

# Possible strategies for anti-fibrotic drug intervention in scleroderma

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**Abstract** There are no approved drugs for treating the fibrosis in scleroderma (systemic sclerosis, SSc). Myfibroblasts within connective tissue express the highly contractile protein  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) and are responsible for the excessive synthesis and remodeling of extracellular matrix (ECM) characterizing SSc. Drugs targeting myofibroblast differentiation, recruitment and activity are currently under consideration as anti-fibrotic treatments in SSc but thus far have principally focused on the transforming growth factor  $\beta$  (TGF $\beta$ ), endothelin-1 (ET-1), connective tissue growth factor (CCN2/CTGF) and platelet derived growth factor (PDGF) pathways, which display substantial signaling crosstalk. Moreover, peroxisome proliferator-activated receptor (PPAR) $\gamma$  also appears to act by intervening in TGF $\beta$  signaling. This review discusses these potential candidates for antifibrotic therapy in SSc.

**Keywords** PDGF · TGF $\beta$  · Endothelin · PPAR $\gamma$

## Introduction

In response to wounding, fibroblasts migrate into the wound and where they produce and remodel extracellular matrix (ECM). These fibroblasts are specialized forms of fibroblasts called myofibroblasts, which express the highly contractile protein  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) which

is organized into stress fibers connected to the ECM via specialized cell surface structures called ‘supermature’ focal adhesions (FAs) (Gabbiani 2003). The  $\alpha$ -SMA stress fibers contract, exerting tension on the ECM ultimately promoting the reorganization of the ECM into functional connective tissue. In normal tissue repair, myofibroblasts disappear from the lesion, likely due to apoptosis; however, myofibroblast persistence is believed to be responsible for scarring disorders and diseases including scleroderma (SSc, Chen et al. 2005). Thus understanding how myofibroblasts arise and function in SSc is likely to be important in understanding how to control the fibrosis in this disorder.

The precise origin of the myofibroblast in fibrotic lesions in SSc is unclear, but several mechanisms are possible (Hinz et al. 2007). One option is that myofibroblasts may arise due differentiation, in response to proteins such as transforming growth factor- $\beta$  (TGF- $\beta$ ) and endothelin-1 (ET-1), of resident fibroblasts within connective tissue (Leask 2008). However, clinical trials assessing the efficacy of drugs combating these pathways in SSc have been disappointing. However, it is possible that activation of microvascular pericytes, which normally express  $\alpha$ -SMA, is principal driving force at least of the cutaneous fibrosis in SSc (Rajkumar et al. 1999). Moreover, recent evidence has elucidated some the mechanisms underlying myofibroblast function. Thus, drugs targeting pericyte recruitment or myofibroblast function may represent the wave of the future in the development of antifibrotic therapies in SSc. This review discusses these issues.

## Transforming growth factor- $\beta$ (TGF- $\beta$ )

The three TGF $\beta$  isoforms (TGF $\beta$ 1, TGF $\beta$ 2 and TGF $\beta$ 3) are initially generated as part of a precursor complex

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containing latent TGF $\beta$ -binding proteins from which active TGF $\beta$  is released by proteolytic cleavage (Leask and Abraham 2004). Liberated, active TGF $\beta$  signals through a heteromeric receptor complex which consists of one type I and one TGF $\beta$  type II receptor. The TGF $\beta$  type I receptor [also known as activin linked kinase (ALK) 5] phosphorylates Smad2 and 3, which then bind Smad4 and translocate into the nucleus to activate gene transcription. The transcriptional cofactor p300 appears to act as a crucial mediator TGF $\beta$  action (see below, Ghosh and Varga 2007). TGF- $\beta$  induces fibroblasts to synthesize ECM by both inducing expression of ECM components such as collagen and fibronectin, but also by suppressing several matrix metalloprotenases and inducing tissue inhibitors of matrix metalloprotenases (Leask and Abraham 2004). Finally, TGF- $\beta$  causes fibroblasts to differentiate into myofibroblasts (Leask and Abraham 2004).

Ample *in vivo* evidence using animal models suggest that the canonical TGF $\beta$ /ALK5/Smad pathway mediates fibrogenesis (Leask and Abraham 2004). However, in human disease, the issue is slightly more complicated. The Smad-responsive element is dispensable for the heightened activity of the CCN2 promoter in SSc fibroblasts (Holmes et al. 2001). Similarly, targeting ALK5 using small molecule inhibitors reverses some fibrotic aspects of lesional dermal scleroderma fibroblasts (such as collagen overproduction), but critically does not reduce  $\alpha$ -SMA or CCN2 protein expression or  $\alpha$ -SMA stress fiber formation in this cell type (Chen et al. 2005, 2006; Ishida et al. 2006). Intriguingly, an anti-TGF $\beta$  antibody was recently tested in a clinical trial for SSc. This trial revealed that that antibody was ineffective, yet caused serious adverse effects (Denton et al. 2007) suggesting that broad inhibition of TGF $\beta$  might not be suitable in SSc. Alternatively, the apparent toxicity related to the study medication may have had more to do with the degree of underlying illness in this patient population than the therapeutic. Moreover, the lack of efficacy could easily have been related to the limited activity of this antibody to neutralizing only TGF $\beta$ 1, and not TGF $\beta$ 2 or TGF $\beta$ 3.

TGF $\beta$  also activates other 'non-canonical' pathways such as the MAP kinase pathways which appear to provide selectivity to TGF $\beta$  responses in cells (Santander and Brandan 2006; Liu et al. 2007; Leask et al. 2003). For example, focal adhesion kinase and JNK are required for myofibroblast differentiation and  $\alpha$ -SMA expression (Liu et al. 2007). Conversely, TGF $\beta$ -induced CCN2 expression is blocked by ERK inhibitors (Leask et al. 2003). Recently it was suggested that TGF $\beta$  might be able to activate Smad1 through endoglin [a coreceptor overexpressed in SSc fibroblasts (Leask et al. 2002)] and that this pathway contributes to CCN2 overexpression in SSc via ERK activation (Pannu et al. 2007). Targeting these alternative

pathways may also represent novel, viable anti-fibrotic approaches.

## PPAR $\gamma$

The transcription factor peroxisome proliferator-activated receptor (PPAR) $\gamma$  appears to control fibrogenesis by attacking the TGF $\beta$  pathway; PPAR $\gamma$  ligands, synthetic versions of which are currently in use to combat type II diabetes, can modify the progression of fibrosis (Sime 2008). PPAR $\gamma$  agonists such as rosiglitazone inhibits the ability of TGF $\beta$  to induce pulmonary fibroblasts to differentiate into myofibroblasts and produce collagen (Burgess et al. 2005), apparently via the transcriptional coactivator p300 (Ghosh et al. 2009). Rosiglitazone suppresses bleomycin-induced skin fibrosis (Wu et al. 2009), and PPAR $\gamma$  knockout mice show enhanced susceptibility to bleomycin-induced skin fibrosis (Kapoor et al. 2009). These results suggest that PPAR $\gamma$  agonists such as rosiglitazone may be useful in the future as antifibrotic agents in SSc.

## Endothelin (ET-1)

Endothelin-1, the significant endothelin in humans, is produced by a wide variety of cell types. Initially secreted as 212-amino acid precursor (prepro-ET-1), active ET-1, a 21-amino acid peptide, is released by proteolytic cleavages (Denton et al. 2006). ET-1 binds to two 7-transmembrane G-protein-coupled receptors (ET $_A$  and ET $_B$ ) (Denton et al. 2006; Clozel and Salloukh 2005). TGF $\beta$  induces ET-1 and ET-1 appears to act downstream of TGF $\beta$  to activate fibrogenic responses (Leask 2008). When added to fibroblasts, ET-1 induces ECM production and contraction, the former via both the ET $_A$  and ET $_B$  receptors and the latter by ET $_A$  (Shi-wen et al. 2004a, b). In a mouse model of lung fibrosis, ET receptor antagonists were found to be effective (Park et al. 1997).

Regarding SSc, lung fibroblasts constitutively overproduce ET-1 in a fashion independent of ALK5 and dependent on JNK (Shi-wen et al. 2006a) and blockade of the ET $_A$  and ET $_B$  receptors ET-1 receptors reverses the persistent fibrotic phenotype of SSc lung fibroblasts (Shi-wen et al. 2004a, 2007). Although endothelin receptor antagonism prevents new digital ulcers and improves mortality of SSc patients with pulmonary arterial hypertension (Korn et al. 2004; Denton et al. 2008), recent evidence suggests that no improvement was observed in exercise and other endpoints in patients with interstitial lung disease secondary to SSc (Siebold et al. 2010). However, despite these observations, a recent intriguing study suggests that ET receptor antagonism reduce the skin score in patients

with diffuse cutaneous SSc (Kuhn et al. 2010). Thus more studies on ET receptor antagonism in SSc may be warranted.

#### Connective tissue growth factor (CTGF, CCN2)

CCN2 is a prototypical member of the CCN (*cyr61*, *ctgf* and *nov*) family of matricellular proteins (Leask and Abraham 2004; Yeger and Perbal 2007). It has long been appreciated that CCN2 is an excellent surrogate marker for the severity of fibrosis including that of SSc (Moussad and Brigstock 2000; Leask et al. 2009). When initially identified, CCN2 was termed connective tissue growth factor; however, it now appears that CCN2 is a matricellular protein that promotes cellular adhesion via integrins and heparin sulfate containing proteoglycans, the precise identity of which changes depending on the cell type examined (Leask et al. 2009).

As CCN2 is potently induced by TGF $\beta$ , CCN2 has been considered in the literature to be a downstream mediator of this protein (Grotendorst 1997). However, the reality is somewhat more complicated. Although CCN2 itself is not a potent mediator of fibrogenesis, CCN2 acts as a cofactor to enhance fibrogenic action of TGF $\beta$  both in vivo and in vitro (Mori et al. 1999; Shi-wen et al. 2006b). In mouse embryonic fibroblasts which express CCN2, CCN2 is not required for TGF $\beta$  to activate Smads, but appears to help TGF $\beta$  activate adhesive signaling (Shi-wen et al. 2006b; Mori et al. 2008). In adult dermal fibroblasts which do not normally express CCN2, CCN2 is not required for TGF $\beta$  to induce type I collagen or  $\alpha$ -SMA mRNA (Liu et al. 2011) suggesting that CCN2 is not required for the ability of TGF $\beta$  to induce myofibroblast differentiation in adult cells.

Direct evidence has recently been provided illustrating that blocking CCN2 action alleviates bleomycin-induced lung and skin fibrosis. For example, an anti-CCN2 antibody or siRNA reduces bleomycin-induced lung fibrosis including collagen and  $\alpha$ -SMA overexpression (Ponticos et al. 2009). Moreover, a conditional knockout strategy has been used to show that CCN2 is required for bleomycin-induced skin fibrosis (Liu et al. 2011). The involvement of pericyte activation in deriving the fibrosis observed in SSc is fairly well-established (Rajkumar et al. 1999); essentially all of the myofibroblasts recruited in response to bleomycin stain positive for NG2, a marker of pericyte activation (Liu et al. 2010). These NG2/ $\alpha$ -SMA-positive cells are absent in CCN2 knockout mice exposed to bleomycin indicating that CCN2 is required for pericyte recruitment in fibrosis (Liu et al. 2010, 2011). In this regard it is interesting that integrin $\beta$ 1, to which CCN2 binds (Chen et al. 2004) and is essential for the fibrotic phenotype of SSc fibroblasts (Shi-wen et al. 2007), is also required for bleomycin-induced skin fibrosis (Liu et al. 2009). Recent data have emerged suggesting that fibrocytes (also known as bone

marrow stem cells), which have been considered to contribute to fibrosis in SSc, might in fact be derived from pericytes (Bianco et al. 2010).

Based on the available data, it is reasonable to conclude that targeting CCN2 may be a useful approach to combating the fibrosis seen in SSc. Moreover, as some members of the CCN family, notably CCN3 and CCN5 block the fibrogenic action of CCN2, it is possible that CCN3 and CCN5 may be used in the future to treat fibrotic diseases such as SSc (Riser et al. 2009; Leask 2009; Yoon et al. 2010).

#### Platelet-derived growth factor (PDGF)

PDGF consists of homo- or hetero-dimers (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD) that bind two different PDGF receptors,  $\alpha$  and  $\beta$  (Bonner 2004). PDGF promotes both fibroblast proliferation and migration and myofibroblast differentiation (Bonner 2004). In vivo evidence links PDGF to pericyte activation. Whereas PDGF $\beta$  receptors are expressed by activated microvascular pericytes in patients with early SSc, these receptors are not seen in abundance in late-stage scleroderma (Rajkumar et al. 1999). In knockout animals, inhibition of PDGF receptor  $\beta$  is linked with failure to recruit pericytes into the wound bed, but was not associated with a failure of myofibroblast differentiation (Rajkumar et al. 2006). In particular, the platelet-derived growth factor receptor (PDGFR)-beta inhibitor imatinib mesylate (which also inhibits c-abl) delayed wound closure, accompanied by a reduction in both myofibroblast numbers and fibronectin ED-A and collagen type I expression (Rajkumar et al. 2006). In a bleomycin-induced skin scleroderma in mice, dual inhibition of c-abl and PDGF receptor signaling using bdasatinib and nilotinib reduced bleomycin-induced dermal thickness, collagen deposition and the appearance of myofibroblasts, consistent with the notion that this drug might be used to treat SSc in the future (Akhmetshina et al. 2008). The precise relationship between CCN2 and PDGF in terms of pericyte activation and recruitment is unclear and warrants further study.

#### Future prospects and conclusions

Evidence thus far suggests a role for TGF $\beta$  and ET-1 in myofibroblast differentiation and for CCN2 and PDGF in pericyte recruitment. PPAR $\gamma$  agonists may be a new approach in SSc. Drugs targeting these pathways alone or in combination may be useful strategies to blocking the fibrosis observed in SSc.

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