Inhibition of signaling cascades in osteoblast differentiation and fibrosis

Krause, C.

Concurrent inhibition of TGF-β and mitogen driven signaling cascades in Dupuytren's Disease – non-surgical treatment strategies from a signaling point of view.

Krause C and Kloen P (2011)

submitted to
J. Medical Hypothesis

Chapter 9

Concurrent inhibition of TGF-β and mitogen driven signaling cascades in Dupuytren's Disease – non-surgical treatment strategies from a signaling point of view

9.1 Summary

Dupuytren's disease (DD) is a benign progressive fibro-proliferative disorder of the fascia palmaris of the hand. Currently, treatment consists of surgical excision with a relatively high recurrence rate and risk of complications. To improve long-term outcome of DD treatment, research focus has shifted towards molecular targets for DD as an alternative to surgery. Therefore, complete and exact understanding of the cause of DD is needed. Transforming growth factor TGF-β is considered a key player in DD. We recently showed that increased TGF-β expression in DD correlates not only with elevated expression and activation of downstream Smad effectors, but also with overactive ERK1/2 MAP kinase signaling. Both TGF-β/Smad and non-Smad signaling pathways increase expression of key fibrotic markers and contractility of Dupuytren's myofibroblasts. What is not yet known is whether these two signaling cascades each accelerate DD autonomously, successively or in conjunction. Elucidation of this mechanism will help develop new potential non-surgical treatments. We hypothesize that TGF-β-induced short-term activation of the MAPK pathway leads to an autonomous non-Smad driven fibrosis. Therefore, succesful treatment strategies will target not only TGF-β/Smad, but also intracellular MAPK signaling. In this review we discuss possible scenarios in which such a drift from TGF-β induced Smad signaling to autonomous non-Smad signaling could be observed in DD. The potential therapeutic effects of small cytokine signaling cascades inhibitors, such as TGF-β type I receptor-, (pan-) tyrosine- or ERK1/2 MAP-kinase inhibitor will be highlighted. To abrogate the fibrotic trait and the recurrence of DD, we speculate on sequential and co-application of such molecules in order to provide possible new non-operative strategies for DD.

Keywords

MAPK, TGF-β, SB-431542, PD-98059, Glivec®, fibrosis

9.2 Introduction

In 1831 Baron Guillaume Dupuytren described a fibrotic contracture of the palmar fascia of the hand which was later eponymously attributed to him. The typical disease progression is the development of nodules and pits in the palmar aspect of the hand and fingers leading to an irreversible contracture. The involved tissue is the fascia palmaris that lies between the skin and tendons of the hand and fingers [7, 24]. The characteristic cell in the nodule is the myofibroblast, a differentiated fibroblast that has properties of smooth muscle cells (expression of α-SMA) and fibroblasts [12]. The formation of the nodules is known as the proliferative phase. As the disease progresses, the nodules give way to the formation of cords [7, 24]. These cords represent characteristics of fibrosis within the involutional and residual stages of the disease and consist mostly of fibroblasts and deposition of excess extracellular matrix (ECM). The proposed aetiological factors of DD are diverse and range from genetic susceptibility, alcohol abuse, epilepsy, smoking, microtrauma, diabetes mellitus to rheumatoid arthritis [7, 24]. On a cellular and molecular level, many growth factors have been implied in Dupuytren's contracture, of which TGF-β and mitogen activated signaling cascades have been proposed to play a prominent role (Figure 1) [13]. More recently, aberrations in the Wnt signalling pathway have been suggested to confer susceptibility to DD [8]. Studies on role of Wnt gene expression on β-catenin upregulation in DD are conflicting [6, 19, 20]. In short, to date, most of the implicated factors are associated with DD but none has been shown to be causative. Interestingly, the histological and biochemical changes seen in DD are similar to those seen in early connective tissue wound repair [24]. It is thought that after an initial trigger such as wounding or inflammation, the release and activation of cytokines stimulate the production of ECM proteins which, ideally, contribute to tissue repair and to the restoration of normal tissue architecture. However, in DD, this mechanism seems to go awry, and excessive TGF-β and mitogen signaling contribute to a pathologic excess of fibrotic tissue that compromises normal hand functionality. This can ultimately lead to the amputation of the affected hand(s) [13, 24]. Currently, surgery is the most common treatment for DD and consists of surgical excision or break-up of the contracted tissue (needle aponeurotomy, open or percutaneous fasciotomy or open fasciectomy). The high recurrence rate after surgical treatment of DD (reported to range between 8-66% (9) drives the ongoing search for the exact underlying cause [7, 24]. The recently introduced collagenase treatment (Collagenase clostridium histolyticum marketed under the name Xiaflex®) is non-surgical, but -similar to surgery- addresses only the result of the disease (excessive EMC and fibrosis) rather than the cause [11]. Long term results of this treatment are still being investigated [7]. As the exact disease process continues to be unravelled, new non-surgical interventions directed at aberrant signaling such as N-acetyl-L-cystein (NAC)/ACE inhibitor are now being proposed [14].

9.2.1 TGF-β signaling in Dupuytren's Disease

TGF-β is a potent modulator of fibroblast and myofibroblast proliferation and differentiation [9, 26]. All key components of the TGF-β/Smad signaling cascade were noted to have increased expression patterns in DD, resulting in accelerated TGF-β signaling [13, 16]. TGF-β transduces a signal through heteromeric complex formation of related type I and type II transmembrane serine/threonine kinase receptors [10]. The signal of the activated type I receptor is mainly transduced into the cytoplasm through phosphorylation and activation of the receptor-regulated Smad2 and Smad3 (R-Smads). Activated R-Smads dissociate from the receptor complex and associate with Smad4 (Co-Smad). These heteromeric Smad complexes accumulate in the nucleus, where they regulate expression of a large array of target genes (Figure 1) [22].

9.2.2 MAPK signaling in Dupuytren's Disease

The mitogen-activated protein kinase (MAPK) pathway is also involved in a wide range of cellular processes such as proliferation, differentiation, and cell survival/apoptosis. MAPKs are serine/threonine-specific protein kinases that respond to a variety of extracellular stimuli (stress, cytokines and growth factors) [12]. Mitogens such as platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and epidermal growth factor (EGF) are also believed to be involved in DD [2, 9, 16]. Although mitogens such as p38 and JNK can activate MAPKs, thus far only a role for extracellular signal-regulated kinases-1/2 (ERK1/2) has been implicated in the disease [16]. To achieve a sustained activation of the ERK/MAPK signaling cascade, a mitogen binds to its respective receptor, which leads to the phosphorylation and activation of signaling enzymes including the MAP kinase kinase kinases (MAP3Ks), which activate the MAP kinase kinases (MEK1/2). These, in turn, activate the MAP kinase (ERK1/2). This sets off ERK1/2 to translocate into the nucleus, where it activates target genes of the *AP-1* family of transcription factors that induce target gene expression (Figure 1) [12].

9.3 Hypothesis

We hypothesize that accelerated TGF-β signaling in DD leads to a signaling drift towards the ERK/MAPK pathway which not only catalyzes the progression but also the recurrence of the disease. We therefore propose a concurrent inhibition of TGF-β and mitogen-driven signaling cascades to abrogate the fibro-proliferative disorder. This might include, for example, a TGF-β type I receptor-, (pan-) tyrosine kinase- and/or a ERK1/2 MAP-kinase inhibitor that specifically interfere with enzymatic activities.

9.4 Evaluation of the hypothesis

At first glance TGF-β and MAPK signaling routes act autonomously from each other. TGF-β is known to sustain an autocrine signaling loop through a Smad2/3 dependent

TGF-β gene expression, whereas the MAPK cascade induces mitogen expression such as PDGF, FGF, EGF and EGFR [4, 5, 15, 16].Interestingly, in recent years a high degree of crosstalk between the TGF-β and MAPK signaling pathway has been described. It is now evident that TGF-β signaling triggers short-term activation of ERK1/2 and also leads in a Smad- dependent manner to increased mitogen expression such as PDGF and FGF [16, 18, 25]. These findings point towards a successive signaling cascade, starting with a TGF-β trigger that further promotes mitogen induced ERK/MAPK signaling. One can speculate that in healthy tissue a balance of both signaling cascades is achieved to avoid unidirectional signaling. Indeed, it is described that mitogen induced MAPKs lead to phosphorylation of the linker region of Smad2/3 which promotes, in concert with other factors, the degradation and attenuation of Smad2/3 dependent TGF-β signaling [1]. Whether these effects also contribute to a balanced signaling network and thus hand functionality is speculative, and in DD specifically it is yet unknown at which point (s) this signaling crosstalk turns awry (Figure 1). With increasing understanding of the respective signaling cascades, the generation of pharmacological molecules that can interfere in signal propagation is rising. The use of small cytokine signaling cascades inhibitors, such as the TGF-β type I receptor kinase inhibitors SB-431542, SD-208, GW788388, and GW66004, the pan-tyrosine kinase inhibitor Glivec® or the MAP kinase kinase (MEK-1) inhibitor PD-98059 have all been reported to be powerful tools to abrogate fibro-proliferation in vitro. In numerous vertebrates the administration of TGF-β type I receptor kinase inhibitors as well as a MEK-1inhibitor have been shown to be non-toxic and capable to abrogate fibrotic traits in lung, liver and kidney models [3, 9, 21, 23, 27]. Glivec® (imatinib mesylate, STI571), on the other hand, is an FDA approved anti-cancer drug that is used clinically to treat patients with chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GISTs). Glivec® specifically binds to the site of tyrosine kinase activity which eliminates mitogen induced signaling at the receptor level.

9.5 Testing the hypothesis

With the known aspects of the TGF-β and ERK/MAPK crosstalk one can now investigate if the accelerated TGF-β initiated signal, as seen in DD, could lead to an autonomous MAPK signaling cascade. Unfortunately there are no animal models of DD to investigate the impact of signaling drifts and resulting pharmacological inhibitor administration *in vivo*. Given this, we used a computational modulation tool for discrete regulatory network with short-term effects and raised the question whether the extra activation of ERK1/2 by TGF-β could have long-term and potentially irreversible effect on the network state [17]. Taken in account that both signaling cascades sustain autonomous loops, we showed *in silico* that accelerated TGF-β signaling can push the network into an autonomous "locked" ERK/MAPK driven fibrosis [17]. Based on this, we suggest that accelerated TGF-β signaling leads to a signaling drift towards the ERK/MAPK pathway, which likely catalyzes not only the progression but also the high recurrence of DD.

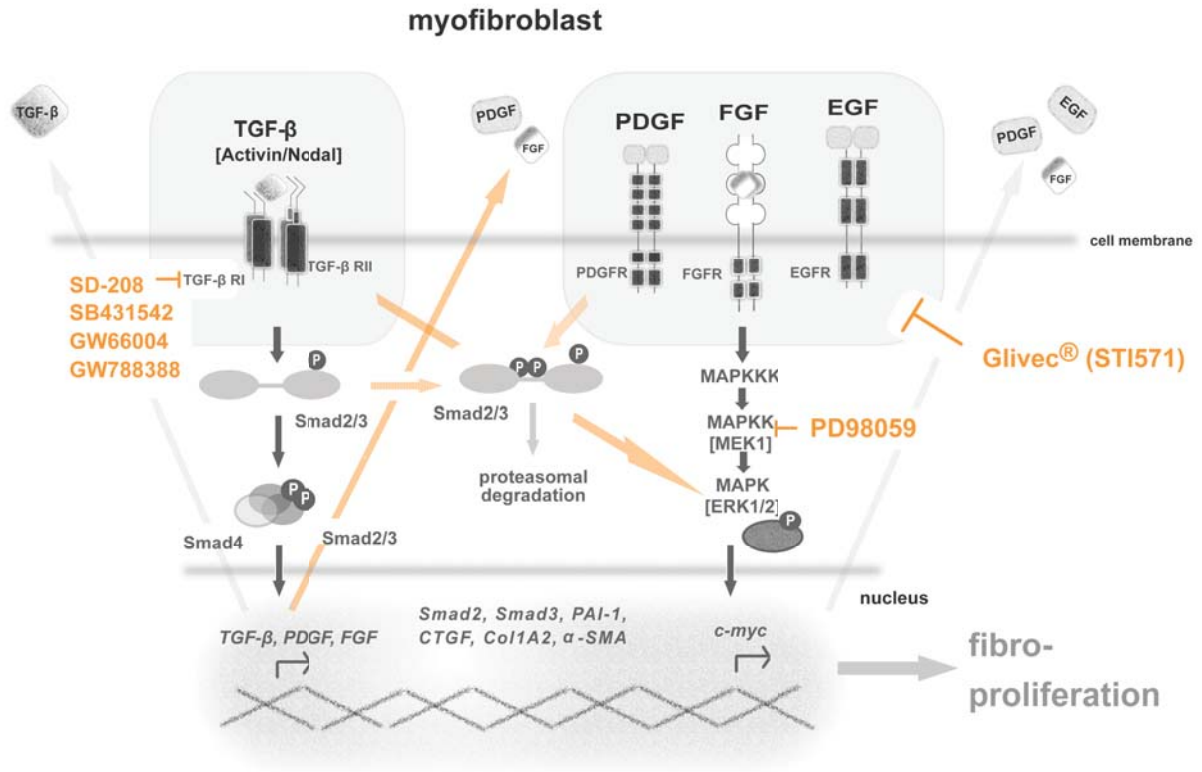

Figure 1: Overview of signaling cascades involved in Dupuytren's Disease. Indicated are small signaling cascade inhibitors (orange) that have been shown to attenuate fibrotic traits.

9.6 Consequences of the hypothesis

Based on our current understanding of the signaling network in DD we believe that a concurrent treatment with an TGF-β type I receptor inhibitor and a MAPK inhibitor will successfully attenuate the TGF-β triggered signaling drift in DD. The TGF-β type I receptor inhibitor will lead to an uncoupling of the TGF-β signaling cascade from ERK/MAPK activation. Eventually, this might eliminate the TGF-β dependent signaling drift that we propose to be the underlying cause for the progression and recurrence of DD. We already know that treatment with Glivec® (a receptor tyrosine kinase inhibitor) eliminates the mitogen initiated MAPK cascades but has no effect on TGF-β induced ERK1/2 activation. Therefore, we propose the local administration of Glivec® in combination with a TGF-β type I receptor inhibitor. Since DD is a progressive disease it will be difficult to distinguish between initial acceleration of TGF-β signaling and consecutive TGF-β actions. Therefore, the use of the MEK-1 inhibitor should be adjuvant in treating DD. Not only is the advantage of an intracellular MAPK inhibitor that it can block various mitogen induced MAPK signals, it can also abrogate consecutive TGF-β stimuli on the ERK/MAPK signaling cascade. Consequently, the MEK-1 inhibitor is the only agent that one could use as a stand alone therapy in treating late stages of DD if they are independent of TGF-β signaling. Whether a systemic treatment with these inhibitors as succesfully performed in fibrotic animal models is also beneficial in humans is still a matter of speculation and debate. The relatively superficial location of DD would make it an ideal candidate for local delivery, either through injection or topical application.

9.7 Acknowledgments

We thank Erik de Vink and Christian Krause for fruitful discussions on computational modulation of cytokine signaling networks. We appreciate suggestions by Peter ten Dijke. This work was supported by the German Diabetes Foundation and the Martin-Keuning Eckhardt Stichting.

9.8 References

[1] C. Alarcon, A. I. Zaromytidou, Q. Xi, S. Gao, J. Yu, S. Fujisawa, A. Barlas, A. N. Miller, K. Manova-Todorova, M. J. Macias, G. Sapkota, D. Pan, and J. Massague. Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-β pathways. *Cell*, 139:757–769, Nov 2009.

[2] K. Augoff, R. Tabo?a, 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.

[3] P. Bonniaud, P. J. Margetts, M. Kolb, J. A. Schroeder, A. M. Kapoun, D. Damm, A. Murphy, S. Chakravarty, S. Dugar, L. Higgins, A. A. Protter, and J. Gauldie.

Progressive transforming growth factor β1-induced lung fibrosis is blocked by an orally active ALK5 kinase inhibitor. *Am. J. Respir. Crit. Care Med.*, 171:889–898, Apr 2005.

[4] B. Chailley-Heu, O. Boucherat, A. M. Barlier-Mur, and J. R. Bourbon. FGF-18 is upregulated in the postnatal rat lung and enhances elastogenesis in myofibroblasts. *Am. J. Physiol. Lung Cell Mol. Physiol.*, 288:43–51, Jan 2005.

[5] A. J. Clark, S. Ishii, N. Richert, G. T. Merlino, and I. Pastan. Epidermal growth factor regulates the expression of its own receptor. *Proc. Natl. Acad. Sci. U.S.A.*, 82:8374–8378, Dec 1985.

[6] I. Degreef, L. De Smet, R. Sciot, J. J. Cassiman, and S. Tejpar. Beta-catenin overexpression in Dupuytren's disease is unrelated to disease recurrence. *Clin. Orthop. Relat. Res.*, 467:838–845, Mar 2009.

[7] S. S. Desai and V. R. Hentz. The treatment of Dupuytren disease. *J Hand Surg Am*, 36:936–942, May 2011.

[8] G. H. Dolmans, P. M. Werker, H. C. Hennies, D. Furniss, E. A. Festen, L. Franke, K. Becker, P. van der Vlies, B. H. Wolffenbuttel, S. Tinschert, M. R. Toliat, M. Nothnagel, A. Franke, N. Klopp, H. E. Wichmann, P. Nurnberg, H. Giele, R. A. Ophoff, C. Wijmenga, A. de Boer, C. K. van der Sluis, H. ter Linden, A. B. Knepper, O. T. Zophel, H. L. de Boer, H. J. Bauer, R. Spicher, P. Bleuler, R. Hoffmann, M. Langer, W. Frank, W. Lenze, A. Meinel, H. E. Nick, J. Rossler, F. Staub, P. Burge, I. McNab, C. Little, P. Critchley, L. Cogswell, V. Teoh, A. Fleming, S. Cerovac, J. Colville, S. Fullilove, I. Chakrabarti, and M. Broadbent. Wnt signaling and Dupuytren's disease. *N. Engl. J. Med.*, 365:307–317, Jul 2011.

[9] A. M. Gonzalez, M. Buscaglia, R. Fox, A. Isacchi, P. Sarmientos, J. Farris, M. Ong, D. Martineau, D. A. Lappi, and A. Baird. Basic fibroblast growth factor in Dupuytren's contracture. *Am. J. Pathol.*, 141:661–671, Sep 1992.

[10] C. H. Heldin, K. Miyazono, and P. ten Dijke. TGF-β signalling from cell membrane to nucleus through SMAD proteins. *Nature*, 390:465–471, Dec 1997.

[11] L. C. Hurst, M. A. Badalamente, V. R. Hentz, R. N. Hotchkiss, F. T. Kaplan, R. A. Meals, T. M. Smith, J. Rodzvilla, E. Akelman, B. Bear, M. R. Belsky, P. Blazar, E. N. Britton, B. Costas, J. L. Frazier, V. Hentz, R. N. Hotchkiss, L. C. Hurst, M. A. Badalamente, F. T. Kaplan, J. Lubahn, S. McPherson, R. Meals, C. A. Peimer, and D. Roeshot. Injectable collagenase clostridium histolyticum for Dupuytren's contracture. *N. Engl. J. Med.*, 361:968–979, Sep 2009.

[12] E. K. Kim and E. J. Choi. Pathological roles of MAPK signaling pathways in human diseases. *Biochim. Biophys. Acta*, 1802:396–405, Apr 2010.

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

[14] K. Knobloch, J. Redeker, and P. M. Vogt. Antifibrotic medication using a combination of N-acetyl-L-cystein (NAC) and ACE inhibitors can prevent the recurrence of Dupuytren's disease. *Med. Hypotheses*, 73:659–661, Nov 2009.

[15] L. G. Komuves, A. Feren, A. L. Jones, and E. Fodor. Expression of epidermal growth factor and its receptor in cirrhotic liver disease. *J. Histochem. Cytochem.*, 48:821–830, Jun 2000.

[16] C. Krause, P. Kloen, and P. Ten Dijke. Elevated transforming growth factor-β and mitogen-activated protein kinase pathways mediate fibrotic traits of Dupuytren's disease fibroblasts. *Fibrogenesis Tissue Repair*, 4:14, 2011.

[17] C. Krause, C. Krause, and E. de Vink. Action-based analysis of discrete regulatory networks with short-term stimuli. In *CMSB'10: Proc. 8th Conference on Computational Methods in Systems Biology*, pages 66–75, New York, NY, USA, 0 2010. ACM.

[18] M. K. Lee, C. Pardoux, M. C. Hall, P. S. Lee, D. Warburton, J. Qing, S. M. Smith, and R. Derynck. TGF-β activates Erk MAP kinase signalling through direct phosphorylation of ShcA. *EMBO J.*, 26:3957–3967, Sep 2007.

[19] D. B. O'Gorman, L. Vi, and B. S. Gan. Molecular mechanisms and treatment strategies for Dupuytren's disease. *Ther Clin Risk Manag*, 6:383–390, 2010.

[20] D. B. O'Gorman, Y. Wu, S. Seney, R. D. Zhu, and B. S. Gan. Wnt expression is not correlated with β-catenin dysregulation in Dupuytren's Disease. *J Negat Results Biomed*, 5:13, 2006.

[21] M. Petersen, M. Thorikay, M. Deckers, M. van Dinther, E. T. Grygielko, F. Gellibert, A. C. de Gouville, S. Huet, P. ten Dijke, and N. J. Laping. Oral administration of GW788388, an inhibitor of TGF-β type I and II receptor kinases, decreases renal fibrosis. *Kidney Int.*, 73:705–715, Mar 2008.

[22] B. Schmierer and C. S. Hill. TGFβ-SMAD signal transduction: molecular specificity and functional flexibility. *Nat. Rev. Mol. Cell Biol.*, 8:970–982, Dec 2007.

[23] S. Segawa, D. Goto, Y. Yoshiga, M. Sugihara, T. Hayashi, Y. Chino, I. Matsumoto, S. Ito, and T. Sumida. Inhibition of transforming growth factor-β signalling attenuates interleukin (IL)-18 plus IL-2-induced interstitial lung disease in mice. *Clin. Exp. Immunol.*, 160:394–402, Jun 2010.

[24] B. Shih and A. Bayat. Scientific understanding and clinical management of Dupuytren disease. *Nat Rev Rheumatol*, 6:715–726, Dec 2010.

[25] F. Strutz, M. Zeisberg, A. Renziehausen, B. Raschke, V. Becker, C. van Kooten, and G. Muller. TGF-β 1 induces proliferation in human renal fibroblasts via induction of basic fibroblast growth factor (FGF-2). *Kidney Int.*, 59:579–592, Feb 2001.

[26] 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.

[27] W. Wei, B. Ma, H. Y. Li, Y. Jia, K. Lv, G. Wang, J. Zhang, S. Zhu, H. Tang, Z. Sheng, and Z. Xia. Biphasic effects of selective inhibition of transforming growth factor β1 activin receptor-like kinase on LPS-induced lung injury. *Shock*, 33:218–224, Feb 2010.