Towards an anti-fibrotic therapy for scleroderma: targeting myofibroblast differentiation and recruitment

Andrew Leask

**Abstract**

**Background:** In response to normal tissue injury, fibroblasts migrate into the wound where they synthesize and remodel new extracellular matrix. The fibroblast responsible for this process is called the myofibroblast, which expresses the highly contractile protein α-smooth muscle actin (α-SMA). In normal tissue repair, the myofibroblast disappears. Conversely, abnormal myofibroblast persistence is a key feature of fibrotic diseases, including scleroderma (systemic sclerosis, SSc). Myofibroblasts can be derived from differentiation of local resident fibroblasts or by recruitment of microvascular pericytes.

**Clinical problem addressed:** Controlling myofibroblast differentiation and persistence is crucial for developing anti-fibrotic therapies targeting SSc.

**Basic science advances:** Insights have been recently generated into how the proteins transforming growth factor β (TGFβ), endothelin-1 (ET-1), connective tissue growth factor (CCN2/CTGF) and platelet derived growth factor (PDGF) contribute to myofibroblast differentiation and pericyte recruitment in general and to the persistent myofibroblast phenotype of lesional SSc fibroblast, specifically.

**Relevance to clinical care:** This minireview summarizes recent findings pertinent to the origin of myofibroblasts in SSc and how this knowledge might be used to control the fibrosis in this disease.

**Conclusions:** TGFβ, ET-1, CCN2 and PDGF are likely to cooperate in driving tissue repair and fibrogenic responses in fibroblasts. TGFβ, ET-1 and CCN2 appear to contribute to myofibroblast differentiation; PDGF appears to be involved with pericyte recruitment. Thus, different therapeutic strategies may exist for targeting the multisystem fibrotic disorder SSc.

**Introduction**

When connective tissue is damaged, fibroblasts migrate into the wound and begin to produce and remodel extracellular matrix (ECM) [1]. These events involve a specific sort of fibroblast termed the myofibroblast, a cell type which expresses the highly contractile protein α-smooth muscle actin (α-SMA) [1]. The α-SMA protein is organized into stress fibres which are connected to the ECM through specialized so-called 'supermature' FAs. As a result, these α-SMA stress fibers can contract and exert mechanical tension on the ECM causing it to be reorganized into functional connective tissue. Myofibroblast persistence is believed to be responsible for fibrotic diseases including scleroderma (SSc; Figure 1) [1,2].

Myofibroblasts have multiple origins, possibly appearing, for example, by differentiation of local resident fibroblasts in response to proteins or by the migration of microvascular pericytes into the lesional area [1] (Figure 1). Understanding how myofibroblasts may originate may be useful in understanding how to combat the fibrosis observed in SSc, and this is the subject of this minireview.

**Transforming growth factor-β (TGF-β)**

Extensive reviews on TGFβ signalling and the contribution of this pathway to experimentally-induced fibrosis have been published elsewhere (for example, see [3]).
Briefly, there are three TGFβ isoforms, TGFβ1, TGFβ2 and TGFβ3. These are initially generated as latent precursors from which active TGFβ is liberated by proteolysis, enabling it to bind to a heteromer complex consisting of one TGFβ type I [termed activin linked kinase 5 (ALK5) in the case of fibroblasts] and one TGFβ type II receptor. ALK5 phosphorylates Smad2 and 3, which can then bind Smad4, translocates into the nucleus and activate transcription. The ALK5/Smad pathway is generally responsible for TGFβ signalling in fibroblasts. In normal fibroblasts, ALK5 appears to mediate the fibrogenic activity of TGFβ [4]. Recombinant TGFβ is fibrogenic in both in vitro and in vivo models of fibrogenesis, acting through ALK5/Smad3 [3]. The contribution of this canonical TGFβ pathway on the persistent fibrotic phenotype of lesional SSC fibroblasts has been evaluated. Targeting ALK5, using small molecule inhibitors, reverses some aspects of lesional dermal scleroderma fibroblasts but, critically, does not reduce α-SMA or collagen type I expression [16,17], p38 appears to be not involved with the fibrogenic activity of TGFβ [4]. Constitutive TAK1 and JNK activation independent of ALK5 is seen in SSC fibroblasts [14,18]; thus, it is likely that signalling pathways are abnormally activated in SSC fibroblasts in a fashion independent of the canonical TGFβ pathway. It is likely that targeting FAK, JNK or TAK1 may be beneficial in alleviating the persistent SSC phenotype of dermal fibroblasts.

Endothelin-1 (ET-1)

There are 3 isoforms of endothelin, namely ET-1, ET-2, and ET-3 [12]. ET-1, the significant isoform in humans, is normally produced by a variety of cell types including endothelial cells, epithelial cells, bone marrow mast cells, macrophages, polymorphonuclear leukocytes, cardiomyocytes, and fibroblasts [12]. Initially, ET-1 is produced in the form of a 212-amino acid precursor (prepro-ET-1) which is enzymatically cleaved to form a biologically active 21-amino acid peptide [12]. ET-1 can then bind its two receptors (ETα and ETβ) [12].

ET-1 induces ECM production in fibroblasts through the ETα and ETβ receptors and MEK/ERK, whereas ET-1 induces myofibroblast formation, migration and ECM contraction through ETα and Akt/rac [19,20] (Figure 1). TGFβ induces ET-1 through JNK, and ET-1 is a downstream mediator of at least some of fibrotic responses of fibroblasts to TGFβ [18,21]. Constitutive ET signalling, operating through TAK1/JNK-dependent and ALK5-independent mechanisms, is responsible for the persistent myofibroblast phenotype of SSC lung fibroblasts [18]. Consistent with the notion that ET-1 contributes to fibrosis in the lung, ET receptor antagonism alleviates bleomycin-induced lung fibrosis and TGFβ-induced skin fibrogenesis in vivo [22,23]. However, the effect of ET
inhibition on SSc dermal fibroblasts has not yet been tested. TGFβ appears to also cooperate with ET-1 to promote myofibroblast differentiation [24]. The ET receptor antagonist bosentan may also be effective at reducing skin fibrosis in patients with SSc [25]. These results suggest that endothelin receptor antagonism might be considered as an appropriate therapy for the fibrosis in SSc, possibly in combination with anti-TGFβ regimens.

**CCN2**

CCN2, a member of the CCN family of matricellular proteins, is an excellent surrogate marker for the severity of fibrosis in SSc [26]. CCN2 signals through a variety of integrins and HSPGs or trkA and promotes cell adhesion and enhances adhesive signaling in response to extracellular ligands [27]. CCN2 is induced by both TGFβ and ET-1 and is considered to be a downstream mediator of these proteins [12]. The CCN2 promoter appears to possess independent TGFβ and ET-1 response elements [16,20] and thus may be a common downstream mediator of the fibrotic effects of these proteins, and thus may represent a more attractive target than either protein alone.

CCN2 acts as a cofactor with TGFβ to induce fibrogenesis but is not considered to be a potent fibrogenic agent on its own [28,29] (Figure 1). However, a recent study revealed chronic overexpression of CCN2 can lead to a fibrotic phenotype [30]. CCN2 is not required for all of the activities of TGFβ actions but appears to be required for TGFβ to maximally induce certain mRNAs including type I collagen and α-SMA and for TGFβ to promote cell adhesion to ECM [31] (Figure 1). CCN2 also can activate ERK by a syndecan 4-dependent mechanism [32]. A CCN2 response element exists in the COL1A2 promoter; blocking CCN2 action using an anti-CCN2 antibody or siRNA reduces some effects of bleomycin-induced lung fibrosis [33]. Overall, the available data suggest that targeting CCN2 may be useful in combating fibrosis in SSc.

**Platelet derived growth factor (PDGF)**

The PDGF family includes PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD. These bind two different PDGF receptors, α and β [34]. PDGF causes neutrophils, macrophages, fibroblasts and smooth muscle cells to proliferate and migrate into the wound site [34]. In vitro, PGDF stimulates fibroblasts to contract collagen matrices and differentiate into myofibroblasts [35].

Studies have revealed that PDGF levels are elevated in the bronchial lavage fluid of SSc patients, as well as elevated levels of the PDGF receptors on SSc fibroblasts [36-38]. Moreover, one study has been reported showing that autoantibodies stimulating the PDGF receptor may be a hallmark of SSc [39].

Mice treated with PDGFβ receptor- inhibitor imatinib mesylate, a tyrosine kinase inhibitor exhibit delayed cutaneous wound closure, diminished numbers of myofibroblast numbers and reduced collagen type I expression [40]. Imatinib mesylate did not prevent the myofibroblast differentiation in vitro but inhibited fibroblast proliferation and migration and appeared to principally act by blocking pericyte recruitment [40] (Figure 1). As a subset (~30%) of myofibroblasts in cutaneous mouse wounds are NG2-positive pericytes, this phenomenon is likely to lead to the reduction myofibroblasts in the wound [41]. Intriguingly, however, the majority (~70%) of myofibroblasts in bleomycin-induced skin fibrosis are derived from pericytes [42]. Tyrosine kinase inhibitors analogous to imatinib mesylate blocked bleomycin-induced dermal fibrosis in mice [43]. It is also interesting to note that imatinib mesylate also blocks the ability of TGFβ to activate Smad 1 and the transcription factor egr-1 via c-abl, emphasizing the potential of signalling crosstalk between PDGF and non-canonical TGFβ signalling and further suggesting that this inhibitor may also work by blocking non-canonical TGFβ signalling [44,45]. Given that pericytes contribute to myofibroblast activation in SSc [46], these results collectively suggest that perhaps targeting PDGF/c-abl might be of benefit in SSc through its ability to block pericycle recruitment. As such, anti-PDGF drugs may represent a different sort of approach to alleviating SSc than blocking growth factor differentiation of resident fibroblasts, which may be of lesser importance than pericycle recruitment in generating a source of myofibroblasts in fibrosis.

**Future Prospects and Conclusions**

TGFβ, ET-1, CCN2 and PDGF are likely to cooperate in driving tissue repair and fibrogenic responses in lesional SSc fibroblasts. However, these proteins seem to be responsible, for somewhat differing activities suggesting that combination therapies may be appropriate for SSc.

**Abbreviations**

α-SMA: α-smooth muscle actin; CTGF: connective tissue growth factor; ECM: extracellular matrix; ERK: extracellular signal-regulated kinase-1; ET: endothelin; FAK: focal adhesion kinase; HSPG: heparan sulphate-containing proteoglycan; PDGF: platelet derived growth factor; SSc: systemic sclerosis; TGFβ: transforming growth factor β; TAK: TGFβ activated kinase 1

**Competing interests**

The author declares that they have no competing interests.

**Acknowledgements**

AL is supported by the Canadian Institute of Health Research, the Ontario Thoracic Society and the Canadian Foundation for Innovation and is an Arthritis Society (Scleroderma Society of Ontario) New Investigator.

**Author Details**

Division of Oral Biology, Department of Dentistry, Schulich School of Medicine and Dentistry, University of Western Ontario, Dental Sciences Building, London ON N6A 5C1 Canada

Received: 3 December 2009 Accepted: 27 May 2010

Published: 27 May 2010
References

1. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbanini G. The myofibroblast: one function, multiple origins. Am J Pathol 2007, 170:1807-1816.

2. Chen Y, Shiwen X, van Beek J, Kennedy L, McLeod M, Renzoni EA, Bou-Gharios G, Woolridge DC, Edmondson A, Black CM. The myofibroblast: a key player in the fibrotic phenotype of scleroderma fibroblasts. Am J Pathol 2006, 167:169-171.

3. Denton CP, Abraham DJ. Transforming growth factor-beta and connective tissue growth factor: key cytokines in scleroderma pathogenesis. Curr Opin Rheumatol 2011, 23:505-511.

4. Kapouzi AM, Gaspar NJ, Wang Y, Damm D, Liu TW, Olyoung G, Quon D, Lam A, Munson K, Tran TT, Ma JY, Murphy A, Dugar S, Chakravarty S, Plotter AA, Wen FQ, Liu X, Rennard SI, Higgins LS. Transforming growth factor-beta receptor type I signaling to the fibrotic phenotype of scleroderma fibroblasts. Arthritis Res Ther 2006, 8:1309-1316.

5. Ishida W, Mori Y, Lakos G, Sun L, Shan F, Bowes S, Josiah S, Lee WC, Singh J, Ling LE, Varga J. Intracellular TGF-beta receptor blockade abrogates Smad-dependent fibroblast activation in vitro and in vivo. J Invest Dermatol 2006, 126:1733-1744.

6. Holmes A, Abraham DJ, SA S, Shiwen X, Black CM, Abraham DJ. Contribution of activin receptor-like kinase 5 (transforming growth factor beta receptor type I) signaling to the fibrotic phenotype of scleroderma fibroblasts. Arthritis Res Ther 2006, 8:1309-1316.

7. Shiwen X, Rodrigues-Pascua F, Lamas S, Holmes A, Howat S, Pearson JD, Shiwen X, Stanton L, Kennedy L, Renzoni EA, Bou-Gharios G, Denton CP, Black CM, Abraham DJ. Endothelin-1 promotes myofibroblast induction through the ETA receptor via a rac/phosphoinositide 3-kinase/Akt-dependent pathway and is essential for the enhanced contractile phenotype of fibroblastic endothelial cells. Mol Biol Cell 2004, 15:2707-2719.

8. Xu SW, Howat SL, Renzoni EA, Holmes A, Pearson JD, Dawash MR, Bou-Gharios G, Denton CP, du Bois RM, Black CM, Abraham DJ, Abraham DJ. Endothelin-1 induces expression of matrix-associated genes in lung fibroblasts through MEK/ERK. J Biol Chem 2004, 279:23098-23103.

9. Shiwen X, Kennedy L, Renzoni E, du Bois R, Denton C, Black C, Abraham D, Leask A, Abraham DJ. Endothelin-1 is a downstream mediator of TGFβ in fibroblasts. Arthritis Rheum 2007, 56:4189-4194.

10. Park SH, Saleh D, Giacch A, Michal RP. Increased endothelin-1 in bleomycin-induced pulmonary fibrosis and the effect of an endothelin receptor antagonist. Am J Respir Crit Care Med 1997, 156:600-608.

11. Lagares D, Garcia-Fernández RA, Jiménez CL, Magán-Marchal N, Buinsidiego O, Lamas S, Rodríguez-Pascual F. Endothelin 1 contributes to the effect of transforming growth factor beta 1 on wound repair and skin fibrosis. Arthritis Rheum 2010, 62:878-889.

12. Shephard P, Hinz B, Smola-Hess S, Meister JJ, Krieg T, Smola H. Dissecting the roles of endothelin, TGF-beta and GM-CSF on myofibroblast differentiation by keratinocytes. Thromb Haemost 2004, 92:262-274.

13. Kuhn A, Hauto M, Ruland V, Weber R, Verde P, Felder G, Ohmann C, Gensch K, Ruzica T. Effect of bosentan on skin fibrosis in patients with systemic sclerosis: a prospective, open-label, non-comparative trial. Rheumatology (Oxford) 2010 in press.

14. Takehara K. Hypothetical pathogenesis of systemic sclerosis. J Rheumatol 2003, 30:755-759.

15. Chen CC, Lau LF. Functions and mechanisms of action of CCN matricellular proteins. J Biochem Cell Biol 2009, 4:711-783.

16. Mori T, Kawara S, Shinohara M, Hayashi N, Kakunuma T, Igarashi A, Takigawa M, Nakashima N, Takehara K. Role and interaction of connective tissue growth factor with transforming growth factor-beta in persistent fibrous: a mouse fibrosis model. J Cell Physiol 2011, 198:153-159.

17. Bonnemann C, Martin G, Margetts PJ, Ask K, Robertson J, Gauldie J, Kolb M. Connective tissue growth factor is crucial to inducing a profibrotic environment in fibrosis-resistant BALB/c mouse lungs. Am J Respir Cell Mol Biol 2004, 31:510-516.

18. Sonnylal S, Shi-Wen X, Leoni P, Naff K, Van Pelt C, Nakamura H, Leask A, Abraham DJ, Bou-Gharios G, de Crombrugge B. Selective expression of connective tissue growth factor in fibroblasts in vivo promotes systemic fibrosis. Arthritis Rheum 2010, 62:1523-32.

19. Shiwen X, Stanton L, Kennedy L, Pala D, Chen Y, Howat SL, Renzoni EA, Carter DE, Bou-Gharios G, Stratton RJ, Pearson JD, Beier F, Lyons RM, Black CM, Abraham DJ, Leask A. CCN2 is necessary for adhesive responses to TGFβ1 in embryonic fibroblasts. J Biol Chem 2006, 281:10715-10726.

20. Kennedy L, Liu S, Shi-Wen X, Carter D, Lyons K, Black CM, Abraham DJ, Leask A. CCN2 is essential for fibroblast function. Exp Cell Res 2007, 313:952-964.

21. Ponticos M, Holmes AM, Shiwen X, Leonl P, Khan K, Rajkumar VS, Hoyles RK, Bou-Gharios G, Black CM, Denton CP, Abraham DJ, Leask A, Lindahl GE. Pivotal role of connective tissue growth factor in lung fibrosis: MAPK-dependent transcriptional activation of type I collagen. Arthritis Rheum 2009, 60:2142-2155.

22. Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. Cytokine Growth Factor Rev 2004, 15:255-273.

23. Rhee S, Grinnell F. P21-activated kinase 1: convergence point in PDGF- and LPA-stimulated collagen matrix contraction by human fibroblasts. J Cell Biol 2006, 172:423-432.

24. Ludwicka A, Ohba T, Trojanowska M, Yamakage A, Strange C, Smith EA, Leroy EC, Sutherland S, Silver RM. Elevated levels of platelet derived growth factor and transforming growth factor-beta 1 in bronchoalveolar lavage fluid from patients with scleroderma. J Rheumatol 1995, 22:1876-1883.

25. Kläreskog L, Gustafsson R, Scheynius A, Hallgren R. Increased expression of platelet-derived growth factor type B receptors in the skin of patients with systemic sclerosis. Arthritis Rheum 1990, 33:1534-1541.

26. Zheng XY, Zhang JZ, Tu P, Ma SQ. Expression of platelet-derived growth factor B-chain and platelet-derived growth factor beta-receptor in fibroblasts of scleroderma. J Dermatol Sci 1998, 18:90-97.
39. Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, Fraticelli P, Sambo P, Funaro A, Kazlauskas A, Avedimento EV, Gabrielli A: Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. N Engl J Med 2006, 354:2667-2676.

40. Rajkumar VS, Shiwen X, Bostrom M, Leoni P, Muddle J, Ivarsson M, Gerdin B, Denton CP, Bou-Gharios G, Black CM, Abraham DJ: Platelet-derived growth factor-beta receptor activation is essential for fibroblast and pericyte recruitment during cutaneous wound healing. Am J Pathol 2006, 169:2254-2265.

41. Kapoor M, Liu S, Huh K, Parapuram S, Kennedy L, Leask A: Connective tissue growth factor promoter activity in normal and wounded skin. Fibrogenesis Tissue Repair 2008, 1:3.

42. Liu S, Taghavi R, Leask A: Connective tissue growth factor is induced in bleomycin-induced skin scleroderma. J Cell Commun Signal 2010, 4:25-30.

43. Akhmetshina A, Dees C, Pileckyte M, Maurer B, Axmann R, Jüngel A, Zwerina J, Gay S, Schett G, Distler O, Distler JH: Dual inhibition of c-abl and PDGF receptor signaling by dasatinib and nilotinib for the treatment of dermal fibrosis. FASEB J 2008, 22:2214-2222.

44. Bhattacharyya S, Ishida W, Wu M, Wilkes M, Mori Y, Hinchcliff M, Leof E, Varga J: A non-Smad mechanism of fibroblast activation by transforming growth factor-beta via c-Abl and Egr-1: selective modulation by imatinib mesylate. Oncogene 2009, 28:1285-1297.

45. Pannu J, Asano Y, Nakaraki S, Smith E, Jablonska S, Blaszczyk M, ten Dijke P, Trojanowska M: Smad1 pathway is activated in systemic sclerosis fibroblasts and is targeted by imatinib mesylate. Arthritis Rheum 2008, 58:2528-2537.

46. Rajkumar VS, Sundberg C, Abraham DJ, Rubin K, Black CM: Activation of microvascular pericytes in autoimmune Raynaud's phenomenon and systemic sclerosis. Arthritis Rheum 1999, 42:930-941.

doi: 10.1186/1755-1536-3-8
Cite this article as: Leask, Towards an anti-fibrotic therapy for scleroderma: targeting myofibroblast differentiation and recruitment Fibrogenesis & Tissue Repair 2010, 3:8