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Translocation of Oncogene c-*sis* from Chromosome 22 to Chromosome 11 in a Ewing Sarcoma-Derived Cell Line

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Somatic cell hybrids, obtained after fusion of translocation (11;22)-positive Ewing sarcoma cells and Chinese hamster fibroblasts, were assayed for the presence of immunoglobulin Cλ, Philadelphia chromosome breakpoint cluster region, and c-*sis* oncogene sequences. It was found that c-*sis* was translocated from chromosome 22 to chromosome 11 in the Ewing sarcoma cells used, indicating that the breakpoint must be proximal to this locus. Moreover, we found that the chromosome 22-linked Cλ and breakpoint cluster region sequences are not translocated. This result confirms an earlier cytogenetic observation that the Ewing sarcoma-associated breakpoint in chromosome 22 is distal to those observed in translocation (8;22)-positive Burkitt lymphoma and in Philadelphia chromosome-positive chronic myeloid leukemia.

During the last two decades, a number of acquired chromosomal abnormalities have been found to be specifically associated with particular human cancers (19, 20). At present, the Philadelphia (Ph^1) translocation (9;22)(q34;q11) in patients with chronic myeloid leukemia and the Burkitt lymphoma-associated translocations (8;14)(q24;q32), (2;8)(p12;q24), and (8;22)(q24;q11) are the best-documented examples. In the Ph^1 translocation, a cellular oncogene, c-*abl*, consistently translocates from chromosome 9 to chromosome 22 (2, 8, 13, 14). In reverse, another oncogene, c-*sis*, translocates from 22 to 9 in the classical Ph^1 translocation (11) or to various other chromosomes involved in Ph^1 variants (3). The breakpoint on chromosome 22, which is relatively far away from the c-*sis* locus, appears to be very consistent in different chronic myeloid leukemia patients (12) and occurs within a so-called breakpoint cluster region (bcr). This region spans no more than 5 kilobases (kb). In t(8;14)-positive Burkitt lymphoma, the c-*myc* oncogene translocates from chromosome 8 to the immunoglobulin heavy chain gene cluster on chromosome 14 (7, 9, 22), whereas in t(2;8)- and t(8;22)-positive BL this gene remains on chromosome 8. Instead, immunoglobulin kappa and lambda light chain constant region sequences translocate from chromosome 2 and chromosome 22 to chromosome 8, respectively (6, 10, and our own unpublished observations). It is supposed (2, 6–10, 12–14, 22) that due to transposition, the involved oncogene becomes activated and that such activation is a crucial step in the induction of the transformed state of the involved cell. Very recently, a specific chromosomal translocation (11;22)(q24;q12) has been reported in primary Ewing sarcomas (ESs) and in various derived in vitro established cell lines (1, 23). Since the oncogene c-*sis* has been localized in a region on chromosome 22 (3) in which the breakpoint in ES cells is observed, the localization of this oncogene, as well as bcr and lambda light chain constant region sequences, relative to the ES-associated breakpoint in 22 was studied.

IARC-EW2 is an in vitro established cell line derived from a blood culture of a patient suffering from ES with circulating metastatic cells (23). This cell line carries the ES-associated chromosomal translocation (11;22)(q24;q12) in 100% of the metaphases. IARC-EW2 cells were fused with thymidine kinase-deficient (TK^-) Chinese hamster a3 fibroblasts (24) by standard procedures, with Sendai virus as fusogen. Hybrids were selected in HAT medium (17). In contrast to the hybrid clones, IARC-EW2 cells grew in suspension and were removed by frequent medium changes. Chinese hamster and derived hybrid cells were grown in F10 medium, and IARC-EW2 cells were grown in RPMI 1640 medium. Cultures were supplemented with 10% fetal calf serum, glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). Air-dried chromosome preparations were analyzed by using R-banding with acridine orange. At least 16 metaphases of each hybrid clone were studied. Cell lysates, derived from the same passage, were assayed by cellulose acetate gel (Cellogel) electrophoresis (18) for the presence of the chromosome 11-encoded isoenzyme lactate dehydrogenase A (EC 1.1.1.27) and the chromosome 22 marker mitochondrial aconitase (EC 4.2.1.3). Parental and hybrid cell DNAs were digested to completion with restriction enzymes according to the suppliers' specifications. The cleaved DNAs were subjected to electrophoresis through 0.7% agarose gels, denatured, and subsequently transferred to nitrocellulose filters essentially as described by Southern (21). Baked filters were hybridized with nick-translated probes, washed to 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 65°C, and exposed to X-ray film. As immunoglobulin lambda light chain constant region (Cλ) probe, a 1.6-kb *Bgl*II-*Hin*dIII fragment (12) isolated from Huλ5 (15) was used. A 1.7-kb *Bam*HI fragment prepared from a c-*sis*-containing cosmid clone (11) was used as probe to detect this oncogene, and a 1.4-kb *Hin*dIII-*Sst*I fragment isolated from the Ph^1 chromosome-associated bcr (12) was used as bcr-specific probe.

Ten hybrid clones were selected for the present study. Only results dealing with chromosomes 11 and 22 and their translocation derivatives are given. Other (aberrant) human chromosomes, present in most of the hybrid clones, are not considered. All 10 hybrids tested (Table 1) had retained chromosome 11 material and they were, as expected, positive for its isoenzyme marker lactate dehydrogenase A. Moreover, all hybrid clones were positive for the isoenzyme

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TABLE 1. Presence (+) or absence (−) of human chromosomes 11, 11q+, 22 or 22q−, the isoenzyme markers lactate dehydrogenase A (LDHA) (chromosome 11) and mitochondrial aconitase (ACO2) (chromosome 22), and c-*sis* oncogene sequences, Cλ sequences, and Ph[1] bcr sequences in human ES × Chinese hamster somatic cell hybrids

No. of hybrid clones	Human chromosomes				Isoenzymes		c-*sis*	Cλ	bcr
	11	11q+	22	22q−	LDHA	ACO2			
1	+	−	+	−	+	+	+	+	+
2	+	+	−	−	+	+	+	−	−
4	−	+	−	−	+	+	+	−	−
1	+	+	−	+	+	+	+	+	+
1	+	−	+	+	+	+	+	+	+
1	−	+	+	+	+	+	+	+	+

marker mitochondrial aconitase (chromosome 22), including those which had retained only the 11q+ chromosome (four clones) or 11 and 11q+ (two clones). The latter result confirms the translocation of chromosome 22 material to chromosome 11 in IARC-EW2 cells. By Southern blot analysis, the location of the chromosome 22 breakpoint in IARC-EW2 was determined relative to breakpoints known to occur specifically in t(8;22)-positive Burkitt lymphoma and t(9;22)-positive (Ph[1]+) chronic myeloid leukemia. The latter two breakpoints have been localized proximal to Cλ sequences and within bcr on chromosome 22, respectively (6, 12). Human, Chinese hamster, and hybrid cell DNAs were digested with *Eco*RI and *Bgl*II and subsequently hybridized to Cλ and bcr probes. Two major fragments of 8 and 16 kb were detected by the Cλ probe in *Eco*RI-digested IARC-EW2 DNA (Fig. 1a, lane D). A 14-kb hybridizing band, which is also present, is unresolved from the stronger hybridizing 16-kb band in Fig. 1a. Such a pattern is in agreement with the presence of non-rearranged λ light chain loci in this cell line. No hybridization with Chinese hamster DNA was observed (Fig. 1a, lane C) under the stringent conditions used. All hybrids (Table 1) containing the 11q+ derivative chromosome in the absence of chromosome 22 or 22q− were negative for Cλ (Fig. 1a, lane B), whereas hybrids containing 22q− (lane A) or 22 were positive for these sequences. This result indicates that Cλ sequences are not included in the translocation to 11q+ and that, therefore, the chromosome 22 breakpoint in IARC-EW2 cells must be distal to this locus. Human K562 DNA showed very strong 8- and 16-kb hybridizing bands (Fig. 1a, lane E), confirming the previously observed amplification of the Cλ locus (in germ-line configuration) in this cell line (14). The 5-kb *Bgl*II bcr fragment in IARC-EW2 is also non-rearranged (Fig. 1b, lane D), whereas in the Ph[1]-positive K562 cell line (lane E) an obvious rearrangement in this region has occurred in one of the chromosome 22 homologs, giving rise to an extra hybridizing fragment of ca. 8 kb. It is apparent that this 8-kb fragment is amplified, which again is in agreement with the known amplification of this Ph[1]-associated breakpoint area (also including c-*abl* sequences [not shown]) in this particular cell line (14). Chinese hamster a3 DNA did not hybridize significantly to the bcr probe (Fig. 1b, lane C) under the stringent conditions applied. Hybrids containing the 11q+ chromosome in the absence of chromosome 22 or 22q− (Table 1) were negative with the bcr probe (Fig. 1b, lane B), whereas hybrids containing both 11q+ and 22q− (lane A) or a normal chromosome 22 did hybridize to this probe. These results indicate that the chromosome 22 breakpoint in IARC-EW2 cells must be distal to that observed in Ph[1] translocation-positive chronic myeloid leukemia since it is known that in the latter cells the break consistently occurs within the 5-kb *Bgl*II bcr fragment, resulting in translocation of at least a part of this area to another chromosome (usually no. 9). Figure 2 shows results obtained with the c-*sis* oncogene probe. In *Eco*RI-digested DNA from IARC-EW2 (and K562) cells, a single 21-kb hybridizing band was found (Fig. 2, lanes E and F), which is in agreement with a non-rearranged configuration of this part of the c-*sis* gene in IARC-EW2 cells. In similarly digested Chinese hamster a3 DNA, a 2.7-kb hybridizing band was observed (Fig. 2, lane D) which clearly resolves from the c-*sis* hybridizing band in human DNA. All hybrids tested (Fig. 2 and Table 1) were positive

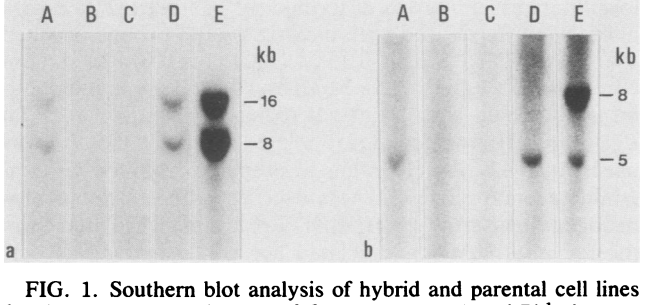

FIG. 1. Southern blot analysis of hybrid and parental cell lines for the presence or absence of Cλ sequences (a) and Ph[1] chromosome bcr sequences (b). The Ph[1]-positive cell line K562 is also included (lanes E). Lanes A, hybrid containing 11q+ and 22q− translocation chromosomes and a normal chromosome 11; B, hybrid containing 11q+ translocation chromosome; C, Chinese hamster a3; D, human IARC-EW2 parental cell lines. (a) *Eco*RI-digested DNAs; (b) *Bgl*II-digested DNAs (ca. 10 μg per lane). Molecular weights were deduced from co-electrophoresed *Hin*dIII- and *Hin*dIII-*Eco*RI-digested λ DNA.

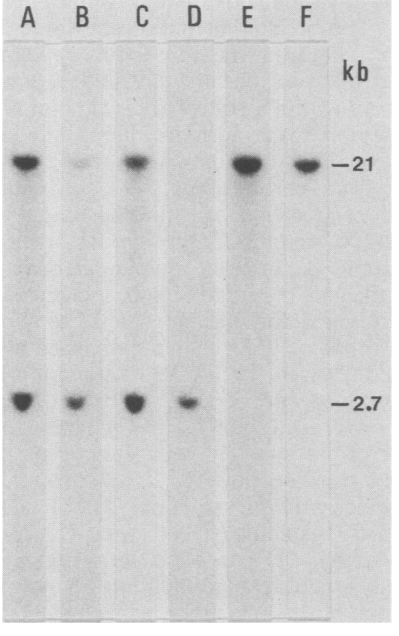

FIG. 2. Southern blot analysis of *Eco*RI-digested hybrid and parental cell DNAs (ca. 10 μg per lane) for the presence of c-*sis* oncogene sequences. Lanes: A, hybrid positive for 11q+ and 22q− translocation chromosomes and a normal chromosome 11; B and C, hybrids containing the 11q+ chromosome; D, Chinese hamster a3; E, human IARC-EW2 parental cell lines; F, Ph[1]-positive K562 cell line. Molecular weights were deduced from co-electrophoresed *Hin*dIII- and *Hin*dIII-*Eco*RI-digested λ DNA.

for human c-*sis* sequences, irrespective whether the 11q+ chromosome alone was present or whether this chromosome was present together with 22q− or a normal chromosome 22. These results indicate that c-*sis* is translocated from chromosome 22 to chromosome 11q+ in IARC-EW2. Similar results with c-*sis* were obtained when the probe was hybridized to *Bam*HI-digested parental and hybrid cell DNAs (data not shown).

The observed transposition of c-*sis* did not result in rearrangement of (at least) the v-*sis* hybridizing region (16) of the c-*sis* locus (21-kb *Eco*RI fragment). A similar observation has been made by Bechet et al. (4). Moreover, these authors found that c-*sis* is not expressed at a significantly high level in ES cells (4). Therefore, despite its transposition, it remains unclear whether c-*sis* is actively involved in the generation of ES. The latter findings, however, do not necessarily exclude an involvement of this oncogene in ES since in the Ph^1-positive cell line K562, for example, it has been shown that the chromosome 9 breakpoint may be located at a distance of over 100 kb upstream from the most 5′ v-*abl* hybridizing sequence of c-*abl* (G. Grosveld et al., unpublished data). It should also be noted that the results described here are based on a single sarcoma derived cell line. Establishment of possible variations in chromosomal breakpoints among ES patients, as has been observed, e.g., in Burkitt lymphomas, obviously requires the investigation of more cell lines or primary tumors.

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