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Micro-RNA and mRNA myocardial tissue expression in biopsy specimen from patients with heart failure☆

Ka-Bik Lai [a], John E. Sanderson [a], Mohammad Bashar Izzat [b,1], Cheuk-Man Yu [a,*]

[a] Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR
[b] Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR

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ABSTRACT

Aims: There is increasing evidence that changes in microRNA (miRNA) expression occur in chronic heart failure and these may be involved in the pathogenesis. In this study we have explored the expression of selected myocyte and fibroblast-related microRNAs and messenger RNAs (mRNAs) that are associated with hypertrophy, apoptosis and fibrosis in biopsy specimens from patients with relatively new onset heart failure compared to a group of patients without heart failure.

Methods and Results: Myocardial biopsy specimens taken from Chinese patients presenting with recent heart failure were compared with a group of patients without heart failure undergoing routine cardiac surgery ($n = 34$). miRNAs (miR-1, -21, -23, -29, -30, -130, -133, -195, -199, -208, and -320) and corresponding mRNA expression were measured by real-time quantitative-PCR method. miR-1, -21, -23, -29, -130, -195 and -199 were significantly up-regulated in the heart failure group when compared to those without heart failure (all $p < 0.01$). However, miR-30, -133, -208 and -320 were not significantly different. Related mRNAs (casp3, coll I, coll III and TGF) were also significantly up-regulated (all $p < 0.05$) in the heart failure group.

Conclusion: Certain selected microRNAs involved in apoptosis, hypertrophy and fibrosis are up-regulated in the myocardium of patients with a clinical history of heart failure compared to those without. These specific miRNAs may be the most suitable for circulating biomarkers in the early stages of chronic heart failure and possibly future therapeutic targets.

1. Introduction

MicroRNAs (miRNAs) are a class of short, highly conserved, small noncoding RNAs which are important for many aspects of homeostasis and diseases [1–4]. miRNAs regulate gene expression at post-transcriptional level by directing protein complexes to binding sites present on the 3′ untranslated region of targeted messenger RNA (mRNA), suppressing translation and/or inducing mRNA degradation. miRNAs can fine-tune mRNA abundance to keep the levels of mRNA within a physiological range [3,4]. More than 2000 miRNAs have been identified in human cells and recently the number of studies on the involvement of miRNAs in cardiac pathology has increased rapidly [4]. In animal models they have been shown to be involved in hypertrophy, fibrosis, and apoptosis and the progression to heart failure (HF) [4–7].

Ventricular remodeling is a major feature of chronic heart failure [8,9]. During the remodeling progress, in response to pathological stimuli, the heart changes its shape and morphology. This process includes cardiomyocyte hypertrophy, apoptosis of cardiomyocyte and other cell types and collagen deposition [10]. There is increasing evidence to suggest that miRNAs are important in these processes of heart failure pathogenesis [11,12]. Most of the published studies have used murine models for investigating the miRNA expression profile [13,14]. In addition, work using whole peripheral blood has suggested that miRNAs are potential biomarkers for systolic heart failure [15,16]. However, there are few data on miRNA expression within the myocardium of patients with heart failure. That which is available comes from studies done on explanted hearts taken at the time of transplantation [17–19] and there appears to be no data derived from myocardial biopsy tissue taken from patients with less extreme disease or early onset heart failure. Therefore in this study we have explored the expression of selected myocyte and fibroblast-specific miRNAs known to be involved in the processes of hypertrophy, apoptosis and fibrosis in myocardial biopsy specimens taken from patients admitted with heart failure compared with a group of patients without heart failure undergoing routine cardiac surgery. In addition specific mRNAs, which are targets of the individual miRNAs, were measured to determine if miRNA expression affected their co-related mRNAs.

☆ The author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

* Corresponding author at: Li Ka Shing Institute of Health and Science, Institute of Vascular Medicine, and Division of Cardiology, Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR.
E-mail address: professorcmyu@gmail.com (C.-M. Yu).
[1] Current address: Damascus University Cardiac Surgery Hospital, Damascus, Syria.

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2. Materials and methods

2.1. Patients

Two groups of Chinese patients admitted into the Prince of Wales Hospital were studied. One group was admitted with symptoms and signs of recent onset heart failure (HF) due to possible myocarditis or dilated cardiomyopathy according to standard criteria [8,9] who had transvenous right ventricular biopsy performed to rule out myocarditis. A second group of patients with or without signs of clinical HF had ventricular biopsies performed at the time of routine cardiac surgery (coronary artery bypass grafting or valve surgery). These were taken from the apex of the left ventricle using a Tru-cut biopsy needle (CareFusion, San Diego, CA). The resulting perforations were oversewn with 3–0 Prolene sutures. All subjects gave informed consent. The study was approved by the institutional clinical research ethics committee.

2.2. Total RNA extraction

Total RNA was extracted using QIAGEN miRNeasy mini-kit (GmbH, Germany) according to the manufacturer's protocol. After extraction, the total RNA samples were stored at −80 °C until used.

2.3. Reverse transcription for mRNA

2 μl of the total RNA was mixed with 1× Taqman RT buffer, 5.5 μM $MgCl_2$, 500 μM dNTP, 2.5 μM random hexamer, 0.4 U Multiscribe reverse transcriptase and made up to 20 μl with H_2O. Reverse transcription was performed at 25 °C for 10 min, 48 °C for 30 min and 95 °C for 5 min. The resulting cDNA were stored in −80 °C until use.

2.4. Reverse transcription for miRNA

3.4 μl of the total RNA was mixed with 1× Taqman RT buffer, 1 mM dNTP (with dTTP), 1 μl specific primers, 3.5 U Multiscribe reverse transcriptase and made up to 10 μl with H_2O. Reverse transcription was performed at 16 °C for 30 min, 42 °C for 30 min and 85 °C for 5 min. The resulting cDNA is stored in −80 °C until use.

2.5. Real-time quantitation PCR

Quantitation of both of the relative mRNA and miRNA abundance was performed using Applied Biosystems Step One Plus (Applied Biosystems, Foster City, USA). In brief, 2 μl of the cDNA was mixed with primers, 100 nM of probe and 1× TaqMan Universal Master Mix in each reaction. TaqMan Gene Expression Assay and TaqMan miR assays were used and purchased from Applied Biosystems. Samples were tested in triplicate, and differences of threshold cycles between target genes and house-keeping genes (GAPDH in mRNA and U6 in miRNA) were calculated using the $2^{-\Delta\Delta CT}$ method using a control group as the calibrator according to the manufacturer's user manual. The value of relative mRNA and miRNA quantity for control group is 1 with arbitrary unit.

2.6. Statistics

Data were expressed as the mean ± standard deviation (SD). Since the data were highly skewed, comparison between the patient group and the control group was performed by Mann Whitey U test using SPSS V20 statistical software. A p value < 0.05 was considered statistically significant.

3. Result

3.1. Patient demographics

Total RNA was isolated from 34 patients (17 with HF and 17 without HF). Mean age for the heart failure patients was 54 ± 10.3 years and for the non-failing heart patients was 59 ± 9.3 years. In both groups, 14 were male and 3 were female. Clinical features of patients with or without heart failure are given in Table 1. All the patients were taking some form of cardiac medication at the time of biopsy. The HF group were mainly on standard HF medications. Five of the HF group and 2 of the non-HF were taking warfarin.

3.2. miRNA expression

The miRNA expression in the patient group compared with the control is shown in Table 2 and Fig. 1. Among the 11 miRNAs measured in this study, seven miRNAs had significant up-regulation in the patient group when compared with the control ($p < 0.05$). These were miR-1, -21, -23, -29, -130, -195 and -199. Whereas four miRNAs were not significantly different between the two groups; these were miR-30, -133, -208 and -320.

3.3. mRNA expression

Eleven mRNAs were investigated in this study. Table 3 and Fig. 2 show their gene expression results in the patient group when compared with the control. It was found that casp3, coll I, coll III and TGF had significant up-regulation ($p < 0.05$) in the patient group whereas CDC42, CDK 9, CTGF, Flk 1, HSP 70, MEF 2 and VEGF were not significantly different between the two groups.

3.4. Potential Confounders

It was not possible to account for medication between groups in view of the variety of medications taken and the relatively small numbers. Similarly, other clinical conditions may be relevant such as diabetes and obesity but again in view of the numbers it was not possible to make any corrections that would be statistically meaningful.

4. Discussion

In this study we have shown that the myocardium of patients with heart failure due to idiopathic dilated cardiomyopathy or ischemic heart disease had evidence of upregulation of certain miRNAs: miR -1, -21, -23, -29, -130, -195 and -199. However, miR-30, -133, -208 and -320 were not significantly different between the heart failure and control group. In addition, Casp3, coll I, coll III and TGF mRNA expression levels were significantly up-regulated in the patient group whereas

Table 1
Patients demographics.

	Heart failure group (n = 17)	Control group (n = 17)
Age	54 ± 10.3	59 ± 9.3
Sex	14 M, 3 F	14 M, 3 F
Diagnosis	DCM (7)	MVR (1)
	Valvular heart disease (1)	ASD closure (1)
	Ischemic heart disease (9)	IHD with CABG (15)
Other relevant conditions	Diabetes (5)	Diabetes (5)
	Hypertension (7)	Hypertension (5)
LVEF mean; (range)	30% (15–45)	>55%
Cardiomegaly	16	0

The abbreviations used are as follows: F, female; M, male; DCM, dilated cardiomyopathy; MVR = mitral valve replacement; IHD = ischemic heart disease; CABG = coronary artery bypass grafting; LVEF = left ventricular ejection fraction.