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Short communication

Aicar treatment reduces interstitial fibrosis in aging mice Suppression of the inflammatory fibroblast

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ABSTRACT

In 2030, elderly people will represent 20% of the United States population. Even now, chronic cardiac diseases, especially heart failure with preserved systolic function (HFpEF), are the most expensive DRGs for Medicare. Progressive interstitial fibrosis in the aging heart is well recognized as an important component of HFpEF. Our recent studies suggested an important pathophysiologic role for reduced TGF-β receptor 1 (TGFβR1) signaling in mesenchymal stem cells (MSCs) and their mesenchymal fibroblast progeny in the development of interstitial fibrosis. This report arises from our previous studies, which suggest that an inflammatory phenotype exists in these mesenchymal fibroblasts as a result of a reduced TGF-β-Smad-dependent pathway but upregulated farnesyltransferase (FTase)-Ras-Erk signaling. In this report we provide evidence for a therapeutic approach that downregulates Erk activation through an adenosine monophosphate-activated kinase (AMPK) pathway. Aging C57BL/6J mice were treated with AICAR (an AMPK activator) for a 30-day period. This treatment suppressed excessive monocyte chemoattractant protein-1 (MCP-1) generation, which diminished leukocyte infiltration and in consequence suppressed the formation of macrophage-derived myeloid fibroblasts. Interestingly, the number of mesenchymal fibroblasts was also reduced. In addition, we observed changes in extracellular matrix (ECM) deposition, specifically that collagen type I and the alternatively spliced variant of fibronectin (EDA) expressions were reduced. These data suggest that the upregulation of AMPK activity is a potential therapeutic approach to fibrosis in the aging heart.

1. Introduction

We have previously described an acute model of cardiac interstitial fibrosis induced by MCP-1 generation resulting in the accumulation of myeloid fibroblasts [1]. The myeloid fibroblast formation was rapidly reduced when MCP-1 was suppressed by TGF-β1 [2].

In contrast to acute models of interstitial fibrosis, the aging mouse (C57BL/6J) developed progressive fibrosis and cardiac dysfunction that was associated with an increasing number of myeloid fibroblasts and increasing MCP-1 and IL-13 synthesis [3]. The age-dependent increase in MCP-1 correlated with an increase in interstitial fibrosis and associated hemodynamic abnormalities [3].

In the aging mouse, we found a defect in endogenous MSCs resulting in marked reduction of TGFβR1 expression and reduced TGF-β responsiveness [4]. Because of reduced responsiveness to TGF-β, these fibroblasts make inflammatory chemokines and cytokines (usually suppressed by TGF-β) [5] that causes an induction of the myeloid fibroblasts [6]. This results in continuous ongoing inflammatory fibrosis in the old heart [6]. In addition, MSCs lack the brake on stem cell differentiation maintained by TGF-β [7] and enter into a differentiation cycle more readily [8], which causes the increased number of mesenchymal fibroblasts found in the aged heart [9]. Finally, despite impaired TGF-β signaling, the mesenchymal fibroblast continued to make collagen via an upregulated FTase-Ras-Erk pathway [9].

We have previously demonstrated that the use of the AMPK agonist, AICAR, increased TGF-β responsiveness to MSCs and their fibroblast progeny in vitro [4] and in a myocardial infarction model in the aging mouse [10]. Therefore, we postulated that AICAR would suppress the cellular phenotype described above via amplified TGF-β signaling. Our data suggest that AICAR treatment reduces the number of myeloid and mesenchymal fibroblasts. Interestingly, in the mesenchymal fibroblasts derived from the aging heart, upregulation

Abbreviations: FN, Fibronectin; MSC, mesenchymal stem cell; TGF-β, transforming growth factor-β; TGFβR1, TGF-β receptor 1; MCP-1, monocyte chemoattractant protein-1.

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of MCP-1 (that is necessary for myeloid fibroblast formation) and some ECM proteins depends on Erk activation, which is reduced by AICAR treatment. The purpose of this study was to evaluate AICAR treatment as a potential therapeutic target in age-dependent cardiac fibrosis.

2. Methods

2.1. Animals

14–21 month-old male C57BL/6J mice were obtained from the National Institute of Aging and aged if necessary. Animals were injected with AICAR (Toronto Research Chemical, 0.5 mg/g of body weight) or saline every day for 7 days or three times a week for 1 month. All animals were treated in accordance with the guidelines of the Baylor College of Medicine Animal Care and Research Advisory Committee.

2.2. Cell isolation

Hearts were cut into 1 mm^3 pieces and digested with Liberase TH Roche Diagnostics, Indianapolis, IN). The resulting non-myocyte cells were immediately used for flow cytometry analysis [6] or for tissue culture.

2.3. Tissue culture

Cells were cultured as previously described [4]. Quiescence was initiated 24 h before each experiment by switching the medium to low glucose DMEM without FBS.

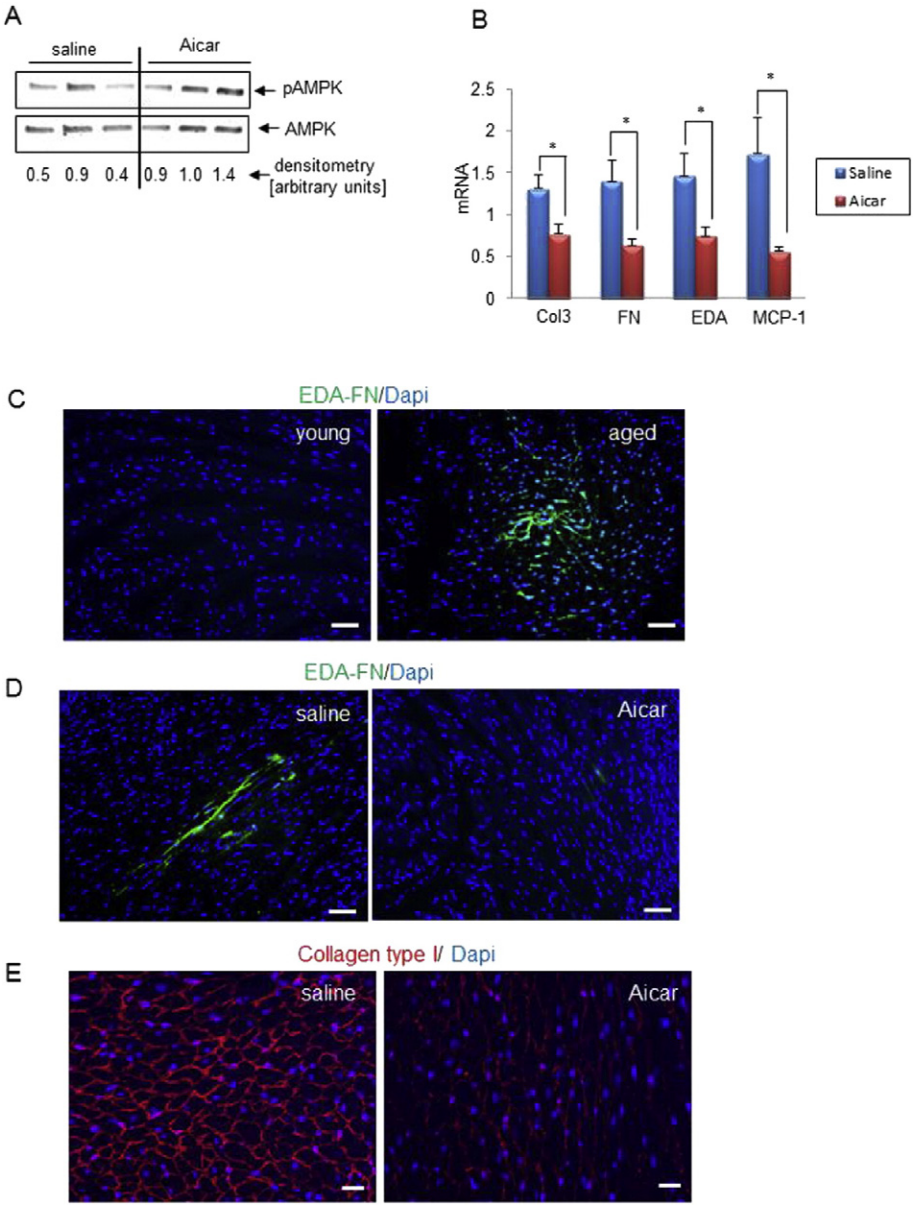

Fig. 1. AICAR treatment reduces levels of injury markers and fibrosis. A. Western blot analysis of phosphorylated AMPK in heart lysates isolated from saline and AICAR-injected mice. B, QPCR analysis of RNA isolated from whole hearts derived from 21 month-old mice injected with saline or AICAR for 4 weeks. Results are represented as mean ± SEM. * denotes $p < 0.05$. C, EDA is present in the aged uninjured heart as shown by immunofluorescence staining. Young denotes 3 month-old and aged denotes 24 month-old mice. Scale bar = 50 μm. D, 7 days AICAR treatment reduces the presence of EDA in the aged heart. 14 month-old mice were injected with saline or AICAR for 7 days. Heart sections were stained with anti-EDA antibody (green) or Dapi (blue). Scale bar = 50 μm. E, 14 month-old mice were injected with saline or AICAR for 7 days and 30 days after the last injection hearts were analyzed using anti-collagen type I antibody (red) or Dapi (blue). Scale bar = 50μm. N = 3 (for A, C), 5 (for D and E), and for B, N = 8, 4 for saline and AICAR treated mice respectively.