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Syndecan-4: A component of the mechanosensory apparatus of cardiac fibroblasts

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Cardiac fibroblasts are the major nonmuscle cells of ventricular myocardium, where they comprise up to 30% of the total cell population of the murine heart [1]. Their lineage remains uncertain, although their ancestry appears to be derived from multiple sources during development and disease [2,3]. Cardiac fibroblasts are the major producers of extracellular matrix (ECM) proteins, and as such have been implicated as the predominant cell type responsible for the interstitial and perivascular fibrosis that develops during ventricular remodeling [4]. As fibroblast lineage varies, it remains unclear whether all subpopulations of cardiac fibroblasts participate in the over-production of fibrillar collagens and other ECM components during cardiac fibrosis. Nevertheless, a complete understanding on the molecular mechanisms responsible for cardiac fibrosis may provide new avenues for therapeutic intervention in heart failure. In a recent issue of the *Journal*, Herum and colleagues [5] now provide new and important information regarding the phenotypic modulation of cardiac fibroblasts, and reveal a previously unrecognized signaling pathway involved in the regulation of cardiac ECM biosynthesis.

1. Cardiac fibroblasts and their transition to myofibroblasts

It is now clear that, regardless of their origin, all cardiac fibroblast subpopulations can exist in either an inactive or active state. In addition to high levels of fibrillar collagen biosynthesis, this “active state” is characterized by expression of smooth muscle markers such as α -smooth muscle actin (SMA), the ED-splice variant of fibronectin [6], and SM22, a protein marker relatively specific for smooth muscle cells [7]. Phenotypic transition of inactive cardiac fibroblasts to activated, cardiac myofibroblasts in vivo accompanies a variety of pathological stimuli, including post-myocardial infarction (MI) ventricular remodeling [8], viral myocarditis [9] and pressure-overload induced left ventricular hypertrophy (LVH) [10]. An array of neuro-hormonal stimuli have been implicated in inducing this transition, but most studies have focused on the pleiotropic cytokine transforming growth factor- β (TGF- β) as playing a central role. Indeed, TGF- β added to the culture medium of quiescent, adult cardiac fibroblasts induced their transition to myofibroblasts [11], and potentiated the production of connective tissue growth factor (CTGF) and other matricellular proteins known to be involved in cardiac fibrosis [12]. Furthermore, cyclic stretch stimulated TGF- β production by both cardiomyocytes and fibroblasts [13], indicating an important role for mechanical factors, mechanotransduction, and autocrine/paracrine release of growth factors in the phenotypic switch. Nevertheless, the

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Disclosure statement

None.

molecular mechanisms responsible for myofibroblast differentiation in response to mechanical load have remained poorly defined.

2. Syndecans are components of the mechanosensory apparatus of cardiac fibroblasts

Focal adhesions are important sites for mechanotransduction in cardiac fibroblasts and other adherent cells [14]. These adhesive organelles are sites for the bi-directional transmission of mechanical forces between the intracellular actin-based cytoskeleton and the ECM, and have long been considered important mechanosensory sites in both cardiomyocytes and fibroblasts. Members of the integrin family of heterodimeric transmembrane receptors predominantly accomplish cellular attachment to the ECM at focal adhesion complexes in cardiac fibroblasts [14]. However, integrins are not the only proteins involved. Cell-surface proteoglycans known as syndecans can also bind ECM proteins via their extracellular heparan sulfate side chains. Typically, structural domains within specific ECM proteins mediate the heparan sulfate binding activity, and these domains are distinct from their integrin binding activity [15]. Thus, both integrins and syndecans contribute to fibroblast adhesion to ECM proteins.

There are 4 members of the syndecan family of heparan sulfate proteoglycans (HSPGs). Syndecan-4 (Syn4), the subject of Herum et al.'s article [5], is widely expressed in mesodermal tissues, including the heart and vasculature. The ectodomain of Syn4 has 3 HS chains that are capable of binding ECM proteins as well as other ligands involved in tissue injury and repair [16]. Like integrins, its cytoplasmic domain also has binding affinity for the actin cytoskeleton through interactions with syndesmos, paxillin and hic-5 [17]. Syn4 co-localizes with integrins in fibroblast focal adhesions, and can recruit focal adhesion proteins to sites of syndecan-specific cellular attachments even in the absence of integrin binding [18]. Furthermore, mechanical deformation of Syn4 binding sites activated the ERK cascade, indicating that Syn4 itself is a mechano-sensitive transmembrane protein that may function cooperatively with integrins at focal adhesion sites to initiate mechanochemical signaling [18].

3. Syndecan-4 is involved in cardiac injury and repair

As discussed in the current paper [5], there has been a great deal of recent interest in the role of Syn4 following cardiac tissue injury and during ventricular remodeling. For example, Finsen et al. [19] first demonstrated that Syn4 was up-regulated in ventricular tissue following MI. Matsui et al. [20] confirmed these findings, and demonstrated that Syn4-knockout mice (Syn4^{-/-} mice) were susceptible to post-MI ventricular rupture due to impaired granulation tissue formation and wound repair. Cardiac fibroblasts isolated from these animals showed fewer fibronectin-induced actin stress fibers, reduced numbers of focal adhesions, and impaired differentiation into myofibroblasts, suggesting a critical role for Syn4 in this process. Fibroblast motility was also significantly affected, and mechanotransduction signaling, including the activation of focal adhesion kinase (FAK), Akt, and RhoA, was substantially blunted [20]. Using a similar strategy, Echtermeyer et al. [21] found that myocardial ischemia-reperfusion injury was greater in Syn4^{-/-} mice as compared to wildtype (WT) animals. The increased damage was attributed to a greater degree of apoptosis 24 h after the is-chemic insult, due in part to reduced activation of the ERK cascade in the cardiomyocyte population of the Syn4^{-/-} hearts. Surprisingly, these authors noted *enhanced* rather than reduced hypertrophic signaling via the calcineurin-NFAT cascade in surviving, Syn4^{-/-} cardiomyocytes, which ultimately translated into improved LV geometry and function 7d post-injury. In contrast, Finsen et al. [22] found that Syn4 was in fact necessary for the development of concentric LVH in response to pressure

overload, and they attributed the reduced hypertrophic response to *reduced* rather than enhanced activation of the cardiomyocyte calcineurin-NFAT pathway. They found that stretch-induced activation of calcineurin was reduced in isolated neonatal cardiomyocytes derived from Syn4^(-/-) mice. Hypertrophic signaling via calcineurin was mediated in part by its direct interaction with the cytoplasmic tail of Syn4, leading to Syn4 dephosphorylation at S179. Thus, although Syn4 appears to play a crucial role in cardiac fibroblast function, its role in mechanical stress-induced hypertrophic signaling in the cardiomyocyte population remains uncertain. This uncertainty may be related to the fact that Syn4 was deficient in both cell populations, thereby potentially affecting autocrine-paracrine release of growth factors required for cardiomyocyte hypertrophy. A cardiomyocyte-specific knockout of Syn4 may help to clarify this issue.

4. Syndecan-4 is involved in mechanical stress-induced cardiac fibroblast differentiation

In contrast to its role in cardiomyocyte mechanotransduction, Herum et al. [5] now demonstrate that molecular markers of the fibroblast-myofibroblast transition were up-regulated in WT mice, but not in Syn4^(-/-) mice within 24 h of thoracic aortic banding. This defect in fibroblast differentiation was confirmed in cultured cardiac fibroblasts isolated from WT and Syn4^(-/-) mice following attachment to fibronectin. Unfortunately, the authors did not demonstrate that exogenous expression of Syn4 could rescue the defect, but they did show that overexpression of Syn4 (by transient transfection of a Syn4 expression plasmid into WT cardiac fibroblasts) increased SMA and SM22 gene expression. Importantly, they also demonstrated that cyclosporine A (CsA, 1 μ mol/L) reduced the number of SMA-positive, WT fibroblasts plated onto fibronectin, thus suggesting that a calcineurin-NFAT pathway might be involved in the Syn4-mediated phenotypic transition. Consistent with these results, fibrillar collagen mRNA levels were also substantially reduced in cultured Syn4^(-/-) cardiac fibroblasts, and in WT fibroblasts treated with either CsA, or the NFAT antagonist A-285222. Other experiments performed in both a noncardiac fibroblast cell line (HT1080 fibroblasts) and in cardiac fibroblasts, identified NFATc4 as the fibroblast NFAT iso-form responsible for the stretch-induced, calcineurin-dependent dephosphorylation and nuclear translocation of NFAT. Indeed, NFATc4 was hyperphosphorylated in Syn4^(-/-) cardiac fibroblasts, and failed to undergo dephosphorylation in response to cyclic stretch. Furthermore, Syn4 and calcineurin co-localized to focal adhesions of WT cardiac fibroblasts, and overexpression of Syn4 in Syn4-deficient cells were sufficient to reduce NFATc4 phosphorylation. Finally, cyclic stretch of Syn4-transfected HT1080 fibroblasts reduced Syn4 phosphorylation at S179, which had been previously proposed by the authors to promote the binding of calcineurin, calmodulin and NFAT to the cytoplasmic tail of Syn4 within cardiomyocyte focal adhesions [22]. Thus, the authors make a strong case for the presence of a Syn4-calcineurin-NFATc4 signaling pathway operative in differentiating cardiac fibroblasts.

5. Syndecan-4 and cardiac fibrosis

It remains unknown how Syn4-dependent signaling might be manipulated to reduce or prevent cardiac fibrosis. Nevertheless, the scaffolding function of Syn4 in fibroblast focal adhesions is reminiscent of similar events that transpire in integrin-dependent signal transduction. Indeed, there may be considerable overlap between both adhesion molecules as they transmit mechanical signals to the cell interior of cardiac fibroblasts. Targeting the cytoplasmic domain of Syn4 to block its interaction with calcineurin during mechanical overload might be a useful approach to reduce myofibroblast differentiation and prevent excess ECM accumulation in some forms of cardiac disease.

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