Published in final edited form as:

J Hand Surg Am. 2010 October ; 35(10): 1580–1588. doi:10.1016/j.jhsa.2010.07.009.

Dupuytren's Fibroblast Contractility by Sphingosine-1-Phosphate is Mediated through Non Muscle Myosin II

Issei Komatsu, MD¹, Jennifer Bond, PhD¹, Angelica Selim, MD³, James J Tomasek, PhD⁴, L Scott Levin, MD², and Howard Levinson, MD^{1,3}

¹Division of Plastic and Reconstructive Surgery, Department of Surgery, Duke University Medical Center, Durham, North Carolina

²Department of Orthopaedics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

³Department of Pathology, Duke University Medical Center, Durham, North Carolina

⁴Department of Cell Biology, University of Oklahoma-Health Sciences Center, Oklahoma City, Oklahoma

Abstract

Purpose—Previous studies suggest that Dupuytren's disease is caused by fibroblast and myofibroblast contractility within Dupuytren's nodules; however, the stimulus for cell contractility is unknown. Sphingosine-1-phosphate (S1P) is a serum-derived lysophospholipid mediator that enhances cell contractility by activation of the S1P receptor, S1P₂. It is hypothesized that S1P stimulates Dupuytren's fibroblast contractility through S1P₂ activation of non muscle myosin II (NMMII). This investigation examines the role of S1P and NMMII activation in Dupuytren's disease progression and suggests potential targets for treatment.

Methods—Dupuytren's fibroblasts were enmeshed into fibroblast populated collagen lattices (FPCL) and S1P stimulated FPCL contraction assayed in the presence of the S1P₂ receptor inhibitor, JTE-013, the Rho kinase (ROCK) inhibitor, Y-27632, the myosin light chain kinase (MLCK) inhibitor, ML-7, and the NMMII inhibitor, blebbistatin. Tissues from Dupuytren's fascia (n=10) and normal palmar fascia (n=10) were immunostained for NMMIIA and NMMIIB.

Results—S1P stimulated FPCL contraction in a dose-dependent manner. Inhibition of S1P₂, and NMMII prevented S1P stimulated FPCL contraction. ROCK and MLCK inhibited both S1P and control FPCL contraction. Dupuytren's nodule fibroblasts robustly expressed NMMIIA and NMMIIB, in comparison to quiescent appearing cords and normal palmar fascia.

Conclusions—S1P promotes Dupuytren's fibroblast contractility through S1P₂, which stimulates activation of NMMII. NMMII isoforms are ubiquitously expressed throughout Dupuytren's nodules suggesting that nodule fibroblasts are primed to respond to S1P stimulation to cause contracture formation. S1P promoted activation of NMMII may be a target for the disease treatment.

© 2010 The American Society for Surgery of the Hand. Published by Elsevier Inc. All rights reserved.

Corresponding author and reprint requests: Howard Levinson, MD, Director of Wound Healing/Fibrosis Laboratory, Assistant Professor of Plastic and Reconstructive Surgery, Assistant Professor of Pathology, Departments of Pathology and Surgery, DUMC, 3181 Durham, NC 27710, Ph 919 684-8661, Howard.levinson@duke.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Dupuytren's disease is an idiopathic fibrocontractile disorder affecting the palmar aponeurosis. Treatment is primarily surgical. Other therapies continue to be investigated.¹ Needle fasciectomy and collagenase injection have shown promising results in the treatment of Dupuytren's disease. However, these are not yet mainstay treatments.^{2–3}

In 1952, Luck first classified Dupuytren's disease as occurring in three stages (proliferative, involutional, and residual).⁴ He suggested that nodules were the source of contractures. His concept was that nodules appear, cause a local contracture and then disappear. This repetitive cycle of flare, contracture, resolution, was thought to occur throughout the palmar aponeurosis leading to a chain of contractures within the natatory cords, pre-tendinous bands, Grayson's ligaments, and central and lateral cords.^{1,4–6} The three stage pathogenesis model proposed by Luck is akin to the three stages of dermal wound healing: inflammation, proliferation and remodeling. Dupuytren's disease and scar contracture formation appear to have similar mechanisms of pathogenesis.^{6–7}

Scar contracture is proposed to result from incremental, progressive tissue remodeling caused by activated contractile fibroblasts and myofibroblasts.⁸ Fibroblast and myofibroblast contractility during wound healing has recently been found to be stimulated by the bioactive lysophospholipid, sphingosine-1-phosphate (S1P).⁹

There are five S1P receptors (S1P_{1–5}). Among the five receptors, studies have indicated that the S1P₂ receptor is a potent agonist of cell contractility and tissue remodeling.^{10–11} S1P₂ mediates cellular contractility by stimulating secondary downstream messengers to promote myosin regulatory light chain (MLC) activation.¹² Activated MLC binds to the neck domain of non muscle myosin II (NMMII) and promotes the kinetic actomyosin interaction, which causes cell contraction and extracellular matrix compaction.^{13–16} Genomic analysis has revealed the existence of at least three different NMMII isoforms in humans termed NMIIA, NMIIIB, and NMIIIC.¹⁷ NMIIA and NMIIIB exist in most tissues and have been investigated at the protein level.^{15–16}

In this study, we examined the mechanism by which S1P signaling may mediate Dupuytren's fibroblast contractility. S1P promoted Dupuytren's contraction was assayed in the fibroblast populated collagen lattice (FPCL) assay in the presence of the S1P₂ receptor inhibitor, JTE-013, the Rho kinase (ROCK) inhibitor, Y-27632, the myosin light chain kinase (MLCK), inhibitor ML-7, and the NMMII inhibitor, blebbistatin. Lastly, the expression profile of NMIIA and NMIIIB was analyzed in Dupuytren's tissue and compared to normal palmar fascia.

Materials and Methods

Reagents and Antibodies

Primary antibodies: Rabbit anti-NMIIA polyclonal IgG, and rabbit anti-NMIIIB polyclonal IgG were purchased from Abcam (Cambridge, MA). Dulbecco's modified eagle's medium (DMEM) was obtained from Sigma (St. Louis, MO). Heat-inactivated fetal bovine serum (FBS) and penicillin/streptomycin were obtained from Invitrogen (Carlsbad, CA).

Inhibitors and Agonists

S1P (Biomol, Plymouth Meeting, PA) was used at 0.01μM to 1μM, which is within the range of its normal concentration in human blood (0.2μM to 5μM).¹⁸ The specific S1P₂ receptor inhibitor, JTE-013 (Cayman biochem, Ann Arbor, MI) was employed at .001μM to 1μM.¹⁹ Rho kinase inhibitor, Y-27632 (Calbiochem, La Jolla, CA), was employed at .01μM to 10μM and the MLCK inhibitor, ML-7 (Biomol, Plymouth Meeting, PA), at 0.22μM to 2.2μM.²⁰ The

NMMII inhibitor, blebbistatin (Calbiochem, La Jolla, CA) was employed at 6.25 μ M to 50 μ M.
21

Human Tissue

Formalin fixed and paraffin embedded Dupuytren's tissues were obtained from Duke University Medical Center, Department of Pathology repository of tissue specimens in accordance with the Duke University Medical Center Institutional Review Board. A total of twelve specimens (mean age: 67.4 years old, nine males and three females) were assessed. Normal palmar fascia from nine cadavers (six males and three females) were obtained from the Duke University Medical Center Human Fresh Tissue Laboratory.

Cell Culture

Dupuytren's fibroblasts were explanted from three patients undergoing surgical fasciectomy (mean age: 63.3 years old, three males). Dupuytren's nodules were dissected from the surrounding cords and palmar fascia. Three cell lines from different patients were used for this study. In brief, tissues were washed, finely minced, and incubated in collagenase type I in DMEM with 1% penicillin/streptomycin, at 37°C for 24h. The cells were subsequently cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Experiments with primary cell cultures were performed when cells were 80–90% confluent between passages 1 and 6.

Fibroblast Populated Collagen Lattices (FPCL)

We employed the FPCL to study the S1P signaling pathway in Dupuytren's disease. Free floating FPCLs (FF-FPCL) and stressed FPCL were synthesized as previously described.^{22–23} The *in vitro* cell based FPCL is a frequently used assay to study three-dimensional tissue remodeling where cell behavior in FPCL contraction closely models what is observed *in vivo*.²⁴ Stressed FPCL contraction is caused by rapid cell contraction, whereas FF-FPCL contraction via fibroblast migration.⁸ For stressed FPCL, the bottoms of each well of a 6-well plate were etched with a 12-mm diameter circle using a compass with 16-gauge needle. Volumes of 200 μ L FPCL (final concentrations of 1.0×10^6 cells/mL, 1.5 mg/mL purified collagen) were added onto the etched areas. The plates were placed in a 37°C incubator with 5% CO₂ to allow the FPCLs to polymerize. After polymerization, 2mL of growth media (10% FBS in DMEM with 50 μ g/mL ascorbic acid) was added to each well. After 24h of incubation, the growth medium was removed, the FPCLs were rinsed with PBS twice and either 0.5% bovine serum albumin (BSA) in DMEM (basal media) or S1P (0.01 μ M, 0.1 μ M, and 1 μ M) in basal media added to the wells. Inhibitors JTE-013, Y-27632, ML-7, and blebbistatin were added to lattices 20 min prior to release. The lattices were released and sizes of collagen lattices were recorded with a digital scanner 1h after release.

For FF-FPCL preparation, the wells were pre-coated with 7.5% BSA for 1h and then washed with PBS to prevent the FPCL from adhering. After polymerization of collagen, 2ml of basal media and basal media with S1P (0.01 μ M, 0.1 μ M, and 1 μ M) were added to the wells, and collagen lattices were released. Collagen lattice sizes were recorded using a digital scanner 4h after release.

Lattice areas were determined using NIH ImageJ software.²⁵ All conditions were performed in triplicate per experiment and these experiments were repeated three times using three different cell lineages. Relative lattice area was obtained by dividing collagen lattice area at each time point by initial lattice area. Percent contraction was determined by subtracting the relative lattice area from one. (Relative lattice area = $1 - \text{lattice area at each time point} / \text{initial lattice area}$)

Immunohistochemistry (IHC)

Consecutive sections of 5µm for each tissue specimen were mounted on silanized charged slides and allowed to dry for 30 min in an incubator at 60°C. After deparaffinization in xylene and rehydration, slides were covered for 10 min with 3% hydrogen peroxide to block endogenous peroxidase. Slides were then placed in citrate antigen-retrieval buffer for 20 min at 80°C, followed by washing in Tris-buffered saline Polysorbate-20 (TBST), 0.5M Tris Base, 9% NaCl, 0.5% Polysorbate-20, pH 8.4) at room temperature. Slides were then placed in a humidified chamber and incubated for 45 min with primary antibodies (anti- NMMIIA at 1:100 dilution, and anti- NMMIIB at 1:100 dilution). After three rinses in TBST, the slides were incubated for 45 min with biotinylated secondary detection antibodies (goat anti-rabbit at 1:200 dilution, Vector, Burlingame, CA). After three rinses in TBST, the slides were incubated with the detection system (Vectastain Elite ABC, Vector) for 30 min. Tissue staining was visualized with a 3,3'-Diaminobenzidine substrate chromogen solution (Innovex, Richmond, CA). Slides were counterstained with hematoxylin, dehydrated, and mounted. Non immune controls were performed by using a rabbit polyclonal IgG (for NMMIIA and NMMIIB) at matched concentration. Sections were analyzed with the assistance by a Dermatopathologist.

Statistical Analysis

All values are presented as mean $\pm$ standard error of mean. Statistical analysis was performed using JMP software (SAS Institute ver. 7, Cary, NC). Differences between groups were compared via Student's t-test and were considered statistically significant at values of $p \leq 0.05$.

Results

Initial experiments were performed comparing Dupuytren's fibroblasts to normal palmar fascia fibroblasts. The cell lines were found to behave the same *in vitro*, in accordance with previous publications.²⁶ This observation indicates that the *in vivo* environment is important for disease progression but it does not preclude the study of Dupuytren fibroblasts alone *in vitro* to determine mechanisms of disease. All further experiments were done with Dupuytren's fibroblasts alone.

Model of S1P Stimulated Fibroblast Contractility in Dupuytren's Disease

Figure 1 depicts the model of S1P stimulated fibroblast contraction in Dupuytren's disease under this investigation.

S1P Promoted Dupuytren's Fibroblast Contractility through S1P₂

S1P promoted stressed-relaxed FPCL contraction in a dose-dependent fashion. At 0.1µM there was a 51±1% contraction and at 1µM there was a 62±1% contraction, as compared to basal media controls (44± 2%), ($p \leq 0.05$, Figure 2). S1P did not stimulate FF-FPCL contraction (Figure 2). Remaining experiments were only done with stressed FPCL.

JTE-013 inhibited S1P (1µM) promoted FPCL contraction in a dose-dependent manner (Figure 3). Inhibition was significant ($p \leq 0.05$) with 0.1µM (44±1%) and 1µM (35±3%) of JTE-013 in comparison to FPCL in S1P alone (65±2%). At 1µM of JTE-013, S1P stimulated FPCL contraction was near totally inhibited to baseline basal media levels.

S1P Promoted FPCL Contraction-ROCK and MLCK

To determine whether S1P stimulated FPCL contraction is calcium-dependent or calcium-independent, S1P stimulated FPCL were treated with the Rho associated kinase (ROCK) inhibitor, Y-27632, or the myosin light chain kinase (MLCK) inhibitor, ML-7. Y-27632 inhibited S1P promoted FPCL contraction in a concentration dependent manner (Figure 4A).

The greatest inhibition of FPCL contraction was observed at 10 μ M of Y27632 (29 $\pm$ 1%) compared to S1P promoted FPCL controls (62 $\pm$ 1%), which was significant ($p \leq 0.05$). At concentrations of 0.22 μ M, 2.2 μ M, and 22 μ M, ML-7 significantly ($p \leq 0.05$) inhibited S1P stimulated FPCL (53 $\pm$ 4%, 48 $\pm$ 2%, and 42 $\pm$ 2%, respectively) compared to S1P promoted FPCL controls (62 $\pm$ 1%) (Figure 4B). Both Y27632 and ML-7 inhibited control lattices to the same extent as S1P stimulated lattices. In comparison with the control data, we cannot determine the roles of calcium-dependent and calcium-independent signaling in S1P stimulated FPCL contraction.

S1P Promoted FPCL Contraction was Blocked by Blebbistatin

To assess the contribution of NMMII in S1P stimulated FPCL contraction, we added the specific NMMII inhibitor, blebbistatin. Blebbistatin at 6.5 μ M significantly ($p \leq 0.05$) inhibited S1P stimulated FPCL contraction but not control FPCL contraction, demonstrating that S1P promotes increased contraction through activation of NMMII (Figure 5). At higher concentrations of blebbistatin inhibition of both S1P and control FPCL contraction was dose dependent (Figure 5).

IHC of Dupuytren's Disease and Normal Palmar Fascia

Routine histologic sections of Dupuytren's nodules, cords and normal palmar fascia were stained for NMMIIA and NMMIIB expression. (Figure 6) Low power magnification reveals robust expression of NMMIIA and NMMIIB in Dupuytren's nodules but little to absent expression of NMMIIA and NMMIIB in cords and normal palmar fascia. High power magnification of the same specimens demonstrates nodules densely populated with a small amount of extracellular matrix separating the cells (Figure 6 **insets**). NMMIIA and NMMIIB are more robustly expressed and widely distributed throughout the cytoplasm of Dupuytren's nodule fibroblasts, as compared to scant peri-nuclear localization in Dupuytren's cords fibroblasts and normal palmar fascia fibroblasts.

Discussion

Dupuytren's disease occurs in 0.2% to 56% of the population, depending on patient demographics.²⁷ There are several co-morbid conditions which suggest an increased likelihood of disease progression, including the following: increasing age, male gender, Northern European descent, Caucasian, having concomitant stigmata of Dupuytren's diathesis (Peyronie's disease, plantar fibromatosis, and dorsal knuckle pads), diabetes and seizures.^{1, 28-34} The pathogenesis of Dupuytren's disease is unknown; however, it does share similarities to wound contraction and scar contracture.⁶⁻⁷ Dupuytren's disease and scar contracture both occur incrementally as a result of fibroblast and myofibroblast contractility.^{6,8} Fibroblast and myofibroblast contractility are activated by two potent lysophospholipid contractile agonists, lysophosphatidic acid (LPA), and S1P.⁹ LPA and S1P are potent mediators of tissue repair and wound healing that have a wide range of biological activities.⁹ They regulate proliferation, migration, and contraction.²⁴⁻³⁵ LPA and S1P are derived from cell membrane sphingomyelin and phosphatidylcholine and they can be internalized to act in an autocrine fashion or they can be secreted into the serum to act in a paracrine fashion.³⁶⁻³⁷ There are five S1P receptors (EDG1/S1P1; EDG5/S1P2; EDG3/S1P3; EDG6/S1P4; and EDG8/S1P5), each S1P receptor stimulates a variety of cellular and biological processes including cell migration, angiogenesis, vascular maturation and neurogenesis.^{9,38} S1P₂ knockout mice have reduced matrix remodeling in response to acute live injury.¹⁰ S1P₂ couples to phospholipase C-beta and Rho kinase activity in smooth muscle cells to mediate initial and sustained contraction.³⁹ S1P₂ also activates smooth muscle myosin and smooth muscle alpha-actin expression in smooth muscle cells by calcium channel mediated activation of ROCK and subsequent serum response factor enrichment of CArG box promoter regions.¹¹ In this in vitro investigation, we demonstrate

that Dupuytren's fibroblast contractility is stimulated by S1P through S1P₂ and activation of NMMII.

S1P increased Dupuytren's fibroblast contractility in the high tensile environment of a stressed FPCL. The ability of S1P to stimulate contractility in a mechanically stiff matrix indicates that mechanical strain enables Dupuytren's fibroblasts to respond to S1P. The response to S1P in the stressed FPCL may also indicate that S1P promotes fibroblast contraction but not fibroblast migration. The reason for this is that stressed FPCL contraction is caused by rapid cell contraction, whereas FF-FPCL contraction via fibroblast migration.⁸

Our data shows that S1P does not promote Dupuytren's fibroblast contractility in the FF-FPCL, which exhibits low mechanical strain (Figure 2). This finding is different to the observation by Grinnell and Syuto using dermal fibroblasts in collagen lattices where S1P stimulated FF-FPCL contraction.²³ These differences in S1P stimulated contractility between dermal fibroblasts and Dupuytren's fibroblasts suggest that the cell lines behave differently when grown in 3-D matrices of low-tension. Since FF-FPCL contraction is primarily mediated by cell migration, it appears that S1P may promote cell migration in dermal fibroblasts under low-tension, but inhibits Dupuytren's fibroblast migration under the same low-tension conditions. This finding leads us to believe that the role of S1P signaling is situational and cell-dependent. This finding is reflected in the works of others who have shown that S1P has pro-migratory and anti-migratory effects.^{40–41} Migration experiments with Dupuytren's fibroblasts under different mechanical stress environments need to be performed to further investigate this observation.

Our studies with stressed FPCL shows that S1P mediated contractility is mitigated by the S1P₂ receptor antagonist, JTE-013 (Figure 3). JTE-013 near completely blocks S1P stimulated FPCL contraction. This suggests that S1P activation of S1P₂ plays a pivotal role in Dupuytren's fibroblast contractility and indicates that S1P₂ inhibition may effectively treat Dupuytren's disease.

Similar to the finding that mechanical stress primes Dupuytren's fibroblasts to respond to S1P, NMMII expression also changes in high-tension versus low-tension conditions. NMMIIA and IIB is upregulated in Dupuytren's nodule fibroblasts but not cord or normal fascia fibroblasts (Figure 6). The reason that NMMIIA and IIB expression may change according to tension is that in high-tensile environments there is a need to generate more contractile force to remodel the tissue. Previous investigations have found that culturing cells on polyacrylamide gels of increasing stiffness induces upregulation of NMMIIA and IIB expression.⁴² This supports the hypothesis that remodeling occurs in Dupuytren's nodules and not in the cords. Although the study did not investigate the distinct roles of NMMIIA and IIB in disease progression, it is clear that NMMIIA and IIB isoforms are working in concert to promote tissue remodeling within the high tensile environment of the nodules. Our data demonstrate that inhibition of NMMII with blebbistatin significantly inhibits S1P stimulated FPCL contraction as compared to controls (Figure 5). NMMII has been suggested as the final common effector of multiple contractile signaling cascades^{15–16} and here we have shown, NMMII may play a central role in S1P signaling pathway in Dupuytren's disease. This is an important observation because it suggests that S1P promoted contractures can be inhibited either through blockade of S1P signaling or through inhibition of NMMII activation.

In summary, we conclude that S1P signaling may contribute to the pathogenesis of Dupuytren's disease. S1P activates the S1P₂ receptor to cause fibroblast contractility. Dupuytren's nodule fibroblasts are primed to respond to these contractile agonists by expressing increased levels of NMMII. Blocking of the S1P receptor or NMMII requires further investigation as potential targets to treat Dupuytren's disease.

Acknowledgments

The authors wish to thank James Aitken, BS for his technical efforts. We are also grateful to Salvatore V Pizzo, MD, PhD for his support.

Grant numbers and sources of support:

The project described was supported by grants from the American Society for Surgery of the Hand, a NIH Mentored Clinical Scientist Award (K08); Grant # GM085562-01, supplemental support from the Divisions of Plastic and Reconstructive Surgery and Departments of Pathology and Surgery at Duke University (Durham, NC) and St. Luke's Life Science Institute (Tokyo, Japan).

References

1. Rayan GM. Dupuytren disease: Anatomy, pathology, presentation, and treatment. *J Bone Joint Surg Am* 2007;89:189–198. [PubMed: 17256226]
2. van Rijssen AL, Werker PM. Percutaneous needle fasciotomy in Dupuytren's disease. *J Hand Surg Br* 2006;31:498–501. [PubMed: 16766101]
3. Hurst LC, et al. Injectable collagenase clostridium histolyticum for Dupuytren's contracture. *N Engl J Med* 2009;361:968–979. [PubMed: 19726771]
4. Luck JV. Dupuytren's contracture; a new concept of the pathogenesis correlated with surgical management. *J Bone Joint Surg Am* 1959;41-A:635–664. [PubMed: 13664703]
5. Al-Qattan MM. Factors in the pathogenesis of Dupuytren's contracture. *J Hand Surg [Am]* 2006;31:1527–1534.
6. Tomasek JJ, Vaughan MB, Haaksma CJ. Cellular structure and biology of Dupuytren's disease. *Hand Clin* 1999;15:21–34. [PubMed: 10050239]
7. Gabbiani G, Majno G. Dupuytren's contracture: fibroblast contraction? An ultrastructural study. *Am J Pathol* 1972;66:131–146. [PubMed: 5009249]
8. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol* 2002;3:349–363. [PubMed: 11988769]
9. Watterson KR, Lanning DA, Diegelmann RF, Spiegel S. Regulation of fibroblast functions by lysophospholipid mediators: potential roles in wound healing. *Wound Repair Regen* 2007;15:607–616. [PubMed: 17971005]
10. Serriere-Lanneau V, et al. The sphingosine 1-phosphate receptor S1P2 triggers hepatic wound healing. *FASEB J* 2007;21:2005–2013. [PubMed: 17341687]
11. Wamhoff BR, Lynch KR, Macdonald TL, Owens GK. Sphingosine-1-phosphate receptor subtypes differentially regulate smooth muscle cell phenotype. *Arterioscler Thromb Vasc Biol* 2008;28:1454–1461. [PubMed: 18535287]
12. Pyne NJ, Pyne S. Sphingosine 1-phosphate, lysophosphatidic acid and growth factor signaling and termination. *Biochim Biophys Acta* 2008;1781:467–476. [PubMed: 18558100]
13. Somlyo AP, Somlyo AV. Signal transduction and regulation in smooth muscle. *Nature* 1994;372:231–236. [PubMed: 7969467]
14. Meshel AS, Wei Q, Adelstein RS, Sheetz MP. Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. *Nat Cell Biol* 2005;7:157–164. [PubMed: 15654332]
15. Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz AR. Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nat Rev Mol Cell Biol* 2009;10:778–790. [PubMed: 19851336]
16. Conti MA, Adelstein RS. Nonmuscle myosin II moves in new directions. *J Cell Sci* 2008;121:11–18. [PubMed: 18096687]
17. Berg JS, Powell BC, Cheney RE. A millennial myosin census. *Mol Biol Cell* 2001;12:780–794. [PubMed: 11294886]
18. Watterson KR, Ratz PH, Spiegel S. The role of sphingosine-1-phosphate in smooth muscle contraction. *Cell Signal* 2005;17:289–298. [PubMed: 15567060]
19. Osada M, Yatomi Y, Ohmori T, Ikeda H, Ozaki Y. Enhancement of sphingosine 1-phosphate-induced migration of vascular endothelial cells and smooth muscle cells by an EDG-5 antagonist. *Biochem Biophys Res Commun* 2002;299:483–487. [PubMed: 12445827]

20. Parizi M, Howard EW, Tomasek JJ. Regulation of LPA-promoted myofibroblast contraction: role of Rho, myosin light chain kinase, and myosin light chain phosphatase. *Exp Cell Res* 2000;254:210–220. [PubMed: 10640419]
21. Abe M, Ho CH, Kamm KE, Grinnell F. Different molecular motors mediate platelet-derived growth factor and lysophosphatidic acid-stimulated floating collagen matrix contraction. *J Biol Chem* 2003;278:47707–47712. [PubMed: 14504290]
22. Rayan GM, Tomasek JJ. Generation of contractile force by cultured Dupuytren's disease and normal palmar fibroblasts. *Tissue Cell* 1994;26:747–756. [PubMed: 9437248]
23. Jiang H, Rhee S, Ho CH, Grinnell F. Distinguishing fibroblast promigratory and procontractile growth factor environments in 3-D collagen matrices. *FASEB J* 2008;22:2151–2160. [PubMed: 18272655]
24. Grinnell F. Fibroblast biology in three-dimensional collagen matrices. *Trends Cell Biol* 2003;13:264–269. [PubMed: 12742170]
25. Abramoff M, Magelhaes PJ, Ram SJ. Image Processing with ImageJ. *Biophotonics International* 2004;11:36–42.
26. Tomasek J, Rayan GM. Correlation of alpha-smooth muscle actin expression and contraction in Dupuytren's disease fibroblasts. *J Hand Surg Am* 1995;20:450–455. [PubMed: 7642925]
27. Hindocha S, McGrouther DA, Bayat A. Epidemiological Evaluation of Dupuytren's Disease Incidence and Prevalence Rates in Relation to Etiology. *Hand (N Y)* 2009;4:256–269. [PubMed: 19145463]
28. Ross DC. Epidemiology of Dupuytren's disease. *Hand Clin* 1999;15:53–62. vi. [PubMed: 10050242]
29. Hindocha S, Stanley JK, Watson S, Bayat A. Dupuytren's diathesis revisited: Evaluation of prognostic indicators for risk of disease recurrence. *J Hand Surg Am* 2006;31:1626–1634. [PubMed: 17145383]
30. Grasso M, Lania C, Blanco S, Limonta G. The natural history of Peyronie's disease. *Arch Esp Urol* 2007;60:326–331. [PubMed: 17601314]
31. Haedicke GJ, Sturim HS. Plantar fibromatosis: an isolated disease. *Plast Reconstr Surg* 1989;83:296–300. [PubMed: 2911628]
32. Caroli A, et al. Epidemiological and structural findings supporting the fibromatous origin of dorsal knuckle pads. *J Hand Surg Br* 1991;16:258–262. [PubMed: 1960489]
33. Arkkila PE, Kantola IM, Viikari JS. Dupuytren's disease: association with chronic diabetic complications. *J Rheumatol* 1997;24:153–159. [PubMed: 9002027]
34. Arafa M, Noble J, Royle SG, Trail IA, Allen J. Dupuytren's and epilepsy revisited. *J Hand Surg Br* 1992;17:221–224. [PubMed: 1588209]
35. Panetti TS. Differential effects of sphingosine 1-phosphate and lysophosphatidic acid on endothelial cells. *Biochim Biophys Acta* 2002;1582:190–196. [PubMed: 12069828]
36. Spiegel S, Milstien S. Exogenous and intracellularly generated sphingosine 1-phosphate can regulate cellular processes by divergent pathways. *Biochem Soc Trans* 2003;31:1216–1219. [PubMed: 14641029]
37. Spiegel S, Milstien S. Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat Rev Mol Cell Biol* 2003;4:397–407. [PubMed: 12728273]
38. Takabe K, Paugh SW, Milstien S, Spiegel S. "Inside-out" signaling of sphingosine-1-phosphate: therapeutic targets. *Pharmacol Rev* 2008;60:181–195. [PubMed: 18552276]
39. Hu W, Mahavadi S, Huang J, Li F, Murthy KS. Characterization of S1P1 and S1P2 receptor function in smooth muscle by receptor silencing and receptor protection. *Am J Physiol Gastrointest Liver Physiol* 2006;291:G605–G610. [PubMed: 16675745]
40. Morii T, Weissbach L. Sphingosine 1-phosphate and cell migration: resistance to angiogenesis inhibitors. *Biochem Biophys Res Commun* 2003;310:884–888. [PubMed: 14550287]
41. Kupperman E, An S, Osborne N, Waldron S, Stainier DY. A sphingosine-1-phosphate receptor regulates cell migration during vertebrate heart development. *Nature* 2000;406:192–195. [PubMed: 10910360]
42. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 2006;126:677–689. [PubMed: 16923388]

Figure 1. Model of S1P Stimulated Fibroblast Contractility in Dupuytren's Disease

S1P is proposed to promote cell contractility through S1P₂ activation of calcium-independent and calcium-dependent signaling pathways. In the calcium-dependent signaling pathway, MLCK is activated to phosphorylate MRLC. In the calcium-independent signaling pathway, ROCK is activated to phosphorylate MRLC and inactivate myosin light chain phosphatase. Activated MRLC bind to the neck domain of NMMII and activate actomyosin contractility. Actomyosin contractility is believed to cause Dupuytren's disease. S1P stimulates fibroblast contractility through S1P₂ receptor mediated calcium-dependent and -independent pathways.

Figure 2. S1P Stimulates FPCL Contraction

Dose dependent effects of S1P on FF-FPCL and stressed FPCL contraction were analyzed. At the concentration of 0.1μM and 1μM, S1P significantly increased in stressed FPCL contraction (*p≤0.05). S1P did not stimulate FF-FPCL contraction through all concentration of S1P tested. All values are mean ± SEM of three set of three lattices from three different cell lineages.

Figure 3. S1P Stimulates FPCL Contraction through S1P₂

JTE-013 inhibits S1P-promoted fibroblast contraction in stressed FPCL. In the presence of 1 μ M of S1P, 1 μ M and 0.1 μ M of JTE-013 significantly inhibited collagen lattice contraction in comparison to S1P control (* $p \leq 0.05$). All values are mean $\pm$ SEM of three set of three lattices from 3 different cell lines.

Figure 4. Figure 4A and 4B. Y-27632 Inhibition of ROCK and ML-7 Inhibition of MLCK Prevents S1P-Promoted Collagen Lattice Contraction in Stressed FPCL to Similar Levels as Control Lattices (A) Y-27632 significantly inhibited S1P stimulated collagen lattice contraction to a similar extent as control lattices (* $p \leq 0.05$). (B) ML-7 significantly inhibited S1P stimulated collagen lattice to a similar extent as control lattices (* $p \leq 0.05$). All data are mean $\pm$ SEM of three set of three lattices from three different cell lineages.

Figure 5. Inhibition of NMMII with Blebbistatin Prevents S1P Stimulated Collagen Lattice Contraction in Stressed FPCL

Blebbistatin significantly inhibited S1P stimulated collagen lattice contraction in all concentrations evaluated in a dose dependent manner (* $p \leq 0.05$). Blebbistatin inhibited S1P stimulated contraction to a greater extent than contraction under control conditions at 6.25 – 25 μM. At 50 μM, blebbistatin near totally inhibited S1P stimulated FPCL contraction. All data are mean $\pm$ SEM of three set of three lattices from three different cell lineages.

Figure 6. IHC Staining of NMMII in Dupuytren's disease and Normal Palmar Fascia
 Histologic sections of Dupuytren's nodules and cords on a representative patient in comparison to normal parmar fascia on a human cadaver Immunohistochemical stains for NMMIIA and NMMIIB are robustly expressed throughout nodules (A and B) but are minimally expressed in cords (C and D) and normal palmar fascia (E and F). High power magnification (100×) shows NMMII is widely distributed throughout the cytoplasm of fibroblast in Dupuytren's nodules (A and B inset) compared to scant cytoplasmic localization in fibroblast in cords (C and D inset) and normal palmar fascia (E and F inset).
 10× magnification: scale bar = 100μm, 100× magnification (inset): scale bar = 10μm