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Short communication

miRNA expression is highly sensitive to a drug therapy in Parkinson's disease

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ABSTRACT

Background: miRNAs may play a role in the pathogenesis of Parkinson's disease. It is necessary to continue the search for new miRNAs that may affect the development of neurodegeneration in Parkinson's disease.
Methods: 20 untreated patients with Parkinson's disease and 18 treated patients with Parkinson's disease (Hoehn and Yahr scores 1–2) were studied. An analysis of the levels of 11 miRNAs in the peripheral blood lymphocytes of patients was carried out using reverse transcription followed by real-time PCR.
Results: The levels of miR-7, miR-9-3p, miR-9-5p, miR-129, and miR-132 were increased by more than three times in treated patients with Parkinson's disease compared with those of the controls.
Conclusions: It is probable that miRNAs are very sensitive to drug therapy and that the effects of therapy observed may be associated with changes in the levels of these miRNAs and their target genes in patients with Parkinson's disease.

1. Introduction

It has been shown that miRNAs are frequently deregulated in human diseases, suggesting that they could serve as possible targets or potential biomarkers for development of miRNA-based therapeutics for the treatment of cancer, heart failure, atherosclerosis and HCV infection [1–4]. There is evidence that microRNAs (miRNAs) may be involved in the Parkinson's disease (PD) pathogenesis [5–8].

A significant reduction in the level of miR-133b compared with healthy controls was shown in the study of the expression profile of 224 miRNA precursors in the brains of patients with PD [9]. Moreover, several studies performed using various model objects have shown that miRNAs may be associated with the development of neurodegeneration in PD [5,10]. However, there is evidence of decreasing in the levels of miR-1, miR-22*, and miR-29a in the peripheral blood of patients with PD [11].

Thus, it is necessary to continue the search for new miRNAs that may affect the development of neurodegeneration in PD. In this regard, we have analyzed changes in the levels of 11 miRNAs in the peripheral blood of patients in the early stages of PD. These miRNAs are preferentially expressed in the brain and may play a role in the pathogenesis of PD.

2. Methods

Two groups of patients with PD (Hoehn and Yahr score of 1–2) were studied: 20 untreated patients with PD and 18 treated patients with PD. The mean age ± SD at disease onset was 54.4 ± 7.6 years (46.8–62), and the mean age at the enrollment was 55.6 ± 7.6 years (47.8–63). Sex ratio was about 1:1 in groups of patients with PD. The form of PD was predominantly mixed.

Patients with PD had no severe concomitant diseases, such as severe cardiovascular disease, cancer, or diabetes mellitus. Treated patients with PD received different medications (dopamine receptor agonists: pramipexole in a dosage of 1.5 mg/day or piribedil in a dosage of 150 mg/day, L-dopa in a dosage of 150–200 mg/day, amantadine in a dosage of 300 mg/day), either as monotherapy or in various combinations.

The following individuals were included in two control groups: the neurological control group consisted of 21 patients with different neurological disorders (amyotrophic lateral sclerosis, Charcot–Marie–Tooth disease, Wilson's disease, cerebellar ataxia, etc.), whereas the control group consisted of 24 neurologically healthy volunteers who were matched to the patients regarding sex, age, and ethnicity.

All patients (Russians residing in the European part of Russia) were diagnosed with PD at the Research Center of Neurology. All patients with PD were selected and studied according to the international Unified Parkinson's Disease Rating Scale and Hoehn and Yahr scores [12,13]. The diagnosis of PD was based on the UK PD Brain Bank Criteria [14].

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Table 1
Relative miRNA levels in the different groups of patients.

miRNA	Expression level in untreated patients Me[a] (25%–75%)[b]	Expression level in treated patients Me (25%–75%)	Expression level in neurological control Me (25%–75%)
miR-7	1.75 (0.66–5.61)	**32.39 (8.91–44.84)**[c]	0.91 (0.05–3.42)
miR-9-5p	1.19 (0.90–5.11)	**6.57 (1.04–19.72)**	1.21 (0.73–1.49)
miR-9-3p	0.86 (0.50–9.52)	**3.27 (0.53–7.90)**	0.61 (0.42–0.95)
miR-129	1.36 (0.83–2.64)	**3.93 (1.28–18.43)**	1.01 (0.54–2.73)
miR-132	1.65 (0.59–5.43)	**3.63 (0.67–11.84)**	1.10 (0.43–2.07)
miR-133b	1.03 (0.63–2.47)	1.65 (0.24–5.73)	–[d]
miR-153	0.76 (0.33–3.11)	3.12 (0.72–10.48)	–
miR-191	2.30 (1.26–4.90)	2.64 (1.19–12.64)	–
miR-346	2.31 (0.40–3.91)	1.59 (0.38–8.76)	–
miR-433	0.98 (0.40–1.60)	2.76 (0.60–9.36)	–
miR-598	2.79 (0.54–9.15)	1.76 (0.92–7.14)	–

[a] Me, median.
[b] 25%–75%, 25–75 percentiles.
[c] The data in bold are statistically significant ($p < 0.05$).
[d] Analysis was not performed in this group.

All blood samples were collected with the informed consent of the persons investigated. The study was approved by the Ethics Committee of the Research Center of Neurology.

miRNA isolation was performed from peripheral blood lymphocytes. Isolation of lymphocytes from the whole blood was carried out using Ficoll-Urografin (DNA Technology, Moscow, Russia). After that fraction containing RNA was isolated from lymphocytes using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA). miRNA isolation from this fraction was performed using the mirVana™ miRNA Isolation Kit (Ambion, Carlsbad, CA, USA). miRNA concentration was measured using a Quant-iT RNA BR Assay Kit and a Qubit fluorimeter (Invitrogen, Carlsbad, CA, USA). All procedures were carried out in accordance with the manufacturer's recommendations.

The analysis of miRNA levels was performed using reverse transcription followed by real-time PCR using TaqMan probes. Reverse transcription was carried out using the MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Analysis of levels of *miR-7*, *miR-9-5p*, *miR-9-3p*, *miR-129*, *miR-132*, *miR-133b*, *miR-153*, *miR-191*, *miR-346*, *miR-433*, *miR-598* and housekeeping RNAs *snRNA U6*, *snRNA U44* and *snRNA U47* was carried out using the Custom TaqMan® Gene Expression Assay and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Real-time PCR was using StepOnePlus™ System (Applied Biosystems, Foster City, CA, USA). All procedures were carried out in accordance with the manufacturer's recommendations. Each blood sample was analyzed in three independent runs for corrections of differences in sample quality and reverse transcription efficiency.

Relative levels of miRNAs were in the test groups were calculated as $R = 2^{-\Delta\Delta Cp}$ [15]. The levels of the miRNAs studied in the group of healthy controls were set as 1. Data were analyzed using the nonparametric Mann–Whitney *U* test with "Statistica for Windows 8.0" (StatSoft, Inc. (2007)) and MS Excel 2010 (Microsoft).

3. Results

The results of the analysis of the levels of 11 miRNAs are shown in Table 1.

In the first stage of our study, we analyzed changes in the miRNA levels in the groups of patients with PD and healthy volunteers. miRNAs that exhibited levels that differed significantly in the patients with PD compared with the control group underwent additional analysis in the neurological control group to assess the specificity of the changes observed in PD (Table 1).

Significant increases in the levels of miR-7, miR-9-5p, miR-9-3p, miR-129, and miR-132 were identified in untreated patients with PD. It should be noted that no difference was observed in miRNA levels between the neurological control group and the healthy volunteers, which may indicate the specificity of the changes of the miRNA levels observed during neurodegeneration in PD.

4. Discussion

The most pronounced change of miRNA levels was observed in treated patients with PD. The levels of miR-9-3p, miR-129, and miR-132 were increased by more than three times, those of miR-9-5p was increased by six times, and those of miR-7 was increased by 32 times in treated patients with Parkinson's disease compared with those of the healthy controls (Table 1). Our data are consistent with the data reported by Margis and coworkers [11], who showed that the use of levodopa treatment led to a change in the expression profile of several miRNAs. Furthermore, it was shown, that several non-coding RNAs change their expression in leucocytes of patients with PD after deep brain stimulation treatment [10,16]. It is probable that miRNAs are very sensitive to the therapy and that the effects of therapy observed may be associated with changes in the levels of these miRNAs and their target genes in patients with PD.

Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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