IJC Heart & Vasculature 8 (2015) 98–102

Contents lists available at ScienceDirect

IJC Heart & Vasculature

journal homepage: http://www.journals.elsevier.com/ijc-heart-and-vasculature

Expression of Let-7 family microRNAs in skin correlates negatively with severity of pulmonary hypertension in patients with systemic scleroderma☆

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ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form 15 June 2015
Accepted 16 June 2015
Available online 18 June 2015

Keywords:
Skin biopsy
MiRNA
Pulmonary hypertension
Systemic scleroderma

ABSTRACT

Background: Pulmonary hypertension (PH) is a serious complication in patients with systemic scleroderma (SSc), therefore it is important to identify the factors that could predict the presence and progression of PH. Skin biopsy is performed in patients with SSc to examine the type and severity of the disease. MicroRNAs (miRNAs) are potential biomarkers for various cardiovascular diseases including PH.

Methods and results: We determined the skin miRNA expression profile in 15 SSc patients with (n = 6) and without PH (n = 9). A mixture of equal amounts of miRNAs from PH and non-PH patients were prepared and used for miRNA PCR array analysis. The analysis identified 591 upregulated miRNAs and 57 downregulated miRNAs in the PH group. Of these, only miRNAs with a Ct value of less than 35 were subjected to further analysis. When a 1.5-fold difference was considered meaningful, 32 miRNAs were upregulated and 14 miRNAs were downregulated in the PH group. Interestingly, 5 out of 14 downregulated miRNAs belonged to the let-7 family. The results were validated by quantitative real-time PCR with specific primer for each miRNA, which showed significant downregulation of five let-7 family members (let-7a, -7d, -7e, -7f, -7g) in 6 PH compared with 9 non-PH skin samples. The expression levels of let-7d and 7b correlated negatively with pulmonary arterial pressure measured by echocardiography.

Conclusions: The results suggest that skin miRNA is a potentially useful marker for the presence and severity of PH in patients with SSc.

1. Introduction

Pulmonary hypertension (PH) is one of the most serious complications in patients with systemic scleroderma (SSc). Meta-analysis indicates that the prevalence of PH is 5 to 14% among SSc patients [1]. The presence of PH worsens prognosis in these patients [2], and thus it is important clinically and therapeutically to identify new factors and biomarkers that can predict the presence and progression of PH.

A number of clinical factors and biomarkers have been implicated in SSc-associated PH [3–5]. Recently, much attention has been paid to microRNAs (miRNAs) as a potential biomarker for PH [6]. miRNA is a short non-cording RNA known to act as a negative regulator of various transcription factors [7]. miRNA was originally thought to function only intracellularly, but recent data suggest that it is also secreted and detected in the circulation [7]. Various miRNAs have been identified to be involved in the pathogenesis of PH [8] and circulating miRNA levels vary according to the severity of PH [9].

In patients with SSc, skin biopsy is performed to examine the type and severity of the disease. Although this procedure is not always required for diagnosis, skin biopsy samples are important to rule out other fibrosing diseases. We have previously shown that miRNA profiling in skin samples was useful to investigate the functional role of miRNAs in the development of SSc [10,11]. However, the roles of miRNAs in the pathogenesis of SSc-associated PH have not been thoroughly investigated. In this study, we used skin biopsy samples to test the hypothesis that skin miRNAs expression profile varies among patients with SSc and depends on the presence or absence of PH.

☆ Acknowledgement of grant support: This work was supported in part by a Grant-in-Aid for Scientific Research C (26461147) to Y.I. from the Ministry of Education, Science, and Culture, Japan.

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http://dx.doi.org/10.1016/j.ijcha.2015.06.006

Table 1
Clinical characteristics of study participants.

	Non-PH group (n = 9)	PH group (n = 6)	p value
Age (years)	73.0 ± 0.8	70.8 ± 4.7	0.59
Females	9 (100%)	6 (100%)	
Body mass index (kg/m^2)	23.6 ± 1.9	24.0 ± 2.6	0.93
Systolic blood pressure (mm Hg)	147.3 ± 4.8	137.2 ± 9.1	0.50
Diastolic blood pressure (mm Hg)	81.7 ± 2.8	73.2 ± 4.8	0.30
Rheumatic factor	15.1 ± 6.3	21.2 ± 3.0	0.51
Ribonucleoprotein (U/mL)	1.09 ± 0.19	1.06 ± 0.24	0.93
Scl-70 (U/mL)	2.73 ± 0.82	3.54 ± 2.40	0.72
Anti-centromere antibody (U/mL)	97.4 ± 33.8	117.9 ± 40.1	0.71
Brain natriuretic peptide (pg./mL)	36.8 ± 8.6	315.9 ± 121.3	0.01
hs-CRP (mg/dL)	0.07 (0.01–0.24)	0.2 (0.04–0.49)	0.07
Creatinine (mg/dL)	0.55 ± 0.04	0.65 ± 0.06	0.19
eGFR	78.3 ± 4.0	69.2 ± 6.0	0.21
HbA1c (%)	5.8 ± 0.1	5.7 ± 0.1	0.69
LVEF (%)	68.2 ± 0.9	65.5 ± 2.80	0.29
LVDd (mm)	40.2 ± 1.4	38.1 ± 0.7	0.33
Left atrium diameter (mm)	31.6 ± 1.7	34.7 ± 2.6	0.33
E/e′	11.0 ± 1.4	15.3 ± 4.3	0.28
Pulmonary arterial pressure (mmHg)	27.4 ± 0.87	66.7 ± 7.94	<0.01

Data are mean ± SEM, median (range) or (percentages).
Scl-70, anti-scleroderma antibody; hs-CRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; LVDd, left ventricular diameter diastolic phase.

2. Materials and methods

2.1. Study population

Data of 15 SSc patients who underwent skin tissue biopsy were available for analysis. They were divided into PH (n = 6) and non-PH groups (n = 9) according to the estimated pulmonary arterial pressure measured by ultrasound echocardiography (UCG). PH was defined as tricuspid valve regurgitation pressure gradient (TRPG) of more than 40 mm Hg. The echocardiographic findings of all study participants using Aplio XG (Toshiba, Tokyo, Japan) ultrasound systems were evaluated by two independent investigators who were blinded to the clinical data. The clinical and laboratory data reported in this study were obtained at the time of skin sampling. The study protocol was approved by the Human Ethics Review Committee of Kumamoto University and a signed consent form was obtained from each subject.

Table 2
List of miRNAs with 1.5-folds up- and down-regulation in the PH group.

miRNA ID	Fold up-regulation PH(+)/PH(−)	miRNA ID	Fold down-regulation PH(+)/PH(−)
hsa-miR-1909*	26.99	hsa-miR-92a	−1.57
hsa-miR-221	8.60	hsa-miR-767-5p	−1.69
hsa-miR-124	7.30	hsa-miR-370	−1.81
hsa-miR-3173-3p	4.22	hsa-let-7b	−1.90
hsa-miR-532-3p	3.49	hsa-miR-628-3p	−1.92
hsa-miR-1307	3.29	hsa-let-7d	−1.98
hsa-miR-4286	2.60	has-let-7f	−2.05
hsa-miR-205	2.55	hsa-miR-433	−2.08
hsa-miR-328	2.48	hsa-miR-23a	−2.13
hsa-miR-200c	2.39	hsa-let-7a	−2.19
hsa-miR-3647-3p	2.25	hsa-miR-203	−2.39
hsa-miR-200b	2.23	hsa-miR-199a-3p	−2.49
hsa-miR-23b	2.08	hsa-let-7 g	−2.64
hsa-miR-4301	2.08	hsa-miR-30c	−2.77
hsa-miR-3607-5p	2.06		
hsa-miR-421	2.04		
hsa-miR-24	1.99		
hsa-miR-125b	1.91		
hsa-miR-125a-5p	1.82		
hsa-miR-1247	1.81		
hsa-miR-27b	1.79		
hsa-miR-490-3p	1.75		
hsa-miR-1280	1.67		
hsa-miR-365	1.66		
hsa-miR-30b	1.65		
hsa-miR-1260	1.60		
hsa-miR-7-2*	1.60		
hsa-miR-720	1.60		
hsa-miR-3607-3p	1.59		
hsa-let-7c	1.58		
hsa-miR-27a	1.51		
hsa-miR-126	1.50		

2.2. miRNA isolation, PCR array and real-time PCR

The miRNA was isolated from human skin tissue using the miRNeasy FFPE kit (Qiagen, Hilden, Germany). For PCR array, the miRNAs were reverse transcribed into first-strand cDNA using an RT2 miRNA First-Strand Kit (Qiagen). The cDNA was mixed with QuantiTast SYBR Green PCR Master Mix, and the mixture was added into 384-well RT2 miRNA PCR Array that included primer pairs for 1066 human miRNAs

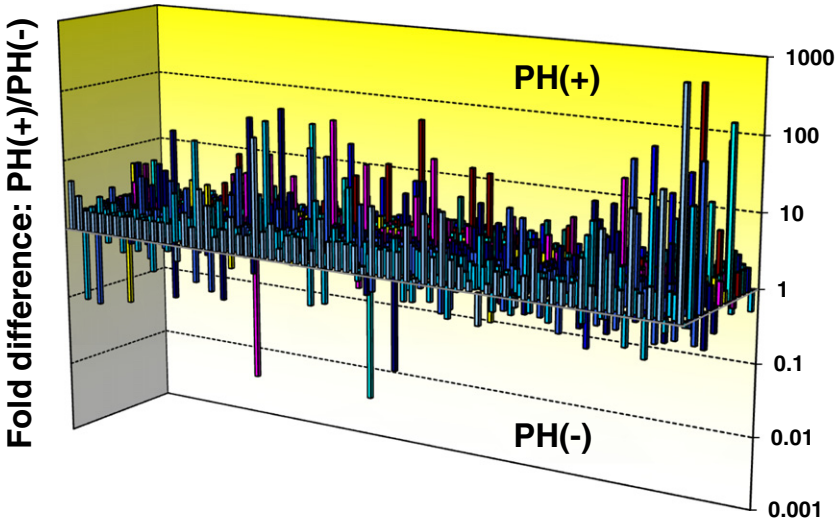

Fig. 1. The 3D-profile graphs of fold differences in the expression levels of each gene between non-PH and PH samples in the PCR Array. Columns pointing up (with z-axis values > 1) indicate up-regulation of gene expression, and columns pointing down (with z-axis values < 1) indicate down-regulation of gene expression in the PH samples, relative to the non-PH samples.