

## Review

# Dupuytren's disease: a localised and accessible human fibrotic disorder

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**We review the biology of Dupuytren's disease (DD), a common localised fibrotic disorder of the hand. The disease develops through a complex interplay of genetic and environmental factors, and epigenetic signalling. The early-stage disease nodules comprise a complex milieu of stromal and immune cells which interact to promote disease development. Recently, inhibition of tumour necrosis factor (TNF) locally resulted in softening and a decrease in nodule size, potentially controlling disease progression. Unlike fibrotic disorders of the visceral organs, the easy access to tissue in DD patients enables dissection of the cellular landscape and molecular signalling pathways. In addition, the study of DD may have wider benefits in enhancing our understanding of less-accessible fibrotic tissues.**

## Dupuytren's disease – a common localised fibrotic disorder

DD is a common fibroproliferative disorder of the hand [1,2]. It is characterised by fibrosis of the palmar and digital fascia leading to flexion deformities of the fingers, most frequently observed in the little and ring fingers [3]. It is most prevalent in Western populations where DD affects ~8% of the general population [4]. The early stages of the disease are manifest as firm nodules in the palm; these later expand into fibrous collagenous cords which extend into the digits [5]. As the disease progresses, the cords thicken, mature, and contract, leading to the permanent flexion deformities of the fingers [6]. Despite increasing knowledge of DD biology, current treatment is restricted to surgical resection or division of the disease tissue when the flexion deformities impair hand function [7]. Effective treatment modalities that control the progression of early-stage disease, or that prevent recurrence, remain to be approved [8].

Fibrosis is defined by the accumulation of excess tissue matrix proteins and contributes to morbidity and mortality across a diverse range of disorders, including tumours, infections, genetic syndromes, and inflammatory conditions [9,10]. There is ever-increasing recognition of the cellular and molecular mechanisms of fibrosis, with collagen-producing stromal cells taking centre stage as core pathogenic mediators [11]. This growing knowledge has highlighted several putative therapeutic targets, but clinical translation has proved to be elusive [12]. Indeed, there are still no truly effective antifibrotic treatments, reflecting the great challenge of modulating aberrant tissue healing to attenuate the fibrotic response in DD and many other fibrotic conditions [13,14]. Moreover, a major challenge in studying fibrosis is the availability of well-characterised patient samples at the early stages of the disease to allow dissection of the molecular landscape of these disorders [15]. A great advantage of studying DD is that it provides a source of highly cellular human fibrotic tissue at the early stage of the disease [16].

This review focuses on the molecular mechanisms that regulate fibrosis in DD. Nodules of DD comprise a complex cellular ecosystem in which the individual cell types act in concert through a well-orchestrated system of molecular signalling. Our review reflects advances in understanding

## Highlights

Single-cell transcriptomic (scRNA-seq) and proteomic [cytometry time of flight (CyTOF) mass spectrometry] approaches have recently been applied to cells isolated from Dupuytren's nodules and have enabled us to comprehensively profile the complex cellular ecosystem within Dupuytren's nodules. This has highlighted the high degree of heterogeneity of both fibroblast and myofibroblast cell states. *In silico* trajectory analysis has facilitated the identification of myofibroblast precursors such as pericytes and resident fibroblasts.

Differential gene analysis along the differentiation trajectory identified novel markers of pathogenic myofibroblasts, including CD82, podoplanin, TNFSF12A, and the transcription factors SCX and DMRT2.

Interleukin 13 (IL-13) has recently been shown to have a role in Dupuytren's disease, where it is expressed by mast cells, and enhances the expression of profibrotic gene expression, which was sensitive to inhibition by the JAK inhibitor, tofacitinib.

Tumour necrosis factor (TNF) has also been shown to be produced by both M2 macrophages and mast cells in DD, and chronic expression was maintained by a positive feedback loop of IL-33. TNF signals through TNFR2 to upregulate the expression of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) and collagen.

These findings led to the assessment of local injection of anti-TNF (adalimumab) into the nodules in a Phase 2b clinical trial, which met the primary endpoint of reduction in nodule hardness and the secondary endpoint of nodule size, suggesting the potential to control the progression of early-stage Dupuytren's disease.



how these pathogenic structures emerge and progress. We also describe how fibrotic pathways in DD are reflected in the pathogenesis of other fibrotic disorders.

### Genetic susceptibility and signalling insights

The exact triggers for the development of DD remain unknown, but growing evidence supports that it develops through a complex interplay between genetic and environmental factors [17,18]. Historically, environmental factors such as alcohol, the antiepileptic drug phenytoin, smoking, and manual labour were proposed to be putative risk factors [19]. However, the evidence to support their association remains unclear. By contrast, the genetic drivers of DD are increasingly being investigated and have been linked to molecular mechanisms of pathogenesis [18]. A twin study from Denmark estimated that the overall heritability of DD is 80%, with strong concordance between monozygotic twins [5]. Moreover, DD cases have been demonstrated to cluster in families, and individuals with a strong family history of first-degree affected relatives can exhibit a more severe disease phenotype and experience onset at a younger age [19,20]. Studies characterizing DD prevalence and phenotype also demonstrate strong ethnic geographic variation, with the highest rate in Northern Europe [21]. Together, these studies demonstrate a complex and dynamic inheritance pattern.

Initial genetic studies in DD proposed several specific associations. These include the *DUPC1* locus at 16q in addition to alterations in the copy number of genes in chromosomal regions 10q22, 16p12.1, and 17p12, and the *HLA-DRB1\*15* allele [22,23]. Subsequently, more distinct genetic associations were uncovered by several large genome-wide association studies (GWAS) [18,24]. One yielded an association with 11 SNPs from nine different loci. Six of these loci contain genes known to be involved in the Wnt signalling pathway, including *WNT2*, *WNT4*, *SFRP4*, *RSPO2*, and *WNT7B*, and in total 26 significant genome-wide SNP associations in 24 independent risk regions were identified [24].

GWAS studies have not only highlighted causal genetic traits but also provided a platform to uncover molecular mechanisms driving fibrosis in DD. Indeed, the strongest GWAS association located in the intron of *EPDR1* revealed an effect on expression and protein secretion of the nearby gene *SFRP4* [24]. From this observation, a decreased accumulation of extracellular SFRP4 (soluble frizzled-related protein) from the high-risk genotype was found, thereby linking a causal genetic variation to a cellular response in DD myofibroblasts. Interestingly, SFRP4 is also a significant marker that distinguishes fibroblast from myofibroblasts in DD [25]. The SFRPs function as modulators of Wnt signalling, which has been shown to control the transcription of key fibrotic genes (*COL1A1* and *ACTA2*) in precursors of DD myofibroblasts [26].

Several SNPs from GWAS are associated with matrix remodelling, including discoid domain receptor (*DDR2*), matrix metalloprotease 14 (*MMP14*), and integrin  $\alpha 11$  (*ITGA11*), supporting the central role of aberrant extracellular matrix (ECM) homeostasis in DD pathogenesis [17,24]. The proteins encoded by these genetic variants have also been described as important regulators of fibrosis in other disease systems where they function to regulate matrix turnover and organization. One interesting example is MMP14 (MT1-MMP) which is associated with a high-risk locus and is a type I transmembrane protein of the MMP family of proteases [27,28]. *MMP14* has also been found to be a significant gene marker of collagen-expressing DD myofibroblasts in single-cellRNA-seq analyses [25]. In addition, MMP14 protein is overexpressed in DD nodules, and broad-spectrum inhibition of MMPs in clinical trials for cancer led to some individuals developing DD and frozen shoulder, which affects ~50% of individuals with DD [29]. MMP gene expression has also been found to correlate with clinical outcomes [30]. At a functional level, MMP14 expression in DD stromal cells enhances both cell contraction and MMP2 activation

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*in vitro* [31]. These findings suggest a function of MMP14 in the stromal cell phenotype in DD. DDR2 is a membrane receptor tyrosine kinase whose ligands include type I and III fibrillar collagen, which are the most prominent ECM proteins found in DD nodules and cords [16,32,33]. DDR2 is associated with a high-risk variant in DD and has also been shown to regulate fibrosis in the lung and liver. In lung fibroblasts, DDR2 potentiated the action of transforming growth factor beta 1 (TGF- $\beta$ 1) and type I collagen in promoting myofibroblast differentiation [34]. It is possible that a similar mechanism promotes the activation of fibroblasts and induces collagen deposition in DD.

### Cellular mechanisms of DD

Myofibroblasts play a key role in tissue repair and fibrosis, and great effort has been devoted to interrogating the myofibroblast phenotype and defining conserved markers since their first description [35,36]. The cytoskeletal protein  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) emerged as the canonical myofibroblast marker and has been used extensively to isolate, define, and study this cell type. Nevertheless, the role of  $\alpha$ -SMA in regulating myofibroblast function *in vivo* remains ill-defined. Previous work has demonstrated that post-transcriptional regulation of  $\alpha$ -SMA and incorporation into actin stress fibres enhanced the contractile phenotype of DD myofibroblasts [37]. In addition,  $\alpha$ -SMA expression in nodules has been shown to correlate with digit flexion deformity, supporting a role in disease progression [32]. More recently, ultrasound assessment of DD nodules demonstrated a correlation between  $\alpha$ -SMA myofibroblast load and sonographic readouts of DD nodule stiffness [38].

Despite growing evidence that  $\alpha$ -SMA is a defining marker of myofibroblasts in DD, we lacked a comprehensive and unsupervised description of this cell type, including its proteomic and transcriptomic signature and of how other cells interact with these central mediators of fibrosis. With the advent of omic technologies these questions have begun to be addressed [39]. Recent single-cell sequencing studies have defined novel gene markers of myofibroblasts, including *CD82*, *PDPN*, *SCX*, *DMRT2*, *COL6A1*, and *TNFSF12A* [25,40]. Moreover, *CD82* has been shown to regulate myofibroblast cell cycle and correlate with  $\alpha$ -SMA protein expression [25]. *SCX* and *TNFSF12A* were also shown to regulate myofibroblast phenotype, and the authors suggested *TNFSF12A* as a potential therapeutic target [40]. In addition, collagen VI emerged as a central regulator of DD myofibroblasts, both directly by influencing cell contraction and indirectly by modulating immune cell chemotaxis [41].

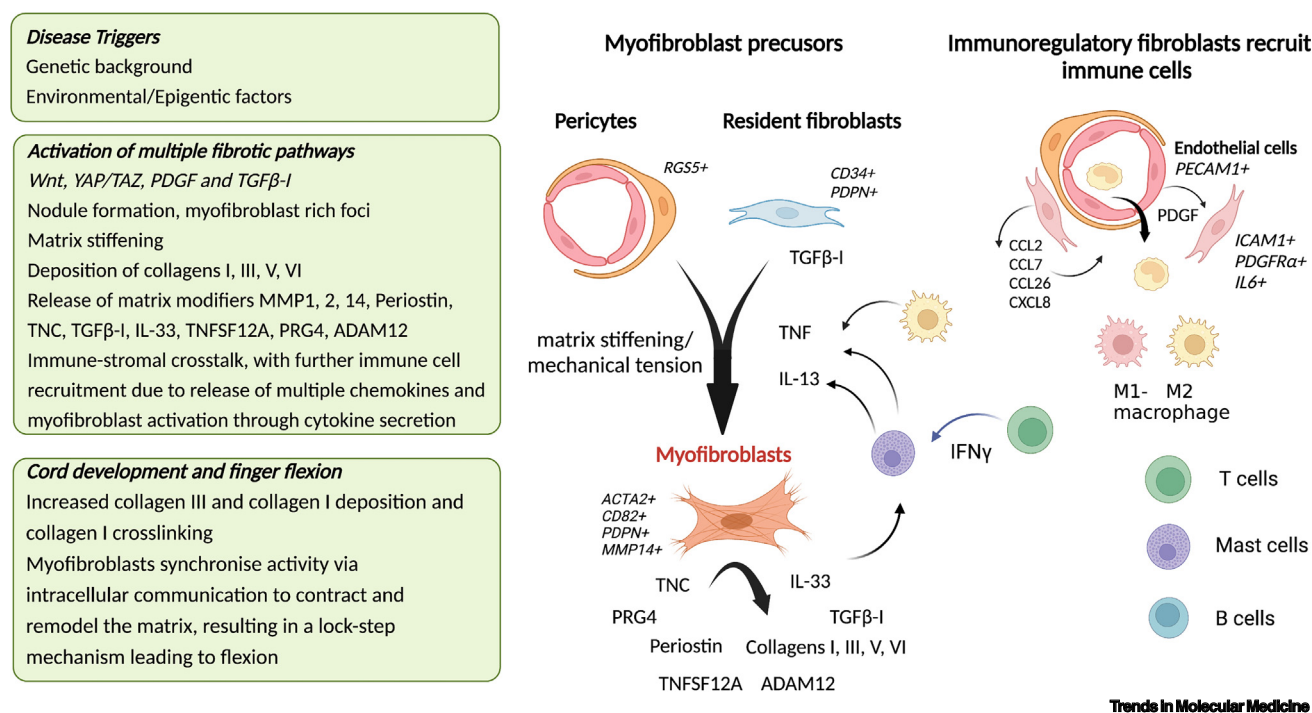
In conjunction with the description of myofibroblast markers, their ontogeny and precursors have been studied extensively [39,42,43]. In diverse models of fibrosis, almost every cell lineage has been designated as a myofibroblast precursor – in renal fibrosis pericytes [44] and resident fibroblasts [45], endothelial cells in liver fibrosis [46], and circulating stromal cells trafficking to the site of scar formation in the heart [47]. Mapping myofibroblast ontogeny has proved challenging in DD, largely reflecting a dearth of representative animal models. Despite this, various precursors have been suggested, including perinodular dermal cells [48], resident stem cells [49], and adipocytes [50]. Single-cell RNA-seq has highlighted nodular RGS5<sup>+</sup> pericytes as a putative source of myofibroblasts [25]. However, the lack of an *in vivo* model for DD means these observations are currently limited to *in silico* modelling with the inherent bias and limitations of these computational methods. Systemic comparisons using comparable lineage-tracing methods across different organs in fibrosis suggest that tissue-specific differences in the sources of myofibroblasts might be partly driven by differences in the primary cellular site of injury [39]. Pseudotemporal trajectory analysis of *COL1A1*, *COL3A1*, *COL5A1*, *COL6A1*, 2, 3, and *COL14A1* transcripts showed that they are all specifically enriched in the podoplanin (PDPN)-positive pathogenic myofibroblast module in DD. This disease-specific PDPN<sup>+</sup>/FAP<sup>+</sup> myofibroblast population is absent in non-

pathogenic fascia or dermis [40]. We found that collagen VI is particularly enriched in the myofibrotic foci in DD nodules, and this particular spatial colocalization is also observed in idiopathic pulmonary fibrosis (IPF), suggesting some shared mechanistic elements between these fibrotic diseases [41]. The growing repertoire of available data may help to define conserved transcriptomic signatures of myofibroblasts to support a precursor in DD, which may in turn help to define the triggers for the development of the disease, as highlighted in Figure 1 (Key figure).

The defining characteristic of myofibroblasts is the formation of actin stress fibres and the incorporation of  $\alpha$ -SMA into these structures [35]. The growing recognition of the role of tissue mechanics and cellular mechanotransduction in fibrosis has stimulated the development of tools to probe myofibroblast force generation [51]. Nodular myofibroblasts in DD are highly

## Key figure

### Stromal and immune cell signalling circuits in nodules of Dupuytren's disease (DD)



**Figure 1.** Schematic illustrating the cellular landscape in DD. The disease is characterised by immune–stromal crosstalk. Environmental triggers and changes in the epigenetic landscape in genetically predisposed individuals lead to the development of DD. A complex low-grade chronic inflammatory milieu of profibrotic factors such as transforming growth factor beta (TGF- $\beta$ ) and cytokines produced by both stromal and immune cells leads to the differentiation of precursors including RGSS<sup>+</sup> pericytes and podoplanin (PDPN)<sup>+</sup> fibroblasts into myofibroblasts. Cytokines such as tumour necrosis factor (TNF) and interleukin 13 (IL-13) stimulate the development of the myofibroblast phenotype, which is promoted by increasing matrix stiffness and extensive matrix remodelling via upregulation of matrix metalloproteinases (MMPs). Myofibroblasts are a significant source of IL-33 which promotes TNF secretion from immune cells, especially M2 macrophages and mast cells. Endothelial cells modulate the activity of immunoregulatory ICAM1<sup>+</sup> fibroblasts via the production of platelet-derived growth factor alpha (PDGF $\alpha$ ), which in turn promote immune cell chemotaxis via the release of chemokines such as CCL2, CCL7, CCL26, and CXCL8 from these specialised immunoregulatory fibroblasts. This leads to further immune cell recruitment of mast cells, M1 and M2 macrophages, and T cells, which further amplifies the activation loop. The sustained activation of myofibroblasts leads to the deposition of collagen I, III, V, and VI, and of the matricellular proteins tenascin C (TNC), periostin, and proteoglycan 4 (PRG4). The matrix is deposited in the form of a cord along the fibres of the palmar fascia. Coordination of myofibroblast contractility via intercellular junctions and remodelling of the matrix results in the development of finger contractures.

contractile, with high expression of *ACTA2*, *MYL9*, and *MLK*. The strong traction force exerted by DD myofibroblasts has been shown to be key for binding to and remodelling the matrix [52]. Myofibroblasts in DD function as a syncytium to coordinate their activities, including contraction, via intercellular junctions [53]. These intercellular forces are modulated through cadherins and gap junctions, and the contracted matrix is then remodelled into a more shortened configuration. This ratchet or lock-step mechanism may help to explain how cells in relatively small fibrous structures can generate force sufficient to induce digital flexion deformities [54].

Mechanical forces also play a key role in promoting the development and progression of fibrosis [54,55]. These forces regulate myofibroblast function in addition to contributing to the structure and mechanics of the matrix [55]. Generally, as fibrosis progresses, matrix protein deposition leads to tissues becoming stiffer. In turn, this promotes further matrix secretion by myofibroblasts, thereby creating a positive feedback loop that maintains the disease pathogenesis. Atomic force microscopy has measured the tissue stiffness in nodules at ~8 kPa [52], in line with fibrotic foci in pulmonary fibrosis, suggesting a shared mechanical environment in the fibrotic milieu [56]. Two important mechanotransduction proteins are the transcriptional coactivators Yes-associated protein 1 (YAP1) and transcriptional coactivator with PDZ-binding motif (TAZ) [57]. These are members of the Hippo pathway and have been shown to regulate global changes in gene expression in response to tissue stiffness. In fibrosis, YAP1 has been shown to coordinate the myofibroblast phenotype and transduce signals from the mechanical environment in several fibrotic conditions [58]. In addition, in an *in vitro* fibroblast spheroid system, TAZ activation resulted in increased cell contraction and ECM expression in response to increasing stiffness [59]. More recently, YAP1 has been shown to be a crucial determinant of the myofibroblast phenotype in DD. Silencing of *YAP1* in DD myofibroblasts demonstrated its key role in the expression of fibrotic genes and cell contraction [60].

Once assumed a homogeneous population, the heterogeneity of fibroblasts in fibrosis has been demonstrated using single-cell technologies to identify functional distinct subclasses and subtypes. Three major subsets have been described in DD, namely ICAM1<sup>+</sup>, CD34<sup>+</sup>, and THY1<sup>+</sup> fibroblasts [25]. DD fibroblasts play a central role in promoting immune cell recruitment and activation. Specifically, ICAM1<sup>+</sup>IL6<sup>high</sup> fibroblasts expressing high levels of chemokines have been shown to modulate immune cell chemotaxis. Interestingly, this population is expanded in developing immune cell-rich stages of fibrosis in Dupuytren's nodules. The demonstration that immune mediators, including TNF, are crucial for myofibroblast development and activity support ICAM1<sup>+</sup>IL6<sup>high</sup> fibroblasts as key drivers of inflammation that sustain stromal activation during fibrosis, as shown in Figure 1.

### DD microenvironment and matrix

The ECM of DD is a complex and dynamic structure that exerts a broad range of effects on the embedded cells [16]. Fibrotic disorders are characterised by aberrant regulation of ECM turnover, and much effort has therefore been devoted to profiling and describing the matrix phenotype in DD [54,61,62]. Overall, the macroscopic matrix structure of DD differs according to the stage of disease, and two distinct structures are present – the cellular nodule and matrix-rich cord [32]. Nodules are composed of dense cellular aggregates with myofibroblast foci reminiscent of the foci seen in IPF [16]. By contrast, cords are relatively acellular and comprise regularly organised parallel collagen bundles, forming a linear structure that extends into the digits. The nodules are the earliest clinical manifestation of the disease, and cords in digits with greater flexion deformities have fewer nodules [32]. This has led to the proposal that the cells within the nodules secrete the matrix components, which are then contracted and remodelled. As the fixed flexion deformity develops, myofibroblast numbers decrease, perhaps through apoptosis,



and the cords become less cellular. The formation and shortening of the cord by the myofibroblasts are necessary for the development of flexion contractures that ultimately compromise hand function [32].

The predominant matrix proteins in DD are collagens, and the subtypes reported include collagen types I, III, V, and VI [16,63,64]. Earlier microarray studies also showed that multiple other proteins are upregulated in DD tissue compared to normal palmar fascia [33,65]. These include matrix metalloproteases (e.g., MMP1, MMP2, and MMP14) [31,66], periostin (POSTN) [67,68], tenascin C (TNC) [69], a disintegrin and metalloproteinase domain 12 (ADAM12), and proteoglycan 4 (PRG4) [70]. More recently, high-throughput transcriptomics has begun to unravel the matrisome in greater detail, and DD myofibroblasts have been shown to express MMP11, FABP5, LOXL1, SPON2, and ITGAV [71]. The exact function of some of these proteins remains to be defined, although others have been studied in detail.

A central function of the matrix is to provide a scaffold to sustain the structure of the fibrotic microenvironment. Unsurprisingly, the matrix has also been found to play a key role in cellular signalling. For example, collagen VI was found to be highly expressed in DD nodules and acts to regulate the myofibroblast phenotype [41]. Selective knockdown of collagen VI protein resulted in inhibition of cell contractility, matrix expression, chemotaxis, and cell migration. Some of these profibrotic activities are likely to be mediated through the activities of proteolytically cleaved fragments of collagen VI, such as endotropin (ETP). These fragments of collagen VI have also been shown to play key signalling roles in other fibrotic diseases, and circulating levels are used as key biomarkers of kidney, liver, and pulmonary fibrosis [72,73]. Multiple enzymes (MMP14, MMP2, 7, 9, and BMP1), have been shown to generate these fragments, possibly in a tissue-specific manner. However, the situation is complex, and simply downregulating one of the enzymes that generates ETP may have deleterious effects because they are responsible for the regulation of multiple peptides involved in cellular metabolism; instead, targeting the receptors of these fragments may represent a more tractable therapeutic approach [74]. A recent genetic study showed that an MMP14 variant expressed in DD myofibroblasts has reduced collagenolytic activity, leading to reduced ECM turnover [27]. The matrix also plays an important role in sequestering molecules that regulate paracrine signalling, of which the TGF- $\beta$  family has been the most extensively studied in DD [75–77]. TGF- $\beta$ 1 signalling has been shown to regulate the activation of DD stromal cells, thereby promoting  $\alpha$ -SMA expression and cell contraction [75,78], as comprehensively reviewed [79]. However, targeting TGF- $\beta$ 1 pathways in fibrosis is fraught with problems, and late-stage clinical trials for other fibrotic diseases have failed to demonstrate effectiveness.

### Inflammation in DD

Inflammation is a central pathological process in the development of fibrosis [11]. The first descriptive studies in DD identified a dense immune cell infiltrate, and since then most immune cell lineages have been reported, including macrophages, dendritic cells, and lymphocytes [15,80]. Recent studies have used high-throughput techniques to profile the immune repertoire in DD, showing that immune cells comprise ~10% of nodular cells [81]. The predominant immune cells in nodules are macrophages (~3.5%), with smaller proportions of T cells, mast cells, and B cells. Both classically activated M1 and alternatively activated M2 macrophages have been described in DD nodules, and the latter together with mast cells play a particularly important role in the pathogenesis of the disease [15]. This immune cell infiltrate is reflected by the chemokines CXCL8, CCL2, CCL7, and CCL26 secreted by nodular cells. In parallel with the diverse immune landscape, an equally broad range of soluble immune mediators have been described in DD. The most abundant nodular cytokines are interleukin 6 (IL-6) and TGF- $\beta$ 1 [15]. Surprisingly, the levels of the cytokines secreted by the nodular cells does not reflect their

### Clinician's corner

Dupuytren's disease (DD) is a common and debilitating fibrotic condition of the hand that has strong genetic heritability.

Currently, treatment is restricted to late-stage disease and includes surgical resection of the fibrotic tissue.

The disease is driven by low-grade chronic inflammation, and recent data show that injection of anti-tumour necrosis factor into the nodules of early-stage disease may control disease progression.

DD mirrors other fibrotic conditions such as idiopathic pulmonary fibrosis (IPF) in its architecture and key effector cells and mechanisms.

importance. Low levels of TNF have been shown to play a key role in the development and maintenance of the myofibroblast phenotype by upregulating the expression of collagens and  $\alpha$ -SMA and by enhancing cell contractility through the Wnt signalling pathway, which has been highlighted in multiple GWAS studies [18]. Interestingly, M2 macrophages and mast cells are the predominant source of TNF in DD from *in vitro* cultures of nodule-derived cells, and TNF exerts its effect via an inducible TNFR2 [82]. These data formed the foundation for a Phase 2a placebo-controlled dose-ranging trial of anti-TNF (adalimumab) [82]. Direct intranodular injection of 40 mg of adalimumab in a concentrated formulation (0.4 ml) resulted in downregulation of procollagen type 1 and  $\alpha$ -SMA proteins at 2 weeks post-injection [82,83]. A subsequent Phase 2b trial comparing four intranodular injections of adalimumab with placebo at 3 month intervals in patients with early-stage DD demonstrated reduction in nodule hardness (primary outcome) and size on ultrasound scan (secondary outcome). Interestingly, both parameters continued to reduce for a further 9 months after the final injection despite the half-life of adalimumab only being 2 weeks, indicating a prolonged biological effect [82]. An increase in nodule and disease surface area has been shown to correlate with development of flexion contractures, and each cm<sup>2</sup> increase raised the likelihood of progressing to Tubiana grade 4 (total flexion deformity >135°) by an odds ratio of 3.2 [84].

Freshly disaggregated nodular cells also secrete low levels of IL-33 [81]. This stromal cell-derived IL-33 stimulated TNF secretion by M2 macrophages and mast cells, which in turn enhanced IL-33 expression. Together, these results not only highlight the identification of novel therapeutic targets and their translation to the clinic but also support the role of targeting stromal-immune circuits in DD to inhibit both inflammation and matrix deposition and remodelling. In another example, nodular T cells produced IFN- $\gamma$  which induced IL-13 secretion from mast cells and upregulated IL-13R $\alpha$ 1 expression on fibroblasts, making them more responsive to IL-13. IL-13 was shown to drive the expression of the key matrix genes *TNC*, *POSTN* and *COL1A1*, as highlighted in Figure 1. This IL-13 and IFN- $\gamma$  pathway is mediated by STAT signalling and was found to be sensitive to the JAK inhibitor, tofacitinib, suggesting the potential for targeting of this pathway in DD [85]. The key role played by chronic, low-grade inflammation in the pathogenesis of DD has been shown to be the case in all other fibrotic diseases [11]. In DD this inflammatory process is confined to the nodules, with no systemic inflammation [81].

The burgeoning field of single-cell technologies has provided a platform not only to resolve cell identity but also to illuminate distinct anatomical and functional compartments in fibrosis [86]. These developments have helped to unravel the architecture of DD. Together with the description of the various cell types in DD nodules, and the soluble factors they produce, recent work has begun to identify distinct compartments that help to explain the function of the various cell types and their interconnectivity [87]. Previous work has demonstrated that, in nodules, T cells are located adjacent to vessels, suggesting a possible microvascular autoantigen trigger to inflammation [80]. More recently, high-resolution tissue imaging has shown that immune cells reside in a perivascular fibroblast-rich niche [87]. In this niche, endothelial cells secrete platelet-derived growth factor (PDGF) and modulate the activities of PDGFR $\alpha$ <sup>+</sup> fibroblasts that reside adjacent to the vessels. These fibroblasts in turn modulate the recruitment of immune cells. The latter secrete cytokines such as TNF to promote the development and maintenance of myofibroblasts in an adjacent compartment, the latter secreting IL-33. The compartments identified in DD nodules are closely reflected in the fibrotic foci of IPF, highlighting the potential for using DD to unravel pathways in other fibrotic diseases.

## Concluding remarks

DD is a common and disabling localised fibrotic disease. Beyond the significant value of identifying novel therapeutic targets in this condition, detailed phenotyping of DD pathology has illuminated distinct and molecular mechanisms in common with other fibrotic diseases. Myofibroblasts play

## Outstanding questions

Why do some nodules remain quiescent whereas others lead to the development of flexion deformities?

How can we better utilise the wealth of GWAS data to define more tractable targets?

What cues from the matrix activate the ICAM1<sup>+</sup>IL6<sup>high</sup> immune regulatory fibroblasts, which are key drivers of inflammation that sustains the activation of stromal cells and promote the progression of fibrosis?

Can we harness spatial transcriptomics to further address the question of what drives and maintains the myofibroblast foci, and what is the physiological relevance of the heterogeneity of the stromal populations identified through recent scRNA-seq studies?

a central role in all forms of fibrosis. The nascent matrix secreted by this population ultimately distorts native tissue architecture leading to impaired organ function. Moreover, there are striking parallels between the IPF and DD microenvironments because both contain dense foci of  $\alpha$ -SMA<sup>+</sup> myofibroblasts surrounded by PDGFR $\alpha$ <sup>+</sup> fibroblasts. Collagen VI is a common marker of IPF and DD myofibroblasts, and distinct cell subsets are also shared across these two diseases, such as COL13A1<sup>+</sup> fibroblasts. By contrast, in many forms of liver fibrosis, matrix deposition and myofibroblast accumulation occur in a zonation pattern organised around a central vascular pedicle. One could speculate that these contrasting features reflect a difference in the underlying drivers of fibrosis: in DD and IPF tissue mechanics related to hand movement and ventilation may be crucial, whereas metabolic dysregulation is key in the pathogenesis of liver fibrosis. Our improved understanding of DD biology has identified tractable therapeutic targets that may help to improve the lives of patients with this condition alongside highlighting central mechanisms driving human fibrosis. Despite a wealth of knowledge of the genetic associations, the precise drivers for the development of the disease remain unclear. Integration of functional genomics together with knowledge of network connectivity maximises the opportunities for target validation and the definition the target-level trait relationships. Using multiple GWAS datasets a subset of (super)enhancers have been shown to be especially important for genes associated with cell identity and risk of disease. The epigenetic regulator EP300 was found to be highly enriched in superenhancers and was very closely associated with genomic regions of eight of 22 SNPs associated with DD. The challenge will be to further rationalise these data to define more tractable drug targets. Furthermore, the reasons why the disease can remain quiescent for many years and progress in only 20–30% of patients remain to be defined. Our understanding of cellular signalling has improved dramatically in recent years. Emerging technologies that allow preservation of tissue architecture while permitting interrogation of cellular activities should shed further light on these processes that are crucial for the development and pathogenesis of the disease (see [Outstanding questions](#)).

### Declaration of interests

No interests are declared.

### References

1. Townley, W.A. *et al.* (2006) Dupuytren's contracture unfolded. *BMJ* 332, 397–400
2. Smith, A.C. (1991) Diagnosis and indications for surgical treatment. *Hand Clin.* 7, 635–642
3. Layton, T. and Nanchahal, J. (2019) Recent advances in the understanding of Dupuytren's disease. *F1000Research* 8, 231
4. Dakin, S. *et al.* (2022) Cost-effectiveness of adalimumab for early-stage Dupuytren's disease: an early economic evaluation based on a randomised controlled trial and individual patient simulation model. *Bone Joint Open* 3, 898–906
5. Larsen, S. *et al.* (2015) Genetic and environmental influences in Dupuytren's disease: a study of 30,330 Danish twin pairs. *J. Hand Surg. Eur.* 40, 171–176
6. Wilburn, J. *et al.* (2013) The impact of Dupuytren disease on patient activity and quality of life. *J. Hand Surg. Am.* 38, 1209–1214
7. Ball, C. *et al.* (2016) Systematic review of non-surgical treatments for early Dupuytren's disease. *BMC Musculoskelet. Disord.* 17, 345
8. Reilly, R.M. *et al.* (2005) A retrospective review of the management of Dupuytren's nodules. *J. Hand Surg. Am.* 30, 1014–1018
9. Wynn, T.A. (2008) Cellular and molecular mechanisms of fibrosis. *J. Pathol.* 214, 199–210
10. Wynn, T.A. and Ramalingam, T.R. (2012) Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat. Med.* 18, 1028–1040
11. Wick, G. *et al.* (2013) The immunology of fibrosis. *Annu. Rev. Immunol.* 31, 107–135
12. Nanthakumar, C.B. *et al.* (2015) Dissecting fibrosis: therapeutic insights from the small-molecule toolbox. *Nat. Rev. Drug Discov.* 14, 693–720
13. Mora, A.L. *et al.* (2017) Emerging therapies for idiopathic pulmonary fibrosis, a progressive age-related disease. *Nat. Rev. Drug Discov.* 16, 755–772
14. Nanchahal, J. and Hinz, B. (2016) Strategies to overcome the hurdles to treat fibrosis, a major unmet clinical need. *Proc. Natl. Acad. Sci.* 113, 7291
15. Verjee, L.S. *et al.* (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, E928–E937
16. van Beuge, M.M. *et al.* (2016) Matrix and cell phenotype differences in Dupuytren's disease. *Fibrogenesis Tissue Repair* 9, 9
17. Becker, K. *et al.* (2016) Meta-analysis of genome-wide association studies and network analysis-based integration with gene expression data identify new suggestive loci and unravel a Wnt-centric network associated with Dupuytren's disease. *PLoS One* 11, e0158101
18. Dolmans, G.H. *et al.* (2011) Wnt signaling and Dupuytren's disease. *N. Engl. J. Med.* 365, 307–317
19. Hart, M.G. and Hooper, G. (2005) Clinical associations of Dupuytren's disease. *Postgrad. Med. J.* 81, 425–428
20. Hindocha, S. *et al.* (2006) The heritability of Dupuytren's disease: familial aggregation and its clinical significance. *J. Hand Surg. Am.* 31, 204–210
21. Gudmundsson, K.G. *et al.* (2000) Epidemiology of Dupuytren's disease: clinical, serological, and social assessment. The Reykjavik Study. *J. Clin. Epidemiol.* 53, 291–296
22. Ling, R.S. (1963) The genetic factor in Dupuytren's disease. *J. Bone Joint Surg. Br.* 45, 709–718
23. Bayat, A. *et al.* (2002) Genetic susceptibility in Dupuytren's disease. TGF-beta1 polymorphisms and Dupuytren's disease. *J. Bone Joint Surg. Br.* 84, 211–215



24. Ng, M. *et al.* (2017) A Genome-wide association study of Dupuytren disease reveals 17 additional variants implicated in fibrosis. *Am. J. Hum. Genet.* 101, 417–427
25. Layton, T.B. *et al.* (2020) Cellular census of human fibrosis defines functionally distinct stromal cell types and states. *Nat. Commun.* 11, 2768
26. Matsushima, K. *et al.* (2010) Secreted frizzled related protein 4 reduces fibrosis scar size and ameliorates cardiac function after ischemic injury. *Tissue Eng. Part A* 16, 3329–3341
27. Itoh, Y. *et al.* (2021) A common SNP risk variant MT1-MMP causative for Dupuytren's disease has a specific defect in collagenolytic activity. *Matrix Biol.* 97, 20–39
28. Zigrino, P. *et al.* (2016) Fibroblast-derived MMP-14 regulates collagen homeostasis in adult skin. *J. Invest. Dermatol.* 136, 1575–1583
29. Hutchinson, J.W. *et al.* (1998) Dupuytren's disease and frozen shoulder induced by treatment with a matrix metalloproteinase inhibitor. *J. Bone Joint Surg. Br.* 80, 907–908
30. Johnston, P. *et al.* (2008) Metalloproteinase gene expression correlates with clinical outcome in Dupuytren's disease. *J. Hand Surg. Am.* 33, 1160–1167
31. Wilkinson, J.M. *et al.* (2012) MMP-14 and MMP-2 are key metalloproteases in Dupuytren's disease fibroblast-mediated contraction. *Biochim. Biophys. Acta* 1822, 897–905
32. Verjee, L.S. *et al.* (2009) Myofibroblast distribution in Dupuytren's cords: correlation with digital contracture. *J. Hand Surg. Am.* 34, 1785–1794
33. Satish, L. *et al.* (2008) Identification of differentially expressed genes in fibroblasts derived from patients with Dupuytren's contracture. *BMC Med. Genet.* 1, 10
34. Zhao, H. *et al.* (2016) Targeting of discoidin domain receptor 2 (DDR2) prevents myofibroblast activation and neovessel formation during pulmonary fibrosis. *Mol. Ther.* 24, 1734–1744
35. Gabbiani, G. (2003) The myofibroblast in wound healing and fibrocontractive diseases. *J. Pathol.* 200, 500–503
36. Hinze, B. and Lagares, D. (2020) Evasion of apoptosis by myofibroblasts: a hallmark of fibrotic diseases. *Nat. Rev. Rheumatol.* 16, 11–31
37. Verjee, L.S. *et al.* (2010) Post-transcriptional regulation of alpha-smooth muscle actin determines the contractile phenotype of Dupuytren's nodular cells. *J. Cell. Physiol.* 224, 681–690
38. Molenkamp, S. *et al.* (2022) Echogenicity of Dupuytren's nodules is correlated to myofibroblast load and nodule hardness. *J. Hand Surg. Eur.* 47, 280–287
39. Kuppe, C. *et al.* (2021) Decoding myofibroblast origins in human kidney fibrosis. *Nature* 589, 281–286
40. Dobie, R. *et al.* (2022) Deciphering mesenchymal drivers of human Dupuytren's disease at single-cell level. *J. Invest. Dermatol.* 142, 114–123.e8
41. Williams, L.M. *et al.* (2020) Identifying collagen VI as a target of fibrotic diseases regulated by CREBBP/EP300. *Proc. Natl. Acad. Sci.* 117, 20753–20763
42. Kanisicak, O. *et al.* (2016) Genetic lineage tracing defines myofibroblast origin and function in the injured heart. *Nat. Commun.* 7, 12260
43. Micallef, L. *et al.* (2012) The myofibroblast, multiple origins for major roles in normal and pathological tissue repair. *Fibrogenesis Tissue Repair* 5, S5
44. Kida, Y. and Duffield, J.S. (2011) Pivotal role of pericytes in kidney fibrosis. *Clin. Exp. Pharmacol. Physiol.* 38, 467–473
45. Li, R. *et al.* (2018) Pdgfra marks a cellular lineage with distinct contributions to myofibroblasts in lung maturation and injury response. *eLife* 7, e36865
46. Pinzani, M. (2011) Epithelial–mesenchymal transition in chronic liver disease: fibrogenesis or escape from death? *J. Hepatol.* 55, 459–465
47. Nagaraju, C.K. *et al.* (2019) Myofibroblast phenotype and reversibility of fibrosis in patients with end-stage heart failure. *J. Am. Coll. Cardiol.* 73, 2267–2282
48. Iqbal, S.A. *et al.* (2012) Identification of mesenchymal stem cells in perinodular fat and skin in Dupuytren's disease: a potential source of myofibroblasts with implications for pathogenesis and therapy. *Stem Cells Dev.* 21, 609–622
49. On, N. *et al.* (2017) Embryonic stem cell-like population in Dupuytren's disease expresses components of the renin–angiotensin system. *Plast. Reconstr. Surg. Glob. Open* 5, e1422
50. Karkampouna, S. *et al.* (2016) Connective tissue degeneration: mechanisms of palmar fascia degeneration (Dupuytren's disease). *Curr. Mol. Biol. Rep.* 2, 133–140
51. Polacheck, W.J. and Chen, C.S. (2016) Measuring cell-generated forces: a guide to the available tools. *Nat. Methods* 13, 415–423
52. Layton, T.B. *et al.* (2020) Single cell force profiling of human myofibroblasts reveals a biophysical spectrum of cell states. *Biol. Open* 9, bio049809
53. Verhoekx, J.S.N. *et al.* (2013) Isometric contraction of Dupuytren's myofibroblasts is inhibited by blocking intercellular junctions. *J. Invest. Dermatol.* 133, 2664–2671
54. Tomasek, J.J. *et al.* (2002) Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* 3, 349–363
55. Wells, R.G. (2013) Tissue mechanics and fibrosis. *Biochim. Biophys. Acta* 1832, 884–890
56. Liu, F. and Tschumperlin, D.J. (2011) Micro-mechanical characterization of lung tissue using atomic force microscopy. *J. Vis. Exp.* 54, 2911
57. Liu, F. *et al.* (2015) Mechanosignaling through YAP and TAZ drives fibroblast activation and fibrosis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 308, L344–L357
58. He, X. *et al.* (2022) Myofibroblast YAP/TAZ activation is a key step in organ fibrogenesis. *JCI Insight* 7, e146243
59. Jorgenson, A.J. *et al.* (2017) TAZ activation drives fibroblast spheroid growth, expression of profibrotic paracrine signals, and context-dependent ECM gene expression. *Am. J. Physiol. Cell Physiol.* 312, C277–C285
60. Piersma, B. *et al.* (2015) YAP1 is a driver of myofibroblast differentiation in normal and diseased fibroblasts. *Am. J. Pathol.* 185, 3326–3337
61. Hinze, B. *et al.* (2004) Myofibroblast development is characterized by specific cell-cell adherens junctions. *Mol. Biol. Cell* 15, 4310–4320
62. Tomasek, J.J. *et al.* (1999) Cellular structure and biology of Dupuytren's disease. *Hand Clin.* 15, 21–34
63. Kozma, E.M. *et al.* (2005) Alterations in the extracellular matrix proteoglycan profile in Dupuytren's contracture affect the palmar fascia. *J. Biochem.* 137, 463–476
64. Chiu, H.F. and McFarlane, R.M. (1978) Pathogenesis of Dupuytren's contracture: a correlative clinical-pathological study. *J. Hand Surg.* 3, 1–10
65. Pan, D. *et al.* (2003) Microarray gene analysis and expression profiles of Dupuytren's contracture. *Ann. Plast. Surg.* 50, 618–622
66. Ulrich, D. *et al.* (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases in sera and tissue of patients with Dupuytren's disease. *Plast. Reconstr. Surg.* 112, 1279–1286
67. Qian, A. *et al.* (2004) Comparison of gene expression profiles between Peyronie's disease and Dupuytren's contracture. *Urology* 64, 399–404
68. Vi, L. *et al.* (2009) Periostin differentially induces proliferation, contraction and apoptosis of primary Dupuytren's disease and adjacent palmar fascia cells. *Exp. Cell Res.* 315, 3574–3586
69. Berndt, A. *et al.* (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
70. Shih, B. *et al.* (2009) Identification of biomarkers in Dupuytren's disease by comparative analysis of fibroblasts versus tissue biopsies in disease-specific phenotypes. *J. Hand Surg. Am.* 34, 124–136
71. Riester, S.M. *et al.* (2015) RNA sequencing reveals a depletion of collagen targeting microRNAs in Dupuytren's disease. *BMC Med. Genet.* 8, 59
72. Sun, K. *et al.* (2014) Endotrophin triggers adipose tissue fibrosis and metabolic dysfunction. *Nat. Commun.* 5, 3485
73. Sparding, N. *et al.* (2021) Endotrophin, a collagen type VI-derived matrine, reflects the degree of renal fibrosis in patients with IgA nephropathy and in patients with ANCA-associated vasculitis. *Nephrol. Dial. Transplant.* 37, 1099–1108
74. Fenton, A. *et al.* (2017) Serum endotrophin, a type VI collagen cleavage product, is associated with increased mortality in chronic kidney disease. *PLoS One* 12, e0175200

75. Badalamente, M.A. *et al.* (1996) The role of transforming growth factor beta in Dupuytren's disease. *J. Hand Surg. Am.* 21, 210–215
76. Baird, K.S. *et al.* (1993) Abnormal growth factor and cytokine expression in Dupuytren's contracture. *J. Clin. Pathol.* 46, 425–428
77. Berndt, A. *et al.* (1995) TGF beta and bFGF synthesis and localization in Dupuytren's disease (nodular palmar fibromatosis) relative to cellular activity, myofibroblast phenotype and oncofetal variants of fibronectin. *Histochem. J.* 27, 1014–1020
78. Satish, L. *et al.* (2011) Reversal of TGF-beta1 stimulation of alpha-smooth muscle actin and extracellular matrix components by cyclic AMP in Dupuytren's-derived fibroblasts. *BMC Musculoskelet. Disord.* 12, 113
79. Meng, X.M. *et al.* (2016) TGF-beta: the master regulator of fibrosis. *Nat. Rev. Nephrol.* 12, 325–338
80. Mayerl, C. *et al.* (2016) Characterisation of the inflammatory response in Dupuytren's disease. *J. Plast. Surg. Hand Surg.* 50, 171–179
81. Izadi, D. *et al.* (2019) Identification of TNFR2 and IL-33 as therapeutic targets in localized fibrosis. *Sci. Adv.* 5, eaay0370
82. Nanchahal, J. *et al.* (2022) Anti-tumour necrosis factor therapy for early-stage Dupuytren's disease (RIDD): a phase 2b, randomised, double-blind, placebo-controlled trial. *Lancet Rheumatol.* 4, E407–E416
83. Nanchahal, J. *et al.* (2018) Anti-tumour necrosis factor therapy for Dupuytren's disease: a randomised dose response proof of concept Phase 2a clinical trial. *EBioMedicine* 33, 282–288
84. Lanting, R. *et al.* (2016) Clusters in short-term disease course in participants with primary Dupuytren disease. *J. Hand Surg. Am.* 41, 354–361
85. Akbar, M. *et al.* (2020) Attenuation of Dupuytren's fibrosis via targeting of the STAT1 modulated IL-13R $\alpha$ 1 response. *Sci. Adv.* 6, eaaz8272
86. Heath, J.R. *et al.* (2016) Single-cell analysis tools for drug discovery and development. *Nat. Rev. Drug Discov.* 15, 204–216
87. Layton, T.B. *et al.* (2022) A vasculature niche orchestrates stromal cell phenotype through PDGF signaling: importance in human fibrotic disease. *Proc. Natl. Acad. Sci.* 119, e2120336119