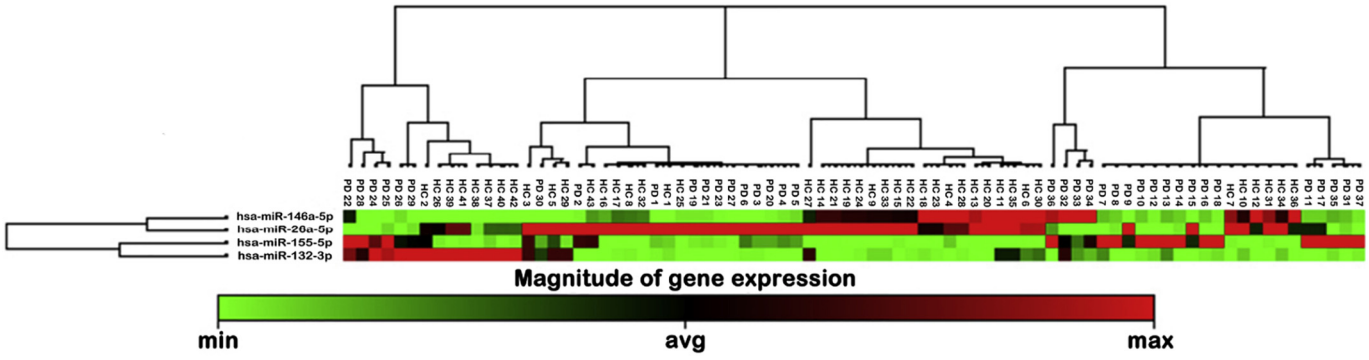

Fig. 1. Heat map of microRNA (miRNA) microarray expression date from plasma samples of PD patients (PD 37) and Healthy controls (HCs 43). The miRNA species are shown on the left. Cluster analysis classified tha samples in groups based on the miRNA expression levels in each samples. The dendogram shows different expression levels of miRNA among samples. Red indicates high expression of miRNA, and green indicates relatively low expression of miRNA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)