

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 b and over-expressed 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., Fibrogenesis Tissue Repair.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.

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 b, endothelin-1, angiotensin and platelet derived growth factor synergize to produce

fibrosis *in vivo* (Krieg et al., 2007; Leask, 2010). 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 (α -SMA)-expressing myofibroblast (Hinz and Gabbiani, 2010). Strategies here 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 not only induced by TGF β , but also endothelin and angiotensin can induce CCN2; 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 a role for CCN2 in fibrosis has lagged behind the hypothesis. The generation of the whole-body CCN2 knockout resulted in mice that possessed peri-natal 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 *Colla2* promoter activity in fibroblasts from bleomycin-treated lungs was observed, and was partly dependent on Smad signaling, whereas CCN2 acted on the *Colla2* 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 TGF β . Indeed, contrary to the initial idea that CCN2 is an essential downstream mediator of TGF β action, transcriptional responses in response to TGF β 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 TGF β . 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, although TGF β and CCN2 individually did not result in sustained fibrotic responses, TGF β and CCN2 together 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 TGF β ; 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 TGF β , suggesting that the presence of basally expressed CCN2 resulted in enhanced TGF β 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 and neutralizing antibody) suggest that CCN2 is indeed essential for fibrosis and may, in fact, represent an excellent, specific target for anti-fibrotic therapies in the future.

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