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Original article

Coronary adventitial cells are linked to perivascular cardiac fibrosis *via* TGFβ1 signaling in the *mdx* mouse model of Duchenne muscular dystrophy

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ABSTRACT

In Duchenne muscular dystrophy (DMD), progressive accumulation of cardiac fibrosis promotes heart failure. While the cellular origins of fibrosis in DMD hearts remain enigmatic, fibrotic tissue conspicuously forms near the coronary adventitia. Therefore, we sought to characterize the role of coronary adventitial cells in the formation of perivascular fibrosis. Utilizing the *mdx* model of DMD, we have identified a population of Sca1+, PDGFRα+, CD31−, and CD45− coronary adventitial cells responsible for perivascular fibrosis. Histopathology of dystrophic hearts revealed that Sca1+ cells extend from the adventitia and occupy regions of perivascular fibrosis. The number of Sca1+ adventitial cells increased two-fold in fibrotic *mdx* hearts *vs.* age matched wild-type hearts. Moreover, relative to Sca1−, PDGFRα+, CD31−, and CD45− cells and endothelial cells, Sca1+ adventitial cells FACS-sorted from *mdx* hearts expressed the highest level of *Collagen1α1* and *3α1*, *Connective tissue growth factor*, and *Tgfβr1* transcripts. Surprisingly, *mdx* endothelial cells expressed the greatest level of the *Tgfβ1* ligand. Utilizing *Collagen1α1-GFP* reporter mice, we confirmed that the majority of Sca1+ adventitial cells expressed type I collagen, an abundant component of cardiac fibrosis, in both wt (71% ± 4.1) and *mdx* (77% ± 3.5) hearts. In contrast, GFP+ interstitial fibroblasts were PDGFRα+ but negative for Sca1. Treatment of cultured *Collagen1α1-GFP*+ adventitial cells with TGFβ1 resulted in increased collagen synthesis, whereas pharmacological inhibition of TGFβR1 signaling reduced the fibrotic response. Therefore, perivascular cardiac fibrosis by coronary adventitial cells may be mediated by TGFβ1 signaling. Our results implicate coronary endothelial cells in mediating cardiac fibrosis *via* transmural TGFβ signaling, and suggest that the coronary adventitia is a promising target for developing novel anti-fibrotic therapies.

1. Introduction

Duchenne muscular dystrophy (DMD) is a genetic X-linked disease characterized by the absence of the dystrophin protein and progressive muscle wasting [1,2]. The majority of DMD patients develop cardiomyopathy and with the advent of ventilators, heart failure is emerging as the leading cause of mortality [3–5]. Cardiomyopathy in DMD is characterized by the accumulation of fibrosis which promotes heart dysfunction [6–8]. Perivascular fibrosis, described in many cardiac disease processes including DMD, has been implicated in heart failure [9,10]. Yet the cellular processes and molecular mechanisms that govern perivascular fibrosis in chronic diseases are poorly characterized [11]. By histopathology, the proximity of perivascular fibrosis to the coronary adventitia is difficult to overlook. Therefore, we set out to define the role of coronary adventitial cells in perivascular fibrosis. Utilizing the *mdx* mouse model of DMD [12], herein this report we have characterized a population of Sca1+, PDGFRα+, CD31−, and CD45− cells that resides in the coronary adventitia, and produces collagen in proximity to perivascular fibrosis. Specifically, in *mdx* hearts we detected Sca1+ cells in regions of severe perivascular fibrosis. In turn, molecular analysis revealed that Sca1+ adventitial cells expressed significant levels of pro-fibrotic genes: *Collagen1α1*, *Collagen3α1*, *Tgfβr1*, and *Connective tissue*

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growth factor (*Ctgf*) [13–15]. Surprisingly, we observed that *mdx* endothelial cells expressed high levels of *Tgfβ1* ligand suggesting that adventitial cells become fibrotic *via* transmural TGFβ1 signaling. Indeed, stimulation of FACS-sorted adventitial cells with TGFβ1 *in vitro*, resulted in increased collagen expression and deposition. In contrast, pharmacological inhibition of TGFβR1 with SB525334

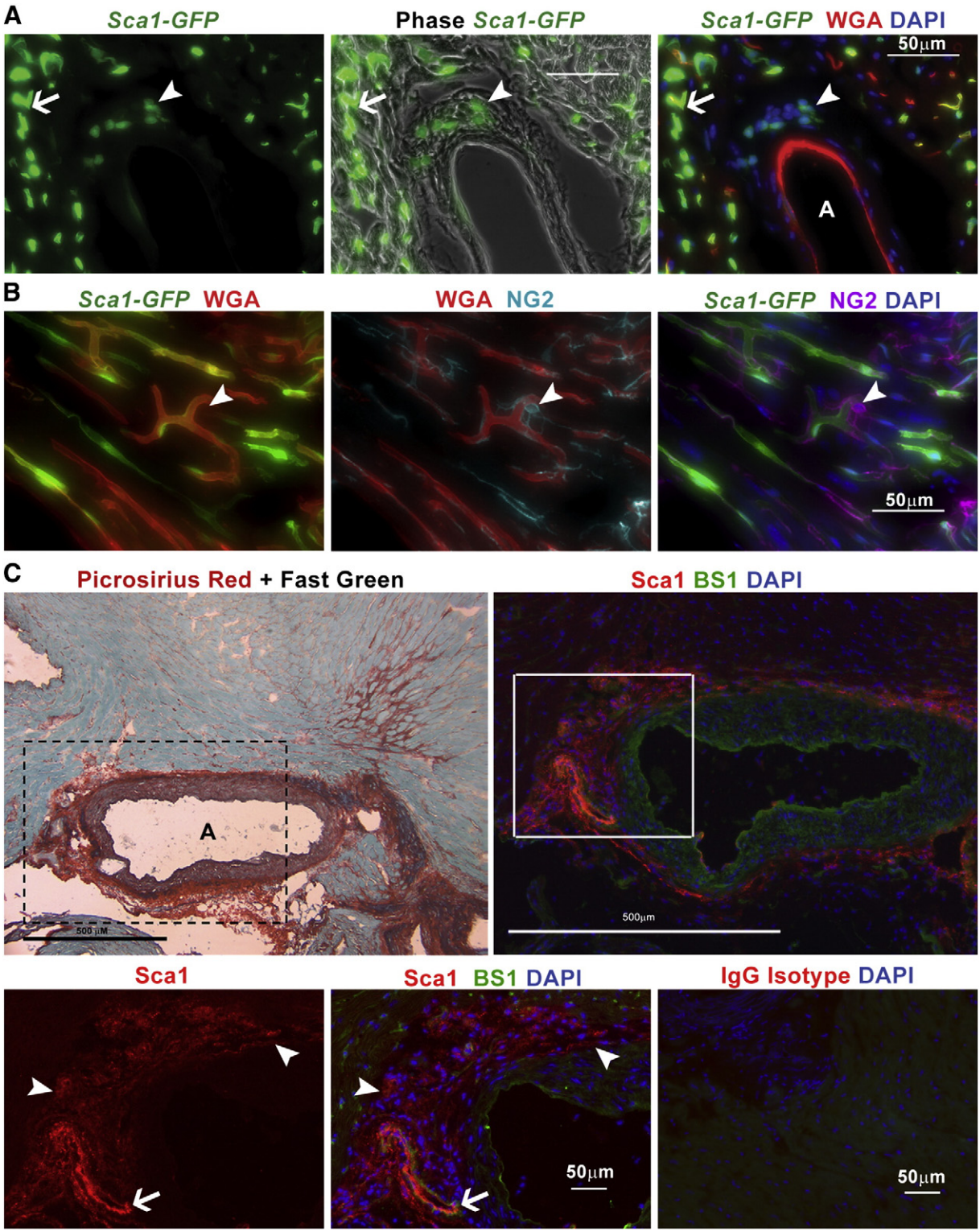

Fig. 1. *Sca1* + cells, distinct from endothelial cells and pericytes, reside in the coronary adventitia. A. Histological analysis of hearts from 11 month old *Sca1-GFP* animals injected intravenously with WGA, reveals that coronary adventitial cells are *Sca1* + (arrowhead) and distinct from GFP +, IV injected-WGA + endothelial cells (arrow) B. Staining for NG2 indicates that pericytes (arrowhead) are negative for *Sca1-GFP* but cover GFP +, IV injected-WGA + vascular endothelial cells. C. Staining with picrosirius red and fast green or Sca1 and BS1 in sections from a 22 month old *mdx* heart, reveals that collagen deposition surrounding the coronary adventitia is occupied by Sca1 + and BS1 − cells. A higher magnification (boxed area represented in bottom row) photo highlights adventitial Sca1 + and BS1 − cells (arrowhead) and Sca1 + and BS1 + endothelial cells (arrow). The bottom right panel shows a serial section of the same *mdx* heart shown in C, stained in parallel with an IgG isotype antibody control. Coronary arteries are denoted with an A.