RAPID COMMUNICATION

Rearrangements of the BCL6 Gene in Diffuse Large Cell Non-Hodgkin’s Lymphoma

By Francesco Lo Coco, Bihui H. Ye, Florigio Lista, Paolo Corradini, Kenneth Offit, Daniel M. Knowles, R.S.K. Chaganti, and Riccardo Dalla-Favera

The pathogenesis of non-Hodgkin’s lymphoma (NHL) with a large cell component (DLLC; including diffuse large cell, DLCL; diffuse mixed cell, MX-D; and immunoblastic, IMB) is unknown. A novel candidate proto-oncogene, BCL6, that is involved in chromosome band 3q27 aberrations in NHL has been recently identified. We have investigated the incidence and disease-specificity of BCL6 rearrangements in a large panel of lymphoid tumors, including acute and chronic lymphoid leukemias (96 cases), various NHL types (125 cases), and multiple myelomas (23 cases). BCL6 rearrangements were found in 16/45 (35.5%) DLLC, more frequently in DLCL (15/32, 47%) than in MX-D (1/10, 10%), in 2/31 (6.4%) follicular NHL, and in no other tumor types. BCL6 rearrangements represent the first genetic lesion specifically and recurrently associated with DLLC and should prove useful for understanding the pathogenesis as well as for the clinical monitoring of these tumors.

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Table 1. Rearrangements of the BCL6 Gene in Lymphoid Tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Histotype</th>
<th>Rearranged/Tested</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>NHL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low grade:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL</td>
<td>0/10</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>SCC-F</td>
<td>2/18</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>MX-F</td>
<td>0/13</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Intermediate grade:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MX-D</td>
<td>1/10</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>DLCL</td>
<td>15/33</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>SCC-D</td>
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<tr>
<td>High grade:</td>
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</tr>
<tr>
<td>IMB</td>
<td>0/2</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>SNCL</td>
<td>0/22</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Others:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CTCL</td>
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<td>0</td>
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<td>ALL</td>
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<tr>
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<td>CLL</td>
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<td>0</td>
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<td>T-lineage:</td>
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</tr>
<tr>
<td>MM</td>
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</tr>
<tr>
<td>0/23</td>
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</table>

Abbreviations: SL, small lymphocytic; SCC-F, follicular small cleaved cell; MX-F, follicular mixed; SCC-D, diffuse small cleaved cell; SNCL, small noncleaved cell lymphoma; CTCL, cutaneous T-cell lymphoma.

* One case showed follicular and diffuse growth patterns.

RESULTS

The tumor panel (Table 1) used for this study is representative of the major categories of lymphoproliferative disease including NHL (125 cases), acute lymphoblastic leukemia (ALL) (45), chronic lymphocytic leukemia (CLL) (51), and multiple myeloma (MM) (23). The NHL series was representative of low- (41), intermediate- (45), and high-grade (24) subtypes according to the Working Formulation. Fifteen cases of cutaneous T-cell NHL were also included.

The presence of BCL6 rearrangements was analyzed by Southern blot hybridization of tumor DNAs using a probe (Sac 4.0) and restriction enzymes (BamH I and Xba I) which, in combination, explore a region of 15.2 kb containing the 5' portion of the BCL6 gene (first exon, 7.5 kb of first intron and 7.4 kb of 5' flanking sequences). This region was previously shown to contain the cluster of breakpoints detected in NHL. No additional rearrangements were found using probes and restriction enzymes exploring approximately 10 kb either 5' or 3' to BCL6 sequences.

The results of this analysis are summarized in Table 1 and representatively shown in Fig 1. All cases of ALL, CLL, and MM showed a normal BCL6 gene. Eighteen of the 125 NHL cases displayed BCL6 rearrangements. Among distinct NHL histologic subtypes, rearrangements were detected in 16/45 (35.5%) DLLC, but significantly less frequently in FL (2/31; 6.4%; P < .001). One of these two FL cases showed both follicular and diffuse patterns of growth. Among DLLC, rearrangements were significantly more frequent in DLCL (15/33, 45.4%) than in MX-D (1/10, 10%) (P < .01), suggesting that these genetic lesions may be specifically associated with the diffuse large cell component of these tumors.

mol/L sodium citrate/0.5% sodium dodecyl sulfate) for 2 hours at 60°C and then subjected to autoradiography for 24 to 48 hours at -80°C using intensifying screens.

All NHL cases were also analyzed for rearrangement of the BCL2 gene using the previously described probes corresponding to the MBR and MCR regions. Immunophenotypic analysis of Ig and cell surface marker expression was performed as previously described. Comparisons of histologic subsets with or without BCL6 rearrangement were made using the method of inferences from proportions.

NHL histologic subtypes, rearrangements were detected in 16/45 (35.5%) DLLC, but significantly less frequently in FL (2/31; 6.4%; P < .001). One of these two FL cases showed both follicular and diffuse patterns of growth. Among DLLC, rearrangements were significantly more frequent in DLCL (15/33, 45.4%) than in MX-D (1/10, 10%) (P < .01), suggesting that these genetic lesions may be specifically associated with the diffuse large cell component of these tumors.
All of the DLLC cases displaying BCL6 rearrangements lacked BCL2 rearrangements that were found in only two DLLC cases (not shown). The status of the BCL2 gene in the two FL cases displaying BCL6 rearrangements was not tested. Although cytogenetic data were not available for the panel of tumors studied, the frequency of BCL6 rearrangements far exceeds that expected for 3q27 aberrations (10% to 12% in DLLC),\(^9\) suggesting that BCL6 rearrangements can occur as a consequence of submicroscopic chromosomal aberrations.

To determine whether the presence of BCL6 rearrangements correlated with distinct immunophenotypic features of DLLC, the entire panel was analyzed for expression of Ig \(\kappa\) and \(\lambda\) light chains, and B-cell–associated antigens CD19, CD20, and CD22.\(^9\) As expected, the expression of these markers was variable in the DLLC cases tested. However, no correlation with the BCL6 rearrangement status was found.

**DISCUSSION**

In this study, we establish BCL6 rearrangement as the most frequent abnormality detectable in DLLC. Previous studies have indicated that MYC and BCL2 rearrangements are detectable in 5% to 20% and 20% of DLLC, respectively.\(^3\) Compared with those lesions, which are also commonly associated with BL (MYC) and FL (BCL2), BCL6 rearrangements appear to be more disease-specific because they were exclusively found in DLLC with the exception of 2 of 45 FL cases. Considering that one of these two FL cases displayed areas of diffuse histology, it is conceivable that BCL6 rearrangements may be occasionally associated with atypical FL cases with mixed follicular and diffuse components. The recurrent and specific association between DLLC and structural lesions of a gene coding for a zinc finger-type transcription factor related to several known proto-oncogenes\(^10\) suggests that these abnormalities may play a role in pathogenesis of DLLC.

Among the heterogeneous DLLC spectrum, BCL6 rearrangements were significantly more frequent in tumors displaying a pure diffuse large cell histology (DLCL), all of which lacked BCL2 rearrangements. Considering that DLCL can originate both “de novo” and from the “transformation” of FL, and that the latter typically carry BCL2 rearrangements, our results suggest that BCL6 rearrangements may be specifically involved in the pathogenesis of “de novo” DLLC. This conclusion is consistent with recent findings indicating that other genetic alterations, namely the inactivation of the p53 tumor suppressor gene, may be involved in the transformation of FL to DLLC.\(^11\)

The results presented herein have relevant diagnostic and prognostic implications. DLLC represent a heterogeneous group of neoplasms that are treated homogeneously despite the fact that only 50% of patients experience long-term disease-free survival.\(^1\) The presence of a marker such as BCL6 rearrangement identifies a sizable subset of cases with a distinct pathogenesis and, possibly, distinct biologic behavior. Additional studies are needed to determine whether this newly identified marker can also identify a clinically and prognostically significant subset of DLLC.

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