Dupuytren's Fibroblast Contractility by Sphingosine-1-Phosphate Is Mediated Through Non-Muscle Myosin II

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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 activating the S1P receptor, $S1P_2$. It is hypothesized that S1P stimulates Dupuytren's fibroblast contractility through $S1P_2$ activation of non-muscle myosin II (NMMII). This investigation examined the role of S1P and NMMII activation in Dupuytren's disease progression and suggests potential targets for treatment.

Methods We enmeshed Dupuytren's fibroblasts into fibroblast-populated collagen lattices (FPCLs) and assayed S1P-stimulated FPCL contraction in the presence of the $S1P_2$ receptor inhibitor JTE-013, the Rho kinase inhibitor Y-27632, the myosin light chain kinase 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 Sphingosine-1-phosphate stimulated FPCL contraction in a dose-dependent manner. Inhibition of $S1P_2$ and NMMII prevented S1P-stimulated FPCL contraction. Rho kinase and myosin light chain kinase inhibited both S1P and control FPCL contraction. Dupuytren's nodule fibroblasts robustly expressed NMMIIA and NMMIIB, compared with quiescent-appearing cords and normal palmar fascia.

Conclusions Sphingosine-1-phosphate promotes Dupuytren's fibroblast contractility through $S1P_2$, which stimulates activation of NMMII. NMMII isoforms are ubiquitously expressed throughout Dupuytren's nodules, which suggests that nodule fibroblasts are primed to respond to S1P stimulation to cause contracture formation. S1P-promoted activation of NMMII may be a target for disease treatment. *(J Hand Surg 2010;35A:1580–1588. Copyright © 2010 by the American Society for Surgery of the Hand. All rights reserved.)*

Key words Dupuytren's disease, non-muscle myosin II, sphingosine-1-phosphate, Rho-associated kinase, myosin light chain kinase.

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The authors thank James Aitken, BS, for technical efforts, and Salvatore V. Pizzo, MD, PhD, for support.

Received for publication February 26, 2010; accepted in revised form July 6, 2010.

Supported by grants from the American Society for Surgery of the Hand; a National Institutes of Health Mentored Clinical Scientist Award (K08), GM085562-01; and 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).

No benefits in any form have been received or will be received related directly or indirectly to the subject of this article.

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0363-5023/10/35A10-0002$36.00/0
doi:10.1016/j.jhsa.2010.07.009

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 1959, Luck first classified Dupuytren's disease as occurring in 3 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, and resolution was thought to occur throughout the palmar aponeurosis, leading to a chain of contractures within the natatory cords, pretendinous bands, Grayson's ligaments, and central and lateral cords.[1,4–6] The 3-stage pathogenesis model proposed by Luck is akin to the 3 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 5 S1P receptors ($S1P_{1–5}$). Among these receptors, studies have indicated that the $S1P_2$ receptor is a potent agonist of cell contractility and tissue remodeling.[10,11] $S1P_2$ 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 3 different NMMII isoforms in humans: NMIIA, NMIIB, and NMIIC.[17] NMIIA and NMIIB 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 $S1P_2$ 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. Finally, we analyzed the expression profile of NMMIIA and NMMIIB in Dupuytren's tissue and compared it with normal palmar fascia.

MATERIALS AND METHODS

Reagents and antibodies

We purchased the primary antibodies rabbit anti-NMMIIA polyclonal immunoglobulin G and rabbit anti-NMMIIB polyclonal immunoglobulin G from Abcam (Cambridge, MA). Dulbecco's modified Eagle medium (DMEM) was obtained from Sigma (St. Louis, MO). Heat-inactivated fetal bovine serum and penicillin-streptomycin were obtained from Invitrogen (Carlsbad, CA).

Inhibitors and agonists

We used S1P (Biomol, Plymouth Meeting, PA) at 0.01 to 1.00 μmol/L, which is within the range of its normal concentration in human blood (0.2–5.0 μmol/L).[18] We employed the specific $S1P_2$-receptor inhibitor JTE-013 (Cayman Biochem, Ann Arbor, MI) at 0.001 to 1.000 μmol/L.[19] We employed the Rho kinase inhibitor Y-27632 (Calbiochem, La Jolla, CA) at 0.01 to 10.0 μmol/L and the MLCK inhibitor ML-7 (Biomol) at 0.22 to 2.20 μmol/L.[20] The NMMII inhibitor blebbistatin (Calbiochem) was employed at 6.25 to 50.00 μmol/L.[21]

Human tissue

We obtained formalin-fixed and paraffin-embedded Dupuytren's tissues from the Department of Pathology, Duke University Medical Center repository of tissue specimens, in accordance with the Duke University Medical Center Institutional Review Board. A total of 12 specimens (9 males and 3 females) were assessed. We obtained normal palmar fascia from 9 cadavers (6 males and 3 females) from the Duke University Medical Center Human Fresh Tissue Laboratory.

Cell culture

We explanted Dupuytren's fibroblasts from 3 patients undergoing surgical fasciectomy (mean age, 63.3 y; 3 males). Dupuytren's nodules were dissected from the surrounding cords and palmar fascia. W used 3 cell lines from different patients 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 24 hours. The cells were subsequently cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. We performed experiments with primary cell cultures when cells were 80% to 90% confluent between passages 1 and 6.