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Comparative Mapping of the Constitutional and Tumor-associated 11;22 Translocations

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Summary

The reciprocal t(11;22)(q23;q11) is the most common non-Robertsonian constitutional translocation in humans. The tumor-associated 11;22 rearrangement of Ewing sarcoma (ES) and peripheral neuroepithelioma (NE) is cytologically very similar to the 11;22 constitutional rearrangement. Using immunoglobulin light-chain constant region, ETS1 probes, and the technique of in situ hybridization, we previously were able to show that the constitutional and ES/NE breakpoints are different. As a first step toward isolating these translocation junctions and to further distinguish between them, we have made somatic cell hybrids. Cells from a constitutional 46,XX,inv(9),t(11;22) carrier and from an ES cell line with a t(11;22) were separately fused to a hypoxanthine-guanine phosphoribosyltransferase–deficient Chinese hamster cell line (RJK88). Resulting clones were screened with G-banding and Southern hybridization. Hybrid clones derived from the constitutional t(11;22) were established which contained the der(22) and both the der(22) and the der(11). Hybrid clones derived from the ES cell line containing the der(11) were isolated. Using the technique of Southern hybridization we have sublocalized the loci; ApoA1/C3, CD3D, ETS1, PBGD, THY1, D11S29, D11S34, and D11S147 to the region between the two breakpoints on chromosome 11 and $V_{\lambda I}$, $V_{\lambda VI}$, $V_{\lambda VII}$, and D22S10 to the region between the breakpoints on chromosome 22. Using anonymous DNA probes, we found that D22S9 and D22S24 map proximal to the constitutional breakpoint and that D22S15 and D22S32 map distal to the ES breakpoint on chromosome 22.

Introduction

Since the advent of chromosome banding (Caspersson et al. 1970), it has been possible to show that cytogenetic abnormalities are associated with human syndromes and malignant disorders. Several of these chromosomal rearrangements are disease specific as well as site specific. Of particular interest are the 11;22 reciprocal translocations with breakpoints in regions 11q23-24 and 22q11-12. These are observed in two categories of chromosomal abnormalities, constitutional (Fraccaro et al. 1980; Zackai and Emanuel 1980; reviewed by Schinzel 1983, pp. 718–746) and acquired (Aurias et al. 1984; Turc-Carel et al. 1984; Whang-Peng et al. 1984). Cytogenetically, even with the application of high-resolution techniques, the breakpoints of these translocations are difficult to distinguish from one another (fig. 1). However, chromosomal in situ hybridization data suggest that the breakpoints for the constitutional and the tumor-associated t(11;22)'s are different at the molecular level (Griffin et al. 1986).

The constitutional t(11;22)(q23;q11) is the most frequent, recurrent, non-Robertsonian translocation and has been described in more than 150 independent families (Fraccaro et al. 1980; Zackai and Emanuel 1980). The carriers of this balanced translocation have the t(11;22) in every cell and are phenotypically normal. Their chromosomal rearrangement is detected either following the birth of a malformed offspring carrying the der(22) as a supernumerary chromosome or as the result of infertility in the balanced carrier. Constitutional t(11;22) carriers do not appear to be at an increased risk for cancer.

The acquired t(11;22) is found in the tumor cells of

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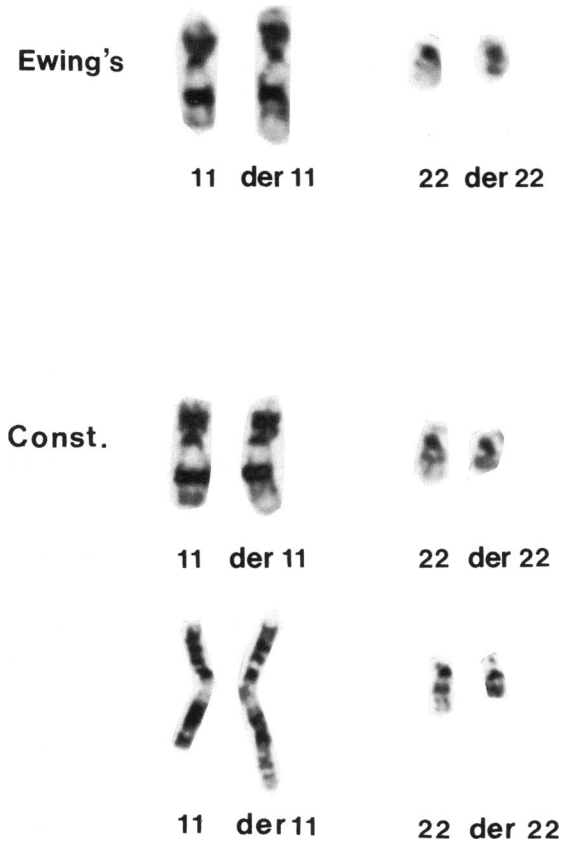

Figure 1 Partial karyotypes of the constitutional and ES-associated t(11;22)'s, showing chromosome-11 and chromosome-22 pairs. The upper two partial karyotypes are shown at similar 400-band resolution. High-resolution examples of the constitutional t(11;22) are shown in the lower panel.

Ewing sarcomas (ES) and peripheral neuroepitheliomas (NE) (Aurias et al. 1984; Turc-Carel et al. 1984; Whang-Peng et al. 1984). Both of these tumor types are composed of primitive, small, round cells and occur in the trunk or extremities. NE is thought to be neuronal in origin. However, the tissue of origin for ES is uncertain. Histologically the two tumor types appear to be somewhat distinct, but a recent study suggests that they both express the same set of protooncogenes and at least one common neuronal enzyme (McKeon et al. 1988). Patients with these tumors have a normal constitutional karyotype, but at least 90% (Heim and Mitelman 1987, p. 229) have the 11;22 translocation in their tumor cells. The consistent finding of this translocation in these tumors suggests that this rearrangement may play an important role in the development of the disease. By analogy with the t(9;22) rearrangement of chronic myelogenous leukemia (CML), where the 3′ region of the ABL oncogene is translocated into the 5′ region of the BCR1 gene on chromosome 22 to form a hybrid gene (Konopka et al. 1984; Shtivelman et al. 1985; Stam et al. 1985), the t(11;22) may cause the rearrangement of two cellular genes to form an oncogenic chimeric gene. Alternatively, the tumor-associated t(11;22) could alter expression of a normal cellular gene by changing its molecular environment, as has been suggested in the t(8;22) rearrangement associated with some cases of Burkitt lymphoma (Haluska et al. 1987).

As a first step in determining what features, if any, the constitutional and acquired translocations share, we have made somatic cell hybrids by fusion of Chinese hamster cells with cell lines containing either a constitutional or an ES-associated t(11;22). We have characterized hybrid clones that contain the relevant derivative 11 and/or 22 chromosomes. These hybrids have been used to sublocalize several genes and anonymous DNA probes which had previously been regionally mapped to distal 11q or proximal 22q. The results of these studies clearly demonstrate that the constitutional and tumor-associated 11;22 translocations are separated by a considerable molecular distance in both 11q23 and 22q11.

Material and Methods

Cells and Media

The Chinese hamster cell line used as a fusion parent was RJK88. This fibroblast cell line is HGPRT deficient as a result of gene deletion (Fuscoe et al. 1983). It was maintained in Dulbecco medium (DMEM) supplemented with 15% FBS.

The constitutional t(11;22) carrier is a phenotypically normal female whose karyotype is 46,XX,inv(9), t(11;22)(q23;q11). We have established fibroblast (GB-F) and lymphoblastoid (GB-L) cell lines from this balanced translocation carrier. Cultures are maintained in RPMI 1640 medium supplemented with 15% FBS.

The patient with the acquired t(11;22) was an 18-year-old female with an undifferentiated small, round cell tumor of mesenchymal origin consistent with ES. A cell line, TW, was established from the tumor cells. It has a karyotype of 53,XX,+4,+5,+8,+9,+13, +del(15)(q11q15),t(11;22)(q23-24;q11-12),+mar. The patient had a normal constitutional karyotype, 46,XX. Tumor cell cultures were maintained in DMEM supplemented with 15% FBS.

GM5565, a normal human fibroblast cell line (Coriell

Institute for Medical Research) with 46,XY karyotype was maintained in RPMI 1640 with 15% FBS and was used as a source of normal human DNA.

Eye F3A6 is a human/hamster hybrid that contains a normal chromosome 22 as the only intact human chromosome (Van Keuren et al. 1987). This cell line was maintained in Ham's F12 with 8% dialyzed FBS.

Isolation of Somatic Cell Hybrids

Lymphocytes from the constitutional t(11;22) carrier (GB lymphocytes) were purified from whole blood by using a Ficoll-Paque gradient (Pharmacia). Lymphocytes (5×10^7) were suspended in Hanks's balanced salt solution (BSS) and were mixed with 10^7 cells from RJK88 also in Hanks's BSS. Cells were centrifuged, and the cell pellet was resuspended and mixed with 50% PEG (PEG 1500; Boehringer Mannheim), and then cells were plated, in DMEM with 15% FBS, into 10 100-mm plates. Selection for hybrids was accomplished in HTZ medium (hypoxanthine, 1×10^{-4} M; azaserine, 3×10^{-5} M; thymidine 2×10^{-5} M). Single clones were isolated and analyzed for presence of the relevant chromosomes. The cell fusions of TW with RJK88 were performed as described for GB × RJK88, with the exception that monolayer cultures of TW were treated with 0.05% trypsin and 0.02% EDTA to obtain a single cell suspension. Ouabain (5×10^{-5} M) was used to select against the human TW parental cells.

Cytogenetic Analyses

G-banding using modified trypsin-Wright's staining (Francke and Oliver 1978) was performed on human cell lines and somatic cell hybrids. Giemsa-11 staining to differentiate between human and hamster chromosomes (Alhadeff et al. 1977) was performed on metaphase chromosome spreads from somatic cell hybrid clones of GB × RJK88 and TW × RJK88.

DNA Isolation

High-molecular-weight DNA was isolated by one of two methods. The first involved isolation of cell nuclei by cell lysis using TX-100 (Aldridge et al. 1984) followed by SDS/proteinase K digestion, phenol extraction, and ethanol precipitation. Alternatively, whole-cell DNA was extracted by direct cell lysis into SDS/proteinase K followed by phenol extraction and ethanol precipitation (Blin and Stafford 1976). DNA was resuspended in 10 mM Tris pH 7.5, 1 mM EDTA (TE) and was stored at 4°C.

Peripheral blood lymphocytes from laboratory personnel were isolated from whole blood, using Ficoll-Paque gradients (Pharmacia, NJ). DNA was extracted as described above. These samples served as control DNA.

Southern Blot Analysis

DNA was digested with the appropriate restriction enzymes, using conditions recommended by the manufacturer (New England Biolabs, Beverly, MA). Digested DNA samples were separated by agarose gel electrophoresis using Tris-borate buffer and were transferred to Zetabind™ (CUNO Inc., Meriden, CT) by using the method of Southern (1975). Plasmids containing human DNA inserts as probe were digested with the appropriate restriction enzymes, and fragments were purified by agarose gel electrophoresis using low-melt agarose. DNA probes, in low-melt agarose, were labeled with [α-^{32}P] dCTP by using the random primer method (Feinberg and Vogelstein 1984). Hybridization of filters was at 42°C, using 50% formamide (Maniatis et al. 1982, pp. 387–389) followed by two high-stringency washes, 0.2 × SSC and 0.1% SDS, at 65°C. Filters were exposed to Kodak XAR-5 film by using DuPont Lightening Plus® screens at −70°C for varying lengths of time.

Probes

Numerous probes for cloned genes and for anonymous DNA segments were used in this study. The specific probes and the appropriate references that describe their derivation are shown in table 1. This list includes probes for 11q and 22q.

Results

Characterization of Constitutional t(11;22) Hybrid Clones

Nineteen clones were obtained from GB × RJK88 fusion, and five were analyzed by G-11 and/or G-banding. Two that appeared to have either the der(11), the der(22), or both in the absence of the relevant normal human homologues will be described. Metaphase spreads of cl 4 showed that the only human chromosomes present appeared to be the X and the der(22). By similar cytogenetic analysis, cl 7-2 was found to have X, der(11), 16, der(22), and inv(9). Southern blot analysis was used as an independent method to check for the presence of der(11) and der(22). The presence of proximal and distal 11q sequences was assessed using BCL1, which maps to 11q13 (Tsujimoto et al. 1985*b*), and ETS1, which maps to 11q23 (de Taisne et al. 1984), respectively. The presence of the chromosome 22 centromere and distal long arm was assessed with p22/1

Table 1

DNA Probes Used for Hybrid Characterization and Subregional Localization

Locus	Probe Name	Insert Size (kb)	Reference
APOAI	pSV2-A-I	pBR322/2.0/*Pst*I	Karathanasis et al. 1982
APOC3	pcIII-606	pKT218/0.4/*Pst*I	Karathanasis et al. 1983
BCL1	pRH3.2	pUC19/2.1/*Sst*I	Tsujimoto et al. 1985*b*
CD3D($T_3\delta$)	pPGBC9	pSP64/0.7 kb/*Pst*I/*Xho*I	van den Elsen et al. 1984
D11S29	L7	pBR322/10.2/*Bam*HI	Warnich et al. 1986
D11S34	phi2-11-2.2	EMBL3/11/*Bam*HI	Malsen et al. 1988
D11S35	phi 2-22	EMBL3/15/*Bam*HI	Maslen et al. 1988
D11S83	p2-25	EMBL3/16.5/*Sal*I	Maslen et al. 1988
D11S85	p6-3	EMBL3/10.3/*Sal*I	Maslen et al. 1988
D11S129	CRI-R365	Ch4A/16/*Eco*RI	Dõnis-Keller et al. 1987
D11S147	pHBI18P2	pUC18/4.0/*Pst*I	Nakamura et al. 1988
D11S148	pYNB3.12	pUC18/2.4/*Acc*I	Leppert et al. 1987
D22S9	p22/34	pUC8/1.85/*Eco*RI	McDermid et al. 1986*a*
D22S10	22C1-18	pUC12/0.5/*Sau*IIIA	Hofker et al. 1985
D22S15	DP22	pBR322/0.6/*Eco*RI	Van Keuren et al. 1987 Rouleau et al. 1988
D22S24	W12G	Ch21A/5.6/*Hin*dIII	Rouleau et al. 1989
D22S32	pEFZ31	pUC18/3.1/*Acc*I	Krapcho et al. 1988
D22Z2	p22/1	pUC8/2.8/*Eco*RI	McDermid et al. 1986*b*
ETS1	pHE5.4	pKH47/5.4/*Eco*RI	de Taisne et al. 1984
IGLC	pλ8	pBR322/8/*Eco*RI	Croce et al. 1983
IGLV I	pHVλ6	pBR322/0.2/*Eco*RI	Tsujimoto and Croce 1984
IGLV VI	pLB1.3	pUC8/0.3/*Alu*I	Anderson et al. 1985
IGLV VII	pHV0.6	pUC8/0.6/*Bam*HI/*Bgl*II	Anderson et al. 1984
PBGD	PBGD 0.9	pGEM1/0.9/*Bam*HI/*Eco*RI	Raich et al. 1986
PDGFB	...	pBR322/1.7/*Bam*HI	Dalla Favera et al. 1982
THY1	pSP64-HT	pSP64/1.0/*Bam*HI/*Pst*I	Seki et al. 1985

(D22Z2) (McDermid et al. 1986*b*) and PDGFB (Sis) probes, which map to 22 centromere and 22q13, respectively. Cl 4 was positive for D22Z2 (22 centromere) and ETS1 (distal 11q) but was negative for BCL1 and PDGFB. This supports the presence of der(22) and absence of normal 11 or 22 in the hybrid. Cl 7-2 was positive for all probes tested, supporting cytogenetic evidence for presence of both derivative chromosomes in the hybrid. Furthermore, using a BCR probe that hybridizes to a family of four genes, all of which map to 22q11 (Croce et al. 1987), we have shown that cl 7-2 gives a hybridization pattern consistent with having a der(22) and a der(11), with the der(22) being present in a higher percentage of the hybrid cells (Budarf et al. 1988*a*).

Characterization of Tumor-associated t(11;22) Hybrid Clones

Two of the clones isolated from TW × RJK88 had the der(11) chromosome on cytogenetic analysis. Cl 1-1 appeared to contain human chromosomes X, 3, 5, 6, 21, and der(11), and cl 5-2 appeared to have human chromosomes X, 6, 6, 7, 8, 9, 13, 17, and der(11). DNA from these clones was examined by Southern hybridization, and probes used to detect the regions of 11 and 22 are the same as those used for the GB × RJK88 hybrids.

Both clones were positive for BCL1 (11q13) and PDGFB (22q13) but were negative for the chromosome 22 centromeric probe p22/1 (D22Z2), indicating that a der(11) was present, in the absence of a normal 22 or a der(22). The two clones are also positive for ETS1. This agrees with previous chromosomal in situ hybridization data, which showed that in four different ES/NE tumor cell lines ETS1 remained on the der(11) (Griffin et al. 1986).

To exclude the presence of a normal chromosome 11, we screened the hybrids by using distal 11q probes.

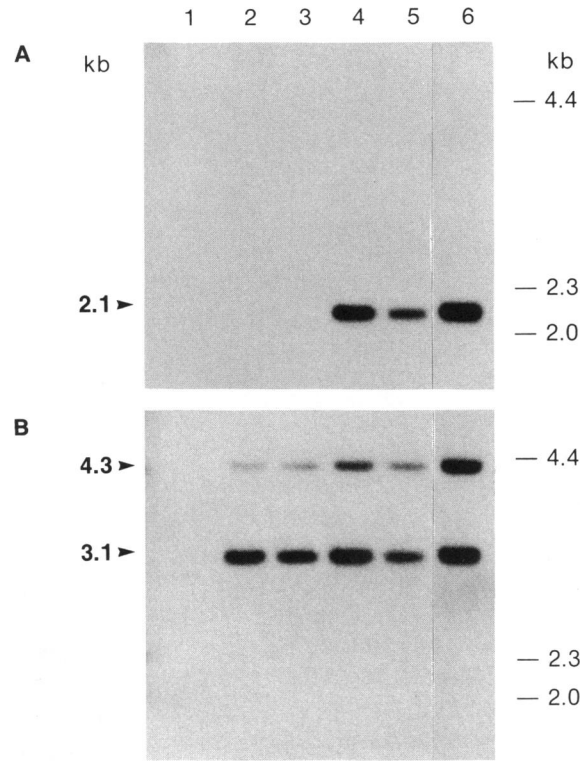

Figure 2 Hybridization of distal 11q probes, R365 (D11S129) and pHBI18P2 (D11S147), to somatic cell hybrid DNA. *Eco*RI-digested DNA from human, hamster, and hybrid cells (~5 μg) was separated on a 0.7% agarose gel. Lane 1, RJK88, the hamster parental; lane 2, cl 1-1 (TW × RJK88); lane 3, cl 5-2 (TW × RJK88); lane 4, TW, the ES tumor cell line; lane 5, cl 4 (GB × RJK88); lane 6, GM5565, normal fibroblast cell line. In panel *A* the probe was a ^{32}P-labeled, 2.0-kb *Hin*dIII fragment from phage R365 (D11S129). In panel *B* the probe was a 4.0-kb *Pst*I fragment from pHBI18P2 (D11S147). λ *Hin*dIII standards are indicated on the right, and the molecular weight of each band is shown on the left.

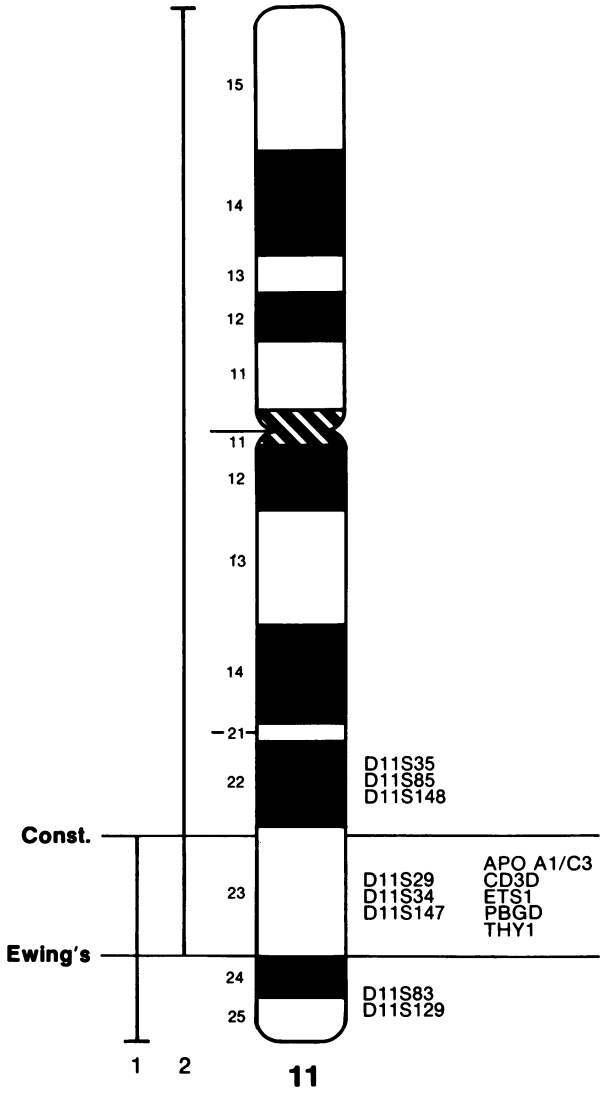

Figure 3 Idiogram showing the location of distal 11q probes with respect to the constitutional and ES t(11;22) breakpoints. Vertical line 1 indicates the region of chromosome 11 translocated to the der(22) of the constitutional t(11;22) and also represents that portion of human chromosome 11 in the somatic cell hybrid cl 4. Vertical line 2 indicates the region of chromosome 11 retained on the der(11) from the ES t(11;22) and also represents that portion of human chromosome 11 in the somatic cell hybrids cl 1-1 and cl 5-2.

Two distal probes, Ø2-25 (D11S83) and R365 (D11S129), did not hybridize to DNA from cl 1-1 and cl 5-2; results of Southern blot analysis using R365 (D11S129) are shown in figure 2*A*. The TW parental (fig. 2*A*, lane 4) hybridizes to R365, while hybrid clones cl 1-1 and cl 5-2 (fig. 2*A*, lanes 2 and 3) do not. Cl 4 (fig. 2*A*, lane 5) from the GB constitutional t(11;22) is shown for comparison. It contains the constitutional der(22) and hybridizes to R365. To show that similar amounts of more-proximal 11q DNA sequences are present in each lane, the filter was stripped and reprobed with pHBI18P2 (D11S147), which maps to 11q23-11q24 (see fig. 3). pHBI18P2 hybridizes with similar intensity to DNA from cl 1-1, cl 5-2, and cl 4 (fig. 2*B*, lanes 2, 3, and 5 respectively). Thus, cl 1-1 and cl 5-2 retain the der(11) in the absence of the other relevant chromosomes.

Mapping of Chromosome-11 and -22 Probes with Respect to t(11;22) Breakpoints

As an initial step in the characterization of the constitutional and ES/NE t(11;22) breakpoints, we used

these hybrids to sublocalize probes, which had been previously mapped to 22q11 and to the distal long arm of chromosome 11. Probes for the following loci on the distal long arm of chromosome 11 were available to us: ApoA1/C3 (11q23→qter), CD3D($T_3\delta$) (11q23→qter), ETS1 (11q23.3-24), PBGD (11q23.2→qter), THY1 (11q22.3), D11S29 (11q23), D11S34 (11q23→qter), D11S35 (11q21-q22), D11S83 (11q23→qter), D11S85 (11q22-23), D11S129 (11q), D11S147 (11q), and D11S148 (11q). Probes to these loci were hybridized to Southern blots of DNA from the hybrid panel; the results are summarized in figure 3. Eight of the probes lie between the constitutional and ES/NE breakpoints, suggesting that these breakpoints are probably separated by a considerable distance. We have performed pulsed-field gel electrophoresis (PFGE) using several restriction enzymes (*Sfi*I, *Not*I, *Bss*HII, *Sac*II, and *Nru*I) and DNA from several t(11;22) cell lines. When probes to six of these loci (ApoA1/C3, CD3D, ETS1, THY1, D11S29, and D11S147) were used, none of the six appeared to be physically linked and none detected translocation-mediated DNA rearrangements in the constitutional or ES/NE cell lines (data not shown).

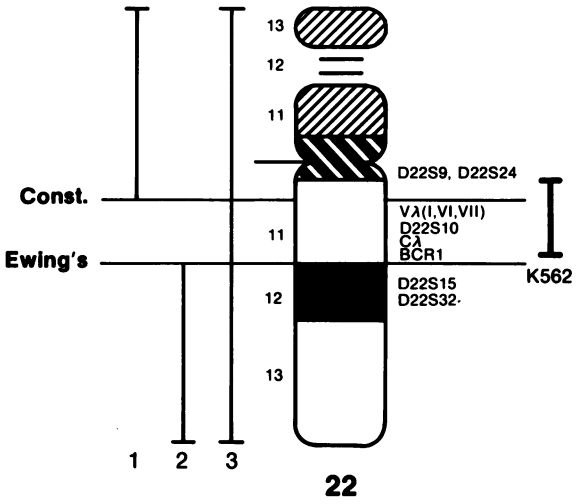

Figure 4 Idiogram showing the location of 22q probes with respect to the constitutional and ES t(11;22) breakpoints. On the left, vertical line 1 represents the region of chromosome 22 retained on the der(22) of the constitutional t(11;22). This also corresponds to the portion of human chromosome 22 in cl 4 (fig. 5, lane 5). Vertical line 2 represents the region of chromosome 22 translocated to the der(11) of the ES t(11;22) and corresponds to the human chromosome 22 sequences present in cl 1-1 and cl 5-2 (fig. 5, lanes 2 and 3). Vertical line 3 represents the entire human chromosome 22 present in the somatic cell hybrid, Eye F3A6 (fig. 5, lane 7). The vertical line to the right of the chromosome is the region of chromosome 22 amplified in K562.

In a similar manner, genes and anonymous DNA sequences that had been mapped to 22q11 were tested on the t(11;22) hybrid panel. With the hybrid DNA it is possible to sublocalize probes to one of three regions on chromosome 22 (fig. 4). K562, a CML-derived cell line, has amplified the BCR-ABL fusion gene and surrounding sequences on a marker chromosome (Heisterkamp et al. 1983; Selden et al. 1983) and gives a greatly increased signal for probes that map in the amplified region. Inclusion of K562 in the panel allows further sublocalization of probes in 22q11 (fig. 4). The results are summarized in figure 4, and examples of autoradiographs for probes to each region are shown in figure 5.

p22/34 (D22S9) is an anonymous DNA probe that

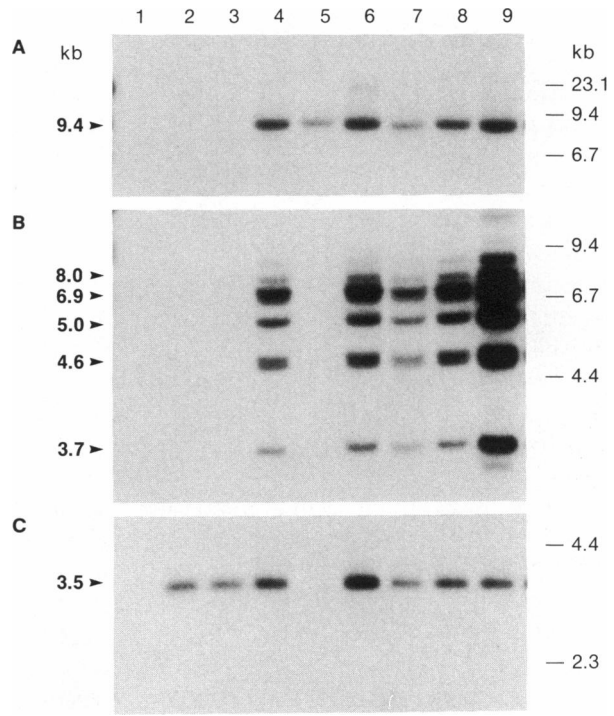

Figure 5 Hybridization of chromosome 22 probes to DNA from the somatic cell hybrid panel. *Eco*RI-digested DNA from human, hamster, and hybrid cells (~5 μg) was separated on a 0.7% agarose gel. In panel *A* the probe used was a 1.8-kb *Eco*RI fragment from p22/34 (D22S9). In panel *B* the probe was a 0.2-kb, *Eco*RI fragment from pHVλ6 (IGLV I). In panel *C* the probe was a 0.6-kb *Eco*RI fragment from DP22 (D22S15). Lane 1, RJK88, the hamster parental; lane 2, cl 1-1 (TW × RJK88); lane 3, cl 5-2 (TW × RJK88); lane 4, TW, the ES tumor cell line; lane 5, cl 4 (GB × RJK88); lane 6, GB-L, lymphoblastoid cell line from constitutional t(11;22); lane 7, Eye F3A6, a human/hamster hybrid that contains a normal chromosome 22 as the only intact human chromosome; lane 8, GM5565, normal human fibroblast cell line; lane 9 K562, CML cell line. λ *Hin*dIII standards are indicated on the right, and the molecular weight of the bands is shown on the left.

has been mapped to 22q11 and has been shown to be present in three to four copies in patients with cat-eye syndrome (McDermid et al. 1986*a*). It is present in cl 4 (fig. 5*A*, lane 5), making the D22S9 locus more centromeric than the constitutional 22q11 breakpoint. p22/34 does not appear to be amplified in K562 (fig. 5*A*, lane 9), placing it centromeric to the amplified region. As expected, it is not present in the ES hybrids that contain 22q11-12→qter (fig. 5*A*, lanes 2 and 3).

Previous work has shown that the variable region of the immunoglobulin λ light-chain locus (IGLV) is centromeric to the constant region (IGLC) (Emanuel et al. 1985). Probes to three IGLV families—i.e., probes for $V_{\lambda I}$, $V_{\lambda II}$, and $V_{\lambda VI}$ (Tsujimoto and Croce 1984; Anderson et al. 1984; Anderson et al. 1985, respectively)—were available to us. None of these IGLV probes hybridized to DNA sequences present in cl 4, as is shown when $V_{\lambda I}$ is used as an example (fig. 5*B*, lane 5). In addition, we have performed PFGE on *Not*I-digested DNA samples from several constitutional t(11;22) carriers and have probed them with $V_{\lambda I}$ and $V_{\lambda VI}$. No rearrangements were detected. All three IGLV families are present on one large *Not*I fragment, >1,400 kb (McDermid and Emanuel 1987). Thus, it is likely that the constitutional t(11;22) is proximal to most, if not all, of the λ light-chain locus. Probes for the IGLV genes as well as for IGLC were also tested on the ES hybrids; the results for $V_{\lambda I}$ are shown in figure 5*B*, lanes 2 and 3. As anticipated on the basis of our previous data, this breakpoint was distal to the entire λ light-chain locus.

D22S10 was absent from cl 4 and also was absent from cl 1-1 and cl 5-2, placing it between the two t(11;22)-associated chromosome 22 breakpoints. Using a hybrid cell line, 514AA2-A2, which has a der(22) from a t(9;22) Ph^+ acute lymphocytic leukemia (ALL) described elsewhere (Erikson et al. 1986), we found that D22S10 mapped proximal to this breakpoint (data not shown). This t(9;22) ALL–associated breakpoint has been mapped to the 5′ region of BCR1 (Fainstein et al. 1987). D22S10 was further sublocalized to the IGLV region by PFGE (H. E. McDermid and B. S. Emanuel, unpublished data). Our sublocalization of D22S10 agrees with the linkage data of Rouleau et al. (1989).

BCR1 has been localized distal to the λ light-chain locus (Emanuel et al. 1984) and hence is not present on the der(22) from this constitutional t(11;22). BCR1 was determined to be proximal to our ES breakpoint (fig. 4). This is consistent with data of Geurts van Kessel et al. (1985), who found that BCR1 is proximal to the 22q breakpoint of another ES.

Probes to D22S15, D22S32, and D22S24 were also tested on the hybrids. Both D22S15 and D22S32 map distal to the ES breakpoint, whereas D22S24 is proximal to the constitutional breakpoint and is not amplified in K562. The hybridization data for DP22 (D22S15) are shown in figure 5*C*. In addition to using these probes (fig. 5), we have also used these somatic cell hybrids to regionally localize the BCR-related genes (BCR2, BCR3, and BCR4) (Budarf et al. 1988*a*), the sodium glucose cotransporter gene (SGLT1) (Hediger et al. 1988), VpreB (Bauer et al. 1988), numerous anonymous probes (Budarf et al. 1988*b*), and human differentiation-stimulating factor (Budarf et al., in press) on chromosome 22.

Discussion

Reciprocal translocations occur in the constitutional karyotype of approximately 1/500–1/750 liveborns (Jacobs et al. 1974; Hamerton et al. 1975; Nielsen and Rasmussen 1976). Although these rearrangements involve numerous chromosomal bands, there is a nonrandom distribution of constitutional chromosomal breakpoints (Aurias et al. 1978; Yu et al. 1978). Certain bands, including 11q23 and 22q11, are more frequently involved as the breakpoints of constitutional translocations than are others. The molecular structure and direct biological consequences of these recombinational hot spots are essentially unknown, especially since these rearrangements usually occur without producing a selective reproductive advantage or distinct phenotype for the balanced translocation carrier.

In contrast, one of the current central tenets of cancer cytogenetics is that the nonrandom chromosomal abnormalities, especially translocations, seen in tumor cells are important in their pathogenesis. This hypothesis has been strengthened by the findings that consistent translocations in Burkitt lymphoma, CML, and other leukemias are near cellular protooncogenes and cause alterations in their expression or function. Thus, the t(11;22) most likely signifies an important mechanism by which genetic alterations play a role in the development of ES. Initially, the mapping of ETS1 to 11q23 implicated it as a potential candidate gene for the t(11;22) of ES. However, our earlier studies of ES tumors and cell lines clearly demonstrated that ETS1 is not rearranged on standard Southern blot analysis (Griffin et al. 1986). We have extended these observations with PFGE and have not detected rearrangement of ETS1. Hence, the absence of a suitable candidate gene in this tumor led us to develop this somatic cell approach to

cloning the breakpoint, which will be the critical step toward understanding the consequences of the t(11;22) rearrangement.

The nonrandom involvement of breakpoints at 11q23 in association with chromosomes other than 22 has also been described for several acquired abnormalities (Trent et al. 1988). These include the t(9;11)(p22;q23), the t(2;11)(p21;q23), the t(11;19)(q23;p13), and the del(11)(q23) of acute nonlymphocytic leukemia, as well as the t(4;11)(q21;q23) of acute lymphocytic leukemia. These translocations and deletions might be taken to implicate a specific oncogenic sequence in cancer-related 11q23 rearrangements. However, the fact that, in the t(4;11) (Sacchi et al. 1986) and t(9;11) (Diaz et al. 1986), the 11q breakpoints are proximal to ETS1 makes these breakpoints distinct from the more distal ES breakpoint. This suggests the involvement of different genes in these different cell types. In addition, the ES 11q23-24 breakpoint is the only solid-tumor-related alteration at 11q23-24 that has been described to date, suggesting that involvement of this region has unique consequences for this cell type.

In an analogous fashion, we and other investigators have demonstrated that the t(8;22) of Burkitt lymphoma, the t(9;22) of CML, and the t(9;22) of acute lymphocytic leukemia involve regions of 22q that are different both from one another and from the region of ES t(11;22)'s. The breakpoint of the t(8;22) is in the λ light-chain locus, and the t(9;22)'s are in the BCR1 locus, which is distal to the λ light-chain locus. Our data have shown that the ES breakpoint on 22 is distal to and distant from the BCR1 locus (PFGE data not shown). Thus, there must be several oncogenic sequences on chromosome 22, each of which is specific for a tumor-associated nonrandom rearrangement.

The existence of multiple 22q breakpoints, for constitutional and acquired abnormalities, suggests that chromosome 22q11-12 is highly recombinogenic. It has been proposed that the λ light-chain locus, which is programmed to rearrange during somatic development, might predispose the 22q11 region to multiple nonrandom changes (Erikson et al. 1981). Our present mapping data, especially our results for the tumor-associated rearrangement, exclude the major portion of the IGLC locus from direct involvement at either of the t(11;22) breakpoints. However, it is important to note that, although our studies to date indicate that the constitutional breakpoint is proximal to most of the IGLV, exclusion of this locus will ultimately require analysis of the breakpoint region to be considered complete. It is also unlikely that the IGLC gene cluster or any gene in proximity to the constitutional breakpoint confers a selective advantage, since this rearrangement takes place during meiosis.

There is evidence for the presence of repetitive DNA elements in regions that engage in chromosomal rearrangement and in mutation-prone regions of the genome. For example, such DNA sequences have been shown to cause intragenic deletions (Lehrman et al. 1986, 1987). It is possible that the recurrent t(11;22) translocations result from physical proximity within the nucleus, coupled with genetic similarity between 11q and 22q. These interchanges reflect interchromosomal—as opposed to intrachromosomal—recombination, which may be mediated by naturally occurring recombinase systems as has been suggested for several other nonrandom tumor-associated changes (Tsujimoto et al. 1985*a*; Haluska et al. 1986). The resolution of such speculations will only be obtained when the breakpoints can be scrutinized in detail. In that context, it is not surprising that our studies show that the distances between the constitutional t(11;22) and the ES-related t(11;22) breakpoints on chromosome 11q23 and on chromosome 22q11 are so large. The rearrangements occur in different cell types and result in distinctly different phenotypes at the cellular level.

Our hybrid-mapping panel has already provided significant information regarding the regional localization of specific genes and of anonymous DNA probes on 11q and 22q. For example, our data do not support the regional localization of THY1 to 11q22.3 (Gatti et al. 1987) but rather to the interval between 11q23→q24, in agreement with and refining its previous localization to 11q23→qter (Glaser et al. 1987). Our data also refine the mapping of APOA1 to the 11q23→q24 interval, for which there previously have been conflicting assignments. PBGD has been more precisely localized, from 11q23.2→qter to 11q23.2→q24 (Wang et al. 1981), as a result of these studies. In addition, this panel has permitted (1) regional localization of anonymous DNA probes isolated from the chromosome 22 libraries from the national laboratory at Los Alamos and from the Lawrence Livermore Laboratory (Budarf et al. 1988*b*), (2) the ordering of the four BCR-related loci in 22q11 (Budarf et al. 1988*a*), and (3) the localization of several genes (Bauer et al. 1988; Hediger et al. 1988; Budarf et al., in press).

These hybrids will also serve as an adjunct to mapping genetic disease loci. Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by immune deficiency, chromosomal instability, and predisposition to neoplasia. A recent study of affected families

by using genetic linkage analysis of probes for THY1 and D11S148 has localized a gene for AT to 11q22-23 (Gatti et al. 1988). The most likely order as determined by this linkage study is THY1–D11S148–AT. Our studies place D11S148 above and THY1 below the 11q23 constitutional breakpoint. As a result of these findings, we would suggest that the AT locus is proximal to the constitutional 11q23 breakpoint.

To elucidate the mechanism by which the 11;22 acquired or constitutional translocations occur and recur will require localization, cloning, sequencing, and detailed analysis of the breakpoints. This hybrid-mapping panel represents a first step toward that goal, which will ultimately answer several biologically important and clinically significant questions. Thus, one would hope to determine, by these efforts, the significance, in the etiology of ES, of the tumor-associated rearrangement between 11 and 22. One would also hope to elucidate the mechanisms that promote or permit the nonrandom involvement between these two chromosomes (*a*) during meiosis, producing the constitutional rearrangement, and (*b*) during mitosis, producing the tumor-associated 11;22 rearrangements.

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