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RESEARCH ARTICLE

CCN2: a mechanosignaling sensor modulating integrin-dependent connective tissue remodeling in fibroblasts?

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Abstract Tensegrity (tensional integrity) is an emerging concept governing the structure of the body. Integrin-mediated mechanical tension is essential for connective tissue function in vivo. For example, in adult skin fibroblasts, the integrin β1 subunit mediates adhesion to collagen and fibronectin. Moreover, integrin β1, through its abilities to activate latent TGFβ1 and promote collagen production through focal adhesion kinase/rac1/nicotinamide adenine dinucleotide phosphate oxidase (NOX)/reactive oxygen species (ROS), is essential for dermal homeostasis, repair and fibrosis. The integrin β1-interacting protein CCN2, a member of the CCN family of proteins, is induced by TGFβ1; yet, CCN2 is not a simple downstream mediator of TGFβ1, but instead synergistically promote TGFβ1-induced adhesive signaling and fibrosis. Due to its selective ability to sense mechanical forces in the microenvironment, CCN2 may represent an exquisitely precise target for therapeutic intervention.

Keywords Mechanotransduction · Fibrosis · CCN2 · CTGF · CCN family

Chronic fibrotic diseases are characterized by the overproduction of scar tissue. These disorders can result in organ failure and death. The importance of mechanical tension in the cellular microenvironment is being increasingly appreciated as contributing to the pathogenesis of fibrotic conditions (Ingber 2003; Huang and Ogawa 2012). Cells such as fibroblasts sense extracellular mechanical forces through cell surface structures termed focal adhesions which contain cell surface receptors called integrins that connect the cytoskeleton to the surrounding extracellular matrix (ECM). Understanding the precise mechanism underlying how fibroblasts sense and communicate local mechanical signals is likely to be of future clinical benefit.

Myofibroblasts, the specialized form of mesenchymal cell responsible for wound repair and fibrosis, are characterized by α-smooth muscle actin (SMA)-containing stress fibers (Hinz 2009). Tissue damage results in elevated mechanical tension exerted by the surrounding microenvironment, causing differentiation of resident fibroblasts to myofibroblasts (Hinz 2009). For example, the potent fibrogenic cytokine TGF-β1 is activated in response to mechanical tension and up-regulates α-SMA in fibroblasts grown on stiff, but not on compliant, surfaces via a focal adhesion kinase (FAK)/src-dependent mechanism (Arora et al. 1999; Liu et al. 2007). In addition, α-SMA is only capable of being incorporated into stress fibers in cells subjected to significant mechanical loading (Goffin et al. 2006; Hinz 2006). Moreover, myofibroblasts inherently exert enhanced mechanical tension on their surrounding ECM through their enhanced adherent and contractile abilities, and hence directly contribute to the tensile strength of scar tissue (Chen et al. 2005; Hinz 2006; Vedrenne et al. 2012). The scar tissue itself exerts strong mechanical forces on the myofibroblast; thus, in fibrotic disease, an autocrine pro-adhesive loop exists that would be expected to be sufficient to result in persistence of the fibrotic phenotype (Hinz 2006, 2009; Leask 2011).

Integrins, the cell surface ECM receptors responsible for sensing mechanical stress, are heterodimers comprised of α and β subunits. The main integrins that actively participate in fibroblast proliferation, collagen contraction, and myofibroblast differentiation include α1β1, α2β1, and α11β1 (Pozzi et al. 1998; Schiro et al. 1991; Carracedo et al. 2010), suggesting that integrin β1 may play a central role in mechanotransduction and fibrogenic responses in dermal

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fibroblasts. This notion is explored extensively elsewhere in a recent review (Leask 2013); the purpose of the present review is to briefly summarize this concept, but to focus on the potential role of CCN2 in mechanotransduction and fibrosis. Integrin β1 knockout dermal fibroblasts are less able to adhere to and contract ECM, and show impaired activation of latent TGFβ1 (Liu et al. 2010a). Fibroblast-specific integrin β1 knockout mice display delayed wound healing that is rescued by addition of active recombinant TGFβ1 (Liu et al. 2010a). Fibroblast-specific integrin β1 knockout mice possess a progressive thinning of the dermis and are resistant to bleomycin-induced skin fibrosis (Liu et al. 2009; Liu and Leask 2013). Integrin β1 knockout fibroblasts also show less collagen and α−SMA production due to reduced in FAK/rac1/NOX/ROS-dependent signaling; the addition of hydrogen peroxide rescues the collagen and α−SMA expression defects of integrin β1 knockout fibroblasts (Liu and Leask 2013). These results are consistent with the hypothesis that antioxidants might be used as anti-fibrotic therapies (Demedts et al. 2005; Behr et al. 2009; Samarakoon et al. 2012).

Integrin-binding matricellular proteins are present within the cellular microenvironment and contribute to tissue plasticity by their ability to enabling a rapid response to changing conditions (Kyriakides and Bornstein 2003). These ECM play minimal roles in matrix structural integrity, but regulate signaling responses through their abilities to directly bind ECM proteins, growth factors and cytokines and also through their ability to trigger integrin signaling. Hence matricellular proteins modulate cellular processes such as cell adhesion and migration, ECM deposition, cell survival, and proliferation. The actual in vivo role of each matricellular protein varies depending based on the particular context and microenvironment.

One frequently studied member of the CCN family of the matricellular proteins is CCN2 (formerly known as connective tissue growth factor). CCN2 is largely absent from normal skin but is selectively upregulated in the dermal fibroblasts during wound healing and fibrosis (Igarashi et al. 1993; Kapoor et al. 2008; Liu et al. 2010b). In fibroblasts, CCN2 supports cell adhesion by binding integrin subunits containing integrin β1 (Leu et al. 2003; Chen et al. 2004). CCN2 also promotes integrin β1-mediated adhesion in other systems (Gao and Brigstock 2005). In vitro and in vivo, CCN2 enhances and alters pro-fibrotic, adhesive signaling responses to ECM components and growth factors (Mori et al. 1999; Chen et al. 2004; Shi-wen et al. 2006; Wang et al. 2011). Although dispensible for development of connective tissue in skin and for normal tissue repair (Liu and Leask 2011; Shangxi Liu, Katherine Thompson and Andrew Leask, unpublished observations), CCN2 is required for bleomycin-induced skin fibrogenesis (Liu et al. 2011). Initially, CCN2 was shown to be upregulated in response to TGFβ, and was considered to be a downstream regulator of this cytokine. A similar situation was also reported for the related protein CCN1 (Chen et al. 2001). However, it appears that TGFβ1 and CCN2/CCN1 are responsible for different yet interacting signaling mechanisms; genes responded in a different manner to the mixture of CCN1 and TGF-β1 than either CCN1 or TGFβ1 alone (Chen et al. 2001). For example, the effects of CCN1 and TGF-β1 on Col1α1 expression were antagonistic; in integrin α_5 expression, the effects of CCN1 and TGF-β1 overlapped; in MMP1 expression, TGF-β1 completely suppressed the strong inducing effect of CCN1; and in PAI-1 expression, effects of CCN1 and TGF-β1 were synergistic (Chen et al. 2011). A similar situation appears to operate with CCN2 (Shi-wen et al. 2006); CCN2 selectively enhances adhesive signaling responses to TGFβ1. Intriguingly, CCN2 appears to act not on affecting myofibroblast differentiation of resident fibroblasts but may act to recruit myofibroblasts (possibly pericytes or mesenchymal precursor cells) to the fibrotic lesion (Liu et al. 2011). In summation, CCN2 may prove in the future to be an exquisitely suitable target to affect mechanotransduction in the fibrotic milieu.

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