The Molecular Pathogenesis of Dupuytren Disease

Review of the Literature and Suggested New Approaches to Treatment

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Background: Ever since the classification of Dupuytren disease into the proliferative, involutorial, and residual stages, extensive research has been performed to uncover the molecular underpinnings of the disease and develop better treatment modalities for patients. The aim of this article is to systematically review the basic science literature pertaining to Dupuytren disease and suggest a new approach to treatment.

Methods: Following Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines, a systematic review was conducted using the MEDLINE database to identify basic science literature on Dupuytren pathophysiology falling under 1 or more of the following categories: (1) Molecular alterations, (2) Structural alterations, and (3) Genetic predisposition.

Results: A total of 177 articles were reviewed of which 77 studies met inclusion criteria. Articles were categorized into respective sections outlined in the study methods.

Conclusion: The pathophysiological changes involved in Dupuytren’s disease can be divided into a number of molecular and structural alterations with genetic predisposition playing a contributory role. Understanding these changes can allow for the development of biologics which may disrupt and halt the disease process.

Key Words: Dupuytren disease, Pathophysiology, Hand

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STUDY METHODS

Of the 385 abstracts that were reviewed for inclusion criteria, only those that discussed the pathophysiological changes in DD and fell under one of the following 3 broad categories were included: (1) Molecular alterations, (2) Structural alterations, and (3) Genetic predisposition. Studies were excluded if they were unrelated to DD or disease recurrence. Articles related to DD treatment and/or recurrence, review articles and case reports were also excluded (C.K., S.C.). To ensure accuracy during this process, each summary and article was read and cross-referenced for completeness (R.S.). In total, 77 articles met the inclusion criteria and were included in the review.

MOLECULAR ALTERATIONS

Several dysfunctional signaling pathways in DD contribute to the proliferation of fibroblasts, their differentiation into myofibroblasts, and the production of ECM. In this section, we review these pathways, specifically focusing on the role of fibroblast growth factor (FGF), wingless/integrated (WNT), and transforming growth factor beta (TGF-β) in disease progression. Fibroblast growth factor has been extensively studied by groups looking to better understand the mechanisms behind dysregulated fibroblastic proliferation in the early stages of the disease. Indeed,
researchers have demonstrated that endothelial cells found within blood vessels promote fibroblast proliferation in DD through the production and secretion of FGF. Under nondiseased conditions, the blood vessel endothelial cells that control the rate of fibroblastic proliferation are subject to negative feedback mechanisms to prevent overproduction of FGF and excessive fibroblast proliferation. However, in Dupuytren's, this negative feedback mechanism is overridden due to abnormally high expression of FGF, TGF-β, and their receptors. As a result, the fibroblasts display increased sensitivity to these growth signals despite the negative feedback systems in place, causing them to consistently proliferate and deposit collagen in the surrounding tissue. Moreover, these proliferating fibroblasts have been shown to aggregate together in the early disease stages due to the presence of high levels of fibrin and low fibrinolytic activity in small nodules. The development of this provisional fibrin matrix with abnormally proliferating fibroblasts sets the stage for the clinical manifestation of DD.

Aside from its effect on fibroblasts and myofibroblasts, FGF also stimulates endothelial cell proliferation within the blood vessels themselves. As a result, the blood vessels become increasingly narrow, leading to a state of hypoxia due to diminished oxygenated blood flow. These hypoxic conditions have significant downstream effects on the surrounding tissue; for instance, reactive oxygen species (ROS) levels increase as adenosine triphosphate is broken down into hypoxanthine and other ROS intermediates in the endothelial cells of narrowed blood vessels. Indeed, investigators have found up to 6-fold increase of hypoxanthine levels in DD tissue, with the highest levels reported in the "nodular" areas. Because DD fibroblasts also display reduced expression of antioxidant proteins, these cells cannot effectively combat elevating ROS levels which may further contribute to their aberrant proliferation. Although the role of ROS in fibroblast proliferation is partly understood, the contribution of hypoxia and ROS on fibroblastic proliferation remains a subject of ongoing research.

FIGURE 1. PRISMA flow diagram: 385 articles were obtained from search terms. After reviewing all 385 abstracts only 177 articles met the inclusion criteria for complete full-text assessment. After full-text assessment only 77 articles were found to fit full inclusion criteria outlined in this review.
contraction in DD remains unclear.22 Moreover, in response to the long-term microvascular narrowing in Dupuytren, compensatory angiogenesis also occurs to improve blood flow in areas affected by the disease. One group of researchers, for instance, found a significant increase in HIF1-α and VEGF receptor 2 expression in Dupuytren tissue when compared with controls.18 HIF1-α is a transcription factor that increases the expression of VEGF, a growth factor that stimulates blood vessel proliferation and angiogenesis.

Fibroblastic proliferation in DD is also influenced by the dysregulation of the WNT signaling pathway. WNT proteins are a family of extracellular signaling glycoproteins which bind to frizzled receptors, lipoprotein receptor-related proteins, or receptor tyrosine kinases.23 Traditionally, the WNT signaling pathway is divided into 2 arms—the canonical pathway (β-catenin dependent) and the noncanonical pathway (β-catenin independent)—both of which are upregulated in DD.23,24 The canonical pathway involves the binding of WNT proteins to frizzled and lipoprotein receptor-related protein receptors to activate β-catenin, a transcriptional coactivator which regulates genes involved in cell proliferation and survival.23 Some authors hypothesized that WNT canonical pathway signaling and increased β-catenin levels may contribute to fibroblastic proliferation.25 One group, Forsman et al25 found a 14-fold upregulation of the receptor tyrosine kinase coreceptor ROR2, which regulates cellular differentiation and apoptosis, in Dupuytren tissue. This finding may help explain the mechanism for the residual phase of DD where marked hypocellularity is presumably caused by apoptosis or programmed cell death.25 On a genomic level, studies have identified that DD tissue has higher expression levels of WNT genes, including WNT2, WNT4, WNT7B, WNT5B, and WNT1, relative to controls.12,26,27 The expression of these genes varies and is likely related to disease stage; for example, authors identified a 9-fold downregulation in WNT2 (P < 0.01), 5-fold upregulation in WNT7B (P < 0.01), and 2-fold upregulation in SFRP4 (P < 0.01) (a protein that stimulates the WNT pathway).27 Therefore, a combination of genetic and molecular alterations may account for changes seen in fibromyoblastic differentiation and proliferation in DD.

Transforming growth factor beta is a growth factor which has been extensively studied in the context of DD because of its role in cellular proliferation and differentiation. It has been shown to work in concert with WNT and other signaling proteins to bring about dysregulated fibroblast proliferation in DD.24 Typically, TGF-β receptor activation can be induced through intracellular SMAD signaling cascades or non-SMAD pathways, such as mitogen-activated protein kinase (MAPK).28–30 Ratkaj et al2 demonstrated that, in DD, TGF-β induces p38MAPK signaling and leads to the expression of regulatory genes involved in cellular proliferation. Inhibition of the p38MAPK pathway also reduces the expression of myofibroblastic cell markers.2 Other researchers have discovered that the addition of TGF-β1 to fibroblast cell cultures from Dupuytren palmar fascia results in an increased concentration of myofibroblasts.25 More specifically, they found a 2.7% to 24.2% increase in total WNT-β-catenin expression, with a 7.2% increase in β-catenin expression and a 23.6% increase in total WNT-β-catenin expression in DD cord cultures, and a 9.7% to 25.4% conversion in those obtained from DD nodules after exogenous addition of TGF-β1 (P < 0.001).31 Furthermore, a TGF-β concentration of 12.5 ng/mL has been shown to yield the greatest increase in both the myofibroblastic cell phenotype and their contraction (P < 0.05).32 Given that TGF-β is clearly involved in the pathogenesis of DD, researchers have focused attention on where, when, and by what cells TGF-β is produced. Studies investigating the levels of TGF-β in DD have identified its presence irrespective of disease stage. Although the exact source for TGF-β production has not been ascertained, some research indicates that immune cells in DD nodules may be responsible.33 Through in situ immunolabeling, Berndt et al34 have shown a spatial correlation between myofibroblasts, TGF-β, and FGF in active proliferative Dupuytren nodules. The locoregional presence of TGF-beta and FGF near myofibroblasts is significant in the context of previous studies because it indicates these growth factors play a role in the phenotypic conversion of fibroblasts into myofibroblasts. Furthermore, with respect to TGF-β2, its levels are particularly elevated in myofibroblasts found in the proliferative and involutional stages of DD and have been shown to stimulate their proliferation, especially when added in vitro to myofibroblast at high plating densities (P < 0.0001).35 Finally, TGF-β may also contribute to collagen contraction and ECM irregularities in DD.36–38 However, the addition of cyclic adenosine monophosphate can dramatically blunt the effects of TGF-β and inhibit abnormal ECM deposition to potentially mitigate DD progression or recurrence (P < 0.0001).36

Bone morphogenetic proteins (BMPs), which belong to the TGF superfamily, are also noteworthy for their role in the pathogenesis of DD. Bone morphogenetic proteins are known to regulate a variety of biological processes, such as cell proliferation, differentiation, determination, and apoptosis. Given both BMPs relationship with TGF and its sphere of cellular influence, it is important to determine its role in the pathogenesis of DD. Unfortunately, very few studies exist on the evaluation of BMP’s role in DD literature. However, in a study performed by Shin et al,39 mRNA expression of various BMPs and their receptors was assessed and compared in both Dupuytren and normal palmar fascia fibroblasts. This group found that Dupuytren fibroblasts expressed reduced levels of BMP 6, 8, 11 and BMP receptors IB, IA, and II as compared with the controls. In addition, a complete lack in expression of BMP-4 was observed in DD fibroblasts.s.39 Given these findings, additional investigations are warranted to ascertain the effect of low BMP levels in DD.

Inflammatory cells and cytokines also take part in the pathogenesis of DD.33 Researchers have found perivascular cuffing of lymphocytes within nodules as well as perivascular clusters of mononuclear cells in DD tissue.7,24 Histochemical analysis of cellular nodules also indicated that the number of macrophages was positively correlated with the number of myofibroblasts, the presence of which is generally considered to be indicative of the active disease state.17 In addition, T and B lymphocytes are involved in the pathogenesis of DD.40,41 As previously mentioned, studies indicate that TGF-β in DD is produced by inflammatory cells.33,42 Immune cells may also be responsible for the increased levels of interleukins IL-6 (P < 0.001), IL-17 (P = 0.031), and IL-1β seen in DD tissue, and it is plausible that these factors play a role in fibrosis.40,43 Similarly, TNF-α, a cytokine involved in systemic inflammation, has been shown to be genetically upregulated in DD tissue and may work with WNT signaling to bring about fibrosis seen in the diseased tissue.13,23,44

In summary, cellular proliferation and differentiation of fibroblasts and myofibroblasts in DD is a complex process involving many genetic and molecular aberrations. The majority of the current body of literature focuses on the dysregulation of FGF, TGF-β, and WNT signaling pathways in DD contracture (Fig. 2). While only a few studies are devoted to the roles of PDGF, IGF, NGF, and androgens, it is important not to overlook their potential contributions to the overall disease process.10,12,13,23,34,44–51 It is plausible that the factors also influence myofibroblastic proliferation and contraction in DD tissue.

**STRUCTURAL ALTERATIONS**

The ECM is an aggregation of connective tissue and extracellular molecules that provide structural and biochemical support to the surrounding cells and tissue. In DD, the normal composition of the ECM is disrupted as certain proteins become overabundant and modified in ways that contribute to palmar contracture. Fibroblasts and myofibroblasts not only contribute to this imbalance of the ECM through their production of excess collagen, but also serve as the main culprits behind the contraction of the palmar fascia. In this section, we review the literature for the main structural and cellular abnormalities of the ECM in DD tissue.

In Dupuytren, the palmar aponeurosis contains excess collagen, one of the main structural proteins of the ECM. DD fibroblasts produce this overabundance of collagen by expressing high levels of collagen-encoding mRNA and downregulating collagen mRNA inhibitor levels.
when compared with controls.\textsuperscript{15,52,53} Collagen within the palmar aponeurosis also undergoes abnormal posttranslational modifications, including increased reducible crosslinking as well as increased hydroxylation and glycosylation of these crosslinks.\textsuperscript{54} These changes contribute to the thickening of the palmar aponeurosis in diseased tissue.\textsuperscript{54,55} The posttranslational modification to collagen may occur due to increased levels of lysyl hydroxylase 2b (LH2b) mRNA, and transglutaminase which are enzymes involved in collagen crosslinkage.\textsuperscript{55,56} After collagen is produced and modified, it undergoes a process of dynamic remodeling by matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). The balance of MMPs and TIMPs is critical for normal collagen turnover. In DD, researchers have found an increase in the ratio of MMPs/TIMPs, and it is this imbalance that leads to dysfunctional collagen breakdown seen in the diseased state.\textsuperscript{7,62,63} Interactions between intracellular microfilaments of myofibroblasts and extracellular proteins affect ECM contraction in DD.\textsuperscript{52,64,65} In DD, myofibroblasts are surrounded by high levels of ECM proteins, including fibronectin, laminin, proteoglycans, and collagen, all of which have the capability to elicit pathologic cellular changes (\(P < 0.05\)).\textsuperscript{7,62,63} Using
electron microscopy, researchers have also identified extracellular filaments on DD myofibroblasts that interact closely with intracellular actin microfilament bundles, suggesting a mechanism by which intracellular forces are transmitted to surrounding tissue during the process of contraction. The ECM proteins that help facilitate force generation through collagen production include fibronectins, laminins, and integrins. Kosmehl et al studied fibronectin and found a positive correlation between the myofibroblast phenotype, cell proliferation, and the presence of increased fibronectin splice variants in Dupuytren tissue. Other proteins also facilitate fibro/myofibroblastic contraction, such as myosin light chain kinase (MLCK), sphenosine-1-phosphate as well as other metabolites. Through immunohistochemistry researchers have found that MLCK, a protein which facilitates contraction once activated, is expressed in the cytoplasm of fibroblasts in DD nodules. This finding suggests that MLCK inhibitors, such as fluorophenazine, may be potential drug targets for the treatment of DD. Other investigators have found that the addition of sphenosine-1-phosphate to DD fibroblast stimulates a dose-dependent increase in cellular contraction. These findings are significant as they may also account for the contractile nature of fibromyofibroblasts in DD. Finally, in the literature, there are various accounts of dysregulated expression of several metabolites, such as leucine, phenylalanine, lysine, cysteine, aspartic acid, and glycero-3-phosphate in DD tissue. It is thought that these metabolites can also directly activate contractile proteins in myofibroblasts or change the structure of the contractile proteins themselves which may contribute to irreversible palmar contracture.

Although there are specific changes in DD ECM that cause myofibroblastic contraction, DD myofibroblasts themselves undergo intracellular structural changes which bring about the disease state. Researchers have found that myofibroblasts in Dupuytren contain elevated levels of intracellular actin microfilaments (P < 0.01). Furthermore, using 3-dimensional collagen lattices from DD tissue, Verhoekx et al demonstrated that DD myofibroblasts express high levels of OB-cadherin, a high strength adhesion molecule. The same group successfully inhibited contraction of collagen matrices through the selective blockade of adhesins and gap junctions of DD myofibroblasts, suggesting that inhibition of intercellular signaling may serve as a therapeutic target for DD. Genomic studies have also identified other proteins involved in cell-cell and cell-matrix adhesion. For example, Staats et al studied the EPDR1 gene, which codes for a transmembrane cell adhesion protein that contributes to palmar fascia contraction in DD. In their study, knockdown of the EPDR1 gene led to a decrease in fibroblast contraction in fibroblast-populated collagen lattices (P < 0.05). These findings are remarkable as the elevated levels of intracellular actin and adhesion molecules may help explain the profound contractile nature of DD contractures from a cellular standpoint. In addition, the interdependence of myofibroblasts in bringing about these pathological changes may be explained by the increased expression of intercellular gap junctions during stages of DD where cellular proliferation is at its maximum. In particular, expression of gap junctions by myofibroblasts is highest during the involutional stage, suggesting that these junctions should be further investigated as a potential therapeutic target for DD.

In conclusion, DD is associated with a pathologic increase in ECM proteins, their linkage and myofibroblast contraction. In the ECM, collagen levels are increased substantially and their posttranslational modifications, as well as long-term remodeling, is disrupted. Dupuytren myofibroblasts, which are residents of the ECM, also contain high levels of actin, adhesins, gap junctions, and ECM proteins which give them the ability to generate an excessive force which leads to palmar contracture (Fig. 2).

**GENETIC PREDISPOSITION**

Genetic analysis of fibroblasts obtained from Dupuytren-affected palmar fascia and non-DD fascial tissue provides evidence for a “two-hit” hypothesis for disease progression and recurrence. In this mechanism proposed by Satish et al, an underlying allelic defect serves as the first “hit” but does not phenotypically manifest until it is followed by a somatic mutation which constitutes the second “hit.” The inherited mutation that constitutes the first hit influences the physiology of the palmar fascia and increases predisposition to DD; however, the defect is necessary but not sufficient to develop nodule/cord formation. A “second hit” to the remaining disease-causing allele within the palmar fascial tissue is also required to develop DD and may occur later in life as mutations accumulate in the body. The same group also found a greater overlap in basal gene expression of diseased and phenotypically unaffected palmar fascia fibroblasts than in those obtained from non-DD fascial tissue. Given the high recurrence rate of DD postsurgical treatment, this finding supports the notion that DD has a genetic predisposition component and that excess, abnormally modified collagen may eventually influence gene expression in the unaffected fascial tissue of DD patients.

Dupuytren disease is also associated with several chromosomal abnormalities that are not typically found in nondiseased tissue. This includes trisomy of chromosome 8 and balanced rearrangements at different genetic loci. Interestingly, trisomy 8 has also been identified in flexor retinaculum cultures obtained from carpal tunnel syndrome patients, suggesting that chromosomal instability in the palmar fascia may possibly serve as a gateway to the development of pathological fibrosis. Genetic analysis has revealed that chromosome 8 contains the c-myc gene, an important cell-cycle regulator whose overexpression due to trisomy may lead to increased proliferation of fibroblasts in Dupuytren. Dupuytren disease is also associated with copy number variations. For instance, Shih et al studied 25 DD patients and found that 87.5% had a high copy number variation in chromosome 7p14.1, and 100% had a high copy number variation in chromosome 14q11.2. In addition, this group found the SFRP4 gene, an activator of the WNT pathway, upstream of chromosome 7p14.1 near a small nucleotide polymorphism that regulates its expression. Finally, they identified a high copy number of MMP 14 in chromosome 14q11.2, a discovery that goes hand-in-hand with the previously mentioned finding that there is an increased ratio of MMPs/TIMPs leading to collagen turnover dysfunction in DD. Altogether, the higher copy number variations at these genetic loci further supports the notion that DD has a strong underlying genetic component.

**TREATMENT APPLICATIONS AND RECOMMENDATIONS**

Dupuytren disease manifests in genetically susceptible individuals due to molecular and structural alterations which disrupt the ECM of palmar fascia. These events clinically translate into the development of a painless nodule on the hand, the subsequent formation of collagen cords in the palm, and finally the irreversible contraction of the fingers. Treatment typically involves invasive surgical excision of the fibrotic cord through either open or limited regional fasciectomy; however, this procedure carries the risk of intraoperative and postoperative complications, including nerve and arterial injury, long recovery periods, and high recurrence rates. Newer methods, such as collagenase injection and percutaneous needle aponeurotomy, are not risk free either. Reports of iatrogenic nerve injury, wound healing complications, tendon rupture, phalangeal fracture and finger dislocation exist in more than sufficient quantity. By elucidating the biological pathways linked to Dupuytren, researchers may be able to devise better treatments for the disease, including reversing ECM changes and myofibroblast contraction through the administration of biologic therapeutics during certain stages of the disease process. Khouri et al, for instance, describe a minimally invasive technique where percutaneous aponeurotomy and lipofilling are performed for treatment of Dupuytren contracture. In this approach, cords are weakened or severed with an 18-gauge needle.
and the resultant subcutaneous space is filled with autologous fat grafts. The success of this procedure could be attributed to the fact that fat grafts are a rich source of adipose-derived stem cells (ADSCs). Furthermore, ADSC have a demonstrated capacity to have the capacity to inhibit the proliferation and contraction of myofibroblasts, one of the principle cell populations implicated in DD contracture. Another mechanism to combat DD may be targeted dedifferentiation of myofibroblasts into precursors cells to reduce the contractility of the Dupuytren tissue. Interestingly, numerous studies identified in this review indicate that an increase in TGF-β expression in DD simulates the process of tissue fibrosis and fibroblast-to-myofibroblast conversion. However, when Dupuytren myofibroblasts are treated with TGF-β1 type 1 receptor inhibitors, the TGF-signaling cascade is inhibited and the contracture of the myofibroblasts is significantly reduced. In addition, certain BMPs, which belong to a subset of the TGF-β superfamily and can have antagonistic effects on the TGF-β pathway, are completely absent (BMP-4) or downregulated (BMP-6, 8, 11) in DD tissue, which gives way for even more potential therapeutic interventions. Following this lead, our previous work in collaboration with the Plikus laboratory at the University of California Irvine, has shown that myofibroblasts can be differentiated and reprogrammed into adipocytes under the influence of recombinant BMP-4 and at high doses. Therefore, there is a potential role for the use of BMPs in the treatment of Dupuytren contracture. Although BMP-4 and 7 can induce myofibroblast dedifferentiation, the direct administration of recombinant BMP for Dupuytren is not currently FDA-approved. However, platelet-rich plasma (PRP), a possible endogenous source of BMPs, falls under the regulation of the FDA’s Center for Biologics and Research and therefore, not under the traditional regulation of the FDA. This opens the door for the use of PRP in the treatment of Dupuytren’s disease. PRP contains extractable levels of BMP, which can bypass the need to produce them via recombinant techniques. While PRP contains extractable levels of BMP, studies have indicated that these BMP levels can be increased even further when PRP is subjected to an acidic pH 4.3 buffer. Furthermore, the addition of ADSCs with PRP synergistically increases BMP levels through ADSC BMP production. In conclusion, DD is associated with both pathophysiological changes and genetic abnormalities. Molecular alterations that take place in the disease process include dysregulated signaling of FGF, WNT and TGF-β, which cumulatively result in abnormal proliferation and differentiation of fibroblasts into myofibroblasts. The effect of FGF on endotheial proliferation results in a pathological cycle that involves vascular narrowing, tissue hypoxia, and increased fibroblastic proliferation. Additionally, increased levels of β-catenin in the WNT pathway also contribute to the proliferation of fibroblasts in DD. TGF-β similarly promotes abnormal proliferation of myofibroblasts and their differentiation from fibroblasts. Increased concentrations of myofibroblasts consequently lead to the overabundance and abnormal posttranslational modification of ECM proteins, namely collagen type III. These effects, in addition to the interactions between intracellular microfilaments of myofibroblasts and extracellular proteins, contribute to the thickening and irreversible contraction of the palmar fascia in Dupuytren. By defining all the biological components that promote the development and progression of DD, we propose to investigate a new, noninvasive, and molecular approach that can potentially alter the disease process.

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Comparison of gene expression profiles between mature smooth muscle myofibroblasts and the response to stimulation by TGF-beta 1.

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