The Extracellular Matrix in Dupuytren Disease

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6.1 The Pathogenesis of Dupuytren Disease

The pathogenesis of Dupuytren Disease remains controversial and poorly understood. This complex condition resembles abnormal wound repair, a process that can be used to provide a contextual framework for understanding Dupuytren Disease development. When normal palmar fascia is wounded, complex arrays of wound healing responses are initiated in local and circulating macrophages, fibroblasts, and other cells that contribute to tissue homeostasis. Under ideal conditions, the signaling pathways that are transiently activated in these cells promote high-fidelity repair that superficially resembles palmar fascia regeneration. Unfortunately, palmar fascia repair also occurs under suboptimal conditions such as chronic microtraumas (Mikkelsen 1978; Liss and Stock 1996), excessive inflammation (Baird et al. 1993; Qureshi et al. 2001; Gudmundsson et al. 1998), and abnormal metabolic (Savas et al. 2007) and/or biomechanical (Verhoekx et al. 2012) stimuli from the extracellular environment. While these conditions alone are often sufficient to modify cellular responses and reduce the quality of subsequent repair processes, their impacts may be amplified when these cells carry heritable, pro-fibrotic genomic traits (Dolmans et al. 2011; Debniak et al. 2013; Bayat et al. 2002). These heritable traits are hypothesized to modify cellular sensitivities to

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adverse conditions and induce chronic activation of signaling pathways that are transiently activated during normal palmar fascia repair and the activation of additional signaling pathways that are normally restricted to development rather than repair (Rehman et al. 2008). The net result is the excessive secretion of collagens (Badalamente and Hurst 1999) and other extracellular matrix molecules by hyper-contractile tissue repair cells known as the myofibroblasts (Tomasek et al. 1999; Vi et al. 2009a). These cells, which have transient but essential roles in normal tissue repair, constantly remodel and contract the collagen-rich matrix they secrete, thereby increasing tissue density and ultimately causing palmar fascia contractures (Meek et al. 1999).

When viewed from this perspective, it is apparent that abnormal environmental stimuli and heritable genomic (genetic and epigenetic) factors act in combination to cause Dupuytren Disease. Ideally, therapeutic interventions to prevent Dupuytren Disease development would target both of these contributing factors simultaneously. Unfortunately, the complex genomic traits that are hypothesized to modify cellular sensitivities to adverse microenvironments and predispose individuals to develop Dupuytren Disease are yet to be clearly defined. Even when they are defined, we may have to await the development of reliable, safe, and approved genome-editing tools before it is feasible to intervene and correct them. However, some of the abnormal environmental stimuli that promote Dupuytren Disease are amenable to therapeutic interventions and are already being targeted in other disease systems. For example, pharmacological inhibitors of some of the inflammatory cytokines hypothesized to induce Dupuytren Disease development, such as TNFα (Verjee et al. 2013), are currently being used to treat inflammatory arthritis (Meroni et al. 2015; Bader and Wagoner 2011). However, to develop effective therapeutic interventions that target these cytokines or other molecules that promote Dupuytren Disease progression and recurrence, we must first take into account the unique palmar fascia microenvironment within which these interventions must take place. This microenvironment consists of a mixture of resident palmar fascia, immune and other cell types embedded in extracellular matrix (ECM) that is unique to this tissue. The central hypotheses of this section are that the ECM in the palmar fascia of patients with Dupuytren Disease is not normal, that it influences disease progression and recurrence, and that it is a viable and readily accessible target for therapeutic interventions to prevent Dupuytren Disease. Before we can determine whether or not the available evidence supports these hypotheses, we must first understand the cellular origins of the Dupuytren Disease ECM, its complexity, and how it interacts with contracture-causing cells.

6.2 The Cellular Origin of the Extracellular Matrix in Dupuytren Disease

The ECM can be described as a complex mixture of proteins and other molecules secreted by cells to provide structural support within a three-dimensional tissue. While this description is accurate, it omits what is arguably the most important role of the ECM, to provide the biochemical and biomechanical feedback that cells require to "sense" their local environment (Aszódi et al. 2006; Piccolo et al. 2014). Dupuytren Disease, like many other fibroses, is characterized by excessive ECM secretion and remodeling. The cells that secrete, condition, and contract the ECM in Dupuytren Disease are hyper-contractile connective tissue myofibroblasts. While circulating and resident macrophages, progenitor/stem, and other cells may play important roles in initiating fibrosis, the substantial overlap between the gene expression profiles of Dupuytren Disease-derived myofibroblasts and of palmar fascia fibroblasts in the adjacent, non-fibrotic palmar fascia strongly suggests that the majority of ECM-secreting myofibroblasts in contracture tissues are derived from the palmar fascia (Satish et al. 2008, 2012). Comparisons between myofibroblasts derived from Dupuytren Disease tissues and fibroblasts derived from normal carpal ligament have revealed

that Dupuytren Disease myofibroblasts not only secrete excessive quantities of ECM, but they also achieve "tensional homeostasis" with their ECM at much higher contractile forces (Bisson et al. 2004). These findings suggest that Dupuytren Disease myofibroblasts either have an impaired ability to recognize normal levels of tensional feedback from their ECM or that the biochemical and biomechanical signals that these cells receive from this ECM actively promote their excessive contractility. There is evidence to support the latter interpretation and, by extension, the hypothesis that the Dupuytren Disease ECM itself promotes disease progression.

6.3 The Complexity of the Extracellular Matrix in Dupuytren Disease

The ECM in Dupuytren Disease is very complex and, as yet, poorly defined. It contains hydroscopic carbohydrate polymers that act in combination with ECM proteins and act as proteoglycans, such as chondroitin sulfate (Bazin et al. 1980; Slack et al. 1982; Flint et al. 1982), and others that act independently of proteins, such as hyaluronan (or hyaluronic acid) (Slack et al. 1982; Andreutti et al. 1999). While ECM proteins are typically divided into structural and nonstructural categories, there is considerable overlap between these categories. For example, the most abundant proteins in contracture cords are type I and type III collagens (Bailey et al. 1977; Brickley-Parsons et al. 1981; Bunker et al. 2000). While their central roles in providing structural integrity to cords are beyond dispute, these proteins also function as signaling molecules (Imamichi and Menke 2007) that induce a variety of cellular responses (Vi et al. 2009b) through well-established cell surface receptors (Naci et al. 2015). Other Dupuytren Disease-associated ECM proteins with structural and signaling roles include other collagens (Magro et al. 1997a), laminin (Tomasek et al. 1986; Magro et al. 1997b; Tomasek et al. 1987), elastin (Neumuller et al. 1994), and insoluble fibronectin, including the alternatively spliced form of fibronectin known as extra domain A (EDA) or "oncofetal" fibronectin (Kosmehl et al. 1995; Berndt et al. 1995; Howard et al. 2004). Nonstructural "matricellular" proteins that interact with structural ECM proteins and regulate growth factor signaling, such as periostin (Vi et al. 2009a; Shih et al. 2009), tenascin C (Shih et al. 2009), CCN2 (connective tissue growth factor) (Satish et al. 2011), and others, are also abundant in the Dupuytren Disease ECM. ECM metalloproteinase (MMP) levels, tissue inhibitors of metalloproteinases (TIMPs), and activities differ between diseased and visibly unaffected palmar fascia, and these differences are likely to contribute to the imbalance in ECM production and degradation in contracture tissues (Verhoekx et al. 2012; Forrester et al. 2013; Tarlton et al. 1998; Johnston et al. 2007; Bunker et al. 2000). Finally, many growth and differentiation factors are associated with the Dupuytren Disease ECM. Some, such as transforming growth factor β (TGFβ) (Kloen et al. 1995; Badalamente et al. 1996; Berndt et al. 1995), bind the ECM in a latent form and can be activated by proteases in the ECM and also by the biomechanical influences imposed by abnormal ECM stiffness/density (Hinz 2009; Wipff et al. 2007). Others, such as basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and insulin-like growth factor-II (IGF-II) (Gonzalez et al. 1992; Raykha et al. 2013; Badalamente and Hurst 1999; Berndt et al. 1995), either bind the ECM directly as active molecules or are bound indirectly through other proteins with the capacity to bind structural components of the ECM. Genes encoding ECM proteins consistently make up the majority of the dysregulated genes identified in gene expression studies of Dupuytren Disease myofibroblasts and tissues (Rehman et al. 2008; Forrester et al. 2013; Qian et al. 2004; Satish et al. 2012), reflecting the extent of ECM remodeling that takes place during Dupuytren Disease development. We do not understand the functions or therapeutic potential of the vast majority of these molecules.

Examples of molecules previously identified in the Dupuytren Disease extracellular matrix		
Structural and nonstructural ECM molecules	Matricellular (nonstructural) ECM molecules	Growth and differentiation factors
Collagens (I, III, + many others) Decorin Vitronectin Hyaluronan (hyaluronic acid) Proteases (MMP/ADAM/ADAMTS) Protease inhibitors (TIMPs) Laminin Elastin Fibronectin (including EDA)	Periostin Tenascin C CCN2 (CTGF) CCN4 (WISP1)	Transforming growth factor ß (TGFß) Tumor necrosis factor α (TNFα) Basic fibroblast growth factor (bFGF) Platelet-derived growth factor (PDGF) Insulin-like growth factor-II (IGF-II)

6.4 Cellular Connections with the Extracellular Matrix

Connective tissue fibroblasts in general, and myofibroblasts in particular, obtain feedback from the biochemical and biomechanical components of their ECM (Hinz 2006; Tomasek et al. 2002) through specialized attachment points known as focal adhesions. While several different types of attachments have been characterized in *in vitro* settings, including focal complexes, fibrillar adhesions, and 3D matrix adhesions (Berrier and Yamada 2007; Harunaga and Yamada 2011), the primary cellular attachments that are formed *in vivo* are focal adhesions (Lock et al. 2008; Christopher and Guan 2000; Hinz et al. 2003) (Fig. 6.1). Focal adhesions are dynamically regulated multi-protein complexes of integrins and other proteins that span the cell membrane and connect cells to proteins in the Dupuytren Disease ECM, which include collagens (Magro et al. 1997a), laminin (Wilbrand et al. 2003), fibronectin (Magro et al. 1995), and matricellular molecules (Vi et al. 2009a). Both biochemical and biomechanical stimuli can activate focal adhesions to facilitate cellular responses. Many of these responses involve the polymerization of globular (G) actin monomers into the filamentous actins (F actin). Actin filaments bind vinculin and other focal adhesion proteins (Hinz and Gabbiani 2003a) to complete the structural link between the ECM and the cytoskeleton and facilitate ECM-induced changes in cellular motility, contractility, and substrate adhesion (Kawaguchi et al. 2003; Yamashiro et al. 1998; Hinz and Gabbiani 2003a).

6.5 Cellular Tensegrity Within the Extracellular Matrix

Myofibroblasts can be distinguished from other fibroblasts by their expression of a distinct form of filamentous actin known as α smooth muscle actin (αSMA) (Darby et al. 1990). When coupled to myosin in "stress" fibers, αSMA allows myofibroblasts to impose much greater contractile forces on the ECM through focal adhesions than connective tissue fibroblasts are normally capable of achieving (Hinz 2006). Myofibroblasts can also form intercellular (cell to cell) connections with other myofibroblasts through adherens junctions, further enhancing their capacity to coordinate ECM contraction in areas of high cell density (Follonier et al. 2008; Hinz and Gabbiani 2003b).

One way of visualizing cellular interactions with their surrounding matrix is to perceive cells as prestressed lattice structures that are stabilized by the combination of tension and compression. This concept, known as the tensegrity principle (Ingber et al. 2014), envisages actin/myosin stress fibers, intermediate filaments, and microtubules (α and β tubulin polymers) as tensional and compression elements that have analogous roles to the cables and columns that maintain the shape of a flexible structure. While a detailed description of cellular tensegrity is beyond the scope of this section (for details, see (Ingber et al. 2014)), it is nonetheless helpful for envisaging how external forces that pull on structural molecules in the ECM can cause integrins in focal adhesions linked to αSMA in stress fibers to distort the shape of a cell and stimulate cellular responses. This concept

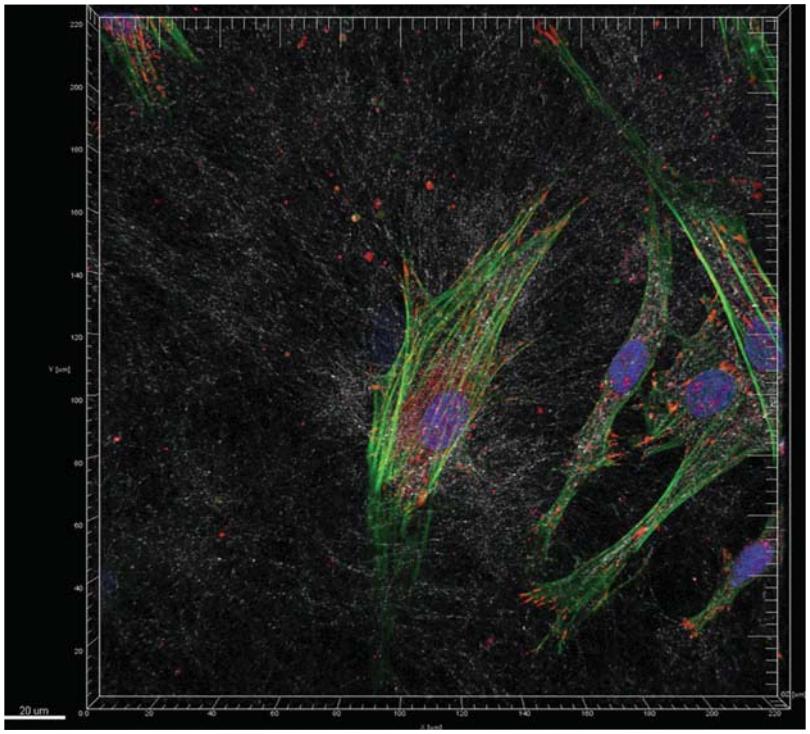

Fig. 6.1 Three-dimensional confocal microscopy of Dupuytren Disease myofibroblasts cultured in type 1 collagen lattices. Stress fibers are stained with green Alexa 488 phalloidin, focal adhesions (vinculin immunoreactivity) are shown in red, and cell nuclei (DAPI staining) are shown in *blue*. The three-dimensional collagen matrix was visualized by laser reflectance microscopy, a technique that utilizes the reflection of laser light by the surfaces of collagen fibers (*white*). Changes in fluorescence intensity within and around these cells indicate cellular processes that are in or out of frame in this two-dimensional rendering of a three-dimensional image. These images illustrate the connections between matrix-associated collagen fibers, focal adhesions, and contractile stress fibers in myofibroblasts. Cells can be envisaged as prestressed lattice structures that are stabilized by the combination of tension and compression in accordance with the tensegrity principle (Ingber et al. 2014). Changes in stress fiber length within cells are translated through focal adhesions to impose changes in collagen fiber density

also works in reverse, allowing cells to contract the surrounding ECM by shortening the actin/myosin stress fibers in their cytoskeleton that are linked to integrins in focal adhesions and to structural molecules, such as collagens, in the ECM.

6.6 Extracellular Matrix Interactions and *In Vitro* Analyses of Dupuytren Disease Cells

Any molecule that promotes the coordinated and excessive contracture of the palmar fascia by myofibroblasts has potential as a therapeutic target. While many of these molecules may reside within palmar fascia fibroblasts or myofibroblasts, others elicit their signals through matricellular and other molecules to induce their effects through focal adhesions to modify cellular proliferation, myofibroblast formation, ECM secretion, or other disease-associated changes. For this reason, *in vitro* studies are performed on palmar fascia fibroblasts or fibrogenic myofibroblasts that do not include a physiologically relevant ECM risk, omitting the contributions of these interactions and, at worst, providing misleading information about cellular responses to treatments.

In practice, culturing cells in a physiologically relevant ECM is technically challenging, especially when most of the constituents of that ECM are yet to be characterized, as is the case in Dupuytren

Disease. One approach to partially overcoming this hurdle is to collect the secretions of primary palmar fascia myofibroblasts and use them to "condition" collagen, hydrogel, or other relatively porous substrates in which cells can be cultured in three dimensions. While this approach can only provide an approximation of an ECM that is continually modified *in vivo,* nonetheless it has advantages over standard tissue culture plastic (TCP) cultures that include little or no ECM components. In addition to providing an increased capacity to bind and act as a reservoir for secreted proteins and other molecules, these substrates can also be designed to have a stress-to-strain ratio, or Young's modulus, that approximates the "stiffness" of normal or fibrotic palmar fascia. The Young's modulus of normal palmar fascia is lower than most tendons (Millesi et al. 1995) and approximates that of the dermis (10–1,000 Pa) (Hinz 2010; Yeung et al. 2005). Fibroblasts begin incorporating αSMA into their stress fibers, indicating their transition from fibroblasts to myofibroblasts, when ECM stiffness approaches 16,000–20,000 Pa (Hinz 2010). Tissues need to achieve a stiffness range of 25,000–50,000 Pa to maintain myofibroblasts in their hyper-contractile state (Hinz 2009). Interestingly, fibroblasts and myofibroblasts can respond to localized changes in the stiffness in their surroundings by migrating toward areas of increased stiffness through a process called durotaxis (Lo et al. 2000; Lange and Fabry 2013). Whether durotaxis contributes to the increased numbers of myofibroblasts in nodules or contracture cords is currently unknown.

TCP has a stiffness of at least 1,000,000,000 Pa (>1 gPa) (Achterberg et al. 2014), which is several orders of magnitude greater than the stiffness of any fibrotic tissue that fibroblasts or myofibroblasts could ever encounter *in vivo.* Under these conditions, fibroblasts spontaneously and robustly transition into αSMA-positive myofibroblasts without the need for any additional treatment interventions (Hinz et al. 2001). While there are applications where comparisons between uniform cultures of myofibroblasts are useful, it should nonetheless be appreciated that the behaviors or responses of cells under these conditions might have little or no similarity to their behaviors or responses on the substrates they normally interact with *in vivo*.

6.7 Interactions Between the Wnt/ß-Catenin Signaling Pathway and the Extracellular Matrix

This point can be illustrated by observing the interactions between Dupuytren Disease fibroblasts and myofibroblasts, cell culture substrates, and the Wnt/ß-catenin signaling pathway. Wnt signaling regulates ß-catenin levels during embryonic development and in a variety of diseases characterized by excessive cellular proliferation (Thompson and Monga 2007; Bowley et al. 2007; Manolagas and Almeida 2007). In the absence of Wnt signaling, ß-catenin is constitutively phosphorylated by casein kinase 1 and glycogen synthase kinase 3ß (GSK3ß), incorporated into a "destruction complex" that includes adenomatous polyposis coli (APC) and axin and degraded through the 26S proteasome (Bowley et al. 2007; Lam and Gottardi 2011). Wnt signaling induces the phosphorylation and inactivation of GSK3ß, thereby allowing ß-catenin to escape the destruction complex, accumulate in the cytoplasm, and translocate to the nucleus. Once in the nucleus, ß-catenin can bind transcription factors and act as a trans-activating factor to regulate gene expression (Bowley et al. 2007) (illustrated in Fig. 6.2).

The discovery of increased levels of ß-catenin in Dupuytren Disease tissues and in primary fibroblasts derived from these tissues (Varallo et al. 2003; Howard et al. 2003) led to the hypothesis that the Wnt/ß-catenin signaling pathway contributed to the pathogenesis of Dupuytren Disease. This hypothesis received indirect support when genome-wide association studies of patients with Dupuytren Disease identified single nucleotide polymorphisms (SNPs) in loci containing genes that encode Wnts or Wnt signaling-associated proteins (Dolmans et al. 2011). These findings led to a more detailed version of the original hypothesis that heritable abnormalities in Wnt gene expression result in dysregulated

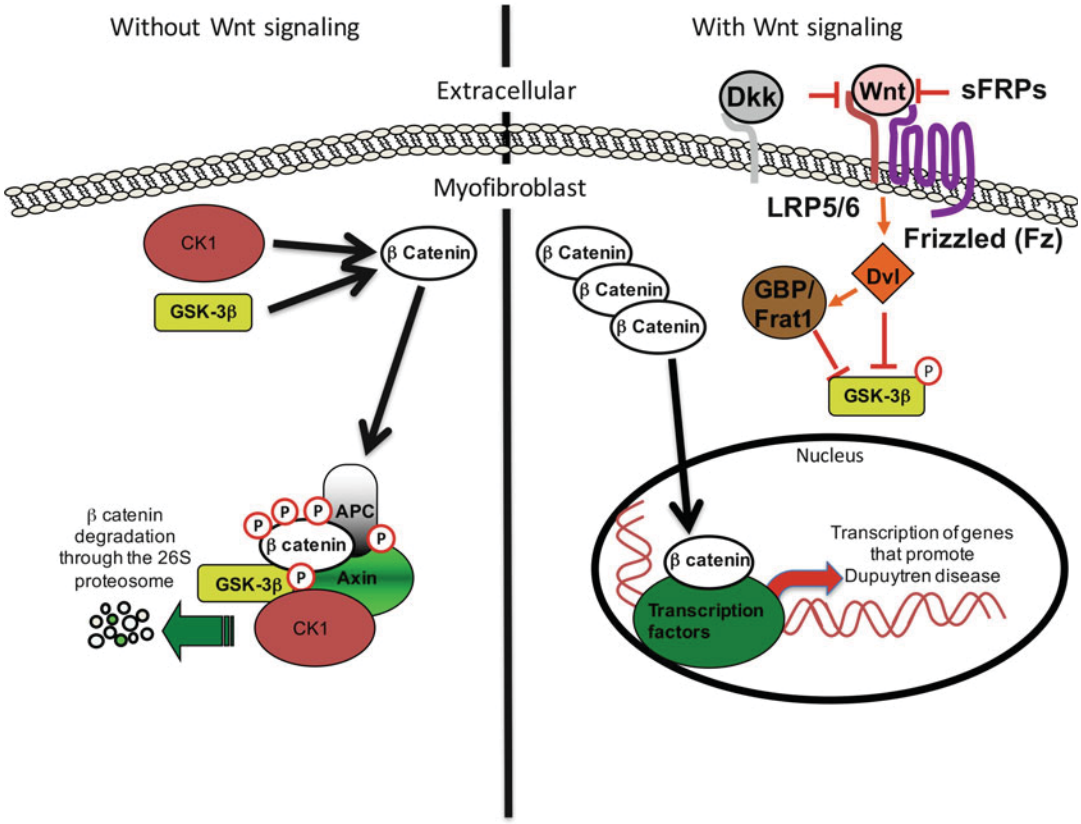

Fig. 6.2 Wnt signaling regulates β-catenin levels. In the absence of wnt signaling (*left*), casein kinase 1 (*CK1*) and glycogen synthase kinase-3β (*GSK-3β*) phosphorylate β-catenin on serine/threonine residues, causing it to be sequestered to a "destruction complex" that includes adenomatous polyposis coli (*APC*) and axin, and its degradation through the 26S proteasome. Wnt signaling from the extracellular environment through the "canonical" frizzled receptor/low density lipoprotein receptor-related protein 5/6 (*LRP5/6*) pathway (*right*) results in phosphorylation of disheveled (*Dvl*), which directly or indirectly (through GSK-3β binding protein, *GBP*) phosphorylates and inactivates GSK-3β. GSK-3β inactivation allows β-catenin to avoid the destruction complex, accumulate within the cytoplasm, translocate to the nucleus, and trans-activate the transcription of genes associated with Dupuytren Disease development

Wnt signaling, ß-catenin accumulation, and the development of Dupuytren Disease. While it is unclear if Wnt expression is dysregulated in Dupuytren Disease-derived fibroblasts or myofibroblasts (O'Gorman et al. 2006), these SNP-associated changes may impact transcriptional responsiveness to biochemical or biomechanical stimuli, gene transcript stability, or enhanced interactions with other pathways that may "cross-talk" with the Wnt/ß-catenin pathway, such as TNFα signaling (Verjee et al. 2013).

In addition to the potential effects of SNPs in or near Wnt or Wnt-related genes, the Dupuytren Disease ECM can independently regulate ß-catenin levels in myofibroblasts. While ß-catenin levels are clearly increased in contracture tissues relative to the levels in syngeneic (genetically identical) fibroblasts in adjacent, macroscopically unaffected palmar fascia (Howard et al. 2003; Varallo et al. 2003), these levels are rapidly normalized and become indistinguishable from those in cells derived from macroscopically unaffected palmar fascia when cultured from explant tissues onto TCP (Varallo et al. 2003). Transferring these cells from TCP into three-dimensional collagen-based cultures under isometric tension in fibroblast-populated collagen lattice assays restores the increased levels of ß-catenin; however, a rapid

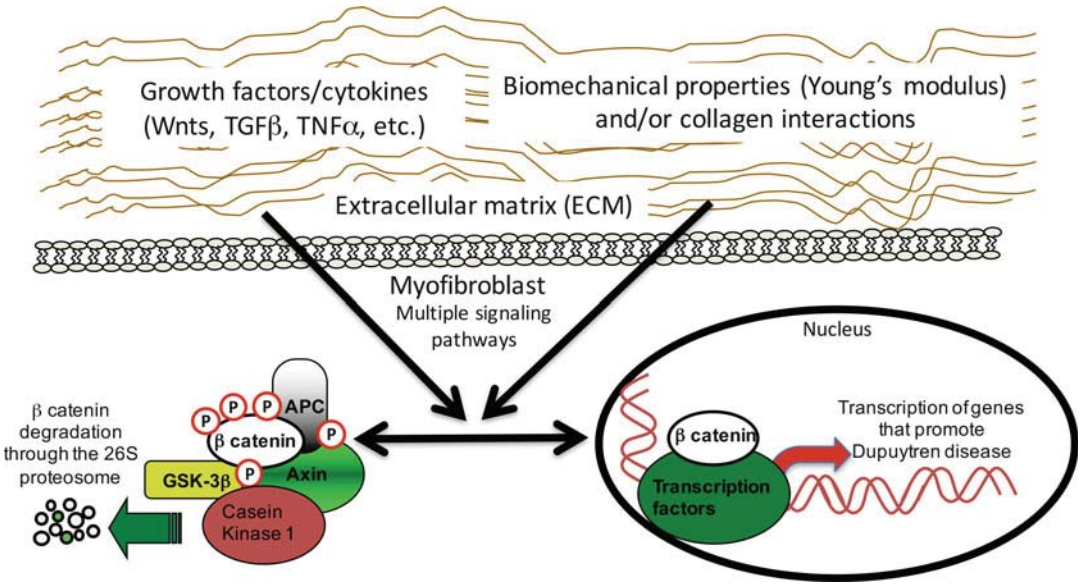

Fig. 6.3 The extracellular matrix regulates β-catenin levels. Many different cytokines, including transforming growth factor-β (*TGFβ*) and tumor necrosis factor α (*TNFα*), can signal through pathways that intersect with the Wnt/β-catenin signaling pathway and increase intracellular β-catenin levels. While the mechanisms are currently unclear, extracellular matrix (*ECM*) factors, such as collagen density, can increase or decrease intracellular β-catenin levels in Dupuytren Disease myofibroblasts, and thereby potentially modify β-catenin signaling in parallel with cytokine-activated pathways. As such, the potential confounding influences of the Dupuytren Disease ECM should be taken into account when assessing the effects of therapeutic interventions designed to modify cytokine or other signaling pathways that regulate β-catenin signaling in Dupuytren Disease

depletion of ß-catenin levels is evident once that tension is released (Varallo et al. 2003). In contrast, the dynamic regulation of ß-catenin levels in Dupuytren Disease myofibroblasts and ß-catenin levels in syngeneic myofibroblasts derived from macroscopically unaffected palmar fascia remain relatively stable under these conditions (Varallo et al. 2003). When cultured in low-density type-1 collagen substrates with little or no isometric tension, ß-catenin levels in myofibroblasts derived from contracture tissues are depleted over 72 h until they are virtually undetectable by immunoblotting (Vi, Njarlangattil, et al. 2009). While these culture conditions also induce some depletion of ß-catenin levels in syngeneic myofibroblasts derived from macroscopically unaffected palmar fascia, the effects are modest and variable between cultures (Vi et al. 2009b). TGFβ-1, the ECM-associated cytokine that is well known to promote the development of myofibroblasts (Badalamente et al. 1996), restores ß-catenin levels in Dupuytren Disease myofibroblasts cultured in low-density type-1 collagen substrates (Vi et al. 2009b). While it is currently unclear whether matrix stiffness, collagen signaling, or both are required to elicit these effects on ß-catenin levels in Dupuytren Disease myofibroblasts, it is clear that these cells are abnormally sensitive to these factors relative to syngeneic myofibroblasts derived from macroscopically unaffected palmar fascia.

These findings predict that the outcomes of Wnt/ß-catenin signaling analyses in Dupuytren Disease will be dependent on the culture substrates used during the analyses. Such effects are predicted to extend beyond the expression of genes that are trans-activated by ß-catenin and translated into fibrosis-associated proteins and may also include the cytokines and ECM-associated signaling molecules that act in parallel to enhance or attenuate Wnt/ß-catenin signaling. Thus, before we perform *in vitro* analyses of therapeutic interventions that modify Wnt/ß-catenin signaling in Dupuytren Disease, it is essential that we make informed choices regarding the

culture substrates in which such analyses take place (Fig. 6.3).

6.8 Targeting the Extracellular Matrix to Attenuate Dupuytren Disease Development

If we accept that the ECM surrounding Dupuytren Disease myofibroblasts modifies their responses and actively promotes disease progression, then the ECM itself can be considered as a therapeutic target. The concept of targeting the ECM in Dupuytren Disease is not new. J. T. Hueston, one of the "founding fathers" of Dupuytren Disease research, originally suggested that "enzymic fasciotomy" (Hueston 1971) could achieve similar outcomes to surgical fasciotomy in select patients. His approach was to use a cocktail of proteolytic enzymes and anti-inflammatory agents to degrade the ECM-associated collagens in contracture cords while simultaneously dampening the effects of pro-fibrotic inflammatory cytokines. While Hueston's approach did not gain broad acceptance at the time, targeting the ECM-associated collagens in contracture cords has now become a therapeutic reality. Xiaflex/Xiapex® (Hurst and Badalamente 1999; Badalamente et al. 2002; Hurst et al. 2009) is a mixture of *Clostridium histolyticum* type I and type II collagenases that specifically target the amino- and carboxy-termini and internal peptide residues of the type I and type III collagens in the Dupuytren Disease ECM. While this approach has many advantages over more invasive treatment options, it is worth noting that degradation of type I and type III collagens in the ECM, while effective for restoring hand function in the short term, is insufficient to prevent Dupuytren Disease recurrence (Watt et al. 2012; Baltzer and Binhammer 2013; Chen et al. 2011). The consequences of disrupting the biochemical and biomechanical signals that myofibroblasts receive from their collagen-enriched ECM under tension remain poorly understood at the molecular level and worthy of detailed investigation. Controlled proteolysis of collagens and other ECM proteins can generate bioactive molecules known as matricryptins (Ricard-Blum and Ballut 2011) that stimulate a wide variety of cellular responses including proliferation, migration, and angiogenesis (Ricard-Blum and Salza 2014). It is currently unclear whether matricryptins or other biologically active factors derived from ECM degradation contribute to Dupuytren Disease recurrence after Xiaflex/Xiapex® treatments.

While Xiaflex/Xiapex® has demonstrated the efficacy of targeting collagens in the Dupuytren Disease ECM, there is considerable potential to expand on this approach and target additional molecules in parallel to more effectively attenuate Dupuytren Disease recurrence. We could revisit the original approach by J. T. Hueston and combine Xiaflex/Xiapex® injections with TNFα inhibitors to dampen pro-fibrotic inflammatory responses. Alternatively, we could combine Xiaflex/Xiapex® injections with other novel interventions reported to prevent the reformation of a pro-fibrotic ECM by myofibroblasts, such as inhibitors of the nuclear factor κB (NFκB) pathway (Mia and Bank 2015) or lysyl oxidase (Barry-Hamilton et al. 2010). Hypothetically, we could also take advantage of the immune response to *Clostridium hystolyticum* type I and type II collagenases that more than 90% of patients develop after they receive Xiaflex/Xiapex® injections (Peimer et al. 2015). It may be possible to use Xiaflex/Xiapex® as a treatment and an adjuvant to promote a robust immune response to peptide antigens. These antigens could be modeled on cell surface or secreted molecules that are specifically expressed or upregulated in Dupuytren Disease myofibroblasts, such as Wilms' tumor 1 (Crawford et al. 2015). These hypothetical possibilities are only the "tip of the iceberg" of potential therapeutic interventions to target molecules that are in, or act in combination with, the Dupuytren Disease ECM. To expand our repertoire of interventions and achieve our goal of preventing Dupuytren Disease progression and recurrence, we need to gain a much more detailed understanding of the complexity of the Dupuytren Disease ECM and to explore its potential as a therapeutic target.

Conflict of Interest The author has no conflicts of interest to declare.

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