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BITS AND BYTES

CCN2: a bona fide target for anti-fibrotic drug intervention

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Abstract CCN2 (formerly known as connective tissue growth factor) was identified by several different laboratories approximately 20 years ago. Almost since its identification as a factor induced in normal fibroblasts by transforming growth factor β and overexpressed in fibrotic disease, CCN2 has been hypothesized to be not only a marker but also a central mediator of fibrosis in vivo. Finally, in vivo data are emerging to validate this key hypothesis. For example, a neutralizing anti-CCN2 antibody was found to attenuate fibrogenesis in three separate animal models (Wang et al. in Fibrogenesis Tissue Repair 4:1–4, 2011). This commentary addresses recent data indicating that CCN2 appears to represent a key central mediator of fibrosis and a good target for anti-fibrotic drug intervention.

Keywords Ctgf · ccn2 · Fibrosis · Bleomycin · UUO · Conditional knockout

Fibroproliferative disease has no treatment in spite of the fact that nearly 45% of all deaths in the developed world are caused by chronic inflammatory and fibrogenic disorders such as cardiovascular disease, pulmonary fibrosis, progressive kidney disease, systemic sclerosis, liver cirrhosis, and inflammatory bowel disease (Pinzani 2008). A possible rationale for why no therapy has been successful is that prior approaches have targeted individual cytokines; it is likely that proteins such as transforming growth factor β, endothelin-1, angiotensin, and platelet-derived growth factor synergize to produce fibrosis in vivo (Krieg et al. 2007; Leask 2010b). Although targeting individual cytokines may prove to be useful, it is perhaps more prudent to target key common downstream mediators of fibrosis. These targets may include those that are responsible for the activity of the cell type responsible for fibrosis, namely the highly contractile α-smooth muscle actin-expressing myofibroblast (Hinz and Gabbiani 2010). Strategies to employ in this regard might involve targeting the actions of integrins or focal adhesions, proteins essential for cell adhesion and matrix contraction, and, hence, fibrogenesis (Liu et al. 2009; Hinz 2009). Or targeting proteins mediating adhesive signaling, such as rac1 (Xu et al. 2009; Liu et al. 2008) or Akt (Kulkarni et al. 2011), might be useful. However, these proteins are expressed and active in normal cells, so it is unclear as to whether these strategies might be realistic.

An alternative approach might be to target a factor which is expressed essentially specifically in fibrosis that acts by modulating adhesive signaling, namely the matricellular signaling modulator CCN2 (also known as connective tissue growth factor, or CTGF). CCN2 was discovered by several groups about 20 years ago (Takigawa et al. 1989; Bradham et al. 1991; Brigstock et al. 1997). CCN2 was found to be induced by the potent fibrogenic protein transforming growth factor (TGF)β (Igarashi et al. 1993; Grotendorst et al. 1996; Abraham et al. 2000) and to be constitutively overexpressed in fibrotic cells but conspicuously absent in normal cells; indeed, CCN2 is considered an excellent surrogate marker for the severity and progression of fibrotic disease (Gressner and Gressner 2008; Leask et al. 2009; Phanish et al. 2010). CCN2 has also been hypothesized, almost since its discovery, to represent a novel specific target for anti-fibrotic approaches (Igarashi et al. 1996; Frazier et al. 1996; Franklin 1997). CCN2 is induced not only by TGFβ

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but also by endothelin and angiotensin; thus, CCN2 may be a common downstream mediator of the fibrotic action of these proteins (Shi-wen et al. 2007; He et al. 2005).

However, the actual in vivo data supporting the notion that CCN2 is essential for fibrosis has lagged behind the hypothesis. Data from several different groups suggests that overexpression of CCN2 in organs results in a susceptibility to develop fibrosis (Brigstock 2010). However, the generation of the whole body CCN2 knockout resulted in mice that possessed perinatal lethality due to rib cage defects and, although extremely interesting from a perspective of the bone field, did not address the key question from the perspective of people studying fibrosis (Ivkovic et al. 2003). However, mice containing a conditional CCN2 allele have now been generated; mice deleted for CCN2 in fibroblasts are resistant to bleomycin-induced skin fibrosis (Liu et al. 2011). Neutralizing antibodies to CCN2 impair fibrogenesis in the unilateral ureteral obstruction (UUO) renal fibrosis model and the bleomycin instillation model of pulmonary fibrosis (Wang et al. 2011; Ponticos et al. 2009). In one study, enhanced Col1a2 promoter activity in fibroblasts from bleomycin-treated lungs was observed and was partly dependent on Smad signaling, whereas CCN2 acted on the Col1a2 promoter by a mechanism that was independent of the Smad binding site, but was, instead, dependent on the ERK-1/2 and JNK MAPK pathways (Ponticos et al. 2009). That is, CCN2 has effects independent of TGFβ. Indeed, contrary to the initial idea that CCN2 is an essential downstream mediator of TGFβ action, transcriptional responses in response to TGFβ are not impaired in cells deleted for CCN2 (in which CCN2 is not normally basally expressed; Liu et al. 2011; Mori et al. 2008). Instead, CCN2 appears to play a key role in myofibroblast recruitment (Liu et al. 2010, 2011).

In fact, the available evidence indicates that CCN2 is a cofactor for TGFβ. A key insight supporting the role of CCN2 in fibrosis was provided what is felt in the field to be a classic study by the Takehara Group, which revealed that when injected subcutaneously, whereas injection of either TGFβ or CCN2 individually did not result in a sustained fibrotic response, simultaneous co-injection of TGFβ and CCN2 resulted in sustained fibrosis (Mori et al. 1999). Consistent with these notions, a recent study obtained similar results using intraperitoneal co-administration of CCN2 and TGFβ; intriguingly, the fibrogenic effect was ablated by co-administration of an anti-CCN2 antibody (Wang et al. 2011). In a similar vein, compared to their wild-type counterparts, mouse embryonic fibroblasts deleted for CCN2 showed impaired adhesive signaling responses to TGFβ, suggesting that the presence of basally expressed CCN2 resulted in enhanced TGFβ signaling (Shi-wen et al. 2006).

These latter data are consistent with the general hypothesis that CCN family members, including CCN2, act as adhesion molecules to modify signaling responses to extracellular ligands (Chen and Lau 2009, 2010).

Overall, the data from several different in vivo models (bleomycin skin and lung, and UUO kidney) and ablation strategies (conditional knockout, small interfering RNA, neutralizing antibody or even using recombinant CCN5) suggest that CCN2 is indeed essential for fibrosis and may, in fact, represent an excellent, specific target for antifibrotic therapies in the future (Brigstock 2009; Leask 2010a; Yoon et al. 2010). Indeed, clinical trials using a neutralizing anti-CCN2 antibody are ongoing and preliminary published Phase I data appear promising (Twigg 2010).

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