**Signaling in fibrosis: Targeting the TGFbeta, endothelin-1 and CCN2 axis in scleroderma**

**Andrew Leask**

*CIHR Group in Skeletal Development and Remodeling, Division of Oral Biology, Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, Dental Sciences Building, London ON N6A 5C1*

**TABLE OF CONTENTS**

## 1. ABSTRACT

Fibrosis affects organs such as the skin, liver, kidney and lung and is a cause of significant morbidity. There is no therapy for fibrosis. Recent significant molecular insights into the signaling underlying fibrosis have been made. Transforming growth factor beta (TGFbeta) signaling is a major contributor to fibrogenesis. The signaling mechanisms through which TGFbeta induces fibrogenic responses have been under intense scrutiny. Moreover, the potent pro-fibrotic proteins endothelin-1 (ET-1) and CCN2 (connective tissue growth factor, CTGF) are believed to play an essential role in this process as downstream regulators or co-factors of TGFbeta signaling. This review summarizes these recent crucial observations with emphasis on the disease scleroderma.

## 2. INTRODUCTION

During normal connective tissue repair, mesenchymal cells such as fibroblasts proliferate and migrate into the wound, where they synthesize, adhere to and contract the extracellular matrix (ECM), resulting in wound closure. Should the normal tissue repair program fail to terminate, scarring results. Excessive scarring characterizes fibrotic diseases, which can affect individual organs, such as the kidney, liver, pancreas and lung, or be systemic, such as in diffuse systemic sclerosis (dSSc, scleroderma) (1-5). Fibrotic disease often culminates in organ failure and death (1,2). There is no treatment for organ fibrosis; identifying the signaling mechanism underlying fibrosis is essential to find appropriate targets around to base selective, anti-fibrotic therapies.

The cells ultimately responsible for the fibrotic phenotype including the disease scleroderma are mesenchymal cells resident within connective tissue. Cells from the fibrotic connective tissue of patients can be readily isolated and cultured. Fibroblasts present within scarred and unscarred areas of patients with scleroderma have been cultured and phenotypically and genotypically analyzed. Intriguingly, both of these cell types possess significantly elevated expression of pro-fibrotic proteins relative to normal healthy fibroblasts (6). Conversely, fibroblasts from scars differ phenotypically from their counterparts, possessing elevated abilities to adhere to and contract ECM relative to both healthy fibroblasts and fibroblasts isolated from unscarred areas of scleroderma patients (6). These results suggest that the excess production of matrix *per se* is insufficient to generate clinically-defined scars. Rather what is essential is to exert mechanical tension on the surrounding tissue (6). These results are consistent with the notion that the presence of a specialized form of fibroblast, called a myofibroblast, which expresses the highly contractile protein alpha-smooth muscle actin (alpha-SMA) is a key feature of scarring (2).

Although the precise origin of the myofibroblast in tissue repair and fibrosis is unclear, signaling downstream of cytokines is a critical driving force in their generation and activity. One of the major cytokines induced during the tissue repair is transforming growth factor-beta (TGF-beta) (7). TGF-beta induces fibroblasts to synthesize and contract ECM (8-10). Thus this cytokine has long been believed to be a central mediator in wound healing and fibrotic responses, including SSc. TGFbeta is required for fibrogenesis in acute animal models; however, the exact contribution of TGFbeta to pathological fibrosis phenotype is unclear. As TGFbeta plays many roles in normal physiology, including as a suppressor of the immune response and epithelial proliferation, broadly targeting TGFbeta signaling for the treatment of disease is anticipated to be problematic (8,10). Thus, much interest exists, from both clinical and pharmaceutical points of views, in identifying increased selectivity. Recently, it has been suggested that key pro-fibrotic proteins such as endothelin-1 (ET-1) and CCN2 (connective tissue growth factor (CTGF)) may operate in tandem with or downstream of TGFbeta in the fibrotic pathway (11). Thus, these may be better targets for anti-fibrotic intervention including in scleroderma.

## 3. TGFBETA SIGNALING

The basic scheme of TGFbeta signaling has been extensively reviewed, and the reader is referred elsewhere for details (9-12). Briefly, there are three TGFbeta isoforms TGFbeta1, TGFbeta2 and TGFbeta3. These are synthesized as latent precursors in a complex with latent TGFbeta-binding proteins. These latter proteins are removed by proteolysis. TGFbeta is then considered activated and can bind to a heteromeric receptor complex, consisting of one TGFbeta type I and one TGFbeta type II receptor. In the case of fibroblasts, the type I receptor is called ALK5 (activin linked kinase 5) (Figure 1). In the presence of TGFbeta ligand, the TGFbeta receptor I kinase phosphorylates the receptor-activated Smads (R-Smads),

Smad2 and 3, which are then able to bind the common mediator Smad, Smad4. The resultant complex can then translocate into the nucleus (Figure 1). The Smad3/Smad4 pair binds promoters at the Smad consensus sequence, CAGAC (13). Smad2, on the other hand, is not believed to bind DNA directly, but rather requires a nuclear DNA-binding protein of the family Fast (Fast-1) to bind DNA (14). Smads then recruit common transcription factors and cofactors to the promoter. A third group of Smad proteins, the inhibitory Smads Smad6 or Smad7, prevent R-Smad phosphorylation and subsequent nuclear translocation of R-Smad/Smad4 heterocomplexes; it appears that Smad7 competes for binding for Smad2 and Smad3 to the TGFbetaR1 (15). TGFbeta induces Smad7 expression through a consensus Smad binding element in its promoter; thus TGFbeta can suppress its own action (16) please include as well here Stopa et al; JBC.

## 4. TGFBETA AS A PRO-FIBROTIC PROTEIN

### 4.1. Genetic models

Evidence supporting the contribution of TGFbeta in fibrotic responses have principally been derived using acute *in vitro* or *in vivo* models. It should be noted that these models, although likely of direct importance in understanding wound healing, may not be of the most relevance to chronic fibrotic disease. It has long been known that mesenchymal cells exposed to TGFbeta acquire a fibrogenic phenotype, including ECM production and contraction (10). This phenotype exists as long as TGFbeta ligand is present; TGFbeta itself is incapable of generating a heritable fibrotic phenotype (17). *In vivo*, treatment of fetal wounds with TGFbeta promotes wound closure and scarring (18,19). Injection of TGFbeta either directly subcutaneously or into metal chambers, results in enhanced deposition of ECM (19-21). Incisional rat wounds treated with anti-TGFbeta antibodies or antisense oligonucleotides show a marked reduction in ECM synthesis and scarring (22,23). Following incisional wounding, animals lacking Smad3 show accelerated wound healing, reduced granulation tissue formation, increased epithelialization, and reduced inflammation possibly due to an impaired chemotactic response (24). Smad3-deficient mice display resistance to cutaneous fibrosis caused by radiation injury or bleomycin; however, there is a Smad3-independent component to this latter model (25, 26). Consistent with these observations, experiments using microarrays and Western blot analyses have compared gene expression profiles of fibroblasts taken from adult *Smad3*^{-/-} and *Smad3*^{+/+} mice. These results have shown that, in the absence of Smad3, TGFbeta was not able to induce gene transcription, including that of matrix and proadhesive proteins such as collagen and CCN2 (27-29).

### 4.2. Fibrotic models

In the case of kidney fibrosis, there is growing evidence indicating that an important source of renal interstitial myofibroblasts is the transdifferentiation of epithelial cells, a process known as epithelial mesenchymal transdifferentiation (EMT). Tubular epithelial cells can phenotypically and ultrastructurally become myofibroblasts (to editorial office: this sentence should be located elsewhere)
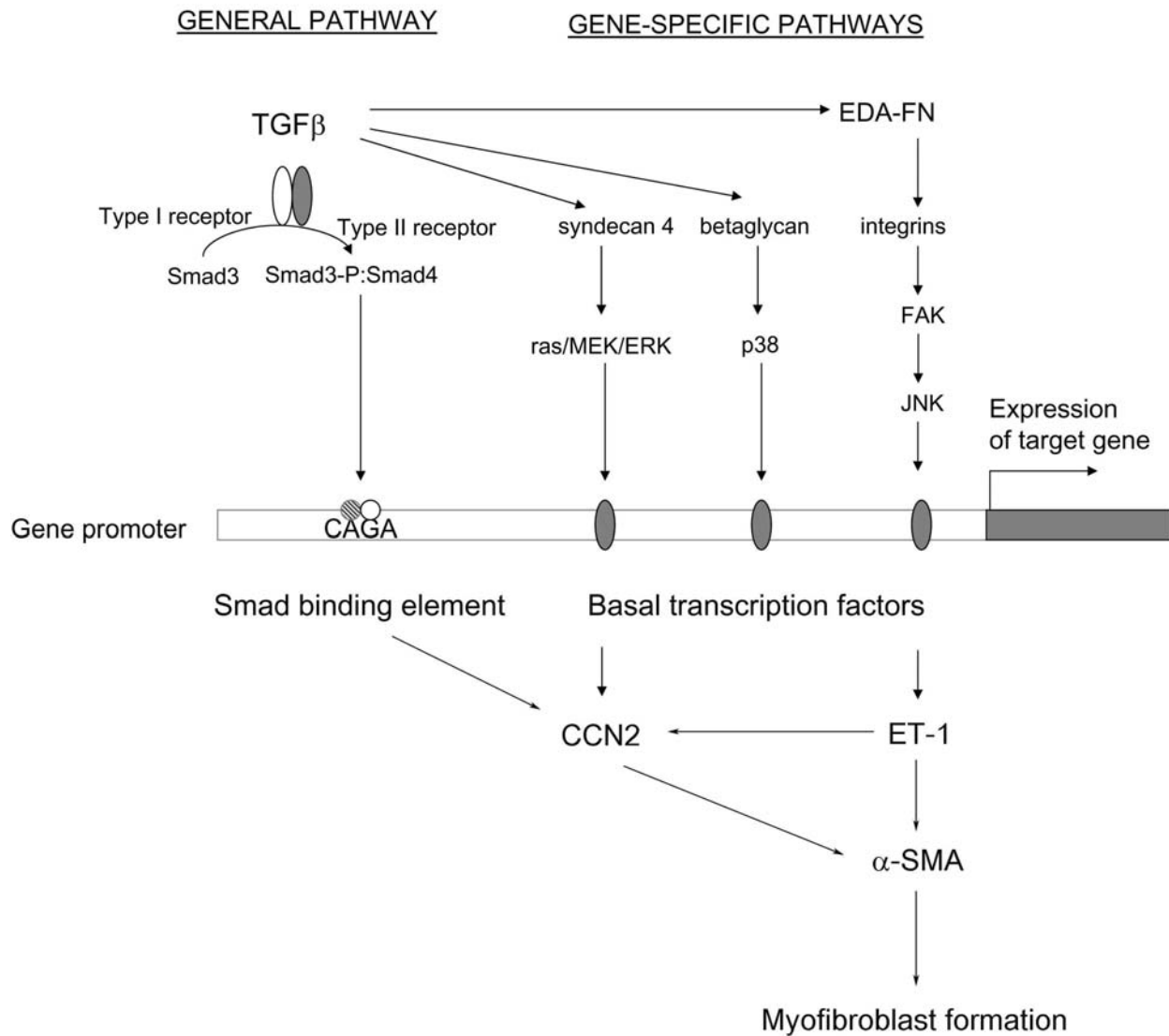
**Figure 1.** Schematic diagram of general and gene-specific TGFbeta signaling in fibroblasts resulting in myofibroblast formation. TGFbeta binds to the TGFbeta type I and type II receptors, activates Smad3, which activates target gene expression by binding the sequence CAGA. This pathway regulates virtually every TGFbeta responsive gene in fibroblasts, but not ET-1 in fibroblasts. Conversely, TGFbeta can act through MAP kinase cascades p38, ERK and JNK requiring betaglycan, syndecan 4 and integrin/FAK, respectively. ERK is required for CCN2 induction, whereas JNK is required for ET-1 production.

in response to TGF-β *in vitro* in a rat remnant kidney model and in progressive human kidney diseases but this does not occur in Smad3-deficient mice (30). Inhibition of Smad2/Smad3 phosphorylation by overexpression of Smad7 inhibited the EMT process induced by TGF-β and prevented TGF-β-induced collagen matrix production (31). Smad-independent signalling pathways, such as RhoA, extracellular signal-related kinase (ERK) 1, ERK2, and p38/mitogen-activated protein kinase (MAPK) have also been reported to play an essential role in the EMT (32-34). Moreover, TGFbeta receptor inhibitors given orally significantly reduced renal fibrosis and decreased the mRNA levels of key mediators of extracellular matrix deposition in kidneys (35). Similar results have been

observed in liver cirrhosis, in which activated hepatic stellate cells play a key role, and in lung fibrosis (36, 37). It is unclear what is the specific origin of the fibrotic cells in scleroderma, but they may arise from EMT, fibroblast differentiation in response to growth factors, pericytes or circulating cells (38).

In humans, TGFbeta signaling through the TGFbeta type I receptor (ALK5) contributes to the pathogenesis of scleroderma (systemic sclerosis, SSc). For example, the over-expression of type I collagen by SSc fibroblasts is blocked by an ALK5 TGFbeta antagonist (39). Similarly, the enhanced ECM contraction and adhesion observed in SSc fibroblasts depends on TGFbeta

type I receptor activity (6, 39). ALK5 inhibition also reduced basal collagen synthesis, adhesion and contraction in normal fibroblasts. These results strongly suggest that the contribution of autocrine TGFbeta and TGFbeta signaling to the phenotype of SSc fibroblasts arises from an exaggeration of processes normally operating in fibroblasts (6, 39). However, ALK5 inhibition had no significant effect on the CCN2, endothelin-1 or alpha-smooth muscle actin overexpression or assembly of an alpha-SMA stress fiber network by SSc fibroblasts (6, 39). These data support to the notions that autocrine TGFbeta signaling contributes to some features of the SSc phenotype. Although there is some evidence that activation of the Smad pathway plays a role in the phenotype of SSc fibroblasts, this activation may be independent of TGFbeta ligand (40). Intriguingly, in SSc there appears to be heightened expression of ALK5 at the expense of the TGFbeta type II receptor and that this phenotype may be responsible for the TGFbeta ligand-independent activation of collagen (41). These data suggest that altering the TGFbeta type I/type II ratio to normal might be a viable therapeutic strategy in SSc. These notions may explain the results recently obtained, where a neutralizing anti-TGFbetaı antibody was used in a multi-dose, multi-center clinical trial to treat SSc patients. In this study, the neutralizing anti-TGFbetaı antibody lacked anti-fibrotic ability, but resulted in increased mortality and serious adverse effects (42) presumably due to the pleiotropic nature of TGFbeta (8).

## 5. ANTI-TGFBETA STRATEGIES IN FIBROTIC DISEASE

### 5.1. Non-Smad signaling

As TGFbeta is a multifunctional protein, for example acting as a potent suppressor of the immune system and of epithelial proliferation, one concern about long-term administration of TGF-β antagonists, including antibody, soluble receptor, small molecular inhibitors, and siRNAs, is unwanted side-effects such as exacerbation of inflammation and increased risk of neoplasia (8,10). Based on these concerns, it is likely that targeting gene- or fibrosis-specific pathways, such as individual MAP kinase pathways, would be more useful anti-fibrotic strategies (11, 43). TGFbeta induces other signaling pathways, such as the MAP kinase pathways, to modify gene expression in a tissue-specific fashion (10) (Figure 1). These pathways include ras/MEK/ERK, which requires the heparan sulfate-containing proteoglycan (HSPG) syndecan 4 as a co-receptor (6), p38, which requires the HSPG betaglycan (17), and JNK which requires focal adhesion kinase and presumably integrin-based signaling (18, 19). These pathways act in a tissue??-specific fashion (6, 17-20). Of these, for example, ERK is important for the contraction of ECM as well as for the expression of CCN2 (connective tissue growth factor, CTGF) (6, 47-49) this is not a general mechanism – the tissue/physiological stage, where this was described, should be mentioned. Moreover, FAK/JNK mediates the induction of key pro-fibrotic proteins such as alpha–smooth muscle actin (SMA), type I collagen and endothelin-1 (ET-1) expression (45, 50, 51) (Figure 1).

### 5.2. Endothelin-1

Direct targeting of downstream mediators or co-factors of TGFbeta is also likely to be of benefit. The vasoconstrictory peptide endothelin-1 (ET-1) is normally produced by endothelial cells, but is overexpressed by fibrotic fibroblasts including in SSc (52, 53). In lung fibroblasts, TGFbeta induces ET-1 by a Smad-independent but ALK5- and JNK-dependent fashion (54; Figure 1) in fibroblasts. TGFbeta induces ET-1 in other cell types including endothelial cells (55). This induction is responsible for many of TGFbeta's profibrotic effects including alpha-SMA and CCN2 production and ECM contraction (56). Consistent with this notion, ET-1 induces ECM synthesis and contraction in fibroblasts (52, 53), and acts synergistically with TGFbeta (48,49). ET-1 signals through two receptors, called the A receptor and the B receptor. The ET-A receptor is responsible for alpha-SMA production and ECM contraction, whereas both the ET-A and ET-B receptors are necessary for ECM production (57, 58). Blockade of the endothelin receptors significantly reduces alpha-SMA, CCN2 and type I collagen overexpression and ECM contraction by SSc fibroblasts (52, 59). ET receptor blockade does not inhibit basal fibroblast activity (52, 59). Overexpression of ET-1 in SSc fibroblasts is ALK5-independent, relying on an autocrine ET-1 loop acting via JNK and TAK1 (53). However, TGFbeta can further induce ET-1 in this cell type (53). In patients with pulmonary arterial hypertension (PAH) exclusively related to connective tissue diseases, bosentan treatment? (description of what bosentan is, is missing) was associated with improvement or stability of clinical status and a 92% estimate for survival at 48 weeks, which was considered to be a significant achievement in this patient population (54). Thus ET-A/B receptor antagonism is well tolerated in patients, and is therefore likely to be of clinical benefit in alleviating at least one aspect of fibrosis in SSc, namely that of the persistently activated fibroblast and the fibrogenic responses to added TGFbeta.

### 5.3 CCN2

The CCN family of cysteine rich matricellular molecules possesses potent adhesive activity acting through integrins and HSPGs. These proteins share a common modular domain structure and modify responses to a variety of extracellular ligands via their adhesive properties. Of this family, the most studied perhaps is CCN2 (CTGF) (60) which is induced by TGFbeta in fibroblasts through protein kinase C and ras/MEK/ERK pathways via Smad and Ets-1 response elements in its promoter (27, 47-49,61). CCN2 is constitutively expressed by mesenchymal cells in development, and by kidney mesangial cells and endothelial cells and is characteristically overexpressed in fibrotic disease, including SSc, in a fashion correlating with severity of fibrosis (62). Anti-CCN2 strategies have been shown to improve the fibrosis in a variety of animal models, including kidney, heart and liver (63-65). As yet, anti-CCN2 strategies have not been attempted in models of SSc. CCN2 by itself is not potently fibrotic, but generates an environment favorable for fibrogenesis (66, 67). Consistent with this notion, CCN2 and TGFbeta synergistically promote sustained fibrosis in rodents, whereas application

of one ligand by itself results in transient fibrotic responses (21). CCN2-deficient embryonic fibroblasts respond to TGFbeta through the Smad pathway; however, these cells show impaired induction of adhesive signaling in response to TGFbeta, as visualized by the induction of FAK and Akt (68). CCN2-deficient fibroblasts show defective induction of alpha-SMA and type I collagen as well as adhesion to matrix (68). These results are consistent with the notion that the CCN2 receptors are integrins (69-71). What was surprising about these studies was that the lack of responses observed in CCN2-deficient embryonic fibroblasts were attributed to the absence of basal CCN2 expression, as the impaired TGFbeta responses did not rely on newly made CCN2 simplify this sentence (68). These results suggest that CCN2 acts as cofactor of TGFbeta to induce adhesive signaling in cells which are already activated and undergoing tissue remodeling (e.g. embryonic and fibrotic fibroblasts). In embryonic fibroblasts, CCN2 was also required for focal adhesion and actin stress fiber formation, as well as adhesion, cell migration and ECM contraction (72). These results suggest that anti-CCN2 therapy may be warranted in fibrotic conditions.

## 6. CONCLUSION

TGFbeta, ET-1 and CCN2 have been shown to mediate fibrogenic responses in fibroblasts. Genetic and pharmacological studies have suggested that although broad targeting of general TGFbeta signaling pathways may be problematic for treating chronic fibrotic disease due to the pleiotropic nature of TGFbeta it is now understood that several pathways operate downstream of TGFbeta and selectively contribute to fibrogenesis. Targeting these pathways, which include MAP kinase cascades and ET-1 or CCN2 production, is likely to be of benefit in combating persistent fibrotic disease such as in SSc.

## 7. ACKNOWLEDGEMENTS

## 8. REFERENCES

1.Eckes B, Zigrino P, Kessler D, Holtkotter O, Shephard P, Mauch C, Krieg T Fibroblast-matrix interactions in wound healing and fibrosis *Matrix Biol.* 19:325-32 (2000)

2.Gabbiani G The myofibroblast in wound healing and fibrocontractive diseases *J Pathol* 200:500-3 (2003)

3.Bedossa P, Paradis V. Liver extracellular matrix in health and disease *J Pathol.* 200: 504-15 (2003)

4.Fogo AB. Mesangial matrix modulation and glomerulosclerosis *Exp Nephrol.* 7: 147-59 (1999)

5.LeRoy EC, Trojanowska MI, Smith EA Cytokines and human fibrosis. *Eur Cytokine Netw.* 1:215-9 (1990)

6.Chen, Y., Shiwen, X., van Beek, J. Kennedy, L., McLeod, M., Renzoni, E.A., Bou-Gharios, G., Wilcox-Adelman, S., Goetinck, P.F., Eastwood, M., Black, C.M., Abraham, D.J., Leask, A Matrix contraction by dermal fibroblasts requires TGFbeta/ALK5, heparan sulfate containing proteoglycans and MEK/ERK: Insights into pathological scarring in chronic fibrotic disease *Am. J. Pathol.* 167: 1699-1711 (2005)

7.Kane CJ, Hebda PA, Mansbridge JN, Hanawalt PC Direct evidence for spatial and temporal regulation of transforming growth factor beta 1 expression during cutaneous wound healing *Cell Physiol.* 148:157-73 (1991)

8.McCartney-Francis NL, Frazier-Jessen M, Wahl SM TGF-beta: a balancing act. *Int Rev Immunol.* 16:553-80 (1998)

9.Massague J. TGF-beta signal transduction. *Annu Rev Biochem.* 67: 753-91 (1998)

10.Leask A, Abraham DJ TGF-beta signaling and the fibrotic response *FASEB J.* 18:816-27 (2004)

11.Leask A. Scar wars: Is TGFbeta the phantom menace in scleroderma? *Arthritis Res. Ther.*, 8 (4):213 (2006)

12.Roberts AB. TGF-beta signaling from receptors to the nucleus *Microbes Infect.* 1:1265-73 (1999)

13.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.* 1:611-7 (1998)

14.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)

15.Nakao A, Afrakhte M, Moren A, Nakayama T, Christian JL, Heuchel R, Itoh S, Kawabata M, Heldin NE, Heldin CH, ten Dijke P Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signalling. *Nature.* 389:631-5 (1997)

16.von Gersdorff G, Susztak K, Rezvani F, Bitzer M, Liang D, Bottinger EP. Smad3 and Smad4 mediate transcriptional activation of the human Smad7 promoter by transforming growth factor beta. *J Biol Chem.* 275:11320-6 (2000)

17.McWhirter A, Colosetti P, Rubin K, Miyazono K, Black C Collagen type I is not under autocrine control by transforming growth factor-beta 1 in normal and scleroderma fibroblasts. *Lab Invest.* 71:885-94 (1994)

18.Mustoe TA, Pierce GF, Thomason A, Gramates P, Sporn MB, Deuel TF Accelerated healing of incisional wounds in rats induced by transforming growth factor-beta *Science* 237: 1333-6 (1987)

19.Lin RY, Sullivan KM, Argenta PA, Meuli M, Lorenz HP, Adzick NS Exogenous transforming growth factor-beta amplifies its own expression and induces scar formation in a model of human fetal skin repair *Ann Surg.* 222:146-54 (1995)

20.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.* 13:1774-86 (1999)

21.Mori T, Kawara S, Shinozaki M, Hayashi N, Kakinuma T, Igarashi A, Takigawa M, Nakanishi T, Takehara K Role and interaction of connective tissue growth factor with transforming growth factor-beta in persistent fibrosis: A mouse fibrosis model. *J Cell Physiol.* 181:153-9 (1999)

22.Shah M, Foreman DM, Ferguson MW Neutralising antibody to TGF-beta 1,2 reduces cutaneous scarring in adult rodents. *J Cell Sci.* 107:1137-57 (1994)

23.Cordeiro MF, Mead A, Ali RR, Alexander RA, Murray S, Chen C, York-Defalco C, Dean NM, Schultz GS, Khaw PT Novel antisense oligonucleotides targeting TGF-beta inhibit *in vivo* scarring and improve surgical outcome *Gene Ther.* 10:59-71 (2003)

24.Ashcroft GS, Yang X, Glick AB, Weinstein M, Letterio JL, Mizel DE, Anzano M, Greenwell-Wild T, Wahl SM, Deng C, Roberts AB Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat Cell Biol.* 1:260-6 (1999)

25.Flanders KC, Sullivan CD, Fujii M, Sowers A, Anzano MA, Arabshahi A, Major C, Deng C, Russo A, Mitchell JB, Roberts AB Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. *Am J Pathol.* 160:1057-68 (2002)

26.Lakos G, Takagawa S, Chen SJ, Ferreira AM, Han G, Masuda K, Wang XJ, DiPietro LA, Varga J Targeted disruption of TGF-beta/Smad3 signaling modulates skin fibrosis in a mouse model of scleroderma. *Am J Pathol.* 165:203-17 (2004)

27.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.* 276:10594-601 (2001)

28.Yang YC, Piek E, Zavadil J, Liang D, Xie D, Heyer J, Pavlidis P, Kucherlapati R, Roberts AB, Bottinger EP Hierarchical model of gene regulation by transforming growth factor beta *Proc Natl Acad Sci U S A* 100:10269-74 (2003)

29.Verrecchia F, Chu ML, Mauviel A Identification of novel TGF-beta /Smad gene targets in dermal fibroblasts using a combined cDNA microarray/promoter transactivation approach. *J Biol Chem.* 276:17058-62 (2001)

30.Sato M, Muragaki Y, Saika S, Roberts AB, Ooshima A. Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction. *J Clin Invest.* 112:1486-94 (2003)

31.Lan HY, Mu W, Tomita N, Huang XR, Li JH, Zhu HJ, Morishita R, Johnson RJ. Inhibition of renal fibrosis by gene transfer of inducible Smad7 using ultrasound-microbubble system in rat UUO model. *J Am Soc Nephrol.* 14:1535-48 (2003)

32.Masaki T, Foti R, Hill PA, Ikezumi Y, Atkins RC, Nikolic-Paterson DJ. Activation of the ERK pathway precedes tubular proliferation in the obstructed rat kidney. *Kidney Int.* 63:1256-64 (2003)

33.Stambe C, Atkins RC, Tesch GH, Masaki T, Schreiner GF, Nikolic-Paterson DJ. The role of p38alpha mitogen-activated protein kinase activation in renal fibrosis. *J Am Soc Nephrol.* 15:370-9 (2004)

34.Fu P, Liu F, Su S, Wang W, Huang XR, Entman ML, Schwartz RJ, Wei L, Lan HY. Signaling mechanism of renal fibrosis in unilateral ureteral obstructive kidney disease in ROCK1 knockout mice. *J Am Soc Nephrol.* 17:3105-14 (2006)

35.Moon JA, Kim HT, Cho IS, Sheen YY, Kim DK. IN-1130, a novel transforming growth factor-beta type I receptor kinase (ALK5) inhibitor, suppresses renal fibrosis in obstructive nephropathy. *Kidney Int.* 70:1234-43 (2006)

36.de Gouville AC, Boullay V, Krysa G, Pilot J, Brusq JM, Loriolle F, Gauthier JM, Papworth SA, Laroze A, Gellibert F, Huet S. Inhibition of TGF-beta signaling by an ALK5 inhibitor protects rats from dimethylnitrosamine-induced liver fibrosis. *Br J Pharmacol.* 145:166-77 (2005)

37.Bonniaud P, Margetts PJ, Kolb M, Schroeder JA, Kapoun AM, Damm D, Murphy A,Chakravarty S, Dugar S, Higgins L, Protter AA, Gauldie J. Progressive transforming growth factor beta1-induced lung fibrosis is blocked by an orally active ALK5 kinase inhibitor. *Am J Respir Crit Care Med.* 171:889-98 (2005)

38.Abraham DJ, Eckes B, Rajkumar V, Krieg T. New developments in fibroblast and myofibroblast biology: implications for fibrosis and scleroderma. *Curr Rheumatol Rep.* 9:136-43 (2007)

39.Chen, Y., Shi-wen, X., Eastwood, M., Black, C.M., Denton, C.P., Leask, A, Abraham D.J. ALK5 (TGFß receptor type I) signaling contributes to the fibrotic phenotype of scleroderma fibroblasts. *Arthr. Rheum.* 54:1309-1316 (2006)

40.Mori Y, Chen SJ, Varga J. Expression and regulation of intracellular SMAD signaling in scleroderma skin fibroblasts. *Arthritis Rheum.* 48:1964-78 (2003)

41.Pannu J, Gore-Hyer E, Yamanaka M, Smith EA, Rubinchik S, Dong JY, Jablonska S, Blaszczyk M, Trojanowska M. An increased transforming growth factor beta receptor type I:type II ratio contributes to elevated collagen protein synthesis that is resistant to inhibition via a kinase-deficient transforming growth factor beta receptor type II in scleroderma. *Arthritis Rheum*. 50:1566-77 (2004)

42.Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, Silliman N, Streisand J, Powell J, Akesson A, Coppock J, Hoogen F, Herrick A, Mayes MD, Veale D, Haas J, Ledbetter S, Korn JH, Black CM, Seibold JR; Cat-192 Study Group; Scleroderma Clinical Trials Consortium. Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. *Arthritis Rheum*. 56:323-33 (2007)

43.Leask A. TGFbeta in cardiac fibrosis. *Cardiovasc. Res*. 74:207-12 (2007)

44. Santander C, Brandan E. Betaglycan induces TGF-beta signaling in a ligand-independent manner, through activation of the p38 pathway. *Cell Signal*. 18:1482-91 (2006)

45.Liu, S., Shi-wen, X., Kennedy, L., Pala, D., Carter, DE, Black, CM, Abraham, DJ, Leask, A. FAK is required for TGFbeta-induced JNK phosphorylation in fibroblasts: implications for acquisition of a matrix remodeling phenotype. *Mol. Biol. Cell*. 18: 2169-2178 (2007)

46.Thannickal VJ, Lee DY, White ES, Cui Z, Larios JM, Chacon R, Horowitz JC, Day RM, Thomas PE Myofibroblast differentiation by transforming growth factor-beta1 is dependent on cell adhesion and integrin signaling via focal adhesion kinase *J Biol Chem*. 278:12384-9 (2003)

47.Chen Y, Blom IE, Sa S, Goldschmeding R, Abraham DJ, Leask A. CTGF expression in mesangial cells: involvement of SMADs, MAP kinase, and PKC. *Kidney Int*. 62:1149-59 (2002)

48.Leask, A, Holmes, A., Black, CM, Abraham, D.J. CTGF gene regulation: requirements for its induction by TGFbeta in fibroblasts. *J. Biol. Chem*. 278, 13008-15 (2003)

49.Stratton R, Rajkumar V, Ponticos M, Nichols B, Shiwen X, Black CM, Abraham DJ, Leask A Prostacyclin derivatives prevent the fibrotic response to TGF-beta by inhibiting the Ras/MEK/ERK pathway *FASEB J*. 16:1949-51 (2002)

50**.** Hayashida T, Wu MH, Pierce A, Poncelet AC, Varga J, Schnaper HW. MAP-kinase activity necessary for TGFbeta1-stimulated mesangial cell type I collagen expression requires adhesion-dependent phosphorylation of FAK tyrosine 397. *J Cell Sci*. 120:4230-40 (2007)

51.Shi-wen, X., Rodrigues-Pascual, F., Lamas, S., Holmes, A., Howat, S., Pearson, J.D., Dashwood, MR., du Bois, RM, Denton, CP, Black, CM, Abraham, DJ, Leask A Constitutive ALK5-indepenent JNK activation contributes to endothelin-1 over-expression in pulmonary fibrosis. *Mol Cell Biol*. 26:5518-27 (2006)

52.Shi-Wen X, Chen Y, Denton CP, Eastwood M, Renzoni EA, Bou-Gharios G, Pearson JD, Dashwood M, du Bois RM, Black CM, Leask A, 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 fibrotic fibroblasts *Mol Biol Cell*. 15:2707-19 (2004)

53.Shi-wen X., Howat SL, Renzoni EA, Holmes A, Pearson JD, Dashwood MR, Bou-Gharios G, Denton CP, du Bois RM, Black CM, Leask A, Abraham DJ. Endothelin-1 induces expression of matrix-associated genes in lung fibroblasts through MEK/ERK. *J Biol Chem*. 279:23098-103 (2004)

54. Denton CP, Pope JE, Peter HH, Gabrielli A, Boonstra A, van den Hoogen FH, Riemekasten G, De Vita S, Morganti A, Berkani O, Guillevin L. Long-term effects of bosentan on quality of life, survival, safety and tolerability in pulmonary arterial hypertension related to connective tissue diseases. *Ann Rheum Dis*. doi:10.1136/ard.2007.079921 (2007)

55.Castañares C, Redondo-Horcajo M, Magán-Marchal N, ten Dijke P, Lamas S, Rodríguez-Pascual F. Signaling by ALK5 mediates TGF-beta-induced ET-1 expression in endothelial cells: a role for migration and proliferation. *J Cell Sci*. 120:1256-66 (2007)

56. Shi-wen, X., Kennedy, L., Renzoni, E., du Bois, R., Denton, C., Black, C., Abraham, D., Leask A. Endothelin-1 is a downstream mediator of the pro-fibrotic effects of TGFbeta in fibroblasts. *Arthritis Rheum*, 56:4189-94 (2007)

57. 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*. 92:262-74 (2004)

58.Horstmeyer A, Licht C, Scherr G, Eckes B, Krieg T. Signalling and regulation of collagen I synthesis by ET-1 and TGF-beta1 *FEBS J*. 272:6297-309 (2005)

59.Shi-wen᾽ X., Renzoni, EA, Kennedy, L., Howat, S, Chen, Y, Pearson, JD Bou-Gharios, G, Dashwood, MR, du Bois, RM, Black, CM, Denton, CP, Abraham, DJ, Leask, A. Endogenous endothelin-1 signaling contributes to type I collagen and CCN2 overexpression in fibrotic fibroblasts. *Matrix Biol*. 26:625-32 (2007)

60.Leask A Abraham DJ. All in the CCN family: essential signaling mediators emerge from the bunker. *J. Cell Sci.* 119:4803-10 (2006)

61.Van Beek JP, Kennedy L, Rockel JS, Bernier SM, Leask A The induction of CCN2 by TGFbeta1 involves Ets-1. *Arthritis Res Ther.* 8:R36 (2006)

62.Dziadzio M, Usinger W, Leask A, Abraham D, Black CM, Denton C, Stratton R N-terminal connective tissue growth factor is a marker of the fibrotic phenotype in scleroderma. *QJM.* 98:485-92 (2005)

63.Okada H, Kikuta T, Kobayashi T, Inoue T, Kanno Y, Takigawa M, Sugaya T, Kopp JB, Suzuki H. Connective tissue growth factor expressed in tubular epithelium plays a pivotal role in renal fibrogenesis. *J Am Soc Nephrol.* 16:133-43 (2005)

64.Uchio K, Graham M, Dean NM, Rosenbaum J, Desmouliere A. Down-regulation of connective tissue growth factor and type I collagen mRNA expression by connective tissue growth factor antisense oligonucleotide during experimental liver fibrosis. *Wound Repair Regen.* 12:60-6 (2004)

65.Lang C, Sauter M, Szalay G, Racchi G, Grassi G, Rainaldi G, Mercatanti A, Lang F, Kandolf R, Klingel K. Connective tissue growth factor: a crucial cytokine-mediating cardiac fibrosis in ongoing enterovirus myocarditis. *J Mol Med.* 86:49-60 (2008)

66.Bonniaud P, 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.* 31:510-6 (2004)

67.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.* 168:770-8 (2003)

68.Shi-Wen X, Stanton L, Kennedy L, Pala D, Chen Y, Howat SL, Renzoni EA, Carter DE, Bou-Gharios G, Stratton R, Pearson JD, Beier F, Lyons KM, Black CM, Abraham DJ, Leask A CCN2 is necessary for adhesive responses to TGFbeta 1 in embryonic fibroblasts *J Biol Chem.* 281:10715-26 (2006)

69.Chen Y, Abraham DJ, Shi-Wen X, Pearson JD, Black CM, Lyons KM, Leask A. CCN2 (connective tissue growth factor) promotes fibroblast adhesion to fibronectin. *Mol Biol Cell.* 15:5635-46 (2004)

70.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.* 19:2958-66 (1999)

71. Gao R, Brigstock DR. Connective tissue growth factor (CCN2) induces adhesion of rat activated hepatic stellate cells by binding of its C-terminal domain to integrin alpha (v)beta (3) and heparan sulfate proteoglycan. *J Biol Chem.* 279:8848-55 (2004)

72. Kennedy, L., Liu, S., Shi-wen, X., Carter, D., Lyons, K., Black, C.M., Abraham, D.J, Leask A. CCN2 is essential for fibroblast function. *Exp. Cell Res* 313: 952-964 (2007)

**Send correspondence to:** Andrew Leask, CIHR Group in Skeletal Development and Remodeling, Division of Oral Biology, Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, Dental Sciences Building, London ON N6A 5C1, Tel: 519-661-2111 Ext 81102, Fax: 519-850-2459, E-mail: Andrew.Leask@schulich.uwo.ca

http://www.bioscience.org/current/volE1.htm