Cloning of the Breakpoint Junction of the Translocation 14;19 in Chronic Lymphocytic Leukemia

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Human B-cell lymphocytic neoplasms are often associated with specific cytogenetic abnormalities that correlate with their histological and immunologic phenotypes. The genes located at the breakpoints of these recurring chromosomal translocations appear to be integrally involved in the pathogenesis of the corresponding B-cell neoplasms. Our laboratory has recently reported that t(14;19)(q32;q13.1) is a recurring translocation in chronic lymphocytic leukemia (CLL). We have analyzed the leukemic cells from two such patients in detail using various probes from the very complex immunoglobulin heavy chain locus (IGH). In both cases, the t(14;19) was part of a three-way translocation with loss of the derivative chromosome containing the q terminus of the affected chromosome 14. Using Southern blot analysis, numerous rearrangements and deletions were found within IGH in these two cases; however, in both, rearrangements were found involving one of the two α constant regions (Cα), which are components of IGH. As described below, these rearrangements result from the 14;19 translocation.

In addition, two internal deletions within IGH have occurred in case D.B., as confirmed by molecular cloning: the μ switch region is largely deleted, and another deletion extends from the switch region of $\alpha 1$ to the switch region of $\gamma 2$. Several additional rearrangements appear to involve the γ gene segments and may be related to a translocation 2;14, which is also present in this patient's malignant cells. Despite the involvement of the α regions in the translocations, the remaining J region, presumably responsible for any immunoglobulin heavy chain produced, is found associated with other constant region segments – Cμ in one patient and C$\gamma 4$ in the other.

In each case, clones containing the rearranged Cα sequences were isolated from a bacteriophage lambda library made using complete *Bgl*II digests. In patient J.L., the rearrangement involved C$\alpha 2$ while C$\alpha 1$ was affected in patient D.B. In both cases, the break in chromosome 14 appears to involve one of the α switch regions. Detailed restriction mapping was used to identify the portion of the clones which was not derived from chromosome 14. Subclones free of repetitive sequences were, in each case, localized to chromosome 19 using a panel of 31 mouse/human somatic cell hybrids. These results were confirmed by in situ hybridization to metaphase chromosomes. By hybridization to somatic cell hybrids containing fragments of chromosome 19, the probes were sublocalized to the q arm of chromosome 19, bands q12 to 13.2.

We have used a human library, prepared from the DNA of a patient without abnormalities of chromosome 19, to isolate clones from the normal sequences on chromosome 19. Restriction mapping of these clones, which encompass about 30 kb of DNA, demonstrates that the breakpoints in the two patients are about 19 kb apart. Such variability is common in other chromosome translocations associated with leukemia or lymphoma. Thus, it is likely that the same gene is affected by the t(14;19) in both patients.

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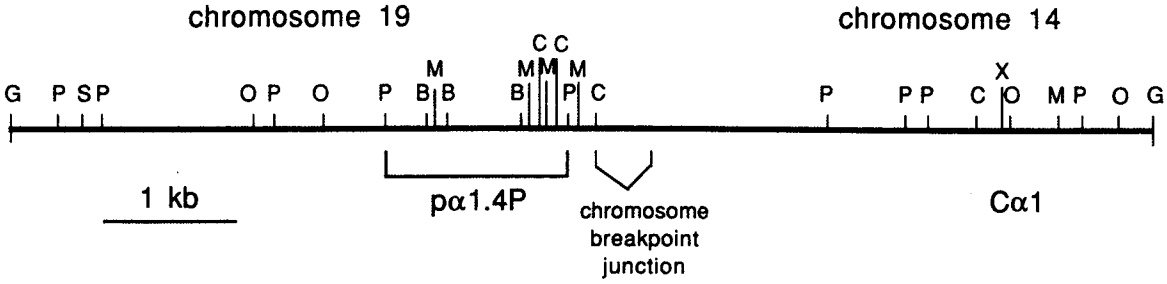

Fig. 1. Restriction map of the cloned *Bgl*II fragment from case 2. The 3′ portion contains Cα1 sequences. A subclone, pα1.4P, was mapped to chromosome 19 using in situ hybridization and hybridization to a panel of somatic cell hybrids. This probe detects a 2.3-kb message in several hematological cell lines. Restriction enzyme sites are illustrated as follows: *B, Bam*HI; *C, Sac*II; *G, Bgl*II; *M, Sma*I; *O, Oxa*NI; *P, Pst*I; *S, Sal*I; *X, Xho*I. Note the cluster of sites for *Sma*I and *Sac*II which partly overlaps pα1.4P. These sites are rare in human DNA except in "CpG islands," which are regions with high C+G content and unusually high frequency of the dinucleotide CpG. These islands are associated with the 5′ ends of many genes. The presence of a CpG island was confirmed by sequencing the pα1.4P segment

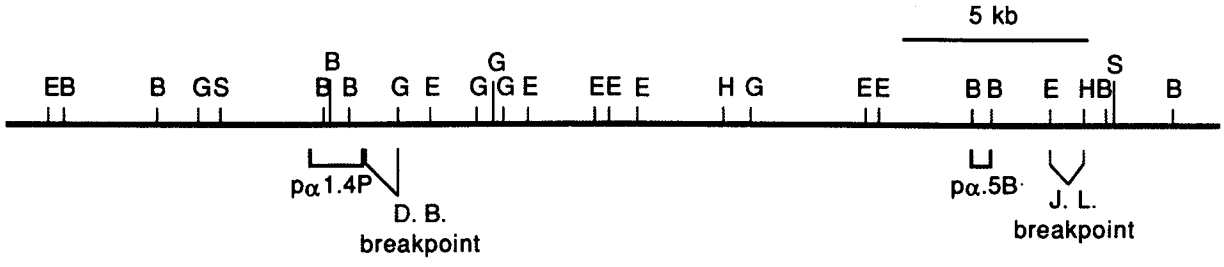

Fig. 2. Composite map of the region on chromosome 19 affected by the t(14:19). This map is based on overlapping bacteriophage clones which were isolated from a normal library, using the two probes pα.5B and pα1.4P adjacent to the translocation breakpoint junctions of the two patients. The locations of the two probes and of the breakpoint junctions are indicated. Restriction sites are as follows: *B, Bam*HI; *G, Bgl*II; *E, Eco*RI; *H, Hind*III; *S. Sal*I

Very recently, we have obtained evidence for a break in the same region in a third patient with the t(14;19).

The region on chromosome 19 near the breakpoint in patient D.B. has an unusually large number of sites for restriction enzymes containing the dinucleotide "CG" as part of their recognition sequence. For example, three *Sma*I sites (one CG) and three *Sac*II (two CGs) are found within a region of about 600 basepairs; *Sac*II sites are generally much less frequent than this, occurring on average about 200 kb apart. Most such restriction sites are associated with "CpG islands," areas of high C+G content and unusually high abundance of the dinucleotide CG, which is otherwise highly depleted in the human genome. CpG islands are found associated with the 5′ portions of all known "housekeeping" genes and many tissue-specific genes as well.

The suspicion that this region is a CpG island was confirmed by DNA sequencing. A 400-basepair region was found to have a high abundance of CG dinucleotides and a C+G content of 80%, which is twice the value for most human sequences. Hybridization of a probe from this region to a 2.3-kb RNA transcript in several lymphoid cell lines has provided further evidence that a gene on chromosome 19 is adjacent to the chromosome breakpoint. We are currently attempting to clone and analyze this gene.