Journal of Cardiology (2011) 57, 338—344

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jjcc

Original article

Altered microRNA expression associated with reduced catecholamine sensitivity in patients with chronic heart failure

Hidehito Funahashi (MD)[a], Hideo Izawa (MD, PhD, FJCC)[b,*], Akihiro Hirashiki (MD, PhD)[a], Xian Wu Cheng (MD, PhD)[a], Yasuya Inden (MD, PhD)[a], Masanori Nomura (MD, PhD, FJCC)[b], Toyoaki Murohara (MD, PhD)[a,*]

[a] *Department of Cardiology, Nagoya University Graduate School of Medicine, 65 Tsurumai, Showa-ku, Nagoya, Japan*
[b] *Department of Cardiology, Fujita Health University Banbuntane-Hotokukai Hospital, Nagoya, Japan*

Received 9 November 2010; received in revised form 5 January 2011; accepted 12 January 2011
Available online 2 March 2011

KEYWORDS
Adrenergic receptor;
Heart failure;
Biopsy;
MicroRNA

Summary
Aims: MicroRNAs (miRNAs) are small non-coding RNAs discovered as potential new gene regulators. Their roles in the development of chronic heart failure (CHF), however, are largely unknown. Reduced catecholamine sensitivity is an early step of CHF. We examined whether altered expression of miRNAs was related to reduced catecholamine sensitivity in patients with CHF.
Methods and results: Maximum first derivative of left ventricular pressure (LV dP/dt_{max}) at baseline and during infusion of dobutamine ($10\,\mu g\,kg^{-1}\,min^{-1}$) were determined in 14 asymptomatic or mildly symptomatic (New York Heart Association class I or II) patients with idiopathic dilated cardiomyopathy (DCM). We performed microarray analysis for a total of 485 miRNAs using endomyocardial biopsy specimens from these 14 patients. Patients were classified into 2 groups based on a percent increase in LV dP/dt_{max} by dobutamine infusion (ΔLV dP/dt_{max}). These are Group I ($n=7$) with ΔLV $dP/dt_{max} > 90\%$, and Group II ($n=7$) with ΔLV $dP/dt_{max} < 90\%$. Out of 485 miRNAs, 32 were differentially expressed in the myocardium with reduced catecholamine sensitivity. Among those, four miRNAs were decreased and one miRNA was increased in the Group II compared to the Group I ($p<0.01$). LVEF measured by left ventriculography at baseline did not differ between the 2 groups. Also there were no differences in plasma norepinephrine levels between the 2 groups.

* Corresponding authors. Tel.: +81 52 744 2149; fax: +81 52 744 2138.
E-mail addresses: izawa@fujita-hu.ac.jp (H. Izawa), murohara@med.nagoya-u.ac.jp (T. Murohara).

0914-5087/$ — see front matter
doi:10.1016/j.jjcc.2011.01.009

Conclusions: Altered expression of several miRNAs was related to the reduced catecholamine sensitivity in mildly symptomatic patients with DCM. These findings shed light on the potential of miRNAs to provide possible etiologic insights as well as therapeutic targets for CHF.

Introduction

Failing human heart is generally characterized by a reduced catecholamine sensitivity that contributes to the loss of cardiac contractility [1—3]. Previous studies demonstrated that the reduced catecholamine sensitivity in idiopathic dilated cardiomyopathy (DCM) patients was associated with poor clinical prognosis [4—7]. The reduced catecholamine sensitivity is associated with an altered myocardial gene expression of contractile proteins even in mildly symptomatic patients with DCM [8]. However, it is still unknown what kind of molecular mechanisms regulate gene expression during the states of reduced catecholamine sensitivity.

MicroRNAs (miRNAs) are considered as one of the key regulators of gene expression [9]. MiRNAs are single-stranded RNAs, typically with 21—24 nucleotides in length, generated from endogenous transcripts and evolutionarily conserved [9,10]. These small non-coding nucleotides hybridize to mRNAs, resulting in the negative regulation of target mRNA stability or translational inhibition of targeted transcripts [9,10]. Although a growing body of evidence over the past few years suggests the importance of miRNAs in oncogenesis, neural diseases, and viral diseases [11—13], the regulatory roles of miRNAs in the pathological process of heart failure have only been recognized more recently [14—16].

In particular, an association of any miRNAs to the catecholamine sensitivity of the heart has not yet been examined.

We hypothesized that miRNAs could regulate and/or be associated with the reduced catecholamine sensitivity in the early stage of chronic heart failure (CHF). Accordingly, we have evaluated myocardial contractility during dobutamine stress testing in patients with idiopathic dilated cardiomyopathy (DCM) and New York Heart Association (NYHA) functional class I or II stages. We have also performed genome-wide miRNA expression profiling of endomyocardial biopsy specimens obtained from these patients.

Methods

Patients

Fourteen DCM patients with a NYHA function class I or II with normal sinus rhythm were enrolled in the present study. The diagnosis of DCM was based on a left ventricular ejection fraction (LVEF) less than 50% as determined by contrast ventriculography, in the absence of coronary artery stenosis > 50% in the diameter as judged by coronary angiography, arterial hypertension, valvular heart disease, and cardiac muscle disease secondary to any systematic diseases [17]. Endomyocardial biopsy at the right ventricular septum was performed to exclude myocarditis or specific diseases. All patients underwent laboratory measurements including echocardiography, neurohumoral factors, and cardiac catheterization. Five patients had been treated with beta-blockers, and nine with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers. The Institutional Ethical Review Board of Nagoya University School of Medicine approved the present study protocols including the analysis of miRNAs in biopsy specimens. All subjects provided written informed consent with regard to the study procedures, potential risks, and the analysis of miRNAs in the biopsy specimens.

Cardiac catheterization

All patients initially underwent routine diagnostic catheterization by a radial artery approach. In brief, a 6-F fluid-filled pigtail catheter with a high-fidelity micromanometer (CA-61000-PLB Pressure-tip Catheter, CD Leycom, Zoetermeer, the Netherlands) was advanced into the LV cavity for the measurement of LV pressure. We calculated the maximum first derivative of LV pressure (LV dP/dt_{max}) as an index of contractility as previously described [18,19]. After collection of baseline hemodynamic data, dobutamine was infused intravenously at incremental doses of 5 and 10 $\mu g\,kg^{-1}\,min^{-1}$, and hemodynamic values were measured at the end of each 10-min infusion period. After hemodynamic values had returned to baseline, right ventricular endomyocardial biopsy was performed. At least 3 endomyocardial samples were obtained from the right ventricular septum in each patient. The biopsy samples for miRNA analysis were snap frozen in liquid nitrogen and stored at −80 °C until the analysis.

miRNA measurements and analysis

Total RNA containing miRNA was extracted from each LV biopsy specimens using mirVana miRNA Isolation Kit according to the manufacturer's instruction (Ambion, Austin, TX, USA). Quality of purified RNA was checked by capillary electrophoresis (Bioanalyser 2100, Agilent Technologies, Tokyo, Japan) and then amplified by NCode miRNA Amplification System (Invitrogen, Carlsbad, CA, USA). Each purified miRNA was labeled with the CyeDye Mono-Reactive dye pack (GE Healthcare Bio-Science, Piscataway, NJ, USA) using the mirVana miRNA Labeling Kit (Ambion). And 1× miRNA hybridization buffer (Ambion) was added to the labeled miRNAs of each subject and the solution was heated to 95 °C for 3 min. Hybridization was carried out on the miRNA microarray (mirVana miRNA Bioarray V9.2, Ambion) containing 485 miRNAs in quadruplicate. After hybridization, each array was washed once with Low Stringency wash (Ambion) and