Brief Definitive Report

CHROMOSOMAL TRANSLOCATION INVOLVING THE β T CELL RECEPTOR GENE IN ACUTE LEUKEMIA

By MICHAEL L. CLEARY,* JULIA D. MELLENTIN,* JESSICA SPIES,* AND STEPHEN D. SMITH‡

*From the *Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, California 94305; and the ‡*Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305*

Chromosomal translocations are implicated as important pathogenetic events in many human malignancies (1). For tumors of B-lymphoid origin, the Ig genes are frequent sites of chromosomal translocations, most commonly resulting in juxtaposition of Ig genes with the c-*myc* or *bcl*-2 genes (2). In rare T cell cancers, the TCR-α gene may be translocated into close proximity of the c-*myc* protooncogene (3, 4). Recent cytogenetic observations demonstrating chromosome 7q32-34 abnormalities in a number of T cell malignancies suggest that the TCR-β gene may be the frequent target for chromosomal translocations in lymphoid cancers, although the donor chromosomes appear to be much more variable than in human B cell malignancies (5–8). We report here molecular data showing involvement of the TCR-β gene by a t(7;19)(q34;p13) chromosomal translocation in acute lymphoblastic leukemia. Nucleotide sequence analysis of cloned breakpoint DNA showed that the point of crossover on chromosome 7 occurred adjacent to joining segment Jβ1.1, suggesting that this translocation resulted from an error in TCR-β gene rearrangement. On chromosome 19, the translocation occurred near or within a previously uncharacterized transcriptional unit, resulting in the production of aberrant sized RNAs.

Materials and Methods

Cell Lines. Cell lines were established from patients with common acute lymphocytic leukemia (SUP-B2), T cell acute lymphoblastic leukemia (SUP-T7, SUP-T8), and B cell lymphoma (SU-DHL-4, SU-DUL-5) as described earlier (6, 9). The A9 and B82 hybrid cell lines have been described earlier (10).

Isolation and Blotting of Nucleic Acids. DNA was extracted from cultured cell lines and leukemia cells, using previously described procedures (11). Total cellular RNA was isolated as described (9) and poly(A)$^+$ RNA was enscribed using oligo-dT–cellulose. Conditions for genomic Southern blot and Northern blot analysis have been described previously (9). DNA probes were labeled with α[^{32}P]CTP by random hexanucleotide priming.

Recombinant DNA. To isolate t(7;19) breakpoint DNA, genomic DNA from the cell line SUP-T7 was digested with either Hind III or Eco RI. The resulting fragments were size fractionated in 0.8% agarose gel, and DNA in the 8 to 9 kb range was purified as

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described earlier (11). The insert DNA was ligated with the Eco RI arms of Charon 35 or Hind III cut λ 590 DNA. After in vitro packaging, each library was screened, using a radiolabeled probe specific for the TCR-β chain constant region gene. To obtain germline chromosome 19 DNA, a human placental DNA library was screened using the 600 bp Eco RI–Xho I chromosome 19 DNA fragment (Fig. 2) as a probe.

Nucleotide Sequencing. Nucleotide sequences were determined by didioxy chain termination on DNA fragments subcloned into M13 vectors. Random deletions of M13RF DNAs were constructed, using DNase I similar to conditions described earlier (9).

Results and Discussion

The TCR-β chain gene has been mapped to chromosome 7, near or within band 7q34 (12–14). Abnormalities involving this chromosome segment have been observed in childhood T cell leukemias, lymphoblastic lymphomas, and cell lines established from these malignancies (5–8). To investigate the possibility that specific chromosomal translocations directly involve the TCR-β gene, molecular cloning studies were carried out on a cell line established from a T cell acute lymphoblastic leukemia (8). Earlier studies have shown that the cell line SUP-T7, which carries a t(7;19)(q34;p13) chromosomal translocation, is representative of the patient's original leukemia cells, based on enzymatic, immunophenotypic, and immunogenotypic criteria (8). The clinical features of a high white blood cell count, a mediastinal mass, and the presence of surface antigens Leu-1–4 were all typical of an acute leukemia of T cell origin.

Southern blot analysis using a probe specific for the TCR-β constant region genes showed two nongermline fragments in both the SUP-T7 cell line and the original leukemia cells (Fig. 1*A*). Each of the rearranged TCR-β genes was molecularly cloned from SUP-T7 DNA using λ phage vectors. The cloned DNAs were mapped for cutting sites of several restriction enzymes, and the deduced maps are shown in Fig. 2. This analysis showed that both fragments contained large portions of the TCR-β locus. Fragment A contained the Cβ1 gene at its 3′ end; whereas fragment B contained the Cβ2 gene at its 3′ end. At their 5′ ends, both fragments diverged from the expected configuration for germline chromosome 7 flanking the β constant region genes.

To test whether one of the cloned DNA fragments contained a t(7;19) crossover, various subfragments of each were used as hybridization probes against DNA isolated from hamster/human hybrid cell lines containing one or a few human chromosomes. A subfragment of A, representing the 5′ 600 bp, hybridized to DNA from a hybrid cell line containing only human chromosome 19; whereas no hybridization was observed with a line containing human chromosome 7 (Fig. 1*B*). The hybridization and mapping results indicated that fragment A spanned the t(7;19) breakpoint. The cytogenetic assignment of the t(7;19) breakpoint to the short arm of chromosome 19 was confirmed by the demonstration that a fragment containing only chromosome 19 DNA hybridized on Southern blot analysis to DNA from the A9 hybrid cell line containing the short arm and part of the long arm of chromosome 19 but not to DNA from a B82 hybrid cell line containing only the long arm of chromosome 19 (data not shown).

The 600 bp Eco RI–Xho I fragment consisting solely of chromosome 19 DNA was used as a probe to screen a human germline DNA library. The resulting phage insert DNAs were used to derive a restriction map of chromosome 19

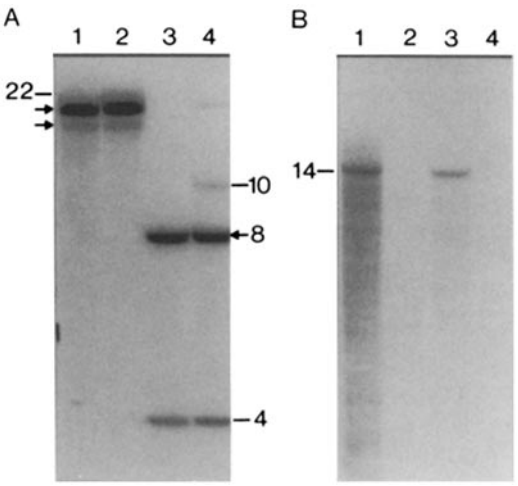

FIGURE 1. (*A*) Southern blot analysis of the TCR-β genes in the SUP-T7 cell line and the leukemic cells from which it was established. After digestion with Bam HI (lanes *1* and *2*) or Eco RI (lanes *3* and *4*), DNA fragments were subjected to Southern blot analysis using a Cβ1 DNA probe. Positions of expected germline β constant region fragments are denoted with dashes; arrows indicate rearranged TCR-β bands. Numbers refer to fragment sizes in kilobases. (Lanes *1* and *3*) SUP-T7 cell line DNA; (lanes *2* and *4*) acute leukemia cell DNA. (*B*) Southern blot analysis of hamster/human hybrid cell line DNAs using a chromosome 19–specific DNA probe. DNAs from hybrid cell lines, CHO cells, and germline human cells were subjected to Southern blot analysis after Eco RI digestion. The DNA probe consisted of the 600-bp Eco RI–Xho I fragment shown in Fig. 2. (Lane *1*) total human germline DNA; (lane *2*) 20HL14-21 (cell line containing human chromosome 7); (lane *3*) 5HL9 (cell line containing human chromosome 19); (lane *4*) CHO hamster DNA.

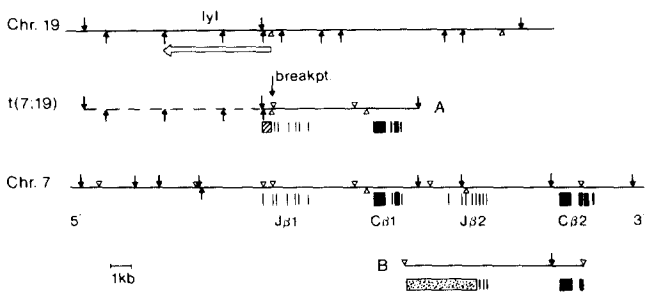

FIGURE 2. Physical maps of the cloned TCR-β genes from the SUP-T7 cell line and germline chromosome 19 DNA flanking the site of t(7;19). The cloned DNA fragments representing rearranged TCR-β genes and containing either a t(7;19) breakpoint (*A*) or the productive TCR gene (*B*) isolated from the SUP-T7 cell line are shown oriented in a 5′ to 3′ direction based on homology with the TCR-β gene. Also shown is a germline segment of chromosome 19 spanning the site where the t(7;19) translocation breakpoint occurred. Solid boxes and vertical lines denote the Cβ sequences and Jβ joining segments, respectively. A crosshatched box denotes the chromosome 19 DNA fragment used as a probe on Southern blots of hamster/human hybrid cell lines. Open horizontal arrow indicates chromosome 19 DNA fragments that detect transcription products on Northern blot analysis and the direction of transcription for the 1.5-kb chromosome 19 mRNA. Restriction enzyme sites: Eco RI (↓), Bam HI (↑), Hind III (▽), Xho I (△).

DNA flanking the region where the t(7;19) breakpoint occurred (Fig. 2). Comparison of the germline 7 and 19 DNA maps with that for t(7;19) DNA (fragment A) suggested that the point of crossover occurred near joining segment Jβ1.1.

Selected nucleotide sequencing was carried out on these DNAs. The results are shown in Fig. 3, where the sequence (14) for germline chromosome 7 is compared with those obtained for the t(7;19) DNA and germline chromosome 19. The sequence of the 3′ end of the breakpoint DNA was identical to that containing joining segment Jβ1.1. Immediately 5′ to Jβ1.1, the homology with chromosome 7 terminated. There was a stretch of 22 nucleotides that did not match that for either chromosome 7 or 19. Beyond this, the nucleotide sequence of the t(7;19) DNA was identical to that for chromosome 19. These data indicated that the point of t(7;19) crossover occurred 5′ to Jβ1.1, but the precise nucleotide where chromosome 7 and 19 had been joined could not be determined accurately due to the insertion at the site of crossover.

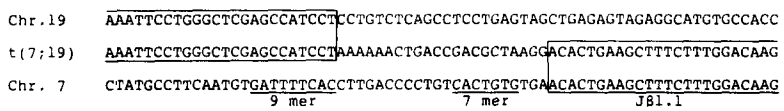

FIGURE 3. Nucleotide sequence of t(7;19) breakpoint DNA and its comparison to germline chromosome 7 and 19 DNA sequences. The nucleotide sequences determined for the t(7;19) breakpoint are displayed along with the DNA sequences determined for germline chromosome 19 and 7 at the sites where the translocation crossover occurred. The 5′ to 3′ orientation is based on that for the TCR genes. Boxes denote stretches of homologous nucleotides shared by adjacent sequences. The heptamer/nonamer recognition sequences are underlined.

All or part of the nonhomologous 22 nucleotides at the site of crossover may represent a TCR D region that has undergone recombination with Jβ1.1. However, the sequence in this region does not match that determined for other human TCR D regions, nor does it contain any consensus sequences that would place it into one of the four proposed TCR D region families (15–18). Alternatively, the 22 nucleotide spacer at the breakpoint may have resulted from random addition of nucleotides by terminal deoxynucleotidal transferase in a fashion analogous to *N*-region diversity. The proximity of the t(7;19) crossover point within 22 nucleotides of a TCR J region suggests that this translocation may have occurred due to an error in TCR gene rearrangement. Similar mechanisms involving cellular recombinase enzymes have been proposed for chromosomal translocations involving Ig heavy chain genes in human B cell malignancies (11) and more recently for the TCR-α gene in a human T cell malignancy (4). Our results suggest that a common mechanism may account for chromosomal translocations involving TCR-α and -β genes and IgH genes, although a search of the germline chromosome 19 DNA sequence flanking the site of t(7;19) did not show any significant homologies with the highly conserved heptamer/nonamer sequences that mediate TCR and Ig gene rearrangements.

Portions of chromosome 19 DNA near the site of t(7;19) contain an active transcriptional unit since chromosome 19 DNA fragments flanking this region detected a moderately abundant 1.5-kb mRNA in several hematolymphoid cell lines (Fig. 4). However, in the SUP-T7 cell line carrying a t(7;19) translocation, mRNAs of reduced size were detected with the chromosome 19 DNA probes. The transcriptional orientation of this 19p13 gene, which we have named *lyl*-1 (for lymphoid leukemia), was determined to be in the opposite direction (*horizontal arrow* in Fig. 2) of that for the TCR-β gene. The transcriptional orientation of the *lyl*-1 gene in conjunction with the presence of smaller mRNAs in SUP-T7 suggests that the t(7;19) results in a 5′ truncation of the *lyl*-1 mRNA; however, further studies are required to determine the implications of this truncation on potential activation of the *lyl*-1 gene. Preliminary studies indicate no homology with known protooncogenes nor with the insulin receptor gene that has been mapped to chromosome region 19p13 by in situ hybridization.

The 7qter region containing the TCR-β gene has received less attention than 14q11-q12 containing the TCR-α genes as an important site for chromosomal translocation in T cell malignancies. However, recent cytogenetic studies of lymphoid blast cells and leukemic cell lines have shown that a high proportion of T cell neoplasms carry abnormalities involving 7q32–34 (5–8). It appears that no single translocation involving 7q32–34 in T cell ALL recurs with a frequency

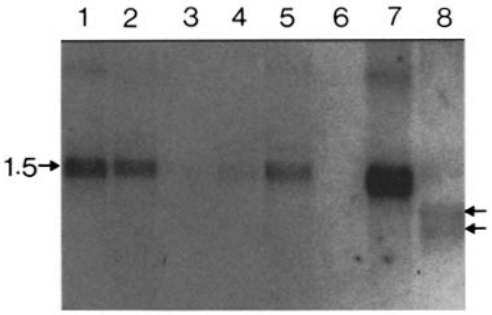

FIGURE 4. Autoradiogram obtained after Northern blot hybridization of RNA transcribed from chromosome 19 DNA near the t(7;19) breakpoint. Poly(A)$^+$ RNA from cultured cell lines SU-DHL-4, SU-DUL-5, IM9, SUP-B6, SUP-B2, SUP-M1, SUP-T8, and SUP-T7 (lanes *1–8*, respectively) were size-fractionated in a formaldehyde agarose gel. Left hand arrow denotes 1.5-kb mRNA present in a wide variety of cell types; right hand arrows denote shorter RNAs present in SUP-T7. The Bam HI fragments overlying open horizontal arrow in Fig. 2 were used as hybridization probes.

of >10%, contrary to the recurring translocations in morphologically distinct B-lymphoid malignancy. In fact, at least nine different chromosome regions (1p32, 1p34, 2p21, 6p21, 6q24, 9q32, 9q34, 15q22, and 19p13) have been reported to be involved in reciprocal exchanges with 7q32–34 in T cell ALL (5–8). A few of these regions coincide with the chromosomal sites for various protooncogenes (e.g., L-*myc* at 1p32, *hpim* at 6p21, and c-*abl* at 9q34); however, to date, there are no molecular data confirming their involvement and studies using pulsed-field gels have shown no alteration of the c-*abl* gene in the t(7;9)(q34;q34) translocation (19), suggesting that most of the chromosome regions involved in 7q32–34 reciprocal exchanges likely encode previously uncharacterized candidate protooncogenes. Our results are consistent with this proposal in that we have identified a novel transcriptional unit juxtaposed with a TCR-β gene at the site of t(7;19) in a T cell malignancy.

Summary

DNA spanning a t(7;19) chromosomal translocation breakpoint was isolated from the human T cell line SUP-T7 established from an acute lymphoblastic leukemia. Nucleotide sequence analysis showed that the point of crossover on chromosome 7 occurred immediately adjacent to joining segment Jβ1.1 within the TCR-β gene, suggesting that this translocation resulted from an error in TCR gene rearrangement. On chromosome 19, the translocation occurred within a previously uncharacterized transcriptional unit for which we propose the name *lyl*-1. An ~1.5-kb RNA is transcribed from this gene in a wide variety of hematolymphoid cell lines. The t(7;19) results in truncation of the *lyl*-1 gene and production of abnormal-sized RNAs, suggesting a role for *lyl*-1 in the pathogenesis of this leukemia.

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