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CLINICAL IMPLICATIONS

CCN2, the connective tissue growth factor

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The role of extracellular matrix proteins in signaling control and regulation of response to injury, particularly in terms of scarring, is a topic of major importance. Blatant clinical examples of disturbed scarring are keloid production and systemic sclerosis, better known as scleroderma [1, 2]. Both these conditions involve the upregulation of a novel extracellular matrix-secreted protein termed connective tissue growth factor (CTGF). In this issue, Lang et al. implicate CTGF in the pathogenesis of cardiac fibrosis in a model of an infectious heart disease [3]. The investigators relied on a murine model of Coxsackie virus B3 (CVB3) myocarditis. They found that CTGF expression was induced in the course of CVB3 myocarditis. Particularly, fibroblasts expressed the cytokine. CTGF upregulation coincided with transforming growth factor-beta (TGF-β) expression and preceded pro-collagen type I mRNA expression. The investigators then used siRNAs. When they knocked down CVB3 replication, CTGF expression was diminished. However, suppression of CTGF had no effect on virus replication. Thus, CTGF is now implicated in the scarring after infectious heart disease. That CTGF is involved in cardiac fibrosis occurring with target-organ damage has been described earlier. The same group has published that the serum and glucocorticoid-inducible kinase (SGK1) is important to cardiac fibrosis occurring in the course of desoxycorticosterone acetate (DOCA salt)-induced hypertension and that CTGF is induced as a downstream target of the kinase [4].

We investigated earlier whether CTGF mediates the profibrotic effects of angiotensin II (Ang II) in the heart and kidney and the role of calcineurin-dependent pathways in *CTGF* gene regulation. We used transgenic rats harboring human renin and angiotensinogen genes. Ang II induced an age-dependent increase in myocardial CTGF expression, which was 3.5-fold greater than in normotensive control rats [5]. CTGF overexpression correlated closely with the Ang II-induced rise in blood pressure. CTGF mRNA and protein were located predominantly in areas with leukocyte infiltration, myocardial, and vascular lesions, and colocalized with TGF-β, collagen I, and collagen III mRNA expressions. Blockade of calcineurin activity with cyclosporine A completely normalized Ang II-induced CTGF overexpression in the heart and kidney, suppressed the inflammatory response, and mitigated Ang II-induced cell proliferation and apoptosis. In contrast, blockade of mTOR (target of rapamycin) pathway with everolimus increased CTGF expression. Apparently, CTGF is an important mediator of fibrosis, and therefore, we need to know about the protein in greater detail.

CTGF belongs to the CCN family of matrix signaling modulators [6]. The family is named after three prototypical members, cysteine-rich protein 61 (*C*yr61), also termed CCN1; *C*TGF, termed CCN2; and the nephroblastoma overexpressed protein (*N*ov), also known as CCN3. The italicized letters show the derivation of the designation "CCN". However, because their description was almost two decades ago, the CCN family has grown. The molecules comprise up to four modules, an insulin-like growth factor binding protein (IGFBP) domain (module I), a Von Willebrand factor domain (module II), a thrombospondin-homology domain (module III), and a cysteine knot, heparin-binding domain (module IV). A schematic view of CTGF and its modules is shown in Fig. 1a.

What do the CCN proteins do? They play key roles in angiogenesis, chondrogenesis, and wound healing. The CCN proteins work by participating in cell proliferation,

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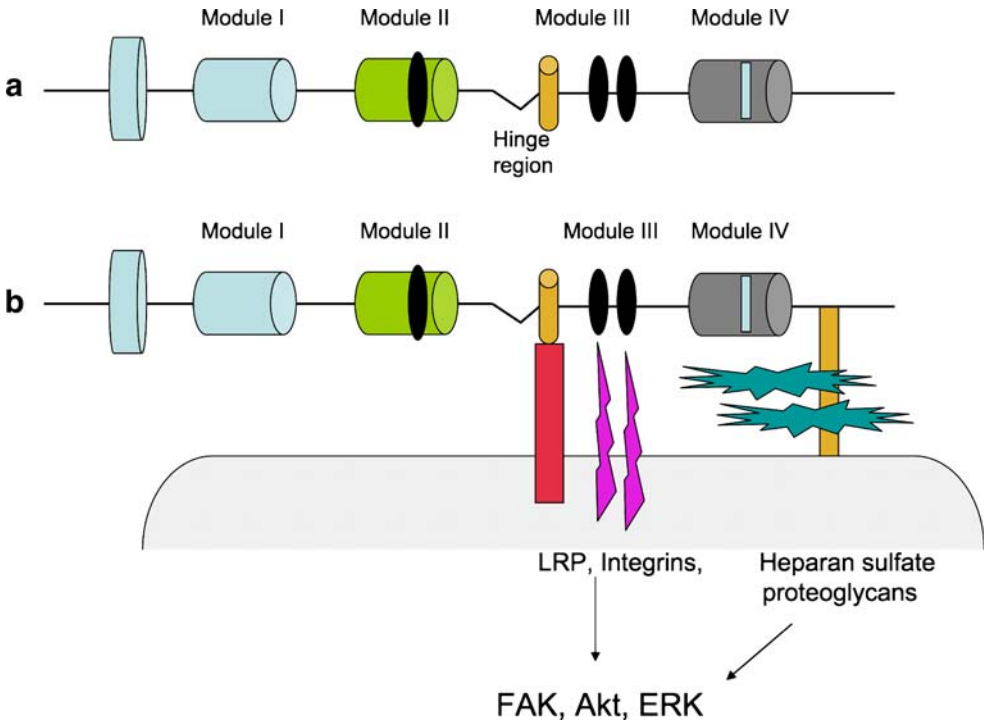

Fig. 1 **a** The modular domains of CTGF are given. **b** These modules interact with TGF-β fibronectin, LRP1 receptor, integrins, and heparan-sulfate proteoglycans; adapted from Leask and Abraham [9]

migration, and differentiation. Although they have common similar structures, they vary greatly in terms of biological functions that are also dependent on their cellular context. CTGF promotes endothelial cell growth, migration, adhesion, angiogenesis, and cell survival. CTGF deletion is lethal [7]. Abrogation of CTGF induced skeletal defects because of impaired chondrocyte proliferation and matrix remodeling. It is interesting to note that a mouse transgenic model for CTGF under the control of the type XI collagen promoter led to dwarfism [8].

Much effort has gone into identifying CCN receptors [9]. CTGF binds to specific integrins through discrete and separate domains. CTGF has as ligands αIIβ3 integrin in platelets and αMβ2 integrin in monocytes. CTGF interacts with heparan-sulfate-containing proteoglycans, including syndecan and perlecan, through a heparin-binding domain. CTGF also binds to the low-density lipoprotein receptor-related protein 1 (LRP1) and to LRP6, which is a Wnt coreceptor. CTGF also binds to the nerve growth factor tyrosine kinase receptor (TrkA). Some CTGF binding possibilities are shown in Fig. 1b.

The relationship between TGF-β and CTGF is a close one [9]. CTGF binds TGF-β through the N-terminal Von Willebrand factor domain (module II) and not only augments TGF-β activity, but also serves as an essential cofactor for the growth factor. In *Ccn2*−/− fibroblasts, TGF-β

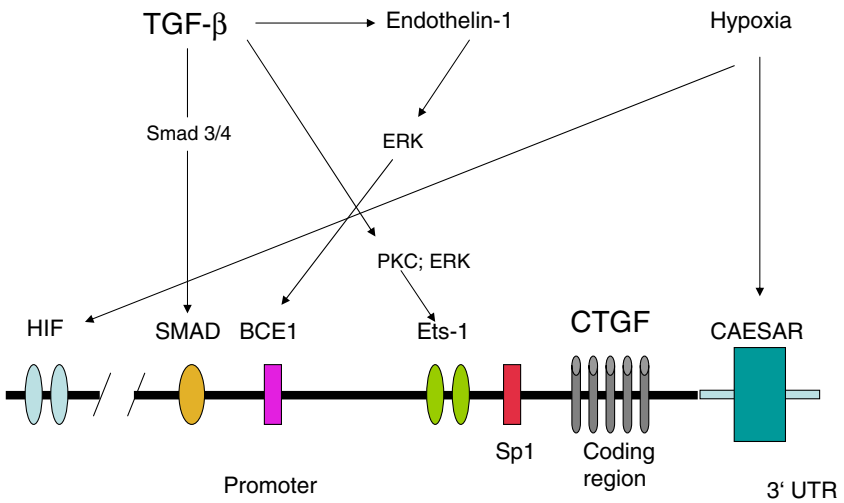

Fig. 2 Regulation of the *CTGF* gene involves Smad, BCE-1, Ets-1, and Sp1. The 3′ UTR of the gene contains a CAESAR; adapted from Leask and Abraham [9]

is able to induce far fewer genes, even though the generic "sons of mothers against decapentaplegic" (Smad) signaling pathways still function. CTGF also binds to fibronectin via the module IV domain. CTGF also enhances the effects of fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and insulin-like growth factor (ILGF).

The synthesis of CTGF is stimulated by specific growth factors, particularly endothelin 1 and TGF-β. Hypoxia and biochemical stimuli also play a role. The CTGF promoter contains recognition sequences for hypoxia-inducible factor (HIF); Smad; basal control element-1 (BCE-1); the transcription factor protooncogene, Ets-1; and the transcription factor, Sp1. A gene schematic is shown in Fig. 2. The 3′ UTR of the gene contains a *cis*-acting element of structure-anchored repression (CAESAR). For TGF-β to activate CTGF, protein kinase C (PKC) and the Ras/MEK/ERK mitogen-activated protein kinase (MAPK) cascade is required.

Is CTGF a potential drug target? CTGF-specific monoclonal antibodies have been tested in a pancreatic tumor cell line and were able to the inhibit growth of these cells [10]. An anti-CTGF treatment was successful in abrogating tumor growth in mice [10]. Also promising is the strategy of downregulating CTGF production. Antisense oligonucleotides have been used in this regard in mouse models of diabetic nephropathy [11]. Our attempt to use cyclosporin A was an indirect method that featured some success [5]. We have discussed almost exclusively only a single member of the fascinating CCN family. The involvement of CCN proteins in many fundamental biological processes suggests that they would be useful targets for molecular medicine with applications in angiogenesis, cartilage and bone formation, nerve conduction, muscle contraction, fibrosing disorders such as scleroderma, and as the report by Lang et al. [3] indicates, fibrosing heart disease.

Respectfully,

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