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Ageing related periostin expression increase from cardiac fibroblasts promotes cardiomyocytes senescent

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ABSTRACT

Periostin, as an extracellular matrix (ECM) protein, plays a critical role in myocardial fibrosis and also might be involved in the heart inflammatory process since it is a downstream molecule of IL4 and IL13. Considering the possible important role of periostin in heart aging, this study explored periostin expression pattern in both rat and human, the effect of periostin expression on cardiomyocyte senescent and expression of three cytokines (IL13, IL4 and IL6) in different age groups of human. This study found heart aging is associated with increased expression of periostin from cardiac fibroblasts and serum inflammatory cytokines (IL13 and IL6). Excessive periostin expression contributed to cardiomyocyte senescent, which could be alleviated through blocking the Ang-II-TGF β1-MAPK/ERK pathway. Thus, periostin might play an important role in a vicious circle (aging-fibrosis-inflammation-aging) of heart through promoting myocardial fibrosis and cardiomyocyte senescent simultaneously. It is a potential aging marker that could be directly measured in serum.

1. Introduction

The process of aging has accumulative effects on cardiovascular disease and is considered as a key risk factor of cardiac morbidity and mortality [1]. Heart aging is associated with progressive fibrosis and inflammation, two of the most important promoters of cellular senescence [2]. In aging heart, excessive mechanical load and reduced arterial compliance lead to the pathogenesis of cardiac fibrosis [1]. At the same time, both acute and chronic heart tissue injury would trigger a local inflammatory reaction, followed by release of fibrogenic cytokines and growth factors which promote fibrosis as a reparative process, such as TGF-β mediated profibrotic responses [3].

In human heart, extracellular matrix (ECM) is the structural fundamental supporting the organ. Periostin is a 90 kDa ECM protein with four repetitive fasci-clin domains [4]. Previous studies observed that periostin could bind to several other ECM proteins such as collagen I, fibronectin and heparin. Their architectural interactions could directly affect collagen synthesis and maturation and thus facilitate ECM deposition and cardiac remodeling [4]. Periostin expression was positively related to myocardial fibrosis. In animal model, significantly higher periostin expression was observed in mouse heart after transverse aortic constriction [5]. Aging related high periostin expression was also observed in rat heart [6]. In human, up-regulated periostin expression and associated higher level of myocardial fibrosis was observed in individuals with cardiac diseases such as myocardial infarction (MI), myocardial hypertrophy and dilated cardiomyopathy (DCM) [7,8]. Beside the role in myocardial fibrosis, periostin was also related to inflammation and was involved in the inflammatory microenvironment of several disorders including skin inflammation, airway inflammation and atherosclerosis [9]. Previous study observed that periostin was a downstream molecule of interleukin (IL)-4 and IL-13 [10]. In mouse heart, age-related inflammation was related to elevated IL-4 and IL-13 expression, which represents a shift to a Th2 phenotype immune response [11]. Thus, periostin also might be an important mediator in aging related inflammation.

However, although there is solid evidence about the role of periostin in myocardial fibrosis, its effect on cardiomyocytes is still not well recognized. In this study, we firstly reported that ageing related periostin expression increase from cardiac fibroblasts could promote cardiomyocytes senescent and age-dependent periostin expression might be related to increased expression of IL6 and IL13.

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2. Materials and method

2.1. Animals

Sprague–Dawley (SD) rats aged 6–8 months (young) and 24–26 months (old) were maintained in a specific pathogen-free facility. Animal based procedures all followed the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington, DC, 1996). Rat serum samples from old and young group were collected to measure serum periostin by using ELISA kit (KAMIYA Biomedical) and used for further study. 10 young rats (6 months) and 10 old rats (24 months) were sacrificed for preparation of rat primary cardiac fibroblasts and cardiomyocytes and myocardial tissues were used for DNA, total RNA and protein extraction.

2.2. Cell culture and preparation of adult rat primary cardiac fibroblasts (CFs) and cardiomyocytes (CMs)

Briefly, ventricles from both young and old SD rats were minced and digested with collagenase II (450 U/mL) (Worthington Biochemical). Cells were pelleted and suspended in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Invitrogen) and plated in culture plates. Non-adherent cells were removed, and CFs were cultured and passaged until they reached confluence. CFs were confirmed by immunostaining for vimentin and DDR2. To isolate cardiomyocytes from young rats, Pierce Primary Cardiomyocyte Isolation Kit (Thermo) was used according to recommended manual. Cardiomyoblast cell line H9C2 (ATCC, CRL-1446) was cultured in T75 flasks. All cells were cultured in Dulbecco's modified Eagle's medium (DEME) medium with 10% v/v fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin and 2 mM glutamine in humidified air (5% CO_2) under 37 °C. Cells were cultured in 6-well plates (1×10^5 cells in 2 mL per well) with serum-free essential medium before treated with target agents. To collect conditioned supernatants from CFs of old and young rat, 1×10^6 CFs were cultivated in 2 mL per well in 6-well plates with serum-free essential medium. 24 h after cultivation, conditioned supernatants were harvested with periostin concentration measured by ELISA (KAMIYA Biomedical).

2.3. Human blood collection

Blood samples were collected from physical examination center of the hospital affiliated to Xi'an Jiaotong University with approval from the Human Experimental Ethics Committee of the University. Inclusion criteria of participants included: healthy individuals: 20–35 (young), 36–54 (middle aged) or 55–85 years of age (old); non-current smoker; body mass index <30 kg/m^2; arterial blood pressure (BP) at rest <140/90 mmHg. A total of 74 samples from participants aged from 20 to 85 were randomly selected in the center. Information consent was obtained from participants before taking blood sample. After the blood was coagulated, the samples were centrifuged at 3500 rpm for 10 min to isolate serum. The serum samples were then used to measure serum periostin and inflammatory cytokines, including IL13, IL4 and IL6, with ELISA kits (R&D systems).

2.4. Periostin overexpression and silencing in H9C2 cell line

2.4.1. Stable transfection for periostin overexpression

The full-length periostin gene with 2340 bp (GenBank NM_001135934) was amplified from a human periostin cDNA clone (OriGene) through PCR with following primers: (F) 5′-GACG AAGCTTACCATGATTCCCTTTTTACCC-3′ and (R) 5′-GACGCTCGAGT CACTGAGAACGACCTTC-3′. The PCR products were digested with *Hind III* and *Xho I* and then purified. The purified PCR products were then cloned into the *Hind III/Xho I* sites of the pcDNA3.1(+) expression vector (Invitrogen). The sequence of this recombinant vector (pcDNA3.1-periostin) was verified through sequencing. H9C2 cells were transfected with pcDNA3.1 empty vectors or pcDNA3.1-periostin by using Lipofectamine 2000 (Invitrogen). 600 μg/mL G418 was applied for over two weeks to select stable transfected clones. Stable drug-resistant clones were used for following studies.

2.4.2. Transient transfection for periostin silencing and overexpression

pLenti-C-mGFP vector expressing mGFP-tagged rat periostin, pGFP-C-shLenti vectors expressing rat periostin shRNA and nontarget control shRNA were purchased from OriGene. To produce lentiviral particles, HEK293T cells were co-transfected with pLenti-C-mGFP or pGFP-C-shLenti vectors and Lenti-vpak packaging kit (OriGene) by using Lipofectamine 2000 (Invitrogen). 48 h after transfection, culture supernatants containing lentiviral particles were harvested. H9C2 cells or primary cardiomyocytes were treated culture supernatants containing lentivirus with 5 μg/mL polybrene (Sigma–Aldrich).

2.5. Quantitative real-time PCR

DNA and total RNA of rat heart tissue were extracted separately for telomere length analysis and periostin mRNA quantification respectively. DNA was extracted by using QIAamp DNA mini kit (Qiagen). Total RNA was isolated with using Trizol reagent (Invitrogen). cDNA was generated from total RNA by use of the RevertAid First Strand cDNA Synthesis Kit (Fermentas). The relative length of telomere of both young and aged rat hearts was measured using real-time PCR, according to the method previously described [12]. This method is based on measurement of ratio between the numbers of telomere repeat copy (T) to a single gene copy-albumin (S) in experimental DNA samples and reference DNA samples separately, assuming that both genes had similar amplification efficiency. The relative T/S ratios are proportional to average telomere length [12]. Primers for telomere and albumin followed Cawthon et al.'s recommendation [12]. Periostin mRNA expression was also quantified by real-time PCR. The primers for periostin (F) 5′-TGCCCTGGT-TATATGAGAATGGAAG-3′ and (R) 5′-GATGCCCAG AGTGCCATAAACA-3′. All qRT-PCR was conducted with ABI Prism 7300 Real Time PCR Sequence Detection System (Applied Biosystems) with the QuantiFast SYBR Green PCR kit (Qiagen).

2.6. Confirmation of Ang-II pathway in inducing periostin expression

To confirm Ang-II pathway in inducing periostin expression, cells were pretreated with Lorsatan (Merck) for 30 min and then stimulated with 1 μmol/L angiotensin II (Ang-II) (Sigma–Aldrich) for 48 h. To explore the effect of TGF-β1 on periostin expression, H9C2 cells were pretreated with 1 μmol/L Ang-II alone for 48 h, TGF-β1 (Sigma–Aldrich) (3 ng/mL) for 30 min or anti-TGF-β1 (Biosource) (3 mg/L) for 30 min and then 1 μmol/L Ang-II for 48 h. To explore the effect TGF-β1 signal on angiotensin induced high periostin expression, H9C2 were pretreated with SB202190 (Sigma–Aldrich), PD98059 (Sigma–Aldrich), or SP600125 (Sigma–Aldrich) (all 10 μmol/L) for 30 min and then stimulated with 1 μmol/L Ang-II for 48 h. Expression of periostin and TGF-β1 was detected by Western blot analysis.

2.7. D-Galactose and H_2O_2 treatment and SA β-gal staining

Normal H9C2 cells and primary cardiomyocytes were pretreated with anti-TGF-β1 (Sigma–Aldrich) (3.0 mg/L) or PD98059