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, the 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 at 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 distantly 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 lymphoplasmocytoid 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 PLZF 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)

Received 2/1/02; accepted 5/17/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by the National Institute of Health Grant CA-37295 (to R. D.-F.); Istituto Superiore di Sanità, III Programma Nazionale di Ricerca sull’AIDS 1999 - Progetto Patologia, Clinica e Terapia dell’AIDS, Rome, Italy (to G. G.); and Fondazione “Piero Pietro e Giovanni Ferrari,” Alba, Italy (to G. G.). M. P. B. was supported in part by the Mater College, Dublin, Ireland, and the Vocational Education Committee Co., Dublin, Ireland. M. P. B. was supported in the PhD program of medicine in University College Dublin, Ireland. D. C. L. S. is supported by a fellowship from Federazione Italiana Ricerca Cancro, Milan, Italy.

2 To whom requests for reprints should be addressed, at Institute for Cancer Genetics, Columbia University, Russ Berrie Science Pavilion, 1150 Saint Nicholas Avenue, Room 303B, New York, NY 10032. Phone: (212) 851-5273; Fax (212) 851-5256; E-mail: rd10@columbia.edu.

3 The abbreviations used are: NHL, non-Hodgkin’s lymphoma; Ig, immunoglobulin; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; MBR, major breakpoint region; ABR, alternative breakpoint region; FISH, fluorescent in situ hybridization; PFGE, pulsed-field gel electrophoresis.
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 HindIII fragment from the \( J_H \) locus (17); and \( C_H \) probe, represented by a 1.3-kb EcoRI fragment from the \( J_H \) locus (17). Some of the \( BCL-6 \) probes used in this study have been used in a previous study (18). For Northern blot analysis, RNA was extracted from cell lines and patient samples using the guanidinium thiocyanate/cesium chloride method, electrophoresed on a 1.0% agarose gel, and then hybridized with PCR-derived probe. The probe used for hybridization was labeled with biotin. 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, Downers Grove, IL).

DNA Sequencing. DNA sequencing was performed by the dyeoxy chain termination method using the ABI 373A automated sequencing system (Applied Biosystems, Foster City, CA).

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 \( B_{CLH} \) and \( H_{INDIII} \) probes on case 1952, represented by an IgM-producing FL. Southern blot analysis of Ig genes using \( B_{CLH} \) and \( H_{INDIII} \) probes showed the presence of three rearranged \( J \) fragments, two of which did not comigrate with any constant gene region probe (data not shown) as observed previously in several cases of chromosomal translocations involving the \( I_{DH} \) locus (20). We then proceeded to clone the rearranged \( B_{CLH} \) fragments containing \( J_H \) and \( C_H \) sequences from a recombinant phage library constructed from \( B_{CLH} \)-digested DNA from case 1952, which was through the hybridization of PCR-derived end clone fragments to somatic cell hybrid filters (panel 2, National Institute of General Medical Sciences Human Genetic Mutant Cell Repository, Coriell Institute, Camden, NJ), as well as by FISH analysis to metaphase spreads of mitogen-stimulated normal human lymphocytes. Insert sizing of \( P1 \) and \( PAC \) clones was achieved through their hybridization with \( NorI/UnI \) and \( NorI \) restriction digestes, 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, Downers Grove, IL).

DNA Sequencing. DNA sequencing was performed by the dyeoxy chain termination method using the ABI 373A automated sequencing system (Applied Biosystems, Foster City, CA).
screened by both J_H and Cμ probes. Restriction mapping and hybridization analysis revealed that the recombinant phage clones contained J_H and Cμ/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 H92611952-2, as well as a 1.3-kb XbaI fragment from H92611952-23 (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 H92611952-2 was then used to screen a genomic placenta library to clone the corresponding normal locus on chromosome 3, represented by phage clones H92611952-15 and H92611952-11 (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 (H9252-15/11) 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 phases 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 EagI 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, probe 19GA10L hybridizes to both 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.
Recurrence 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 \(H92611952-11\), 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.

**Table 1** Rearrangement analysis at ABR in NHL cases with 3q27 chromosomal translocations

<table>
<thead>
<tr>
<th>Case</th>
<th>Histology</th>
<th>3q27 aberration</th>
<th>ABR</th>
<th>MBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1952</td>
<td>FL</td>
<td>t(3;14)(q27;q32)</td>
<td>R</td>
<td>G</td>
</tr>
<tr>
<td>1562</td>
<td>DLBCL</td>
<td>t(2;3)(q21;q27)</td>
<td>R</td>
<td>G</td>
</tr>
<tr>
<td>591</td>
<td>DLBCL</td>
<td>t(3;22)(q27;q11)</td>
<td>R</td>
<td>G</td>
</tr>
<tr>
<td>95-625</td>
<td>DLBCL</td>
<td>t(3;14)(q27;q32)</td>
<td>R</td>
<td>G</td>
</tr>
<tr>
<td>1188</td>
<td>DLBCL</td>
<td>add(3q27)</td>
<td>R</td>
<td>G</td>
</tr>
<tr>
<td>498</td>
<td>DLBCL</td>
<td>t(3;9)(q27;p13)</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>1187</td>
<td>FL</td>
<td>t(2;3)(q21;q27)</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>770</td>
<td>DLBCL</td>
<td>add(3q27)</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>1879</td>
<td>FL</td>
<td>t(3;14)(q27;q32)</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>95-517</td>
<td>DLBCL</td>
<td>t(2;3)(q21;q27)</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>95-89</td>
<td>DLBCL</td>
<td>t(6;14)(q27;p35;q32)</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>91-153</td>
<td>DLBCL</td>
<td>t(3;14)(q27;q32)</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

Fig. 3. Southern blots showing rearrangement at the BCL-6 ABR in three representative lymphoma cases. Rearrangements are depicted in lymphoma cases 1188, 1562, and 95-625, confirmed by two DNA digests.

**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...
region of the translocation breakpoint (23). An alternative mode of BCL-6 deregulation by ABR translocations consists in the removal of cis-acting regulatory domains in a fashion similar to that suggested for some rearrangements involving the c-MYC locus (24). In the case of NHL cases rearranged at the ABR but not involving the Ig genes, it is conceivable that BCL-6 is deregulated by enhancer substitution of genes other than Ig genes. Finally, it cannot be formally excluded that ABR breaks affect genes mapping to 3q27 but unrelated to BCL-6. However, the genomic region surrounding ABR analyzed to date failed to reveal any functionally proven gene.

Our survey of BCL-6 ABR breakpoints in B-cell lymphoma defines that the frequency of ABR rearrangements is substantially lower than that of BCL-6 MBR. In fact, MBR rearrangements seem to be involved in ~75% of DLBCL cases with cytogenetically detectable 327 breakpoints (3q27+) (3), whereas ABR rearrangements account for only a fraction (4 of 7, >50% in this study) of the remaining MBR-negative 3q27+ cases. Thus, ABR rearrangements are expected to account for ~12% of all 3q27 rearrangements, corresponding to an expected frequency of 4% in the overall DLBCL populations and consistent with the actual frequency observed in this initial study (2 of 84). Conversely, it is curious that the frequency of ABR rearrangements in FL appears to be similar, or possibly higher, than that of MBR rearrangements. Future studies on large panels of cases are needed to define whether BCL-6 ABR rearrangements preferentially associate with FL.

From a diagnostic standpoint, the results of this study point to the need of long range DNA analysis for the correct identification of all BCL-6 breakpoints occurring in lymphomas. In this respect, FISH provides a powerful tool to detect both MBR and ABR BCL-6 breakpoints, thereby surpassing the capabilities of conventional Southern blot hybridization analysis. In particular, highly predictive analysis of BCL-6 rearrangements can be performed by FISH using as a probe a 650-kb clone contig designed to detect breakpoints occurring both at the MBR and ABR, as well as those which scatter around the BCL-6 locus. Because a fraction of 3q27 breaks cannot be currently explained by BCL-6 breaks at MBR or ABR, FISH studies may also lead to the discovery of additional BCL-6 breakpoint clusters that, together with MBR and ABR, will recapitulate the molecular pathology of 3q27 breaks in B-cell lymphoma.

**REFERENCES**


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

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


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/14/4089

Cited articles
This article cites 23 articles, 13 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/14/4089.full#ref-list-1

Citing articles
This article has been cited by 11 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/62/14/4089.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/62/14/4089.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.