Alternative Translocation Breakpoint Cluster Region 5’ to BCL-6 in B-cell Non-Hodgkin’s Lymphoma


Institute for Cancer Genetics, and the Departments of Pathology and Genetics & Development, Columbia University, New York, NY 10032 [M. P. B., S. I., R. D. F.]; Hematology Unit, Division of Internal Medicine, Department of Medical Sciences, Amedeo Avogadro University of Eastern Piedmont, 28100 Novara, Italy [D. C., D. R., G. G.]; Cell Biology and Genetics Programs, Memorial Sloan-Kettering Cancer Center, New York, New York 10021 [P. H. R., P. N., D. C. L., S. C., R. S. K. C.]; and the Department of Pathology, British Columbia Cancer Agency, Vancouver, V5Z 4E6 Canada [T. A., R. D. G.]

ABSTRACT

Chromosomal translocations involving band 3q27 with various different partner chromosomes represent a recurrent cytogenetic abnormality in B-cell non-Hodgkin’s lymphoma. In a fraction of these translocations, the chromosomal breakpoint is located within the 5’ non-coding region of the BCL-6 proto-oncogene where the BCL-6 major breakpoint region (MBR) maps. As a result of the translocation, BCL-6 expression is deregulated by promoter substitution. However, between 30 and 50% of lymphomas with cytogenetically detectable translocations affecting band 3q27 retain a germ-line configuration within the BCL-6 locus. To identify possible additional breakpoint clusters within 3q27, we cloned a t(3;14)(q27;q32) lymphoma without MBR rearrangement and found a novel breakpoint site located between 245 and 285 kb 5’ to BCL-6. Breakpoints within this newly described region, which we called the alternative breakpoint region (ABR), were found to be recurrent in lymphomas carrying t(3;27) chromosomal translocations but devoid of BCL-6 MBR rearrangements. Comparative analysis of multiple lymphomas carrying rearrangements within the ABR showed that the breakpoints cluster within a 20-kb distance. Translocations involving the ABR may juxtapose BCL-6 to distinctly acting, heterologous transcriptional regulatory elements which cause deregulation of the proto-oncogene. The identification of BCL-6-ABR provides new tools for the diagnosis of lymphomas carrying aberrations at 3q27 and deregulated BCL-6 genes.

INTRODUCTION

B-cell NHL form a heterogeneous group of neoplasms that arise as a result of the clonal expansion of a single cell arrested at a specific stage of B-cell differentiation (1). The uncovering of the genetic elements involved in lymphomagenesis has been aided by cytogenetic analysis, which has revealed that nonrandom chromosomal translocations are found in association with specific subtypes of NHL (1–2). The molecular cloning of the breakpoint junctions of these translocations has led to the identification of several proto-oncogenes that undergo transcriptional deregulation because of their juxtaposition to regulatory elements of genes constitutively expressed in mature B cells, most often represented by Ig genes (1). The B-cell NHL-associated oncogenes discovered to date include BCL-1 at 11q13 involved in ~95% of mantle cell lymphomas, BCL-2 at 18q21 involved in 70–90% of FLs (1), c-MYC at 8q24, which is found to be rearranged in 100% of Burkitt’s lymphoma (1), PAX-5 at 9p13 involved in 50% of lymphoplasmacytoid lymphomas (1), and BCL-6 at 3q27 rearranged in 30–40% of DLBCLs and in 4–15% of FL cases (3–5).

The BCL-6 gene encodes a nuclear phosphoprotein characterized by six COOH-terminal Kruppel-type zinc finger motifs, and an NH2-terminal POZ motif, shared by several zinc finger molecules, including the Drosophila developmental regulators Tramtrak and Broad-Complex, as well as the human KUP, ZID, and PIzf proteins (3). BCL-6 has been shown to function as a potent transcriptional repressor of promoters linked to its DNA target sequence (6–8). The BCL-6 protein is expressed in mature B-cells within germinal centers but not in immature B-cell precursors or in differentiated plasma cells (9). Targeted disruption of the BCL-6 gene shows that BCL-6 is essential for germinal center formation, being involved in the control of Th2-type immune responses (10, 11).

Rearrangements involving the BCL-6 gene at 3q27 cluster mainly in a 4-kb genomic region, termed the MBR, which spans the first noncoding exon of the gene (3–5). Translocations affecting band 3q27 in NHL are not limited to the Ig loci but may involve numerous different partner chromosomes. The molecular characterization of several 3q27 chromosomal translocations has shown that the coding domain of the translocated BCL-6 gene becomes fused downstream to heterologous promoters. These include the Eμ and Ig promoters of Ig genes in the case of t(3;14) as well as promoters of other genes, namely TTF, BOB-1, H4, in the case of t(3;4), t(3;11), and t(3;6), respectively (12–15). A general feature of promoters juxtaposed to translocated BCL-6 is their constitutive expression in B-cells, which leads to the inappropriate expression of BCL-6 (12–15).

Between 30 and 50% of the 3q27 chromosomal breakpoints found in lymphomas do not occur within the MBR of BCL-6, suggesting the presence of alternative breakpoints at 3q27 (3). To address this question, we cloned a t(3;14)(q27;q32) occurring in a FL case shown to be negative for rearrangement at the MBR of BCL-6. We detected a novel breakpoint cluster located 245–285 kb upstream of the first exon of the BCL-6 gene and termed ABR. This breakpoint site was shown to be recurrent in several lymphomas with 3q27 chromosomal translocations that lacked BCL-6 MBR rearrangement.

MATERIALS AND METHODS

Tumor Biopsies and Cell Lines. Lymph node biopsies from NHL cases were obtained from the Memorial Sloan-Kettering Hospital. Tumors were classified histologically according to the Revised European-American Lymphoma Classification (16). The t(3;14)(q27;q32)-positive NHL case 1952, which served for BCL-6 ABR cloning, was diagnosed as having a FL demonstrating an add (8)(q24) but no karyotypic evidence of a t(14;18)(q32;q21).
translocation. Immunohistochemical analysis demonstrated that this tumor expressed surface IgM.

Southern and Northern Blot Analysis. High molecular weight DNA was extracted by the SDS/proteinase K method followed by “salting out” and ethanol precipitation. Southern blot analysis was performed following standard procedures. The following probes were used for Southern blot analysis of Ig gene rearrangements: \( J_H \) probe, represented by a 6.6-kb BamHI/HindIII fragment from the \( I_g H \) locus (17); and \( C_H \) probe, represented by a 1.3-kb EcoRI fragment from the \( I_g H \) locus (17). Some of the \( BCL-6 \) ABR probes used in this study have been used in a previous study (18). For Northern blot analysis, RNA was extracted by the SDS/proteinase K method followed by “salting out” and ethanol precipitation.

Cloning of Chromosomal Breakpoints. High molecular weight DNA from NHL case 1952 was digested with BamHI and size fractionated by running on a 0.7% low-melting agarose gel. Fragments ranging from 12 to 23 \( BamHI \)-digestion.

RESULTS

Cloning of t(3;14) from a FL. Cloning of t(3;14)(q27;q32) was performed on case 1952, represented by an IgM-producing FL. Southern blot analysis of Ig genes using BamHI and HindIII digestions and probes for \( J_H \) and \( C_H \) sequences from a recombinant phage library (panel 2, National Institute of General Medical Sciences Human Genetic Mutant Cell Repository, Coriell Institute, Camden, NJ), as well as FISH analysis to metaphase spreads of mitogen-stimulated normal human lymphocytes. Insert sizing of P1 and PAC clones was achieved through their digestion with NotI and NotI restriction digests, respectively. For YAC clones, yeast cells were embedded in agarose plugs by a standard method and analyzed by PFGE on the CHEF-DR II (Bio-Rad, Richmond, CA) after restriction digestion.

FISH. Briefly, probes were labeled by nick translation with biotin-16-dUTP or digoxigenin-11-dUTP and detected by indirect immunofluorescence using fluorescein isothiocyanate-conjugated avidin and rhodamine-conjugated anti-digoxigenin, respectively. Chromosomes were counterstained with 4',6-diamidino-2-phenylindole. Images were captured with a cooled charge-coupled device camera (Photometrics, Tucson AZ) attached to a Nikon Microphot-SA microscope and processed using Smart capture imaging system (Vysis, Downsner Grove, IL).

DNA Sequencing. DNA sequencing was performed by the dyeodeoxy chain termination method using the ABI 373A automated sequencing system (Applied Biosystems, Foster City, CA).
screened by both J_H and C_H probes. Restriction mapping and hybridization analysis revealed that the recombinant phage clones contained J_H and C_H/H9262 sequences juxtaposed to sequences unrelated to the Ig H locus. To determine the chromosomal origin of these sequences, a 2.1-kb BamHI-HindIII fragment from 1952-2, as well as a 1.3-kb XbaI fragment from 1952-23 (Fig. 1), were hybridized to DNA of somatic-cell hybrids representative of individual human chromosomes. Both probes identified human sequences only on somatic cell hybrids containing chromosome 3 (data not shown). Probe 2.1 B/H from 1952-2 was then used to screen a genomic placenta library to clone the corresponding normal locus on chromosome 3, represented by phage clones 1952-15 and 1952-11 (Fig. 2).

Restriction mapping and partial sequence analysis of der(3) and der(14) indicated that the clones represented a reciprocal translocation (Fig. 1). The position of the breakpoints was mapped by sequencing of derivatives (3) and (14) junctions regions, which revealed that the recombination involved a deletion of 63 bp on chromosome 3 (Fig. 1). FISH analysis of metaphase spreads prepared from case 1952 using the phage contig (1952-15/11) as a probe revealed signals on chromosomal derivatives (3) and (14) as well as normal chromosome 3, band q27, thus confirming the derivation of these phages from a t(3;14) (data not shown).

By sequencing analysis, the typical Ig switch pentameric repeat motifs were seen in the immediate vicinity of the cloned breakpoint. No apparent homology was detectable between chromosome 14 and chromosome 3 sequences near the breakpoint.

Relationship between the 3q27 Breakpoint of Case 1952 and the BCL-6 Gene. To define the position of the newly identified 3q27 breakpoint, a contig of P1, PAC, and YAC clones was constructed linking the newly cloned ABR to the BCL-6 locus (Fig. 2). A restriction map of the YAC clone 19GA10 is given in Fig. 2, showing MluI and XhoI restriction enzyme sites identified by double enzyme digestion and by hybridization analysis using YAC/PAC clone ends. By Southern blot hybridization analysis, probe 1.3 X from ABR (Fig. 2) hybridized only to YAC clone 19GA10 in the contig. It hybridized specifically to a 220-kb MluI restriction fragment of YAC clone 19GA10. An adjacent ABR probe, termed 2.5 B/H, hybridized to a different MluI restriction fragment of 85 kb (see Fig. 2). This indicates that the ABR is located 220 kb from the left YAC arm terminus, 19GA10L. By Southern blot hybridization analysis, the 5' marker of P1371O8 and PAC133M19. The 5' marker of P1371O8 (T7 end) is positioned ~65 kb 5' to exon 1 of BCL-6 by a combination of PFGE and hybridization analysis (Fig. 2). Marker 133M19T7 maps 25 kb 5' to BCL-6 as determined by restriction fingerprint analysis and hybridization analysis (data not shown). Overall, these results indicate that ABR is positioned at a distance of 245–285 kb 5' to BCL-6.
Recurrency of the BCL-6 ABR in Lymphomas. To assess the frequency of ABR, we collected a series of lymphomas (n = 12) with cytogenetic evidence of t(3q27) translocations but absence of rearrangement involving the MBR of BCL-6. The histological and partial cytogenetic profiles of these NHL cases are presented in Table 1. Southern blots of HindIII-, BglII-, and BamHI-digested tumor DNAs were prepared and hybridized sequentially with probes derived from phages λ9261-15/11, exploring a genomic distance of ~40 kb surrounding the breakpoint of case 1952. All rearrangements reported were confirmed by analysis with a second enzyme digest. Representative results of hybridization analyses with probes from the ABR on Southern blots of t(3q27) NHL cases are shown in Fig. 3. Using probes 1.3 X and 0.4 HaeIII on HindIII, BglII, and BamHI Southern blot digests of lymphoma cases (Fig. 3), rearrangements were seen in 42% (5 of 12) of NHL cases that had cytogenetic evidence of t(3q27) chromosomal translocations and that showed germ-line configuration of the MBR (Table 1).

Subsequently, we investigated a cytogenetically uncharacterized series of FL (n = 20) and DLBCL (n = 84) that were known to be devoid of BCL-6 MBR rearrangements. Rearrangements of the BCL-6 ABR were detected in 10% (2 of 20) FL and 2.4% (2 of 84) DLBCL, including one case arising in an immunocompetent host and one case associated with AIDS. Mapping studies showed that all ABR rearrangements of BCL-6 clustered within a 20-kb region.

**BCL-6 Expression Levels in NHL Cases with ABR Rearrangement.** To analyze the effect of ABR rearrangement on BCL-6 gene expression, we examined the levels of BCL-6 expression in lymphoma cases 1952 (FL) and 1188 (DLBCL) (both rearranged at ABR), as compared with two control FLs (cases 2065 and 1992) showing no rearrangement at the ABR or MBR of BCL-6 (Fig. 4). BCL-6 RNA levels were similar, independent of the BCL-6 genomic status. In addition, BCL-6 expression levels of case 1952 were also comparable with those of DLBCL cell line LY18 with germ-line BCL-6, as well as of immunoblastic lymphoma LY8 carrying a MBR rearrangement. Overall, these results indicate that

<table>
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<th>Case</th>
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<th>MBR</th>
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<td>DLBCL</td>
<td>add(3q27)</td>
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cases with ABR rearrangement express BCL-6 at levels comparable with phenotypically similar cases with MBR rearrangement or lacking 3q27 abnormalities.

DISCUSSION

B-cell NHL are typically characterized by recurrent chromosomal translocations, which deregulate proto-oncogenes found at or in close proximity to the chromosomal breakpoint junction areas (1). In some translocations, exemplified by the BCL-1 and BCL-2 translocations, the proto-oncogene may be involved by multiple, distinct breakpoint clusters that are recurrent in different patients (21, 22). In the case of 3q27 translocations involving the BCL-6 proto-oncogene, only one breakpoint cluster has been identified to date (3). This breakpoint cluster, termed BCL-6 MBR, is detectable at the molecular level in ~50–60% of lymphoma cases carrying the 3q27 translocation (3–5). The occurrence of lymphomas harboring 3q27 breaks but devoid of BCL-6 MBR rearrangements point to the putative existence of other BCL-6 breakpoint clusters. Here we report the identification of a novel ABR, which, together with the BCL-6 MBR, accounts for ~70% of the total number of 3q27 chromosomal breakpoints detectable in NHL cases.

The BCL-6 ABR is located at a distance of ~280 kb telomeric to BCL-6, raising the question of how the translocation induces BCL-6 deregulation. On the basis of the model of several other lymphoma translocations, the ABR breaks may exert several possible modes of BCL-6 deregulation. These include substitution of enhancer regions, a translocations, the ABR breaks may exert several possible modes of deregulation. On the basis of the model of several other lymphoma

REFERENCES


Fig. 4. Northern blot analysis of the t(3;14) positive lymphoma case 1952 hybridized with the BCL-6 probe. Other lymphoma cases are also included for comparison. A 10 μg aliquot of total RNA was loaded onto each lane, blotted, and hybridized with the full-length BCL-6 cDNA probe together with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe to control for the amount of RNA loading, as well as RNA degradation for the tumor RNAs. LVY8 and LVY17 are DLBCL cell lines; LV8 is an immunoblastic lymphoma cell line; primary cases 2065, 1992, and 1952 are FL; case 1188 is a DLBCL. The genomic status of each sample is indicated as ABR; MBR; G (for germ-line configuration at the ABR and MBR of BCL-6).


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Marion P. Butler, Shinsuke Iida, Daniela Capello, et al.


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