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Cloning of the Chromosome Breakpoint of Neoplastic B Cells with the t(14;18) Chromosome Translocation

Abstract. *From an acute B-cell leukemia cell line, a DNA probe was obtained that was specific for chromosome 18 and flanked the heavy chain joining region of the immunoglobulin heavy chain locus on chromosome 14. This probe detected rearrangement of the homologous DNA segment in the leukemic cells and in follicular lymphoma cells with the t(14;18) chromosome translocation but not in other neoplastic or normal B or T cells. The probe appears to identify* bcl-2, *a gene locus on chromosome 18 (band q21) that is unrelated to known oncogenes and may be important in the pathogenesis of B-cell neoplasms with this translocation.*

Recently, we obtained a cell line, 380, derived from a 16-year-old male with acute pre–B-cell leukemia (L2 in the FAB classification) (*1*). Chromosome analysis of the leukemic cells indicated that they are pseudodiploid and carry the two chromosome translocations, t(8;14) (q24;q32) and t(14;18) (q32;q21), that are characteristic of Burkitt lymphoma (*2*) and follicular lymphoma (*3, 4*), respectively. We now describe our study with 380 cells used to clone the chromosome joining region between chromosomes 14 and 18 on one of the two $14q^+$ chromosomes resulting from these translocations.

Only the t(8;14) and t(14;18) translocations in the 380 leukemic cells, resulting in two $14q^+$ chromosomes, were detected in the karyotype (Fig. 1). The DNA's from the 380 cells were digested with Bam HI and analyzed by Southern blot transfer; the results indicated that the DNA's carry two rearranged heavy chain joining (J_H) segments of 18.5 and 14 kb (kilobases) (*1*). Since the same two DNA fragments hybridized to a C_μ (C, constant region) DNA probe (*1*), we inferred that the rearrangements occurred either within or close to the J_H segment of the two heavy chain loci. No expression of heavy chains was detected in the 380 cells (*1*), thus we also concluded that both C_μ rearrangements in these cells are unproductive. However, the t(8;14) translocation might have involved the productively rearranged μ gene leading to its activation (*1*). Deletion of both C_K genes and rearrangements of both C_λ loci were also detected in 380 cells.

A full genomic library was prepared from the DNA of the 380 cells. Genomic DNA was partially digested with the restriction enzyme Sau 3A, and DNA fragments 14 to 23 kb in length were purified by sucrose gradient centrifugation. The fragments were then ligated with DNA of the λ phage vector EMBL3A (*5*), which was cut with Bam HI. After packaging in vitro, 420,000 independent recombinant phages were screened with a probe specific for the J_H DNA segment (pHj) (Fig. 2) essentially as described for the cloning of the t(11;14) chromosome joining from neoplastic human B cells (*6*). Nine recombinant clones were obtained, and restriction map analysis allowed their classification into two groups that presumably represented sequences derived from the two $14q^+$ chromosomes.

To establish which of the two groups of recombinant clones contained the joining between chromosomes 8 and 14, we subcloned DNA fragments 5′ of both cloned J_H segments (p380j-2RR and p380j-9SS) (Fig. 2) that were free of repetitive sequences. These subclones were then used as probes in Southern blot hybridization of DNA from human cells and rodent × human hybrid cells containing either human chromosome 8, 14, or 18. Probe p380j-9SS hybridized to human DNA and to the DNA of a Chi-

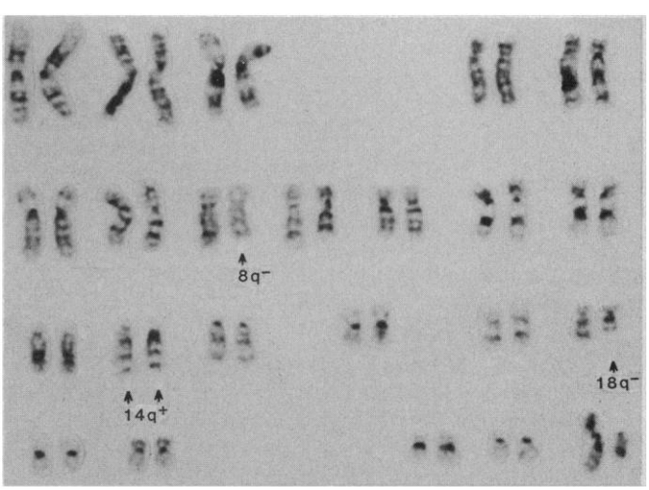


Fig. 1. Representative G-banded karyotype of the 380 cells: 46, XY, t(8;14) (q24;32), and t(14;18) (q32;q21). As a result of the two reciprocal translocations, the 380 cells have one rearranged chromosome 8 ($8q^-$), two abnormal chromosomes 14 ($14q^+$), and one abnormal chromosome 18 ($18q^-$).

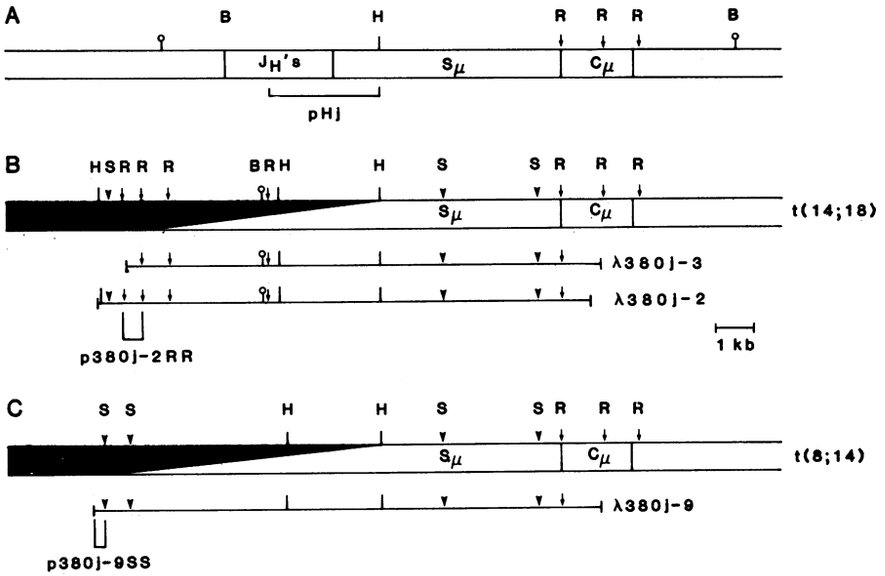


Fig. 2. Restriction maps of the germ line C_μ gene (A) and of the two classes of recombinant clones from the $14q^+$ chromosomes resulting from the t(14;18) (B) and the t(8;14) (C) translocations. Abbreviations: H, Hind III; R, Eco RI; B, Bam HI; and S, Sst I. The black bars represent the chromosome 18–derived sequences in (B) and the chromosome 8–derived sequences in (C). The open bar represents the chromosome 14–derived sequences.

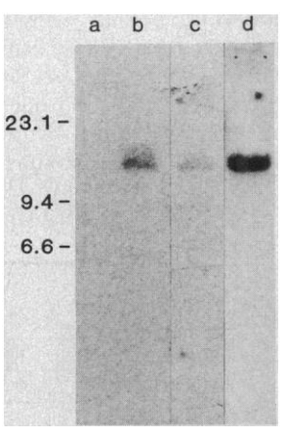
nese hamster × human hybrid containing only human chromosome 8 and no other human chromosome (*7*) (Fig. 3). The same probe did not hybridize to rodent × human hybrids containing only human chromosome 14 (data not shown). Therefore, we concluded that the class of recombinants containing the p380j-9SS DNA segment carry the joining between chromosomes 8 and 14 on the $14q^+$ chromosome (Fig. 2).

The p380j-9SS probe detected only a single germ line–hybridizing fragment in the DNA of a T-cell line (545T) derived from the same patient from whom the leukemic 380 cell line was obtained and also in various other human DNA's (Fig. 4, lanes a to d). However, this probe detected a germ line and a rearranged DNA fragment in the 380 leukemic cell DNA (Fig. 4, lane f), indicating that the translocation between chromosomes 8 and 14 was a somatic event during the development of the patient's leukemia. Because the p380j-9SS probe did not hybridize to DNA from chromosome 14, and because the pHj probe did not hybridize to the DNA segment on the left of the most 5′ Hind III site of clone λ380j-9 (Fig. 2), the chromosome 14 breakpoint involved in the t(8;14) translocation must be between the most 5′ Sst I site and the second Hind III site (Fig. 2C).

Because the clone shown in Fig. 2C is derived from the $14q^+$ chromosome of the t(8;14) translocation, clones λ380j-2 and λ380j-3 (Fig. 2B) should be derived from the $14q^+$ chromosome of the t(14;18) translocation. Therefore, we hybridized the DNA's of mouse × human and Chinese hamster × human hybrid cells containing human chromosomes 14 or 18 or both with the p380j-2RR probe. This probe hybridized to human DNA and to that from hybrids containing human chromosome 18 but not to that from hybrids containing human chromosome 14 (data not shown). This indicates that clones λ380j-2 and λ380j-3 contain the joining between chromosomes 14 and 18.

On the basis of these findings, the p380j-2RR probe might be expected to detect a germ line DNA fragment in human cellular DNA's and to detect both a germ line and a rearranged DNA fragment in the DNA of 380 cells. All human DNA's tested except that from 380 and LN128 cells showed a single hybridizing fragment representing the germ line sequence, while the DNA of cell line 380 and of LN128 had an additional DNA fragment (Fig. 5). A single band representing a germ line in DNA from the 545T cell line was also detected (lane e). Therefore, the t(14;18) translocation and the DNA rearrangement (lane f) was a somatic event during the development of this leukemia. Furthermore, since the p380j-2RR DNA does not contain sequences from chromosome 14 and since the pHj probe did not hybridize to the DNA fragment on the left of the second 5′ (left) Hind III site (data not shown), the chromosomal breakpoint involving the t(14;18) translocation must have occurred between the third 5′ (left) Hind III site and the second Eco RI site on the left (Fig. 2B).

The t(14;18) chromosome translocation is known to be common in follicular lymphoma (*3*, *4*). A t(14;18) translocation has been detected in 12 of 15 patients with this disease (*4*). We have asked



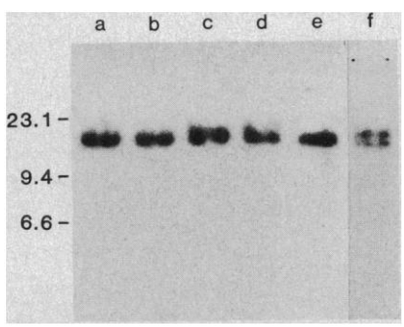


Fig. 3 (left). Hybridization with the p380j-9SS probe of Hind III–digested DNA's from rodent × human hybrid cells containing human chromosome 8. The DNA samples were fractionated on 0.7 percent agarose gel. The Southern blot filter was hybridized in 50 percent formamide and 4× standard saline citrate (SSC) at 37°C with a ^{32}P-labeled p380j-9SS probe and was finally washed with 0.2× SSC at 65°C. (Lane a) DNA from CHO (Chinese hamster ovary) cell; (lane b) DNA from 706B6-40 Cl17 (CHO × human hybrid cell containing only human chromosome 8); (lane c) DNA from 280AG8Ce4 (mouse × human hybrid containing human chromosome 8 but not human chromosome 14); (lane d) DNA from 545T human cell line. The molecular size of the Hind III–digested λ marker DNA is in kilobases. Fig. 4 (right). Southern blot hybridization of 380 leukemic cell DNA with sequence of p380j-9SS. The cellular DNA's were digested with Bam HI. The hybridization and washing were performed as described in the legend to Fig. 3. (Lane a) DNA from normal peripheral blood lymphocytes; (lane b) DNA from PAF (SV40-transformed human fibroblast); (lane c) DNA from human T-cell lymphoma cells; (lane d) DNA from cells of CLL271 [a chronic lymphocytic leukemia with a t(11;14) chromosome translocation] (*6*); (lane e) DNA from 545T T-cell line; (lane f) DNA From 380 leukemic cell line. The size of marker DNA is in kilobases.

whether the same chromosome 18–specific DNA segment cloned from a t(14;18) translocation in a case of acute lymphocytic leukemia could detect DNA rearrangements in follicular lymphomas with the same translocation. DNA from neoplastic cells of a 35-year-old male with follicular lymphoma (LN128) showed both a germ line and a rearranged DNA fragment hybridizing to the p380j-2RR DNA probe (Fig. 5). A lymph node biopsy specimen from this patient showed a histologic picture of typical follicular small-cleaved cell lymphoma, and all ten mitoses examined from this specimen showed the chromosome complement: 47, XXY, dup(1) (q21qter), and t(14;18) (q32.3;q21.3). Thus, the same DNA segment from chromosome 18 is rearranged in both the 380 and LN128 cell populations carrying the t(14;18) chromosome translocation, suggesting that the same gene, called *bcl-2* (B-cell lymphoma/leukemia 2), may be consistently involved in B-cell malignancies with this translocation (Fig. 6).

These results indicate that immunoglobulin heavy chain DNA probes can be used to clone sequences from segments of human chromosomes 8 and 18 translocated to band q32 of human chromosome 14, as has been shown in the case of neoplastic B cells carrying the t(11;14) chromosome translocation (*6*). In the 380 cells, the translocated c-*myc* gene, which is normally located on band q24 of human chromosome 8 (*7–9*), was still in its germ line configuration more than 14 kb upstream from the immunoglobulin heavy chain locus (*1*). The DNA segment derived from chromosome 18 was not only rearranged in the parental leukemic 380 cells but was also rearranged in a follicular lymphoma with the t(14;18) chromosome translocation. These findings suggest that the *bcl-2* gene, which is normally located on band q21.3 of human chromosome 18, may be moved into close proximity to a rearranged heavy chain locus. Since this translocation involves the J_H segment of the immunoglobulin chain gene, the enhancer located between the J_H and the switch region of the human μ chain gene (*10*) must be brought close to the translocated segment of chromosome 18. Thus, it seems possible that this enhancer may have an important role in the activation of the *bcl-2* gene in neoplastic B cells carrying the t(14;18) chromosome translocation. Since no human homolog of known retrovirus oncogenes is located on the long arm of human chromosome 18, we can infer that the postulated *bcl-2* gene is not homologous with any of the known human oncogenes.

In considering the pathogenesis of the acute B-cell leukemia from which the 380 cell line was derived, it is possible that the first step was the t(14;18) translocation resulting in a relatively low-grade neoplasm, analogous to follicular lymphoma. Subsequently, a second translocation, t(8;14), in one of the neoplastic cells could have led to activation of the c-*myc* oncogene (*11*) and expansion of a neoplastic subclone with the aggressive clinical characteristics of an acute leukemia.

The molecular cloning of the t(14;18) breakpoint in neoplastic B cells is important not only because it allows the study of the structure, organization, and expression of the postulated *bcl-2* gene, but also because it provides a molecular probe for the t(14;18) chromosome translocation that could prove useful in the diagnosis of follicular lymphoma in humans.

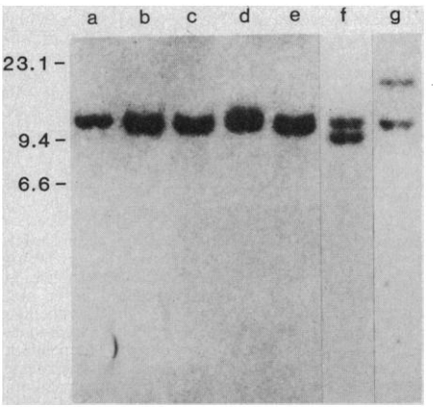


Fig. 5. Southern blot hybridization with human chromosome 18–specific probe p380j-2RR of DNA from 380 leukemic cells and LN128 lymphoma cells. Human DNA's were cut with Sst I and run on 0.7 percent agarose gel. The Southern blot filter was hybridized with ^{32}P-labeled p380j-2RR and washed as described in the legend to Fig. 3. (Lane a) DNA from normal human peripheral blood lymphocytes; (lane b) DNA from human T-cell lymphoma cell line; (lane c) DNA from cells of CLL271 [a chronic lymphocytic leukemia with a t(11;14) chromosome translocation]; (lane d) DNA from Burkitt lymphoma cell line (Daudi) with t(8;14) translocation; (lane e) DNA from 545T T-cell line; (lane f) DNA from 380 leukemic cell line; (lane g) DNA from LN128 cells [human follicular lymphoma with a t(14;18) chromosome translocation].

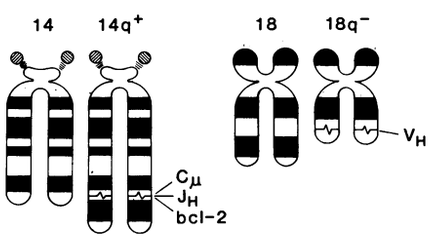


Fig. 6. Diagram of the t(14;18) translocation in human follicular lymphomas. The breakpoint on chromosome 14 directly involves the heavy chain locus. The V_H genes are translocated to the involved chromosome 18 (18q$^-$). The *bcl-2* locus translocated from its normal position on band q21.3 of human chromosome 18 to the heavy chain locus on chromosome 14.

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