



Review

The Role of TGF- β Signaling in the Pathogenesis of Fibrosis in Scleroderma

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Abstract. Excessive extracellular matrix (ECM) deposition in the skin, lung, and other organs is a hallmark of systemic sclerosis (SSc). The pathogenesis of SSc is still poorly understood, but increasing evidence suggests that transforming growth factor (TGF)- β is a key mediator of tissue fibrosis as a consequence of ECM accumulation in pathological states such as systemic sclerosis. TGF- β regulates diverse biological activities including cell growth, cell death or apoptosis, cell differentiation, and ECM synthesis. TGF- β is known to induce the expression of ECM proteins in mesenchymal cells and to stimulate the production of protease inhibitors that prevent enzymatic breakdown of the ECM. This review focuses on the possible role of TGF- β in the pathogenesis of fibrosis in SSc.

Key words: TGF- β ; fibrosis; systemic sclerosis; signal transduction.

Introduction

Scleroderma, or systemic sclerosis (SSc), is a generalized connective tissue disease that involves sclerotic changes in the skin and many other organ systems⁵². Although the pathogenesis of SSc is still unknown, the basic mechanism appears to involve endothelial cell injury, overproduction of extracellular matrix (ECM), and aberrant immune activation^{45, 51}.

Increasing evidence suggests that activation of lesional fibroblasts contributes to the fibrotic process^{42, 47}. Numerous differences between cultured SSc and healthy skin fibroblasts that may contribute to excessive ECM deposition *in vivo* have been demonstrated, such as elevated expression of collagen types I, III, VI, and VII, fibronectin, and glycosaminoglycans^{16, 42, 50, 54, 63, 81}, elevated expression of tissue inhibitor of metalloproteinases⁴⁴, and elevated expression of intercellular adhe-

sion molecules³¹. SSc and healthy skin fibroblasts also differ in their response to transforming growth factor (TGF)- β . SSc fibroblasts are less sensitive to the stimulatory effects of cytokines that regulate collagen production, such as TGF- β , oncostatin M (OSM) and interleukin (IL)-4^{13, 33, 49}.

The mechanism of fibroblast activation in SSc is currently unknown. However, many of the characteristics of SSc fibroblasts resemble those of healthy fibroblasts stimulated by TGF- β ^{53, 55}, suggesting that TGF- β is a key mediator of tissue fibrosis in SSc.

Fibrosis is a complex biological process involving an acute inflammatory response. Transient activation of fibroblasts to proliferate and produce elevated quantities of ECM is essential to fibrosis. Transient fibroblast activation is likely regulated by a variety of cytokines produced by infiltrating platelets, monocytes, T lymphocytes and other inflammation-associated

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cells⁹. Numerous *in vitro* and *in vivo* studies have suggested that some cytokines such as TGF- α and - β , platelet-derived growth factor (PDGF), epidermal growth factor (EGF), IL-1 α and - β , tumor necrosis factor (TNF)- α and - β , IL-4, IL-6, and OSM, regulate dermal fibroblast proliferation and ECM deposition^{3, 4, 10, 14, 15, 21, 65, 69}.

One of the most potent profibrotic stimuli to fibroblasts is TGF- β . The TGF- β superfamily includes the prototypic factor TGF- β 1 and other TGF, bone morphogenic protein and activin families. The family has a shared structure, similar signaling pathways and an overlap of biological effects. TGF- β is a 25 kDa homodimeric polypeptide which participates in a broad array of biological activities, such as normal development, wound repair and pathological processes². TGF- β regulates multiple cellular functions, including inhibition and stimulation of cell growth, cell death or apoptosis, and cellular differentiation. This review discusses the function, the signal transduction and the expression of TGF- β in the pathogenesis of fibrosis in SSc.

TGF- β Superfamily

The TGF- β superfamily includes the various forms TGF- β , bone morphogenic protein, nodals, activin, the anti-Mullerian hormone and many other structurally related factors⁵⁶. There are three mammalian isoforms of TGF- β , TGF- β 1, - β 2 and - β 3, which are structurally identical. TGF- β are secreted as latent precursor molecules as a large latent complex covalently binding between the latency-associated peptide region and the latent TGF- β -binding protein, which serves to bind TGF- β to the ECM and to enable its proteolytic activation⁶¹. Activation of TGF- β is a complex process involving conformational changes of latent TGF- β -binding protein, induced by either cleavage of the latency-associated peptide by various proteases such as plasmin, thrombin, plasma transglutaminase, or endoglycosylases, or by physical interactions of the latency-associated peptide with other proteins, such as thrombospondin-1, leading to the release of bioactive and mature TGF- β . TGF- β has been shown to increase expression of collagen types I, III VI, VII, and X, fibronectin, and proteoglycans⁵⁵. Stimulation of ECM production by TGF- β is further enhanced by its inhibitory effect on matrix degradation, decreasing the synthesis of proteases and increasing levels of protease inhibitors⁵⁵.

Enhanced expression of TGF- β has been well demonstrated in the tissue of fibrosis, especially in systemic

sclerosis⁴⁷. However, the production of TGF- β from fibroblasts from fibrosis was not increased^{37, 60}.

TGF- β Receptors

TGF- β exerts its multiple biological actions by the interaction with two transmembrane serine/threonine kinase receptors, types I and II, that are coexpressed by most cells, including mesangial and endothelial cells. Initiation of signaling requires the binding of TGF- β to TGF- β receptor type II, a constitutively active serine/threonine kinases, resulting in the recruitment and phosphorylation of TGF- β receptor type I to produce a heteromeric complex that activates downstream signaling pathways⁸⁰. To date, at least six distinct type I receptors of the TGF- β superfamily, named activin receptor-like kinases (ALKs), have been cloned. Type I receptors have a region between the transmembrane and the kinase domains containing a conserved TSGSGSG motif, called the GS domain. The phosphorylation of serine and threonine residues in the GS domain of the type I receptor by the type II receptor was shown to be essential for TGF- β signaling⁷⁰. TGF- β receptor type I is thought to determine the specificity of the cellular response to TGF- β , whereas TGF- β receptor type II is thought to determine the ligand specificity. TGF- β receptor type I alone is unable to bind TGF- β , and TGF- β receptor type II is unable to signal without TGF- β receptor type I⁸⁰. Betaglycan, a transmembrane proteoglycan also known as TGF- β receptor type III, allows high-affinity binding of TGF- β to TGF- β receptor type II, but does not itself transduce signal.

Enhanced expression of TGF- β receptors has been well demonstrated in fibrosis^{37, 43, 46, 68}. The overexpression of TGF- β receptors induced collagen transcription in cultured dermal fibroblasts⁴³, which indicates a potential role of an enhanced expression of TGF- β receptors in fibrosis. Furthermore, overexpression of the dominant negative TGF- β receptor type II improved liver fibrosis⁶⁶. The effects of overexpression of the dominant negative TGF- β receptor type II has also been investigated in SSc⁷⁹.

Smad Proteins

Following ligand activation, signaling from TGF- β receptor type I to the nucleus occurs predominantly by phosphorylation of cytoplasmic proteins belonging to the Smad family⁵⁷. TGF- β receptor type I specifically recognizes and phosphorylates the ligand-specific re-

ceptor activated Smad (R-Smad). R-Smads include Smad1, Smad5 and Smad8 downstream of bone morphogenic protein, and Smad2 and Smad3 downstream of TGF- β and activin. They all consist of two Mad-homology (MH) domains and a linker region⁷⁴. The N-terminal MH1 domain has DNA-binding activity, whereas the C-terminal MH2 domain has protein-binding properties. Phosphorylation of an R-Smad by TGF- β occurs principally on serine residues within the C-terminus⁵⁷. After phosphorylation by TGF- β receptor type I, R-Smads form heteromeric complexes with co-Smad, Smad4. The R-Smad/Smad4 complex is then translocated into the nucleus, where it functions as a transcription factor, binding DNA either directly or in association with other DNA-binding transcription factors⁵⁷. The Smad3/Smad4 complex binds to DNA via the consensus sequence "CAGAC"⁸³. However, Smad2 does not bind DNA directly, requiring other DNA-binding transcription factors and Smad4 to activate transcription in response to TGF- β . The inhibitory Smad (I-Smad), such as Smad6 or Smad7, binds to TGF- β receptor type I and prevents R-Smad phosphorylation and subsequent nuclear translocation of the R-Smad/Smad4 heterocomplex³⁸. Recent studies focused on the role of Smad in tissue fibrosis.

As for the type I collagen gene, which is excessively deposited in fibrosis, Smad has been reported to be involved in the transcriptional regulation of the gene. The human α 2(I) collagen gene is up-regulated at the transcriptional level in fibrotic lesion³³ and its promoter is known to be regulated by transcription factors Sp1/Sp3 and CCAAT-binding factor (CBF)^{28-30, 32, 35}. Earlier studies identified a TGF- β response element containing an Sp1 binding site in the human α 2(I) collagen⁴¹ and Sp1 was shown to be required for the response of the gene to TGF- β ²². Further studies showed the important role of the Smad3/Smad4 complex binding to the CAGACA motif near the Sp1 binding site in the human α 2(I) collagen promoter for the full TGF- β response^{7, 8, 59}. In addition, recent studies showed that synergistic cooperation between Sp1 and Smad3/Smad4 are required for the TGF- β response of the collagen gene^{40, 64, 84}. Further detailed analyses showed the cooperation of p300/CBP with Smad in the TGF- β response of the collagen gene^{19, 20}. Another study indicates that the interaction of Ets with Smad is also involved in the TGF- β response of the collagen gene¹¹. Furthermore, MMP-1 down-regulation by TGF- β is also shown to involve Smad3⁸².

Several studies showed that mice lacking Smad3 have a significantly reduced cutaneous as well as pulmonary fibrotic response^{17, 85}, which indicate an im-

portant role of Smad3 in fibrosis. Smad2, Smad3 and Smad4 were reported to contribute in liver fibrosis *in vitro* and *in vivo*^{39, 48, 72, 77}. Gene transfer of Smad7 was shown to prevent renal fibrosis⁷⁸. These not only suggest the potential role of Smad7 in fibrosis, but also may lead to the development of novel approaches for treating fibrosis.

The expression levels of Smad proteins were investigated in SSc fibroblasts²⁴. Some SSc fibroblasts expressed increased levels of Smad3 and Smad7, but others did not²⁴. However, another study reported a decreased expression of Smad7 in scleroderma fibroblasts¹². In that study, SSc fibroblasts showed increased phosphorylation of Smad2 and Smad3 compared with normal fibroblasts after TGF- β treatment¹². Further studies are needed to clarify these controversial results.

The Mitogen-Activated Protein Kinase

The mitogen-activated protein kinase (MAPK) is a major signaling system used by eukaryotic cells to transduce extracellular signals to intracellular responses⁷³. Three major subgroups of the MAPK superfamily members have been identified to date: the extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2), also known as p44/p42 MAPKs, respectively; the c-jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK); and the p38 MAPK⁷³.

The signal transduction cascades involved in the activation of MAPKs require a well-coordinated cascade of three protein kinase reactions that transduce signals by sequential phosphorylation and activation of the next kinase in their respective pathways. The MAPKs require dual phosphorylation at the threonine and tyrosine sites by MAPKs. The MAPKs are considered to play essential roles in the signal transduction of many biological events, such as the regulation of cell growth, differentiation, apoptosis and cellular responses to environmental stresses. ERK1 and ERK2 were shown to be involved mainly in fibroblast proliferation³⁴. The p38 MAPK was reported to be involved mainly in ECM production by fibroblasts³⁶.

TGF- β has been demonstrated in various cell types to activate each of the three major MAPK members. Recent studies showed that p38 MAPK is involved in type I collagen synthesis by TGF- β stimulation, which suggests the possible role of p38 MAPK in fibrosis⁷¹. However, p38 was reported not to be involved in the increased expression of the type I collagen gene in SSc fibroblasts⁷¹. Fibronectin synthesis was shown to be up-regulated via the JNK pathway²³. On the other hand,

activation of ERK was reported to inhibit type I collagen expression⁶⁷.

Connective Tissue Growth Factor

Connective tissue growth factor (CTGF) is induced by TGF- β and modulates fibroblast cell growth and ECM secretion^{5, 27}. CTGF has been demonstrated in experimental and human fibrosis^{58, 75}, in which its expression appears to correlate with the degree of fibrosis^{25, 26}. These results suggest that CTGF mediates many of the profibrotic actions of TGF- β .

CTGF is a 36 to 38 kDa cysteine-rich peptide containing 349 amino acids. It belongs to the CCN (CTGF, Cyr 61/cef 10, nov) family of growth factors. The gene for CTGF was originally cloned from a human umbilical endothelial cell cDNA library⁵. CTGF has been detected in endothelial cells, fibroblasts, cartilaginous cells, smooth muscle cells and some cancer cell lines. Earlier studies revealed that TGF- β 1 increases CTGF mRNA markedly in human foreskin fibroblasts²⁷. PDGF, EGF and FGF were also shown to induce CTGF expression, but their effects were only transient and weak²⁷.

CTGF has diverse bioactivities. Depending on cell types, CTGF was shown to trigger mitogenesis, chemotaxis, ECM production, apoptosis, and angiogenesis. In earlier studies, CTGF was noted to have mitogenic and chemotactic effects on fibroblasts⁵. CTGF was also reported to enhance the mRNA expressions of α 1(I) collagen, fibronectin, and α 5 integrin in fibroblasts¹⁸. The findings that TGF- β increases CTGF synthesis and that TGF- β and CTGF share many functions are consistent with the hypothesis that CTGF is a downstream mediator of TGF- β .

The mechanism by which CTGF exerts its effects on cells, especially its signal transduction, is still unclear. CTGF was reported to bind to the surface of fibroblasts with high affinity, and this binding was completed with recombinant PDGF BB⁵. This suggested that CTGF binds to a certain class of PDGF receptor or there is some cross reactivity of PDGF BB with CTGF receptors.

CTGF mRNA has been detected in fibroblasts of sclerotic lesions of patients with systemic sclerosis²⁶. In patients with localized scleroderma, CTGF mRNA was detected in fibroblasts in tissues of sclerotic stage more than of inflammatory stage, which suggested a close correlation between CTGF and fibrosis²⁵. Similar results were also obtained in keloid and other fibrotic diseases²⁵. Subsequently, expression of CTGF has been

reported in a variety of fibrosis, such as liver fibrosis, pulmonary fibrosis and heart fibrosis^{1, 6, 62, 86}. CTGF is also implicated in dermal fibrosis of scleroderma⁷⁶. However, the detailed role of CTGF in fibrosis is still unclear. Further studies are needed to clarify this point.

Conclusions

Great progress has been accomplished over the past several years in the understanding of TGF- β signaling. The identification of Smad proteins and other signal pathways, such as p38 MAPK, as downstream signal transduction mediators in TGF- β signaling has led to the elucidation of the molecular mechanisms of gene activation by TGF- β . However, further detailed analyses are required to clarify the pathogenesis of fibrosis in SSc.

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