Involvement of the bel-2 Gene in Human Follicular Lymphoma

Abstract. Recombinant DNA probes were cloned for the areas flanking the breakpoint on chromosome 18 in cells from a patient with acute lymphocytic leukemia of the B-cell type; cells of this line carry the t(14;18) chromosomal translocation. Two of the probes detected DNA rearrangements in approximately 60 percent of the cases of follicular lymphoma studied. Most of the breakpoints in band q21 of chromosome 18 were clustered within a short stretch of DNA, approximately 2.1 kilobases in length. Chromosome 18-specific DNA probes for the areas flanking the breakpoints also detected RNA transcripts 5 kilobases in length in various cell types. The gene coding for these transcripts (the bel-2 gene) seems to be interrupted in most cases of follicular lymphomas carrying the t(14;18) chromosomal translocation.

Follicular lymphoma is one of the most common human B-cell neoplasms; in most patients the lymphomas cells carry a translocation between chromosomes 14 and 18 (1,2). By taking advantage of an established cell line, 380, derived from a patient with acute lymphocytic leukemia of the pre-B-cell type (3), we cloned the DNA region joining chromosomes 14 and 18 on the 14q chromosome of line 380 cells (4). Using chromosome 18-specific DNA probes flanking the chromosome breakpoint of line 380 cells, we also showed DNA rearrangements of the homologous DNA segments in follicular lymphoma cells with the t(14;18) chromosome translocation (5).

In the present study we have investigated the on the region of chromosome 18 that is involved in chromosome rearrangements in B-cell neoplasms to map the breakpoints in follicular lymphoma and to identify the putative bel-2 gene. The study may provide a role in the pathogenesis of follicular lymphoma and other B-cell neoplasms carrying the t(14;18) chromosomal translocation.

By using probes that mapped close to the t(14;18)-associated breakpoint on chromosome 18 in line 380 leukemic cells, we were able to

8. L. V. Ensar et al., ibid. p. 628.
10. Synthetic peptides H(Ala-Asn-Pro) - H(Ala-Asn-Pro), H(Ala-Asn-Pro), H(Ala-Asn-Pro), were synthesized by the stepwise solid-phase method of B. Merrifield (I. Am. Chem. Soc. 82, 3440 (1961)). The attachment of the COOH-terminal amino acid residue, Glycyl-L-lysine (GlyLys), into a hydroxyethyl polylysylaminoethyl (HEPAM) resin (polylysine and 1 percent divinylbenzene) support (A. S. Mitchell et al., J. Am. Chem. Soc. 98, 7257 (1976)), is possible because of high yield of peptide chains during the synthesis.
Figure 1A. DNA's were extracted from 17 randomly selected follicular lymphomas and screened for DNA fragments by Southern blot hybridization with chromosome 18-specific c, b, c, and d (Fig. 1A). Probe c detected rearranged DNA fragments in normal DNA (Fig. 1B) and also in DNA from LN129 follicular lymphoma cells with the t(14;18) chromosome translocation (4). This probe detected a rearranged DNA fragment in only one (FL1018) of the additional 16 cases of follicular lymphoma tested (Fig. 1B). The rearranged DNA fragment was not due to polymorphisms in restriction fragment length because hybridization of FL1018 follicular lymphoma DNA from different preparations from the same patient revealed unrearranged bands of various intensities; these variations were caused by the variable numbers of normal cells contaminating the neoplastic specimen.

Probe b detected rearrangements in 9 of 17 follicular lymphoma DNA's digested with Bam HI (Fig. 1C) and Sts I (Fig. 1D). Two or three (FL993) rearranged fragments and one unrearranged frag-
ment (FL1281) were observed after Sst I digestion. This suggested that the breakpoint in these eight cases occurred within the hybridization of follicular lymphoma (kiolobases) in length (Fig. 2). The two rearranged fragments may represent probe b-hybridizing sequences that were split by the chromosome breakpoints. Thus one of the two fragments may represent the segment of chromosome 18 that is translocated to the 14q" chromosome, and the other may represent the segment of chromosome 18 that remains on the 14q" chromosome. In the other rearranged fragment in Bam HI digestion of follicular lymphoma DNA's can be explained by comigration of one rearranged fragment with one of the other two hybridizing fragments. Hybridization of 20 normal human DNA's with probe b revealed no DNA rearrangement, which confirms that the rearranged fragments in follicular lymphoma DNA's reflect chromosome translocation and not restriction site polymorphism.

No rearrangements were observed after hybridization of follicular lymphoma DNA's with either DNA probe a or d. Thus DNA probes b and c, which are only 13 kb apart, detected rearrangements in 11 of 18 follicular lymphoma DNA's (87%) (Fig. 2). Only sample LN128 was karyotyped before DNA analysis (5) (Fig. 2). Thus, these probes can be used to detect a t(14;18) translocation in B-cell neoplasms. Our data also indicate that, in most follicular lymphomas showing rearrangements, the chromosome breakpoints are clustered within a very short segment of DNA (2.1 kb in size).

We have previously shown that the breakpoint on chromosome 14 in the t(14;18) chromosome translocation of line 380 cells is close to the JH region of the immunoglobulin heavy-chain locus (4). By comparing the detailed restriction maps of the recombinant clone representing normal chromosome 18 sequences (A380-18-4; Fig. 1A) and of the clone representing the breakpoint (A380-2-1; Fig. 1A), we conclude that the breakpoint on chromosome 14 occurs within the JH region.

To determine whether the chromosome 18 sequences involved in follicular lymphomas with the t(14;18) chromosome translocation are also translocated to the JH region of the heavy-chain locus, we hybridized Sst I-digested DNA's with probe b and with a JH probe (pPHJ) (4, 5). In five cases tested, some of the rearranged fragments that hybridized with probe b also hybridized with the pPHJ probe, suggesting that the breakpoint on chromosome 14 map to the JH region (Fig. 3, A and B). We have also cloned the joining region between chromosome 14 and 18 in four (FL966, FL1003, FL1032, and FL1144) of the nine cases described in Fig. 1C and have detected both JH and probe b sequences within the same genomic clones.

Thus the breakpoints on chromosome 14 in the cases we analyzed are also clustered and involved the JH region of the heavy-chain locus. Clustering of breakpoints (within a region of 0.9 kb) has been observed on chromosome 11 in cells having the t(11;14) translocation from a patient with chronic lymphocytic leukemia (CLL) of the B-cell type and a patient with diffuse B-cell lymphoma (5, 6). In the case of the t(11;14) chromosome translocation in CLL of the B-cell type, the translocation seems to be sequence-specific and to involve the enzyme that takes part in VDJ joining (6). DNA sequence analysis of the breakpoints in the t(14;18) translocation may provide new findings concerning the mechanisms of this translocation.

We have proposed that a gene, for which we suggested the name bel-2, has a role in the t(14;18) translocation in follicular lymphomas (4) and in other B-cell neoplasms such as acute B-cell leukemia, from which the 380 cell line was established (6) and that this gene is important in the pathogenesis of these diseases (3, 6).

To determine whether a cellular gene (the putative bel-2 gene) is involved in the t(14;18) chromosome translocation, we used probe b to detect RNA transcripts in polyadenylated (poly(A)) RNA isolated from line 380 leukemic cells with the t(14;18) translocation. We detected a 6-kb RNA transcript that hybridized to the nick-translated b probe (4, A and C). The smaller RNA band hybridizing with the probe might represent cross-hybridization to contaminating 28S ribosomal RNA. The levels of bel-2 transcripts in line 380 cells (pre-B cells) were higher than those in line 697 cells, which are also derived from a pre-B-cell line, but carry a t(11;19) chromosome translocation (Fig. 4A). Hybridization of the same filter with a P-labeled human phosphoglycerate kinase (PGK) complementary DNA probe (pHPGK-7e) showed approximately the same levels of transcripts (Fig. 4B). Northern blot hybridization of RNA from an Epstein-Barr virus-transformed lymphoblastoid cell line (GM1500), a chronic myelogenous leukemia cell line (Bv173), an erythroleukemia cell line (K562), and a T-cell lymphoma cell line (Jurkat) indicated the presence of the bel-2 transcripts, although in lower amounts than in line 380 leukemic cells carrying the t(14;18) chromosome translocation (Fig. 4C). This suggests that transcription of the bel-2 gene of line 380 cells is deregulated by its translocation to the heavy-chain locus. We have shown a similar phenomenon in the case of Burkitt lymphoma, where the c-myc oncogene is deregulated by its close proximity to one of the three immunoglobulin loci [see (8)]. Because the breakpoint on chromosome 14 in line 380 cells maps within the JH region, the immunoglobulin enhancer element of the c-myc locus may be required to promote expression of a gene in the breakpoint region. This enhancer may have a role in bel-2 gene activation in line 380 cells.

Because most of the chromosome breakpoints on chromosome 18 in follicular lymphomas showing DNA rearrangement occur within probe b, and because this probe contains an extra 6-kb exon transcribed into the 6-kb bel-2 RNA transcripts, many of the breakpoints in follicular lymphoma must involve or be very close to the bel-2 gene.
Heat Shock Genes: Regulatory Role for Differentiation in Parasitic Protozoa

Abstract. The parasitic protozoa Trypanosoma brucei and Leishmania major are transmitted by insect vectors to their mammalian hosts. The temperature difference between the hosts (25°C and 37°C) may induce a heat shock response in the parasite. Transcripts of heat shock genes (homologous to Hsp70 and Hsp83) were 25 to 100 times more abundant in Trypanosoma brucei bloodstream forms (trypanosomes) than in insect (procyclic) stages. In Leishmania major the patterns of heat shock gene expression in promastigotes (insect-adapted) and amastigotes (mammal-adapted) were different. A temperature shift in vitro induced differentiation of Leishmania major from promastigotes to amastigotes. Therefore, heat shock genes may be responsible for differentiation of these vector-borne parasites.

Heat shock genes are activated when a cell responds to stress, such as an increase in temperature (37°C to 42°C) or exposure to inhibitors of oxidative phosphorylation (I). The heat shock response involves the immediate activation of several heat shock genes, resulting in extensive synthesis of heat shock proteins (Hsp's), a rapid decrease in transcription of most other genes, and a cessation in the synthesis of most other proteins (I, 2). In many organisms heat shock-related genes are expressed during certain stages of cell development: in mice during embryogenesis (3), in erythropoiesis (4), and in yeast at sporulation (5). These heat shock gene responses indicate that Hsp's, which have a nuclear location (6, 7), may be involved in differentiation.

Many parasitic protozoa have biphasic life cycles that involve an insect vector and a mammalian host. Adaptation of the protozoa to either of its hosts involves differentiation with extensive morphological alterations, often including a sexual life cycle in the insect vector and a switch from oxidative phosphorylation in the insect to anaerobic respiration in the mammalian host (8). Trypanosoma brucei in addition loses its protective cell-surface coat when entering the fly gut, where it differentiates into the non-infecctive procyclic trypanosome (9, 10). The kinetoplastid protozoa T. brucei and Leishmania major (=Leishmania tropica major) are transmitted by the tsetse fly and the sand fly, respectively. These insects are restricted to habitats with a very narrow temperature range (22°C to 28°C) (11, 12). In nature, transfer of the parasite from its poikilothermic (temperature-regulated) insect vector to the homeothermic (temperature-regulated) mammalian host might trigger a heat shock response that could play a role in the adaptation of the parasite to life in mammalian host tissues.

References and Notes

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