REVIEW

Transcriptional profiling of the scleroderma fibroblast reveals a potential role for connective tissue growth factor (CTGF) in pathological fibrosis

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Abstract. The cause of fibrotic disease is unknown. We have undertaken transcriptional profiling of dermal fibroblasts cultured from patients with the fibrotic disease scleroderma (systemic sclerosis, SSc) to identify genes overexpressed in fibrosis and have explored their contribution to the fibrotic phenotype. Connective tissue growth factor (CTGF, CCN2), a member of the CCN family of proteins, is overexpressed in SSc fibroblasts. In adult skin, CTGF is not normally expressed in dermal fibroblasts. However, CTGF is induced during the wound healing response and is constitutively overexpressed by fibroblasts present in fibrotic lesions. The overexpression of CTGF present in fibrotic lesions contributes to the phenotype of scleroderma in that CTGF promotes matrix deposition, and fibroblast adhesion and proliferation. In animal models, whereas either TGFβ or CTGF alone produce only a transient fibrotic response, CTGF and TGFβ act together to promote sustained fibrosis. Thus the constitutive overexpression of CTGF by fibroblasts present in fibrotic lesions would be expected to directly contribute to chronic, persistent fibrosis. (Keio J Med 53 (2): 74–77, June 2004)

Key words: CTGF, scleroderma, fibrosis, TGFβ

Introduction

De novo synthesis of connective tissue occurs during the wound healing process. Normally this response is appropriately terminated; however, if the wound healing process occurs unabated, excessive deposition of extracellular matrix (ECM) occurs resulting in the formation of scar tissue.[1] Excessive scarring results in the progressive, pathological scarring characteristic of fibrotic disease. One example of such a connective tissue disease is systemic sclerosis (scleroderma; SSc) which affects the skin as well as internal organs.[2,3] There is no effective treatment for this disorder, in part because the etiology of this disease is unknown. Thus to identify appropriate targets for therapeutic intervention, it is necessary first to identify proteins overexpressed in fibrotic disease and then to assess the role that these proteins might have in the fibrotic phenotype.

CTGF is Overexpressed in Scleroderma Fibroblasts

By differential display and Western blot analyses, we found that connective tissue growth factor (CTGF, CCN2) is constitutively overexpressed in dermal fibroblasts isolated from SSc lesions.[4] *In vitro*, CTGF promotes fibroblast proliferation, matrix production, and granulation tissue formation.[5–7] CTGF also promotes cell adhesion and migration in a wide variety of cell types.[8] Human foreskin fibroblasts adhere to CTGF through integrinα6β1.[8] In addition, a heparin-binding domain of CTGF, present in the carboxy-terminal region of CTGF, is important for CTGF-mediated fibroblast proliferation and adhesion.[7,9] Therefore, CTGF promotes adhesion in a seemingly unique integrin- and HSPG-dependent fashion.

One of the most compelling pieces of evidence showing CTGF can independently induce matrix is that an expression vector encoding CTGF transfected into

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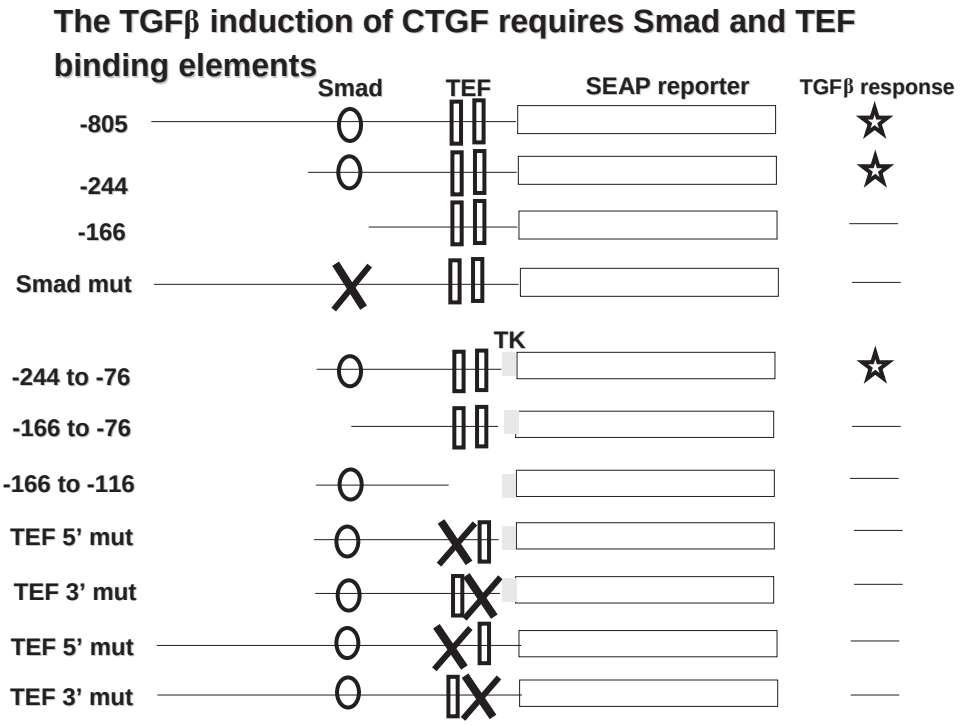

Fig. 1 Mapping of response elements in the CTGF promoter necessary for its induction by TGFβ. Smad = Smad binding element, TEF = TEF binding element, Star = construct which is TGFβ responsive, X = mutated binding site, TK = minimal herpes simplex virus thymidine kinase promoter, SEAP = secreted enhanced alkaline phosphatase reporter gene. NIH 3T3 fibroblasts were transfected with CTGF promoter/reporter constructs, and reporter gene expression was determined. The Smad and TEF elements are required for the ability of the CTGF promoter to respond to TGFβ. For details, see refs (13, 14).

fibroblasts can activate a cotransfected reporter construct driven by the type I collagen promoter.[4] *In vivo*, CTGF acts with TGFβ to induce a sustained fibrotic response, as although subcutaneous injection of TGFβ into neonatal mice caused a transient fibrotic response and injection of CTGF alone had little effect, co-injection of CTGF and TGFβ resulted in persistent fibrosis.[10]

CTGF Gene Regulation: CTGF Overexpression in Scleroderma Fibroblasts is Independent of Its TGFβ Response Element but Dependent on Sp1

CTGF is not normally expressed in skin unless induced, for example during the normal wound repair process.[11] The profibrotic protein TGFβ induces CTGF expression in dermal fibroblasts, but not in epidermal keratinocytes.[6,11–13] Although the TGFβ induction of CTGF requires Smad3 and a functional Smad binding element in the CTGF promoter acting with a consensus TEF binding element (Fig. 1),[13,14] the elevated expression of the CTGF promoter in SSc fibroblasts seems to be independent of its Smad response element[14] and TGFβ response element.[13] Instead, the elevated expression of the CTGF promoter in SSc fibroblasts requires a Sp1 binding element in the CTGF promoter, which is not required for the ability of CTGF to respond to TGFβ (Fig. 2).[13,15] Sp1 regulates a wide variety of matrix genes[16] suggesting that elevation of matrix genes in lesional SSc fibroblasts may be due to elevated Sp1 binding activity.

Hypothesis: CTGF and TGFβ Act to Promote a Sustained Fibrotic Phenotype

Histological studies examining the distribution of CTGF and TGFβ mRNAs in skin sections showed that in diffuse SSc lesions TGFβ is overexpressed in the leading inflammatory edge of the lesion, but not in the lesional area itself.[17] CTGF expression shows an expression pattern correlating with the severity of fibrosis; that is, CTGF expression is abundant in fibrotic lesions, even in the absence of markedly elevated amounts of TGFβ ligand.[18] Taken together, in contrast to the situation in normal dermal fibroblasts whereby CTGF is not normally expressed unless induced by TGFβ through the TGFβ response element, the persistent level of CTGF observed in lesional SSc fibroblasts

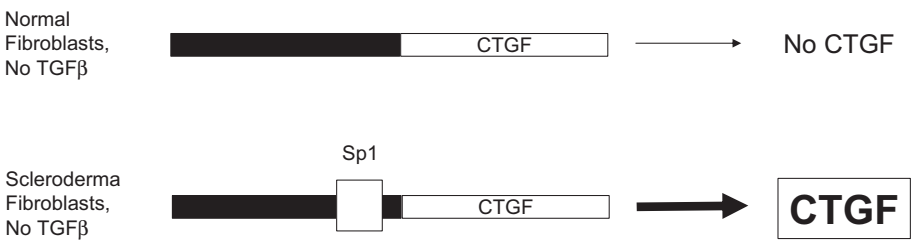

Fig. 2 Sp1 is required for the overexpression of the CTGF promoter in SSc fibroblasts. The minimal CTGF promoter containing only a functional Sp1 element is required for the elevated level of CTGF promoter expression in SSc fibroblasts. Removal of the Sp1 element in the promoter or blockade of Sp1 activity by mithramycin suppresses elevated CTGF promoter and protein expression in SSc fibroblasts. For details, see ref (15).

seems to be independent of TGFβ ligand.

Based on these results, we hypothesize that in the normal wound healing response, the up-regulation of CTGF is dependent on the TGFβ response element of the CTGF promoter, and is therefore subject to the controls that normally negatively regulate the TGFβ-induced wound healing response.[19] In pathological fibrosis, however, fibroblasts display an elevated level of CTGF expression that is independent of the TGFβ response element of the CTGF promoter.[14,15] Thus we believe that, whereas TGFβ is essential for the initiation of fibrosis, the persistent, TGFβ-independent CTGF expression characteristic of fibrotic lesions perpetuates the fibrotic response.

Conclusion

The observations concerning CTGF expression and function have led to the intriguing notion that CTGF represents a novel, molecular target for therapeutic intervention in fibrotic disease.[20–23] As further research is conducted, the precise functional role of CTGF in cellular function as well as the mechanism of CTGF action should emerge. This process should 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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