Insights Into the Molecular Mechanism of Chronic Fibrosis: The Role of Connective Tissue Growth Factor in Scleroderma

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Connective tissue growth factor (CCN2), a member of the CCN family of proteins, is a cysteine-rich matricellular protein. Connective tissue growth factor is not normally expressed in dermal fibroblasts unless induced. The most potent inducer of connective tissue growth factor thus far identified is transforming growth factor β. Connective tissue growth factor, however, is constitutively overexpressed by fibroblasts present in skin fibrotic lesions, including scleroderma. The overexpression of connective tissue growth factor present in fibrotic lesions contributes to the phenotype of scleroderma in that connective tissue growth factor promotes matrix deposition, and fibroblast adhesion and proliferation. In animal models, whereas either transforming growth factor β or connective tissue growth factor alone produce only a transient fibrotic response, connective tissue growth factor and transforming growth factor β act together to promote sustained fibrosis. Thus the constitutive overexpression of connective tissue growth factor by fibroblasts present in fibrotic lesions would be expected to contribute directly to chronic, persistent fibrosis. This review discusses recent information regarding insights into connective tissue growth factor biology and, using scleroderma as a model system, the part connective tissue growth factor might play in fibrotic disease.

Key words: TGF beta/fibrosis/CTGF/scleroderma
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The extracellular matrix (ECM) is essential in establishing and maintaining the structural and mechanical integrity of skin (Nimni, 1974; Kielty and Shuttleworth, 1997). Virtually every person at some point in their life suffers skin damage, and tissue repair occurs effectively. If the tissue repair process is not appropriately terminated, however, excessive deposition of ECM occurs resulting in the deposition of excessive scar tissue (Silver and Glasgold, 1995). These scars are generally limited in their nature; however, although relatively rare, progressive, pathologic, fatal scarring (fibrotic) disorders can occur. One example of such a connective tissue disease is systemic sclerosis (scleroderma; SSc), which affects the skin as well as internal organs (Simms and Korn, 2002; Valentini and Black, 2002). There is no effective treatment for this disorder, in part because the etiology of this disease is unknown. Thus, in order to develop effective anti-scarring therapies it is necessary to understand the molecular players in the normal wound healing process and how these proteins normally interact to co-ordinate the proper repair of the epidermis and dermis, and to understand how this process goes awry, leading to scarring and fibrosis.

One of the major cytokines involved with tissue repair and fibrosis in general is transforming growth factor (TGF)-β. TGF-β promotes deposition of the ECM by inducing expression of matrix genes (Ignotz and Massague, 1986; Quaglino *et al*, 1991) and decreasing the expression of matrix metalloproteinases while increasing that of tissue inhibitors of metalloproteinases (Overall *et al*, 1989, 1991; Yuan and Varga, 2001). Anti-TGF-β strategies, such as an anti-TGF-β antibody and an adenovirus overexpressing Smad7, are effective at blocking fibrosis in animal models (McCormick *et al*, 1999; Nakao *et al*, 1999; Yamamoto *et al*, 1999; Terada *et al*, 2002; Lan *et al*, 2003). In addition, mice knocked out for TGF-β or Smad 3 show alterations in wound healing, such as enhanced re-epithelialization and suppressed matrix deposition; however, these mice also show an impaired inflammatory response (Letterio and Roberts, 1996; Ashcroft *et al*, 1999; Crowe *et al*, 2000; Flanders *et al*, 2002) consistent with the notion that TGF-β controls a wide range of physiologic functions (McCartney-Francis *et al*, 1998). Thus using a global anti-TGF-β strategy as a prolonged treatment to combat chronic fibrosis might be expected to have harmful consequences for human health.

The protein connective tissue growth factor (CTGF) was initially discovered in 1991 as a secreted protein in the conditioned media of cultured human umbilical vascular endothelial cells (Bradham *et al*, 1991). In adult skin, however, CTGF is not normally expressed unless induced, for example during the normal wound repair process (Igarashi *et al*, 1993). One of the most exciting early observations concerning CTGF was that TGF-β induced CTGF expression in dermal fibroblasts, but not in epidermal keratinocytes (Igarashi *et al*, 1993; Frazier *et al*, 1996; Grotendorst *et al*, 1996). In contrast to the situation in normal fibroblasts, CTGF is constitutively overexpressed in dermal fibrotic lesions, such as in scleroderma (Igarashi *et al*, 1996; Abraham *et al*, 2000; Shi-wen *et al*, 2000; Holmes *et al*, 2001). These observations suggest that CTGF

may act as a downstream effector of at least some of the pro-fibrotic effects of TGF-β and therefore that blockading CTGF might be expected to alleviate the symptoms of fibrosis, but TGF-β-dependent, CTGF-independent events unaltered (Grotendorst, 1997). This review focuses on the role that connective tissue growth factor (CTGF, CCN2) may play as marker and a mediator of the fibrotic phenotype of the disease scleroderma.

CTGF functions

CTGF, a member of the CCN family of matricellular proteins (Bork, 1993; Lau and Lam, 1999; Moussad and Brigstock, 2000; Perbal, 2001), promotes fibroblast proliferation, matrix production, and granulation tissue formation (Bradham *et al*, 1991; Frazier *et al*, 1996; Brigstock *et al*, 1997). In addition, CTGF promotes cell adhesion and migration in a wide variety of cell types (Kireeva *et al*, 1997; Babic *et al*, 1999; Shimo *et al*, 1999; Crean *et al*, 2002). During development, CTGF is strongly expressed in mesenchyme such as in hypertrophic chondrocytes in the growth plate of cartilage, the developing glomerulus and connective tissue surrounding developing muscle (Yamamoto *et al*, 2002; Friedrichsen *et al*, 2003). In addition, CTGF is expressed in the placenta during embryo implantation (Surveyor *et al*, 1998) and during ovarian follicogenesis (Slee *et al*, 2001). Recently, CTGF knockout mice were generated. The phenotype of these mice is consistent with a role for CTGF in matrix synthesis and remodeling and cell proliferation and migration. Mice homozygous for a deletion of the CTGF gene die soon after birth, displaying an inability of the rib cage to ossify properly, including a failure of the embryo to induce expression of bone-specific matrix, such as aggrecan, and a failure of chondrocyte proliferation (Ivkovic *et al*, 2003). CTGF knockout mice also display a reduction in the number of capillaries invading cartilage (Ivkovic *et al*, 2003). CTGF knockout animals also show a decreased expression of the pro-angiogenic factor vascular endothelial growth factor (Ivkovic *et al*, 2003). These activities are consistent with the notion that recombinant CTGF induces chondrocyte differentiation and proliferation *in vitro* (Nakanishi *et al*, 2000) through p38 and mitogen-activated protein kinase, respectively (Yosimichi *et al*, 2001), and angiogenesis *in vivo* and *in vitro* (Babic *et al*, 1999; Shimo *et al*, 1999, 2001).

CTGF receptors

In spite of substantial efforts expended by several laboratories, a specific CTGF receptor has not yet been identified. Rather, CTGF promotes many of its activities by binding to integrins (Lau and Lam, 1999). For example, CTGF promotes angiogenesis endothelial cells through integrin αvβ3 (Babic *et al*, 1999), and adhesion to human foreskin fibroblasts through integrin α6β1, to human platelets through integrin αIIβ3, to endothelial cells through αvβ3 and to blood monocytes through integrin αMβ2 (Babic *et al*, 1999; Jedsadayanmata *et al*, 1999; Chen CC *et al*, 2001; Schober *et al*, 2002). CTGF directly binds integrin α6β1 through a motif in the carboxyl-terminal half of CTGF (Leu *et al*, 2003), in the so-called thrombospondin motif (for review, see Perbal, 2001). Functional studies, however, have emphasized the essential nature of the heparin-binding domain of CTGF, which is carboxy-terminal of the thrombospondin motif, in CTGF-mediated fibroblast proliferation and attachment (Brigstock *et al*, 1997; Ball *et al*, 2003a). These results suggest that the ability of CTGF to bind cell surface heparin sulfate proteoglycans is essential for CTGF activity. Indeed, CTGF-mediated adhesive signaling possesses both integrin- and heparin sulfate proteoglycan-mediated components (Chen CC *et al*, 2001). Although the specific heparin sulfate proteoglycans and heparin side chains that bind CTGF have not been fully elucidated, a recent report found that the ability of CTGF to induce chondrocyte proliferation and migration required the heparin sulfate proteoglycan perlecan (Nishida *et al*, 2003). Thus, the available evidence suggests that CTGF mediate its effects through integrin- and heparin sulfate proteoglycan-dependent mechanisms. Iodinated CTGF has also been shown to interact with the low-density lipoprotein receptor (Segarini *et al*, 2001). This interaction has not yet been shown to result in a CTGF-mediated signaling event, but is involved with CTGF internalization and degradation in the endosome (Chen Y *et al*, 2001; Segarini *et al*, 2001).

CTGF and TGF-β synergize to promote a sustained fibrotic response

CTGF has been directly implicated in the excessive matrix deposition characteristic of scleroderma lesions. Experiments using recombinant CTGF and neutralizing antibodies targeting CTGF have suggested that CTGF mediates at least some of the effects of TGF-β on fibroblast proliferation, adhesion, and ECM production (Grotendorst, 1997; Duncan *et al*, 1999; Lau and Lam, 1999). One of the most compelling pieces of evidence showing CTGF can independently induce matrix is that an expression vector encoding CTGF transfected into fibroblasts can activate a cotransfected reporter construct driven by the type I collagen promoter (Shi-wen *et al*, 2000). These results suggest that a CTGF response element exists in the type I collagen promoter. Studies are currently underway to identify the *cis*-acting sequences, *trans*-acting factors and signal transduction mechanisms involved with this response.

Physiologically, the effect of CTGF induced by TGF-β might result in a prolonged induction of expression of collagen mRNA. In fact, CTGF seems act with TGF-β to promote a sustained fibrotic response. Mori *et al* (1999) showed that subcutaneous injection of TGF-β into neonatal mice caused a transient fibrotic response, which dissipated when ligand was no longer injected. Conversely, although injection of CTGF alone had little effect, coinjection of CTGF and TGF-β resulted in sustained fibrosis that persisted for at least a week after cessation of injection (Mori *et al*, 1999). Other experiments have shown that CTGF alone causes an increase in matrix deposition when injected subcutaneously (Frazier *et al*, 1996; Ball *et al*, 2003b). Recently it was shown, using an adenoviral delivery system, that overexpression of CTGF in lung tissue caused a modest,

transient fibrosis, suggesting that another stimulus might be required for sustained fibrosis (Bonniaud *et al*, 2003). Alternatively, it was recently suggested that CTGF may bind to TGF-β and potentiate TGF-β binding to the TGF-β type II receptors and thus contribute to the effects of TGF-β on cells, for example, by promoting activation of Smad, at least in *Xenopus* (Abreu *et al*, 2002). The relevance of these latter observations to mammalian systems is currently under investigation. The available data, however, suggest that, whereas TGF-β or CTGF alone may only result in transient fibrosis, TGF-β in the presence of high levels of CTGF may result in a sustained fibrotic response.

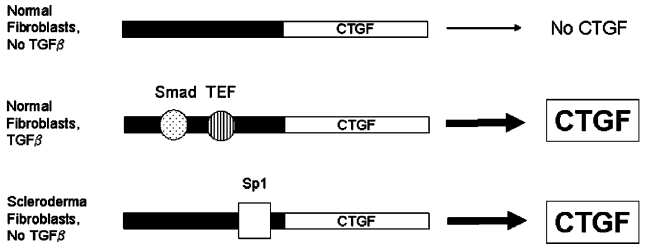

Figure 1
The regulation of CTGF in normal and scleroderma fibroblasts. A diagram of the CTGF promoter is shown, with the elements that are required for the expression of CTGF in normal and scleroderma fibroblasts. The expression of CTGF in normal fibroblasts requires induction by TGF-β and its TGF-β response element, namely Smad and TEF recognition sequences (circles with stippled and vertical lines, respectively; Holmes *et al*, 2001; Leask *et al*, 2003). The expression of CTGF in scleroderma fibroblasts requires an Sp1-binding element (open box; Holmes *et al*, 2003) and is independent of the TGF-β response element of the CTGF promoter.

Elevated levels CTGF expression in scleroderma fibroblasts are independent of the TGF-β response element of the CTGF promoter but dependent on Sp1

It has long been known that TGF-β induction of collagen occurs directly in an immediate early fashion, requiring action of the general TGF-β signaling mediators the Smad (Chen *et al*, 1999). The Smad family of proteins has been extensively reviewed (for example, Roberts, 1999; Attisano and Wrana, 2002; Verrecchia and Mauviel, 2002). Briefly, in the presence of TGF-β ligand, the receptor-activated Smad, Smad2 and 3, are phosphorylated directly by the TGF-β receptor I kinase and then translocate to the nucleus complexed to the common mediator, Smad4. Maximal affinity of recombinant Smad3 and Smad4 to DNA is observed with the 5 bp sequence, CAGAC (Zawel *et al*, 1998). Smad2, on the other hand, does not bind DNA directly, requiring a nuclear DNA-binding protein of the Fast family to bind DNA, in association with Smad4, and activate transcription in response to TGF-β and activin (Liu *et al*, 1999). A third group of Smad proteins, the inhibitory Smad (I-Smad), such as Smad6 or Smad7 prevent R-Smad phosphorylation and subsequent nuclear translocation of R-Smad/Smad4 heterocomplexes (Nakao *et al*, 1997).

Smad 3 is essential for the induction of matrix (Verrecchia *et al*, 2001b). Indeed, a functional Smad element resides within the CTGF promoter; the TGF-β induction of CTGF is grossly impaired in embryonic fibroblasts taken from Smad3 knockout mice (Holmes *et al*, 2001; Fig 1). In addition to the Smad binding element, the TGF-β-induction of CTGF also requires a consensus TEF binding element (Leask *et al*, 2003; Fig 1).

Smad3 is essential for the initiation of fibrosis; radiation-induced fibrosis does not develop in Smad3 knockout animals (Flanders *et al*, 2002). Consistent with this idea, elevated levels of Smad3 and phosphorylated Smad3 are observed during bleomycin-induced skin fibrosis for 3 wk after injection of bleomycin (Takagawa *et al*, 2003). Another study from the same group examined the level of unphosphorylated and phosphorylated Smad3 present in SSc fibroblasts cultured from the leading edge of the fibrotic lesion (Mori *et al*, 2003); that is, in the area of the fibrotic lesion involved with the initiation and expansion of the fibrotic response. In this latter study, elevated levels of phosphorylated Smad3 and expression of a generic Smad3-responsive promoter were observed in leading edge SSc fibroblasts relative to normal fibroblasts (Mori *et al*, 2003). Collectively, these results suggest that an elevated level of Smad3 activation is involved with the initiation of the fibrotic response in SSc. When we examined Smad expression in SSc fibroblasts cultured from established fibrotic lesions, however, we were not able to show a consistent pattern of Smad3 overexpression (Holmes *et al*, 2001). Thus, it is not clear whether a generally activated Smad3-dependent signaling pathway is sufficient to result in prolonged pathologic fibrosis. For example, it remains to be seen if every transcript that is Smad3 responsive is overexpressed in lesional SSc fibroblasts, or if every gene overexpressed in lesional SSc fibroblasts is Smad3 responsive in normal fibroblasts. In addition, it remains to be seen if activity of a generic Smad3-responsive promoter is upregulated in SSc fibroblasts cultured from established lesions.

In contrast to the situation in normal dermal fibroblasts, CTGF is constitutively overexpressed in dermal fibrotic lesions, such as in scleroderma (Igarashi *et al*, 1996; Abraham *et al*, 2000; Shi-wen *et al*, 2000; Holmes *et al*, 2001). As CTGF is thus an effective marker of an activated, sclerotic fibroblast, examining the mechanism underlying the overexpression of CTGF in fibroblasts cultured from fibrotic lesions is likely to provide insights into understanding the mechanism underlying persistent fibrosis.

Thus to probe the molecular mechanism underlying the maintenance of the fibrotic phenotype and to investigate the role of Smad-dependent and Smad-independent signaling pathways in the maintenance of the fibrotic phenotype in established SSc lesions, we have identified elements in the CTGF promoter required for its overexpression in lesional SSc fibroblasts. We found that the elevated expression of the CTGF promoter in SSc fibroblasts seems to be independent of its Smad response element (Holmes *et al*, 2001) and TGF-β response element (Leask *et al*, 2003). Instead, the elevated expression of the CTGF promoter in SSc fibroblasts was dependent, at least in part, on an Sp1

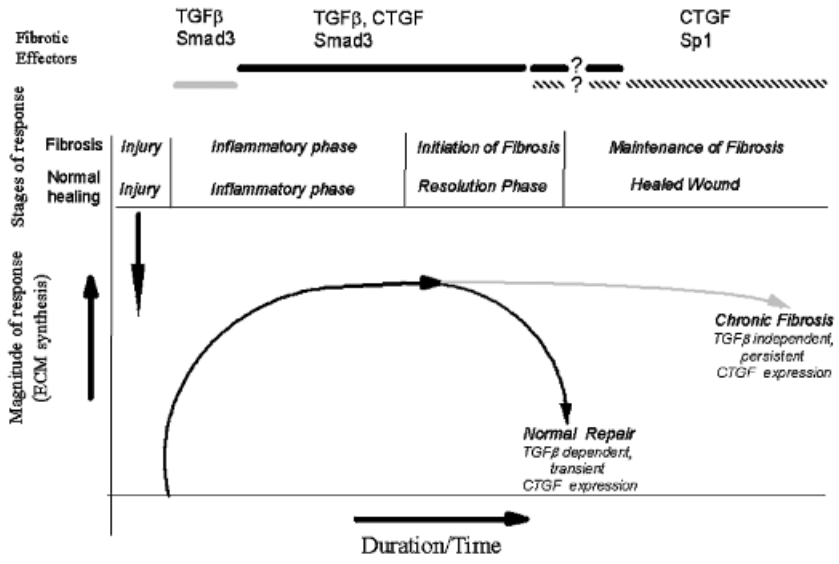

Figure 2
Model of sustained, chronic fibrosis. TGF-β is necessary for the initiation of the normal wound healing response, including the expression of ECM and CTGF in dermal fibroblasts. In the normal wound healing response, the action of TGF-β, including the induction of CTGF, is suppressed leading to the proper termination of the wound healing response (*black arrow*). Conversely in pathologic fibrosis, the expression of CTGF in the lesion is independent of TGF-β action. Thus CTGF expression is not subject to controls that normally terminate the wound healing response. The fibrotic response persists, leading to sustained, chronic fibrosis (*gray arrow*). The phases of the normal wound healing and fibrotic response are shown, and the effectors of the fibrotic response are shown at the top of the diagram.

binding element in the CTGF promoter and an elevated level of Sp1 DNA binding present in nuclear extracts prepared from scleroderma fibroblasts (Holmes *et al*, 2003; Fig 1). The Sp1 element of the CTGF promoter was not required for the ability of the CTGF promoter to respond to TGF-β (Holmes *et al*, 2003). Previously, it was shown that Sp1 regulates a wide variety of matrix genes (Verrecchia *et al*, 2001c). Intriguingly, the transcription factor fli-1, which suppresses collagen promoter activity through an Sp1-dependent mechanism (Czuwara-Ladykowska *et al*, 2001), was recently found to be markedly downregulated in SSc lesions (Kubo *et al*, 2003). Collectively, these results suggest that elevation of matrix genes in lesional SSc fibroblasts may involve elevated Sp1 activity.

The divergence between the requirement of TGF-β for the induction of CTGF in normal fibroblasts and the constitutive, elevated level of CTGF expression in scleroderma dermal fibroblasts is supported by other observations. Histologic studies examining the distribution of CTGF and TGF-β mRNA in skin sections showed that in diffuse SSc lesions TGF-β expression is only overexpressed in the leading inflammatory edge of the lesion, but not in the lesional area itself (Querfeld *et al*, 1999). In contrast, CTGF shows an expression pattern that correlates with the severity of fibrosis; that is, CTGF expression is abundant in fibrotic lesions, even in the absence of markedly elevated amounts of TGF-β ligand (Sato *et al*, 2000). Additional data suggesting that the elevated level of CTGF expression in fibrotic lesions is TGF-β-independent comes from data showing that tumor necrosis factor α, which is believed to contribute to the downregulation of the fibrotic response *in vivo* in part through the suppression Smad activity in fibroblasts (Verrecchia *et al*, 2000), blocks the ability of TGF-β to induce CTGF in normal and SSc fibroblasts, but does not affect the basal, constitutive levels of expression of CTGF observed in scleroderma fibroblasts (Abraham *et al*, 2000). Taken together, in contrast to the situation in normal dermal fibroblasts whereby CTGF is not normally expressed unless induced by TGF-β through the TGF-β response element, the persistent level of CTGF observed in lesional SSc fibroblasts seems to be independent of TGF-β ligand.

Hypothesis: The elevated, TGF-β-independent levels of CTGF promotes a sustained fibrotic response in scleroderma

TGF-β and the TGF-β signaling mediators, the Smad, are necessary for the induction of fibrosis in animal models (McCormick *et al*, 1999; Nakao *et al*, 1999; Yamamoto *et al*, 1999; Terada *et al*, 2002; Lan *et al*, 2003). In animal models, however, whereas subcutaneous injection of TGF-β or CTGF alone only causes a transient fibrotic response, simultaneous injection of TGF-β and CTGF—that is, when CTGF is present in levels over and above those normally induced by TGF-β—causes a sustained fibrotic response (Mori *et al*, 1999). We hypothesize that in the normal wound healing response, the upregulation of CTGF is dependent on the TGF-β response element of the CTGF promoter, and is therefore subject to the controls that normally negatively regulate the TGF-β-induced wound healing response, such as tumor necrosis factor α (Abraham *et al*, 2000; Fig 2). In pathologic fibrosis, however, fibroblasts display an elevated level of CTGF expression that is independent of the TGF-β response element of the CTGF promoter (Holmes *et al*, 2001, 2003). Thus, the elevated level of CTGF expression observed in the scleroderma fibroblast is therefore not subject to the negative regulatory controls that normally suppress the TGF-β-induced wound healing response. In fact, it is tempting to speculate that, whereas TGF-β is essential for the initiation of fibrosis it is the persistent, TGF-β-independent CTGF expression characteristic of fibrotic lesions perpetuates the wound healing response, resulting in sustained, chronic, pathologic fibrosis (Fig 2).

In conclusion, the observations concerning CTGF expression and function have led to the intriguing notion that CTGF might be a novel, molecular target for therapeutic

intervention in fibrotic disease (Grotendorst, 1997; Perbal, 2001; Leask *et al*, 2002; Rachfal and Brigstock, 2003). As further research is conducted, the precise functional role of CTGF concerning the regulation of cellular function should emerge, and result in the development of novel methods for anti-fibrotic intervention to prevent dermal scarring and alleviate the symptoms of fibrosis.

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References

Abraham DJ, Shiwen X, Black CM, Sa S, Xu Y, Leask A: Tumor necrosis factor alpha suppresses the induction of connective tissue growth factor by transforming growth factor-beta in normal and scleroderma fibroblasts. J Biol Chem 275:15220–15225, 2000

Abreu JG, Ketpura NI, Reversade B, De Robertis EM: Connective-tissue growth factor (CTGF) modulates cell signaling by BMP and TGF-beta. Nat Cell Biol 4:599–604, 2002

Ashcroft GS, Yang X, Glick AB, *et al*: Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. Nat Cell Biol 1:260–266, 1999

Attisano L, Wrana JL: Signal transduction by the TGF-beta superfamily. Science 296:1646–1647, 2002

Babic AM, Chen CC, Lau LF: Fisp12/mouse connective tissue growth factor mediates endothelial cell adhesion and migration through integrin alphavbeta3, promotes endothelial cell survival, and induces angiogenesis in vivo. Mol Cell Biol 19:2958–2966, 1999

Ball DK, Rachfal AW, Kemper SA, Brigstock DR: The heparin-binding 10 kDa fragment of connective tissue growth factor (CTGF) containing module 4 alone stimulates cell adhesion. J Endocrinol 176:R1–R7, 2003a

Ball DK, Moussad EE, Rageh MA, Kemper SA, Brigstock DR: Establishment of a recombinant expression system for connective tissue growth factor (CTGF) that models CTGF processing in utero. Reproduction 125:271–284, 2003b

Bonniaud P, Margetts PJ, Kolb M, Haberberger T, Kelly M, Robertson J, Gauldie J: Adenoviral gene transfer of connective tissue growth factor in the lung induces transient fibrosis. Am J Respir Crit Care Med 168:770–778, 2003.

Bork P: The modular architecture of a new family of growth regulators related to connective tissue growth factor. FEBS Lett 327:125–130, 1993

Bradham DM, Igarashi A, Potter RL, Grotendorst GR: Connective tissue growth factor: A cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product: CEF-10. J Cell Biol 114:1285–1294, 1991

Brigstock DR, Steffen CL, Kim GY, Vegunta RK, Diehl JR, Harding PA: Purification and characterization of novel heparin-binding growth factors in uterine secretory fluids. Identification as heparin-regulated Mr 10,000 forms of connective tissue growth factor. J Biol Chem 272:20275–20282, 1997

Chen CC, Chen N, Lau LF: The angiogenic factors Cyr61 and connective tissue growth factor induce adhesive signaling in primary human skin fibroblasts. J Biol Chem 276:10443–10452, 2001

Chen SJ, Yuan W, Mori Y, Levenson A, Trojanowska M, Varga J: Stimulation of type I collagen transcription in human skin fibroblasts by TGF-beta: Involvement of Smad 3. J Invest Dermatol 112:49–57, 1999

Chen Y, Segarini P, Raoufi F, Bradham D, Leask A: Connective tissue growth factor is secreted through the Golgi and is degraded in the endosome. Exp Cell Res 271:109–117, 2001

Crean JK, Finlay D, Murphy M, Moss C, Godson C, Martin F, Brady HR: The role of p42/44 MAPK and protein kinase B in connective tissue growth factor induced extracellular matrix protein production, cell migration, and actin cytoskeletal rearrangement in human mesangial cells. J Biol Chem 277:44187–44194, 2002

Crowe MJ, Doetschman T, Greenhalgh DG: Delayed wound healing in immunodeficient TGF-beta 1 knockout mice. J Invest Dermatol 115: 3–11, 2000

Czuwara-Ladykowska J, Shirasaki F, Jackers P, Watson DK, Trojanowska M: Fli-1 inhibits collagen type I production in dermal fibroblasts via an Sp1-dependent pathway. J Biol Chem 276:20839–20848, 2001

Duncan MR, Frazier KS, Abramson S, Williams S, Klapper H, Huang X, Grotendorst GR: Connective tissue growth factor mediates transforming growth factor beta-induced collagen synthesis: Down-regulation by cAMP. FASEB J 13:1774–1786, 1999

Flanders KC, Sullivan CD, Fujii M, *et al*: Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. Am J Pathol 160:1057–1068, 2002

Frazier K, Williams S, Kothapalli D, Klapper H, Grotendorst GR: Stimulation of fibroblast cell growth, matrix production, and granulation tissue formation by connective tissue growth factor. J Invest Dermatol 107:404–411, 1996

Friedrichsen S, Heuer H, Christ S, Winckler M, Brauer D, Bauer K, Raivich G: CTGF expression during mouse embryonic development. Cell Tissue Res 312:175–188, 2003

Grotendorst GR: Connective tissue growth factor: A mediator of TGF-beta action on fibroblasts. Cytokine Growth Factor Rev 8:171–179, 1997

Grotendorst GR, Okochi H, Hayashi N: A novel transforming growth factor beta response element controls the expression of the connective tissue growth factor gene. Cell Growth Differ 7:469–480, 1996

Holmes A, Abraham DJ, Chen Y, Denton C, Shi-wen X, Black CM, Leask A: Constitutive CTGF expression in scleroderma fibroblasts is dependent on Sp1. J Biol Chem 278:41728–41733, 2003

Holmes A, Abraham DJ, Sa S, Shiwen X, Black CM, Leask A: CTGF and SMADs, maintenance of scleroderma phenotype is independent of SMAD signaling. J Biol Chem 276:10594–10601, 2001

Igarashi A, Okochi H, Bradham DM, Grotendorst GR: Regulation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair. Mol Biol Cell 4:637–645, 1993

Igarashi A, Nashiro K, Kikuchi K, *et al*: Connective tissue growth factor gene expression in tissue sections from localized scleroderma, keloid, and other fibrotic skin disorders. J Invest Dermatol 106:729–733, 1996

Ignotz RA, Massague J: Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 261:4337–4345, 1986

Ivkovic S, Yoon BS, Popoff SN, *et al*: Connective tissue growth factor coordinates chondrogenesis and angiogenesis during skeletal development. Development 130:2779–2791, 2003

Jedsadayanmata A, Chen CC, Kireeva ML, Lau LF, Lam SC: Activation-dependent adhesion of human platelets to Cyr61 and Fisp12/mouse connective tissue growth factor is mediated through integrin alpha(IIb) beta(3). J Biol Chem 274:24321–24327, 1999

Kielty CM, Shuttleworth CA: Microfibrillar elements of the dermal matrix. Microsc Res Techn 38:413–427, 1997

Kireeva ML, Latinkic BV, Kolesnikova TV, Chen CC, Yang GP, Abler AS, Lau LF: Cyr61 and Fisp12 are both ECM-associated signaling molecules: Activities, metabolism, and localization during development. Exp Cell Res 233:63–77, 1997

Kubo M, Czuwara-Ladykowska J, Moussa O, *et al*: Persistent down-regulation of Fli1, a suppressor of collagen transcription, in fibrotic scleroderma skin. Am J Pathol 163:571–581, 2003

Lan HY, Mu W, Tomita N, *et al*: Inhibition of renal fibrosis by gene transfer of inducible Smad7 using ultrasound-microbubble system in rat UUO model. J Am Soc Nephrol 14:1535–1548, 2003

Lau LF, Lam SC: The CCN family of angiogenic regulators: The integrin connection. Exp Cell Res 248:44–57, 1999

Leask A, Holmes A, Abraham DJ: CTGF: A new and important player in the pathogenesis of fibrosis. Curr Rheum Rep 4:136–142, 2002

Leask A, Holmes A, Black CM, Abraham DJ: Connective tissue growth factor gene regulation. Requirements for its induction by transforming growth factor-beta 2 in fibroblasts. J Biol Chem 278:13008–13015, 2003

Letterio JJ, Roberts AB: Regulation of immune responses by TGF-beta. Annu Rev Immunol 16:137–161, 1996

Leu SJ, Liu Y, Chen N, Chen CC, Lam SC, Lau LF: Identification of a novel integrin alpha 6beta 1 binding site in the angiogenic Inducer CCN1 (CYR61). J Biol Chem 278:33801–33808, 2003

Liu B, Dou CL, Prabhu L, Lai E: FAST-2 is a mammalian winged-helix protein which mediates transforming growth factor beta signals. Mol Cell Biol 19:424–30, 1999

McCartney-Francis NL, Frazier-Jessen M, Wahl SM: TGF-beta: A balancing act. Int Rev Immunol 16:553–580, 1998

McCormick LL, Zhang Y, Tootell E, Gilliam AC: Anti-TGF-beta treatment prevents skin and lung fibrosis in murine sclerodermatous graft-versus-host disease: A model for human scleroderma. J Immunol 163:5693–5699, 1999

Mori T, Kawara S, Shinozaki M, *et al*: Role and interaction of connective tissue growth factor with transforming growth factor-beta in persistent fibrosis: A mouse fibrosis model. J Cell Physiol 181:153–159, 1999

Mori Y, Chen SJ, Varga J: Expression and regulation of intracellular SMAD signaling in scleroderma skin fibroblasts. Arthritis Rheum 48:1964–1978, 2003

Moussad EE, Brigstock DR: Connective tissue growth factor: What's in a name? Mol Genet Metab 71:276–292, 2000

Nakanishi T, Nishida T, Shimo T, *et al*: Effects of CTGF/Hcs24, a product of a hypertrophic chondrocyte-specific gene, on the proliferation and differentiation of chondrocytes in culture. Endocrinology 141:264–273, 2000

Nakao A, Afrakhte M, Moren A, *et al*: Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signalling. Nature 389:631–635 389, 1997

Nakao A, Fujii M, Matsumura R, Kumano K, Saito Y, Miyazono K, Iwamoto I: Transient gene transfer and expression of Smad7 prevents bleomycin-induced lung fibrosis in mice. J Clin Invest 104:5–11, 1999

Nimni ME: Collagen: Its structure and function in normal and pathological connective tissues. Semin Arthritis Rheum 4:95–150, 1974

Nishida T, Kubota S, Fukunaga T, *et al*: CTGF/Hcs24, hypertrophic chondrocyte-specific gene product, interacts with perlecan in regulating the proliferation and differentiation of chondrocytes. J Cell Physiol 196:265–275, 2003

Overall CM, Wrana JL, Sodek J: Independent regulation of collagenase, 72-kDa progelatinase, and metalloendoproteinase inhibitor expression in human fibroblasts by transforming growth factor-beta. J Biol Chem 264:1860–1869, 1989

Overall CM, Wrana JL, Sodek J: Transcriptional and post-transcriptional regulation of 72-kDa gelatinase/type IV collagenase by transforming growth factor-beta 1 in human fibroblasts. Comparisons with collagenase and tissue inhibitor of matrix metalloproteinase gene expression. J Biol Chem 266:14064–14071, 1991

Paradis V, Perlemuter G, Bonvoust F, *et al*: High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: A potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology 34:738–744, 2001

Perbal B: NOV (nephroblastoma overexpressed) and the CCN family of genes: Structural and functional issues. Mol Pathol 54:57–79, 2001

Quaglino D Jr, Nanney LB, Ditesheim JA, Davidson JM: Transforming growth factor-beta stimulates wound healing and modulates extracellular matrix gene expression in pig skin: Incisional wound model. J Invest Dermatol 97:34–42, 1991

Querfeld C, Eckes B, Huerkamp C, Krieg T, Sollberg S: Expression of TGF-beta 1, -beta 2 and -beta 3 in localized and systemic scleroderma. J Dermatol Sci 21:13–22, 1999

Rachfal AW, Brigstock DR: Connective tissue growth factor (CTGF/CCN2) in hepatic fibrosis. Hepatol Res 26:1–9, 2003

Roberts AB: TGF-beta signaling from receptors to the nucleus. Microbes Infect 1:1265–1273, 1999

Sato S, Nagaoka T, Hasegawa M, Tamatani T, Nakanishi T, Takigawa M, Takehara K: Serum levels of connective tissue growth factor are elevated in patients with systemic sclerosis: Association with extent of skin sclerosis and severity of pulmonary fibrosis. J Rheumatol 27:149–154, 2000

Schober JM, Chen N, Grzeszkiewicz TM, *et al*: Identification of integrin alpha(M)beta(2) as an adhesion receptor on peripheral blood monocytes for Cyr61 (CCN1) and connective tissue growth factor (CCN2): Immediate-early gene products expressed in atherosclerotic lesions. Blood 99:4457–4465, 2002

Segarini PR, Nesbitt JE, Li D, Hays LG, Yates JR 3rd, Carmichael DF: The low density lipoprotein receptor-related protein/alpha2-macroglobulin receptor is a receptor for connective tissue growth factor. J Biol Chem 276:40659–40667, 2001

Shimo T, Nakanishi T, Nishida T, *et al*: Connective tissue growth factor induces the proliferation, migration, and tube formation of vascular endothelial cells *in vitro*, and angiogenesis *in vivo*. J Biochem 126:137–145, 1999

Shimo T, Nakanishi T, Nishida T, *et al*: Involvement of CTGF, a hypertrophic chondrocyte-specific gene product, in tumor angiogenesis. Oncology 61:315–322, 2001

Shi-wen X, Pennington D, Holmes A, *et al*: Autocrine overexpression of CTGF maintains fibrosis: RDA analysis of fibrosis genes in systemic sclerosis. Exp Cell Res 259:213–224, 2000

Silver FH, Glasgold AI: Cartilage wound healing: An overview. Otolaryngol Clin North Am 28:847–864, 1995

Simms RW, Korn JH: Cytokine directed therapy in scleroderma: Rationale, current status, and the future. Curr Opin Rheumatol 14:717–722, 2002

Slee RB, Hillier SG, Largue P, Harlow CR, Miele G, Clinton M: Differentiation-dependent expression of connective tissue growth factor and lysyl oxidase messenger ribonucleic acids in rat granulosa cells. Endocrinology 142:1082–1089, 2001

Surveyor GA, Wilson AK, Brigstock DR: Localization of connective tissue growth factor during the period of embryo implantation in the mouse. Biol Reprod 59:1207–1213, 1998

Takagawa S, Lakos G, Mori Y, Yamamoto T, Nishioka K, Varga J: Sustained activation of fibroblast transforming growth factor-beta/Smad signaling in a murine model of scleroderma. J Invest Dermatol 121:41–50, 2003

Terada Y, Hanada S, Nakao A, Kuwahara M, Sasaki S, Marumo F: Gene transfer of Smad7 using electroporation of adenovirus prevents renal fibrosis in post-obstructed kidney. Kidney Int Suppl 61 (Suppl. 1):94–98, 2002

Valentini G, Black C: Systemic sclerosis. Best Pract Res Clin Rheumatol 16:807–816, 2002

Verrecchia F, Mauviel A: Transforming growth factor-beta signaling through the Smad pathway: Role in extracellular matrix gene expression and regulation. Invest Dermatol 118:211–215, 2002

Verrecchia F, Pessah M, Atfi A, Mauviel A: Tumor necrosis factor-alpha inhibits transforming growth factor-beta/Smad signaling in human dermal fibroblasts via AP-1 activation. J Biol Chem 275:30226–30231, 2000

Verrecchia F, Tacheau C, Schorpp-Kistner M, Angel P, Mauviel A: Induction of the AP-1 members c-Jun and JunB by TGF-beta/Smad suppresses early Smad-driven gene activation. Oncogene 20:2205–2211, 2001a

Verrecchia F, Vindevoghel L, Lechleider RJ, Uitto J, Roberts AB, Mauviel A: Smad3/AP-1 interactions control transcriptional responses to TGF-beta in a promoter-specific manner. Oncogene 20:3332–3340, 2001b

Verrecchia F, Rossert J, Mauviel A: Blocking sp1 transcription factor broadly inhibits extracellular matrix gene expression *in vitro* and *in vivo*: Implications for the treatment of tissue fibrosis. J Invest Dermatol 116:755–763, 2001c

Yamamoto T, Takagawa S, Katayama I, Nishioka K: Anti-sclerotic effect of transforming growth factor-beta antibody in a mouse model of bleomycin-induced scleroderma. Clin Immunol 92:6–13, 1999

Yamamoto Y, Furukawa K, Ueyama K, Nakanishi T, Takigawa M, Harata S: Possible roles of CTGF/Hcs24 in the initiation and development of ossification of the posterior longitudinal ligament. Spine 27:1852–1857, 2002

Yosimichi G, Nakanishi T, Nishida T, Hattori T, Takano-Yamamoto T, Takigawa M: CTGF/Hcs24 induces chondrocyte differentiation through a p38 mitogen-activated protein kinase (p38MAPK), and proliferation through a p44/42 MAPK/extracellular-signal regulated kinase (ERK). Eur J Biochem 268:6058–6065, 2001

Yuan W, Varga J: Transforming growth factor-beta repression of matrix metalloproteinase-1 in dermal fibroblasts involves Smad3. J Biol Chem 276:38502–38510, 2001

Zawel L, Dai JL, Buckhaults P, Zhou S, Kinzler KW, Vogelstein B, Kern SE: Human Smad3 and Smad4 are sequence-specific transcription activators. Mol Cell 1:611–617, 1998