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Short Communication

Ring Chromosomes in Dermatofibrosarcoma Protuberans Are Composed of Interspersed Sequences from Chromosomes 17 and 22

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Ring chromosomes are found in most dermatofibrosarcoma protuberans (DFSPs), and recent reports demonstrate that portions of the DFSP ring chromosomes derive from chromosome 17. In this study we characterized ring chromosomes in three DFSPs using a combined approach of karyotyping, chromosome painting, and comparative genomic hybridization. Chromosome painting demonstrated that the ring chromosomes in each DFSP were composed of discontinuous, interwoven sequences from chromosomes 17 and 22. Amplification of chromosomes 17 and 22 sequences was confirmed in each of these cases by comparative genomic hybridization, and over-representation of chromosomes 17 and 22 sequences was also demonstrated by comparative genomic hybridization in 1 of 2 cytogenetically unremarkable DFSPs. We conclude that amplification of chromosomes 17 and 22 sequences, in ring form, is a characteristic aberration in DFSP. (Am J Pathol 1995, 147:1553–1558)

Dermatofibrosarcoma protuberans (DFSPs) are nodular cutaneous tumors in which the most distinctive histological feature is a storiform or cartwheel-like organization.[1] Although considered generally to be of medium grade, DFSPs are associated with substantial risk of local recurrence. Distant metastases are rare, however, in the absence of dedifferentiation to a high grade fibrosarcoma. DFSPs are of uncertain histogenesis; derivation from a fibrohistiocytic precursor has been suggested by most investigators, but a neural origin has also been hypothesized.[1]

Cytogenetic analyses have been reported for 13 DFSPs,[2–9] including 11 cases with ring chromosomes.[2–6,9] Karyotypes for most DFSPs with ring chromosomes were noncomplex, containing either supernumerary ring chromosomes as solitary cytogenetic aberrations or ring chromosomes accompanied by simple chromosome trisomies. This cytogenetic profile is particularly notable because ring chromosomes in the context of noncomplex karyotypes have been associated with only three other neoplasms,[10] including myxoid malignant fibrous histiocytoma,[11] atypical lipomatous tumor,[12,13] and paraosteal osteosarcoma.[5,14] Pedeutour et al[15] demonstrated that the ring chromosomes in two DFSPs were composed partially of chromosome 17 sequences, and this observation has now been confirmed in two additional cases.[6,9] Other sequences in these same ring chromosomes did not appear to derive from chromosome 17, however, and the origin of the non-chromosome-17 components was not identified. In the present study we used a combination of cytogenetic and molecular cytogenetic ap-

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Table 1. *Clinical Data, Cytogenetic, FISH, and CGH Results for 5 DFSP*

Case	Age/sex	Site*	Size (cm)	Cytogenetics	Ring components (FISH)	CGH
1	38/F	Forehead (R)	8.5	47,XX,+r[32]/46,XX[18]	Chr 17 & 22	+17 /+22q
2	47/M	Shoulder (R)	1.9	47,XY,del(3)(q21),+r[6]/46,XY[7]	Chr 17 & 22	+17 /+22q
3	32/M	Infraclavicular (P)	2.5	49,XY,+5,+8,+r[10]†	Chr 17 & 22	+17q/+22q
4	46/F	Abdominal Wall (P)	9.5	46,XX[12]	No ring	Negative
5	52/M	Neck (P)	2.0	46,XY[20]	No ring	+17 /+22q

*R, recurrent tumor; P, primary tumor.
†Ring was closed in six cells and open in four cells (see text).

proaches to determine the origin of ring chromosomes in three DFSPs. These studies revealed an identical chromosomal derivation in each DFSP ring chromosome, and this mechanism is potentially unique to DFSP.

Materials and Methods

Five primary DFSPs were obtained immediately after resection and processed for cytogenetic and molecular cytogenetic analyses. Viable tumor material was transported to the Cytogenetics Laboratory where half of the material was frozen at −80°C. The other half of each specimen was disaggregated to single cells and established in tissue culture as described previously.[16] Metaphase cells were harvested after 3 to 7 days in culture. Harvesting conditions, slide making, and trypsin-Giemsa chromosome banding were as described previously.[16] Approximately 30% of the harvested metaphase cells were analyzed cytogenetically from each case, whereas the remaining metaphase material was stored in 3:1 methanol: acetic acid (−80°C) for subsequent fluorescent *in situ* hybridization (FISH) studies.

FISH was performed with biotin- and digoxigenin-labeled whole chromosome paints (Oncor, Gaithersburg, MD) or spectrum green- and spectrum orange-labeled whole chromosome paints (Imagenetic, Naperville, IL) according to the manufacturer's protocols. Comparative genomic hybridization (CGH) was performed, as described previously,[17] by cohybridization of biotin-labeled DFSP DNA and digoxigenin-labeled normal control DNA (200 ng of each) against normal male metaphase cells. CGH probe cohybridization was carried out in the presence of unlabeled Cot-1 DNA (20 μg) for competition of repetitive sequences. Locations of aberrant CGH signals were determined through correlation with DAPI banding and were confirmed by FLpter analysis[18] in at least five metaphase cells from each case.

Results

Cytogenetic analyses revealed ring chromosomes in three of five DFSPs (Table 1, Figure 1). A ring chromosome was the sole clonal cytogenetic aberration identified in case 1, whereas the ring chromosomes in cases 2 and 3 were accompanied by additional clonal aberrations (Table 1). Case 3 was unusual in that the ring chromosome appeared to have opened into a linear form in 4 of the 10 metaphase cells that were karyotyped (Figure 1). Because chromosome 17 sequences had been reported in ring chromosomes from three DFSPs,[6] we next evaluated ring chromosomes in our three cases using FISH with a whole chromosome 17 paint. Each DFSP ring chromosome was partially composed of chromosome 17 sequences that were interspersed with non-chromosome-17 sequences (Figure 2a). The same pattern of interspersed chromosome 17 sequences was also observed in the linearized ring chromosomes from case 3 (Figure 2b). We then evaluated the non-chromosome-17 constituents in the ring chromosomes using biotinylated whole chromosome paints (Oncor) corresponding to chromosomes 1, 3, 7, 9, 11, 12, and 22. Sequences from chromosomes 1, 3, 7, 9, 11, and 12 were not identified in any ring, whereas each ring contained sequences from chromosome 22. Dual-color FISH was then performed through cohybridization of a biotin-labeled chromosome 17 paint (fluorescein isothiocyanate (FITC) detection) and a digoxigenin-labeled chromosome 22 paint (rhodamine detection). These studies demonstrated that each DFSP ring chromosome was entirely composed of interspersed chromosome 17 and chromosome 22 sequences (Figure 2c). Although the ring chromosomes in different DFSPs varied in size, no correlation was observed between ring size and chromosome content.

CGH was performed to localize the regions on chromosomes 17 and 22 over-represented as a result of supernumerary ring chromosome formation.

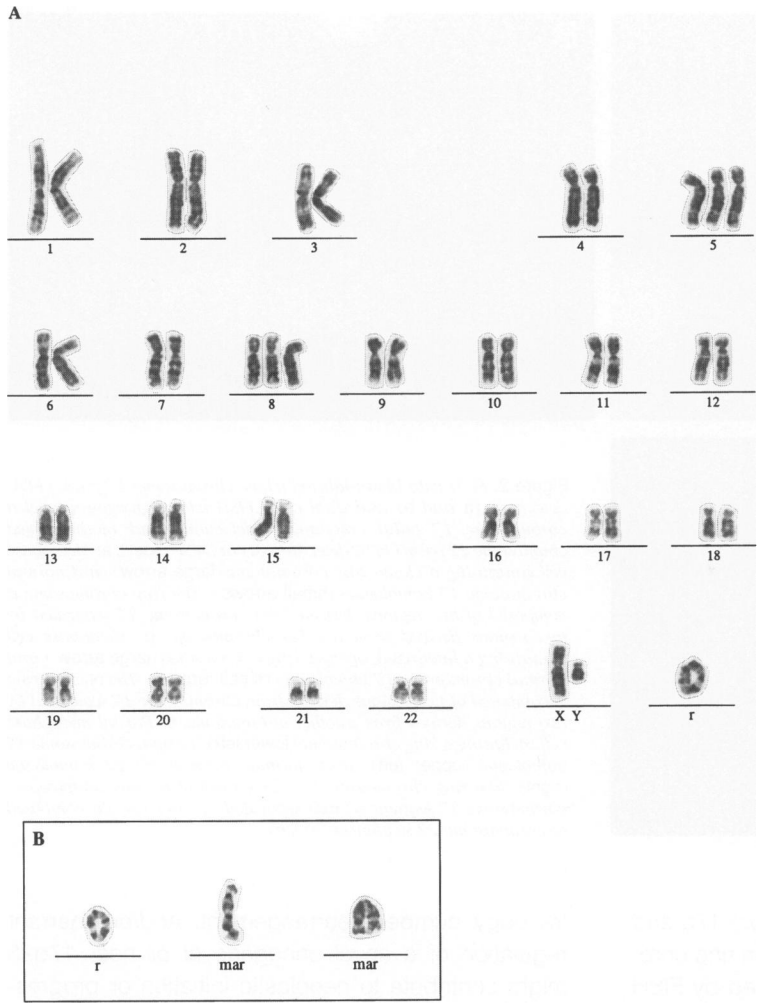

Figure 1. *Giemsa-trypsin banded karyotype of DFSP case 3 showing ring chromosome and trisomies for chromosomes 5 and 8.* Inset *at bottom shows the closed and opened ring chromosomes from three additional metaphase cells in case 3.*

Amplification of chromosome 17 and chromosome 22q sequences was found in each DFSP with demonstrable ring chromosomes (cases 1 to 3) and in one DFSP that had a diploid karyotype (case 5). The remaining DFSP had a normal karyotype and had no evidence of chromosome 17 or 22 amplification by CGH. The amplified chromosome 17 and 22q sequences could be sublocalized in DFSP case 3; in this DFSP the chromosome 17 amplification was most pronounced in the q21.3→qter region, and the chromosome 22 amplification was most pronounced in the q12→qter region (Figure 3).

Discussion

The first DFSP karyotypes were reported just 5 years ago,[2,3] and both of these karyotypes were notable for the presence of ring chromosomes. A growing body of cytogenetic data has since confirmed the striking occurrence of ring chromosomes in DFSP. Eighteen DFSP karyotypes have been reported, including those in the present study, and fourteen of these karyotypes demonstrated ring chromosomes.[2–9] The derivation of DFSP ring chromosomes was established in part by Pedetour et al,[6,15] who demonstrated chromosome 17 sequences in ring chromosomes from three DFSPs. Interestingly, however, the chromosome 17 sequences in these ring chromosomes were interrupted by sequences from another, unidentified chromosome(s). In the present study we demonstrated that each of three DFSP ring chromosomes was composed of interspersed sequences from chromosomes 17 and 22 (Figure 2). Our findings confirm those in a study by Pedeutour et al,[19] published after submission of this manuscript, in which ring chromosomes from seven DFSPs were evaluated by cytogenetic banding, FISH, and CGH. Each of the ring chromosomes in that study, as in our own series, was composed of sequences from the long arms of both chromosome 17 and chromosome 22. These findings reveal an extremely consistent genetic mechanism involving both extra copy num-

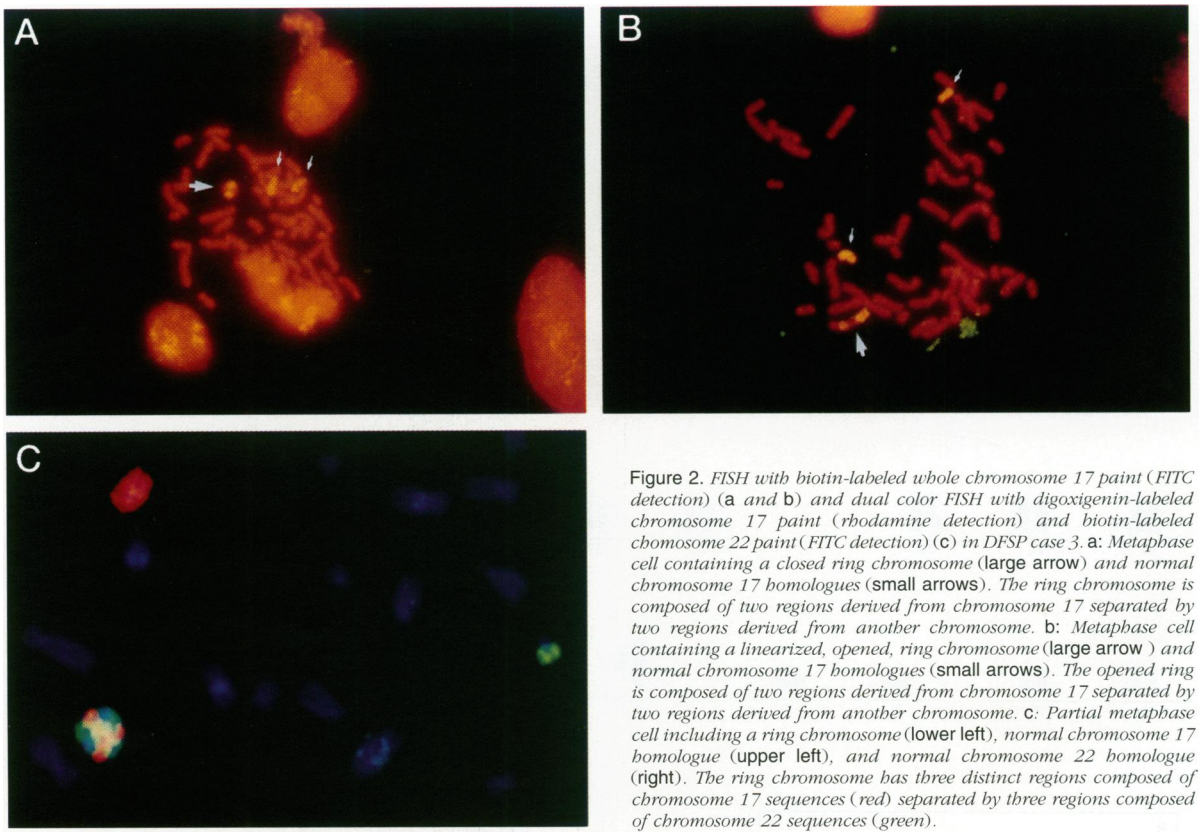

Figure 2. *FISH with biotin-labeled whole chromosome 17 paint (FITC detection)* (a *and* b) *and dual color FISH with digoxigenin-labeled chromosome 17 paint (rhodamine detection) and biotin-labeled chomosome 22 paint (FITC detection)* (c) *in DFSP case 3.* a: *Metaphase cell containing a closed ring chromosome* (large arrow) *and normal chromosome 17 homologues* (small arrows). *The ring chromosome is composed of two regions derived from chromosome 17 separated by two regions derived from another chromosome.* b: *Metaphase cell containing a linearized, opened, ring chromosome* (large arrow) *and normal chromosome 17 homologues* (small arrows). *The opened ring is composed of two regions derived from chromosome 17 separated by two regions derived from another chromosome.* c: *Partial metaphase cell including a ring chromosome* (lower left), *normal chromosome 17 homologue* (upper left), *and normal chromosome 22 homologue* (right). *The ring chromosome has three distinct regions composed of chromosome 17 sequences (red) separated by three regions composed of chromosome 22 sequences (green).*

ber and rearrangement of sequences from 17q and 22q. This mechanism, now documented in ring chromosomes from each of ten DFSPs studied by FISH and CGH, presumably contributes to deregulated cell proliferation and has not been described in any other type of neoplasia. Ring chromosomes are found in the majority of atypical lipomatous tumors,[12,13] but these ring chromosomes are composed invariably of sequences from the long arm of chromosome 12.[20–22] Ring chromosomes have also been reported in association with noncomplex karyotypes in myxoid malignant fibrous histiocytoma[11] and paraosteal osteosarcoma,[5,14] but the ring chromosomes in these entities have not yet been characterized.

The biological implications of ring chromosome 17/22 in DFSP are unknown. However, clues to potential oncogenetic mechanisms are suggested by the CGH findings in the present studies and by cytogenetic findings in some DFSPs and fibrosarcoma. CGH in DFSP case 3 demonstrated marked overrepresentation of material from the distal long arm of chromosome 17 (Figure 3); this region encompasses the 17q25 translocation breakpoint reported in one of two cytogenetically abnormal DFSPs lacking a ring chromosome.[7] These findings suggest that extra copy number, rearrangement, and/or aberrant regulation of a novel oncogene at or near 17q25 might contribute to neoplastic initiation or progression in some DFSPs. A mechanism involving extra copy numbers of gene(s) on chromosome 17 is also supported by the frequent finding of trisomy or tetrasomy 17 in infantile fibrosarcoma.[23–25]

Although supernumerary ring chromosomes are certainly the most consistent aberration in DFSP, several other cytogenetic anomalies occur nonrandomly. Two DFSPs, including case 3 in the present series, have now been reported with an identical karyotype including trisomy 5, trisomy 8, and a supernumerary ring chromosome.[6] Two other DFSPs had a supernumerary ring chromosome and trisomy 8 but lacked trisomy 5.[3,9] The latter two DFSPs both contained a cell population with trisomy 8 alone and another cell population with both trisomy 8 and the supernumerary ring chromosome. These findings suggest that trisomy 8 is a nonrandom aberration that might be acquired before formation of the ring chromosome in some DFSPs. Trisomy 8 is a relatively nonspecific aberration that has been described in many cases of infantile fibrosarcoma,[23–26] Ewing's sarcoma,[27] myxoid liposarcoma,[28] and desmoid tumor.[29,30] Thus, it is possible that extra copy

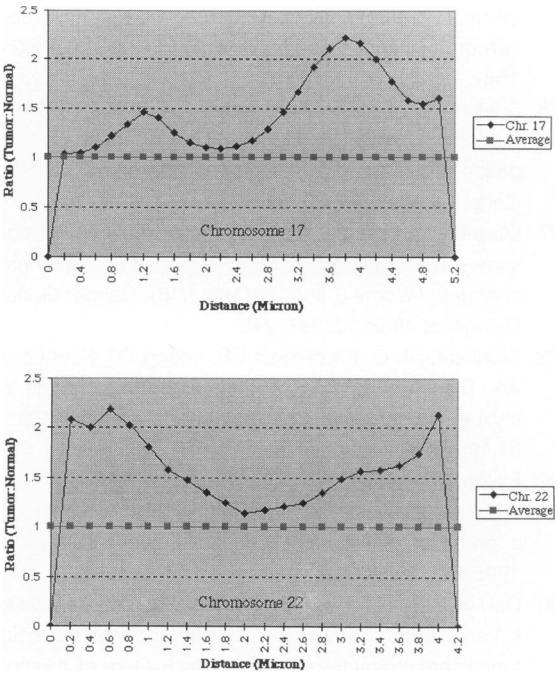

Figure 3. *CGH profiles, with pter at left and qter at right, for chromosomes 17 and 22 in DFSP case 3. Black line shows ratio of DFSP DNA (FITC) to control DNA (rhodamine) signal intensity across the designated chromosomes, whereas the gray baseline was established from average FITC:rhodamine signal intensity readings in nonamplified chromosomes from the same metaphase. Amplification of chromosome 17 and 22 sequences is most pronounced in regions corresponding to 17q21→qter and 22q12→qter, respectively. Spurious amplification is also seen in the chromosome 22 pericentromeric region as a result of incomplete competition of repetitive satellite sequences.*

numbers of gene(s) on chromosome 8 leads to enhanced proliferation in a wide variety of mesenchymal cell types.

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