Evidence for a Polyclonal Etiology of Palmar Fibromatosis

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X chromosome inactivation patterns at the androgen receptor locus were evaluated to determine clonality in microdissected lesional tissue and in leukocytes from 2 women with Dupuytren's disease. The tissue from both patients generated a polyclonal pattern of X chromosome inactivation of the human androgen receptor gene. This finding supports a polyclonal reactive process as the underlying etiology for palmar fibromatosis. (J Hand Surg 1999;24A:339–344. Copyright © 1999 by the American Society for Surgery of the Hand.)

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Palmar fibromatosis (Dupuytren's disease) is a disorder of unknown etiology that most commonly affects the palmar fascia in white, middle-aged men. The lesion is often bilateral and occasionally involves the plantar fascia or the penis (Peyronie's disease). There is a hereditary component to the disease as well as an association with trauma, smoking, epilepsy, diabetes mellitus, and alcoholism.[1–6] The lesions are progressive nodular proliferations of cytologically benign fibroblastic or myofibroblastic cells within the subcutaneous palmar tissue. A dense, pseudotendinous, collagenous matrix is formed that results in secondary contractures of the digits.[7]

The histologic appearance of palmar fibromatosis is nearly the same as that of a desmoid tumor (aggressive fibromatosis) or even a well-differentiated fibrosarcoma.[7] Plump fibroblast-like cells with occasional mitotic figures envelop adjacent structures. As with these other clinical entities, the treatment of Dupuytren's disease is usually surgical and recurrent disease is not uncommon. Exacerbation and remission may occur, although frank regression is rare.[8]

There have been numerous theories suggesting that Dupuytren's disease may be an inflammatory process, the result of trauma, or a benign neoplasm. Several lines of evidence support any or all of these theories of pathogenesis. Several growth factors, including insulin-like growth factor-1, transforming growth factor-β, and basic fibroblast growth factor, have been demonstrated by immunohistochemistry or *in situ* hybridization to exist at higher levels within the Dupuytren's tissue than in the surrounding normal aponeurotic tissue.[9–13] Other studies have found decreased expression of the Rb tumor suppressor gene and expression of oncofetal variants of fibronectin within the hyperplastic tissue.[14,15]

The biological hallmark of a neoplasm is monoclonality.[16,17] Although the development of a neoplasm is a multistep process in which more than one cell clone may be involved, with rare exception, all cells of a true neoplasm originate from a single transformed cell. In contrast, a reactive nonneoplastic proliferative lesion is of polyclonal origin. One method that can be used to determine the clonality of tumors relies on the analysis of patterns of X chromosome inactivation in tumor tissue. During embry-

ogenesis, all but one X chromosome is inactivated in each cell. Because this activity status is maintained during mitosis, all progeny of a particular cell will have the same X chromosome inactivated. Normal tissues are composed of a mixture of cells, some having the paternal X chromosome active and others having the maternal X chromosome active. It has been shown that the patterns of X chromosome activity are similar in different normal tissues from an individual.[18] In contrast, in a tissue that has a clonal development, all cells will have the same active parental X chromosome. To study the clonality of a lesion one must have a female subject who is heterozygous for the particular X-linked marker to be analyzed. Many epithelial and several mesenchymal conditions of uncertain etiology have been studied by analysis of X chromosome inactivation patterns.[19–32]

Several techniques have been used in the determination of patterns of X chromosome inactivation. The first technique to be widely used was the analysis of polymorphisms of the glucose-6-phosphate dehydrogenase gene. However, its usefulness is limited because the majority of females are homozygous for this polymorphism. Vogelstein et al[33] developed an assay that used restriction fragment length polymorphism analysis to determine the difference in methylation patterns in X-linked genes. More recently, analysis of a polymorphism in the human androgen receptor alpha gene (HUMARA) located on the X chromosome has been used as a marker of clonality. One advantage of studying this polymorphism is that it can be performed by polymerase chain reaction (PCR) methods and does not require preparation of Southern blots.[34] This gene contains a hypervariable CAG repeat region adjacent to two HpaII restriction sites. Approximately 90% of females are heterozygous for this marker. The restriction enzyme HpaII selectively cuts only the unmethylated DNA of the active HUMARA gene. The PCR is then used to amplify the DNA segment containing both the CAG repeats and the HpaII restriction sites on the inactive gene. The sequence on the active X chromosome will not be amplified because the forward and reverse primers will not bind to the same DNA fragment. Assuming that the tissue being examined is purely lesional, a monoclonal proliferation will yield a PCR product of a single length, whereas a reactive proliferative process will reveal its polyclonal nature by yielding PCR products of 2 different lengths. Normal tissue is evaluated to document the relative activities of the 2 alleles (Fig. 1).

The purpose of this study was to determine whether palmar fibromatosis is a monoclonal or polyclonal process. X chromosome inactivation patterns were analyzed to evaluate clonality in microdissected lesional tissue and in leukocytes from 2 women with Dupuytren's disease.

Case Reports

Patient 1

A 50-year-old woman of Scandinavian ancestry presented with a 2-year history of Dupuytren's disease and a 60° flexion contracture of the proximal interphalangeal (PIP) joint. There was at least one family member known to have the disease. At the time of examination there appeared to be no other sites symptomatically involved; however, early formation of knuckle pads on the PIP joints of the index and little finger were apparent. Findings at the time of surgery were grossly consistent with Dupuytren's disease. A nodule (8 × 10 mm), which was palpable before surgery, was located at the level of the PIP joint within, but distinct from, the subcutaneous tissue. Following excision, histologic examination of the tissue showed a fibrocytic proliferation within a dense collagenous matrix, confirming the clinical diagnosis of Dupuytren's disease.

Patient 2

A 48-year-old woman presented with a 7-year history of flexion contractures of the small and ring fingers of both hands and of the left index finger. There was a positive family history of Dupuytren's disease (her mother and one of her sisters). The patient had previously undergone 2 operations on the right hand for release of contractures. Examination revealed a 30° contracture of the metacarpophalangeal joint and a 60° contracture of the index finger PIP joint on the left hand. Disease was also felt in the palm between the ring and small finger metacarpophalangeal joints. A Dupuytren's nodule was present on the dorsum of the ring finger PIP joint with some extension into the web space between the ring and middle finger. There was also extensive disease between the index finger and thumb. A partial palmar fasciotomy was performed, with histologic confirmation of the diagnosis of Dupuytren's disease. Tissue from a spiral cord at the level of the metacarpophalangeal joint was selected for analysis.

Materials and Methods

A small portion of grossly representative lesional tissue, snap-frozen in liquid nitrogen and stored at

Human Androgen Receptor Gene

A
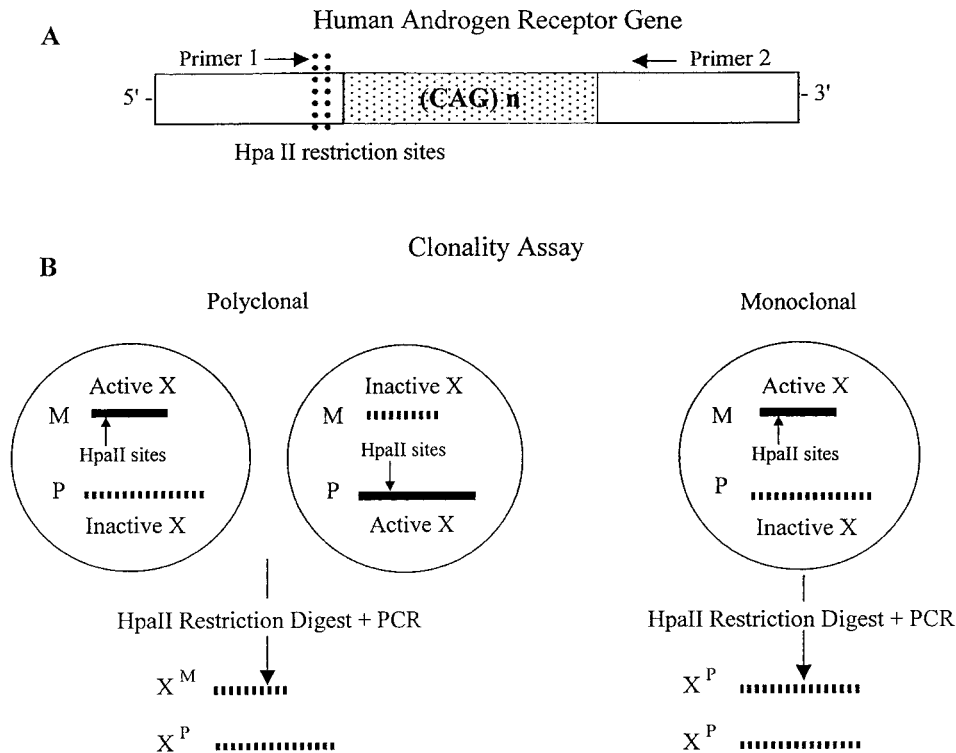
Figure 1. (A) Schematic of the X-linked human androgen receptor gene. The methylation-sensitive restriction enzyme, HpaII, will cleave the active gene 5′ to the polymorphic CAG repeat ([CAG]n). Primers 1 and 2 permit amplification of the methylation sites and the CAG repeat only in the intact gene. (B) Analysis of clonality by X chromosome inactivation. The maternal (M) gene has a shorter CAG repeat than the paternal (P) gene. In a polyclonal population of cells, the maternal or paternal genes are randomly methylated and thereby inactivated (broken lines). The active genes are nonmethylated and thus digested by the HpaII restriction enzyme. A monoclonal population of cells has the same methylated (inactive) allele in every cell. The amplified inactive genes are visualized in a gel. Alleles of 2 different lengths are seen in the polyclonal cell population but only 1 length is seen in the monoclonal population.

−80°C, and 5 mL peripheral blood from each patient were set aside for analysis of clonality. The tissue was examined with light microscopy and a small portion of lesional tissue, not contaminated by blood, fat, or skin, was teased off the slide. Separated polymorphonuclear leukocytes from a patient with polycythemia vera were used as a positive monoclonal control.[35] A salting out procedure was used to prepare DNA from lesional tissue, buffy-coated blood samples, and polymorphonuclear leukocytes.[36,37] Eight hundred nanograms of DNA was digested overnight with 30 U HpaII (New England Biolabs, Beverly, MA; 50 U/μL) and 4 U of RsaI in 20 μL volumes in tightly sealed PCR tubes. To minimize evaporation resulting from vapor condensation under the lids, digests were performed in a 37°C incubator in polycarbonate 96-well Concord PCR trays (MJ Research, Watertown, MA) tightly sealed with Seal Plate sealing film (PGC Scientific, Gaithersburg,

MD). Four microliters of each restriction digest (containing approximately 150 ng DNA) was amplified (MJ Research; PTC-1000) in 20 μL PCR reactions containing 0.5 U Taq polymerase (AmpliTaq; Perkin-Elmer, Norwalk, NJ), 200 μmol/L dNTPs, 0.3 μmol/L primers (AR.PCR1.1/AR.PCR1.2) including 4.5 pmol of forward primer end-labeled with 0.4 μCi [32]P. The thermal profile was as follows: preheat cycler to 95°C; initial denaturation, 94°C for 3 minutes; 30 cycles of 94°C for 45 seconds, 65°C for 30 seconds, and 72°C for 30 seconds; final extension, 72°C for 7 minutes.[34,38] Samples were run for 4 hours at 50°C in 7 mol/L urea 6% polyacrylamide denaturing sequencing gels, dried, and autoradiographed.

Results

Histologic analysis of lesional tissue verified the diagnosis of palmar fibromatosis. Figure 2 shows the
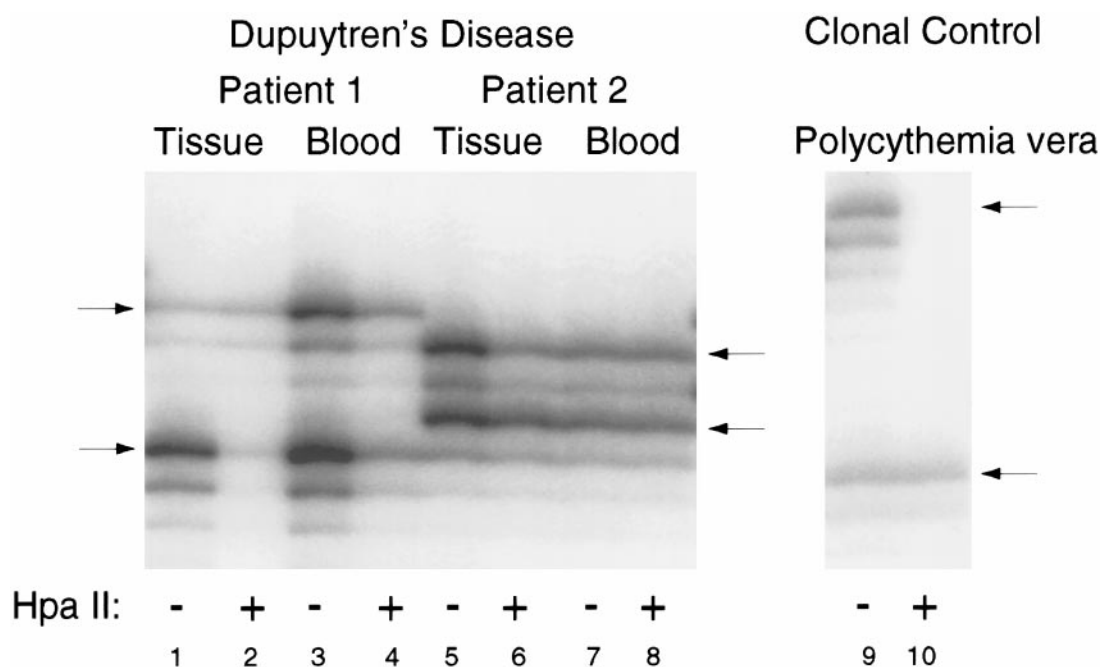
Figure 2. Analysis of clonality by X chromosome inactivation. Patients 1 and 2 are subjects with Dupuytren's disease. The control subject has polycythemia vera, a clonal hematopoietic disease. Lanes 1, 2, 5, and 6 contain DNA from Dupuytren's lesions; lanes 3, 4, 7, and 8 contain DNA from unseparated white blood cells; lanes 9 and 10 contain DNA from polymorphonuclear cells. Lanes marked "+" contain DNA that has been digested by the restriction enzyme HpaII; DNA in lanes marked − has not been digested by HpaII. Arrows point to the alleles at the human androgen receptor alpha (HUMARA) locus. All 3 subjects are heterozygous for HUMARA; thus, 2 dominant bands result from amplification of DNA not previously digested with HpaII (lanes 1, 3, 5, 7, and 9). Amplification of HpaII-digested DNA from the patient with polycythemia vera yields only a single band, indicating that the polymorphonuclear cells are monoclonal. In contrast, polyclonality of the Dupuytren's samples is demonstrated by the persistence, after HpaII digestion, of 2 dominant bands in lanes 2 and 6 (lesional tissue) with the same relative intensities as the bands in lanes 4 and 8 (blood).

genotypes and X chromosome inactivation patterns for the HUMARA locus in normal and lesional tissue from patients 1 and 2 and in blood cells from a person with polycythemia vera, a clonal myeloproliferative disorder. As is always observed for short tandem repeat sequences, amplification of each allele generated one dominant band and a series of bands that were each progressively weaker and shorter by one repeat unit. This pattern is thought to result from slippage of the polymerase. The analysis was based on the amplification pattern of the dominant bands in each lane. Tissue from both patients with Dupuytren's disease and the patient with polycythemia vera yielded 2 major HUMARA bands after amplification of undigested DNA (− lanes) showing that they were heterozygous for the CAG repeat number. After digestion with HpaII, the tissue from both patients still clearly demonstrated 2 different major bands (+ lanes). As expected, this pattern was also observed in the control DNA obtained from peripheral blood leukocytes. Thus, both cases exhibited a polyclonal pattern of X chromosome inactivation, in contrast to the monoclonal pattern demonstrated in granulocytes from the patient with polycythemia vera. The differences were apparent with visual inspection of the gels, obviating the need for densitometry.

Discussion

The etiology of palmar fibromatosis remains uncertain. Various theories have been proposed to explain the histologic and biological features of the disease. While most investigators support the concept that palmar fibromatosis is a reactive process involving myofibroblasts, the histologic appearance of the diseased tissue can be nearly indistinguishable from that of aggressive fibromatosis or even low-grade fibrosarcoma.

Several studies support a clonal origin for

aggressive fibromatosis[20,28,39,40]; however, the clonality of palmar fibromatosis has not been studied. This analysis of 2 patients supports a polyclonal reactive origin for palmar fibromatosis. It appears that the proliferating fibroblastic/myofibroblastic cells responsible for the clinical manifestations of Dupuytren's disease are not neoplastic. Although it is formally possible that the fibroblastic proliferation is a secondary event related to an underlying clonal neoplastic etiology, as has been shown for the marrow fibrosis in agnogenic myeloid metaplasia,[41] no other primary process has been identified in Dupuytren's disease. It seems more likely that potent modulators of fibroblast and myofibroblast proliferation and differentiation, such as basic fibroblast growth factor and transforming growth factor-β may be stimulating nontransformed cells to form hyperproliferative, contractile nodules.[42–44] These mitogenic and growth factors have been demonstrated in the proliferative tissue of Dupuytren's disease and also may be responsible for recruiting surrounding normal aponeurotic fibroblasts, thus explaining the locally invasive nature of the disease.

Only 2 subjects were included in our study; additional cases will need to be evaluated before definitively concluding that palmar fibromatosis is a reactive process and not a benign clonal neoplasm. Several factors hinder the ability to obtain these specimens. Dupuytren's disease requiring surgical intervention is less common in women, thus limiting the number of fresh specimens suitable for analysis by X chromosome inactivation. X chromosome inactivation assays can be performed on archival paraffin-embedded specimens by comparing diseased tissue with normal tissue, but this technique is problematic since high-quality DNA is needed to achieve informative results.

We were careful to select homogeneous, representative tissue for analysis while excluding contaminating blood cells and normal tissue. With this technique, however, there is still potential contamination of lesional tissue with blood vessels or inflammatory cells derived from normal surrounding tissue or distant cellular precursors. One refinement of our technique would be to select specific cells from a sectioned specimen for analysis. While in theory this would yield highly accurate results, practical difficulties arise as many cells are needed to prevent artifactual skewing of data in a polyclonal sample.

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