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Original Article

Circulating microRNAs levels in Chinese heart failure patients caused by dilated cardiomyopathy

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ABSTRACT

Background: Previous studies demonstrated dysregulated expression of microRNAs (miRNAs) in the myocardium of patients with dilated cardiomyopathy (DCM). This study investigated levels of miRNAs in the circulation of DCM patients, and the value of miRNAs as biomarkers for DCM.

Methods and materials: In 45 DCM patients and 39 age- and sex-matched controls, circulating miR-423-5p, miR-126, miR-361-5p, miR-155, and miR-146a concentrations were measured and correlated to cardiac functional parameters, including left ventricular ejection fraction (LVEF) and N-terminal pro-brain natriuretic peptide (NT-proBNP).

Results: Plasma levels of miR-126 and miR-361-5P did not differ between the DCM and control groups ($p = 0.331$ and $p = 0.784$, respectively). Plasma levels of the immunity-associated miRNAs, miR-146a and miR-155, did not differ between the DCM and control groups ($p = 0.437$ and $p = 0.702$, respectively). Levels of circulating miR-423-5p were significantly greater in the DCM group ($p = 0.003$). Further, there was a positive correlation between plasma levels of miR-423-5p and NT-proBNP ($r = 0.430$, $p = 0.003$). MiR-423-5p distinguished DCM cases from controls with an area under the curve (AUC) receiver operating characteristic (ROC) curve of 0.674 (95% CI, 0.555–0.793).

Conclusions: Patients with DCM have elevated plasma miR-423-5p levels. The plasma concentration of miR-423-5p was positively correlated with the level of NT-proBNP. Circulating levels of miR-423-5p could be served as a diagnostic biomarker for heart failure caused by DCM. Plasma levels of immunity-associated miR-146a, -155, and -126 were not significantly different between DCM and control groups.

1. Introduction

MicroRNAs (miRNAs) are endogenous small RNAs that are 21–25 nucleotides in length.[1] Dysregulation of intracellular miRNA expression has been described in various diseases, including a number of cardiovascular conditions.[2] Many studies have indicated that miRNAs are detectable and highly stable in plasma and other biological fluids. Furthermore,

Abbreviations: AUC, area under the receiver–operator characteristic curve; NT-proBNP, N-terminal pro-brain natriuretic peptide; DCM, dilated cardiomyopathy; EF, ejection fraction; HF, heart failure; miRNA, microRNA; ROC, receiver–operator characteristic.

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levels of circulating miRNAs have been shown to correlate with disease, thereby suggesting that miRNAs, might be valuable diagnostic biomarkers.[2]

Previous reports demonstrated a potential pathophysiological link between expression of miRNAs (miR-126, miR-155, miR-146a) and immune response for humans with dilated cardiomyopathy (DCM).[3,4] Other studies showed up-regulated expression of miR-423-5p and miR-361-5p in DCM myocardium.[5] The purpose of this study was to characterize levels of cardiac-associated miRNAs in the circulation of DCM patients and to determine their value as biomarkers for DCM.

2. Materials and methods

2.1. Participants

We recruited 45 DCM patients with New York Heart Association (NYHA) function classes I–IV, and a control group of 39 healthy age- and gender-matched volunteers, at the First Affiliated Hospital (Nanjing Medical University) after obtaining their written informed consent. Inclusion criteria for the DCM group were: a left ventricular ejection fraction (LVEF) less than 45%, as determined by two-dimensional and Doppler echocardiography (GE Vivid VII scanner with 1.7–3.4 MHz transducers); Exclusion criteria were: coronary heart disease (stenosis >50% of the luminal diameter in a major branch), as judged by coronary angiography; arterial hypertension (>160/100 mmHg); primary valvular heart disease; cardiomyopathy secondary to any systematic disease; clinical, sustained and rapid supraventricular arrhythmias; pericardial diseases; congenital heart diseases; cor pulmonale.[6] Inclusion criteria for the control group were: absence of known coronary, valvular, or myocardial disease. Exclusion criteria for all participants were: pregnancy, dialysis, and known or treated malignancies. The Institutional Review Board of the First Affiliated Hospital approved the study protocol.

2.2. N-terminal pro-brain natriuretic peptide (NT-proBNP) measurements

NT-proBNP was measured using an electrochemiluminescence immunoassay (E170 Roche Diagnostics, Mannheim, Germany).

2.3. Plasma RNA purification

Blood samples were collected in tubes containing EDTA (Gong Dong EDTA K_2), and plasma was isolated by centrifugation at 1500 $\times g$ for 15 min at 4 °C. Total RNA was isolated from 400 μL of plasma using the mirVana PARIS kit (AM1556, Ambion) according to the manufacturer's instructions. Before RNA isolation, 25 fmol of synthetic *Caenorhabditis elegans* MicroRNA-39 (cel-miRNA-39; Qiagen, Germany) was added to the mixture as an internal reference.[7] RNA isolated from plasma was subsequently re-suspended in 100 μL of RNase-free water, and then stored at −80 °C.

2.4. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Reverse transcription of miRNAs was performed using the TaqMan® MicroRNA Reverse Kit (Applied Biosystems, Foster, CA) according to the manufacturer's recommendations. The 15 μL RT reaction system contained 5 μL of RNA extract, 0.15 μL of 100 mM dNTPs (with dTTP), 1 μL of multiscribe reverse transcriptase (50 U/μL), 1.5 μL of 10 × RT buffer, 0.19 μL of RNase inhibitor (20 U/μL), 4.16 μL of RNase-free water, and 3 μL of 5 × miRNA-specific stem-loop RT primer (Applied Biosystems, Foster, CA). For real-time quantitative PCR (qRT-PCR), 1.33 μL of the cDNA product was used as a template in 20 μL reactions containing 1 μL of TaqMan® MicroRNA Assay, 7.67 μL of RNase-free water, and 10 μL of TaqMan® 2× Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems, Foster, CA). qRT-PCR was performed with 7900HT real-time PCR system (Applied Biosystems, Foster, CA). Triplicate measurements were obtained for each sample on a 384-well plate (Applied Biosystems, Foster, CA). This reaction contained a miRNA-specific forward primer, and a TaqMan® probe complementary to the 3′-end of the specific miNA sequence. Data were analyzed with SDS Relative Quantification Software version 2.2.2 (Applied Biosystems, Foster, CA), with the automatic Ct setting for assigning baseline and threshold for Ct determination. The relative expression level of each individual miRNA after normalization to cel-miRNA-39 was calculated using the $2^{-\triangle\triangle Ct}$ method.[8]

2.5. Statistical analyses

Continuous data are presented as mean ± standard deviation (SD). Categorical data are presented as counts and proportions. Values were log-transformed when appropriate for statistical analysis. Between group comparisons were examined using unpaired Student's *t*-tests and χ^2 tests for continuous and categorical variables, respectively. One-way ANOVA was used if more than two groups were compared. For correlation, Pearson's or Spearman's correlation coefficient was calculated for continuous and categorical data, respectively. Receiver Operating Characteristic (ROC) curve analysis was used to assess the diagnostic accuracy of miRNAs. The area under the ROC curve (AUC) was used as diagnostic index. Statistical significance was assumed at $p < 0.05$ and was two-sided. For all statistical analyses, the statistical software SPSS 17.0 (Statistical Package for the Social Sciences, Chicago, IL) for Windows was used.

3. Results

No significant differences in the clinical characteristics of the study populations were observed (Table 1). NT-proBNP measurements were only performed on DCM patients, most of whom were receiving diuretics, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers and β blockers. Plasma levels of miR-126 and miR-361-5P did not differ between the DCM and control groups (3.59 vs. 3.21, $p = 0.331$; 5.81 vs. 5.63; $p = 0.784$, respectively). Plasma levels of the immunity-associated miRNAs, miR-146a and miR-155, did