Dupuytren's Disease: Physiologic Changes in Nodule and Cord Fibroblasts through Aging in Vitro

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The pathogenesis of the fibrotic disease Dupuytren's contracture remains unclear. The disease process includes two structurally distinct fibrotic elements, the nodule and the cord. It has been proposed that as the disease progresses, nodules develop into cords. To corroborate that hypothesis, the authors took advantage of cultured fibroblast differences found between gap junction intercellular communication and fibroblast-populated collagen lattice contraction. Paired fibroblast cell lines of nodules and cords derived from four patients with Dupuytren's disease were maintained in culture for at least eight passages. The presence of gap junction intercellular communication in nodule- and cord-derived fibroblasts was documented and reported as a coupling index. The contraction of free-floating nodule- or cord-derived collagen lattices was also documented and reported. Early passage (passage 4) cord-derived fibroblasts showed a significant increase in coupling index compared with passage 4 nodule-derived fibroblasts (4.0 ± 0.4 versus 2.5 ± 0.3, respectively), where $p \leq 0.01$. However, late passage (passage 8) nodule- and cord-derived fibroblasts were equivalent in their coupling index (4.1 ± 0.4 versus 4.4 ± 0.4, respectively). Early passage nodule-derived fibroblast-populated collagen lattices contracted by 64 percent, whereas late passage nodule-derived lattices showed less contraction, at only 40 percent. Early and late passage cord-derived lattices contracted 46 and 37 percent, respectively. All nodule- and cord-derived cell lines were statistically equivalent at lattice contraction by passage 8. These in vitro studies support the hypothesis that fibroblasts derived from Dupuytren's contracture nodules change their phenotype after undergoing repeated cell passage, acquiring a cord-like fibroblast phenotype. Dupuytren's nodules represent the early, active form of fibrosis in which cells are more proliferative, better at fibroblast-populated collagen lattice contraction, and display less gap junction intercellular communication. The speculation is that alterations in gap junction intercellular communication may be involved in the progression of Dupuytren's nodules to cords as the disease progresses. (*Plast. Reconstr. Surg.* 110: 187, 2002.)

Dupuytren's disease is a fibrotic process that leads to a flexion deformity of the hand.[1,2] The disease is progressive and results in contracture of the palmar fascia, affecting the metacarpophalangeal and proximal interphalangeal joints.[3,4] Recent reports reflect efforts to uncover the etiologic basis of Dupuytren's disease and possibly develop new treatment strategies. A treatment that results in an improved outcome over operative treatment has yet to be discovered. Therefore, operative treatment continues to remain the best option for Dupuytren's disease.[5,6]

In 1959, Luck[7] classified the progression of Dupuytren's disease into three stages. The first stage is the proliferative stage, marked by the proliferation and differentiation of fibroblasts. The second stage is the involutional stage. During this stage, the fibroblasts align themselves along lines of tension. The third and final stage is the residual stage represented by a reduction in cell density and the presence of thick collagen cords. Over the years of investigating Dupuytren's disease, these three biologic stages are still accepted and are considered the standard through which the disease is discussed.

Characterized as a fibromatosis, Dupuytren's disease contains two fibrotic structures that are clearly identifiable, the nodule and the cord.[4,7,8] The nodule contains a dense population of fibroblasts, with a high proportion being myofibroblasts, a highly vascularized tissue.[1,4,9,10] Myofibroblasts are specialized fibroblasts, identified by their expression of the

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α-smooth muscle isoform of actin.[11] In contrast to the nodule, the cord is a collagen-rich structure that is relatively avascular, acellular, and devoid of myofibroblasts. Different schools of thought exist regarding the development of cords. One scheme is that the nodule develops into the cord as the disease progresses over time.[12] It implies that the phenotype of the nodule fibroblast changes into the phenotype of the cord fibroblast. Another view is that the two structures represent different stages of the disease and arise independently.[13] The implication here is that both the nodule cell phenotype and the cord cell phenotype are derived from a separate precursor cell.

We wish to demonstrate that the cord fibroblast phenotype is derived from nodule fibroblasts. To quantify that possibility, two phenotypic markers were selected and studied. The first involves changes in the contraction of fibroblast-populated collagen lattices.[14] The second involves changes in gap junctional intercellular communication between cultured nodule and cord fibroblasts in monolayer cell culture. Intercellular communication between gap junctions involves the development of a channel structure between two cells that allows the passage of molecules with a molecular weight less than 1000.[15] The proposition is that in Dupuytren's disease, immature proliferative cells (nodules fibroblasts) will have less intercellular communication between gap junctions compared with more mature cord-derived fibroblasts.[16] It should follow that the maturation of nodule-derived fibroblasts into cord fibroblasts will be associated with an increase in gap junctional intercellular communication.

Methods

Cell Culture

Nodules and cords from four patients undergoing primary surgery for Dupuytren's disease were collected. Each patient had at least a 30-degree metacarpophalangeal joint contracture and a 20-degree proximal interphalangeal joint contracture. Each individual nodule and cord tissue specimen was cut into sixteen 1 × 1 mm pieces. The specimens were then placed in 35-mm tissue culture dishes. Four pieces of either the nodule or the cord were placed per dish, resulting in a total of four nodule-designated and four cord-designated 35-mm dishes. A glass coverslip was placed over tissue pieces and 1 ml of Dulbecco modified Eagle medium, supplemented with 10% fetal bovine serum (referred to as complete Dulbecco modified Eagle medium), was added. Medium was changed twice a week until the cells had reached confluence. From each confluent 35-mm dish, the glass coverslip was removed, and the cells were freed by trypsin and passed into a 60-mm tissue culture dish. When the 60-mm plates reached confluence, they were each passed into a 10-cm dish. Each 10-cm dish was passed by equally distributing trypsin-freed cells between two 10-cm dishes (about 500,000 cells per dish). Each 10-cm dish contained about 10^6 fibroblasts when it reached confluence. By passage 4, there were a pair of 10-cm dishes derived from each initial 35-mm dish. Eight parallel pairs of nodule- and cord-cultured cell lines were therefore established. All cell lines were regarded as being in early passage at passage 4. A 10-cm plate from each cell line at passage 4 was saved for study. Cell cultures were maintained for four more passages. At passage 8, which was designated as late passage in these experiments, a 10-cm plate from each cell line was saved for study. Because these fibroblast cell lines were derived from individuals over the age of 65 years, the four cell lines became senescent between passages 10 and 12.

Two groups of cell lines were studied: an early passage group represented by nodule and cord fibroblast cell lines at passage 4 and a late passage group represented by nodule and cord fibroblast cell lines at passage 8. Established cell lines of normal dermal fibroblasts were used for comparison with the nodule and cord cell lines. The normal dermal cell line used in these studies was between passage 20 and 24 and has been previously described.[17]

Scrape Loading

Fibroblasts harvested in their fourth or eighth passage were plated in 35-mm dishes and grown near confluence, approximately 250,000 cells. The medium was removed, and the cell monolayer was rinsed in phosphate-buffered saline. To each dish, approximately 1 ml of dye solution, 20 mg/ml Lucifer yellow in phosphate-buffered saline, and 5 mg/ml rhodamine dextran (Molecular Probes, Eugene, Ore.) was added. A commercial glass cutter with a keen cutting wheel was used to scratch a fine linear wound in the cell monolayer. The row of injured fibroblasts in the monolayer were permeable to both dyes, which enter dam-

aged cells only. Intact, uninjured cells were not capable of taking up either dye in the absence of gap junctions. Within injured cells, only the Lucifer yellow dye could pass through patent gap junctions established between the injured cell and its uninjured neighbors. The large-size rhodamine dextran particles remained trapped within the scrape-injured cell. After a 2-minute incubation period at room temperature, the dye solution was removed, and the cells were rinsed with phosphate-buffered saline.[18,19] The scratched monolayer was fixed in buffered 4% paraformaldehyde/phosphate-buffered saline, pH 7.2.

Only cells injured by the glass cutter wheel were labeled with both rhodamine dextran and Lucifer yellow dyes. Uninjured cells linked to injured cells by gap junction channels were labeled with Lucifer yellow dye but not rhodamine dextran. A coupling index was determined and reported by viewing the fibroblasts at the scratch mark with a fluorescent microscope equipped with appropriate green and red fluorescent filters. The ratio of Lucifer yellow–labeled cells, which appear as yellow-green fluorescent cells, to the rhodamine dextran–labeled cells, which appear as red fluorescent cells, is the coupling index. Figure 1 is representative of such an experiment. The greater the ratio of yellow-green cells to red cells, the

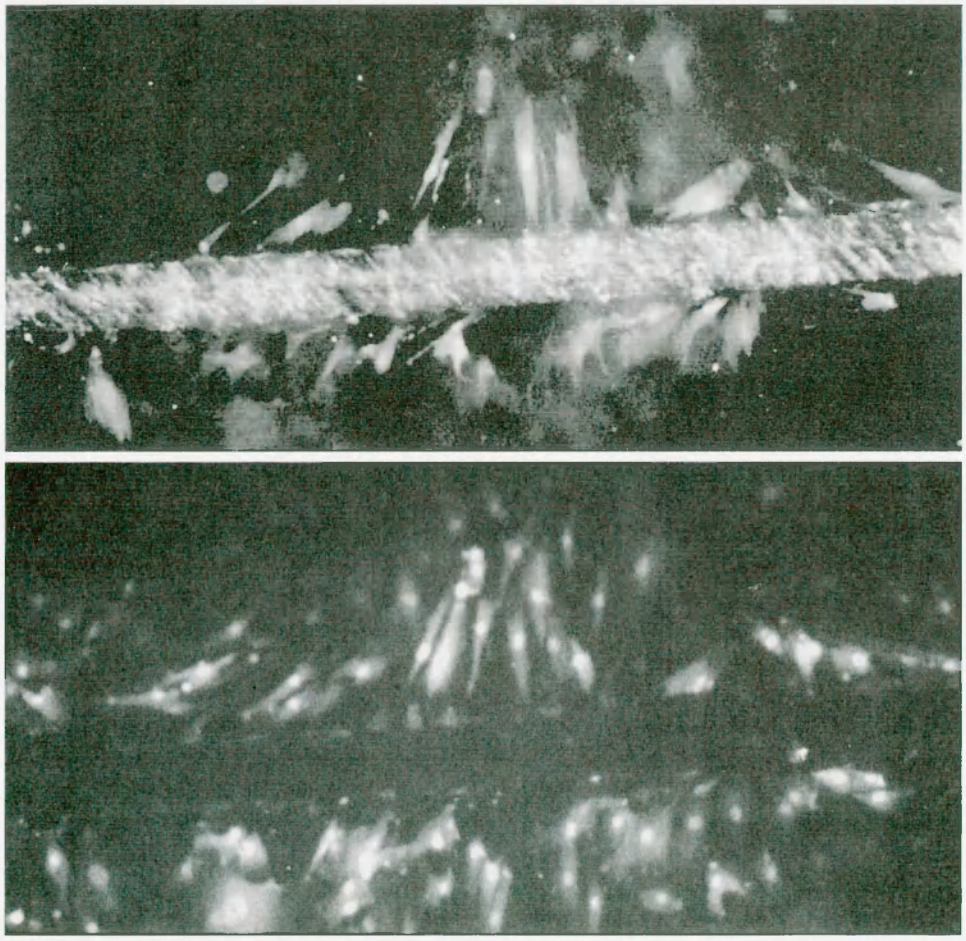

FIG. 1. Scrape loading of a fibroblast cell culture monolayer. A monolayer of early passage nodule fibroblasts was injured by the wheel of a glass cutter, which created a scratch or narrow scrape through the cell layer. In the panel *above*, visualized with a filter for rhodamine, the scrape is the path going right to left. The fibroblasts residing in the path of the wheel of the glass cutter take up rhodamine dextran, which stains those cells. In the panel *below*, visualized with a filter for Lucifer yellow, is the same field shown in the panel above. There is a greater number of cells staining for Lucifer yellow (*below*) than for rhodamine dextran (*above*). This difference demonstrates that both injured and uninjured cells take up Lucifer yellow, but rhodamine dextran is restricted to the injured cells (*above*). The Lucifer yellow entered the uninjured cells through gap junction channels formed between injured and neighboring uninjured cells. Original magnification, 35×.

higher the coupling index and the greater the number of cell-sharing gap junction channels.

Fibroblast-Populated Collagen Lattice Contraction

Native soluble collagen from rat tail tendons was isolated by acetic acid extraction and salt precipitated with NaCl.[17] Briefly, tendons were teased from rat tails and stirred in ice-cold 0.5 M acetic acid for 2 days. All subsequent steps were carried out at 4°C. The solubilized collagen was cleared by centrifugation. The collagen was precipitated by the addition of 10% wt/vol NaCl, stirred for 4 hours, and the collagen was collected by centrifugation. The collagen pellet was resuspended in 100 mM acetic acid and then exhaustively dialyzed against 1 mM HCl, frozen, lyophilized, weighted, resuspended in sterile 1 mM HCl at 5 mg/ml, and stored at 4°C until needed.

Fibroblast-populated collagen lattices were manufactured with fibroblasts from both early and late passage paired nodule and cord cell lines. Fibroblasts (100,000) were suspended in 1.5 ml of complete Dulbecco modified Eagle medium and combined with 2.5 mg of collagen solution (0.5 ml), making a total volume of 2.0 ml. The solution was rapidly mixed, after which a 0.5-ml aliquot was placed in a 16-mm-diameter well (area, 201 mm^2) within a 24-well tissue culture plate (Corning Cell Wells No. 25820, Corning, N.Y.). Plates were placed in a 37°C incubator in a water-saturated atmosphere with 95% air and 5% CO_2, where the collagen polymerized in less than 90 seconds. Thirty minutes after casting, the lattices were detached from the walls of the wells with a glass rod. A stereomicroscope with a fixed ruler in place was used to measure the diameters of each lattice. From the diameter measurements, the area of each lattice was calculated and recorded. Statistical analysis was completed through Student's *t* test.

RESULTS

Scrape Loading

Fibroblasts derived from early passage (passage 4) nodules from patients with Dupuytren's disease had a coupling index of 2.5 ± 0.3. Fibroblasts from passage 4 cords from patients with Dupuytren's disease had a coupling index of 4.0 ± 0.4. The coupling index difference between early passage cord-derived and nodule-derived fibroblasts was significant $p \leq 0.01$. The greater coupling index of cord-derived fibroblasts demonstrated greater gap junctional intercellular communication between these cells compared with early passage nodule-derived fibroblasts.

After eight passages, the coupling index for the late passage nodule-derived fibroblasts had increased to 4.1 ± 0.4. The coupling index for late passage cord-derived fibroblasts remained unchanged at 4.4 ± 0.4, which was the same as the coupling index for early passage cord cells. Hence, cord-derived fibroblasts from patients with Dupuytren's disease had no change in their coupling index through continued cell passage. However, nodule-derived fibroblasts from patients with Dupuytren's disease increased their coupling index through continued cell passage (Table I). As a point of reference, normal dermal fibroblasts had a coupling index of 3.8 ± 0.8. Hence, the coupling index of cord-derived fibroblasts was similar to normal dermal fibroblasts, whereas the coupling index of nodule-derived fibroblasts was lower than normal dermal fibroblasts.

Fibroblast-Populated Collagen Lattice Contraction

The fibroblast-populated collagen lattice contraction data are summarized in Table II. Early passage fibroblasts derived from nodules had reduced their lattice area from 201 mm^2 to 72 ± 20 mm^2 at 24 hours, a 64 percent decrease in area ($p \leq 0.01$). Early passage cord-derived fibroblasts had reduced their lattice area to 108 ± 6 mm^2, a 46 percent decrease in area. The difference between early passage nodule and early passage cord lattice contraction was significant ($p \leq 0.01$).

Late passage nodule-derived fibroblasts became less effective at lattice contraction. The passage 8 nodule-derived lattice contracted to 120 ± 30 mm^2, a 40 percent decrease in area. The late passage cord-derived lattice had contracted to 126 ± 6 mm^2, a 37 percent decrease in area. Late nodule-derived and cord-derived

TABLE I
Fibroblast Coupling Index

	Coupling Index, Passage 4	Coupling Index, Passage 8
Dupuytren's nodule fibroblasts	2.5 ± 0.3*	4.1 ± 0.4
Dupuytren's cord fibroblasts	4.0 ± 0.4	4.4 ± 0.4
Foreskin dermal fibroblasts	3.8 ± 0.8	3.8 ± 0.8

Mean ± SD of four pairs of cell lines derived from surgically excised Dupuytren's nodules and cords are presented. Two culture dishes from each cell line were subjected to scrape loading.

* Significant difference as determined by Student's *t* test ($p \leq 0.01$).

TABLE II
Fibroblast Populated Collagen Lattice Contraction at 24 Hours

	Area from Passage 4	Area from Passage 8
Dupuytren's nodule fibroblasts	72 ± 20 mm²*	120 ± 30 mm²
Dupuytren's cord fibroblasts	108 ± 6 mm²*	126 ± 6 mm²

Mean ± SD of four pairs of cell lines derived from surgically excised Dupuytren's nodules and cords. Four fibroblast populated collagen lattices for each cell line were measured; hence, each mean ± SD represents 16 measurements.

* Significant difference as determined by Student's *t* test ($p < 0.01$).

lattices were equal in lattice contraction. The normal dermal-derived lattice contraction was 64 ± 0, a 68 percent reduction in area. Normal dermal lattice contraction was therefore equivalent to early nodule-derived lattice contraction, which was greater than cord and late nodule-derived lattice contraction.

DISCUSSION

There are different schools of thought of how a Dupuytren's cord develops during the progression of the disease. Hueston[12] suggests that cords advance from nodules. However, Gosset[13] suggests that the two structures represent different stages of the disease and arise independently. Hueston favors that a precursor cell develops into a nodule phenotype, which undergoes a change, leading to the development of a cord phenotype. Gosset puts forward the notion that a precursor cell develops into a nodule phenotype and another precursor cell develops into a cord phenotype. The in vitro findings from this report support Hueston's theory that the nodules develop into the cords, representing a progression of the disease state. Because it is too difficult to study sequential palmar fascia specimens within patients afflicted with Dupuytren's disease, the in vitro investigation of fibroblasts derived from nodules and cords is a practical approach.

Early passage nodule-derived fibroblasts seemed to be representative of the diseased state of Dupuytren's contracture. Cells in a proliferative state demonstrate reduced cell coupling through intercellular communication between gap junctions.[16] The nodule is formed during the proliferative phase of Dupuytren's disease, and therefore, gap junctional intercellular communication in fibroblasts derived from nodules is initially reduced. As the nodule-derived cells undergo continued passage in culture, they seem to lose their proliferative phenotype and develop into a more mature cell phenotype. The mechanism that links decreased intercellular communication between gap junctions and cell proliferation is clear. Another possibility is that nodule-derived fibroblasts represent a diseased state in which they are defective in intercellular communication between gap junctions. During their maintenance in culture, these nodule fibroblasts undergo a change that restores their ability to create functional gap junction channels.

Early passage nodule-derived fibroblasts were equivalent to normal dermal fibroblasts at fibroblast-populated collagen lattice contraction. Early passage nodule-derived fibroblasts were better at lattice contraction when compared with both late passage nodule fibroblasts and all cord-derived fibroblasts. It seems that cord fibroblasts and late passage nodule fibroblasts are poor at lattice contraction, suggesting that aging in Dupuytren's disease reduces the capacity of fibroblasts to reorganize collagen. It is reported that late passage, normal human dermal fibroblasts are better at lattice contraction than early passage fibroblasts.[14] This suggests that late passage nodule fibroblasts may be losing some of their capacity to interact with collagen, leading to the assumption that nodule fibroblasts in Dupuytren's disease have an altered phenotype that is maintained in cell culture.

There is a relationship between optimal fibroblast-populated collagen lattice contraction and gap junctional intercellular communication. The inclusion of intercellular communication between gap junction uncouplers in the casing of lattices inhibits lattice contraction.[17] The elimination of connexin 43 expression, the protein responsible for gap junction channel structures in transfected rat osteoblasts, also leads to the inhibition of populated collagen lattice contraction.[20] However, with fibroblasts derived from nodules in Dupuytren's disease, which show retarded gap junctional intercellular communication, there is no inhibition of lattice contraction. Nodule-derived fibroblasts that have undergone continued cell passage in vitro and cord-derived fibroblasts demonstrate defective lattice contraction associated with normal levels of intercellular communication between gap junctions. In the case of late passage nodule and cord fibroblasts, gap junctional intercellular communication seems to be independent of optimizing lattice contraction.

There are reports that suggest the nodule of

Dupuytren's disease represents the contractile element of the disease process.[1,4,21] Populations of myofibroblasts, as defined by their expression of α-smooth muscle actin, reside in nodules but not in cords. Dupuytren's cords are relatively acellular in comparison to the nodules and do not contain myofibroblasts. However, a patient will commonly show nodules in the palm of the hand and not demonstrate Dupuytren's contractures. The development of Dupuytren's contracture occurs after the appearance of cords. This in vivo picture creates a dilemma in associating the nodule with the contractile state of the disease. It is suggested that myofibroblasts contained within nodules are not responsible for generating the forces of Dupuytren's contracture.[6,14] In the free-floating lattice contraction model, myofibroblasts are absent; hence, there is no experimental evidence for a role of myofibroblasts in lattice contraction.[22] In vitro, early passage nodule-derived fibroblasts, which are not myofibroblasts, seem to be the contractile cell of Dupuytren's disease. It is unclear what role myofibroblasts have in the progression of the disease.

One thought is that the Dupuytren's disease process represents a maturation of fibrotic tissue. The nodule contains immature cells representing an early stage of the disease. The maturation of these cells leads to the endstage of the disease and the formation of a fibrotic cord. The concept follows the maturation of granulation tissue into a scar during wound healing. Immature granulation tissue has an increased cell density and increased myofibroblasts; like the nodule, as granulation tissue matures into a scar, cell density decreases and myofibroblasts disappear, much like the cord.[23,24] However, Dupuytren's contracture and the process of wound healing by contraction have some contrasts. Wound contraction is a rapid process that proceeds soon after injury, whereas Dupuytren's contracture is a slow contractile process that occurs long after the appearance of fibrotic tissue. Wound contraction will continue as long as the wound is not completely epithelialized. In contrast, Dupuytren's contracture occurs under an intact epidermis. It suggests that fibroblasts derived from patients with Dupuytren's disease have an unusual fibroblast phenotype that contributes to the disease state.

The role of growth factors on the pathogenesis of Dupuytren's disease has been reported.[25–28] In particular, transforming growth factor-β (TGF-β) in vitro induces fibroblast proliferation, increases α-smooth muscle actin expression, and increases the deposition of collagen, all fundamental to the progression of Dupuytren's disease.[26,29] With other mesenchymal cell types, TGF-β increases intercellular communication between gap junctions.[30,31] If TGF-β is responsible for the initiation of the proliferative stage of Dupuytren's disease, nodule-derived fibroblasts would be expected to show increased cell coupling. However, the opposite is seen in nodule fibroblasts, in which there is less gap junctional intercellular communication. Of interest, there is a report that shows TGF-β leading to a decrease in intercellular communication between gap junctions. Transfected osteoblastic cell lines result in an altered phenotype and show reduced cell coupling when treated with TGF-β.[32] On the basis of these experimental findings, nodule fibroblasts behave like a cell with an altered phenotype and not like normal dermal fibroblasts.

The proliferative stage of Dupuytren's disease represents the development of the nodule, and through in vitro aging of nodule fibroblasts, the cord-like fibroblast phenotype develops. The dilemma of the clinical characteristics of palmar contracture and how it relates to nodules and cords is compounded by the findings presented here. The presence of both nodules and cords in the disease state seems to be necessary for the generation of palmar contracture.[1] It suggests that the nodule, combined with the cord structure, represents the contractile unit of Dupuytren's disease. On the basis of cell phenotype, the nodule is responsible for generating the contractile forces, which lead to the characteristic flexion deformity of Dupuytren's disease.[1,9,10] On the other hand, it seems that the cord is needed to transmit those generated forces. It is suggested that the cord radiates out and makes contact with distant regions of the fascia. As the nodule generates contractile force, that force is radiated out and is linked to the distant fascia by means of the cords. The concept is that nodules develop into a complex contractile unit by the generation of cords that radiate out into the palmar fascia. Hence, in Dupuytren's disease, the nodule-cord complex is the contractile unit responsible for the generation of palmar contracture.

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Discussion

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Since the original description of palmar fibromatosis by the infamous Guillaume Dupuytren on December 5, 1831, at the Hotel Dieu in Paris,[1] physicians have recognized the intriguing characteristic features of this form of fibroproliferative disorder as a progressive contractile fibrosis that involves the tissues of the palmar fascia and the deep dermis of the skin. It occurs in genetically predisposed racial groups and their family members, particularly blue-eyed Celtic descendants of the Vikings.[2] Dupuytren's contracture is often associated with other forms of fibromatosis in fascial structures of other regions of the body. Extensive clinical experience has demonstrated that it responds to surgical removal of the diseased tissues, but it usually responds best when the wounds are closed with full-thickness skin grafts to avoid the major and most frequent surgical complication, that is, recurrence in the site of resection.[3] Despite this familiarity with Dupuytren's disease and major advances in cellular and molecular biology, the pathogenesis of Dupuytren's contracture and other fibroproliferative disorders continues to escape us. Thus our approach to treatment remains crude and not totally effective. Many questions remain unanswered and include the following: What is the genetic link that predisposes some racial groups and families to fibroproliferative disorders and not others? What is the basic initiating event that triggers the selective activation of some regions of previously quiescent tissues? Which cells are responsible for the pathophysiology? Why, after extensive removal of these locally activated abnormal cells and tissues, does the disease readily recur? Unfortunately, these questions are common to all forms of fibroproliferative disorders, including hypertrophic scarring and keloids familiar to plastic surgeons,[4] but involve virtually all tissues and organs that sustain injury from disease or trauma. They respond with a wound healing response that is excessive and compromises function, including hepatic fibrosis, atherosclerosis, proliferative retinal diseases, glomerulosclerosis, and many other forms of fibroproliferative disorders.

In this article, Dr. Paul Ehrlich and his colleagues continue an accomplished, career-long investigation of the cellular features of fibroblasts in contractile disorders.[5] Despite the limitations of the in vitro environment, they suggest that a subset of fibroblasts, those in the nodular regions of Dupuytren's contracture, are activated to contract their extracellular matrix environment by means of an energy-dependent, cytoskeletal-mediated process. Once activated, they intercommunicate through intracellular gap junctions to a lesser extent than fibroblasts located in the normal dermis of the skin. These findings are consistent with the recognized importance of intercellular communication through specific membrane channel proteins, connexins, which are under connexin gene regulation. They appear to impart tissue interdependence and homeostasis through growth control of interconnected cells.[6] Deletion of different connexin genes from mice has provided in vivo evidence for the importance of gap junction intercellular communication in the control of abnormal

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cell growth in carcinogenesis[7] and now, possibly, fibroproliferative disorders.[8] In addition, their findings are consistent with the recognition of the role of transforming growth factor-β, one well-recognized immunosuppressive and fibrogenic family of cytokines that have been identified in fibrotic tissues of Dupuytren's contracture,[9] hypertrophic scars,[10] and other fibroproliferative disorders. Transforming growth factor-β is known to deplete gap junction intercellular communication in osteoblasts.[11] The floating fibroblast-populated dermal lattice model given to us by Bell and Ehrlich is a useful in vitro model of the contracting matrix in which transforming growth factor-β_1 is known to stimulate matrix-lattice contraction.[12] This article is also consistent with the growing understanding that the myofibroblast is a cell with prominent fibronectin fibrils and fibronexus junctions distinct from the basement membrane. It stains positive for vimentin and α-smooth muscle actin in stress fibers but is likely not as contractile as other "activated" fibroblasts observed in different fibroproliferative disorders.[13,14] It likely resembles the cord or aged fibroblast of Dupuytren's contracture previously activated, now dormant and quiescent in the stabilized matrix that may be committed to apoptosis as the matrix remodels and cellularity is reduced. The subset of activated fibroblasts in the diseased tissue seems to be proliferative and moves in the tissues expressing matrix metalloenzymes necessary for advancement through the matrix, dragging the trailing components through the tissues until physical forces of resistance prevent further movement and contraction. The cell then reverts to the dormant phenotype typical of the cord fibroblast or the mature scar fibroblast.[14,15]

Unfortunately, this evolving understanding of the activated fibroblast, though consistent with the earlier paradigm of Lewis, is focused on likely late events in response to earlier triggers. It does not expand our understanding of the unanswered questions posed earlier regarding the initial inciting event, the genetic component in the response to injury of this disease, and other fibroproliferative disorders. In addition, it does not answer why the disease recurs often quickly and as extensively or more extensively after local removal of the activated fibroblasts. In the next decade, Drs. Moyer et al. and others will likely increase our understanding of how inflammatory cells responding to injury may direct the fibroblast in tissues through direct and indirect paracrine signaling in normal or aberrant ways and supply physicians with new intracellular growth factors[16] and gene therapy regimens that are emerging to increase our supply of more sophisticated therapies. Similarly, the recent appreciation of systemic, circulating, immunologically active cells (which are derived from bone marrow) that move to injured tissues and differentiate into activated fibroblasts where they produce matrix and likely perform many other fibroblast functions that may link the systemic immune response to injury with local wound healing.[17] To date, such mature stem cells termed fibrocytes may account, in part, for some of the unique genetic aspects of wound healing in fibroproliferative disorders, including recurrence of disease, and may contribute to the cellular origin of the active fibroblast in Dupuytren's contracture[18] and other fibroproliferative disorders.[10] They may also offer the potential to broaden our approach from direct treatments for localized disease to include novel, local, and systemic therapies that more favorably influence wound healing responses to injury.

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