Universiteit Leiden
The Netherlands

Inhibition of signaling cascades in osteoblast differentiation and fibrosis

Krause, C.

Citation
Krause, C. (2011, October 5). *Inhibition of signaling cascades in osteoblast differentiation and fibrosis*. Retrieved from https://hdl.handle.net/1887/17892

Version:	Corrected Publisher's Version
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Note: To cite this publication please use the final published version (if applicable).

Signal transduction cascades controlling fibrosis in Dupuytren's Disease

Krause C.

2011

Chapter 7

Signal transduction cascades controlling fibrosis in Dupuytren's Disease

7.1 Dupuytren's Disease

Dupuytren's Disease (DD) is a progressive and irreversible fibro-proliferative disorder of unclear etiology and pathogenesis that affects the palmar aponeurosis and rarely the plantar or penis fascia. The progression of the disease mainly causes permanent flexion contracture of the digits [38]. It is named after Baron Guillaume Dupuytren, the surgeon who described for the first time in 1834 the disease and the corresponding operation to correct the affliction. Treatment to date is mostly surgical, but there is a high recurrence rate [15].

7.1.1 Epidemiology

DD predominantly affects older men of northern European descent and is generally uncommon in the black population and in Asia [24, 30, 13]. The overall incidence of DD varies from 2% to 42% [24, 19]. Men are six times more likely than woman to present with the condition, and children are rarely affected [15]. The incidence rises dramatically for men in their fifth decade and for woman in their sixth decade [35]. Both hands are involved with equal frequency, and the condition can occur bilateral [19]. It is usually more severe in one hand, but there is no relation to handedness [28]. The ring finger; the index finger and the thumb are rarely involved [38, 19]. Disease recurrence is equal in both sexes [35].

7.1.2 Etiology

The etiology of DD is diverse and not at all uniform. A genetic susceptibility to the disease is uniquely found in the white population [37, 53]. The highest prevalence can be found in Northern Scotland, Iceland, Norway and Australia [23, 18, 29, 20]. An autosomal dominant pattern of inheritance with incomplete penetrance has also been

reported [24]. Additionally, chronic alcoholic patients with subsequent liver cirrhosis have a prevalence of 66% and alcoholic patients without cirrhosis of 27% [11]. Furthermore, a strong association of DD with diabetes mellitus has been recorded, with a prevalence of 3% to 32%, and an average of around 20% [33]. Epilepsy, trauma, chronic pulmonary disease and rheumatoid arthritis are additional etiological factors for DD [19].

7.1.3 Pathogenesis

Fibrosis is defined as the result of abnormal development of excess fibrous connective tissue formation in organs which including skin, heart, lung, kidney and vessels [16, 51]. Fibrosis is not always pathological and fibrotic processes are key events in the normal wound healing process [12]. However, uncontrolled fibrosis which most commonly is induced through chronic inflammation may result in organ dysfunction [12]. Although the mechanism of fibrosis varies in each organ, as a common pathogenesis,transiently activated extracellular matrix (ECM)-producing cells that proliferate and produce increased amount of ECM are often detected [1]. The activation of cellular proliferation, migration, adhesion and extracellular matrix production is governed by growth factors among which transforming growth factor-β (TGF-β), connective tissue growth factor (CTGF), interleukin-1 (IL-1), IL-4, platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and tumor necrosis factor-α (TNF-α) are reported to have an impact on fibrotic processes in DD [21].

DD begins as a nodule and slowly progresses to contracture of the fingers (Figure 2) [46]. Disease progression is classified using a grading system (Table 1) [46].

Grading	Clinical Presentation
Grade 1	a thickend nodule and a band in the palmar aponeurosis; the band progresses to skin tethering, puckering and pitting
Grade 2	a peritendinous band and extention of the affected finger is limited
Grade 3	flexion contractur

Table 1: Grades of Dupuytren's Disease

The disease usually progresses in severity, although approximately 10% of the cases regress without treatment [46, 19]. Histologically, the cords of DD consist of a dense collagenous matrix containing fibroblasts, arranged along the longitudinal lines of stress [46]. Nodules, which occur within the cords, contain a high concentration of intensively proliferating cells, most of which are highly specialized myofibroblasts that are rich in α-smooth muscle actin (α-SMA) [46]. Initially, there is a proliferative stage characterized by an increase in myofibroblasts [46]. The subsequent involutional stage involves alignment of these cells along the longitudinal lines

of tension [46]. The abnormal tissue contains increased levels of active growth factors, glycosaminoglycons, collagen [46]. It has been suggested that DD is a result of local hypoxia and chronic ischaemia [46, 19]. High levels of free radicals have also been found, which can induce fibroblast proliferation *in vitro* [46, 19, 21].

7.2 Treatment of Dupuytren's Disease

Up to date there is no effective cure for DD but most patients gain significant functional improvement after corrective surgery (Figure 2) [21, 46, 19]. Many surgical operations of different magnitude are available and range form limited fasciectomy (removal of involved fascia), fasciotomy (simple division of contracted tissue) to hand amputation [8]. The high recurrence rate of DD makes a strong demand on more effective treatment strategies and led surgeons and researchers to investigate alternative non-surgical treatment options. Injection of steroids, radiotherapy or clostridial collagenase have been shown to prevent disease progression for earlier stage DD [19]. Nevertheless, there is a high rate of recurrence which is regardless of the technique [21, 46, 19, 8].

7.3 Growth factor signaling in Dupuytren's Disease

7.3.1 TGF-β signaling

TGF-β is known to control the proliferation, migration, differentiation and survival of many different cell types [50]. Dysregulation of TGF-β signaling pathways is associated with serious human diseases such as fibrosis [16, 51]. There are three TGF-β isoforms know: TGF-β1, TGF-β2 and TGF-β3 [50]. TGF-β family members activate canonical signaling cascades via binding to specific sets of heteromeric receptor complexes, which comprise of type II and type I serine/threonine kinase receptors. Up to date seven different human type I receptors, also termed activin receptor-like kinases (ALKs)-1 to ALK-7, and five type II receptors have been identified. Additionally, accessory receptors, so called type III receptors, include Endoglin and β-glycan [25, 27, 41, 14].

TGF-β signals via TGF-β receptor type II in complex with ALK-5 [42]. Interestingly, the mode of binding of TGF-β2 differs from other TGF-β isoforms. TGF-β2 binds the TGF-β receptor type II with 100 to 1000 fold lower affinity, and a differing sequential binding pattern was reported [26]. In fact, TGF-β2 is bound to β-glycan which presents TGF-β2 to the type II receptor [26]. Activin and nodal, two additional members of the TGF-β superfamily, signal via activin receptor type II (ActRII) or ActRIIB and either ALK-4 or ALK-7 [42, 50]. Upon activation, type I receptors activate through phosphorylation their intracellular mediators, the receptor-activated (R)-Smads, namely Smad-2 and Smad-3. In complex with the common-mediator (Co-) Smad, Smad-4, the signal is transduced into the nucleus where the complex in syn-

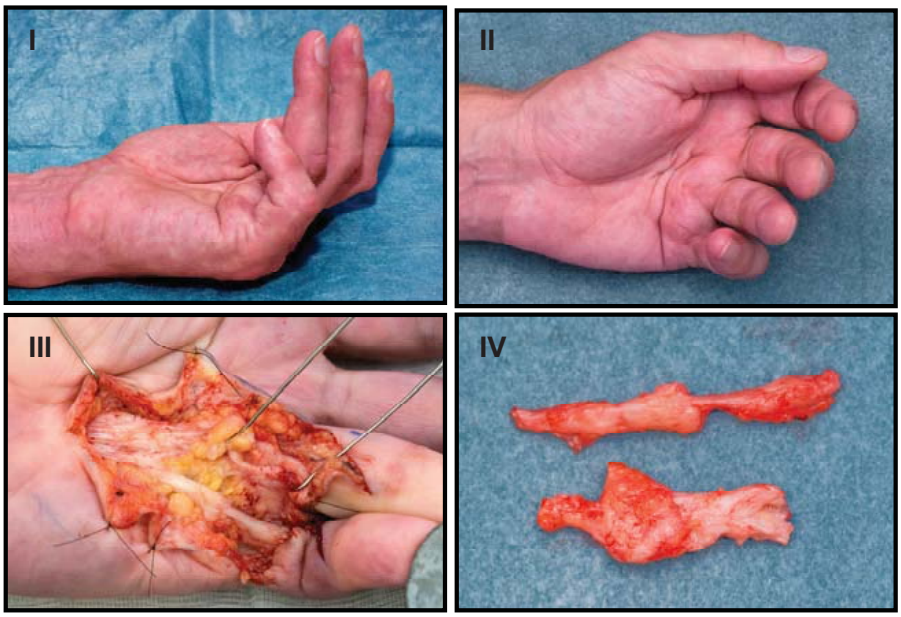

Figure 1: Treatment of Dupuytren's Disease. I-II Preoperative pictures of a Dupuytren's cord, causing metacarpal and proximal inter-phalangeal contracture of the little finger; III intra-operative view of the cord; IV resected specimens showing the cord (top) and the nodule (bottom).

ergy with other transcription factors regulates transcriptional activation or repression of a diverse array of target genes [50, 25, 27, 41, 14].

In addition to the canonical Smad pathway, TGF-β can also signal via Smad-independent pathways. Among those are the rapid activation of mitogen activated protein kinases (MAPK), such as extracellular-signal regulated kinase-1/2 (ERK-1/2), p38 MAPK, c-jun N-terminal kinase (JNK), Rho-like small GTPase pathways, and phosphatidylinositol-3-kinase (PI3K/AKT) pathways [32, 56]. Signals are propagated through auto-phosphorylation of TGF-β receptor type II at tyrosine residues, which leads to trans-tyrosine phosphorylation of ALKs and subsequent activation of down-stream targets [32, 56, 52]. It becomes more and more evident that non-canonical, non-Smad pathways co-operate with the canonical, Smad pathway to determine the final outcome of cellular responses to TGF-β [56].

TGF-β is a pro-fibrotic factor that acts on several stages of fibrotic disease progression. In patients with DD, upregulated TGF-β1 and TGF-β3 mRNA and protein has been detected in all three phases of DD. TGF-β2 on the other hand, was only de-

tected in the proliferative and involutional but not the residual phase of DD. TGF-β is thought to be secreted by monocytes, macrophages and nodule derived cells. TGF-β is thought to be predominantly responsible for the fibrotic progression of DD [21]. TGF-β is secreted in an inactive complex with latency associated peptide (LAP) which is proteolytically cleaved and locally activated through thrombospondin-1, matrix metalloproteases, $\alpha v\beta 6$ integrin, plasmin or cathepsin. Once activated TGF-β initiates, in synergy with other growth factors, expression of a set of genes among which *laminin*, *fibronectin*, *proteoglycan*, *heparan sulfate* and *type-I, -III, -IV collagen* contribute towards sustained extracellular matrix deposition [16, 43, 22]. Thereby TGF-β does not only directly trigger ECM deposition but also initiates *connective tissue growth factor* (CTGF), *plaminogen activator inhibitor-1 (PAI-1)* and *inhibitor of metalloproteases (TIMPs)* expression. CTGF further contributes to ECM accumulation through up-regulated expression of fibronectin and type-III and-IV collagen. PAI-1 and TIMPs supress plasminogen activator and MMPs,respectively, whith the net effect of ECM accumulation [43, 22]. An additional cellular patho-mechanism, whereby the TGF-β signaling pathway contributes to the progression of DD is the induction of α-smooth muscle actin (α-SMA) expression which plays an essential role in DD fibroblast contractility and finger flexion, respectively [21]. A recent study demonstrated the association between DD and a polymorphism on the 5'-untranslated region of the *TGF-β2* gene: the variability of the gene could modulate myofibroblast activity, proliferation and induction of ECM. This results in fibromatous tissue [7].

7.3.2 BMP signaling

Bone Morphogenetic Proteins (BMPs) which belong to the TGF-β superfamily, represent one of the major classes of metabologens. More then 15 BMPs have been identified which all have in common that they transduce a signal via binding as dimers to type II and type I serine/threonine receptor kinases, forming an oligomeric complex. Thus far four BMP type I receptors (ALK-1 – ALK-6) and three BMP type II receptors (BMPR-II), ActRII and ActRIIB have been described. Upon oligomerization, the constitutive active type II receptor phosphorylate and consequently activates the type I receptor. Subsequently, the activated type I receptor phosphorylates BMP R-Smads, namely Smad1, -5 and -8. These R-Smads associate with co-Smad, Smad4, and translocate into the nucleus, where they together with other transcription factors regulate distinct target gene expression. Like TGF-β, BMPs have the ability to use the canonical, Smad pathway but additionally the non-canonical, Smad-independent p38, MAPK/ERK-1/2, JNK and PI3K pathway to relay a signal to the nucleus [31].

It is up to date not known whether BMPs play a role in DD. Compared to normal fascia-derived cells, Dupuytren-derived cells do not express BMP-4 and exhibit decreased BMP-6 and BMP-8 expression [39]. A previous study found that there is decreased BMP receptor expression and, apparently, reduced BMP responsiveness in DD tissue [39]. Even though BMPs, in particular exogenous provided BMP-7 has been shown to antagonize TGF-β induced fibrosis in the kidney, lung and liver no

investigations have been conducted to investigate the role of BMPs in TGF-β driven DD [54, 49, 55].

7.3.3 PDGF signaling

Platelet-derived growth factor (PDGF) is a potent mitogen that regulates cell growth and division. PDGF is a dimeric glycoprotein that is composed of two similar chains, i.e. PDGF-A, PDGF-B, PDGF-C and PDGF-D, or a combination of two distinct chains, e.g. PDGF=AB. The receptor for PDGF, PDGFRα and PDGFβ are classified as receptor tyrosine kinases (RTKs) and bind with varying affinity to the distinct PDGF ligands. Upon activation by PDGF, the receptors dimerise and auto-phosphorylation serves as a mediator for binding of cofactors that initiate signal transduction cascades such as RAS-RAF-MEK-ERK-1/-2 MAP kinase pathway [40].

In DD increased PDGF-B and PDGF receptor expression was detected. In particular in the proliferative and involutional stage of DD enhanced PDGF binding to the myofibroblast cell membrane was described. Upon PDGF challenge Dupuytren derived cells demonstrate an increase in proliferation. Additionally, PDGF stimulate collagen production and its increased PDGF expression was found to coincide with increased ECM deposition [21, 5].

7.3.4 bFGF signaling

Basic Fibroblast Growth Factor (bFGF), alias FGF-2 or FGF-β, is a member of the fibroblast growth factor family, which plays a crucial role in angiogenesis, wound healing and embryonic development. In humans, 22 members of the family have been identified, all of which are structurally related signaling molecules. The FGFs are heparin-binding proteins and interact with the cell-surface associated heparin sulfate proteoglycans. Additionally FGF ligands bind to fibroblast growth factor receptors (FGFR), a family comprised of 4 members: FGFR-1, FGFR-2, FGFR-3 and FGFR-4. A signaling complex is believed to be a ternary complex formed between two identical FGF ligands, two identical FGFR subunits and either one or two heparin sulfate chains. Upon activation, the RAS-RAF-MEK-ERK-1/-2 MAP kinase pathway transduces a signal into the nucleus which can lead to activation and repression of target genes [48].

FGFR and bFGF mRNA and protein are highly expressed in Dupuytren derived cells and tissue. Upon exogenous bFGF treatment normal fascia derived fibroblasts and Dupuytren derived cells respond with increased proliferation rates. Thereupon, Collagen production was elevated 20% in Dupuytren derived cells and 30% inhibited in normal fascia by FGF treatment [21, 9, 47].

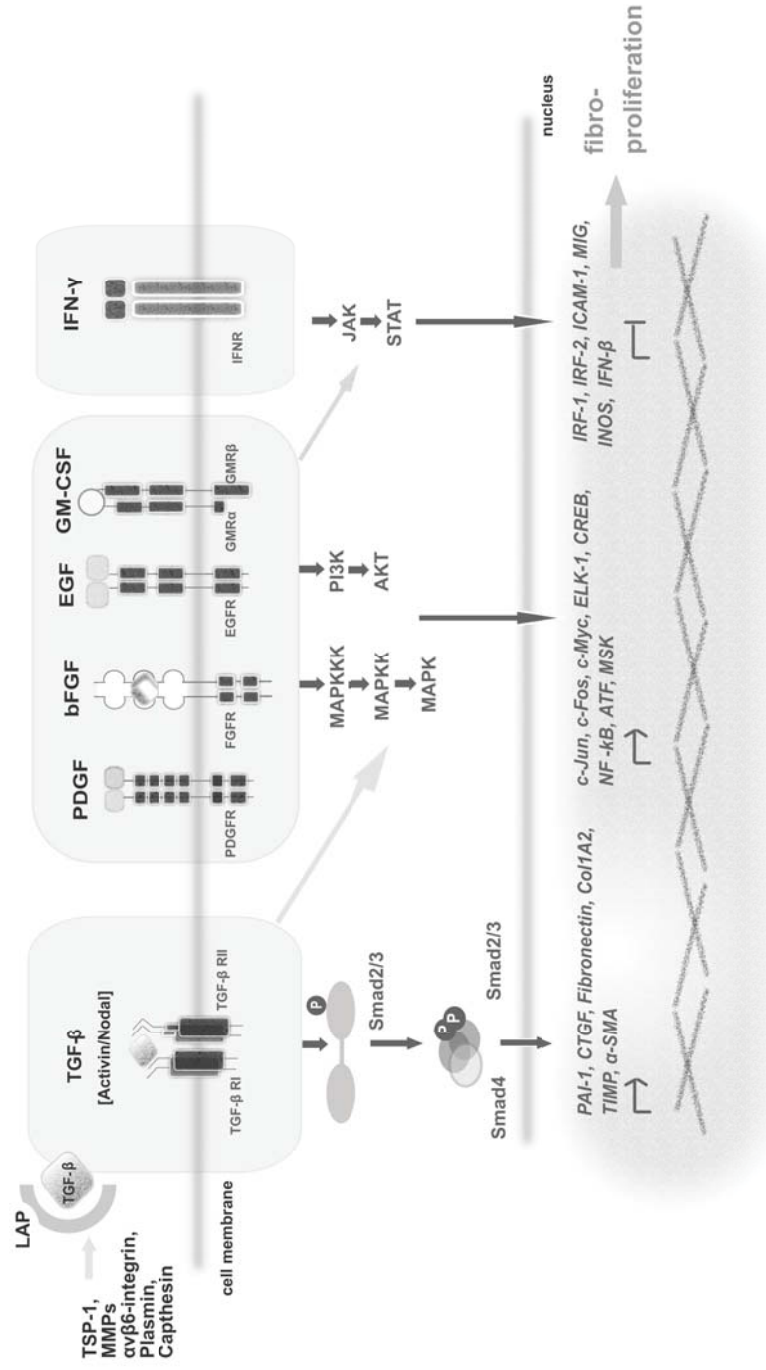

Figure 2: Schematic model of signaling pathways involved in the progression of fibro-proliferation of Dupuytren's Disease.

7.3.5 EGF signaling

Epidermal growth factor (EGF) is the founding member of the EGF-family of proteins, which are described to mediate proliferation and other responses. EGF binds on the cell surface to the EGF receptor (EGFR, also known as ErbB-1 or HER-1). Thereby, EGFR undergoes a transition from inactive monomeric to active homodimer that transduces its activation via phosphorylation of downstream signaling proteins of which members of the MAPK, Akt and JNK pathway are commonly described to be involved in DNA synthesis of EGF target genes [10].

Recently, it was shown that different expression level of EGF exist in different stages of DD. The concentration of EGF in pathological tissues from grade-1 and -3 was lower as compared to control tissue. Nevertheless, an increase of EGF expression level was detectable during advanced stages of DD which coincide with neoangiogenesis and apoptosis of myofibroblasts. EGF may conducts an active role in control of apoptosis, angiogenesis and connective tissue cell migration, division and differentiation [21, 4, 3].

7.3.6 GM-CSF signaling

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that functions as a white blood cell growth factor. It is secreted by macrophages, T-cells, mast cells, endothelial cells and fibroblasts. GM-CSF binds to GM-CSF receptor (GM-CSFR, also known as CD116). Upon dimerization of the α and β subunit, the β subunit becomes phosphorylated on tyrosine residues by members of the Janus kinase (JAK) family. This leads to association with the Shc adaptor protein that then facilitates interactions with the GRB-2/SoS complex for downstream molecule activation via the RAS-RAF-MEK-ERK-1/-2 pathway [6].

GM-CSF expression has been shown to be elevated in DD. It has been shown to induce proliferation of mesenchymal cells through the formation of granulation tissue containing myofibroblasts rich in a lung fibrosis model. Additionally, GM-CSF induces α-SMA rich myofibroblasts in the skin *in vivo* [21, 36, 34]. *In vitro* experiments have shown that GM-CSF does not directly stimulate α-SMA expression when added to the culture medium of rat or human fibroblasts. Indeed, after GM-CSF local treatment, the appearance of α-SMA-rich myofibroblasts is preceded by a characteristic cluster-like accumulation of macrophages, suggesting that such macrophages are important in the stimulation of α-SMA synthesis by myofibroblasts [36, 34]. Moreover, GM-CSF induces the expression of TGF-β1 mRNA by macrophages [2]. These data support the possibility that GM-CSF participates in the initial steps of leading to granulation tissue formation in normal healing and to fibrosis in pathologic situations, perhaps through a stimulation of TGF-β1 production.

7.3.7 IFN-γ signaling

Interferon-γ (IFN-γ) is a cytokine that is important for innate and adaptive immunity. Aberrant IFN-γ expression is associated with a number of auto-inflammatory and auto-immune diseases. Interferon-γ is the only member of the type II class of interferons that transduces its signal via binding and activation of a heteromeric receptor consisting of Interferon gamma receptor-1 (IFNGR-1) and IFNGR-2. Binding to the receptor activates the JAK-STAT pathway. IFN-γ also binds glycosaminoglycan and heparan sulfate which is known to inhibit the biological activity of the latter [17].

In DD IFN-γ is known to decrease cell proliferation and α-SMA expression *in vitro* and *in vivo*. The outcome of intra-lesional injection of IFN-γ are decreased size of Dupuytren lesions [21, 44, 45].

7.4 References

[1] D. Abraham. Connective tissue growth factor: growth factor, matricellular organizer, fibrotic biomarker or molecular target for anti-fibrotic therapy in SSc? *Rheumatology (Oxford)*, 47 Suppl 5:8–9, Oct 2008.

[2] D. Andreutti, G. Gabbiani, and P. Neuville. Early granulocyte-macrophage colony-stimulating factor expression by alveolar inflammatory cells during bleomycin-induced rat lung fibrosis. *Lab. Invest.*, 78:1493–1502, Dec 1998.

[3] K. Augoff, J. Kula, J. Gosk, and R. Rutowski. Epidermal growth factor in Dupuytren's disease. *Plast. Reconstr. Surg.*, 115:128–133, Jan 2005.

[4] K. Augoff, R. Taboa, J. Kula, J. Gosk, and R. Rutowski. Epidermal growth factor receptor (EGF-R) in Dupuytren's disease. *J Hand Surg Br*, 30:570–573, Dec 2005.

[5] M. A. Badalamente, L. C. Hurst, S. K. Grandia, and S. P. Sampson. Platelet-derived growth factor in Dupuytren's disease. *J Hand Surg Am*, 17:317–323, Mar 1992.

[6] S. Barrientos, O. Stojadinovic, M. S. Golinko, H. Brem, and M. Tomic-Canic. Growth factors and cytokines in wound healing. *Wound Repair Regen*, 16:585–601, 2008.

[7] A. Bayat, A. Alansar, A. H. Hajeer, M. Shah, J. S. Watson, J. K. Stanley, M. W. Ferguson, and W. E. Ollier. Genetic susceptibility in Dupuytren's disease: lack of association of a novel transforming growth factor $\beta(2)$ polymorphism in Dupuytren's disease. *J Hand Surg Br*, 27:47–49, Feb 2002.

[8] A. Bayat, E. J. Cunliffe, and D. A. McGrouther. Assessment of clinical severity in Dupuytren's disease. *Br J Hosp Med (Lond)*, 68:604–609, Nov 2007.

[9] A. Berndt, H. Kosmehl, U. Mandel, U. Gabler, X. Luo, D. Celeda, L. Zardi, and D. Katenkamp. TGF β and bFGF synthesis and localization in Dupuytren's disease (nodular palmar fibromatosis) relative to cellular activity, myofibroblast phenotype and oncofetal variants of fibronectin. *Histochem. J.*, 27:1014–1020, Dec 1995.

[10] A. Citri and Y. Yarden. EGF-ERBB signalling: towards the systems level. *Nat. Rev. Mol. Cell Biol.*, 7:505–516, Jul 2006.

[11] C. S. DAVIDSON, W. H. SUMMERSKILL, and S. J. WOLFE. Thickening and contraction of the palmar fascia (Dupuytren's contracture) associated with alcoholism and hepatic cirrhosis. *N. Engl. J. Med.*, 255:559–563, Sep 1956.

[12] R. F. Diegelmann and M. C. Evans. Wound healing: an overview of acute, fibrotic and delayed healing. *Front. Biosci.*, 9:283–289, Jan 2004.

[13] T. Egawa, H. Senrui, and A. Horiki. Epidemiology of the oriental patient. *Dupuytren's Disease Biology and Treatment*, 1:239–245, Jun 1999.

[14] X. H. Feng and R. Derynck. Specificity and versatility in TGF-β signaling through Smads. *Annu. Rev. Cell Dev. Biol.*, 21:659–693, 2005.

[15] D. G. Permanent retraction of the fingers, produced by an affection of the palmar fascia. *Lancet*, ii:222–225, ... 1884.

[16] K. J. Gordon and G. C. Blobe. Role of transforming growth factor-β superfamily signaling pathways in human disease. *Biochim. Biophys. Acta*, 1782:197–228, Apr 2008.

[17] D. J. Gough, D. E. Levy, R. W. Johnstone, and C. J. Clarke. IFNgamma signaling-does it mean JAK-STAT? *Cytokine Growth Factor Rev.*, 19:383–394, 2008.

[18] K. G. Gudmundsson, R. Arngrimsson, N. Sigfusson, A. Bjornsson, and T. Jonsson. Epidemiology of Dupuytren's disease: clinical, serological, and social assessment. The Reykjavik Study. *J Clin Epidemiol*, 53:291–296, Mar 2000.

[19] M. G. Hart and G. Hooper. Clinical associations of Dupuytren's disease. *Postgrad Med J*, 81:425–428, Jul 2005.

[20] J. T. HUESTON and B. DUPUYTREN. Baron Dupuytren. *Med. J. Aust.*, 47(1):808–812, May 1960.

[21] P. Kloen. New insights in the development of Dupuytren's contracture: a review. *Br J Plast Surg*, 52:629–635, Dec 1999.

[22] A. Leask and D. J. Abraham. TGF-β signaling and the fibrotic response. *FASEB J.*, 18:816–827, May 2004.

[23] I. A. Lennox, S. R. Murali, and R. Porter. A study of the repeatability of the diagnosis of Dupuytren's contracture and its prevalence in the grampian region. *J Hand Surg Br*, 18:258–261, Apr 1993.

[24] R. S. LING. The Genetic Factor In Dupuytren's Disease. *J Bone Joint Surg Br*, 45:709–718, Nov 1963.

[25] J. Massague. TGFβ in Cancer. *Cell*, 134:215–230, Jul 2008.

[26] J. Massague and Y. G. Chen. Controlling TGF-β signaling. *Genes Dev.*, 14:627–644, Mar 2000.

[27] J. Massague, J. Seoane, and D. Wotton. Smad transcription factors. *Genes Dev.*, 19:2783–2810, Dec 2005.

[28] O. A. Mikkelsen. Dupuytren's disease–the influence of occupation and previous hand injuries. *Hand*, 10:1–8, Feb 1978.

[29] O. A. Mikkelsen, H. M. Hyeraal, and L. Sandvik. Increased mortality in Dupuytren's disease. *J Hand Surg Br*, 24:515–518, Oct 1999.

[30] A. Mitra and R. Y. Goldstein. Dupuytren's contracture in the black population: a review. *Ann Plast Surg*, 32:619–622, Jun 1994.

[31] K. Miyazono, Y. Kamiya, and M. Morikawa. Bone morphogenetic protein receptors and signal transduction. *J. Biochem.*, 147:35–51, Jan 2010.

[32] A. Moustakas and C. H. Heldin. Non-Smad TGF-β signals. *J. Cell. Sci.*, 118:3573–3584, Aug 2005.

[33] J. Noble, J. G. Heathcote, and H. Cohen. Diabetes mellitus in the aetiology of Dupuytren's disease. *J Bone Joint Surg Br*, 66:322–325, May 1984.

[34] B. Pittet, L. Rubbia-Brandt, A. Desmouliere, A. P. Sappino, P. Roggero, S. Guerret, J. A. Grimaud, R. Lacher, D. Montandon, and G. Gabbiani. Effect of gamma-interferon on the clinical and biologic evolution of hypertrophic scars and Dupuytren's disease: an open pilot study. *Plast. Reconstr. Surg.*, 93:1224–1235, May 1994.

[35] D. C. Ross. Epidemiology of Dupuytren's disease. *Hand Clin*, 15:53–62, Feb 1999.

[36] L. Rubbia-Brandt, A. P. Sappino, and G. Gabbiani. Locally applied GM-CSF induces the accumulation of α-smooth muscle actin containing myofibroblasts. *Virchows Arch., B, Cell Pathol.*, 60:73–82, 1991.

[37] A. P. Saboeiro, J. J. Porkorny, S. I. Shehadi, K. S. Virgo, and F. E. Johnson. Racial distribution of Dupuytren's disease in Department of Veterans Affairs patients. *Plast. Reconstr. Surg.*, 106:71–75, Jul 2000.

[38] R. B. Shaw, A. K. Chong, A. Zhang, V. R. Hentz, and J. Chang. Dupuytren's disease: history, diagnosis, and treatment. *Plast. Reconstr. Surg.*, 120:44e–54e, Sep 2007.

[39] S. S. Shin, C. Liu, E. Y. Chang, C. S. Carlson, and P. E. Di Cesare. Expression of bone morphogenetic proteins by Dupuytren's fibroblasts. *J Hand Surg Am*, 29:809–814, Sep 2004.

[40] M. Tallquist and A. Kazlauskas. PDGF signaling in cells and mice. *Cytokine Growth Factor Rev.*, 15:205–213, Aug 2004.

[41] P. ten Dijke and C. S. Hill. New insights into TGF-β-Smad signalling. *Trends Biochem. Sci.*, 29:265–273, May 2004.

[42] P. ten Dijke, H. Yamashita, H. Ichijo, P. Franzen, M. Laiho, K. Miyazono, and C. H. Heldin. Characterization of type I receptors for transforming growth factor-β and activin. *Science*, 264:101–104, Apr 1994.

[43] J. J. Tomasek, G. Gabbiani, B. Hinz, C. Chaponnier, and R. A. Brown. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.*, 3:349–363, May 2002.

[44] J. J. Tomasek, M. B. Vaughan, and C. J. Haaksma. Cellular structure and biology of Dupuytren's disease. *Hand Clin*, 15:21–34, Feb 1999.

[45] W. A. Townley, R. Baker, N. Sheppard, and A. O. Grobbelaar. Dupuytren's contracture unfolded. *BMJ*, 332:397–400, Feb 2006.

[46] T. H. Trojian and S. M. Chu. Dupuytren's disease: diagnosis and treatment. *Am Fam Physician*, 76:86–89, Jul 2007.

[47] R. Tse, J. Howard, Y. Wu, and B. S. Gan. Enhanced Dupuytren's disease fibroblast populated collagen lattice contraction is independent of endogenous active TGF-β2. *BMC Musculoskelet Disord*, 5:41, Nov 2004.

[48] N. Turner and R. Grose. Fibroblast growth factor signalling: from development to cancer. *Nat. Rev. Cancer*, 10:116–129, Feb 2010.

[49] S. Wang and R. Hirschberg. BMP7 antagonizes TGF-β -dependent fibrogenesis in mesangial cells. *Am. J. Physiol. Renal Physiol.*, 284:F1006–1013, May 2003.

[50] M. Y. Wu and C. S. Hill. TGF-β superfamily signaling in embryonic development and homeostasis. *Dev. Cell*, 16:329–343, Mar 2009.

[51] T. A. Wynn. Cellular and molecular mechanisms of fibrosis. *J. Pathol.*, 214:199–210, Jan 2008.

[52] M. Yamashita, K. Fatyol, C. Jin, X. Wang, Z. Liu, and Y. E. Zhang. TRAF6 mediates Smad-independent activation of JNK and p38 by TGF-β. *Mol. Cell*, 31:918–924, Sep 2008.

[53] J. Yost, T. Winters, and H. C. Fett. Dupuytren's contracture; a statistical study. *Am. J. Surg.*, 90:568–571, Oct 1955.

[54] M. Zeisberg, C. Bottiglio, N. Kumar, Y. Maeshima, F. Strutz, G. A. Muller, and R. Kalluri. Bone morphogenic protein-7 inhibits progression of chronic renal fibrosis associated with two genetic mouse models. *Am. J. Physiol. Renal Physiol.*, 285:F1060–1067, Dec 2003.

[55] M. Zeisberg, A. A. Shah, and R. Kalluri. Bone morphogenic protein-7 induces mesenchymal to epithelial transition in adult renal fibroblasts and facilitates regeneration of injured kidney. *J. Biol. Chem.*, 280:8094–8100, Mar 2005.

[56] Y. E. Zhang. Non-Smad pathways in TGF-β signaling. *Cell Res.*, 19:128–139, Jan 2009.