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REVIEW

Update on the role of molecular factors and fibroblasts in the pathogenesis of Dupuytren's disease

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Abstract The mechanism by which the fibroblast is able to trigger palmar fibromatosis is still not yet fully understood. It would appear certain that the "abnormal" fibroblasts continuously synthesise profibrotic cytokines which are able to determine the activation to myofibroblasts, to stimulate them to the further proliferation and synthesis of other cytokines, to modify the cells' differentiation and ultrastructural characteristics, as well as the production of matrix and other proteins. Several fibroblast growth factors have been suggested to be responsible of an abnormal cell activation with an aberrantly elevated collagen synthesis and extracellular deposition in Dupuytren's disease, as TGF-Beta, TNF-Alfa, PDGF, GM-CSF, free radicals, metalloproteinases, sex hormones, gene modified expression, mechanical stimulation. The Authors review the current state of knowledge in the field, by analyzing the role of these cytokines in the palmar fibromatosis.

Keywords Cytokines · Fibroblast · Dupuytren's disease

Introduction

Growth factors are produced within a cell and then secreted into the extracellular space where they subsequently bind to the specific receptors exposed either on their own surface (autocrine control) or on those of neighbouring cells (paracrine control). Following traumatic or treatment-induced damage of a tissue, a complex, balanced system of cytokines at different serum concentrations, at different activation time points and with different modes of action- either individually or combined with others - lead to the repair of the damaged tissue. A classic example of this process is wound scarring repair, in which these growth factors intervene on various levels to control haemostasis, cell proliferation and collagen synthesis. One characteristic of the normal cytokine-induced repair process is that once homeostasis has been achieved, the autocrine and/or paracrine cell growth control mechanisms return to their normal conditions, consequently, the extracellular concentration of these cytokines tends to drop gradually together with their function (Mutsaers et al. 1997). The scarring process, which is similar to the palmar fibromatosis mechanism, is influenced by constitutional factors (age, sex, hereditary/familial genetic characteristics) and local and systemic factors (anatomical location of the wound, infections, health conditions, previous radiation treatment, chemotherapy). It is believed that with different intensities and mechanisms that are yet to be fully understood, these factors are able to cause palmar fibromatosis. Many scientific studies would appear to show that Dupuytren's disease is nothing more than the result of the prolonged expression of "abnormal" cytokines that are thought to cause an abnormal and continuous proliferation of fibroblasts, responsible for the production and deposition of collagen fibres in the extracellular space (Kuhn et al. 2001).

A milestone in our histopathological knowledge of Dupuytren's disease, and, in particular, concerning the aetiological role played by fibroblasts, were the studies by Gabbiani et al. (1971) and Gabbiani and Majno (1972), who were the first to describe the ultrastructure of an adapted type of fibroblast within fibromatous nodules that was given the name myofibroblast. Previously, Meyerding et al. (1941) had described the disease as benign fibromatosis of the palmar aponeurosis that can spread to the subcutaneous tissue and dermis. However, it was Luck (1959) who devised the

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histopathological classification of the condition into three consecutive stages: proliferative, involutive and residual, based upon the morphofunctional changes in the fibroblast, as will be described in greater detail in the next chapter.

Biology

The fibroblast is the type of cell most representative of connective tissue and the superficial palmar fascia (Schürch et al. 1990) (Fig. 1). It is involved in various inflammatory and damaged tissue repair processes and also synthesises profibrotic cytokines and components of the extracellular matrix: elastic fibres (vimentin, desmin, alpha actin) glycosaminoglycans, and type I-III-IV and V collagen. Morphologically, it has three different evolutionary stages: fibroblast, myofibroblast and fibrocyte. It has been observed that the abnormal proliferation of this cell underlies many types of organ fibromatosis, such as those affecting the liver and kidneys. This cell's role in the genesis of Dupuytren's disease is now undisputed. The fibroblast is indeed the only type of cell able to synthesise type III collagen, which is present in high concentrations in the fibromatous tissue, but is not present in healthy palmar fascia (Gelberman et al. 1980; Brickley Parson et al. 1981; Pasquali Ronchetti et al. 1993). In vitro studies have shown that adding certain cytokines to culture media containing fibroblasts stimulates the multiplication of the cells and their ability to synthesise and deposit collagen fibres. The origin of myofibroblasts is not fully known. Potential precursors include immature fibroblasts, monocytes, mesenchymal cells and pericytes. Using four macrophage antibodies (27E10 = acute tissue inflammation indicator, RM3/1 = intermediate inflammation phase indicator, 25F9 = late inflammatory phase indicator, CD68 = Mitchell macrophage indicator), it has been shown that fibromatous palmar tissue contains myofibroblasts at different morphological stages, presumably of an inflammatory origin (Brenner et al. 1996). Ultrastructural cytoskeleton studies performed using monoclonal antibodies made it possible to classify myofibroblasts into four morphological types:

1) phenotype "V", vimentin-positive myofibroblasts. These cells are derived directly from fibroblasts (Fig. 2);
2) "VAD" phenotype: in this class, myofibroblasts are positive for vimentin, desmin and alpha actin. These myofibroblasts are thought to derive from fibroblasts, pericytes and non-muscle mesenchymal cells such as endothelial cells.
3) "VA" phenotype: myofibroblasts positive for vimentin and alpha actin.
4) "VD" phenotype: myofibroblasts positive for vimentin and desmin.

Culture tests performed on samples of fibromatous palmar tissue are reported as showing that the myofibroblasts undergoing active multiplication in Dupuytren's disease are derived from fibroblasts (Skalli et al. 1989). (Moyer et al. 2002) proved that the cells derived from fibromatous palmar tissue and then cultivated, possess an appearance and behaviour that is halfway between those of normal fibroblasts and "transformed", "neoplastic" fibroblasts. During the advanced stage of the disease, the myofibroblasts no longer express on their surface the epidermal growth factor receptor (EGF-R) that is present during the early phases, as we will see later. This suggests that fibromatous tissue myofibroblasts do not derive from palmar skin

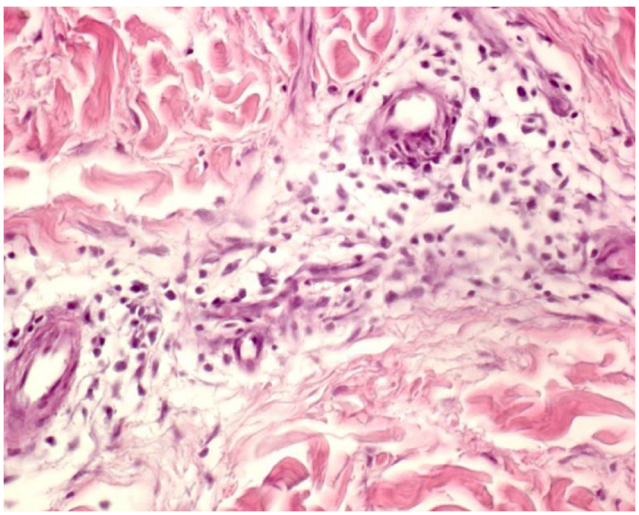

Fig. 1 The fibroblast is the type of cell most representative of connective tissue and the superficial palmar fascia. The figure shows a section of the superficial palmar fascia showing the immunohistochemical H&E-stained fibroblasts, 50 x

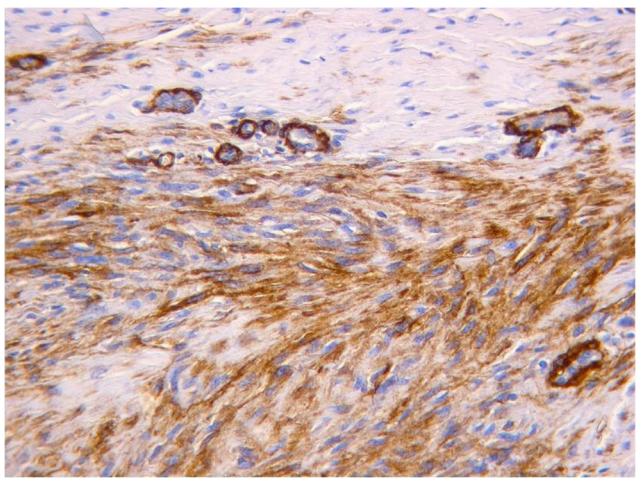

Fig. 2 Hystological and immunohistochemical studies on cytoskeleton performed using monoclonal antibodies made it possible to classify myofibroblasts into four morphological types. The figure shows "V" phenotype fibroblasts (*azure*) and vimentin-positive fibroblasts (*brown*) (immunohistochemical vimentin stain, 20x)

fibroblasts (which always express EGF-R, even when activated to myofibroblasts, rather that they originate from connective fibroblasts present in the subcutaneous tissue above the superficial palmar fascia. Tomasek et al. (1986) used immunofluorescence techniques to show that myofibroblasts, unlike fibroblasts and fibrocytes, possess characteristics typical of smooth muscle cells, for example, they express alpha actin on their surface. However, their basement membrane is positive for anti-fibronectin and anti-non-muscle myosin antibodies and could therefore be considered to be of non-muscular derivation. Tomasek also observed the complex interactions between the myofibroblast, fibronectin and alpha actin in the extracellular matrix, suggesting that a sort of contractile signal is transmitted between the intracellular and extracellular environments (Tomasek and Haaskma 1991; Tomasek and Rayan 1995; Tomasek et al. 1999). Through the bond with its receptor, the serum-derived phospholipid sphingosine-1-phosphate is able to activate non-muscle myosin, which triggers a transmembrane signal, responsible for the contractility of nodule myofibroblasts (Komatsu et al. 2010).

Pathogenesis

The mechanism by which the fibroblast is able to trigger palmar fibromatosis is not yet fully understood; however, it would appear certain that the "abnormal" fibroblasts continuously synthesise profibrotic cytokines that bind specific receptors to the surface of the fibroblasts, determining the activation to myofibroblasts and stimulating them to the further proliferation and synthesis of other cytokines. Subsequently, "inhibitor" growth factors that are yet to be identified are thought to interrupt the differentiation into myofibroblasts and their intense proliferation, thereby deactivating the myofibroblasts to "fibrocytes". These results could have enormous importance in research into a mechanism that makes it possible to inhibit the progression or recurrence of palmar fibromatosis. An animal model has been created to evaluate potential nonsurgical treatments of Dupuytren's disease (Satish et al. 2015). The dorsal skin of laboratory rats divided into two groups was implanted with healthy palmar fascia cells taken from patients undergoing carpal tunnel surgery and cells from pathological palmar fascia harvested from fibromatosis patients. Eight weeks later, the animal tissues were removed and analysed using immunohistochemical techniques. The alpha actin protein content was seen to be far higher in the tissue obtained from the pathological fascia than in the skin of rats that were implanted with the fragment of healthy palmar fascia. This confirms that myofibroblasts are able to maintain their "activation" and continue to synthesise alpha actin protein even after implantation on healthy tissue. One recent study on the contractile properties of myofibroblasts extracted from palmar nodules showed that the increase in alpha actin is able to maintain the myofibroblast's contractile capacity in the long term, and that this does not appear to be influenced by dermal fibroblasts (Verjee et al. 2010). According to this study, in the presence of keratinocytes derived from hypertrophic scar dermis, normal human fibroblasts synthesise and excrete into the extracellular space a great variety of cell growth factors that, conversely, do not appear when the cells come into contact with keratinocytes harvested from normal tissue. This result is a further confirmation of the complex interaction on various levels that takes place between cells and biomolecular factors in fibromatosis. In one recent study, an animal model was created on which to investigate certain genetic aspects associated with palmar fibromatosis (Satish et al. 2012). Fibroblasts derived from the superficial palmar fascia of patients undergoing carpal tunnel surgery and fibroblasts derived from the palmar fascia of patients with Dupuytren's contracture were cultivated in agarose gel, enriched with saline solution containing lipophilic substances. Three weeks later, the cells were injected into the abdominal skin of rats. Eight weeks after implantation, the rats were sacrificed and the abdominal skin removed for ultrastructural analysis. It was seen that both groups of fibroblasts, those derived from normal fascia and those obtained from patients with palmar fibromatosis were successfully developed in the abdomen of the rats. In addition, the concentration of messenger RNA levels in relation to alpha actin protein (calculated using the PCR) and the concentration of type I collagen were significantly higher in the pathological palmar fascia than in the normal fascia. This shows that the fibroblasts derived from the pathological fascia are more readily activated to myofibroblasts and, therefore, have a greater capacity to synthesise alpha actin and type I collagen. In the future, this animal model could make it possible to analyse in vivo the complex mechanisms leading to the formation of fibromatous tissue starting from myofibroblasts.

The role of cytokines

The cytokines responsible for controlling palmar fascia fibroblasts are the so-called fibrogenic cytokines: TGF-β, TGF-α, EGF, PDGF, GM-CSF, TNF, CTGF, bFGF, and IGF-2. Many of these cytokines are able to stimulate the synthesis of other cytokines in a paracrine manner and to induce the proliferation of a great variety of epithelial and endothelial immune system cells which allows another important process for tissue growth and repair, namely angiogenesis (Cordova et al. 2005) (Table 1).

TGF-β

TGF-β belongs to a family of proteins that regulate the proliferation, proliferative inhibition and differentiation of a great variety of cells. Structurally, it is a 25 kiloDalton protein containing 112 amino acids. By its synthesis, the gene is identified on chromosome 19q13. There are seven isoforms of the

Table 1 Factors which control the palmar fascia fibroblasts' proliferation

TGF-BETA	TGF-ALFA and EGF-R	PDGF	GM-CSF	CTGF, bFGF, IGF-2,.	TNF	Free radicals	Metalloproteinases	Sex hormones	Gene expression alteration	mechanical stimulation

protein, however, the best known, in terms of its role in fibromatosis, are TGF-β 1 and TGF-β 2. TGF-β plays a role in fibroblast proliferation, differentiation and apoptosis, in the activation of proteolytic enzymes, in chemotaxis, in the control of vasomotor regulation and in the synthesis and deposition of collagen fibres in the extracellular space (Montesano and Orci 1988; Vaughan et al. 2000). It also acts as the main mechanoregulator growth factor, increasing the expression of integration and stimulating the up-regulation of certain cytoskeleton components involved in the transformation from fibroblast to myofibroblast. Many studies have investigated the role of this growth factor in Dupuytren's contracture (Kloen et al. 1995).

One study conducted on pathological and healthy palmar tissue (Badalamente et al. 1996) showed that:

1. TGF-β 1 is present at high concentrations in myofibroblasts and fibroblasts in all stages of Dupuytren's contracture.
2. Normal palmar fascia fibroblasts also express TGF- Beta 1, but at lower concentrations.
3. TGF-β 2 is present inside patients' myofibroblasts during the proliferative phase, but is not present in the fibroblasts of the residual phase and those of normal palmar fascia.
4. Adding TGF-β 1, TGF-β 2 or both to culture media containing myofibroblasts, significantly increases the proliferation of these cells, particularly in those areas with the highest cell density. In any case, TGF-β 2 has the most efficacious action on proliferation.

A study was recently conducted on the polymorphism of the TGF-β 2 gene's extremity 5[1] and its increased synthesis in fibromatous palmar fascia samples (Bayat et al. 2002a, b). More specifically, an abnormal insertion of a group of four amino acids known as "ACAA" (adenin, cytosin, adenin, adenin) was observed, between positions 1249 and 1250 of the amino acid chain. This allele abnormality is reported to be present in 65 % of patients with palmar fibromatosis analysed in this study and to cause the hyperactivation of the transcription factor HNF-3b with a consequent increase in the synthesis of TGF-β 2. The authors also researched a TGF-β 1 gene polymorphism that may be related to palmar fibromatosis (Bayat et al. 2002a, b). The comparison between 153 patients and 200 controls performed on codons 10, 25, 509, and 800 of the TGF-β 1 amino acid chain did not reveal any significant allele variation; therefore, at least for these known sites, it is not possible to identify a polymorphism related to the disease. Allele polymorphisms were also identified for the TGF-β receptor in patients with palmar fibromatosis (Bayat et al. 2003). A genetic analysis on TGF-β gene expression in myofibroblasts extracted from fibromatous cords and nodules showed that TGF-β 2 has a greater concentration in cords, whereas TGF-β 1 and 3 have a greater concentration in nodules Zhang et al. 2008). Research into genetic predisposition for Dupuytren's disease has yielded interesting results concerning the relationship between zinc finger protein ZF9 and TGF-β. Protein ZF9 is a transcription factor synthesised inside the cell nucleus whose main role consists in binding to the promoter sequence of the TGF-β 1 and TGF-β 2 genes and their respective receptors, promoting the transcription of the messenger RNA (mRNA) of these factors, thereby controlling the synthesis of all these molecules. In one study conducted on 138 subjects (119 men, 19 women) all with palmar fibromatosis, it was seen that the protein ZF9 gene synthesises an unstable form of this molecule, which is thought to be directly responsible for an increased synthesis of TGF-β 1, TGF-β 2, and their receptors, in both the serum and tissues. (Igarashi et al. 1996) demonstrated that the hyperexpression of the TGF-β 1 gene is responsible for the increase in the synthesis of the mRNA of connective tissue growth factor (CTGF), which is involved in the genesis of systemic scleroderma and nodular fasciitis, conditions characterised by soft tissue fibrosis. Once again this data shows the close relationship between TGF-β and the mechanism underlying palmar fibromatosis. TGF-β is known to have significant effects both in vitro and in vivo in scar repair models, such as an increase in type III collagen and glycosaminoglycan synthesis, abnormal formation of fibromatous tissue and angiogenesis stimulation, similar to those observed in Dupuytren's disease. Recent studies showed that when TGF-β 1 is added to culture media containing fibroblasts derived from pathological palmar fascia it has a mechano-transducer role in the triggering of reactions that lead to cell proliferation (Bisson et al. 2009). In vitro this cytokine has shown an ability to activate fibroblasts to myofibroblasts and to activate cell binding and contractility and could, therefore, in the future represent a potential target for the medical treatment of palmar fibromatosis. Research performed on fibroblasts extracted from fibromatous palmar fascia samples have shown that these cells synthesise HSP47 (heat-shock protein 47) and fibronectin, two factors associated with tissue scarring, with a higher concentration than in controls (Howard et al. 2004). Ultrastructural studies on palmar tissue samples from patients with Dupuytren's disease have revealed that myofibroblasts extracted and cultivated with type I collagen and TGF-β 1, increase their capacity to synthesise beta-catenin and alpha actin, thereby simulating the

biocellular reactions that in vivo lead to the healing of damaged tissue (Vi et al. 2009a, b).

In a recent study it has been shown that TGF-β 1 is able to induce p38 MAPK phosphorylation, expecially MK2 in primary nonDupuytren's disease cells (ND) grown from macroscopically unaffected palmar fascia adjacent to diseased tissue from Dupuytren's disease patients (DD) (Bujak et al. 2015). This kinase is exclusively phosphorylated by activated p38 and was previously implicated in myofibroblast differentiation and fibrotic processes other than Dupuytren's disease. Primary ND cells were isolated from macroscopically unaffected palmar fascia adjacent to diseased tissue obtained from patients diagnosed with the last stage of Dupuytren and cultured in vitro. Gene expression, collagen gel contraction assay and analysis of secreted proteins were performed on ND cells treated with TGF-β1 and/or inhibitor of p38 phosphorylation. The author detected stable endogenous expression of phosphorylated MK2 form in cells grown from macroscopically unaffected palmar fascia adjacent to diseased tissue prior to and upon treatment with TGF-β 1. In cells co-incubated with TGF-β1 and p38 phosphorylation inhibitor, the levels of phosphorylated MK2 diminished. The active form of MK2 was detected in untreated ND fibroblasts as well, which could be attributed to the well-known role of p38 MAPK signaling in homeostasis of palmar fascia fibroblasts or in the intrinsic predisposition of normal palmar fibroblasts in DD patients for disease development. Activation of p38 MAPK signaling by TGF-β 1 in ND cells has been shown to influence directly the expression of a number of tested genes, including genes encoding for chemokine (C-C motif) ligand 11 (*CCL11*), interleukin 6 (*IL-6*), chemokine (C-C motif) ligand 2 (*CCL2*), and interleukin 1 receptor accessory protein (*IL1RAP*) which were found to be over-expressed. Inhibition of p38 phosphorylation partially reversed this process and significantly down-regulated genes *CCL11* and *IL1RAP*. Similar down-regulation was also observed for *IL-6*. A number of genes had lower expression levels in ND cells activated by TGF-β 1 including those coding for complement component (*C3*), fms-related tyrosine kinase 3 ligand (*FLT3LG*), interleukin 6 receptor (*IL6R*), lymphocyte antigen 96 (*LY96*), toll-interleukin 1 receptor (*TIR*) domain containing adaptor protein (*TIRAP*), toll-like receptor (*TLR3*), toll like receptor 5 (*TLR5*), and histone deacetylase 4 (*HDAC4*). The majority of the genes whose expression levels were altered by TGF-β 1 showed reversed expression pattern upon treatment with the inhibitor of p38 phosphorylation. According to this study, a dual targeting of inflammation and p38 MAPK signaling in DD should be considered as a novel strategy for advancing the management of DD patients and prevention of disease recurrence.

In conclusion, a novel approach to the treatment of palmar fibromatosis could involve the inhibition of TGF-β -induced myofibroblast proliferation, using direct antibodies against TGF-β itself or against its receptor, in the early phases or recurrence of the disease. This type of approach may be possible once we have achieved full knowledge of the receptor for myofibroblast-expressed TGF-β.

TGF-α and EGF-R

Abnormal expression of TGF-α and EGF-R occurs during the various phases of palmar fibromatosis. Both are present in the myofibroblasts of the hypercellular nodules of the proliferative phase, but are absent in the hypercellular and fibrotic nodules of the involutive and residual phases. This explains why the myofibroblasts produce and release TGF-α, which, with an autocrine and paracrine mechanism (binding to EGF-R), stimulates cell proliferation (proliferative phase), with a consequent increase in the number of cells and formation of hypercellular areas; subsequently, TGF-alpha-inhibitors, which are as yet unknown, reduce its expression and cause hypocellularity. The myofibroblasts' contractile capacity will cause palmar retraction (Magro et al. 1997).

PDGF

The relationship between PDGF and myofibroblasts has been known for some time. Some authors believe that it is able to induce the proliferation of these cells. One ultrastructural study on fibroblasts extracted from fibromatous palmar fascia showed that, subject to tensile stress in the culture, these cells were able to synthesize PDGF, whereas the fibroblasts extracted from healthy palmar fascia did not have this characteristic (Alman et al. 1996). An increase in the expression of this growth factor has been experimentally proven in the fibrous areas of Dupuytren's disease and other forms of fibromatosis, in which high TGF-β 1 expression is observed. The use of anti-PDGF and anti-TGF-β 1 monoclonal antibodies seeded on culture media with myofibroblasts also revealed a reduction in the proliferation of these cells and the synthesis of collagen (Li et al. 2001). This has provided the basis for the hypothesis that the mitogenic effects of PDGF are controlled by means of a paracrine mechanism, by TGF-β itself, which suggests once again that this growth factor plays a central role. An ultrastructural study on samples of fibromatous tissue extracted from palmar fascia showed that PDGF creates a stable bond with dermatan sulphate, thereby generating a molecular core to which other extracellular matrix structures, such as type III collagen and fibronectin, bond. The inhibition of this binding could, in the future, represent a non-surgical approach to the treatment and prevention of palmar fibromatosis (Koźma et al. 2009).

GM-CSF

Studies performed on hepatic and renal fibrosis have suggested that a role may be played by GM-CSF, which is known for its proliferative effect on haematopoietic cells and the inflammatory process. More specifically, it has been observed that the transfer of the gene for modified GM-CSF synthesis to 12-week-old rat embryos causes an increase in the pulmonary synthesis of this cytokine and an increased concentration of TGF-β 1 in extrapulmonary fibroblasts. This in turn seems to increase the synthesis and deposition of collagen in the interstitial spaces with consequent pulmonary fibromatosis (Xing et al. 1997).

TNF

A recent study on TNF has proposed this cytokine as a therapeutic target to down-regulate myofibroblast differentiation and activity (Verjee et al. 2013). The study has compared the effects of TNF on contraction and profibrotic signaling pathways in fibroblasts derived from the palmar and nonpalmar dermis of patients with Dupuytren's disease and palmar fibroblasts from non-Dupuytren's patients. It has been demonstrated that the exogenous addition of TNF - but not other cytokines, including IL-6 and IL-1β - promotes differentiation into myofibroblasts, specifically in the palmar dermal fibroblasts of Dupuytren's patients. TNF acts via the Wnt signalling pathway and seems to drive contraction and profibrotic signalling in these cells. The authors have also examined the effects of targeted cytokine inhibition, and have demonstrated that neutralizing antibodies to TNF inhibited the contractile activity of myofibroblasts derived from Dupuytren's patients, reduced their expression of α-smooth muscle actin, and mediated disassembly of the contractile apparatus.

CTGF, bFGF, IGF-2

Connective tissue growth factor (CTGF) also known as CCN2 is a member of the CCN family- six matricellular proteins (CCN1-6) dynamically regulated and believed to contribute to tissue remodeling and repair, as well as the abnormal activation of connective tissue in pathological diseases such as fibrosis and cancers. One main reason for the interest in CTGF is its induction by TGF-β and its role as mediator of at least some of the fibrogenic actions of TGF-β.In adult connective tissue, CTGF is not normally expressed unless induced. For many years, CTGF has been known to be induced in wound healing and in fibrogenesis. However, until recently the precise in vivo role of CTGF has remained elusive. Tsang and Leask (2015) analyzed the role of CTGF in skin fibrosis. Mice lacking CTGF in cells expressing a fibroblast-specific type I collagen promoter/enhancer showed resistance to bleomycin-induced fibrosis and the progressive skin fibrosis caused by loss of phosphatase and tensin homolog (PTEN) expression in fibroblasts. In bleomycin-induced skin fibrosis, the overwhelming number of myofibroblasts stained positive with the pericyte/progenitor cell marker. These cells were also largely positive for CTGF and the pericyte/progenitor cell marker Sox2. CTGF expression by type I collagen- or Sox2-expressing cells appeared to contribute to bleomycin-induced skin fibrosis by being essential for the recruitment of these pericyte-like progenitor cells to fibrotic tissue. CTGF appeared to contribute to fibrosis by being required for dermal progenitor cells to differentiate into myofibroblasts. Such a mechanism seems not to contribute substantially to cutaneous tissue repair, a process dependent on TGF-β signalling likely due to its ability to induce myofibroblast differentiation of resident fibroblasts. Specifically, the authors found that although loss of CTGF resulted in failure to recruit cells derived from Sox2-expressing progenitors to the wound area, the overall impact of this defect was not detected. According to the experimental data, it is possible that CCN3, a member of the CCN family that antagonizes CTGF action, might block the recruitment of progenitor-derived cells to the wound. These studies have revealed that CTGF expression by progenitor cells is responsible for the development of skin fibrosis in the bleomycin model but not cutaneous tissue repair. Thus, CTGF may be a selective anti-fibrotic target.

Bruno et al. (2015) verified that in cultured myoblasts CTGF cause their transdifferentiation into myofibroblasts by up-regulating the expression of fibrosis marker proteins α-smooth muscle actin and transgelin. It was also found that the profibrotic effect exerted by CTGF was mediated by the sphingosine kinase (SK)-1/$S1P_3$ signalling axis specifically induced by treatment with the profibrotic cue. Another significative finding is that CTGF silencing prevented the TGFβ-dependent up-regulation of SK1/S1P3 signaling axis and strongly reduced the profibrotic effect exerted by TGFβ, pointing at a crucial role of endogenous CTGF generated following TGFβ challenge in the transmission of at least part of its profibrotic effect. These results provide new insights into the molecular mechanism by which CTGF drives its biological action and strengthen the concept that SK1/S1P3 axis plays a critical role in the onset of fibrotic cell phenotype.

A recent study analyzing the potential molecular determinants that might contribute to the formation of Dupuytren's tissue has demonstrated that specific areas of the fibrous tissue could sustain cell proliferation (Viil et al. 2015). The authors have studied the expression pattern of cell proliferation marker Ki67, phosphorylated AKT (Ak mouse strain thymoma) kinase, connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF), insulin-like growth factor 2 (IGF-2) and extracellular matrix components (laminins, fibronectin, collagen IV) in Dupuytren's disease tissue and normal palmar fascia, using immunofluorescence microscopy and quantitative real-time polymerase chain reaction (qPCR). Proliferative

cells in the fibrous nodules are concentrated in the immediate vicinity of small blood vessels and are localized predominantly in the myofibroblast layer. Correspondingly, the Dupuytren's disease-associated blood vessels contained increased levels of phosphorylated AKT, a hallmark of activated growth factor signalling. When studying the expression of potential activators of AKT signalling, the authors found that the expression of bFGF was confined to the endothelium of the small blood vessels, IGF-2 was present uniformly in the fibrous tissue and CTGF was expressed in the Dupuytren's tissue associated with sweat gland acini. In addition, the blood vessels in fibrous nodules contained increased amounts of laminins 511 and 521, which are well known to promote the proliferation and stem cell properties of different cell types. According to the study, in Dupuytren's nodule-small blood vessels, the presence of growth factors in combination with favorable extracellular matrix composition provide a supportive environment for sustained proliferation of myofibroblasts and, thus, the blood vessels play an important role in Dupuytren's disease pathogenesis.

Autophagy is a highly conserved catabolic pathway activated in response to stress or starvation. Several studies have highlighted a correlation between dysregulated autophagy and the development of fibrosis. However, the signalling pathways and central mediators linking autophagy to myofibroblast differentiation and fibrosis remain largely undefined. In the past decade, a large body of evidence has demonstrated that CTGF, is another central mediator of myofibroblast differentiation and fibrosis. CTGF expression is activated downstream of TGFB-dependent SMAD signalling and various reports point to CTGF as a key activator of myofibroblast differentiation. Autophagic fibroblasts showed increased expression and secretion of CTGF, and CTGF silencing prevented myofibroblast differentiation. Bernard et al. (2014) demonstrated temporal relations between sustained autophagy and myofibroblast differentiation, and also the central role for autophagy in triggering myofibroblast differentiation, by use of the MTOR inhibitor rapamycin, a classical inducer of autophagy, in models of renal, pulmonary, and skin fibrosis. This inhibitor has been shown to prevent or to decrease fibrotic indices including myofibroblast differentiation. According to Bernard et al., in a pure fibroblast system, autophagy enhances myofibroblast differentiation through TGFB-independent pathways. Also, neutralizing antibodies against all active isoforms of TGFB failed to prevent autophagy-induced myofibroblast differentiation while effectively blocking TGFB-induced differentiation. This study has shown MTORC2 can act as an upstream regulator of stress-induced proteins and identify CTGF as a novel downstream product of MTORC2 signalling. These results do not, however, rule out a potential contribution of TGFB in vivo where the gathering of immune cells to the sites of tissue remodelling could accentuate myofibroblast differentiation via TGFB-dependent pathways.

Role of free radicals

The relationship between free radicals and palmar fibromatosis has been known for some time now and is associated with the cascade of biochemical reactions that these molecules are able to trigger in the tissues in which they accumulate. Traumas, smoking and uncompensated metabolic diseases, such as diabetes and dyslipidaemia, are responsible for the necrosis of pericytes, structural cells found in blood vessel walls. This causes endothelial cell activation with a thickening of the basement membrane, followed by a reduction in the lumen of the capillaries and localised hypoxia. The reduction in tissue oxygen causes an increase in the levels of xanthine oxidase, an enzyme that catalyses the conversion of hypoxanthine to xanthine, from which uric acid and free radicals are produced. As a consequence, in the event of ischaemia the concentration of these molecules increases exponentially. Free radicals are molecules made up of one or more atoms with a free electron, which makes them extremely unstable, reactive and able to bind other radicals or take an electron from other neighbouring molecules. The ability of free radicals to stimulate fibroblast proliferation and the deposition of collagen leading to the formation of fibromatous tissue has been extensively proven. Studies on seeding culture media containing fibroblasts with allopurinol, a molecule that inhibits xanthine oxidase and prevents the formation of free radicals, have not, however, provided conclusive results concerning the possibility of inhibiting fibroblast proliferation. The administration of high-pressure oxygen (hyperbaric therapy), applied in the initial stages of the condition, by reducing the concentration of free radicals, could slow down the progression of fibromatosis; however, the data obtained from the literature is inadequate to establish the true efficacy of such treatment Yildiz et al. (2004).

Molecular genetics

Metalloproteinases

Recent studies have analysed the potential role of matrix metalloproteinases (MMP), proteolytic enzymes whose structure contains zinc atoms, in the genesis of Dupuytren's disease (Townley et al. 2008). These enzymes are expressed by fibroblasts, endothelial cells, macrophages, keratinocytes and certain tumour cells that, thanks to the lytic effect these enzymes have on the extracellular matrix, reach the lymphatic and blood systems and are carried to other areas (metastasis mechanism). Metalloproteinase activity is controlled by a specific inhibitor known as TIMP (tissue inhibitor of metalloproteinases). The activity of metalloproteinases is increased after

tissue damage and they play an important role in tissue repair by facilitating cell movement and the consequent contraction of the matrix. One analysis on metalloproteinase gene expression in myofibroblasts extracted from relapsed palmar fibromatous tissue revealed an abnormal activation of the MMP-2, MMP-13, MMP-14, MMP-16, MMP-19 and for ADAMT-S2, ADAMT-S4, ADAMT-S5, ADAMT-S14, ADAMT-S16 genes compared to controls (Johnston et al. 2008; Shih et al. 2012). An analysis of the levels of MMP-2 performed on fibromatous and healthy tissue samples revealed a higher concentration of this molecule in the pathological fascia than in control samples, although it would appear that MMP-2 levels do not vary with the stage of the disease. This suggests that it plays a role in the trigger mechanisms of palmar fibromatosis, rather than in its progression or recurrence (Qian et al. 2004; Augoff et al. 2006). Studies conducted on fibromatous nodules and cords have shown an increase in the activity of TIMP1 compared to controls, and a consequent blockage of MMP2 and MMP9. The increased deposition of collagen on the palmar fascia, which is no longer balanced by the degradation by the MMPs, appears to be responsible for the formation of fibromatous tissue (Ulrich et al. 2003; Johnston et al. 2008; Ulrich et al. 2009). Metalloprotease inhibitors could represent a critical target in the prevention of contracture. Indeed the potency of their action causes a reduction in the contraction of the extracellular matrix. Ilomastat, a broad-spectrum matrix metalloproteinase inhibitor, is able to slow down tissue contracture in cultures of fibroblasts obtained from both fibromatous nodules and cords, with more significant activity on nodules. In vitro studies involving seeding ilomastat on culture media containing fibromatous tissue fibroblasts have shown this molecule's ability to regulate the contractile force of these cells (Townley et al. 2009).

Gene expression alteration

Cells isolated from cords and nodules have been seen to present altered expression of various genes, which would appear to affect cell proliferation, differentiation and apoptosis and on the synthesis of extracellular matrix proteins and, therefore, also on the development of the mitochondrial cytoskeleton, lipid metabolism and the inflammatory cascade (Vi et al. 2009a, b; Yenidunya et al. 2009). Some of the most extensively analysed genes are POSTN, ADAM12 and TNC. These markers have already been associated with other cell growth disorders and in particular with other fibrotic diseases (Le Pabic et al. 2003; Kraljević Pavelić et al. 2009a, b).

Despite being a challenging arduous path, this is nevertheless one which researchers must take in order to clarify, once and for all, how decisive the role of the genome is in the genesis of the disease and to what extent familiarity influences the incidence of fibromatosis.

Sex hormones

Research into sex-related aetiological factors has led to a re-evaluation of the role played by sex hormones in myofibroblast proliferation. One case control study on androgen receptors present on the surface of myofibroblasts (Pagnotta et al. 2002) showed that the number of these receptors is far higher on myofibroblasts derived from the fibromatous fascia of male patients, than on the myofibroblasts obtained from the normal fascia of subjects undergoing carpal tunnel surgery. A subsequent study analysed the role of the biologically-active metabolite of testosterone, 5 α-dihydrotestosterone (5αDHT), in myofibroblast proliferation (Pagnotta et al. 2003). It was shown that seeding 5αDHT on culture media containing myofibroblasts derived from fibromatous fascia increased its multiplication and collagen synthesis with a greater intensity than on myofibroblasts derived from the palmar fascia. However, oestrogens are also thought to be capable of activating myofibroblast multiplication as shown in one study on tamoxifen, an anti-oestrogenic metabolite used in hormone therapy for cancer (Kuhn et al. 2002). In the presence of tamoxifen, the myofibroblasts of patients with palmar fibromatosis appears to present a lesser capacity for TGF-β2 synthesis, with a consequent reduction in collagen proliferation and synthesis. The findings illustrated above show that we still have a great deal to learn about the complex mechanisms regulating the control of cell growth and collagen synthesis, aetiopathogenetic moments that are fundamental to the onset of palmar fibromatosis.

The effects of mechanical stimulation

One important fibroblast function consists in the capacity to exert cytomechanical forces on the surrounding extracellular matrix. Human fibroblasts tend to maintain tensional homoeostasis. It has been shown on a three-dimensional collagen matrix that the cells, when subject to a uniaxial increase in force, respond to stimulation mechanically, i.e. by reducing the force exerted on the matrix, to take the system to an equilibrium. The opposite cell response is observed if there is a reduction in the force applied (Bisson et al. 2004). In Dupuytren's disease, on the other hand, the fibroblasts present force generation abnormalities and, therefore, escape this tensional homoeostasis mechanism. They respond by generating force both to an increase and to a decrease in tension (thus, regardless of the type of mechanical stimulus applied) and present a contractile activity on the extracellular matrix that is far greater than that of normal fibroblasts. The cells derived from cords and nodules are phenotypically similar, despite presenting certain different antigens (Hindocha et al. 2011), and they do not show any qualitative or quantitative differences in the generation of force. The above alterations are enhanced by the addition of TGF-β 1, which increases the

force generated and exacerbates the contractile response to the increase in tension. Its action would appear to be related to an up-regulation of the extracellular matrix – cell interaction molecules or to a direct effect on the cytoskeleton; in addition, in this phase, TGF-β 1 could regulate certain intracellular transduction pathways involved in the recognition of and response to external forces.

These alterations are thought to have important pathophysiological repercussions given the continuous mechanical stimuli the palmar fascia is subject to. The continuous microtrauma are thought to stimulate the differentiation of fibroblasts into myofibroblasts, under the action of fibrogenetic cytokines. Subsequently, the matrix, and in particular the collagen fibres, are remodelled by the cells, as shown by the increased expression of matrix metalloproteinase in nodules and cords.

The role of the extracellular matrix

Research into the interactions between cells and their microenvironment is of fundamental importance to the understanding of the pathophysiology of Dupuytren's disease. The molecular mechanisms underlying the condition, especially those associated with cell growth and differentiation, could, in the future, represent an important target for nonsurgical treatments. Attention has recently focused on various factors able to modify the interactions between cells and the extracellular matrix. As we will see later, fibroblasts respond to chemical signals, cytokines and growth factors, but also to mechanical stimulation through a process of *mechanotransduction* (Brown et al. 1998), which is able to modify the cells' differentiation and ultrastructural characteristics, as well as the production of matrix and other proteins (Satish et al. 2008).

The connective tissue is constituted by cellular components (10 %) and extracellular components (90 %). Cells can be classified according to their migratory behaviour: fibroblasts and fibrocytes are *fixed* i.e. stationary cells, whereas histiocytes, monocytes, macrophages and lymphocytes are *mobile* (Krstic 1988). The most important stationary connective cell is the fibroblast, the *totipotent* cell element that has been most extensively researched by man (Tomasek et al. 1999). The fibroblast, with its pseudopods, forms a mesh structure inside which the mobile cells move. It is a biosynthetically active cell, i.e. one that is able to secrete various molecules that it transfers into the extracellular space; fibrocytes, on the other hand, stable cell elements derived from fibroblasts by differentiation, are inactive and unable to secrete cytokines. Further details on the fibroblast and its role in the genesis of Dupuytren's disease will be provided later. Collagen is the best known structural protein of the matrix. The fibroblasts synthesise its simple components, peptides, which combine to form molecules of pro-collagen from which tropocollagen derives. This latter precursor is converted into a three-dimensional triple-helix structure and finally into molecules of collagen, by means of the removal of special peptides. Hydroxyproline and hydroxylysine are the constant peptides in the structure of collagen and are rarely identified in the structure of other proteins. Their presence is an indirect indicator of collagen synthesis. The overall collagen content is more or less the same as that of hydroxyproline. Under an electron microscope, collagen fibres lose their flexibility and transparency and appear thicker and three-dimensionally interwoven. It has been calculated that in the superficial palmar fascia not affected by fibromatosis the amount of collagen present is 25 % higher than in the other fascia tissues. 1 mg of healthy palmar fascia is thought to contain 73.4 μg of collagen; in the fibromatous fascia of the intermediate stages of the condition, its concentration is thought to rise to 87.4 μg/mg, and to 91.3 μg/mg in the fibrous cords of the advanced stages. One of the main biochemical characteristics of Dupuytren's disease is the excessive synthesis and deposition of this protein in the palmar fascia. Ultrastructural research has shown that there are approximately 12 genetically-different types of tissue collagen, of which the first five are the best known. Type I collagen is characteristic of normal superficial palmar fascia, whereas type III is thought to be present at higher concentrations in pathological palmar fascia (Bailey et al. 1994; Melling et al. 2000). The type III: type I collagen ratio is thought to increase as the disease progresses, but is not due to a greater synthesis of type III collagen than type I collagen by the fibroblasts, rather to an increase in fibroblast density (Murrel and Hueston 1990). The same thing has also been observed for fibroblasts generated by other pathological tissues such as keloids, hypertrophic scars, and also by foetal skin. However, it is not clear how the increase in cell density in these tissues causes a reduction in the synthesis of type I collagen.

Binding proteins

The sites of binding to the extracellular matrix on surface of the fibroblasts described by Ryan et al. (1974) were studied in greater detail in 1979 by Majno (1979), who named them *binding proteins*. Fibronectin is the best known of these surface proteins. Its metabolism occurs primarily in the myofibroblasts of the nodular phase of palmar fibromatosis. Its concentration remains high throughout the initial phases of the disease and gradually decreases in the advanced stages. The role of this protein in Dupuytren's disease consists in guaranteeing fibroblast binding to the surrounding type I and III collagen. It is also able to transmit the mechanical contraction signal from the intracellular environment to the extracellular one (Tomasek et al. 1987; Berndt et al. 1994; Hoch et al. 2002), making Fibronectin essential to the connective tissue remodelling process. Transforming growth factor-beta1 (TGF-β 1), which we will describe later, is able to stimulate

fibroblast synthesis of fibronectin and its receptor (Tomasek et al. 1999).

The fibroblasts' basement membrane contains high concentrations of a protein known as *laminin,* which facilitates the binding of these cells to type IV collagen, glycosaminoglycans, heparin and heparan sulphate (Yurchenco 1989).

Desmin and vimentin are binding proteins that act as markers of muscle cell differentiation, they are also present on the surface of the fibroblasts and myofibroblasts, which they can be synthesised by, in line with the hypothesis that myofibroblasts can be obtained from undifferentiated muscle cells, or that, like the latter, have contractile abilities that explain the shortening of the fibromatous cords.

Another characteristic of myofibroblasts is the presence inside them and on a transmembranous level of microfilaments made up of cytoskeletal binding proteins, in particular, *myosin* and *actin*. There are five actin isoforms: skeletal alpha actin, smooth muscle alpha actin, cardiac alpha actin, beta actin and gamma actin. Smooth muscle alpha actin is present in far greater concentrations in the myofibroblasts during the early stages of Dupuytren's disease, and decreases in the advanced faces, in which the myofibroblasts stop multiplying. This relationship could explain the increase in fibroblast density in the early stages of palmar fibromatosis. One ultrastructural study on the synthesis of this protein showed that adding TGF-β 1 to culture media containing myofibroblasts extracted from fibromatous nodules and cords increases alpha actin synthesis compared to cells extracted from normal palmar fascia (Bisson et al. 2003). Myosin partners with actin in regulating fibroblast binding to the proteins of the extracellular matrix and in particular to collagen (Tomasek et al. 1999). Beta-catenin is a protein involved in the stabilisation of the cytoskeleton and intercellular junctions. High cytoplasm and nuclear concentrations of beta-catenin have been observed in fibromatous cords and nodules and some authors believe they may be the cause that triggers fibroblast proliferation (Bowley et al. 2007; Degreef et al. 2008; Vi et al. 2009a, b). Its accumulation is caused by TGF-β 1 and by changes in isometric tension and interactions between cells and the extracellular matrix (Howard et al. 2003; Wong and Mudera 2006; Vi et al. 2009a, b). The change in beta-catenin levels may be one of the causes of the increased density of the myofibroblasts and their contractile ability mentioned above (Vi et al. 2009a, b). The relationship between transcription factor Zic1 and the synthesis of beta-catenin and alpha actin was recently analysed (Degreef et al. 2009). In myofibroblasts extracted from the palmar fascia of 20 patients with Dupuytren's disease, it was observed that Zic1 hyperexpression does not occur in all myofibroblasts; however, the concentration of the two cytoskeleton proteins is in any case high, at least in the early stages of the disease. The lack of a direct proportional relationship between the concentration of Zic 1 and that of beta-catenin and alpha actin would appear to be related to a kind of phenotypical heterogenicity of the myofibroblasts.

One recent study showed that the altered synthesis of this molecule is related to a certain genetic pattern observed in patients with fibromatosis (Mosakhani et al. 2010).

Integrins and disintegrins are binding proteins present on different types of mobile cells (monocytes, lymphocytes and histiocytes) and on myofibroblasts. High levels of α5β1, a TGF-β 1-induced integrin, have been observed in fibromatous palmar tissue (Magro et al. 1997); its interaction with collagen fibres would appear to favour cytoplasmatic beta- catenin accumulation in certain tumour cells, by breaking bonds. A similar mechanism has been suggested for collagen-rich fibromatous cords (Vi et al. 2010).

The POSTN gene encodes a matrix protein known as *periostin*, which is involved in cell binding and angiogenesis. Its up-regulation would appear to be an effect of the disease's development rather than a cause. Indeed, the POSTN gene has a negative influence on the growth of certain tumours, by promoting the synthesis of extracellular proteins that, through cell binding, are thought to cause the formation of a kind of peritumoural capsule. It is possible that the high proliferation of myofibroblasts in the nodules leads to a higher expression of this gene with an increase in the synthesis of cytoskeleton proteins and the formation of further nodules (Vi et al. 2009a, b).

Protein ADAM12 (Disintegrin and metalloproteinase domain-containing protein 12) belongs to a family of membrane *proteases* containing disintegrin and metalloprotease domains; it is involved in myofibroblast activation through a TGF-β -induced mechanotransduction reaction (Kveiborg et al. 2005; Frohlich et al. 2006; Rocks et al. 2008). Abnormal gene activation by this protein has been observed in a number of fibrotic conditions. According to one recent study conducted on palmar fibromatous tissue samples compared with samples of healthy palmar fascia, it was seen that the subcutaneous and adipose tissues covering the fibromatous nodules hyperexpress the ADAM12-encoding gene, with a consequent accumulation of it in the extracellular space (Shih et al. 2009).

The TNC gene encodes tenascin C, a glycoprotein of the extracellular matrix, involved in the regulation of TGF-β expression. Tenascin C has been seen to play a role in the binding of fibroblasts and myofibroblasts to the collagen of the extracellular matrix during scarring (Qian et al. 2004); this protein is therefore also being studied in fibromatous palmar fascia.

Glycosaminoglycans of the extracellular matrix

Glycosaminoglycans (GAGs), which were previously known as mucopolysaccharides, are long unbranched chains made up of disaccharide units that are repeated in a given order, alternating an saccharide amino acid (containing an amine instead

of a simple –OH group) with a monosaccharide that is usually acidic (i.e. it contains one or more carboxylic acids and/or sulphonic acids and therefore negative charges). Being hydrophilic, GAGs bind very readily to water molecules, creating hydrated structures and contributing to the stability of the extracellular matrix. The type, composition and characteristics of the glycosaminoglycans vary depending on the type of connective tissue. The matrix's chemical and physical properties differ with their composition. GAGs perform primarily support and protection functions for most tissues, i.e.:

- they create and keep constant extracellular turgor pressure;
- they contain large quantities of reservoir water;
- they give cartilage its shock absorbing properties, as GAGs are able to change their volume rapidly by releasing or binding water molecules;
- they perform lubrication functions inside the synovial membrane;
- they transport water-soluble molecules that are able to distribute rapidly inside the porous structure of the GAG;
- they provide glycoside units during the synthesis of the glucidic component of glycoproteins;
- they control the viscosity and permeability of the extracellular matrix;
- they can bind with one another and with proteins to obtain proteoglycans

Glycosaminoglycans can be divided into four classes, according to their biochemical characteristics and varying metabolism: 1) hyaluronic acid, 2) chondroitin and dermatan sulphate, 3) heparan-sulphate and heparin, 4) keratan sulphate. Heparin isomers and keratan sulphate are well known anticoagulants. Only the first two classes would appear to be involved in the aetiopathogenesis of Dupuytren's disease.

Hyaluronic acid The structural simplicity of this molecule has made it the best known of all glucosaminoglycans. It is synthesised by many different types of cell, but primarily by fibroblasts. It modulates the consistency of the gel of the fundamental extracellular substance and is responsible for its viscosity and plasticity (Bertheim and Hellström 1994); it also facilitates cell mobility during tissue morphogenesis and takes part in scarring repair processes, making it an indicator of connective remodelling. When the remodelling process is complete, the excess hyaluronic acid is broken down by the enzyme *hyaluronidase* (Mast et al. 1992; Brenner et al. 1994). Its daily turnover is estimated to be 150 mg, its urinary concentration is approximately one tenth that of its serum concentration and it is eliminated primarily by the liver (Endo et al. 1979). High hyaluronic acid serum concentrations are typical of people with rheumatoid arthritis, particularly early in the morning (Trelstad 1989).

Chondroitin sulphate The most extensively studied of the glycosaminoglycans, its characteristics are similar to the dermatan sulphate group, from which it differs by the epimerisation of glucuronic acid to irudonic acid. Its average metabolism varies from 7 to 10 days (Gurr et al. 1985; Karlson 1998; Trelstad 1989).

The dermatan group The main isomers of this glycosaminoglycan are: non-sulphated dermatan, dermatan 4-sulphate and 6-sulphate. High concentrations of this macromolecule have been observed in human skin. It is a natural anticoagulant, with therapeutic potential as an alternative to heparin (Dawes and Pepper 1992; Silvestro et al. 1992). Glycosaminoglycan biosynthesis starts with hyaluronic acid and proceeds to the synthesis of chondroitin 4-sulphate and chondroitin 6-sulphate. One ultrastructural study on samples of fibromatous fascia tissue showed that the dermatan sulphates undergo structural alterations due to changes in the glucuronil epimerisation process, which is thought somehow to alter the signals between the matrix and fibroblasts, causing an abnormal production of fibrils and an accumulation of collagen in the extracellular space (Koźma et al. 2007).

Proteoglycans

Proteoglycans are very large molecules that constitute the main glucosamine component of the fundamental substance, the gelatinous matter in which the matrix is immersed and in which the main chemical and physical reactions of the extracellular environment take place. The term proteoglycan was initially used to indicate molecules containing chondroitin sulphate bound to non-collagen proteins. Proteoglycans are now a group of macromolecules constituted by a protein core to which a disaccharide is bound. Proteoglycan synthesis starts with the transcription and translation of the protein core in the endoplasmic reticulum. The polypeptide and polysaccharide are added to the core in the Golgi organ, followed by exocytosis into the extracellular matrix. 1 g of hyaluronic acid is able to bind 5 mL of water (Varma et al. 1983). The amount of water in the extracellular matrix increases when the normal palmar fascia becomes cords and, ultimately, fibromatous nodules. In healthy fascia tissue it is usually 54.3 %, in cords 60.7 %, and in nodules 62.1 % (Bazin et al. 1980).

Fascia glycosaminoglycans in Dupuytren's disease

Fibromatous fascia tissue contains small proteoglycans (PG-S) and large proteoglycans (PG-L), as well as glycosaminoglycans of the dermatan and chondroitin sulphate group. Structural analysis suggests that in Dupuytren's disease, PG-L have a longer protein core and a lower glycosaminoglycan concentration. Compared to normal fascia, these macromolecules are not merely quantitatively different, they are also

structurally different (Tunn et al. 1988; Gurr et al. 1985; Schmidtchen and Fransson 1992; Scott 1994). When analysing the proximal, distal, radial and ulnar sections of pathological palmar aponeurosis, an increase is observed in the concentration of hyaluronic acid in a proximodistal and ulnoradial direction. Its synthesis is eight times greater than in normal palmar fascia. Chondroitin 4-sulphate is the glycosaminoglycan present in the highest concentrations in fibromatous palmar fascia (between 41.5 and 63.7 %), and it increases in a proximodistal and proximoradial direction. Dermatan 4-sulphate is the second most common glycosaminoglycan in pathological fascia tissue, with a concentration of 17.9 – 24.1 %, and increases in a proximodistal, proximoradial and proximoulnar direction. We could conclude by stating that in primarily radial Dupuytren's disease chondroitin 4-sulphate is the glycosaminoglycan present in the highest concentrations (Koźma et al. 2007), whereas dermatan 4-sulphate is synthesised to a greater extent in the ulnar region of the fibromatous fascia. The repeated repair processes undergone by the extracellular matrix during palmar fibromatosis explain why these glycosaminoglycans are synthesised in greater quantities than in normal fascia tissue, but do not explain the reason for different concentrations between individual glycosaminoglycans. The data could be explained as follows: an endogenous mediator, non-muscle myosin, is thought to trigger a kind of "contraction" by the myofibroblasts (Ryan et al. 1974), followed by a change in three-dimensional structure of the extracellular matrix that these cells are bound to and the remodelling of the collagen fibres. These changes are thought to cause the "contracture" of the superficial palmar fascia. Collagen fibrillogenesis would appear to be associated with certain glycosaminoglycans, in particular small-diameter collagen fibrils are related to a higher local concentration of hyaluronic acid, those with an intermediate calibre to a local increase in chondroitin 4-sulphate and those with a large calibre to dermatan 4-sulphate. According to one histochemical study, inside the collagen fibrils, newly-synthesised glycosaminoglycans are thought to bind to specific sites (Scott 1994) that trigger the release of alpha actin from the myofibroblasts into the extracellular space. Similarly they are thought to make cytochemical mediators such as TGF-β, gamma-interferon and heparin. Alpha actin is thought to stimulate the further "contraction" of the myofibroblasts and the three-dimensional change in the extracellular matrix (Tomasek and Rayan 1995). Histological studies on myofibroblasts extracted from fibromatous nodules and cultivated *in vitro* have shown that these cells, once they have been "transformed" from fibroblasts into myofibroblasts, maintain their ability to synthesise alpha actin, even outside the environment in which they are activated (Dave et al. 2001). According to recent studies, recurrence of the condition is associated precisely with these cells that, being adjacent to pathological palmar fascia, are not removed during aponeurectomy procedures. The theory of a *myofibroblastic migration* from the skin would no longer appear to be valid. Many of the details of these biochemical processes and ultrastructural changes are not known and do not yet allow us to fully understand the complex aetiopathogenesis of Dupuytren's disease.

Current therapies

Dupuytren's disease is a benign fibroproliferative disorder that causes the fingers to be drawn into the palm via formation of new tissue under the glabrous skin of the hand. This disorder causes functional limitations, but it can be treated through a variety of surgical techniques. As a chronic condition, it tends to recur. Different treatments can be adopted as needle fasciotomy, segmental fasciectomy, z-plasty, skin-grafting. All these procedures are associated to different recurrence rate and complications. In the needle fasciotomy, the less invasive procedure, a small hypodermic needle is used to divide and sever the contracting bands in the diseased areas of the palm and fingers, releasing the contracture and avoiding the extra surgical trauma associated with resecting it, including possible skin grafts. Patients benefit from rapid healing and are able to return to normal activity after 48 h, with no need for physical therapy. However, not all cases of Dupuytren's disease can be corrected with needle fasciotomy. Fasciectomy to date represents the gold standard procedure, especially for advanced stage Dupuytren's disease or in case of several recurrences in which the skin is contracted and involved by fibrous process. The goal of limited fasciectomy is to excise the diseased fascia to avoid progression of the disease. A patient should be referred to a hand surgeon if the MCP contracture is more than 30° or if any contracture of the PIP is present. Functional disability may be an indication for surgery if the patient accepts the associated morbidity and understands that surgery, as the other procedures, may not be curative. As with all elective surgeries, the patient's age, comorbid conditions, and ability to comply with postoperative care and rehabilitation also determine whether surgery is appropriate. In February 2010, the first nonsurgical treatment was approved by FDA: enzymatic fasciotomy by collagenase Clostridium histolyticum at a dose of 0.58 mg per injection for the treatment of Dupuytren's disease in a single digit (Gilpin et al. 2010). The rate of recurrence is similar to those of needle fasciotomy, adverse events observed as tendon ruptures, anaphylactic reactions, or nerve or ligament injuries, are statistically not significant (Arora et al. 2016). In affected PIP joints, collagenase seems significantly worse residual contracture in patients treated with collagenase compared with those underwent limited fasciectomy (Zhou et al. 2015).

Summary

Given the above, it goes without saying that palmar fibromatosis is a complicated process that involves a cascade of molecular and cellular events, in which the cytokine TGF-β plays a fundamental role. Once started, this process is thought to be followed by feedback with TGF-β auto-induction. Secondly, the increase in fibromatosis appears to lead to localised hypoxia, which amplifies TGF-β expression. This complex mechanism may also involve free radical synthesis, induced by tissue hypoxic secondary to continuous microtrauma, smoking and uncompensated metabolic diseases. However, the studies conducted to date on the aetiological role of biomolecular factors in palmar fibromatosis have not allowed the identification of a potential nonsurgical treatment for Dupuytren's disease.

Conclusions

Many processes are still unclear and numerous questions are still waiting to be answered. For example, it is not known for certain what triggers the cascade of events that causes an excessive proliferation of fibroblasts; researchers are still asking themselves whether a genetic defect underlies altered fibroblast apoptosis and whether a molecule exists that is able to modulate this mechanism. Moreover, it is still not clear whether the fibromatous process starts with local hypoxia, as it would seem, or with an inflammatory response to external or internal stimuli, followed by the activation of macrophages and/or platelets producing specific fibrogenic cytokines. Research is currently focusing on local gene therapy with which, in the near future, it may be possible to control the expression of certain growth factors, by using antibodies that inhibit their binding with their respective receptors, or, when binding has already taken place, by blocking the molecule – receptor complex. The local application of anti-TGF-β antibodies has been seen to cause a decrease in the evolution of scarring in rats and the histological evaluation of tissue has shown a reduction in the number of macrophages, reduced angiogenesis and lesser collagen deposition. Many substances that act on the proliferation and differentiation mechanisms are currently being studied in view of their potential therapeutic repercussions in the early stages of the disease. Amidine-dihydrotyenothyenyl-quinolone hydrochloride has been seen to have an anti-proliferative effect that appears to be associated with an action on DNA and a specific action on cytoskeleton modifications in cells undergoing differentiation. This compound specifically inhibits the activity of topoisomerase in mammals and interferes with tubulin polymerisation. These modifications would appear to prevent fibroblasts from acquiring the myofibroblastic phenotype (Kraljević Pavelić et al. 2009a, b).

Dupuytren's disease is a disease confined to humans, and a major limiting factor in investigating this disorder has been the lack of a faithful animal model that can recapitulate its distinct biology. In a recent study Dupuytren's disease (DD)- and control carpal tunnel (CT)-derived fibroblasts were transplanted in the forepaw of the nude rats. Both fibroblasts survived for a period of 62 days, but DD-derived cells showed a significantly greater level of persistent fluorescent signal at the end of this time than did CT-derived cells. mRNA expression levels of α-smooth muscle actin (α-SMA), type I- and type III- collagens were all significantly elevated in the forepaw receiving DD cord-derived fibroblasts in comparison to CT-derived fibroblasts. Masson's trichrome stain confirmed increased collagen deposition in the forepaw that was injected with DD cord-derived fibroblasts. DD-derived fibroblasts showed persistently elevated expression of multiple genes involved in fibrosis and contraction after transfer to the forepaw. In conclusion this animal model could be used as a baseline for investigating novel therapeutic regimens to determine an effective strategy in treating palmar fibromatosis.

Compliance with ethical standards

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References

Alman BA, Greel DA, Ruby LK, Goldberg MJ, Wolfe HJ (1996) Regulation of proliferation and platelet-derived growth factor expression in palmar fibromatosis (Dupuytren contracture) by mechanical strain. J Orthop Res 14(5):722–728

Arora R, Kaiser P, Kastenberger TJ, Schmiedle G, Erhart S, Gabl M (2016) Injectable collagenase Clostridium histolyticum as a nonsurgical treatment for Dupuytren's disease. Oper Orthop Traumatol 28: 30–37

Augoff K, Ratajczak K, Gosk J, Tabola R, Rutowski R (2006) Gelatinase A activity in Dupuytren's disease. J Hand Surg [Am] 31(10):1635–1639

Badalamente MA, Sampson SP, Hurst LC, Dowd A, Miyasaka K (1996) The role of transforming growth factor beta in Dupuytren's disease. J Hand Surg [Am] 21(2):210–215

Bailey AJ, Tarlton JF, Van der Stappen J, Sims TJ, Messina A (1994) The continuous elongation technique for severe Dupuytren's disease: a biochemical mechanism. J Hand Surg [Am] 19:522–527

Bayat A, Alansar A, Hajeer HA, Shah M, Watson JS, Stanley JK, Ferguson MW, Ollier WE (2002a) Genetic susceptibility in Dupuytren's disease: lack of association of a novel transforming

growth factor B2 polymorphism in Dupuytren's disease. J Hand Surg (Br) 27(1):47–49

Bayat A, Watson JS, Stanley JK, Alansari A, Shah M, Ferguson MW, Ollier WE (2002b) Genetic susceptibility in Dupuytren's disease. TGF-beta1 polymorphisms and Dupuytren's disease. J Bone Joint Surg (Br) 84(2):211–215

Bayat A, Stanley JK, Watson JS, Ferguson MW, Ollier WE (2003) Genetic susceptibility to Dupuytren's disease: transforming growth factor beta receptor (TGFbetaR) gene polymorphisms and Dupuytren's disease. Br J Plast Surg 56(4):328–333

Bazin S, Le Lous M, VC D (1980) Biochemistry and histology of the connective tissue of Dupuytren's disease lesion. Eur J Clin Invest 10:166–171

Bernard M, Dieudé M, Yang B, Hamelin K, Underwood K, Hébert MJ (2014) Autophagy fosters myofibroblast differentiation through MTORC2 activation and downstream upregulation of CTGF. Autophagy 10(12):2193–2207

Berndt A, Kosmehl H, Katenkamp D, Tauchmann V (1994) Appearance of the myofibroblastic phenotype in Dupuytren's disease is associated with a fibronectin, laminin, collagen type IV and tenascin extracellular matrix. Pathobiology 62:55–58

Bertheim U, Hellström S (1994) The distribution of hyaluronan in human skin and mature, hypertrophic meloi scars. Br J Plast Surg 47:483–489

Bisson MA, McGrouther DA, Mudera V, Grobbelaar AO (2003) The different characteristics of Dupuytren's disease fibroblasts derived from either nodule or cord: expression of alpha-smooth muscle actin and the response to stimulation by TGF-beta1. J Hand Surg (Br) 28: 351–356

Bisson MA, Mudera V, McGrouther DA, Grobbelaar AO (2004) The contractile properties and responses to tensional loading of Dupuytren's disease-derived fibroblasts are altered: a cause of the contracture? Plast Reconstr Surg 113(2):611–621

Bisson MA, Beckett KS, McGrouther DA, Grobbelaar AO, Mudera V (2009) Transforming growth factor-beta1 stimulation enhances Dupuytren's fibroblast contraction in response to uniaxial mechanical load within a 3-dimensional collagen gel. J Hand Surg [Am] 34(6):1102–1110

Bowley E, O'Gorman DB, Gan BS (2007) Beta-catenin signaling in fibroproliferative disease. J Surg Res 138:141–150

Brenner P, Grassler N, Berger A (1994) Epidemiology of Dupuytren's disease. In: Pathobiochemistry and clinical management. Springer, Berlin, pp 244–254

Brenner P, Sachse C, Reichert B, Berger A (1996) Expression von diversen monoklonalen anticörpern im knotenund strangstadium des morbus Dupuytren. Hand-Chir Mikrochir Plast Chir 28:322–327

Brickley Parson D, Glimcher MJ, Albin R (1981) Biochemical changes in the collagen of the palmar fascia in patients with Dupuytren's disease. J Bone Joint Surg 63(5):787–797

Brown RA, Prajapati R, McGrouther DA, Yannas IV, Eastwood M (1998) Tensional homeostasis in dermal fibroblasts: mechanical responses to mechanical loading in three-dimensional substrates. J Cell Physiol 175:323–332

Bruno G, Concetti F, Pertici I, Japtok L, Bernacchioni C, Donati C, Bruni P (2015) CTGF/CCN2 exerts profibrotic action in myoblasts via the up-regulation of sphingosine kinase-1/S1P3 signaling axis: implications in the action mechanism of TGFβ. Biochim Biophys Acta 1851:194–202

Bujak M, Ratkaj I, Markova-Car E, Jurišić D, Horvatić A, Vučinić S, Lerga J, Baus-Lončar M, Pavelić K, Kraljević PS (2015) Inflammatory gene expression upon TGF-β1-induced p38 activation in primary Dupuytren's disease fibroblasts. Front Mol Biosci 8: 2–68

Cordova A, Tripoli M, Corradino B, Napoli N, Moschella M (2005) Dupuytren's contracture: an update review of biomolecular aspects and therapeutic perspectives. J Hand Surg (Br) 30(6):557–562

Dave SA, Banducci DR, Graham WP 3rd, Allison M, Ehrlich HP (2001) Differences in alpha smooth muscle actin expression between fibroblasts derived from Dupuytren's nodules or cords. Exp Mol Pathol 71:147–155

Dawes J, Pepper DS (1992) Human vascular endothelial cell catabolise exogenous glycosaminoglycans by a novel route. Thromb Haemost 67:468–472

Degreef I, Steeno P, De Smet L (2008) A survey of clinical manifestations and risk factors in women with Dupuytren's disease. Acta Orthop Belg 74(4):456–460

Degreef I, De Smet L, Sciot R, Cassiman JJ, Tejpar S (2009) Beta-catenin overexpression in Dupuytren's disease is unrelated to disease recurrence. Clin Orthop Relat Res 467(3):838–845

Endo M, Yamamoto R, Namiki O, Satake S, Yosizawa Z (1979) Comparison of glycosaminoglycans (GAG) in normal human plasma and urine. Tohoku J Exp Med 128:89–99

Frohlich C, Albrechtsen R, Dyrskjot L, Rudkjaer L, Orntoft TF, Wewer UM (2006) Molecular profiling of ADAM12 in human bladder cancer. Clin Cancer Res 12:7359–7368

Gabbiani G, Majno G (1972) Dupuytren's contracture: fibroblast contraction? an Ultrastructural study. Am J Pathol 66:131–146

Gabbiani G, Ryan GB, Majno G (1971) Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experimentia 27:549–550

Gelberman RH, Amiel D, Rudolph RM, Vance RM (1980) Dupuytren's contracture. An electron microscopic, biochemical and clinical correlative study. J Bone Joint 62:425–432

Gilpin D, Coleman S, Hall S, Houston A, Karrasch J, Jones N (2010) Injectable collagenase clostridium histolyticum: a new nonsurgical treatment for Dupuytren's disease. J Hand Surg [Am] 35:2027–2038

Gurr E, Pallasch G, Tunn S, Tamm C, Delbrück A (1985) high performance liquid chromatographic assay of disaccharides and oligosaccharides produced by the digestion of glycosaminoglycans with chondroitin sulphate lyases. J Clin Chem Clin Biochem 23:77–87

Hindocha S, Iqbal SA, Farhatullah S, Paus R, Bayat A (2011) Characterization of stem cells in Dupuytren's disease. Br J Surg 98(2):308–315

Hoch J, Felouzis E, Meyer-Walters O, Nebe B, Notbohm H (2002) Fibronectin-chemotaxis and collagen-gel contraction of the palmar aponeurosis in morbus dupuytren. Handchir Mikrochir Plast Chir 34:292–297

Howard JC, Varallo VM, Ross DC, Roth JH, Faber KJ, Alman B, Gan BS (2003) Elevated levels of beta-catenin and fibronectin in three-dimensional collagen cultures of Dupuytren's disease cells are regulated by tension in vitro. BMC Musculoskelet Disord 16:16–21

Howard JC, Varallo VM, Ross DC, Faber KJ, Roth JH, Seney S, Gan BS (2004) Wound healing-associated proteins Hsp47 and fibronectin are elevated in Dupuytren's contracture. J Surg Res 117(2):232–238

Igarashi A, Nashiro K, Kikuchi K, Ihn H, Fujimoto M, Grotendorst GR, Takehara K (1996) Connective tissue growth factor gene expression in tissue sections from localized scleroderma, keloid, and other fibrotic skin disorders. J Invest Dermatol 106:729–733

Johnston P, Chojnowski AJ, Davidson RK, Riley GP, Donell ST, Clark IM (2007) A complete expression profile of matrix-degrading metalloproteinases in Dupuytren's disease. J Hand Surg [Am] 32(3): 343–351

Johnston P, Larson D, Clark IM, Chojnowski AJ (2008) Metalloproteinase gene expression correlates with clinical outcome in Dupuytren's disease. J Hand Surg [Am] 33(7):1160–1167

Karlson P (1998) Proteoglykane. In: Karlson P (ed) Kurzes lehrbuch der biochemie für mediziner und naturwissenschaftler. Thieme, Stuttgart, pp 253–255

Kloen P, Jennings CL, Gebhardt MC, Springfield DS, Mankin HJ (1995) Transforming growth factor-beta: possible roles in Dupuytren's contracture. J Hand Surg [Am] 20:101–108

Komatsu I, Bond J, Selim A, Tomasek JJ, Levin LS, Levinson H (2010) Dupuytren's fibroblast contractility by sphingosine-1-phosphate is mediated through non-muscle myosin II. J Hand Surg [Am] 35(10):1580–1588

Koźma EM, Głowacki A, Olczyk K, Ciecierska M (2007) Dermatan sulfate remodeling associated with advanced Dupuytren's contracture. Acta Biochim Pol 54(4):821–830

Koźma EM, Wisowski G, Olczyk K (2009) Platelet derived growth factor BB is a ligand for dermatan sulfate chain(s) of small matrix proteoglycans from normal and fibrosis affected fascia. Biochimie 91(11): 1394–1404

Kraljević Pavelić S, Bratulic S, Hock K, Jurisic D, Hranjec M, Karminski-Zamola G, Zinic B, Bujak M, Pavelic K (2009a) Screening of potential prodrugs on cells derived from Dupuytren's disease patients. Biomed Pharmacother 63(8):577–585

Kraljević Pavelić S, Sedic M, Hock K, Vucinic S, Jurisic D, Gehrig P, Scott M, Schlapbach R, Cacev T, Kapitanovic S, Pavelic K (2009b) An integrated proteomics approach for studying the molecular pathogenesis of Dupuytren's disease. J Pathol 217(4):524–533

Krstic RV (1988) In: Die gewebe des menschen und der säugetiere. Springer, Berlin

Kuhn MA, Payne WG, Kierney PC (2001) Cytokine manipulation of explanted Dupuytren's affected human palmar fascia. Int J Surg Invest 2:443–456

Kuhn MA, Wang X, Payne W, Ko F, Robson MC (2002) Tamoxifen decreases fibroblast function and downregulates TGF-β2 in Dupuytren's affected palmar fascia. J Surg Res 103:146–152

Kveiborg M, Frohlich C, Albrechtsen R, Tischler V, Dietrich N, Holck P, Kronqvist P, Rank F, Mercurio AM, Wewer UM (2005) A role for ADAM12 in breast tumor progression and stromal cell apoptosis. Cancer Res 65:4754–4761

Le Pabic H, Bonnier D, Wewer UM, Coutand A, Musso O, Baffet G, Clement B, Theret N (2003) ADAM12 in human liver cancers: TGF-β-regulated expression in stellate cells is associated with matrix remodeling. Hepatology 37:1056–1066

Li C, Nguyen Q, Cole WG (2001) Potential treatment for clubfeet based on growth factor blockade. J Pediatr Orthop 21:372–377

Luck JV (1959) Dupuytren's contracture: a new concept of the pathogenesis correlated with surgical management. J Bone Joint Surg 41:635–664

Magro G, Lanteri E, Micali G, Paravizzini G, Travali S, Lanzafame S (1997) Myofibroblasts of palmar fibromatosis co-express transforming growth factor-alpha and epidermal growth factor receptor. J Pathol 181:213–217

Majno G (1979) The story of fibroblasts. Am J Surg Pathol 3:535–542

Mast BA, Hayness JH, Krummel TM, Diegelmann RF, Cohen KI (1992) In vitro degradation of fetal wound hyaluronic acid results in increase fibroplasias, collagen deposition and neovascularisation. Plast Reconstr Surg 89:503–509

Melling M, Karimian-Teherani D, Mostler S, Behnam M, Sobal G, Menzel EJ (2000) Changes of biochemical and biomechanical properties in Dupuytren disease. Arch Pathol Lab Med 124:1275–1281

Meyerding HW, Black JR, Broders AC (1941) The etiology and pathology of Dupuytren's contracture. Surg Gynecol Obstet 72(3):582–590

Montesano R, Orci L (1988) Transforming growth factor beta stimulates collagen-matrix contraction by fibroblasts: implications for wound healing. Proc Natl Acad Sci U S A 85:4894–4897

Mosakhani N, Guled M, Lahti L, Borze I, Forsman M, Pääkkönen V, Ryhänen J, Knuutila S (2010) Unique microRNA profile in Dupuytren's contracture supports deregulation of β-catenin pathway. Mod Pathol 23:1544–1552

Moyer KE, Banducci DR, Graham WP 3rd, Ehrlich HP (2002) Dupuytren's disease: physiologic changes in nodule and cord fibroblasts through aging in vitro. Plast Reconstr Surg 110:187–193

Murrel GAC, Hueston JT (1990) Aetiology f Dupuytren's contracture. Aust N Z Surg 60:247–252

Mutsaers SE, Bishop JE, McGrouther G, Laurent GJ (1997) Mechanisms of tissue repair: from wound healing to fibrosis. Int J Biochem Cell Biol 29:5–17

Pagnotta A, Specchia N, Greco F (2002) Androgen receptors in Dupuytren's contracture. J Orthop Res 20:163–168

Pagnotta A, Specchia N, Soccetti A, Manzotti S, Greco F (2003) Responsiveness of Dupuytren's disease fibroblasts to 5 –alpha dihydrotestosterone. J Hand Surg [Am] 28(6):1029–1034

Pasquali Ronchetti I, Guerra D, Baccarani Contri M, Fornieri C, Mori G, Marcuzzi A, Zanasi S, Caroli A (1993) A clinical ultrastructural and immunohistochemical study of Dupuytren's disease. J Hand Surg (Br) 18:262–269

Qian A, Meals A, Rajfer J, Gonzalez-Cadavid NF (2004) Comparison of gene expression profiles between Peyronie's disease and Dupuytren's contracture. Urology 64:399–404

Rocks N, Paulissen G, El Hour M, Quesada F, Crahay C, Gueders M, Foidart JM, Noel A, Cataldo D (2008) Emerging roles of ADAM and ADAMTS metalloproteinases in cancer. Biochimie 90:369–379

Ryan GB, Cliff WJ, Gabbiani G, Irlé C, Montandon D, Statkov PR, Majno G (1974) Myofibroblasts in human granulation tissue. Hum Pathol 5:55–67

Satish L, LaFramboise WA, O'Gorman DB, Johnson S, Janto B, Gan BS, Baratz ME, Hu FZ, Post JC, Ehrlich GD, Kathju S (2008) Identification of differentially expressed genes in fibroblasts derived from patients with Dupuytren's Contracture. BMC Med Genom 23: 10–15

Satish L, LaFramboise WA, Johnson S, Vi L, Njarlangattil A, Raykha C, Krill-Burger JM, Gallo PH, O'Gorman DB, Gan BS, Baratz ME, Ehrlich GD, Kathju S (2012) Fibroblasts from phenotypically normal palmar fascia exhibit molecular profiles highly similar to fibroblasts from active disease in Dupuytren's Contracture. BMC Med Genom 5:15

Satish L, Palmer B, Liu F, Papatheodorou L, Rigatti L, Baratz ME, Kathju S (2015) Developing an animal model of Dupuytren's disease by orthotopic transplantation of human fibroblasts into athymic rat. BMC Musculoskelet Disord 16:138–148

Schmidtchen A, Fransson LA (1992) Analysis of glycosaminoglycan chains from different proteoglycan populations in human embryonic skin fibroblasts. Eur J Biochem 208:537–546

Schürch W, Skalli O, Gabbiani G (1990) Cellular biology. In: McFarlane RM, McGrouther DA, Flint MH (eds) Dupuytren's disease: biology and treatment, vol 5, The hand and upper limb series. Churchill Livingstone, Edinburgh, pp 31–47

Scott JE (1994) Proteoglycan-collagen interactions in connective tissues. In: Berger A, Delbrück A, Brenner P, Hinzmann R (eds) Dupuytren's disease. Pathobiochemistry and clinical management. Springer, Berlin, pp 171–177

Shih B, Brown JJ, Armstrong DJ, Lindau T, Bayat A (2009) Differential gene expression analysis of subcutaneous fat, fascia, and skin overlying a Dupuytren's disease nodule in comparison to control tissue. Hand (NY) 4:294–301

Shih B, Tassabehji M, Watson J, Bayat A (2012) DNA Copy number variations at chromosome 7p14.1 and chromosome 14q11.2 are associated with Dupuytren's disease: potential role for MMP and Wnt signaling pathway. Plast Reconstr Surg 129:921–932

Silvestro L, Viano J, Naggi A, Torri G, Da Col R, Baiocchi C (1992) High-performance liquid chromatographic-mass spectrometric analysis of oligosaccharides from enzymatic digestion of glycosaminoglycans. J Chromatogr 591:225–232

Skalli O, Schürch D, Seemayer TA, Lagacé R, Montandon D, Pittet B, Gabbiani G (1989) Myofibroblasts from diverse pathologic settings are heterogeneous in their contento f actin isoforms intermediate filament proteins. Lab Investig 60:275–285

Tomasek JJ, Haaskma CC (1991) Fibronectin filaments and actin microfilaments are organized into a fibroneous in Dupuytren's disesed tissue. Anat Rec 230:175–182

Tomasek JJ, Rayan GM (1995) Correlation of alpha-smooth muscle actin expression and contraction in Dupuytren's disease fibroblasts. J Hand Surg [Am] 20(3):450–455

Tomasek JJ, Schultz RI, Episalla CW, Newman SA (1986) The cytoskeleton and the extra-cellular matrix of the Dupuytren's disease "myofibroblast": an immune-fluorescence study of a non-muscle cell type. J Hand Surg [Am] 11(3):365–371

Tomasek JJ, Shultz RJ, Haaksma CJ (1987) Extracellular matrix-cytosckeletal connections at the surface of the specialized contractile fibroblast (myofibroblast) in Dupuytren's disease. J Bone Joint 69: 1400–1407

Tomasek JJ, Vaughan MB, Haaksma CJ (1999) Cellular Structure and biology of Dupuytren's disease. Hand Clin 15:21–34

Townley WA, Cambrey AD, Khaw PT, Grobbelaar AO (2008) Matrix metalloproteinase inhibition reduces contraction by dupuytren fibroblasts. J Hand Surg [Am] 33(9):1608–1616

Townley WA, Cambrey AD, Khaw PT, Grobbelaar AO (2009) The role of an MMP inhibitor in the regulation of mechanical tension by Dupuytren's disease fibroblasts. J Hand Surg Eur Vol 34(6):783–787

Trelstad RL (1989) Matrix glycoproteins. In: Kelley WN, Harris ED, Ruddy S, Sledge CB (eds) Textbook of rheumatology. Saunders, Philadelphia, pp 42–53

Tsang M, Leask A (2015) CCN2 is required for recruitment of Sox2-expressing cells during cutaneous tissue repair. J Cell Commun Signal 9(4):341–346

Tunn S, Gurr E, Delbruck A, Buhr T, Flory J (1988) The distribution of unsulphated and sulphated glycosaminoglycans in palmar fascia from patients with Dupuytren's disease and healthy subjects. J Clin Chem Clin Biochem 26:7–14

Ulrich D, Hrynyschyn K, Pallua N (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases in sera and tissue of patients with Dupuytren's disease. Plast Reconstr Surg 112:1279–1286

Ulrich D, Ulrich F, Piatkowski A, Pallua N (2009) Expression of matrix metalloproteinases and their inhibitors in cords and nodules of patients with Dupuytren's disease. Arch Orthop Trauma Surg 129(11): 1453–1459

Varma R, Varma RS (1983) In: Mucopolysaccharides, glycosaminoglicans of body fluids in health and disease. De Gruiter, Berlin

Vaughan MB, Howard EW, Tomasek JJ (2000) Transforming growth factor beta-1 promotes the morphological and functional differentiation of the myofibroblast. Exp Cell Res 257:180–189

Verjee LS, Midwood K, Davidson D, Eastwood M, Nanchahal J (2010) Post-transcriptional regulation of alpha-smooth muscle actin determines the contractile phenotype of Dupuytren's nodular cells. J Cell Physiol 224(3):681–690

Verjee LS, Verhoekx JS, Chan JK, Krausgruber T, Nicolaidou V, Izadi D, Davidson D, Feldmann M, Midwood KS, Nanchahal J (2013) Unraveling the signaling pathways promoting fibrosis in Dupuytren's disease reveals TNF as a therapeutic target. Proc Natl Acad Sci U S A 110(10):E928–E937

Vi L, Feng L, Zhu RD, Wu Y, Satish L, Gan BS, O'Gorman DB (2009a) Periostin differentially induces proliferation, contraction and apoptosis of primary Dupuytren's disease and adjacent palmar fascia cells. Exp Cell Res 315(20):3574–3586

Vi L, Njarlangattil A, Wu Y, Gan BS, O'Gorman DB (2009b) Type-1 Collagen differentially alters beta-catenin accumulation in primary Dupuytren's Disease cord and adjacent palmar fascia cells. BMC Musculoskelet Disord 19(10):72–77

Vi L, Gan BS, O'Gorman DB (2010) The potential roles of cell migration and extra-cellular matrix interactions in Dupuytren's disease progression and recurrence. Med Hypotheses 74:510–512

Viil J, Maasalu K, Mäemets-Allas K, Tamming L, Lõhmussaar K, Tooming M, Ingerpuu S, Märtson A, Jaks V (2015) Laminin-rich blood vessels display activated growth factor signaling and act as the proliferation centers in Dupuytren's contracture. Arthritis Res Ther 17(1):144–153

Wong M, Mudera V (2006) Feedback inhibition of high TGF-beta1 concentrations on myofibroblast induction and contraction by Dupuytren's fibroblasts. J Hand Surg (Br) 31(5):473–483

Xing Z, Tremblay GM, Sime PJ et al (1997) Overexpression of granulocyte-macrophage colony-stimulating factor induces pulmonary granulation tissue formation and fibrosis by induction of transforming growth factor B1 and myofibroblast accumulation. Am J Pathol 150:59–66

Yenidunya MO, Yenidunya S, Seven E (2009) Pacinian hypertrophy in a type 2A hand burn contracture and Pacinian hypertrophy and hyperplasia in a Dupuytren's contracture. Burns 35(3):446–450

Yildiz S, Karacaoğlu E, Pehlivan O (2004) Hyperbaric oxygen for the treatment of early-phase Dupuytren's contracture. Microsurgery 24(1):26–29

Yurchenco PD (1989) Laminin polymerization and binding to glycosaminoglycans: a hypothesis for modulation of basement membrane structure. In: Aebi U, Engel J (eds) Cytoskeletal and extracellular proteins. Structure, interactions and assembly. Springer, Berlin, pp 357–366

Zhang AY, Fong KD, Pham H, Nacamuli RP, Longaker MT, Chang J (2008) Gene expression analysis of Dupuytren's disease: the role of TGF-beta2. J Hand Surg Eur Vol 33(6):783–790

Zhou C, Hovius SE, Slijper HP, Feitz R, Van Nieuwenhoven CA, Pieters AJ, Selles RW (2015) Collagenase clostridium histolyticum versus limited fasciectomy for Dupuytren's contracture: outcomes from a multicenter propensity score matched study. Plast Reconstr Surg 136(1):87–97