The (4;11)(q21;q23) chromosome translocations in acute leukemias involve the VDJ recombines (cancer/oncogene activation/RNA alterations/gene rearrangement)


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ABSTRACT Chromosomal region 11q23 is frequently rearranged in acute lymphocytic leukemia (ALL) and in acute myeloid leukemias (AMLs), mostly in reciprocal exchanges with various translocation partners. The most common of these translocations is t(4;11)(q21;q23). It is present in ~10% of ALL patients, most frequently in very young children. We have recently cloned a region of chromosome 11, the ALL-I locus, found to be rearranged in malignant cells from patients with the t(4;11), (t;9;11), (t;1;11), (t;4;11), (t;10;11), and del(11q23) chromosomal abnormalities. Here we report the cloning and characterization of chromosomal breakpoints from leukemic cells with t(4;11) aberrations. The breakpoints cluster in regions of ~7–8 kilobases on both chromosomes 4 and 11. The presence of heptamer- and nonamer-like sequences at the sites of breakage suggests that the VDJ recombinase utilized for immunoglobulin gene rearrangement is also directly involved in these translocations. We also show that leukemic cells with (t;4;11) express altered RNAs transcribed from the derivative chromosomes 11 and 4.

Specific reciprocal chromosome translocations are the cytogenetic hallmark of lymphomas and leukemias. These chromosomal abnormalities have a molecular basis in alterations of normal cellular genes leading to their deregulation (1). Chromosome 11 region 11q23 is frequently rearranged in acute lymphocytic leukemia (ALL) and in acute myelogenous leukemia (AML). Reciprocal translocations t(4;11)(q21;q23), t(11;19)(q23;p13), and t(11;11)(p32;q23) are found in up to 10–15% of ALL cases (2). Translocations between 11q23 and chromosomal regions 9p23, 6q27, 1p21, 2p12, 10p11, 17q23, and 19p13 are found in 5–6% of AML cases. In addition, interstitial deletions in 11q23 have been detected both in ALL and in AML (2). Chromosome 11q23-associated leukemias, in particular those with t(4;11), often appear in very young children and are characterized by heavy tumor load, poor prognosis, and frequent expression of both lymphoid and myeloid markers. A 350-kilobase (kb) human DNA fragment containing the CD3 gene was cloned from a yeast artificial chromosome (YAC) library and shown by fluorescence in situ hybridization to span several 11q23 chromosome breakpoints (3). From the same YAC clone we obtained a DNA segment that detected on Southern blots rearrangements in leukemic cells from patients with the t(4;11), (t;9;11), (t;11;19), (t;1;11), (t;6;11), (t;10;11), or del(11q23) chromosomal abnormalities. Probes from both sides of the breakpoint cluster region detected transcripts in a variety of cell lines (5, 6). One probe also identified altered RNAs in cells with t(4;11) (5). We have now extended these studies to clone and sequence translocation junction fragments and to further characterize the altered RNAs.

MATERIALS AND METHODS

Leukemic cells with the t(4;11) chromosome translocation were obtained from three patients and three cell lines. The latter included MV4:11 (7), RS4:11 (8), and B-1 (9). Other cell lines used were GM1500 (Epstein-Barr-virus-immortalized B lymphocytes), obtained from the National Institute of General Medical Sciences Cell Repository (Camden, NJ); 380, a pre-B-ALL (10); and T98G, a glioblastoma (11).

Somatic cell hybrids containing derivative chromosomes 4 and 11 were obtained by fusing Chinese hamster ovary (CHO) cells with the leukemic cells of two patients with the t(4;11) aberration (12, 13).

High molecular weight DNA was obtained and analyzed by standard procedures. RNA was extracted by the guanidinium thiocyanate method (14). Aliquots of 5–10 μg of polyadenylated RNA were electrophoresed in 1.1% agarose gels containing formaldehyde, blotted onto nitrocellulose filters, and hybridized to probes radiolabeled by the random primer method (Boehringer Mannheim kit).

For amplification of the junction fragment present in the der(11) of the RS4:11 cell line, we used the primers 5'-GG-TGT-AAG-GCA-AAT-AGG-GTG-TG-3' and 5'-GAG-GGA-AAG-ATC-TAC-TAC-GG-3', designed on the basis of sequence analysis of regions on chromosomes 11 and 4, respectively. To amplify the junction fragment located on der(4) of the same cell line, we used the primers 5'-CAG-GGC-AAG-CTC-TCT-CCG-3' and 5'-GAA-GCC-AAG-TGG-TGT-AG-3', corresponding to sequences within chromosomes 11 and 4, respectively. The amplified fragments were electrophoretically purified and sequenced with an ABI automatic sequencer.

RESULTS

Physical Mapping and Nucleotide Sequencing of t(4;11) Breakpoints. We first cloned the breakpoint from the MV4:11 cell line (7) by using a rearranged BamH1 fragment that was identified with the FA4 (Dde I) probe derived from chromosome 11 (5). The fragment was enriched by gel electrophoresis and cloned into the L47 λ phage vector (15). A chromosome 4-derived probe (YS4.5), a 0.3-kb Xho I-EcoRI fragment, was then obtained from the MV4:11 junction fragment and was used in Southern blotting to examine DNAs from three cell lines and three primary tumors with the t(4;11) chromosome aberration (Fig. 1). In each case, rearranged fragments were identified with either this probe

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; TCR, T-cell antigen receptor.

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(termed Y in Fig. 1) or the chromosome 11-specific probe (termed F in Fig. 1). Probe YS4.5 was then used to clone the corresponding normal region from chromosome 4, allowing mapping of the breakpoints in the six tumors (Fig. 2). The breakpoints clustered within regions of 7–8 kb on both chromosomes 4 and 11. This tight clustering suggested recombination-favoring sequences within the two clusters and/or close proximity to exons of genes directly affected by the translocation.

To try to elucidate the enzymatic mechanism involved in the recombination process associated with the translocations, we determined the sequences at the breakpoints as well as within the relevant segments of chromosomes 4 and 11 (Fig. 3). The sequence of the derivative chromosome 4 of the MV4:11 cell line showed perfect homology to sequences of normal chromosomes 11 and 4 except for 4 base pairs (bp), GGGT, inserted between the two joined chromosomes. The 4-bp insert is reminiscent of the extra nucleotides, termed the N region, that are frequently added during immunoglobulin gene rearrangements (16, 17). A heptamer sequence highly related (6 of 7 nucleotides) to the heptamer signal associated with rearrangements of the immunoglobulin and T-cell antigen receptor (TCR) genes, CACACAGTG (18–20), was present 2 bp from the breakpoint on chromosome 11. A nonamer-like sequence (8 of 9 nucleotides of the consensus GGGTTTTGT; the inverted consensus is ACACACAGC) was identified 2 bp from the breakpoint on chromosome 4. This nonamer was preceded, 27 bp upstream, by a heptamer-like signal (7 of 7 nucleotides with an extra base pair included).

To analyze a second t(4;11) junction, we sequenced some of the cloned genomic DNAs at regions predicted to be adjacent to the breakpoints in the RS4:11 cell line (case 2 in Fig. 2). Based on this sequence information it was possible to design primers to amplify the junction regions from both der(4) and der(11) chromosomes of RS4:11. The sequences of these junctions are shown in Fig. 3. The translocation in this cell line was found to involve two consecutive breakages on each chromosome. Breaks in position 52 on the two chromosomes (Fig. 3 Middle) resulted in simple joining to form

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**Fig. 1.** Chromosome 4- and chromosome 11-specific probes detect DNA rearrangements in leukemic cells from patients with t(4;11). Genomic DNAs from the cell lines MV4:11 (lanes 1), RS4:11 (lanes 2), and B-1 (lanes 3) and from cells of three patients (lanes 4–6) were digested with the indicated restriction enzyme and analyzed by Southern blotting and consecutive hybridizations to the FA4 probe (F), derived from chromosome 11, and to the YS4.5 probe (Y), originating from chromosome 4. Numbers at the sides represent kilobases. N, germ-line fragment. In lanes 1, 4, and 6, a single rearranged fragment reacted with both probes.

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**Fig. 2.** Clustering of 4;11 breakpoints on both chromosomes 11 and 4. Numbered arrowheads correspond to lane numbers in Fig. 1. Tel and Cen denote the direction of the telomeres and centromeres of the two chromosomes. Restriction sites: H, HindIII; B, BamHI; G, Bgl II; X, Xba I; R, EcoRI; V, EcoRV.
the der(11). The breakpoint on chromosome 11 was 1 bp 5' to a nonamer-like sequence (6 of 9 nucleotides of the consensus) separated by a spacer of 23 bp from a heptamer-like signal (5 of 7 nucleotides with addition of 1 bp). A structure of nonamer (6 of 9)–23-bp spacer–heptamer (5 of 7) occurred also on chromosome 4. The der(4) chromosome was formed (Fig. 3 Bottom) by subsequent deletion of nucleotides 52–60 on chromosome 4, a break at position 19 on chromosome 11, and insertion of a segment of 43 bp duplicated and inverted from nucleotides 9–51 of chromosome 11. Also inserted was a single base, T. These secondary breaks also occurred 1–3 bp from the heptamer/nonamer signals.

**Altered RNAs Encoded by the der(4) and der(11) Chromosomes.** Using as a probe a 1-kb genomic EcoRI fragment composed of chromosome 11 sequences derived from the terminus of cosmid 20 (4) and located 10 kb centromeric to the breakpoint cluster region, we previously identified an 11-kb altered RNA in the RS4:11, MV4:11, and B-1 cell lines established from patients with the t(4;11) chromosome translocation (5); we now estimate the size of this RNA to be 12.7 kb. In addition, this probe detected a major transcript of 12.5 kb, estimated now to be of 15–16 kb, in all cell lines with or without the translocation (5). We have now extended this analysis and utilized a cDNA probe originating from the telomeric side of the translocation. This 6-kb cDNA contained chromosome 11 sequences included in cosmids 53 and 43 (4), and it detected in the T98G, 380, and GM 1500 cell lines transcripts of 15–16 kb (Fig. 4, lanes a–c). In the three cell lines with t(4;11), we observed a major new transcript of 14 kb, and a major transcript of 15 kb similar to the one observed in the cell lines without the translocation (Fig. 4, lanes d–f).

To examine whether the RNAs unique to the cell lines with t(4;11) were transcribed from the derivative chromosomes, we conducted a Northern analysis of RNAs of somatic cell hybrids (CHO cells) containing the der(4) or der(11) chromosome. The probe that contained sequences telomeric of chromosome 11 breakpoints detected in a hybrid with der(4) a 14-kb RNA species, a diffuse band of smaller molecular size, and a larger RNA, which was presumably of CHO origin (Fig. 4, lanes g and h). Similarly, the probe composed of sequences located centromeric to the breakpoint cluster region identified in a hybrid with der(11) a major transcript of

![Fig. 3](image-url)  
**Fig. 3.** Nucleotide sequence at chromosome 11 and 4 breakpoints in the MV4:11 and RS4:11 cell lines. Heptamer- and nonamer-like sequences are boxed.

![Fig. 4](image-url)  
**Fig. 4.** Northern blot analysis of RNAs from cell lines (A) and somatic cell hybrids (B and C). The probes used were a 6-kb cDNA (T.N., unpublished work) containing chromosome 11 sequence telomeric of the breakpoint cluster region (A and B) and a 1-kb EcoRI genomic fragment derived from cosmid 20 (4) and located 10 kb centromeric of the breakpoint cluster region (C). Cell lines included T98G, 380, GM 1500, RS4:11, MV4:11, and B-1 (lanes a–f, respectively). Lanes g and i, RNA from CHO cells; lanes h and j, RNA from CHO cells with der(4) and der(11) chromosomes, respectively.
12.7 kb (Fig. 4, lane j); a larger transcript was observed both in this hybrid and in control cells (lanes 1 and j). Thus, it appears that the two major RNAs that are characteristic of t(4;11) cells are transcribed from the two derivative chromo-

somes.

DISCUSSION

Our results indicate a tight clustering of breakpoints on both chromosomes 4 and 11. Such clustering might reflect a proximity of the breakpoints to genes on the two chromo-
somes. These genes may have to be fused in a distinct way so as to give rise to a deregulated protein. A productive fusion might require juxtapositioning of specific exons (or an exon and a control region) of the two genes. Alternatively, the two genes are not fused, but the translocation affects the structure of their transcripts through gene truncation or modified splicing.

The structure of the DNA in both cluster regions might favor recombination. Indeed, in each of the 4;11 breakpoints analyzed in the MV4:11 and RS4:11 cell lines, a heptamer-
like or a nonamer-like sequence occurred 1–3 bp from the junction. The signals identified showed similarities with the consensus sequences of the immunoglobulin and TCR loci, with homology ranging from 6/9 to 8/9 and from 5(7 + 1) to 6/7. Signals with similar fit have been shown to be functional in joining of variable (V), diversity (D), and joining (J) gene segments of immunoglobulin genes (20). In one breakpoint (chromosome 11 of MV4:11), we observed a single heptamer. Previous reports have suggested that isolated heptamers can participate in joining elements of the immunoglobulin and TCR loci (21–23) as well as in promoting chromosome abnormalities (24–26). In five other breakpoints analyzed in MV4:11 and RS4:11 DNAs, we detected adjacent heptamers and nonamers separated by 23 or 27 bp. Within the structures of these heptamer–spacer–nonamer, the 5‘ to 3‘ orientation of the two signals varied from the orientation encountered in the immunoglobulin and TCR loci. However, the similarity between the length of these spacers and the length of bona fide VDJ spacers suggests that this is not a coincidence and that the VDJ recombinase can recognize such structures. The identification of extra nucleotides in the MV4:11 junction, and of a complex rearrangement which required the action of an endonuclease and DNA polymerase in one RS4:11 junction, extends the similarities between normal VDJ joining (27) and (t4;11) recombinations.

Is there a high concentration of heptamer- and nonamer-like sequences within the breakpoint clusters? This is presently not known, although in a region of 200 bp 3‘ of the RS4:11 breakpoint on chromosome 4, we identified seven heptamer or nonamer-like sequences (data not shown). Similarly, within 200 bp 5‘ of the chromosome 4 breakpoint in MV4:11 we observed six such sequences. Finally, a high concentration of Alu repeats [there are six such repeats within the DNA spanning break sites 1, 2, 4, 5, 6, on chromosome 11 (Fig. 2)] might play a role in making these regions accessible to enzymes involving recombination.

Our suggestion that 4;11 chromosome translocations are catalyzed by the VDJ recombinase is consistent with the classification of the relevant leukemias as B-precursor cell ALL. Not with the detection of immunoglobulin gene rear-

rangement in most or all (t4;11) cases determined, including the six analyzed in this report (refs. 7–9 and unpublished data). Previously, involvement of the recombinase in chromosomal translocations was shown in B-cell malignancies carrying t(11;14) (28), (t4;18) (29), and (t8;14) (26) and in a variety of T-cell malignancies (23, 25, 26). Our results indicate that the VDJ recombinase participates in rearrangement of genes other than the immunoglobulin or TCR genes.

The RNA analysis presented here extends previous inves-
tigations (5, 6). Normal RNAs of 15–16 kb were detected in all cells examined, by probes from both sides of the break-
point cluster region. Probes from the two sides of the break-
point cluster each detected an altered RNA in cell lines with t(4;11). These RNAs appeared to be expressed by the deri-

vative chomosomes. Thus, a 12.7-kb RNA present only in cell lines with t(4;11) (5) was transcribed from der(11). A transcript of 14 kb previously identified (estimated then as 11.5 kb) in the RS4:11 cell line (6) was detected by us in two additional cell lines with t(4;11) and appears to be transcribed from der(4). Although a minor transcript of similar size was observed in cell lines without the translocation, the two transcripts probably correspond to different RNA species. It is still to be determined which of these transcripts (if any) plays a crucial role in the disease.

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10. Pegoraro, L., Palumbo, A., Erikson, J., Falda, M., Gian-