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EXPERT OPINION

Dupuytren's disease therapy: targeting the vicious cycle of myofibroblasts?

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Introduction: Dupuytren's disease (DD) is a proliferative fibromatosis of the hand, which causes permanent flexion contracture of the digits and, ultimately, loss of function. The treatment of DD is complex and involves surgical and nonsurgical approaches, with the goal of removing the affected tissue. New biological targets are under investigation in order to develop innovative therapies.

Areas covered: The etiology of DD is still unknown. Several authors who focused their studies on the genetics of DD recognized an inherited autosomal dominant pattern. Actually, DD is a multifactorial and complex disease. Myofibroblasts are thought to play a crucial role in its pathogenesis, although their origin is not clear.

Expert opinion: There is a general consensus that a better understanding of cellular and molecular mechanisms of DD will lead to the design of more specific and effective treatment alternatives. In this review, the authors hypothesize a new biological model for DD pathology, where myofibroblasts enhance the reservoir of the disease acting as if in a vicious cycle. This could help, ultimately, in identifying new therapeutic strategies to treat this common and disabling fibroproliferative disorder.

Keywords: Dupuytren's disease, molecular mechanisms, myofibroblasts, therapeutic strategies

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1. Introduction

Dupuytren's disease (DD) is a proliferative fibromatosis of the hand, which causes permanent flexion contracture of the digits and, ultimately, loss of function [1,2]. DD is a slowly progressive and irreversible pathology characterized by a high rate of recurrence after surgical intervention [3].

The etiology of DD is so far unknown. From a clinical point of view, there is no proven evidence that hand injuries or specific occupational exposures are linked to a higher risk of developing the disease. Some theories connect DD onset or progression to physical trauma such as over-exertion of the hands or unrelenting manual work. Suspected, but unproven, causes of DD include trauma, diabetes [4,5], alcoholism [6], epilepsy therapy with phenytoin [7] and liver diseases [8]. An association between DD and human immunodeficiency virus (HIV) has been also proposed. Particularly, a high prevalence of DD has been described in a group of 50 men affected by HIV infection [9].

DD is probably due to a defect in the wound healing process or an abnormal response to wounding [10]. Usually, the disease evolves slowly starting with the formation of highly vascularized nodules, which then develop into avascular collagen-rich cords. Myofibroblasts, having characteristics of both smooth muscle cells and fibroblasts, play a critical role in DD. These cells are thought to be mainly

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Article highlights.

- Dupuytren's disease (DD) is a proliferative fibromatosis of the hand, which often causes permanent flexion contracture of the digits and, ultimately, loss of function.
- The etiology of DD is so far unknown.
- The treatment of DD is complex and involves surgical and nonsurgical approaches, with the goal of affected tissue eradication.
- A defect in a wound healing process or an abnormal response to wounding is thought to be the main events of DD pathophysiology.
- DD complexity may constitute an invitation to consider it as a multifactorial disorder.
- Myofibroblasts are thought to play a crucial role in the pathogenesis of DD, although their origin is still unclear.
- A deeper knowledge of cells origin and activated pathways in DD will lead to design more specific and effective treatment alternatives.
- A new biological model for the pathology, where DD myofibroblasts enhance the reservoir of the disease acting as in a vicious cycle has been hypothesized.

This box summarizes key points contained in the article.

responsible for the formation of nodules and subsequent contracture [11]. Several, cytokines and growth factors that may participate in the development of the disease have been identified [12,13], together with altered expression of different genes [14].

Since an animal model for DD has not been developed yet, the possibility to investigate animal models of similar or related diseases must be considered to better understand the pathophysiology of DD [15].

The genetics of DD has been widely studied and recognized as an autosomal dominant pattern. However, up to date, there is greater evidence among researchers to reconsider DD as a multifactorial and complex disease, where different pathways are altered. Understanding the biological and molecular mechanisms of DD may improve its diagnosis and management. Moreover, the higher risk of surgical treatment failure and the consequent recurrence, highlight the necessity of additional research. Indeed, particular attention should be paid on different aspects of this common fibroproliferative disorder, from clinics to biology, focusing on originating cells and their mechanism of action.

This review explores the pathophysiology of DD and proposes a new model where cells enhance the reservoir of the disease acting as in a vicious cycle. Thus, contributing to the understanding of the etiology, defining new therapeutic targets toward potential future therapies.

2. Anatomy and histopathology: the cellularity of the nodules is strictly related to the activity of the disease

Studying the physiological anatomy of the palmar fascia is essential to understand pathologic changes that occur in DD. The palmar fascia is a thick aponeurotic layer localized between the dermis and the flexor muscles of the hand. It stabilizes the palmar skin during movement, and protects the deeper neurovascular bundle. Clinically, DD appears as an alteration of the physiological palmar fascia. Indeed, it is characterized by the formation of nodules from the fibers of the palmo-digital fascia, which gradually develop into cords, the affected structures of the digital aponeurosis, whereas Grapow fibers, connections of the dermis to the palmar fascia, induce skin pitting. Cords cross joints and, once contracted, induce deformities. Indeed, flexion contracture at the metacarpophalangeal (MP) joint is caused by the pretendinous cord, whereas the flexion of the proximal interphalangeal (PIP) joint is caused by the lateral digital sheet that becomes the lateral cord. The role of the spiral cord is clinically crucial, it inserts distally into the lateral digital sheet and when affected it leads to PIP contracture and superficial displacement of the neurovascular bundle, with higher risk of accidental injury during surgery.

Several authors have focused their attention on the histopathology of DD. Meyerding *et al.* first assumed that the cellularity of the nodules is strictly related to the activity of the disease [16]. Moreover, Luck *et al.* classified the disease into three stages: proliferative, involutional and residual [17]. The first stage is characterized by the nodule, a highly vascularized structure containing high concentrations of fibroblasts, with a large predominance of myofibroblasts, cells characterized by expression of smooth muscle α-actin (α-SMA), no cytonuclear atypia with small and pinpoint nucleoli. The second stage is characterized by both highly cellular nodules and areas with reduced cellularity but abundant collagen fibrosis (higher amount of collagen type III compared to type I). The absence of mitoses is the main difference between the first two stages. As the disease progresses, nodules change into cords, which are quite avascular, acellular, collagen-rich and have few myofibroblasts. In the third phase comes the complete involution of the nodule and fibrocytes represent the main cell line. Other researchers revised this classification. Rombouts *et al.* found a relationship between recurrence rate and histological modifications based on different stages of Luck's classification [18]. Recently, Lam *et al.* edited the same classification creating a new staging system. Based on the amount of type III collagen, they differentiated stage I > 35%, stage II > 20% but < 35%, and stage III < 20% [19].

Several researchers confirmed that DD is linked to various cells type and concentrations according to different stages of the disease. Moreover, in the same specimen, different phases with predominant cell populations can be recognized. Indeed, nodules are highly cellular whereas fascial cords, made of packed collagen fibers, are relatively acellular [20,21]. Gabbiani and Majno showed that myofibroblasts are present in DD and are phenotypically similar to those of the granulation tissue [22]. The authors assumed that myofibroblasts are the main actors of wound contracture, determining the retraction

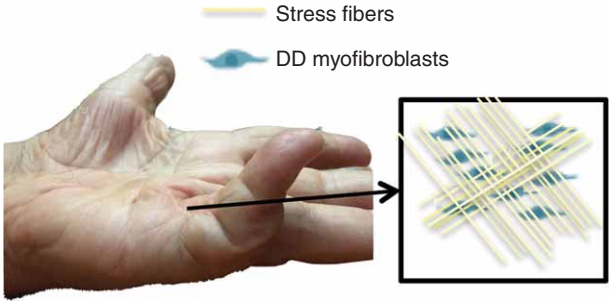

Figure 1. Dupuytren's disease nodules formed under the skin by myofibroblasts develop into tight bands of tissue causing the fingers to curl.
DD: Dupuytren's disease.

mechanisms. Thus, myofibroblasts could be responsible of the palmar retraction in DD (**Figure 1**).

3. Biology of DD: the central role of myofibroblasts

Numerous studies highlight the central role of myofibroblasts in tissue contraction of DD [23-25]. Myofibroblasts express α-SMA and synthesize fibronectin with distinctive contractile forces and ability to create cell-to-cell and cell-to-stroma connections. These characteristics may explain the contractures occurring in DD. Moreover, myofibroblasts form and deposit collagen type I and type III into the extracellular matrix (ECM). ECM and remodeling by myofibroblasts has an important effect on their own behavior and that of other cell types found in the same microenvironment [26].

As DD is characterized by the abnormal deposition of collagen into the fascial structures of the palm and fingers, it is likely that, with continued collagen deposition, myofibroblasts progressively shorten the ECM, inducing contraction of the affected structures [27]. The resulting cords are relatively acellular and predominantly composed of type III over type I collagen, in contrast to the normal palmar fascia in which type III collagen is absent [28,29]. Collagen in DD has a different configuration and a shorter wavelength than in the normal fascia. Furthermore, a relationship between clinical severity of DD and myofibroblasts activity, expressed as myofibroblasts ATPase activity, has been shown in severe cases of the disease [30,31].

DD is often characterized by the appearance of fibronectin in the palmar fascia. There are several isoforms of fibronectin that belong to the same family of glycoproteins and each of them derives from differential splicing of a single gene transcript [32]. The fibronectin extra domain A (ED-A), but not plasma fibronectin, is crucial for myofibroblastic differentiation induced by TGF-β1 [33,34], and it is, therefore, a fundamental part of myofibroblast ECM.

The source of myofibroblasts in the disease remains unknown. Some candidates could be resident fibroblasts, fibrocytes or mesenchymal stem cells (MSCs). Several authors have demonstrated that in early stages, fibroblasts proliferate and, after activation, differentiate into myofibroblasts [24]. Under physiological conditions fibroblasts exhibit few or no actin-associated, cell–cell and cell–matrix, contacts and little ECM production [35]. They are activated after tissue injury and migrate into the damaged tissue where cytokines are released by inflammatory cells [36]. In injured tissues, mechanical stress generated by disruption of the ECM activates fibroblasts to produce contractile stress fibers composed of cytoplasmic actins [35]. Moreover, in DD tissues, macrophages and T lymphocytes have also been observed [37]. Apparently, the factor that induces differentiation of original fibroblasts into myofibroblasts with contractile ability is the TGF-β, a fibrogenic factor essential in numerous fibroproliferative disorders [38].

Even though, the pathogenesis of tissue injuries may be immunologically similar for all pathologies, the causes are often different. Indeed, a dominant Th1 response is a feature of effective healing, while in chronic inflammation a predominant Th2 response and an increase in Th17 cells are present resulting often in fibrosis [39].

DD is also similar to Peyronie's disease being characterized by stress-protein expression (e.g., HSP47, HSP60, HSP70), early perivascular accumulation of naive and activated CD3+ T cells, antigen-presenting DCs, and proinflammatory cytokines (e.g., macrophage migration inhibitory factor-1, myeloid-related protein-8/14) near occluded vessels [40].

Moreover, the parallelism with similar pathologies has suggested fibrocytes as precursor cells of myofibroblasts. In fact, in renal fibrosis, even though it is a different pathology where renal tubular cells are able to assume mesenchymal phenotype, myofibroblasts derive from fibrocytes that circulate in the blood and migrate to specific tissues where they differentiate [41,42].

Fibrocytes have similar features to both stromal and hematopoietic cells and originate from peripheral blood mononuclear cells [43-46]. They are not only precursors of myofibroblasts but also release a great amount of growth factors, cytokines and other ECM proteins that are increased in DD tissues such as TGF-β, TNF-α, IL-6, VEGF, platelet-derived growth factor, type I collagen, α-SMA and fibronectin [47]. Fibrocytes play a crucial role in wound healing and tissue regeneration and, whenever their function is impaired or proliferation increased, tissue fibrosis may develop [48-53]. Injured tissue leads to fibrocytes differentiation into myofibroblasts determining an increased production of collagen [47], which may be the cause of further progression of the disease.

Since fibrocytes and fibroblasts are distinct cell types, the former differentiate from hematopoietic stem cells (HSCs) and the latter derive from MSC/stromal, Hindocha *et al.* supposed that MSCs and HSCs may be involved in DD [54]. They compared stem cells in the cord, nodule, perinodular fat and skin of DD patients to controls. Progenitor cells were identified in DD patients in the skin overlying the

nodule and perinodular fat. Moreover, there was greater expression of MSCs markers including CD13 and CD29 in the fat surrounding the nodule. The origin of myofibroblasts as such may be the skin overlying the nodule or the perinodular fat.

It is clear that myofibroblasts play a key role in the pathophysiology of DD. Further studies on the biology of DD, from the origin to the mechanism of action of these cells, would help in improving prognosis and diagnosis.

4. Genetics and molecular pathways of DD: a cross between different roads

4.1 Genetics

The genetic susceptibility is usually considered an important etiological factor. Some theories on the genetics of DD recognized an autosomal dominant pattern linked to a single 6 cM region on chromosome 16 [55]. Other authors suggested the presence of chromosomally aberrant fibroblasts, or clones of trisomic cells for either chromosome 7 or 8 [56-58]. Kaur *et al.* didn't notice any cytogenetic abnormalities using DNA extracted directly from nodules as opposed to fibroblasts expanded *in vitro* used in previous studies. They demonstrated possible effects of artifacts in culture [59]. Recent studies demonstrated that localized changes in gene copy number, as opposed to gross cytogenetic changes, may predispose to the development of the disease (specifically at the 7p14.1 and 14q11.2 loci) [60].

However, there is no statistically significant link to a single genetic modification, probably due to different molecular mechanisms involved in this pathology. Several sporadic cases of DD and few familiar cases have been described and genetically analyzed. Moreover, the limited number of members of families affected makes the determination of the mode of inheritance difficult and the late age of the onset of the disease limits examination of two generations [61].

Northern Europeans are the most affected by the pathology, and it is supposed that it initially arose in Vikings or a precursor tribe [62], and was then spread throughout the surrounding regions. However, it is less prevalent in certain ethnic groups, including the Japanese [63] and Black Africans [64].

Recent studies have demonstrated that the cause of this disorder is more complex and evidently not associated to mutations in a single gene, but likely to the effects of multiple genes in association with environmental factors. On this basis, an integrated approach of genomics, transcriptomics and proteomics could be ideal in order to reveal novel insights of DD etiology.

Indeed, numerous genes have been found altered at the mRNA expression level in a study comparing DD fibroblasts with controls. Quantitative PCR analysis confirmed the downregulation of proteoglycan, fibulin and the α1 chain of collagen type XV [65]. Another study showed the amplification of mRNAs of metalloproteases and tissue inhibitors of metalloproteases in DD tissue [66]. Considering the multifactorial origin of this disease, Rehman *et al.* performed a new optimized transcriptome analysis, highlighting the two distinct structures of the nodule and the cord and adding a pathway-oriented approach. They compared DD tissue biopsies with corresponding healthy tissues from the same patients establishing that the expression of some genes is altered in DD [67]. These genes encode molecules involved in immune-response, cell cycle, angiogenesis, apoptosis, cell proliferation, differentiation and transcription [68]. Moreover, histological studies have showed varying levels of collagen production, myofibroblasts and myoglobins in DD tissue. Mitochondrial dysfunction and oxidative stress (reactive oxygen species [ROS] production) have been also suggested as involved and affected pathways [69]. It has been supposed that a combination of mitochondrial DNA mutations and increased levels of ROS could lead to higher expression of TGF-β, greater myofibroblast activity, fascial contraction and DD. A mutation at position C2839A in the mitochondrial genome 16s rRNA region has been identified in 90% of patients affected by DD [70]. However, another study did not support the reported incidence of the mitochondrial mutation reported by Bayat *et al.* [71].

Therefore, it is clear that DD is a disorder characterized by an altered expression of different genes involving multiple pathways regulation. Furthermore, DD is often defined as a quasi-neoplastic fibromatosis, since it matches with cancer, which etiology is known to be multifactorial.

The identification of different pathways involved in DD is crucial for its research, diagnosis and management in order to identify new targets for intervention. In particular, the importance to investigate all of the molecular phases, such as transcriptomic, post-transcriptomic, protein activation and metabolic release of molecules, at the same time would contribute to better understand the complexity of this pathology. Thus, a 360° vision around the intricate network of DD represents a necessity and goal for novel and targeted research in order to improve prognosis and therapy.

4.2 Molecular pathways

Understanding the molecular mechanisms of DD could lead to more specific and effective treatments. Different molecular pathways have been found altered in DD contributing to its progression. TGF-β is considered to play a primary role in the pathophysiology of DD, where local production and sensitivity to TGF-β increase. TGF-β is able to upregulate α-SMA and collagen both *in vitro* and *in vivo* and is thought to be the master inducer of myofibroblast transdifferentiation phenotype [72]. Krause *et al.* used two protein kinase inhibitors on cell culture preparations of DD palmar fascia to block the main pathways of TGF-β. The inhibitors were able to stop the fibroproliferative process and induce myofibroblasts dedifferentiation to fibroblast phenotype [73].

High levels of pro-inflammatory cytokines have been detected in tissue of DD patients. It was demonstrated that neutralization of TNF-α by antibodies decreased the

contractile activity of myofibroblasts, showing the promising outcome of this therapeutic approach [74].

Moreover, several dysregulated genes in DD that encode secreted ECM proteins may play a role in disease progression or recurrence. Structural components of the ECM such as collagens, laminin, fibronectin and elastin have been found altered in DD. Proteases like metalloprotease ADAM-12 and disintegrin, proteoglycans and other ECM components, including tenascin C and periostin, as well as specific members of the MMP family (MMP-2 and MMP-9), have been also found abnormally regulated. Periostin is able to induce normal fibroblasts proliferation, apoptosis and differentiation in myofibroblasts. Due to this potential, periostin is able to initiate different responses in DD cells and adjacent fibroblasts and may have different roles in DD progression and recurrence. Therefore, the characterization of ECM proteins may be fundamental to identify new therapeutic targets for DD therapy [75].

Susceptibility to the disease may also be conferred by alterations in genes that encode proteins involved in the Wnt-signaling pathway. An association with genes involved in the Wnt-signaling pathway such as *WNT4*, *WNT2 RSPO2* and *WNT7B* has been found in 35 single nucleotide polymorphisms strongly associated with DD [76]. In addition, VEGFR-2 and hypoxia-inducible factor-1α resulted expressed in the involutional phase, when nodules have high concentrations of α-SMA-positive myofibroblasts, concluding that hypoxia and (subsequently) angiogenesis may also have a role in the pathophysiology of DD [77]. VEGF expression is upregulated by HIF-1α binding to the hypoxia response element in the gene promoter region of the VEGF gene. VEGF has a crucial role stimulating endothelial cells to migrate, proliferate, and create new vessels. These new vessels invade the provisional wound matrix, made of collagen type III, proteoglycans fibrin and fibronectin, where fibroblast and myofibroblasts are embedded. Overexpression of VEGF enhances wound healing of the skin in transgenic mice [78].

It has been shown that in several pathologies, under hypoxic stimuli, fibroblasts convert to myofibroblasts through a MMP-2-mediated pathway [79]. Indeed, cells cultured under hypoxia condition have relevant morphological differences compared to cells cultured under normoxia. Hypoxia could deeply influence morphological changes occurring in DD cells.

The possibility to target cells responsible for DD pathogenesis would represent one more step to understand its evolution: superficial markers would be promising in that sense. In myofibroblasts linked to DD an encouraging marker is CD90. In particular, stem-like $CD90^+$ subpopulation has been identified as contributor of DD development. In a recent study, it has been also revealed the involvement of the p38 MAPK pathway as a possible signaling cascade in the pathogenesis of DD [80].

Finally, microRNAs (miRNAs) have been identified to participate in DD pathogenesis. miRNAs are small, single-stranded noncoding endogenous RNAs consisting of 20 – 23 nucleotides that play key roles in the regulation of gene expression and are implicated in several diseases including a variety of cancers. miRNAs involved in DD have been identified using an Agilent's miRNA microarray platform. miRNA-29c, miRNA-130b, miRNA-101, miRNA-30b and miRNA-140-3p were found to regulate important genes related to the β-catenin pathway (Wnt5A, ZIC1 and TGF-β) and thus they could be used as a therapeutic targets [81].

A schematic summary of the main pathways involved in DD is shown in **Figure 2**.

5. Treatment of DD: understanding the possibilities

5.1 Surgical approaches

The treatment of DD includes surgical and nonsurgical approaches, with the goal to remove affected tissue in order to avoid the deformity to become irreversible [82,83]. The most effective treatment is the surgical removal of fibrous cords causing patients' symptoms, by fasciectomy (excision of the contracture, the most widely used because associated with lower rate of recurrence) [83], fasciotomy (cut the fascia without excision to relieve tension), or dermofasciectomy (discarding both DD tissue and the overlying skin) [84]. The indication for surgery is based on the Tubiana's staging system and the contracture that interferes with hand function. In that sense, PIP contractures are treated earlier and more aggressively. There is no general consensus on the best procedure; therefore, the choice of the proper approach should be based on the necessities of the single patient. New techniques such as distraction devices or preoperative external fixator may be associated to surgery in severe stages of disease; however, clinical outcomes are still controversial [85]. The major benefit of surgery is restoration of function through correction of deformity but full recovery can take up to 3/6 months, as power grip is likely to be reduced until the scar or graft is mature in the palm. However, there are no significant ways to prevent DD or limit its progression. Regardless of technique used, the patient should be told that surgery only corrects the deformity but does not remove the tendency of the disease to produce contractures. Hence, recurrence at the same or different sites in the hand can still occur in a high rate after surgery.

5.2 Nonsurgical approaches

Nonsurgical treatments can obviate surgical risks but do not give durable benefits. They are mainly based on the use of radiotherapy, physiotherapy and steroid injections.

More recently, Phase II and III clinical trials have shown promising results of new therapies in some DD patients [86,87]. Collagenase clostridium histolyticum (CCH), sold as Xiaflex® (Auxilium Pharmaceuticals, Inc.), is an enzymatic treatment for adult patients with DD approved by the US FDA in February 2010. CCH injected into DD cords lyses collagen inducing cords rupture [87,88]. Since its introduction, the use of CCH has been progressively

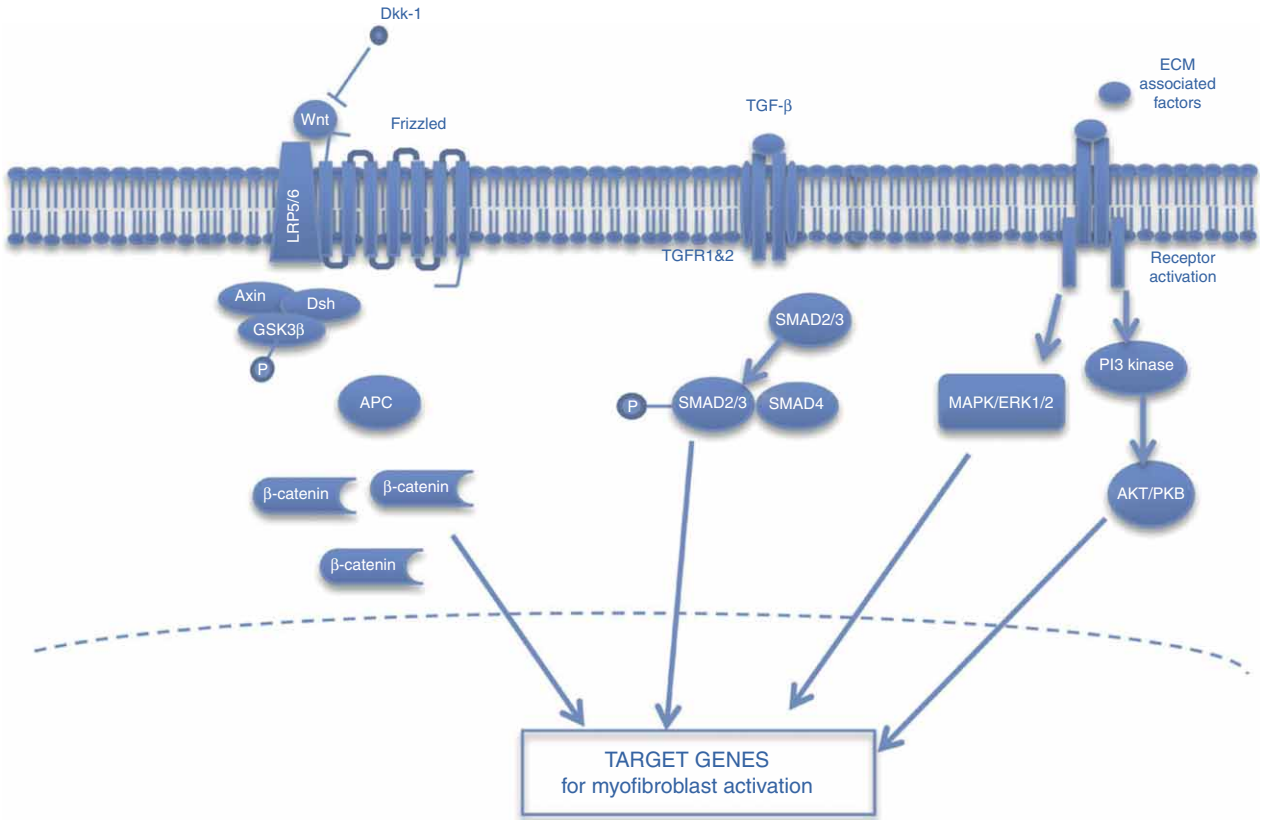

Figure 2. Proposed scheme of the etiology of Dupuytren's disease, leading to dysregulation of myofibroblasts differentiation. Main signaling pathways involved in the molecular pathogenesis of DD.

AKT/PKB: Protein kinase B; APC: Adenomatous polyposis coli; DD: Dupuytren's disease; Dkk-1: Dickkopf-related protein 1; Dsh: Dishevelled; ECM: Extracellular matrix; GSK3β: Glycogen synthase kinase 3 β; LRP5/6: LDL receptor-related protein 5 and 6; MAPK/ERK1/2: Mitogen-activated protein kinases/ Extracellular signal-regulated kinase 1/2; PI3 kinase: Phosphoinositide 3-kinase; SMAD 2/3: Small mother against decapentaplegic 2/3; SMAD 4: Small mother against decapentaplegic 4; TGFR1 or 2: Transforming growth factor receptor 1 or 2.

increased with reduction of the percentage of surgical procedures. This approach is an injectable treatment that does not include hospitalization, surgery, or anesthesia and is characterized by low pain intensity of short duration, low risk of complications such as infection or other wound healing problems that, if present, are mainly localized. Contraindications are coagulation disorders, anticoagulant therapy, or chronic hand muscular, neurologic, or neuromuscular disorders. Patients who are not candidates for surgical intervention according to the clinical presentation may benefit from this less invasive option. However, the lack of long-term follow-up results makes its use as a procedure in place of surgery debated.

MESNA (Uromixetan) (sodium 2-mercaptoethane sulfonate), which is a clear liquid mucolytic agent with the ability to dissolve connections between tissues, has also been used for the treatment of DD [89]. Several experimental and clinical studies have been performed to evaluate tolerability and toxicity of the drug, establishing that intravenous MESNA administration is rarely related to side effects (e.g., nausea, vomiting, diarrhea, allergic reactions, hypertension).

However, even though different surgical and nonsurgical approaches are available, the risk of treatment failure and disease recurrence is still high. Additional research on the causes and factors related to disease relapse is crucial in order to find treatment alternatives and improve short- and long-term outcome of patients.

5.3 New therapeutic approaches

Recent research has been carried out to identify potential molecular and cellular targets as DD nonsurgical therapies. Along this line, the analysis of molecular mechanisms activated in DD cells and identification of key proteins involved in disease onset and progression are fundamental.

Transcriptomic and proteomic analyses on DD cells showed promising results. New drugs for DD treatment have been proposed to essentially targeting the excessive pathway activation. A promising treatment of DD cells is represented by the inhibition of p38 and Akt kinases through the SB203580, which is able to induce downregulation of several fibrotic genes [90]. In a similar way, block of proliferation and contraction of Dupuytren's fibroblasts was obtained using

PD98059 able to inhibit mitogen-activated protein kinase 1 and SB-431542, inhibitor of TGF-β type I receptor kinase [73]. However, the clinical use of such small molecules is still difficult, thus the continue research of new target pathway and new drugs. Targeting downstream effectors of TGF-β1, especially those of nonclassic signaling pathways, might be more selective.

Considered the crucial role of ECM as a mediator of fibrosis, it could represent the target of anti-fibrotic drugs. Among all, fibronectin could be for sure a suitable target to control myofibroblast development and survival, focusing on the EDA domain which is more attractive as a potential therapeutic target for the treatment of fibrotic diseases [26].

A biological agent used to inhibit cell proliferation and inflammation in DD is δ-interferon. Studies on cells derived from DD patients showed that δ-interferon could reduce cell proliferation, collagen production and expression of α-SMA [91,92].

Moreover, considered the role of stem cells in preventing fibrosis, a cell therapy has been also proposed for DD. Verhoekx *et al.* demonstrated that adipose-derived stem cells are able to inhibit contractile myofibroblasts in DD. These findings support the potential benefit of lipo-grafting in conjunction with aponeurotomy as a novel strategy for the treatment of DD [93].

Clinical translation of basic knowledge of the molecular and cellular mechanisms of fibrosis in order to develop diagnostic, preventive and therapeutic measures is often challenging. Even though, diseases and molecular pathways that initiate the fibrotic process may be different, the biochemical and cellular mechanisms leading to fibrosis are often similar [94].

However, *in vitro* and *in vivo* results from animal models of fibrosis cannot always translate into effective therapy in humans. Therefore, further clinical trials are needed.

6. Expert opinion: the vicious cycle of myofibroblasts

The DD is a hand deformity that causes the palmar fascia to thicken and contract slowly. Over years, daily activities are complicated by fingers being progressively affected until the complete loss of function. This pathology is a common and disabling fibroproliferative disorder with an important social and economic impact in terms of lifestyle changes and loss of working hours.

The etiology of DD is still unknown. A genetic susceptibility to the disease is the only generally agreed etiological factor. It has been recognized as a disease inherited in an autosomal dominant pattern. However, up to date, there are more evidences to reconsider DD as an evident multifactorial pathology, as different molecular pathways are altered. Defect in the wound healing process or an abnormal response to wounding are thought to be the main event in the pathogenesis of DD [10]. Several studies demonstrate that myofibroblasts, expressing α-SMA, are the cells responsible for DD tissue contraction [21-23], although their origin and mechanism of action still need to be investigated.

The most effective treatment for DD is the surgical removal of the fibrous cords causing patients' symptoms [84]. Nonsurgical treatments can prevent surgical risks but have failed to give long-lasting benefits. Despite recent advances in understanding the pathophysiology of DD, the therapeutic approach is still palliative and not curative [95]. In most cases, evolution of DD is progressive and irreversible, and the risk of relapse after surgical excision is high [96].

Recently, it has been highlighted that the best option for DD therapy could be analyzing the molecular mechanisms activated in DD cells and identifying the key proteins involved in disease onset and progression. Ideal treatment for DD would involve modulation of the cellular mechanisms to prevent or control the development of this fibroproliferative disorder.

However, there are still several open questions.

6.1 How could we identify an efficacious treatment for DD?

Considering DD as a multifactorial disease as due to genetic, biological and mechanical factors, we can advocate that myofibroblasts play a key role in the formation of nodules/cords and mechanisms that involve their function must be explored.

6.2 What are the progenitor cells of DD myofibroblasts?

This point needs to be addressed, taking into account that some candidates could be MSCs, fibrocytes or resident fibroblasts. Further *in vitro* and *in vivo* studies are necessary in order to precisely identify DD myofibroblasts and progenitors. Isolation and characterization of these cells by surface markers to find the ideal target therapy could be an option. However, the choice of the better culture condition is crucial, as it has to be as similar to the physiological state as possible in order to avoid morphological and molecular changes in the cells.

6.3 Which mechanisms are involved in cell communication?

It is crucial to investigate the type of communication that these cells use to exponentially grow. We propose the existence of cell-to-cell interactions and, most importantly, the release of factors upside or inside specialized vesicles such as exosomes. This mechanism could be essential to recruit cells from other sites to injured ones. As the disease starts with some initial events leading to cells transdifferentiation in DD myofibroblasts, it could be possible that these cells transmit signals to recruit other cells from close and distant sites, as in a vicious cycle (**Figure 3**).

Identifying the players of this vicious cycle, their mechanism of action and the signal molecules released from cells could clarify the origin, onset and progression of this pathology.

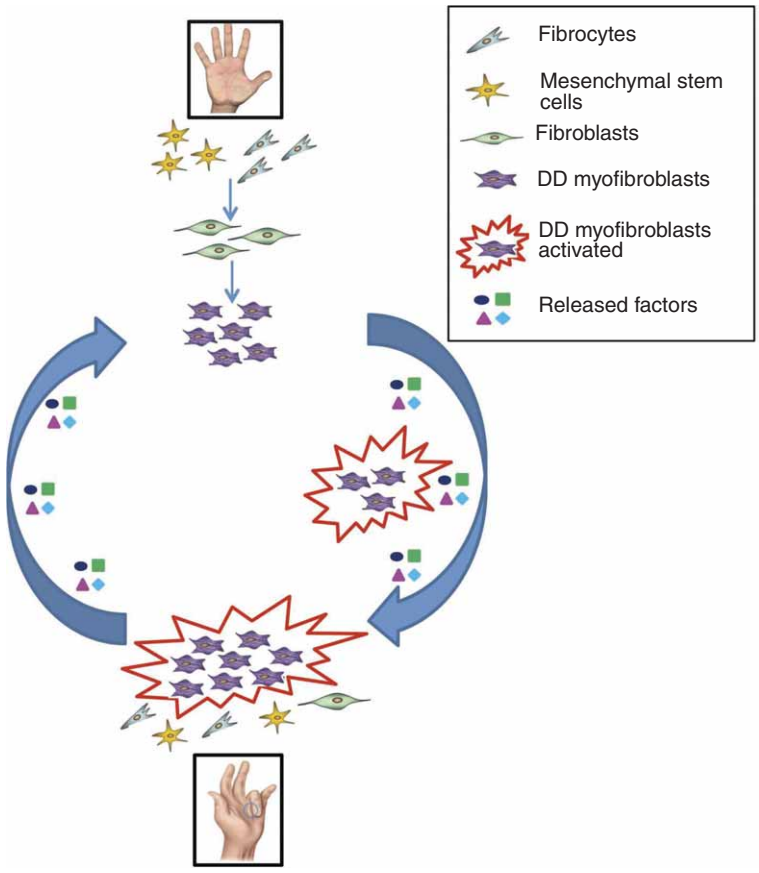

Figure 3. The proposed vicious cycle of Dupuytren's disease. Myofibroblasts, upon activation, release factors to attract other cells from close and distant sites, enhancing the reservoir of the pathology.
DD: Dupuytren's disease.

Furthermore, a better knowledge of the molecular mechanisms involved in the disease onset and progression, not only in DD cords but also in the rest of the palmar fascia, could contribute to a better treatment and prevention of post-surgical relapse.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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