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Cardiac extracellular matrix tenascin-C deposition during fibronectin degradation

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ABSTRACT

Tenascin-C (TN-C) might aggravate left ventricular remodeling after myocardial infarction (MI). Our previous study demonstrated that ventricular remodeling after MI is linked with the degradation of fibronectin (FN). The aim of the present study was to determine whether cardiac extracellular matrix TN-C deposition after MI requires FN degradation. We found that treatment with angiotensin (ANG) II significantly down-regulated FN while remarkably up-regulated TN-C in co-cultured cardiomyocytes and fibroblasts. Inhibitors of matrix metalloproteinase (MMP)-2, MMP-3 or MMP-9 significantly attenuated ANG II-induced loss of FN and obviously blunted ANG II-induced re-expression of TN-C in co-cultured cells. Moreover, FN fragments dose-dependently induced the deposition of TN-C. In addition, MI induced a significant reduction of FN protein expression and a marked elevation of TN-C expression level at day 7 after MI compared with the sham group. The present findings suggest that cardiac TN-C matrix deposition after MI is induced by FN degradation, which is dependent on the activation of MMPs. These findings might contribute to gain mechanistic insights into the regulation of TN-C formation after MI.

1. Introduction

Heart failure remains a major clinical problem with increasing prevalence partially attributed to better survival from acute myocardial infarction (MI) and increased longevity of the population [1]. The progression of heart failure is associated with myocardial remodeling, which manifests as gradual increase in left ventricular end-systolic volume and a change in chamber geometry to a more spherical and less elongated shape [2]. It is now becoming apparent that dynamic changes occur within the cardiac extracellular matrix (ECM) that directly contributes to adverse myocardial remodeling following MI [3–5]. ECM remodeling has been recognized as an important part in the development of ventricular remodeling [4].

Tenascin-C (TN-C), a major component of the cardiac ECM, was found to be the first member of the family expressed during embryonic development and wound healing [6]. TN-C is not expressed in healthy adult hearts, but is re-expressed or up-regulated under some pathological conditions, such as acute MI [7,8]. The previous study demonstrated that targeted deletion of TN-C improved cardiac function and myocardial stiffness after MI [9]. This finding suggests that the up-regulation of TN-C after MI may accelerate the progression of adverse ventricular remodeling. However, little is known about the factors that regulate the re-expression of TN-C.

Fibronectin (FN) is another major component of cardiac ECM. Breakdown of FN by matrix metalloproteinases (MMPs) facilitates myocardial remodeling [10]. Our previous study demonstrated that ventricular remodeling after MI is closely associated with the degradation of FN. Interactions between FN and TN-C are complex [11,12]. Thus, we hypothesized that the FN-degraded fragments could stimulate the deposition of TN-C, which may consequently accelerate ventricular remodeling. The present study was designed to determine whether cardiac ECM TN-C deposition after MI is associated with FN degradation.

2. Materials and methods

2.1. Cardiac cell culture

Experimental procedures were approved by the Institutional Animal Care and Use Committee of General Hospital of PLA Chengdu Military Area Command. Cardiac fibroblasts and cardiomyocytes were isolated by proteolytic dissociation of ventricular tissue and cultured using standard protocols as previously described [13]. Briefly, hearts were removed aseptically from neonatal (2–3 days old) Wistar rats. The ventricles were cut into pieces and incubated for 30 min in KB buffer, consisting of (mM) 70 KCl, 30 K_2HPO_4, 5 $MgSO_4$, 0.5 EGTA, 22 glucose, 20 taurine, 5 creatine, 10 succinic acid, 2 pyruvic acid, 5 ATP, 2 butyric acid, pH 7.4, and 115 U/mg collagenase. The cell suspension was layered over a Percoll gradient in a 15-ml conical tube and subjected to centrifugation

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(3000 rpm, 4 °C, 30 min). The top band of cardiac fibroblasts and lower band of cardiomyocytes were separately collected.

2.2. Co-culture model

The cardiomyocytes and fibroblasts were co-cultured to develop structured cardiac tissue model as previously described [14]. Angiotensin (ANG) II was added to stimulate the remodeling of cardiac ECM. MMP-2 Inhibitor I (sc-204092), MMP-3 Inhibitor (sc-311431) and MMP-9 Inhibitor I (sc-311437) were used to inhibit the activity of MMP2, MMP3 and MMP9, respectively. All the MMP inhibitors were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA).

2.3. FN fragments

Rat FN was purchased from Sigma–Aldrich (St. Louis, MO, USA). FN was digested for ten-minute at 37 °C with 1 U/mL α-chymotrypsin (Calbiochem, La Jolla, CA). The FN fragments were collected as previously described [15]. The co-cultured cells were treated

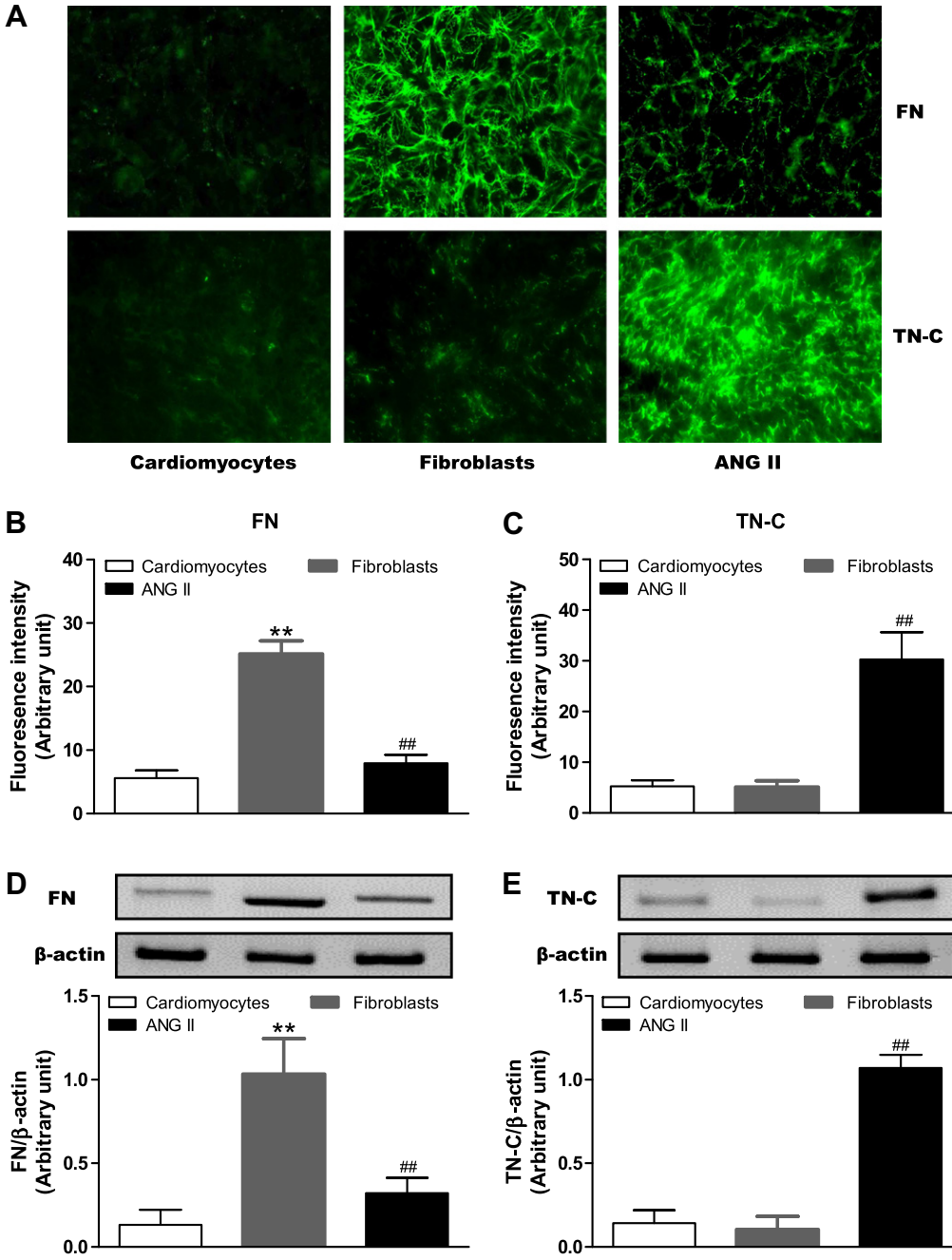

Fig. 1. FN degradation is linked with TN-C deposition *in vitro*. (A) Evaluation of FN (upper panel) and TN-C (lower panel) deposition by immunofluorescence microscopy. Cardiomyocytes (left), co-cultured cardiomyocytes and fibroblasts (medium) and ANG II-treated co-cultured cells (right) were grown on glass coverslips for 24 h, fixed and processed for immunofluorescence microscopy using anti-FN or anti-TN-C antibodies (magnifications: 200×). (B and C) Quantification of the fluorescence intensity for FN and TN-C. $^{**}p < 0.01$ vs. cardiomyocytes group, $^{\#\#}p < 0.01$ vs. fibroblasts group ($n = 6$). (D and E) Western blot analysis of FN and TN-C deposition. The protein was isolated from the cardiomyocytes, co-cultured cardiomyocytes and fibroblasts and ANG II-treated co-cultured cells and analyzed by Western blot with anti-FN and anti-TN-C antibodies. Equal protein loading was confirmed using β-actin antibody. Target proteins/β-actin is shown in the bar graph. $^{**}p < 0.01$ vs. cardiomyocytes group, $^{\#\#}p < 0.01$ vs. fibroblasts group ($n = 6$).