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Identification of a panel of five serum miRNAs as a biomarker for Parkinson's disease

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ABSTRACT

Background and objective: Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder after Alzheimer's disease. The aim of this work was to determine whether the differences of serum miRNAs profiling could distinguish PD patients from healthy individuals.
Methods: We collected serum samples from 106 sporadic PD patients and 91 age/gender-matched healthy controls. Serum miRNAs were analysed by Solexa sequencing followed by a qRT-PCR examination. The qRT-PCR assay, which was divided into two phases, was used to validate the expression of miRNAs screened by Solexa sequencing. Receiver operating characteristic (ROC) curve analysis and clustering analysis were performed to determine the diagnostic usefulness of the selected miRNAs for PD.
Results: In this study, we generated a profile of 5 serum miRNAs: miR-195 was up-regulated, and miR-185, miR-15b, miR-221 and miR-181a were down-regulated.
Conclusion: This group of five miRNAs can precisely distinguish PD patients from health individuals and may be used as a potential serum-based biomarker for the diagnosis of PD.

1. Introduction

Parkinson's Disease (PD), which is characterized by resting tremor, bradykinesia, rigidity and postural instability, is the second most common age-related neurodegenerative disorder [1]. Currently, a PD diagnosis mainly depends on neuroimaging and clinical manifestations using the UK PDS Brain Bank Criteria, Unified Parkinson's Disease Rating Scale and the modified Hoehn–Yahr stage [2].These diadynamic criteria are subjective and can be applied only when motor features appear. However, PD clinical manifestations do not appear until 50%–70% of the dopaminergic neurons have been lost, causing patients to lose the opportunity for early treatment [3]. Therefore, seeking novel biomarkers that are objective and can be quantified may contribute to the diagnosis of PD, especially at the early stages of the disease process.

MicroRNA (miRNA), an abundant class of small non-coding RNA, primarily cause the degradation or translational suppression of target mRNAs. It is reported that PD patients and patients with other neurodegenerative diseases have significantly different tissue miRNA profiles [4]. Several miRNAs (miR-133b, miR-34c, miR-107, miR-433 and miR-205) have been observed to be aberrantly expressed in the brain tissue and are involved in neuron differentiation, apoptosis and neurite outgrowth [5]. Therefore, these miRNAs are considered as novel biomarkers and potential therapy targets. The brain tissues from patients with neurodegenerative disorders are normally collected at autopsy; however, this invasive technique makes sample collection from living patients impossible. Recent reports have also demonstrated that miRNAs remain stable in the blood and may be used as novel non-invasive biomarkers for several types of diseases; thus, we supposed that there was a unique serum miRNA expression profile in PD patients that could be a new indicator [6]. The aim of this study was to investigate the

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profile of serum miRNAs and to explore its clinical value as a novel biomarker for PD.

2. Methods

2.1. Study population and blood sampling

The present study included 106 PD patients and 91 healthy individuals. All PD patients were diagnosed at the Jiangsu province hospital and the Nanjing brain hospital. Patients with cancer, significant cardiac dysfunction or diabetes were excluded from this study. UPDRS-III scores and a modified Hoehn–Yahr scale were used to evaluate the disease stages and the living quality of the PD patients. Ninety-one individuals from a large pool of individuals seeking a routine health checkup at the Jiangsu province people's hospital and showing no evidence of disease were selected as healthy controls. All samples were collected from consenting individuals according to protocols approved by the ethics committee of each participating institution. The controls were matched to the patients by age and sex (Supplementary Tables 1–2). The persons who performed the following experiments were blinded to the diagnostic result. All blood samples were collected, centrifuged and then stored according to previous report [7].

2.2. RNA isolation

For the Solexa sequencing of the serum samples, equal volumes of serum from each sample (6 mL each) were pooled separately to form patient and control sample pools (each pool contained 90 mL), and total RNA was extracted with TRIzol reagent (Life Technologies, Carlsbad, CA, USA) according to a previous report. The resulting RNA pellet was dissolved in 20 μL of RNase-free water and then stored at −80 °C for following examination. For the qRT-PCR assay, total RNA was extracted from 100 μL of the serum sample with a one-step phenol/chloroform purification protocol as previously described [8].

2.3. Solexa sequencing technology

Solexa sequencing was performed according to a previous report. Briefly, after PAGE purification of small RNA molecules (<30 nucleotides) and ligation of a pair of Solexa adaptors to the 5′ and 3′ RNA ends, the RNA molecules were amplified using primers to the adaptor regions for 17 cycles. Fragments that were approximately 90 bp (small RNA + adaptors) were isolated from an agarose gel. Purified DNA was used for cluster generation and sequencing analysis using Illumina's Solexa sequencer according to the manufacturer's instructions. Image files were generated by the sequencer and were processed to produce digital-quality data. After masking the adaptor sequences and removing the contaminated reads, clean reads were processed for in silico analysis as previously reported [8].

2.4. Individual qRT-PCR assay of serum miRNAs

A TaqMan probe-based qRT-PCR assay was performed to quantify the serum miRNA levels according to the manufacturer's instructions (7500 Sequence Detection System, Applied Biosystems) as described previously [8]. All reactions, including no-template controls, were performed in triplicate. It was proved that let-7d, let-7g and let-7i (let-7d/g/i) were more stable than other reference genes (U6, miR-16, RNU48 and RNU44) and the more accurate data could be obtained after normalization to a combination of let-7d/g/i [9]. We also observed the low variability of let-7d/g/i between PD patients and healthy controls (Supplementary Fig. 1), so we used a combination of let-7d/g/i as an endogenous reference gene for the normalization of serum miRNAs. The relative levels of miRNAs were calculated using the $2^{-\Delta\Delta Cq}$ method.

2.5. Data analysis

All of the statistical analyses were performed using the Statistical Analysis System software SPSS 19.0. The data were presented as the mean ± SEM for miRNAs or mean 6 SD for other variables. A Student's t-test or two-sided χ^2 test was used to compare the differences in variables between the two groups. A P *value* ≤ 0.05 was considered as statistically significant. For miRNAs, we constructed Receiver operating characteristic (ROC) curves and calculated the area under the ROC curves (AUC) to evaluate the predictive power of the candidate miRNA for PD.

3. Results

3.1. Solexa screening of serum miRNAs in PD

A multiphase case-control study was constructed to find markedly changed levels of serum miRNAs for PD patients (Fig. 1). In the initial biomarker screening stage, two pooled serum samples from 15 PD patients and 15 controls underwent Solexa sequencing to identify miRNAs that showed significant differences between the PD cases and matched controls. Supplementary Table 1 summarizes the demographic and clinical features of the patients for Solexa sequencing. According to the miRbase 16.0 version,a total of 1123 miRNAs were screened by Solexa sequencing. 138 and 159 miRNAs could be detected in healthy controls and PD patients, respectively

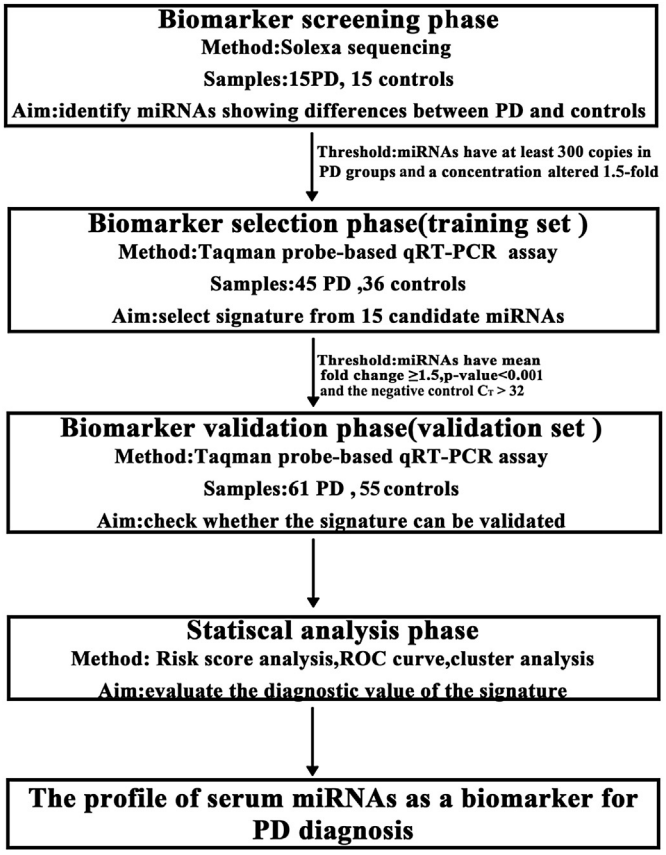

Fig. 1. The overview of the experiment design.