Haematology and Blood Transfusion Vol. 31
Modern Trends in Human Leukemia VII
Edited by Neth, Gallo, Greaves, and Kabisch

Human Follicular Lymphomas: Identification of a Second t(14;18) Breakpoint Cluster Region

N. Galili, M. L. Cleary, and J. Sklar

A. Introduction

Most follicular lymphomas, which comprise nearly two-thirds of the non-Hodgkin's lymphomas occurring in U.S. adults, have been shown by cytogenetic analyses to contain a t(14;18) translocation [1, 2]. This breakpoint on chromosome 14 has been localized to band 14q32, the site of the immunoglobulin heavy chain genes. It was thus possible to use human *Ig* gene fragments to clone out a breakpoint DNA fragment (pFL-1) from tissue biopsy specimens of these lymphomas and from cell lines containing this translocation. When used as a hybridization probe, rearranged pFL-1 containing DNA was detected in approximately 60% of follicular lymphomas. Thus, a significant percentage of follicular lymphomas failed to show a breakpoint on chromosome 18 within 15–20 kb on either side of the breakpoint region. We describe here the cloning of a chromosome 18 DNA fragment (pFL-2) that detects t(14;18) rearranged DNA in most of these negative follicular lymphomas.

B. Materials and Methods

I. 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 HL 21-27 (human chromosomes 4, 8, and 21) have been described previously [3]. The cell line SU-DUL-5 was provided by Jean Jang and Dr. H. Kaplan (Stanford University).

II. Genomic Southern Blot Analyses

DNA was extracted from lymph node biopsy specimens and cultured cell lines and subjected to Southern blot analysis using procedures previously described [4].

III. Construction and Screening of Genomic DNA Libraries

To isolate rearranged IgH genes, follicular lymphoma DNA was digested to completion 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 [4]. The recombinant DNAs were packaged in vitro and $\sim 10^6$ recombinant phages were plated and screened using a radiolabeled J_H hybridization probe according to methods previously described [4]. Hybridizing plaques were purified by three successive platings.

Laboratory of Experimental Oncology, Department of Pathology Stanford University, Stanford, California 94305, USA

IV. Nucleotide Sequencing

Nucleotide sequences were obtained using the dideoxy chain termination method [5], using DNA fragments subcloned into M13 phages [6].

C. Results and Discussion

All t(14;18) breakpoints that have been analyzed [4, 7–9] occur in chromosome 14 DNA adjacent to or within the heavy chain *J* region segment. Thus, using the probe specific for the human J_H region, the breakpoint of a follicular lymphoma lacking the previously described breakpoint cluster region pFL-1 chromosome 18 rearrangement was molecularly cloned from genomic DNA.

The cloned DNAs are shown in Fig. 1. As expected, each was homologous in part to the human J_H region. The homology is terminated 5′ of the joining segment J4 for both alleles. In addition, C_μ has been deleted from one allele and replaced by C_γ sequences. Since the malignant cells of the lymphoma expressed μ-containing Ig, the C_γ allele must represent the nonproductive (i.e., translocated) Ig gene. This apparent class switch is, in our experience [2], a frequent but unexplained finding in follicular lymphomas.

In order to see whether this nonproductive allele DNA fragment contained 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. 2 B). The 3′ half of this 5 kb DNA probe contained sequences derived from the J_H region, while the 5′ end contained sequences of unknown origin. Two *Eco*RI fragments were detected in human germline DNA (lane 1). The 19 kb band corresponds to the expected *Eco*RI germline J_H region. The 4 kb *Eco*RI band resulted from hybridization with the 5′ end of this probe. No cross-hybridization was seen with the parent hamster cell line UV20 (lane 2). The human/hybrid cell line UV20HL21-7 DNA in lane 3 lacks the human chromosome 14, but has chromosome 18 [3]. This lane correspondingly lacks the 19 kb band but contains the 4 kb band. The hybrid UV20HL21-27 in lane 4 has lost chromosome 18 and thus no hybridizing sequences can be detected. These results indicated, therefore, that the 5′ half of this probe contains DNA from chromosome 18. The nonproductive IgH allele thus contained a t(14;18) breakpoint.

Restriction enzyme mapping of the cloned breakpoint DNA fragment indicated that the site of t(14;18) fusion had occurred near the joining segment J4. Nucleotide sequencing was thus carried out on fragments subcloned into M13 phages. As seen in Fig. 3, the breakpoint DNA sequence diverged from germline J_H immediately 5′ of J4. The sequence also showed that the D-J joint in

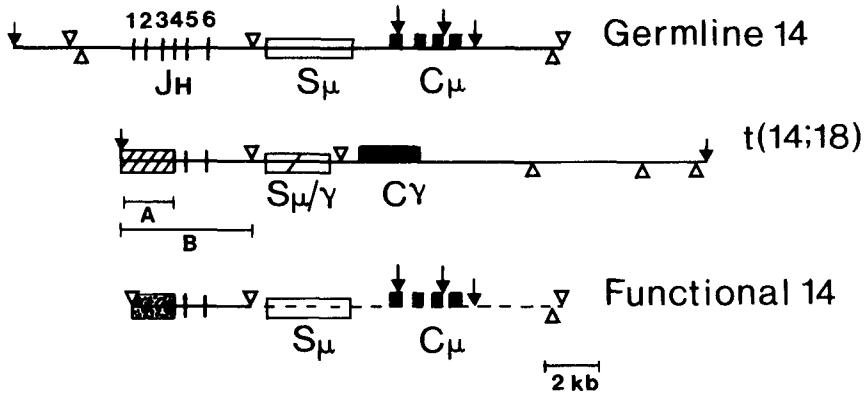

Fig. 1. Graphic depiction of the cloned DNA fragments representing the two rearranged heavy chain alleles shown along with the germline configuration for comparison. The cloned DNAs are oriented 5′ to 3′. *Solid boxes* represent C_H-containing regions, the *open boxes* denote switch region sequences, and the *stippled box* represents V_H-containing sequences of the productive *Ig* gene. The *cross-hatched box* represents DNA sequences derived from chromosome 18. **A** indicates the chromosome 18-specific probe pFL-2; **B** indicates the breakpoint DNA fragment used as a probe on the human/hamster cell lines. Restriction sites as follows: *Eco*RI (↓), BamHI (△), *Hind*III (▽)

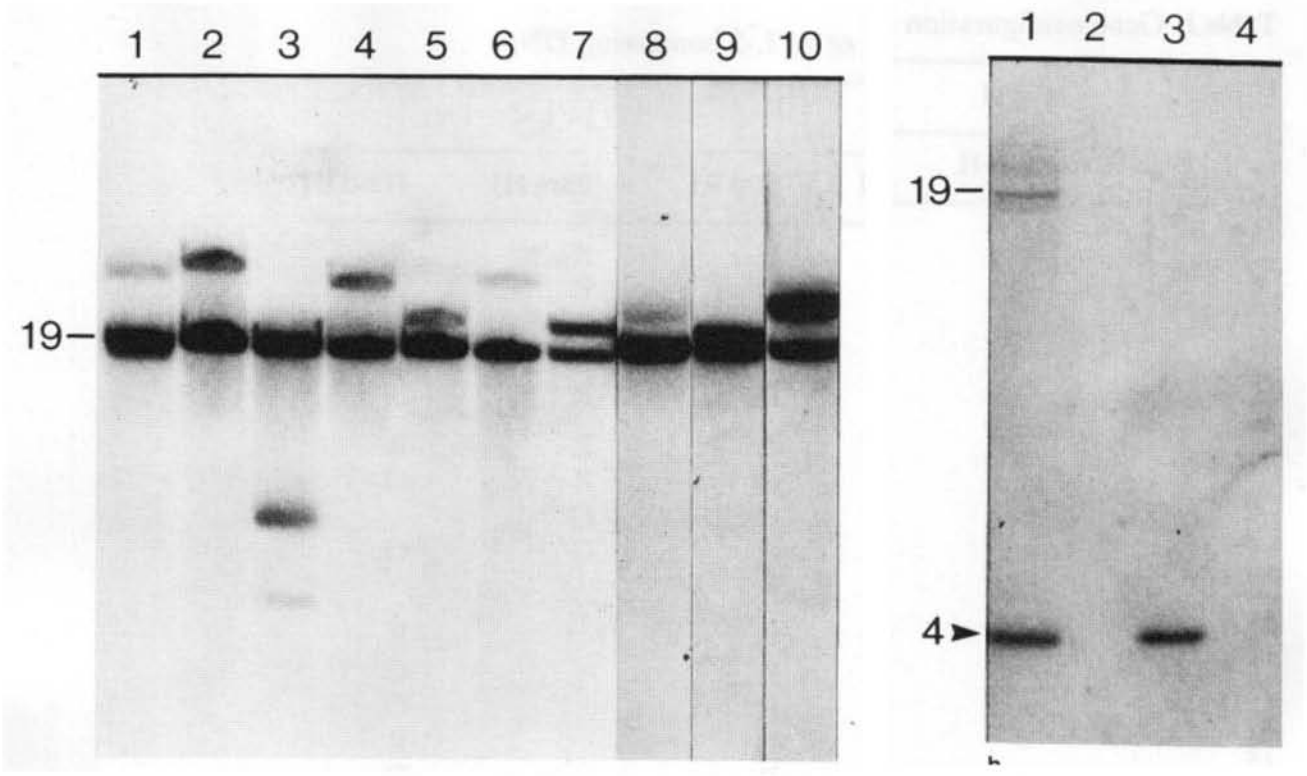

Fig. 2. a Southern blot analyses of genomic DNAs containing pFL-2 (fragment A) as shown in Fig. 1. Germline *Bam*HI and *Hind*III containing pFL-2 bands are ~17 kb each. *Lane 8,* lymphoid cell line SU-DUL-5; all *other lanes,* follicular lymphomas. **b** Southern blot analyses of hamster/human hybrid cell line DNAs digested with *Eco*RI and probed with a t(14;18) DNA probe. *Lane 1,* germline human DNA; *lane 2,* UV20; *lane 3,* UV20HL21-7; *lane 4,* UV20HL21-27

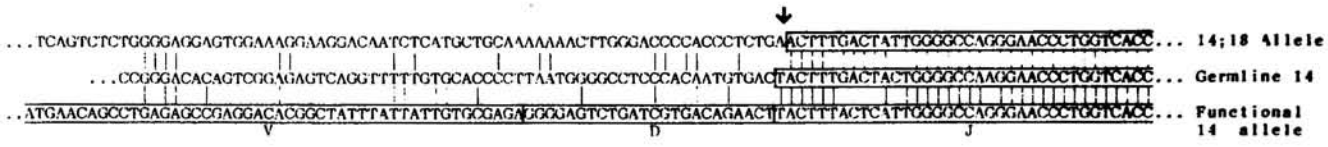

Fig. 3. Nucleotide sequences of the t(14;18) breakpoint DNA and the productive V-D-J joint isolated from a follicular lymphoma. Orientation is 5′ to 3′. *Arrow* indicates presumed point of translocation

the functional allele has occurred at J4 in a position nearly identical to the breakpoint on the translocated allele. We have reported similar patterns of nearly identical t(14;18) breakpoint and D-J joint in the productive allele for two other lymphomas using the pFL-1 probe [4]. These structural similarities implicate D-J recombination enzymes as mediators of t(14;18) translocations.

Follicular lymphomas negative for rearranged pFL-1 containing DNA fragments were screened by genomic Southern analyses using the subcloned pFL-2 fragment (fragment A in Fig. 1) as the probe. Ten of these lymphoma DNAs are shown in Fig. 2A. A significant fraction of follicular lymphomas contained a chromosome 18 DNA rearrangement detectable with pFL-2, thereby defining a second breakpoint cluster region for this translocation. Table 1 summarizes our results with 30 follicular lymphoma DNA biopsy specimens and cell lines examined with pFL-1 and/or pFL-2. More than 90% of these randomly selected samples contained a chromosome 18 DNA rearrangement falling within one or the other cluster region. This correlates well with the reported [2] frequency of cytologic t(14;18) translocations.

In order to determine a possible linkage relationship of pFL-1 and pFL-2 on chromosome 18, the pFL-2 fragment was used as

Table 1. 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	R	
4			G	R	
5		G	G	R	R
6		G	G	R	R
7		G	G	R	G
8		G	G	G	
9		G	G	G	G
10			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	R	R		G	G
SU-DUL-5	G	G		R	R

R, rearrangement of pFL-1 or pFL-2 containing DNA, respectively; G, germline configuration.

a probe against recombinant phages whose inserts contained ~40 kb of germline chromosome 18 DNA flanking pFL-1 [4]. No hybridization was detected (not shown), indicating that the cluster region defined by pFL-2 is not within 20 kb on either side of pFL-1.

The finding of two major cluster regions for the t(14;18) translocation suggests two different outcomes of such crossovers. Breakpoints in either region may, in some as yet unknown way, affect the transcription of the same gene product despite the distance (>20 kb) between them. Alternatively, each cluster region may affect different transcriptional units. The use of the two DNA probes pFL-1 and pFL-2 will enable further study of the biological or clinical significance of these two classes of t(14;18) translocations and may be useful for the diagnosis of this type of lymphoma.

References

1. Fukuhara S, Rowley JD, Variakojis D, Golomb HM (1979) Chromosome abnormalities in poorly differentiated lymphocytic lymphoma. Cancer Res 39:3119

2. Yunis JJ, Oken N, Kaplan ME, Ensrud KM, Howe RR, Theoligides A (1982) Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. N Engl J Med 307:1231
3. Thompson LH, Mooney CL, Burkhart-Schultz K, Carrano AV, Siciliano MJ (1985) Correction of a nucleotide-excision-repair mutation by human chromosome 19 in hamster-human hybrid cells. Somatic Cell Mol Genet 11:87
4. Cleary ML, Sklar J (1985) Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. Proc Natl Acad Sci USA 82:7439
5. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463
6. Norrander J, Kempe T, Messing J (1983) Construction of improved M13 vectors using oligodeoxy-nucleotide-directed mutagenesis. Gene 26:101
7. Bakhshi A, Jensen JP, Goldman P, Wright JJ, McBride DW, Epstein AL, Korsmeyer SJ (1985) Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around J_H on chromosome 14 and near a transcriptional unit on 18. Cell 41:899
8. Tsujimoto Y, Finger LR, Yunis JJ, Nowell P, Croce CM (1984) Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 226:1097
9. Tsujimoto Y, Gorham J, Cossman J, Jaffe E, Croce CM (1985) The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. Science 229:1390