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PERSPECTIVES IN BASIC SCIENCE

Connective tissue growth factor: Potential role in glomerulosclerosis and tubulointerstitial fibrosis

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Connective tissue growth factor: Potential role in glomerulosclerosis and tubulointerstitial fibrosis. Transforming growth factor beta (TGF-β) is a pivotal driver of glomerulosclerosis and tubulointerstitial fibrosis in renal diseases. Because TGF-β also plays important anti-inflammatory and antiproliferative roles in mammalian systems, there has been a recent drive to elucidate downstream mediators of TGF-β's pro-fibrotic effects with the ultimate goal of developing new anti-fibrotic strategies for treatment of chronic diseases. Connective tissue growth factor (CTGF) belongs to the CCN family of immediate early response genes. Several lines of evidence suggest that CTGF is an important pro-fibrotic molecule in renal disease and that CTGF contributes to TGF-β bioactivity in this setting. CTGF expression is increased in the glomeruli and tubulointerstium in a variety of renal disease in association with scarring and sclerosis of renal parenchyma. In model systems in vitro, mesangial cell CTGF expression is induced by high extracellular glucose, cyclic mechanical strain and TGF-β. Recombinant human CTGF augments the production of fibronectin and type IV collagen by mesangial cells and the effects of high glucose on mesangial cell CTGF expression and matrix production are attenuated, in part, by anti-TGF-β antibody. In aggregate, these observations identify CTGF as an attractive therapeutic target in fibrotic renal diseases.

Regardless of the initiating insult, progression to end-stage renal failure in proteinuric kidney disease appears to involve a final common pathway culminating in glomerulosclerosis and tubulointerstitial fibrosis. Unfortunately, this relentless loss of residual renal function proceeds, albeit at a slower rate, despite blockade of the renin-angiotensin system and control of systemic hypertension. The development of novel therapeutic agents that interrupt maladaptive fibrotic responses to renal injury requires, at first, the comprehensive elucidation of the molecular drivers of tissue fibrosis, their interrelation, and their relative contribution to the ultimate clinical outcome of end-stage renal disease.

A key cytokine, intimately involved in the pathogenesis of glomerulosclerosis and tubulointerstitial fibrosis, is transforming growth factor-β (TGF-β) [1–4]. TGF-β augments matrix protein production while simultaneously abrogating matrix degradation [2]. Sustained activation of TGF-β, as occurs in renal scarring, results in a self-perpetuating cycle of matrix deposition and relentless tissue injury. Inhibition of TGF-β bioactivity attenuates extracellular matrix (ECM) deposition and progression of fibrosis in experimental renal injury [2, 5, 6]. Long-term direct inhibition of TGF-β in the clinical setting, however, presents several theoretical problems. In addition to its profibrotic activity, TGF-β has also antiproliferative and anti-inflammatory effects [6, 7]. These considerations have focused investigators on downstream or parallel mediators of tissue fibrosis that may yield to pharmacological intervention without adverse effects on cell cycle control [6].

Connective tissue growth factor (CTGF) is one such mediator that is induced by TGF-β, among other stimuli, and modulates fibroblast cell growth and fibroblast and mesangial cell ECM secretion. CTGF was initially reported in renal cells in vitro (originally reported in abstract; Mason et al, *J Am Soc Nephrol* 8:642A, 1997) and has recently been demonstrated in experimental and human renal fibrosis, in which its expression appears to correlate with the degree of tubulointerstitial fibrosis. CTGF may mediate many of the profibrotic actions of TGF-β. This review discusses the structure and function of CTGF, its relationship to TGF-β, its expression in disease, and the potential for pharmacological intervention.

CTGF: STRUCTURE AND FAMILY MEMBERS

Connective tissue growth factor is a 36 (nonreduced) to 38 (reduced) kD cysteine-rich peptide containing 349 amino acids. It belongs to the emerging *CCN* (*C*TGF, *c*yr 61/cef 10, *n*ov) family of growth factors that share a

Key words: transforming growth factor-β, hyperglycemia, cyclic mechanical strain, diabetic nephropathy, mesangial cells, extracellular matrix.

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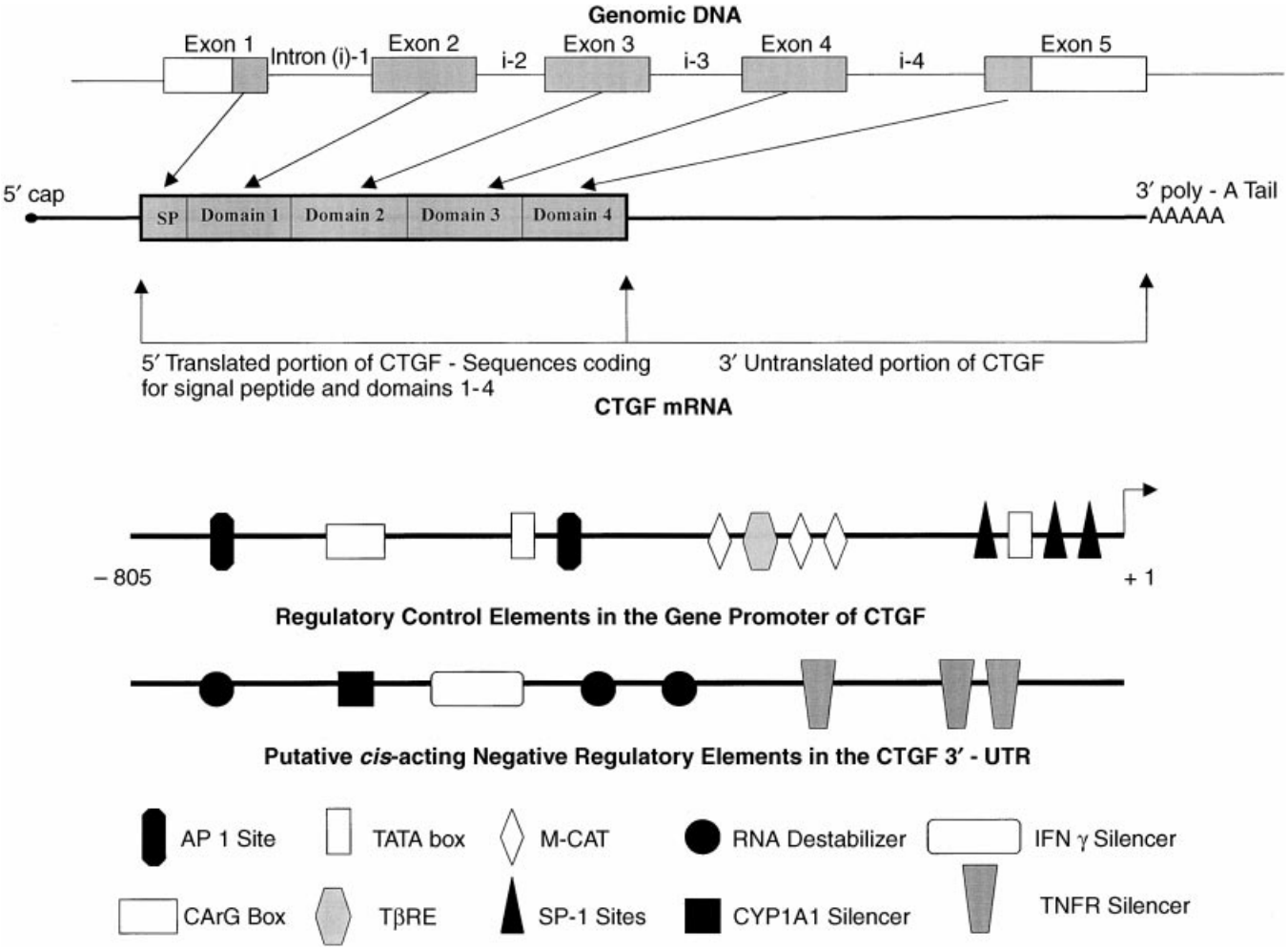

Fig. 1. Structure of the human connective tissue growth factor (CTGF) gene (modified from Grotendorst [22], Kubota [49], Oemar [89], and Brigstock [68]).

conserved multimodular structure but exhibit distinctive functional features. The gene for human CTGF that resides on chromosome 6q23.1, proximal to c-myb, comprises five exons and four introns (Fig. 1) and was originally cloned from a human umbilical vein endothelial cell (HUVEC) cDNA library [8]. CTGF is also termed fisp-12 (murine CTGF/mCTGF [9] or βIG-M2 [10]). Other CCN family members include cysteine-rich 61 (cyr 61; also termed βIG-M1 [10, 11] or cef 10 [12]), nov (nephroblastoma overexpressed gene) [13], wisp-1 [14] (also termed elm1 [15]), CTGF-L [16] (also termed rCop-1 [17] or wisp-2 [14]), and wisp-3 [14]. Cyr 61/cef 10 and CTGF/fisp-12 stimulate chemotaxis, adhesion, proliferation, and ECM formation by some cells, whereas nov, CTGF-L (wisp-2/rCop-1), wisp-3, and wisp-1 (elm1) exhibit features of negative regulators of growth. The *Drosophila* genes, twisted gastrulation (tsg), and short gastrulation (sog) display weak homology to the CCN family (approximately 19%) and are involved in dorsal-ventral axis pattern formation, that is, the development of dorsal structures during embryogenesis (Table 1) [18, 19].

CCN family members are cysteine-rich and possess a secretory signal peptide at the *N* terminus. All family members have four domains: an insulin-like growth factor (IGF)-binding domain, a von Willebrand factor (VWF) type C repeat domain (wisp-3 has 6 instead of 10 cysteine residues in this domain), a thrombospondin (TSP) type 1 repeat domain, and the C-terminal (CT) domain. An exception is CTGF-L/rcop-1/wisp-2, which lacks the CT domain. Connecting the VWF type C repeat domain to the TSP type 1 repeat domain is a region that is highly charged, variable, and devoid of cysteines, and it acts as a hinge connecting the N- and C-terminal halves of the proteins (Fig. 2). Tsg and sog only contain sequences that resemble domains 1 and 2, respectively. Within family members, hCTGF has a 60% homology to CTGF-L [16], a 53% homology to human nov (novH) [20], a 43% homology to human cyr 61 (hcyr 61) [21], and a 36 to 44% homology

Table 1. Summary of species distribution and structural features of CCN family members and other CCN-like molecules

Abbreviation	Full name	Alternative nomenclature	Species	Modular structure
CCN family members				
CTGF	Connective tissue growth factor (CTGF)	Fibroblast-inducible secreted protein-12 (FISP-12)	Human, mouse, pig, rat, frog, and cow	4 Domains
		Transforming growth factor-β-inducible early gene in mouse AKR-2B cells-2(βIG-M2)		
CYR61	Human cysteine-rich 61 (hCYR61)	Transforming growth factor-β-inducible early gene in mouse AKR-2B cells-1 (βIG-M1)	Human, mouse, and chicken	4 Domains
		CEF-10		
NOV	Human nephroblastoma overexpressed (novH)		Human, chicken, mouse, quail, and *Xenopus*	4 Domains
WISP-1	WISP-1	Expressed in low-metastatic type 1 cells (ELM-1)	Human, mouse, monkey, rat, dog, and cow	4 Domains
CTGF-L	Connective tissue growth factor-like cDNA (CTGF-L)	rCOP-1	Human, rat, and mouse	3 Domains (lacking CT domain)
		WISP-2		
WISP-3	WISP-3		Human	4 Domains (lacking 4 cysteines in domain 2)
CCN-like molecules				
TSG	Twisted gastrulation (TSG)		*Drosophila*	Contains sequences resembling domain 1
SOG	Short gastrulation (SOG)		*Drosophila*	Contains sequences resembling domain 2

to wisp-2/rCop-1, wisp-1/elm1, and wisp-3. With regard to differences between species, human CTGF displays 94% homology [22] to murine CTGF (mCTGF)/fisp-12/βIG-M2 (gene for mCTGF resides in chromosome 10), a 96% homology [23] to rat CTGF (rCTGF), and a 92% homology [24] to pig CTGF (pCTGF).

Connective tissue growth factor is also a member of the IGF binding protein (IGFBP) superfamily and was initially classified as IGFBP-8 [25] on the basis that it is 30 to 38% homologous to IGFBPs 1 to 6 and specifically binds to IGF. However, this nomenclature was subsequently revised at the Fourth International Symposium on IGFs because of its relatively low affinity binding for IGFs. CTGF is now termed IGFBP-related protein 2 (IGFBP-RP2) [26–28].

Recently, 10, 12, 16, 18, 19, 20 and 24 kD forms of CTGF have been demonstrated in different cell types, tissues, and body fluids [24, 27, 29–34]. The possible significance of these smaller forms is discussed later in this article.

CELL SOURCES AND REGULATION OF EXPRESSION OF CTGF

Connective tissue growth factor was first isolated in media conditioned with HUVECs [8]. Subsequently, it has been detected in various fibroblasts [23, 29, 33, 35–40], endothelial cells [30, 39, 41], cartilagenous cells and chondrocytes [32, 42–44], cancer cell lines [27, 45, 46] and smooth muscle cells (Table 2) [39].

Early studies revealed that TGF-β1 increases CTGF mRNA levels markedly in human foreskin fibroblasts (hFFs) [35]. This effect of TGF-β1 with hFFs was seen within 30 minutes, and CTGF mRNA levels remained elevated after 24 hours [35]. In contrast, platelet-derived growth factor (PDGF), epithelial growth factor (EGF), and fibroblast growth factor (FGF) provoked only a transient and weak response [35]. CTGF mRNA expression in response to TGF-β1 was enhanced in the presence of cycloheximide, indicating that the induction process did not require de novo protein synthesis [35]. These data suggest that TGF-β1 is a direct stimulus for CTGF gene transcription [35]. Subsequent analysis of the CTGF promoter revealed a TGF-β response element (TβRE) [47] (Fig. 1). This element is distinct from other reported TGF-β–responsive elements and is not present in other genes regulated by TGF-β [47]. Moreover, this element is preserved in fisp-12 and is not found in other CCN family members [47].

In other in vitro studies, TGF-β increased expression of CTGF in bovine microvessel endothelial cells [30], in HCS (a human chrondrosarcoma-derived chondrocytic cell line) cells [42], and in pancreatic cell lines (Panc-1 and HPAF, human pancreatic adenocarcinoma cell lines of ductal organ) [45]; TGF-β1 induced CTGF message

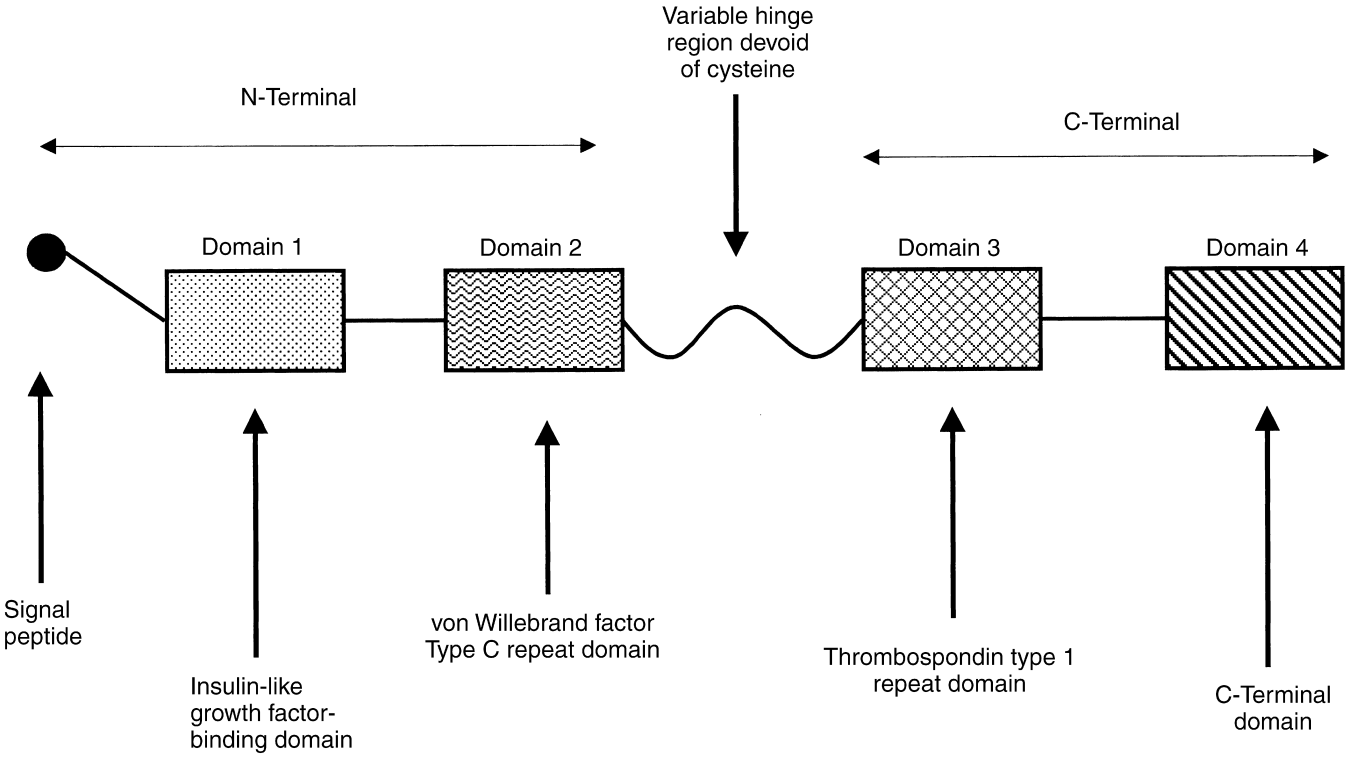

Fig. 2. Modular structure of connective tissue growth factor.

in human and mouse lung fibroblasts [23], in human breast cancer cell line MCF-7, and in cultures of fibroblasts from patients with scleroderma [40], and TGF-β2 up-regulated CTGF expression in Hs578T breast cancer cells [27]. Surprisingly, Boes et al reported that TGF-β1 or TGF-β2 did not stimulate CTGF mRNA or protein levels in bovine large vessel endothelial cells [30]. Indeed, TGF-β1 stimulated CTGF breakdown in bovine microvessel endothelial cells [30]. In addition to TGF-β, BMP 2 [42], TGF-α and EGF [45], conditioned monocyte medium (an atherogenic stimulus) [48] and dexamethasone (a potent anti-inflammatory agent) [38] have each been detected to induce CTGF expression in HCS cells, pancreatic cell lines, human endothelial cells, and fibroblasts, respectively. The inducing effect of dexamethasone on CTGF overexpression in fibroblasts was not mediated by TGF-β1, and this effect was attenuated by TNF-α [38]. TNF-α has also been shown to down-regulate CTGF expression in bovine endothelial cells, fibroblasts, and smooth muscle cells [39].

Recently, analysis of the 3′-untranslated region of the human *CTGF* gene has revealed a negative regulatory element that was highly homologous to known negative regulatory *cis* elements [49]. These elements are likely to be involved in differential regulation of CTGF expression (Fig. 1).

Connective tissue growth factor is a cysteine-rich secreted peptide present in media conditioned with HUVECs [8] and serum-stimulated NIH 3T3 cells [50] and undergoes microsomal processing [9]. In hFFs and mouse fibroblasts, the 38 kD CTGF is reportedly the cell-associated, long-lived, and insoluble form of CTGF, which is secreted inefficiently [29], whereas the 10 kD CTGF described by Steffen is soluble and biologically active with these fibroblasts [29]. mCTGF (fisp-12), on the other hand, is secreted efficiently by NIH 3T3 fibroblasts [9, 37] and is present in the cellular fraction, ECM, and media of these fibroblasts [37]. The presence of mCTGF in the culture medium of these cells and its weak association with the ECM implies that mCTGF may be able to act at areas distant from its site of synthesis and secretion [37].

The signal transduction events that regulate CTGF synthesis and secretion in response to TGF-β and other mediators are still being appreciated. As mentioned previously in this article, the CTGF promoter contains a TβRE. Initial studies indicate that inhibitors of protein kinase A, but not of tyrosine kinase or protein kinase C, block TGF-β–stimulated CTGF transcription and that CTGF gene expression is modulated by cell cAMP levels [51]. Raised cAMP levels or cAMP analogues inhibit TGF-β's induction of CTGF in normal rat kidney (NRK) fibroblasts and NIH 3T3 cells, but not TGF-β–induced changes in cell morphology [51]. Raised cAMP levels or

Table 2. Some cell sources and regulation of CTGF

	Cell type	Reference
Stimuli		
TGF-β	Fetal bovine aortic smooth muscle cells	[47]
	Human vascular smooth muscle cells	[60]
	Pancreatic cancer cell lines: Panc-1 and HPAF	[45]
	Human chondrosarcoma-derived chondrocytes	[42]
	Human lung fibroblasts	[81]
TGF-β1	Human foreskin fibroblasts	[35]
	Mouse NIH 3T3 cells	[35]
	Skin fibroblasts from patients with scleroderma	[40]
	Human lung fibroblasts	[23]
	Mouse lung fibroblasts from BALB/c mice	[23]
	Human gingival fibroblasts	[33]
	Transfected newt blastema B1H1 cells	[65]
	Human breast cancer cell line MCF-7	[54]
	Primary human mesangial cells	[76]
TGF-β2	Human Hs578T breast cancer cells	[27]
	Rat mesangial cells	[34]
TGF-α	Pancreatic cancer cell lines: Panc-1, Patu8988t, and HPAF	[45]
EGF	Pancreatic cancer cell lines: Panc-1, Patu8988t, and HPAF	[45]
High glucose	Human mesangial cells	[76]
	Rat mesangial cells	[34]
Cyclical mechanical strain	Rat mesangial cells	[34]
BMP 2	Human chondrosarcoma derived chondrocytes	[42]
Dexamethasone	Mouse BALB/c 3T3 fibroblasts	[38]
Inhibitors		
cAMP analogues	NRK fibroblasts, NIH 3T3 cells	[51, 52]
TNF-α	Bovine aortic endothelial cells	[39]
	BALB/c 3T3 fibroblasts	[38]

cAMP analogues also inhibited TGF-β's induction of anchorage-independent growth (AIG) of NRK fibroblasts [51] and TGF-β–induced collagen synthesis in NRK fibroblasts and hFFs [52]. The inhibition of AIG but not of collagen synthesis was reversed by the addition of CTGF [51, 52]. Subsequently, cAMP and its analogues were observed to reduce CTGF message and protein in hFFs [52]. In contrast to this observation, cAMP increased CTGF protein (without an increase in mRNA level) by inhibiting its breakdown in bovine large vessel endothelial cells [30]. In the same article, in bovine microvessel endothelial cells, cAMP stimulated CTGF degradation, suggesting differential regulation of CTGF in microvessel versus large vessel endothelial cells.

CTGF BIOACTIVITIES IN VITRO

Connective tissue growth factor has diverse bioactivities in vitro (Table 3). Depending on the cell types, CTGF may trigger mitogenesis, chemotaxis, or matrix production, prevent proliferation, stimulate apoptosis, or modulate angiogenesis. In early reports, CTGF was noted to have mitogenic and chemotactic effects with NRK fibroblasts and NIH 3T3 cells, respectively, similar to PDGF [8]. CTGF's mitogenic activity on NRK fibroblasts is augmented by heparin and EGF, and not by TGF-β [36]. The 10 kD form of porcine CTGF is also mitogenic for BALB/c 3T3 cells, vascular smooth muscle cells, and endometrial stromal cells, but not endothelial cells [24]. This mitogenic activity is potentiated by IGF, PDGF, EGF, and basic FGF (bFGF). In NIH 3T3 cells and HUVECs, mCTGF (fisp-12) is not mitogenic by itself, but enhances bFGF-induced DNA synthesis in these cells [37]. CTGF is likewise mitogenic for cultured fibroblasts from patients with scleroderma [40]. CTGF also promotes proliferation and differentiation of chondrocytes in culture [44].

Connective tissue growth factor enhances mRNA levels for α1-type 1 collagen, fibronectin, and α5 integrin in NRK fibroblasts [36], suggesting a role in matrix production. Overexpression of CTGF, by transfection of CTGF mRNA into human aortic smooth muscle cells (HASCs), inhibits PDGF-stimulated cell proliferation and increases apoptosis [53]. The same authors reported a similar apoptotic effect with a human breast cancer cell line [54].

Recently, mCTGF (fisp-12) and cyr 61 have been reported to regulate angiogenesis [55]. Specifically, purified mCTGF protein stimulates adhesion and migration and promotes survival (by inhibiting apoptosis) of primary human dermal microvascular endothelial cells (HMVECs) [56]. Recombinant CTGF also induces proliferation, adhesion, migration, and remodeling of bovine aortic endothelial (BAE) cells [41, 57].

With the exceptions of mitogenesis, stimulation of matrix production and apoptosis, the various bioactivities of CTGF described earlier in this article, are not all shared by TGF-β. Other effects of TGF-β not shared by CTGF include AIG of fibroblasts and inhibition of epithelial cell growth [36].

The finding that TGF-β can induce CTGF synthesis and that TGF-β and CTGF share many functions is compatible with the hypothesis that CTGF is a downstream mediator of many of TGF-β's bioactivities in many systems. As discussed later in this article, this hypothesis is further supported by studies employing anti-CTGF antibodies to inhibit TGF-β–induced effects. CTGF is essential for TGF-β's induction of collagen synthesis [52] and AIG in fibroblasts [58], although CTGF does not induce AIG on its own. This suggested that TGF-β–stimu-

Table 3. Some actions of CTGF in vivo and in vitro

In vitro actions of CTGF		
Cell type	Bioactivity	Reference
NRK fibroblasts	Mitogenic	[8]
NIH 3T3 cells	Chemotactic	[8]
NRK fibroblasts	Mitogenic effect enhanced by EGF, heparin and TGF-β	[36]
NRK fibroblasts	No action on AIG growth	[36]
NRK fibroblasts	Requirement for TGF-β induced AIG growth	[58]
NRK fibroblasts	Enhances α1 type 1 collagen, fibronectin and α5 integrin formation	[36]
NRK fibroblasts and human foreskin fibroblasts	TGF-β mediated collagen synthesis and dose-dependent increases of collagen	[52]
NRK fibroblasts	Modulation of cell-cycle progression in cAMP-arrested cells	[51, 59]
Bovine endothelial cells	Stimulates proliferation, migration and tube formation	[41, 57]
BALB/c 3T3 cells and bovine smooth muscle cells	Mitogenesis (10 kDa fragment of pCTGF)	[24]
Human microvascular endothelial cells	Promotes cell and migration via $\alpha_v \beta_3$ integrin	[56]
HUVECs, NIH 3T3 & Mv1Lu cells	Promotes cell attachment	[37]
HUVECs	Enhances mitogenic effect of bFGF	[37]
Chondrocytic cell lines, rabbit growth cartilage cells	Proliferation and differentiation	[44]
Human aortic smooth muscle cells (HASC)	Inhibition of cell proliferation stimulated by PDGF and enhanced apoptosis of cells	[53]
Human breast cancer cell line	Apoptosis	[54]
Rabbit growth cartilages	Increased aggrecan and collagen types II and X	[44]
Primary human mesangial cells	Increased types I and IV collagen and fibronectin levels	[76]
Rat mesangial cells	Increased type 1 collagen and fibronectin	[34]
In vivo actions of CTGF		
Tissue	Bioactivity	Reference
Skin of NIH Swiss mice	Nodule formation, increased connective tissue and ECM material	[36]
Subcutaneous tissue of BALB/c mice	CTGF on its own, causing some edema and cell infiltration, simultaneously with and serially after TGF-β, causing fibrotic tissue formation, and serially before TGF-β, causing granulation tissue only	[72]
Subcutaneous tissue of Wistar rats	Angiogenesis	[57]
Corneal micropockets of Sprague-Dawley rats	Neovascularization	[56]

lated AIG of fibroblasts requires activation of both CTGF-dependent and CTGF-independent pathways [58]. Kinetic studies of the induction of DNA synthesis revealed that the CTGF-dependent restriction point of the NRK fibroblast cell cycle was late in G1 and that CTGF controlled cell cycle progression through late G1- and S-phase entry of these cells [51]. Subsequent to this, CTGF was reported to trigger S phase entry by reducing p27 (Kip 1) levels and thereby increasing cyclin A levels. These changes, in turn, resulted in hyperphosphorylation of pRb and release of E2F, suggesting that CTGF mediates TGF-β–induced fibroblast proliferation by modulating cyclin-dependent kinase (cdk) activities.

CTGF EXPRESSION AND ACTIONS IN VIVO

Connective tissue growth factor has been detected in many human tissues, including heart, brain, placenta, lung, liver, muscle, kidney, and pancreas [60], and in a variety of human biological fluids, for example, serum, pregnancy serum, amniotic, follicular, peritoneal, and cerebrospinal fluids [27, 61]. CTGF has also been detected in human pancreatic islet and ductal cells [45, 62] and in portal tracts of human livers [63]. In animals, CTGF has been isolated in various tissues of mice embryos [37, 64], in limb blastemas of newts [65], and in various cells of the rat central nervous system [66].

A recent study has demonstrated that CTGF is expressed in uterine luminal flushings (ULFs) of pigs [24]. There were 10, 16, and 20 kD forms of CTGF (the 38 kD CTGF was not detected in the ULF) that were isolated by specific heparin binding. These forms were highly truncated and showed that the N-terminal two thirds of the primary translational product (domains I to III, that is, IGF, VWF, and TSP modules) was not required for mitogenic activity or heparin binding. Ball et al presented evidence that the 16, 18, and 20 kD forms of CTGF are intermediate mass forms of CTGF and are produced with 10 kD CTGF by proteolysis of 38 kD CTGF, which is a normal physiological process in utero [67]. CTGF protein levels were also found in pregnant and normal uteri of the pigs, suggesting several roles in regulating cell function in the uterine tract. Another study supported these roles of CTGF in uterine cell growth, migration, adhesion, and ECM production during the oestrous cycle and early pregnancy in mice, as well as in the early development of the embryo [31]. The presence of these CTGF products in various body fluids suggests that they may be reservoirs of CTGF in vivo [68].

Since its discovery in 1991, the expression of CTGF

Table 4. Patterns of increased expression of CTGF in disease in vivo

Tissue	Reference
Fibroblasts from patients with systemic sclerosis and localized scleroderma	[69, 70]
Fibroblasts from human keloid, scar tissue, eosinophilic fasciitis and Dupuytren's contracture	[70]
Skin fibroblasts from NIH 3T3 mice treated with TGF-β	[36]
Skin fibroblasts from BALB/c mice treated with CTGF or TGF-β3	[72]
Skin fibroblasts of BALB/c mice treated with TGF-β2	[82]
Fibroblasts and endothelial cells from dermatofibromas and endothelial cells from pyogenic granulomas	[83]
Fibroblasts of patients within desmoplastic malignant melanoma	[73]
Fibrous stroma of human mammary infiltrating tumors and fibroblasts of murine mammary tumor models	[84]
Pancreatic fibroblasts of patients with chronic pancreatitis	[62]
Fibrotic areas in patients with pancreatic cancers	[45]
Fibrotic areas of chronic human hepatitis	[63]
Centrilobular and fibrous septa of models of liver fibrosis in rats	[63]
Sera of patients with biliary atresia	[61]
Inflammatory areas of patients with Crohn's disease and ulcerative colitis, and fibrotic stenotic areas of patients with Crohn's disease	[85]
Lung fibrosis model of bleomycin-sensitive mice	[23]
Transbronchial biopsies of patients with idiopathic pulmonary fibrosis	[86]
Bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis	[28]
Cardiac myocytes and mesenchymal cells in infarct zone of rat myocardium	[87]
Lens epithelial cells of patients with anterior polar and nuclear cataracts	[88]
Fibrotic areas of atherosclerotic plaques	[60]
Heart, kidney & skin of BALB/c mice injected with dexamethasone	[38]

has also been explored in various human diseases and animal models of disease. CTGF mRNA has been detected in fibroblasts of sclerotic lesions of patients with systemic sclerosis by in situ hybridization [69]. In patients with localized scleroderma, more CTGF mRNA was detected in fibroblasts in tissue from the sclerotic stage than the inflammatory stage, indicating a close correlation between CTGF and fibrosis. This was also true for keloid tissue and fibrotic areas of eosinophilic fasciitis, nodular fasciitis, and Dupuytren's contractures [70]. Subsequently, expression of CTGF has been reported in a variety of diseases (Table 4).

In a model of wound healing in which stainless steel mesh chambers were implanted into the flanks of Fisher rats [35], TGF-β expression preceded CTGF expression by about six days, suggesting that, in vivo CTGF may be a downstream mediator of TGF-β. This effect of TGF-β was again proposed to be with mesenchymal-derived cells, that is, endothelial cells and fibroblasts. In another model of wound healing in which the skin and panniculus carnosus of the back of BALB/c mice was excised [38], CTGF mRNA was maximally expressed at 12 to 24 hours after wounding and returned to basal levels within seven days. A similar pattern was observed for induction of TGF-β [71].

Intradermal injections of CTGF into NIH Swiss mice [36] induced granulation tissue formation and fibrosis similar to that observed at the injection site of TGF-β. In another recent study of a mouse skin fibrosis model [72], induction of persistent fibrosis was demonstrated to require interaction of several growth factors: TGF-β, for example, being an induction factor, and CTGF, being a maintenance factor. CTGF mRNA levels remained elevated in those areas of persistent fibrosis, as determined by in situ hybridization.

Intradermal cAMP analogue injections into transgenic mice with a human CTGF promoter/lacZ reporter transgene [52] suppressed TGF-β's activation of this transgene. These analogues were then used to block TGF-β–induced collagen deposition in Schilling-Hunt chambers inserted into wounds of Sprague-Dawley rats and to reduce dermal fibroblast populations induced by injecting TGF-β into the dermis of NIH Swiss mice [52]. Collectively, these observations indicate that CTGF mediates TGF-β–induced fibroblast collagen synthesis, and that in vivo blockade of CTGF synthesis or action reduces TGF-β–induced granulation tissue formation by inhibiting collagen synthesis and fibroblast accumulation.

In two other in vivo models, CTGF was confirmed to play a role in angiogenesis where CTGF placed on chorioallantoic membranes of chick embryos formed small vessels and injection of CTGF into the backs of Wistar rats promoted vessel formation [57].

CTGF SIGNAL TRANSDUCTION

The mechanisms by which CTGF exerts its effects on cells are still being appreciated. In initial reports, CTGF bound to the surface of NIH 3T3 cells with relatively high affinity, and this binding could be competed with increasing concentrations of recombinant PDGF BB [8]. CTGF has also been reported to interact with a PDGF α receptor in scleroderma fibroblasts [40]. This suggested that the CTGF peptide binds to a certain class of PDGF receptor (PDGF-R) or there is some cross reactivity of PDGF BB with CTGF receptors. mCTGF, as described earlier, is present in the media of NIH 3T3 cells and was shown to be internalized and degraded through lysosomal pathways, implying cell surface receptor interactions [37].

The soluble 10 kD form of CTGF does not bind to the PDGF-R, however, but stimulates mitosis in fibroblasts [29]. This observation points to the existence of multiple and possibly CTGF-specific cell surface receptors.

A human chondrocyte cell line expresses another putative CTGF receptor [32] that is larger (240 kD) than the PDGF type β receptor previously reported by Brad-

Table 5. CTGF expression by renal cells in vitro

Cell type	Species	Stimulus	Reference
Glomerular endothelial cells	Not reported	Not reported	Not reported
Glomerular mesangial cells	Human	30 mmol/L glucose	[76]
	Human	TGF-β1	[76]
	Rat	20 mmol/L and 35 mmol/L glucose	[34]
	Rat	TGF-β2	[34]
	Rat	Cyclical mechanical strain	[34]
	Rat	CTGF	[34]
Renal epithelial BSC cells	African green monkey	Scrape wounding	[79]
	African green monkey	Calcium oxalate crystals	[80]

Table 6. CTGF expression in renal disease in vivo

Disease	Species	Distribution	Reference
Streptozotocin-induced diabetes	Rats	Cortex and glomeruli	[76]
DbDb diabetes	Mice	Glomeruli	[34]
IgAN	Human	Crescents, glomeruli, and tubulointerstitium	[75]
CGN	Human	Crescents, glomeruli, and tubulointerstitium	[75]
DN	Human	Glomeruli	[75]
Diffuse LN	Human	Crescents, glomeruli, and tubulointerstitium	[75]
MPGN	Human	Glomeruli and tubulointerstitium	[75]
FGS	Human	Glomeruli, extracapillary lesions and tubulointerstitium	[75]
CTR	Human	Glomeruli and tubulointerstitium	[75]
MN with fibrosis	Human	Glomeruli, extracapillary lesions and tubulointerstitium	[75]

Abbreviations are: IgAN, immunoglobulin A nephropathy; CGN, crescentic glomerulonephritis; DN, diabetic nephropathy; diffuse LN, diffuse lupus nephritis; MPGN, mesangioproliferative glomerulonephritis; FGS, focal glomerulosclerosis; CTR, chronic transplant rejection; MN, membranous nephritis.

ham et al [8] and Kubo et al [73], and than the PDGF type α receptor described by Kikuchi et al [40].

mCTGF and cyr 61 appear to modulate angiogenesis via integrin receptors. Specifically, the effects of mCTGF on endothelial cell adhesion, migration, survival, and angiogenesis appear to involve an $\alpha_5\beta_3$ (integrin receptor)-dependent pathway [56]. This integrin connection is further supported by another study whereby activation-dependent adhesion of platelets to mCTGF is mediated through integrin $\alpha_{IIb}\beta_3$ [74].

PUTATIVE ROLE IN GLOMERULOSCLEROSIS AND TUBULOINTERSTITIAL FIBROSIS

Whereas CTGF has been described in various human tissues, it is most abundant in the kidney [60]. In normal renal tissue, CTGF mRNA has been demonstrated by in situ hybridization in visceral and parietal epithelial cells, in some interstitial cells of the periglomerular and peritubular areas, in endothelial cells of peritubular capillaries, and probably in interstitial fibroblasts [75]. Increased CTGF mRNA expression has been reported in a variety of inflammatory glomerular and tubulointerstitial lesions [75] in association with cellular proliferation and matrix deposition, including IgA nephropathy (IgAN), chronic transplant rejection (CTR), crescentic glomerulonephritis (CGN), focal glomerulosclerosis (FGS), class IV lupus nephritis (LN), and membranoproliferative glomerulonephritis (MPGN). CTGF was only minimally up-regulated or remained normal in minimal-change nephrotic syndrome (MCNS), idiopathic membranous nephropathy (MN), and acute post-infectious glomerulonephritis.

In glomerular lesions, CTGF expression was increased in cellular crescents of CGN, IgAN, and diffuse LN (proliferating epithelial cells and not macrophages or fibroblasts were the main components of these crescents), as well as in the fibrocellular crescents. In addition, CTGF was increased in other extracapillary lesions, such as areas of segmental sclerosis with adhesion to Bowman's capsule in FGS and MN with fibrosis. CTGF was highly expressed in glomeruli of patients with IgAN, diabetic nephropathy (DN), and diffuse LN where there was mesangial proliferation. With regard to the tubulointerstitium, CTGF mRNA was markedly increased in patients with CTR and chronic tubulointerstitial damage associated with glomerulonephritis, and this expression was localized to α-smooth muscle actin-positive cells (myofibroblasts), as well as in areas of periglomerular fibrosis. CTGF-positive cells, however, were rarely seen in the tubulointerstitium of patients with MCNS, FGS, and MN without fibrosis. In vessels, CTGF expression was increased in smooth muscle and endothelial cells.

Recent in vivo studies have highlighted increased expression of CTGF mRNA in glomeruli of rats with streptozotocin-induced DN [76] and the db/db diabetic mice [34], implicating CTGF in the development of diabetic glomerulosclerosis (Tables 5 and 6).

Studies with cultured human cells in vitro have shed light on the mechanisms by which CTGF is induced and on its functional significance in this setting. CTGF message is up-regulated in primary human mesangial cells exposed to high glucose and TGF-β1 [76]. Recombinant CTGF, when added to mesangial cells induces expression of ECM proteins (fibronectin, collagen I, and collagen IV), mimicking mesangial matrix accumulation in diabetic glomerulosclerosis [76]. Under high-glucose conditions, the induction of mesangial cell CTGF mRNA levels was partially suppressed by TGF-β antibodies and protein kinase C (PKC) inhibitors, suggesting that CTGF expression by high glucose is mediated by both TGF-β– and PKC-dependent pathways [77, 78]. The role of high glucose and TGF-β as a stimulus for CTGF expression, at both the mRNA and protein level, has been confirmed by Riser et al using rat mesangial cells [34]. CTGF expression, but not protein, is increased by cyclical mechanical strain in rat mesangial cells [34].

Interestingly, induction of CTGF expression has been noted in an in vitro model of wound healing using renal epithelial cells of the nontransformed African green monkey BSC-1 line [79]. This system is a model of cell migration, independent of proliferation, as may occur during renal regeneration after acute tubular necrosis. CTGF was expressed as early as one hour after wounding and was maximal at four hours. Levels returned to baseline within 24 hours, and high concentrations were found in cells of the wound edge and cells away from the wound. Intriguingly, expression of TGF-β1 and TGF-β2 was unaltered in these experiments. In the model used by Hammes et al, exploring the effects calcium oxalate monohydrate crystals on a monkey kidney epithelial cell line (BSC-1; a model for crystal-induced interstitial fibrosis), CTGF was one of the growth factors up-regulated at 6 to 24 hours, but was not evident in basal conditions [80]. TGF-β1 was present constitutively, but not up-regulated on exposure to crystals, demonstrating again that CTGF expression may occur in the absence of TGF-β1 activity.

SUMMARY

In summary, CTGF is a member of the CCN family, which also includes *cyr 61*, *nov*, and *wisp* genes. CTGF is generated in vitro in multiple cell types, including mesangial cells and renal tubule epithelial cells, by a variety of stimuli, including high glucose, mechanical strain, and TGF-β. Emerging evidence suggests that CTGF may be an important downstream mediator of TGF-β's profibrotic activities. CTGF appears to play a role in the development and progression of glomerulosclerosis and tubulointerstitial fibrosis as part of TGF-β–dependent and TGF-β–independent pathways. The elucidation of the receptors and signaling events that exert CTGF's bioactivities should suggest strategies for interference with its profibrotic effects, and may antecede the development of novel antifibrotic therapies that target the integral CTGF system.

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