Journal of Cardiology 63 (2014) 373–378

Contents lists available at ScienceDirect

Journal of Cardiology

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

Original article

Periostin expression is upregulated and associated with myocardial fibrosis in human failing hearts

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ARTICLE INFO

Article history:
Received 18 March 2013
Received in revised form 30 August 2013
Accepted 30 September 2013
Available online 9 November 2013

Keywords:
Heart failure
Transplantation
Periostin
Myocardial fibrosis
MMPs

ABSTRACT

Background and purpose: Periostin, a matricellular protein, plays an important role in cardiac development and remodeling. Its expression profile and the association with myocardial fibrosis have not been investigated in human failing hearts. This work aimed to explore the behavior and pathologic significance of periostin in signifying collagen fibrogenesis in human hearts from patients with end-stage heart failure.
Methods and subjects: Tissues were collected from heart transplant recipients and the control hearts were from unmatched donors. Periostin mRNA and protein were detected using quantitative real-time polymerase chain reaction and Western blotting. Immunohistochemistry staining was employed to directly detect the protein level and distribution of periostin in heart tissues. The extent of myocardial fibrosis was expressed by the percentage of Masson's trichrome staining. Gelatin zymography was used to detect the activities of matrix metalloproteinase (MMP)2 and MMP9.
Results: A low level of periostin mRNA expression was found in control hearts while not detectable at the protein level. Periostin mRNA was increased significantly in failing myocardium compared to that of controls. Periostin was distributed extensively in left ventricle and interventricular septum of the failing hearts. Correlation analysis showed periostin protein expression was positively associated with myocardial fibrosis as well as left ventricular diastolic dimension. The distribution and extent of periostin was consistent with that of myocardial fibrosis. MMP2 activity has an obvious increase about fourfold in heart tissues from HF patients. But there is no quantitative association with the expression of periostin.
Conclusions: Periostin, the distribution and expression of which were consistent with the extent of myocardial fibrosis, might be a potential biomarker of cardiac remodeling in heart failure patients.

Introduction

Heart failure (HF) is a complex clinical syndrome arising from heart or blood vessel abnormalities. It is a common, potentially deadly condition of the cardiovascular system and the cause of a large financial burden and cost to the whole world [1]. The development of HF is a chronic process of myocardium and cardiac extracellular matrix remodeling. There is extensive comprehension of cardiac remodeling in the properties of cardiomyocytes. Also, cardiac extracellular matrix remodeling, especially tissue fibrosis, plays a pivotal role in promoting the development of HF. It might decrease heart compliance and increase the susceptibility to chamber dilatation and cardiac dysfunction [2,3]. In recent years, periostin, an extracellular matrix protein, has been proposed as a regulator of cardiac remodeling, it might be a marker for diagnosis of the severity of HF, and a therapeutic target for heart failure as well [4].

Periostin is a 90-kDa secreted protein with 4 repetitive fasciclin domains that are similar in sequence to the insect protein fasciclin-1. It was reported that periostin plays an important role in promoting collagen fibrogenesis and regulating atrioventricular valve maturation during cardiac development [5]. Periostin expression is minimal in adult hearts but essential to retain the

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http://dx.doi.org/10.1016/j.jjcc.2013.09.013

biomechanical properties of adult myocardium. It is upregulated in several heart diseases such as dilated cardiomyopathy (DCM), myocardial hypertrophy, or myocardial infarction (MI). But the role of periostin in myocardial regeneration remains controversial [6]. Kuhn et al. found that periostin induced reentry of adult cardiomyocytes into the cell cycle in vitro and in vivo [7], whereas Lorts et al. did not show an increase in mitosis of myocytes by periostin [8]. Furthermore, it has been demonstrated that periostin might also be a regulator of cardiac hypertrophy [9]. Cheng et al. [10] reported that in patients 3 months after an acute MI, periostin levels were negatively correlated with the left ventricular ejection fraction (LVEF), but positively correlated with the left ventricular end-diastolic and end-systolic dimensions. Multivariate analysis showed the periostin levels were independently associated with the LVEF [10]. Although partial functions of the extracellular matrix protein have been defined, the expression profile of periostin in human failing heart and the associations with myocardium remodeling have not been established. The present study aimed to investigate the change in periostin behavior and the pathological significance in signifying collagen fibrogenesis in the human failing heart.

Materials and methods

Sample collection

Human failing heart tissues were collected from explanted hearts of heart transplant recipients. Control hearts were obtained from unmatched donors whose hearts were not suitable to be transplanted. The etiology of HF included idiopathic DCM, hypertrophic cardiomyopathy (HCM), rheumatic heart disease (RHD), and viral myocarditis (VMC). The hearts were excised and transported in ice-cold, oxygenated cardioplegic solution, as well as flash frozen in liquid nitrogen. And then they were kept at −80 °C until processing. Sample collection and study design were performed as the protocols approved by the medical ethical committee, First Affiliated Hospital of Nanjing Medical University, PR China.

Quantitative real-time polymerase chain reaction

Total RNA was prepared from human myocardial tissues by using TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) as the manual instructed. cDNA was achieved from total RNA by use of the PrimeScript RT reagent Kit according to the manufacturer's instruction (TaKaRa, Shiga, Japan). And then cDNA was used as template for quantitative real-time polymerase chain reaction (PCR) with gene specific primers (periostin-sense TGCCCTGGTTATATGAGAATGGAAG, antisense GATGCCCAGAGTGCCATAAACA; GAPDH-sense GCACCGTCAAGGCTGAGAAC, antisense TGGTGAAGACGCCAGTGGA) and SYBR Green reagent (TaKaRa). Transcription RNAs were quantified by the comparative cycle threshold method, normalized to GAPDH. All real-time PCRs were performed in triplicate.

Western blotting

Western blotting was used to examine the protein expression of periostin. A recombinant human periostin protein produced in our laboratory was used as positive control [11]. The polyvinylidene fluoride membrane containing periostin was incubated with primary antibodies, anti-periostin (1:1000, Abcam, Cambridge, UK), and then with secondary antibodies, horseradish peroxidase-conjugated antibody (1:2000, Sigma–Aldrich, St. Louis, MO, USA). Proteins were revealed using an enhanced chemiluminescence detection method following to the manufacturer's instructions (Pierce, Rockford, IL, USA). Semi-quantification of the positive bands in the images was performed using Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Histological stains and immunohistochemistry

Heart tissues were fixed in 10% phosphate-buffered formalin and embedded in paraffin. De-paraffined sections with 4 μm thickness were stained according to Masson's trichrome staining protocol. The percentage of blue staining indicated the extent of myocardial fibrosis. Alternatively, the de-paraffined sections were permeabilized by 0.2% Triton X-100 in phosphate-buffered saline solution. And then they were incubated with anti-periostin antibody (ab14041, Abcam) overnight at 4 °C and subsequently with secondary antibodies for 2 h at 37 °C. Cell nuclei were stained with hematoxylin (Sigma–Aldrich). Periostin-positive areas were quantified by use of Image-Pro plus (IPP) 6.0 software (Media Cybernetics, Silver Spring, MD, USA). Protein level was expressed as the ratio of periostin-positive area to myocardial tissue area. For correlation analysis of periostin expression and myocardial fibrosis, consecutive sections were obtained for Masson staining and immunohistochemistry. All images were obtained at 200× magnification using inverted optical microscope (Eclipse 50i; Nikon, Corp., Tokyo, Japan).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis zymography

Protein concentrations were analyzed with a BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). Equal amounts of protein (20 μg/lane) for each sample were mixed with 1 sample buffer and loaded on a 7.5% polyacrylamide gel incorporated with 0.1% gelatin for electrophoresis under non-reducing conditions. Following electrophoresis the gels were washed twice in 2.5% Triton X-100 for 30 min at room temperature to remove sodium dodecyl sulfate. The gels were then incubated at 37 °C overnight in substrate buffer containing 50 mM Tris–HCl, 10 mM $CaCl_2$, 50 mM NaCl, and 0.05% Brij35 at pH 7.6 and stained for 30 min with 0.1% Coomassie brilliant blue. Gelatinolytic activity was visualized as a transparent band against a blue background. Zymography was measured for quantification analysis by lane density measurement using Image Lab 2.0 Software (Bio-rad).

Statistical analysis

Data are expressed as means ± SE. Multiple-group comparison was performed by one-way analysis of variance. Comparison between two groups under identical conditions was performed by the two-tailed Student's *t*-test. Spearman' correlation was used to assess the relation of periostin levels with left ventricle diastolic diameter (LVDd) and the extent of myocardial fibrosis. Values of $p < 0.05$ were considered statistically significant. All statistical analyses were 2-sided and performed using SPSS software (version 12.0; Chicago, IL, USA).

Results

Clinical characteristics

Baseline characteristics of the enrolled patients are summarized in Table 1. Briefly, a total of 15 patients with orthotopic heart transplantation were included in the study. Of them, 12 were males and 3 females, with a mean age of 44.5 ± 9.8 years (range, 17–55 years). There were 3 patients in New York Heart Association functional class III and 12 in class IV. Among these failing hearts, 11 were from patients with primary DCM, 2 from HCM, 1 from RHD, and 1 from VMC. These subjects with end stage heart