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Ring 22 Chromosomes in Dermatofibrosarcoma Protuberans Are Low-Level Amplifiers of Chromosome 17 and 22 Sequences[1]

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ABSTRACT

Ring chromosomes have been found with some regularity as solid tumors have come increasingly under cytogenetic study. The full genetic content and significance of these rings remain unclear. Dermatofibrosarcoma protuberans, a tumor of the deep dermis, consistently has supernumerary ring chromosomes, sometimes as the sole detectable cytogenetic change. Using a modified method for comparative genomic hybridization and fluorescent *in situ* hybridization with a panel of various probes, we found that these ring chromosomes consistently contain the chromosome 22 centromere along with interstitial sequences from chromosomes 17 and 22, specifically from regions 17q23–24 and 22q11–12. The ring chromosomes in dermatofibrosarcoma protuberans are vehicles for a particular pattern of relatively low-level genomic amplification of selected sequences.

INTRODUCTION

The earliest record of a human ring chromosome was supplied by Levan (1) in 1956. The ring was in a tumor cell. It was thus perfectly clear from the start of human ring chromosome research that a ring could occur in a tumor cell. However, much was not immediately clear about ring chromosomes in tumor cells. Constitutional ring chromosomes usually replace normal rod chromosomes, and so they can often be easily identified. Rings in tumor cells may be supernumerary and be far more difficult to decipher. Rings are not particularly prevalent in neoplasia. Only about 3% of abnormal karyotypes in tumors contain rings (2). In given types of tumors, rings have seemed inconsistent, and, by inference, of no special consequence. However, ring chromosomes have been found with some regularity as solid tumors have come increasingly under cytogenetic study.

Rings are a feature of a group of bone and soft tissue tumors of "low or borderline malignancy" (3). The analysis of these rings has been aided by FISH[3] and related techniques. In well-differentiated liposarcoma, for instance, the characteristic rings and giant marker rods have been found to contain consistent chromosome sequences and correlate with genomic amplification (4). The studies reported here are of DP, a soft tissue tumor typically located deep in the dermis (5). DP is locally invasive and can be classed among the tumors of low or borderline malignancy with ring chromosomes (3).

The rings from six DP cases have been found, using a WCP probe to chromosome 17, to contain a consistent contribution from that chromosome (cases 1–5 in Ref. 6, case 6 in Ref. 7). Judging from the patterns of fluorescence with the WCP 17 probe, the DP rings must also contain an "alien" chromosome contribution: a contribution from some chromosome other than chromosome 17. The alien contribution was identified to be from chromosome 22 in the ring in another DP case (case 2 in Ref. 7). This result prompted us to reevaluate the presence of chromosome 22 in the cases published in Ref. 6. We have extended our knowledge of the content of these rings by determining the origin of the centromere by searching for amplified sequences using mCGH and FISH with single copy probes from targeted regions in seven DP cases, including an unpublished DP case. We have found remarkable regularity in the chromosome sequences contributing to these rings. We have also documented an unusual pattern of low-level genome amplification due to these rings.

MATERIALS AND METHODS

Tumor Samples and Karyotypes. Tumors and karyotypes from cases 1 (T91125), 2 (T91127), 3 (T92338), 4 (PM 2315), 5 (T93456), and 6 (2314) have been described elsewhere (6, 7). All cases displayed supernumerary ring chromosomes. Case 7 (T93575) was a primary tumor of the right cheek diagnosed as a grade I DP. It was removed in September 1993 from a 41-year-old male. Twenty R-banded metaphases were analyzed: 19 were normal and 1 was polyploid with two ring chromosomes.

FISH Techniques. WCP was done with probes to chromosome 22 from Oncor (Gaithersburg, MD). Centromeric probes were from Oncor. Single-copy probes from chromosomes 17 and 22 used for direct FISH on metaphase chromosomes are listed in Fig. 1. Labeling, hybridization, and detection for direct FISH were done as described (4, 8).

DNAs from tumor samples and lymphocytes from normal human males and females were extracted using standard protocols and used for mCGH analysis (9). In case 1, the low amount of extracted DNA led us to do a round of amplification by means of a degenerate-oligonucleotide-primer PCR (10) before labeling. Tumor DNAs were labeled with biotin-14-dATP (Bionick; GIBCO BRL, Eragny, France) and normal DNA with digoxin-11-dUTP (Boehringer Mannheim, Mannheim, Germany) using a nick translation kit (GIBCO BRL). Tumor and normal DNAs (200–400 ng of each) were precipitated with 100X Cot-1 DNA (GIBCO BRL), denatured 10 min at 70°C in a hybridization mixture, reannealed 2 h at 37°C, put on a slide, and a coverslip applied. After 4 days of hybridization, slides were washed and revealed with avidin-FITC, allowing detection of amplified tumor sequences exclusively. Slides were mounted with propidium iodide and diamidinophenylindole, examined using a Zeiss Axiophot microscope, and photographed on Kodak 400 EL Colorslide film or analyzed with an image processor (Perceptive Scientific International).

RESULTS

A summary of the results is presented in Fig. 1, which also includes the WCP 17 data previously reported on cases 1–6 (6, 7).

Because of lack of metaphases (growth of DP cells is often very difficult), FISH studies with centromere and single-copy probes were not done on cases 4, 6, and 7. For the same reason, cases 4 and 6 were not painted with a chromosome 22 probe.

Since ring chromosomes have been shown to result in gene amplification in well-differentiated liposarcoma (4), mCGH was done to detect amplified regions in DP rings from all cases. Amplified regions were observed in all cases except case 7. In each of four cases, (cases

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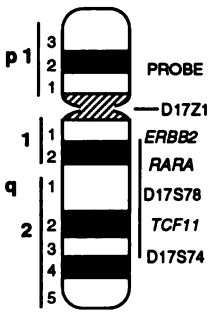
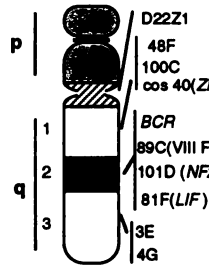
[3] The abbreviations used are: FISH, fluorescent *in situ* hybridization; DP, dermatofibrosarcoma protuberans; WCP, whole chromosome painting; mCGH, modified comparative genomic hybridization.



		CASE N°							
		1	2	3a	3	3b	4	5	6	7
PROBE	REFERENCE	FISH								
D17Z1	Oncor	0	3	0	0		ND	0	ND	ND
ERBB2	Oncor	ND[a]	ND	0	0		ND	0	ND	ND
RARA	Oncor	0	0	0	ND		ND	ND	ND	ND
D17S78	8	0	0	ND	ND		ND	ND	ND	ND
TCF11	8	ND	ND	0	0		ND	0	ND	ND
D17S74	8	5	1	2 to 5	1 to 2		ND	3	ND	ND
		WCP[b]								
		banded	diffuse	diffuse	diffuse		diffuse	banded	banded	banded
		mCGH								
		0	17q23-q24		17q23-q24		17q23-q24	17q23-q24	0	NI[c]



		FISH							
D22Z1	Oncor	1	1 or 2	1	1	ND	1	ND	ND
48F	14	2 or 3	ND	2	1	ND	1	ND	ND
100C	14	3	ND	2	1	ND	1	ND	ND
cos 40(*ZNF 74*)	15	3	1	3	2	ND	3	ND	ND
BCR	Oncor	3	ND	2	1	ND	1	ND	ND
89C(VIII F2)	16	ND	ND	ND	ND	ND	3	ND	ND
101D (*NF2*)	17	2	1 or 2	2	1	ND	1	ND	ND
81F(*LIF*)	16	1 to 3	1	0	0	ND	1	ND	ND
3E	18	0 or 1	0	0	0	ND	0	ND	ND
4G	Sanson, unpublished	ND	ND	0	0	ND	0	ND	ND
		WCP							
		banded	1 band	diffuse	diffuse	ND	banded	ND	banded
		mCGH							
		22q11-12	22q11-12		22q11-12	22q11-12	22q11-12	22q11-12	NI

Fig. 1. Molecular cytogenetics from seven DP cases. On the ring chromosomes: copy number status of probes from 17q and 22q and summarized results obtained after WCP with chromosomes 17 and 22 probes. In case 3, *3a* and *3b* indicate the two different rings positive with the WCP 17 and 22 probes. Chromosomal localization of amplified regions as shown by mCGH. Footnote *a*: *ND*, not done; footnote *b*: see WCP 17 data in Refs. 6 and 7 (cases 1–6); footnote *c*: *NI*, not informative (see text).

2–5), there were two bright fluorescent regions corresponding to overrepresentation of 17q23–24 and 22q11–12 (Fig. 2), while in two cases (case 1 and 6) only the bright region corresponding to 22q11–12 was evident. In case 7, mCGH did not detect an amplified region on any chromosome. This negative result was considered uninformative since we believe that the very low ratio of abnormal cells:normal cells (1:20 as shown by the cytogenetic study) reduced amplification to an undetectable level.

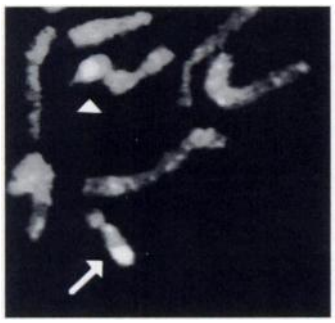


Fig. 2. Representative chromosomes 17 and 22 after mCGH with DNA from case 3, showing amplified regions 17q23–24 (*arrow*) and 22q11–12 (*arrowhead*). The tumor DNA was revealed by FITC. The chromosomes were counterstained by propidium iodide.

To determine the chromosomal content and organization of sequences from chromosome 22 in the rings, WCP was done and compared to the chromosome 17 painting previously done (Refs. 6 and 7; Fig. 1). The WCP 22 probe gave a positive signal on rings in all five cases tested. In case 3, a small unidentified marker rod chromosome was also found to be positive with a WCP 22 probe and proved to be a chromosome 22 with partial deletion of the long arm: del (22)(q12). WCP 22 probes sharply delineated some regions in rings, producing a banded pattern (cases 1, 5, and 7) or, as in case 2, a unique band confined to the region adjacent to the 22 centromere. However, in case 3, the WCP 22 probe produced a fuzzy diffuse pattern superimposed on WCP 17 regions. The precise positions of chromosome 17 and chromosome 22 areas in these regions were difficult, if not impossible, to determine. Therefore, we cannot rule out the possible presence on rings of sequences from chromosomes other than 17 and 22.

All tested cases displayed a positive signal to chromosome 22 centromere. Only one (case 2) had centromeres from both chromosomes 17 and 22.

To begin to investigate the content and the borders of the amplified regions, we performed FISH with probes to 17q and 22q on the metaphases of cases 1, 2, 3, and 5 (Fig. 1). None of the 17q probes proximal to D17S74 were present on the rings. D17S74 probe, located

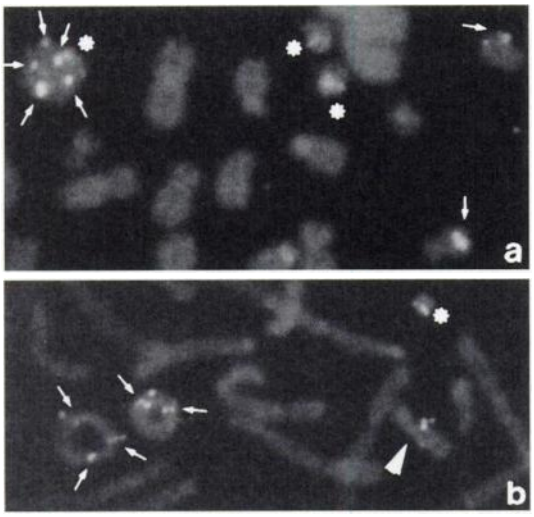


Fig. 3. *a*, FISH with D17S74 probe (*small arrows*) and centromeric D14Z1/D22Z1 probe (*asterisks*) on a partial metaphase from case 1. Five copies of D17S74 probe are shown on the ring. *b*, FISH with cos 40 (*ZNF74*) probe on a partial metaphase from case 3. Two and three copies of *ZNF74* (*small arrows*) are shown on the two-ring chromosomes. Normal chromosome 22, *arrowhead*; del(22)(q12), *asterisk*.

in 17q23 (Fig. 3*a*), was consistently present on the rings. In case 2 only one spot per ring was observed. Other cases showed an amplification (three to five copies per ring). With regard to chromosome 22 probes, the cos 40 probe containing the *ZNF74* gene located in the 22q11 region was the one corresponding to the highest number of copies (one to three copies per ring, which means five extra copies in case 3 which consistently has two rings; Fig. 3*b*). Probes to the distal region 22q (probes 4G and 3E) were not observed on the rings, except 2 rings/10 with one spot for probe 3E in case 1.

DISCUSSION

Shared features of the rings include the presence of chromosome 22 centromere sequences, the association in the rings of amplified chromosome 17 and 22 sequences, multiple copies of D17S74 (17q23) and probes located in 22q11–12, and loss of the distal region from 22q. Aside from these common characteristics, some differences in the cases were observed: 81F was not present on the rings and on the deleted chromosome 22 from case 3. It was present in one copy in cases 2 and 5 and in one to three copies in case 1. Case 2 illustrates the complexity of the ring chromosomes in DP. Although it contains the chromosome 17 centromere and the 17q23–24 region appeared to be overrepresented by means of mCGH, this ring did not contain probes from the 17q21–22 region such as *RARA* or *TCF11* (17q21).

In most cases, gene amplification was detected at a low level, with a maximum of five copies per ring and a rather good agreement between mCGH and FISH results. An exception was case 1, which showed the greatest level of amplification (using the D17S74 probe), but no evidence for chromosome 17 overrepresentation by mCGH. An explanation for this discrepancy may be that, in spite of a low 5-fold amplification, the detection of the amplified region was under the limit of detection of mCGH because the amplification unit was too small. The converse involved case 2, which gave a positive signal by mCGH at 17q23–24 but only one spot by FISH with the D17S74 probe. Such a result may be due to the borderline localization of D17S74, present in chromosome 17 sequences but not belonging to the amplification units.

The noteworthy association between chromosomes 17 and 22 in ring chromosomes from DP suggests that the primary event could be a translocation (or insertion) associating two genes respectively from 17q and 22q followed by ring formation and duplications within the rings. Recently, Minoletti *et al.* (7) have described the karyotype of a DP case with loss of a chromosome 22 and two copies of a derivative chromosome 22 containing chromosome 17 sequences. Another translocation t(2;17)(q33;q25) investigated by conventional cytogenetics has been reported in a DP case (11). It should be worthwhile to investigate this case for involvement of chromosome 22 at the molecular level.

Rings in DP containing part of chromosomes 17 and 22 are obviously constituted by mechanisms other than simple circularization of one chromosome (like the classical description of constitutional rings, with loss of telomeres). In tumors, ring chromosomes can be a means of gene amplification. For example, in well-differentiated liposarcoma, rings that appear acentric produce amplification of *MDM2* (sometimes up to 50 copies) along with various nonsyntenic sequences (4). In DP, the ring structure appears to be different from that observed in well-differentiated liposarcoma: (*a*) the level of amplification detected is low in DP, (*b*) the DP rings bear at least one 22 centromeric sequence, and (*c*) chromosome 17q23–24 and chromosome 22q11–12 sequences are consistently associated in DP rings.

The proximal 22q region has often shown alterations in tumors, particularly from tumors of neural origin (12). The 17q22–24 region has been recently shown to be strongly amplified by comparative genomic hybridization in breast cancer (8, 13). Their juxtaposition and overrepresentation in DP rings and translocations could reflect a rearrangement involving two specific sequences.

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