DETECTION OF A SECOND t(14;18) BREAKPOINT CLUSTER REGION IN HUMAN FOLLICULAR LYMPHOMAS

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Malignant cells from most follicular lymphomas contain a t(14;18) chromosomal translocation detectable in cytogenetic analyses (1, 2). Fragments of DNA containing a breakpoint for this translocation have been cloned out of DNA isolated from tissue biopsies of follicular lymphomas and also from lymphoma cell lines (3–6). Chromosome 18 DNA probes flanking these breakpoints have been used (3–6) to detect t(14;18) DNA rearrangements in ~60% of follicular lymphoma DNA specimens, thereby defining a t(14;18) breakpoint cluster region. However, a significant minority of follicular lymphomas lacked detectable breakpoints on chromosome 18 within 15–20 kb on either side of this cluster region. We describe here the cloning and characterization of a chromosome 18 DNA probe that detects t(14;18) DNA rearrangements in most of the follicular lymphomas that previously failed to show detectable breakpoints. Our findings indicate that almost all t(14;18) breakpoints occur within one of two cluster regions, and suggest two potentially different pathogenetic consequences of t(14;18) translocations.

Materials and Methods

Tumor Tissues and Cell Lines. Lymphoma tissues serving as a source of DNA for cloning were obtained from a single patient with follicular lymphoma. The hybrid cell lines UV20 HL21-7 (containing human chromosomes 4, 8, 18, and 21) and UV20 HL21-27 (human chromosomes 4, 8, and 21) have been described previously (7). Cell lines SU-DHL-4 and SU-DUL-5 were provided by Jean Jang and Dr. H. Kaplan (Stanford University).

Genomic Southern Blot Analyses. DNA was extracted from lymph node biopsy specimens and cultured cell lines and subjected to Southern blot analysis using previously described procedures (8, 9).

Construction and Screening of Genomic DNA Libraries. To isolate rearranged IgH genes, follicular lymphoma DNA was digested with the appropriate restriction enzyme, and size-fractionated in 0.8% agarose gel. Regions of the gels that contained DNA fragments of 3–6 kb for Hind III (productive Ig allele) and from 20–23 kb for Eco RI (translocated allele) were excised. DNA was electroeluted from the gel slices, purified, and ligated into appropriate phage vectors as described previously (3, 8). The recombinant DNAs were packaged in vitro and ~10^6 recombinant phages were plated and screened using a radiolabeled JH hybridization probe according to previously described methods (3, 8, 10). Hybridizing plaques were purified by three successive platings.

Nucleotide Sequencing. Nucleotide sequences were obtained by the dideoxy chain termination method (11), using DNA fragments subcloned into M13 phages (12).
Results and Discussion

All t(14;18) breakpoints that have been analyzed in detail (3–6) lie in chromosome 14 DNA within or adjacent to one of the heavy chain J region segments. In these cases, t(14;18) translocations appeared as rearranged Ig DNA fragments in genomic Southern blots, and could thus be cloned from the DNA of malignant lymphomas using a probe specific for the human JH region. It was of interest therefore to examine the rearranged Ig fragments of a follicular lymphoma representing the 40% of cases lacking a chromosome 18 DNA rearrangement detectable by our previously described breakpoint cluster region DNA probe (pFL-1). To this end, the rearranged Ig genes were molecularly cloned from the genomic DNA of such a lymphoma using λ phage vectors and a JH-specific hybridization probe.

The cloned DNAs are shown in Fig. 1. As expected, each was homologous in part to the human JH region. However, for both alleles, this homology abruptly terminated 5' of joining segment J4. In addition, Cμ had been deleted from one allele and replaced by Cv sequences. Because the malignant cells of this lymphoma expressed μ-containing Ig, the Cv allele must represent the nonproductive Ig gene. As we reported earlier (3), deletion of Cμ on the excluded (i.e., translocated) allele by an apparent class switch mechanism is a frequent but unexplained finding in follicular lymphomas.

To test whether the excluded allele represented by the Eco RI fragment cloned from this follicular lymphoma might contain a t(14;18) breakpoint, a 5' subclone (fragment B in Fig. 1) was used as a hybridization probe on genomic Southern blots of DNA from a series of hamster/human hybrid cell lines (Fig. 2B). The 3' half of this 5 kb DNA probe contained sequences derived from the JH region; the 5' half contained sequences of unknown origin. Using this probe, two Eco RI fragments were detected in human germline DNA (Fig. 2B, lane 1). The 19 kb band corresponds to the expected Eco RI configuration of the germline JH region. The 4 kb Eco RI band resulted from hybridization with the 5' end of the probe DNA. DNA from the parent CHO cell line UV20 gave no detectable
FIGURE 2. A. Southern blot analysis of various genomic DNAs containing pFL-2 DNA rearrangements. DNA was extracted from lymph node biopsy tissues and cultured cell lines and subjected to Southern blot analysis. The hybridization probe consisted of the chromosome 18-specific subclone pFL-2 (fragment A) as shown in Fig. 1. The germline Bam HI and Hind III bands containing pFL-2 correspond to fragments of ~17 kb each. All other bands represent rearrangements of pFL-2-containing DNA. Lanes 1–6 and 8–10, follicular lymphoma DNAs; lane 7, lymphoid cell line SU-DUL5. B. Southern blot analysis of hamster/human hybrid cell line DNAs with a t(14;18) DNA probe. DNAs from hybrid cell lines and germline cells were purified and subjected to Southern blot analysis. DNAs were digested with Eco RI. Dashes indicate the 19 kb Eco RI germline configuration of JH and 4 kb germline configuration of pFL-2. Faint bands above and below the 19 kb germline JH band result from hybridization with residual C,-containing fragments present in the DNA probe preparation. Lane 1, germline human DNA; lane 2, UV20; lane 3, UV20HL21-7; lane 4, UV20HL21-27.

signal (lane 2), indicating that no crosshybridizing sequences were present in the CHO line under stringent hybridization conditions. However, DNA of the UV20HL21-7 human/hamster hybrid cell line (containing human chromosomes 4, 8, 18, and 21 [7]) contained the 4 kb band (lane 3). No germline JH band of 19 kb could be detected, confirming that chromosome 14 was not present in this hybrid. DNA from the hybrid line UV20HL21-27, which had lost chromosome 18 but still retained 4, 8, and 21, contained no hybridizing sequences (Fig. 2B, lane 4). These results indicated that the 5' half of this probe contained DNA originating from chromosome 18. Therefore, the excluded IgH allele from this follicular lymphoma contained a t(14;18) breakpoint.

Restriction enzyme analyses of the cloned breakpoint DNA fragment suggested that the site of t(14;18) fusion had occurred near joining segment J4. To confirm this, nucleotide sequence analyses were carried out on DNA fragments subcloned into M13 phages (11, 12). As shown in Fig. 3, the breakpoint DNA sequence diverged from that of germline JH immediately 5' of J4. This is the presumed site of fusion, since the sequence does not match that of any published human D segment. The sequence data also indicated that the D-J joint in the productive Ig allele occurred at J4 in a position nearly identical to the breakpoint on the translocated allele. As discussed previously (3), the similar sites for the t(14;18)
breakpoint and D-J joint in the respective J4 alleles strongly implicates D-J recombination enzymes in the mechanism of t(14;18) translocation.

A series of follicular lymphoma DNAs was screened by genomic Southern analyses for possible rearrangement of chromosome 18 DNA using the subcloned fragment pFL-2 (fragment A in Fig. 1) as a hybridization probe. The results indicated that a significant fraction of follicular lymphomas (Fig. 2 A) had t(14;18) breakpoints detectable with the pFL-2 probe, thereby defining a second breakpoint cluster region for this translocation. Table I summarizes our results from 30 follicular lymphoma DNA biopsies and cell lines examined with either or both of the translocation probes pFL-1 and pFL-2. Almost all have breakpoints falling within one or the other cluster region. Altogether, >90% of the DNA samples from this randomly selected set of follicular lymphomas contained a chromosome 18 DNA rearrangement detectable with pFL-1 or pFL-2. This correlates well with the reported (2) frequency of cytologic t(14;18) translocation for this histologic subtype of lymphoma. It is not clear whether the lymphomas in Table I that lacked chromosome 18 DNA rearrangements also lacked a cytologic t(14;18) translocation, since karyotype data were not available for these tumors.

Karyotype analyses of cell lines containing translocations detectable with the pFL-1 or pFL-2 probes (e.g., SU-DHL-4 and SU-DUL-5, respectively) implicate the same cytologic region (18q21) on chromosome 18 as the site of t(14;18) translocation (14 and our unpublished observations). To determine the potential linkage relationship of pFL-1 and pFL-2, the chromosome 18-specific fragment pFL-2 was used as a hybridization probe against recombinant phages whose inserts represent ~40 kb of germline 18 DNA flanking pFL-1, as described previously (3). No hybridization was detected (data not shown), demonstrating that the cluster region defined by pFL-2 is not located within 20 kb on either side of the cluster region defined earlier by us and others (3–6).

It is becoming increasingly apparent that chromosomal translocations in hematological neoplasias cluster near or within cellular protooncogenes. We and others have previously shown (3, 4, 15) that cell lines and lymphomas containing pFL-1 DNA rearrangements also expressed transcripts encoded by chromosome 18 DNA flanking the t(14;18) breakpoint cluster region. We wished, therefore, to examine RNA isolated from SU-DUL-5, which contains a pFL-2-detectable translocation, for similar transcription products. On Northern analyses, no transcripts could be detected in polyadenylated RNA from SU-DUL-5 using the pFL-2 probe. Furthermore, no RNA could be detected in these cells using several
Table I

Gene Configuration of pFL-1- or pFL-2-Containing DNA

| Patient | pFL-1 | pFL-2 |
|---------|-------|-------|
|         | Bam HI | Hind III | Eco RI* | Bam HI | Hind III |
| 1       | G      | G       | G       | R      | R       |
| 2       | G      | G       | G       | R      |         |
| 3       | G      | G       | R       |        |         |
| 4       | G      | R       |        |        |         |
| 5       | G      | G       | R       | R      |         |
| 6       | G      | G       | R       | R      |         |
| 7       | G      | G       | R       | G      |         |
| 8       | G      | G       | G       | G      |         |
| 9       | G      | G       | G       | G      |         |
| 10      | G      | R       |        |        |         |
| 11      | G      | G       | R       |        |         |
| 12      | R      | R       | G       |        |         |
| 13      | R      | R       | G       |        |         |
| 14      | G      | G       | R       |        |         |
| 15      | R      | R       | G       |        |         |
| 16      | G      | R       | G       |        |         |
| 17      | R      | G       | G       |        |         |
| 18      | R      | R       | G       |        |         |
| 19      | G      | R       |        |        |         |
| 20      |       |         | R       |        |         |
| 21      | R      | R       | G       |        |         |
| 22      | R      | R       | G       |        |         |
| 23      | R      | G       | G       |        |         |
| 24      | R      | R       | G       |        |         |
| 25      | R      | R       | G       |        |         |
| 26      | R      | R       | G       |        |         |
| 27      | G      | R       | G       |        |         |
| 28      | R      | G       | G       | G      |         |

Cell Line

|         | SU-DHL-4 | SU-DUL-5 |
|---------|----------|----------|
|         | R       | R       | G       | G       |
|         | G       | G       | R       | R       |

R, rearrangement. G, germline configuration.
* For Eco RI determinations, the adjacent downstream Eco RI fragment was used as a probe (9).

Chromosome 18 DNA probes flanking both sides of the pFL-1 cluster region (data not shown) and capable of detecting transcription products in cell lines with pFL-1 DNA rearrangements.

Summary

Our results indicate that there are two major breakpoint cluster regions in chromosome 18 DNA for t(14;18) translocations in follicular lymphomas. The absence of a pFL-1 homologous transcript in a cell line containing a pFL-2-detectable translocation suggests that there may be two different pathogenetic consequences of t(14;18) translocations. One possibility is that, despite the distances between them (>20 kb), breakpoints in the two cluster regions in some way affect transcription of the same gene product, which has not yet been identified. Alternatively, two separate transcriptional units may be involved. The availability of DNA probes for each of the two t(14;18) breakpoint cluster regions...
will allow further studies regarding the biologic significance of these two genetically distinct classes of t(14;18) translocations.

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