Expression of LMO2 Is Associated With t(14;18)/IGH-BCL2 Fusion but Not BCL6 Translocations in Diffuse Large B-Cell Lymphoma

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Abstract

Diffuse large B-cell lymphoma (DLBCL) can be separated for prognostic purposes using gene expression profiling (GEP) into 2 subgroups: germinal center B-cell (GCB) and activated B-cell phenotypes. However, GEP is impractical for routine clinical use, and immunophenotyping is an imperfect surrogate. Therefore, we studied the relationship between expression of the purported germinal center marker LMO2 and the presence of IGH-BCL2 fusions, BCL6 translocations, and LMO2 translocations. In addition, we investigated the usefulness of LMO2 expression as a marker of GCB subtype in DLBCL. Immunohistochemical and fluorescence in situ hybridization studies were successfully performed on 101 cases of de novo DLBCL that had been incorporated into a tissue microarray. There was a statistically significant association between IGH-BCL2 fusion and LMO2 protein expression (P = .02) but not between BCL6 translocations and LMO2 expression. LMO2 translocations were not identified. Although uncommon, all cases that had both IGH-BCL2 fusion and BCL6 translocations expressed LMO2. The findings suggest LMO2 as a potential marker for the GCB phenotype.
and Burkitt lymphomas. The mechanism for increased LMO2 expression is not known. Reciprocal translocations involving LMO2 have been identified in T-acute lymphoblastic leukemia (T-ALL), resulting in increased expression of LMO2, although increased expression of LMO2 has also been identified in T-ALL cases that lack an LMO2 translocation. The incidence of LMO2 translocations in other hematolymphoid malignancies is also unknown.

We studied the relationship between IGH-BCL2, BCL6, and LMO2 translocations and LMO2 expression in DLBCL to better clarify their relationship and the usefulness of LMO2 expression as a marker of the GCB subtype in DLBCL.

**Materials and Methods**

For the study, 131 consecutive cases of de novo DLBCL were selected from the Stanford University Medical Center, Stanford, CA, that contained sufficient tissue for inclusion in a tissue microarray (TMA). The specimens met the morphologic and immunophenotypic criteria of DLBCL according to the World Health Organization classification, and the patients consented to research use of their tissue. Seven normal tonsil specimens were also studied. The TMA was constructed from formalin-fixed, paraffin-embedded tissue using 0.6-mm punches from each case in triplicate. Monoclonal anti-LMO2 antibody was applied to the TMA with immunohistochemical staining of LMO2 in lymphoma cell nuclei scored as follows: staining in 30% or more of lymphoma cells was scored as positive and staining in fewer than 30% as negative. Results were considered acceptable as long as 1 core yielded a result. If cores showed discordant staining results, the core with the highest value was used (eg, if ≥30% of lymphoma cells showed nuclear staining in 2 cores and <30% of lymphoma cells showed nuclear staining in 1 core, the case was considered positive for LMO2 expression). Cases were excluded if no tissue was remaining in the TMA sections, no lymphoma cells were present in the cores, or if necrosis or technical artifacts precluded staining and its interpretation.

Sections of the TMA were also screened by interphase fluorescence in situ hybridization (FISH) for the following translocations: t(14;18)/IGH-BCL2 using a dual fusion probe (Vysis, Downers Grove, IL), BCL6 using a break-apart probe (Vysis), and LMO2, also using a break-apart probe (LMO2 break-apart probe at 11p15; bacterial artificial chromosomes [BACs] RP11-102C11 and RP11-646J21 distal; BACs RP11-98C11 and RP11-734J23 proximal). If there were no readable signals present, the hybridization was deemed a failure because all of the cells studied should have at least 1 copy of each of the genes. The FISH assay was performed in replicate to minimize the number of test failures due to insufficient tissue quantity or poor hybridization and, in addition, to verify the results. The presence or absence of LMO2 protein expression was then compared with the FISH results.

**Results**

Of the 131 consecutive cases of DLBCL, immunohistochemical analysis and FISH were both successful in 101
cases (77.1%). Of the 101 cases, 50 (49.5%) stained positively with the anti-LMO2 antibody. By interphase FISH, 14 DLBCL cases (13.9%) had an IGH-BCL2 fusion (Table 1). Durnick et al. / LMO2 vs Cytogenetics in DLBCL

LMO2 expression was analyzed in relation to translocation status. All 3 cases with both IGH-BCL2 fusion and a BCL6 translocation expressed LMO2. When the presence of IGH-BCL2 fusion and BCL6 translocation were considered separately, there was a statistically significant association between an isolated IGH-BCL2 fusion and LMO2 expression (P = .02; Fisher exact test) Table 2. In contrast, there was no association between the presence of an isolated BCL6 translocation and LMO2 expression (P = .36; Fisher exact test).

Discussion

LMO2 is highly expressed at the messenger RNA and protein levels in normal GCBs and in many B-cell lymphomas that are thought to be of GCB origin, such as follicular lymphoma and Burkitt lymphoma. LMO2 expression has been shown to be the strongest predictor of good outcomes in patients with DLBCL and is independent of rituximab therapy, suggesting that LMO2 expression may be independent of DLBCL subtype as a prognostic marker. LMO2 expression in our cohort of DLBCL was about 50%, which is similar to the reported rate of 55%. (14;18)/IGH-BCL2 is a characteristic feature of follicular lymphoma, and, although it is less common in DLBCL, it has been specifically associated with the GCB subgroup as determined by gene expression profiling. The incidence of IGH-BCL2 fusion in DLBCL in our study (~14%) is similar to previously published results in DLBCL (17%). We showed a statistically significant association between the presence of an IGH-BCL2 fusion and LMO2 expression (P = .02; Table 2). However, 3 (21%) of 14 cases of IGH-BCL2 fusion-positive DLBCL lacked LMO2 expression, suggesting that a subset of DLBCL cases of germinal center derivation undergo some unexplained biochemical alteration that leads to the loss of LMO2 expression. This is in keeping with the findings in follicular lymphoma, in which the frequency of IGH-BCL2 fusion (~90% of cases) exceeds that of LMO2 expression (~50% of cases), and suggests that LMO2 expression is an imperfect surrogate for GCB origin.

Because many other lymphomas demonstrate translocations and juxtaposition to an enhancer region leading to increased expression of a particular gene, we decided to investigate whether the LMO2 gene was involved by structural rearrangement. All 101 DLBCL cases studied lacked a translocation involving the LMO2 gene as indicated by the lack of split signals using break-apart probes in interphase FISH.

Table 1
Frequency of LMO2 Expression and Detection of IGH-BCL2, BCL6, and LMO2 Translocations in DLBCL

<table>
<thead>
<tr>
<th></th>
<th>FISH Detection Rate</th>
<th>LMO2 Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>All DLBCL cases</td>
<td>—</td>
<td>50/101 (49.5)</td>
</tr>
<tr>
<td>IGH-BCL2 only</td>
<td>11/101 (10.9)</td>
<td>8/11 (73)</td>
</tr>
<tr>
<td>BCL6 translocation only</td>
<td>8/101 (7.9)</td>
<td>4/8 (50)</td>
</tr>
<tr>
<td>Both</td>
<td>3/101 (3.0)</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>Negative</td>
<td>79/101 (78.2)</td>
<td>35/79 (44)</td>
</tr>
<tr>
<td>LMO2 translocation</td>
<td>0/101 (0.0)</td>
<td>—</td>
</tr>
</tbody>
</table>

DLBCL, diffuse large B-cell lymphoma; FISH, fluorescence in situ hybridization. * Data are given as number/total (percentage).

Table 2
Relationship Between Translocation Status (IGH-BCL2 Fusion and BCL6 Rearrangement) and LMO2 Expression

<table>
<thead>
<tr>
<th></th>
<th>LMO2+</th>
<th>LMO2−</th>
<th>Total</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGH-BCL2 fusion</td>
<td></td>
<td></td>
<td></td>
<td>.02</td>
</tr>
<tr>
<td>Yes</td>
<td>11 (22)</td>
<td>3 (6)</td>
<td>14 (14)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>39 (78)</td>
<td>48 (94)</td>
<td>87 (86)</td>
<td></td>
</tr>
<tr>
<td>BCL6 translocation</td>
<td></td>
<td></td>
<td></td>
<td>.36</td>
</tr>
<tr>
<td>Yes</td>
<td>7 (14)</td>
<td>4 (8)</td>
<td>11 (11)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43 (86)</td>
<td>47 (92)</td>
<td>90 (89)</td>
<td></td>
</tr>
</tbody>
</table>

* Data are given as number (percentage).
* Fisher exact test.
Despite this finding, about 50% of the cases showed up-regulated expression of LMO2 by immunohistochemical analysis, suggesting that a mechanism other than up-regulation due to juxtaposition to an enhancer region (eg, IGH) is responsible for LMO2 expression in the LMO2+ cases. Increased expression of LMO2 has also been identified in a subset of T-ALL cases that lack an LMO2 translocation.5

The frequency of BCL6 translocations in our study (~11%) was somewhat lower than that described in previous studies (range, 15%-31%8,13-17); the reason for the lower frequency is unknown. Not surprisingly, LMO2 expression was not significantly different between the BCL6 fusion–positive and BCL6 fusion–negative cases. This finding is in keeping with the observation that the BCL6 translocation is not specific for a particular DLBCL subtype.

Only 3 cases had an IGH-BCL2 fusion and a BCL6 translocation (dual-positive cases). Despite the small number of cases, all 3 were LMO2+, raising the possibility that the presence of these 2 translocations together is a sensitive and specific marker for the GCB-like subtype of DLBCL.

We have demonstrated a statistically significant association between LMO2 expression and IGH-BCL2 fusion, in keeping with the previously demonstrated association between LMO2 expression and GCB subtype in DLBCL by gene expression profiling. Additional study will be necessary to understand the biologic basis of the survival advantage of LMO2 expression in DLBCL and to understand the relationship between IGH-BCL2 and BCL6 translocation status and CD10, BCL2, BCL6, and MUM1 protein expression in DLBCL.

References