**BCL6** gene translocation in follicular lymphoma: a harbinger of eventual transformation to diffuse aggressive lymphoma

Takashi Akasaka, Izidore S. Lossos, and Ronald Levy

Follicular lymphoma (FL) is characterized by a relatively indolent clinical course, but the disease often transforms into a more aggressive large cell lymphoma with a rapidly progressive clinical course. In the present study, we analyzed 41 cases of FL known to have subsequently transformed to aggressive lymphoma and an additional 64 FL samples from patients not subsequently transformed. We studied **BCL6** gene rearrangement by the methodology of long-distance inverse polymerase chain reaction (LDI-PCR). Of the 41 cases known to transform, 16 (39.0%) harbored **BCL6** translocation or deletion at the time of FL diagnosis. Among 64 cases not known to transform, **BCL6** translocation was detected in 9 (14.1%). The prevalence of **BCL6** translocation in the group known to transform was significantly higher (P = .0048). Among the transformation cases, the partners of the **BCL6** translocation were identified in 13 cases and included **IGH**, **CIITA**, **U50HG**, **MBNL**, **GRHPR**, **LRMP**, **EIF4A2**, **RhoH/TTF**, and **LOC92656** (similar to **NAPA**), whereas in the control group the **BCL6** partners were **IGH**, **CIITA**, **SIAT1**, and **MBNL**. In 13 cases paired specimens before and after transformation were available. Among these paired specimens, a loss (3 cases) or a gain (1 case) of **BCL6** translocation was observed after the transformation. Analysis of clonality showed that all of these cases represented the evolution of a subclone of the original tumor population. Our study demonstrated that **BCL6** translocation in FL may constitute a subgroup with a higher risk to transform into aggressive lymphoma. (Blood. 2003;102:1443-1448)

© 2003 by The American Society of Hematology
morphologic transformation of FL. Some of these mutations were reported to be associated with increased BCL6 mRNA expression; however, increased BCL6 mRNA expression is not uniformly necessary for the transformation.

The aim of this study was to explore the prevalence of BCL6 gene translocations in FL which eventually transformed to DLBCL. We searched by long-distance inverse polymerase chain reaction (LDI-PCR) for BCL6 translocations in FL tumors. Our study demonstrates a high prevalence of BCL6 gene translocations in FL destined to transform.

Patients and methods

Tumor specimens

Specimens were selected from patients with FL observed at Stanford University Medical Center between 1974 and 2002. According to World Health Organization (WHO) classification, FL cases of grade 1 (follicular small cleaved) or grade 2 (follicular mixed) at the time of diagnosis were selected. Grade 3 FL (follicular large cell) cases were excluded from this study. We defined FL cases as “known to transform” if a subsequent biopsy was ever performed that confirmed transformation to DLBCL. By contrast, FL “not known to have transformed” was defined as FL at the time of initial diagnosis with adequate clinical follow-up and either no subsequent biopsy or subsequent biopsy not showing transformation. Samples from 41 patients known to have undergone subsequent transformation to diffuse aggressive lymphoma were selected. These samples included 13 on whom specimens were available both from the initial FL diagnosis and from the transformed phase. These 13 cases have been included in our previous studies.

Molecular cloning and nucleotide sequencing

All PCR products, which showed a nongermline band, were purified by gel extraction (QIAquick Gel Extraction Kit; Qiagen, Hilden, Germany). The PCR products in some cases were cloned into the pGEM-T Easy plasmid (Promega, Madison, WI). Transformation and extraction of DNA were performed by established methods. In the case of BCL6 translocation-derived PCR products, 3 or 4 molecular clones per sample were subjected to sequencing. Nucleotide sequencing of the PCR products or cloned DNA was performed with a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), and the sequencing reactions were resolved on an ABI 377 automated sequencer (Applied Biosystems).

Statistical analysis

Correlations between 2 groups were examined by Fisher exact test. Overall survival was calculated from the date of diagnosis until the patient’s death or last follow-up. Time to transformation was calculated between the time of diagnosis and the biopsy date of morphologic transformation. Survival
Figure 3. Representative ethidium bromide–stained gel electrophoresis of LDI-PCR of the *BCL6*. (A) The sizes of the LDI-PCR products are unique to each case. (B) A gain or a loss of partner/BCL6 fusion was observed in the paired diagnosis-transformation samples. The LDI-PCR products represent fusions either on the der(3) or der(partner), and partner genes or loci identified by sequencing analysis of the products are indicated at the bottom. An aliquot of 2 to 10 μL was loaded in each lane and electrophoresed through a 0.7% agarose gel. HindIII-digested DNA was used as a molecular weight marker. D indicates at the diagnosis; T, at the transformation.

Diverse partners of *BCL6* translocation in the transformation cases

The LDI-PCR products encompassing junctional points were directly subjected to nucleotide sequencing or were cloned into plasmids and sequenced with primers from the known *BCL6* sequences. The sequences appearing beyond the artificial *Xho*I or *Bam*HI site represented those from the partners. Homology search of the GenBank database revealed that 14 cases harbored *BCL6* translocations with diverse partners, and 2 additional cases harbored a deletion of more than 1 kb of the 5′ *BCL6* gene. Among the 14 cases with *BCL6* translocations, all but 2 harbored non-IG partners. The remaining 2 cases had a point mutation leading to the generation of a new restriction site. The partner loci located in the vicinity of the breakpoints included known genes such as immunoglobulin heavy chain (*IGH*) gene on 14q32, major histocompatibility complex (MHC) class II transactivator (*CIITA*), *BCL6* gene on 16p13, eukaryotic translation initiation factor 4A, isoform 2 (*EIF4A2*) gene on 3q23.3, Ras homolog gene family, member H gene (*ARHH*, *RhoH/TF*) on 4p13, U50 small-nucleolar-RNA host gene (*U50HG*) on 6q15, muscleblind-like protein (*MBNL*) gene on 3q25, glyoxylate reductase/hydroxypyruvate reductase (*GRHPR*) gene on 9p11.2, lymphoid-restricted membrane protein (*LRMP*) gene on 12q12.1, and LOC92656 (similar to napsin A gene, *NAPA*) on 19q13.33 (Table 1). Among them, the partner genes of 5 kinds, *IGH*, *CIITA*, *EIF4A2*, *RhoH/TF*, and *U50HG*, have been reported from our and/or other laboratories, and those of the other 4 cases, *MBNL*, *GRHPR*, *LRMP*, and LOC92656 (similar to *NAPA*), were novel partners for *BCL6*. In the remaining 3 partner loci, responsible genes were not identified; they were localized at 2p21, at 3q28, and at 6q16.1.

Prevalence of *BCL6* translocation in FL cases not known to transform

The prevalence of the *BCL6* translocation in the 41 transformation cases was significantly higher than the *BCL6* translocation prevalence previously reported in FL tumors (11, 27, 34) (Table 2).

Table 1. Partner loci of *BCL6* translocations determined by LDI-PCR

<table>
<thead>
<tr>
<th>Partner</th>
<th>Chromosomal locus</th>
<th>Group known to transform</th>
<th>Group not known to transform</th>
<th>Case no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Involved gene</td>
<td>17 (14)*</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IGH</em></td>
<td>14q32</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>CIITA</em></td>
<td>16p13</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>RhoH/TF</em></td>
<td>4p13</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>MBNL</em></td>
<td>3q25</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>EIF4A2</em></td>
<td>3q27.3</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>U50HG</em></td>
<td>6q15</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>GRHPR</em></td>
<td>9p11.2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>LRMP</em></td>
<td>12q12.1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>LOC92656 (similar to <em>NAPA</em>)</td>
<td>19q13.33</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>SIAT1</em></td>
<td>3q26.33</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Currently uncharacterized</td>
<td>†</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Deletion of <em>BCL6</em></td>
<td>NA</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mutation of <em>BCL6</em></td>
<td>NA</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>21</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

NA indicates not applicable.

* Three cases had two independent *BCL6* partners (see Table 3).
† Chromosomal loci for currently uncharacterized partners are described in the text.
We applied LDI-PCR to an additional randomly selected group of 64 FL cases followed for similar time periods but not known to have undergone subsequent transformation. The median follow-up was 5.4 years from the time of diagnosis (range, 0.2-13.4 years) compared with that of the transformation group (median time to documented transformation, 5.5 years) (Figure 1).

Of a total of 64 cases not known to transform, 11 (17.2%) showed nongermline-altered PCR bands. Nucleotide sequencing of the PCR products confirmed that 8 cases involved BCL6 translocation, including IGH on 14q32, CIITA on 16p13, and sialyltransferase 1 (SIAT1) gene on 3q26.33, each in 2 cases, and MBNL on 3q25 in 1 case. SIAT1 was a novel recurrent partner for BCL6. In the remaining one partner locus, the responsible gene was not identified; it was localized at 6q25. One case had a deletion of 4.7-kb segment involving the MTC of the BCL6 gene. The remaining 2 cases had a point mutation leading to the generation of a new restriction site. Therefore, the prevalence of BCL6 translocation in the group not known to transform by our method of LDI-PCR was similar to the previously reported prevalence of BCL6 translocation in nonselected FL (Table 2) and significantly lower than the BCL6 translocation prevalence in FCL cases that transformed to DLBCL.

**Alterations of BCL6 translocation status in the transformation process**

This study included sequential biopsies from 13 patients prior to and after morphologic transformation from FL to DLBCL. All these sequential biopsies were clonally related, as demonstrated by analysis of immunoglobulin (IG) gene rearrangements, BCL2 translocations, and BCL6 and IG gene mutations (data not shown).

In 4 cases, the results of LDI-PCR were inconsistent between the paired samples; 3 cases (cases IL114, IL125, and IL126) lost the original nongermline-altered PCR amplification at the time of morphologic transformation, and the remaining case, IL122, newly acquired another PCR product at the transformation (Table 3 and Figure 3B).

This group with BCL6 translocation in the 13 paired samples harbored t(14;18) in 6 (75%) of 8 cases (Table 3), similar to the prevalence of this translocation at initial diagnosis in general FL.9-11

**Discussion**

This study involved 2 groups of cases of FL, one known to have transformed to diffuse aggressive lymphoma and one followed for a similar time but not known to have transformed. The clinical suspicion of transformation is based on the observation either of an asynchronous pattern of growth or a change in the overall pace of growth of the tumor. Such clinical events lead to the performance of repeated biopsies. It is possible that all cases of FL undergo transformation at some time and some location. Therefore, the distinction between the 2 groups of cases studied here is ultimately based on clinical behavior that caused the clinician to perform a biopsy to prove transformation.

The aim of this study was to assess the effect of BCL6 gene translocations in higher-grade transformation of FL. Our study demonstrates that FL patients harboring BCL6 translocation at the time of diagnosis may be prone to subsequent higher-grade transformation.

**Table 3. Molecular features of 13 paired diagnosis-transformation samples**

<table>
<thead>
<tr>
<th>Case</th>
<th>At diagnosis</th>
<th>BCL6 partner</th>
<th>At transformation</th>
<th>BCL6 partner</th>
<th>At transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL105</td>
<td>A deletion</td>
<td>A deletion</td>
<td>A 5' mcr</td>
<td>A 5' mcr</td>
<td></td>
</tr>
<tr>
<td>IL114</td>
<td>A MBNL</td>
<td>G</td>
<td>A Far 3'-MBR</td>
<td>A Far 3'-MBR</td>
<td></td>
</tr>
<tr>
<td>IL115</td>
<td>G</td>
<td>G</td>
<td>A 150-bp MBR</td>
<td>A 150-bp MBR</td>
<td></td>
</tr>
<tr>
<td>IL116</td>
<td>A similar to NAPA</td>
<td>A similar to NAPA</td>
<td>A 150-bp MBR</td>
<td>A 150-bp MBR</td>
<td></td>
</tr>
<tr>
<td>IL117</td>
<td>G</td>
<td>G</td>
<td>A 150-bp MBR</td>
<td>A 150-bp MBR</td>
<td></td>
</tr>
<tr>
<td>IL119</td>
<td>A GRHPR, 6q16.1</td>
<td>A GRHPR, 6q16.1</td>
<td>A mcr</td>
<td>A mcr</td>
<td></td>
</tr>
<tr>
<td>IL120</td>
<td>G</td>
<td>G</td>
<td>A 150-bp MBR</td>
<td>A 150-bp MBR</td>
<td></td>
</tr>
<tr>
<td>IL121</td>
<td>G</td>
<td>G</td>
<td>A Far 3'-MBR</td>
<td>A Far 3'-MBR</td>
<td></td>
</tr>
<tr>
<td>IL122</td>
<td>A 3q28</td>
<td>A 3q28, IGH</td>
<td>A 150-bp MBR</td>
<td>A 150-bp MBR</td>
<td></td>
</tr>
<tr>
<td>IL123</td>
<td>G</td>
<td>G</td>
<td>A mcr</td>
<td>A mcr</td>
<td></td>
</tr>
<tr>
<td>IL124</td>
<td>A CIITA</td>
<td>A CIITA</td>
<td>A 3'-MBR</td>
<td>A 3'-MBR</td>
<td></td>
</tr>
<tr>
<td>IL125</td>
<td>A USO09G</td>
<td>G</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>IL126</td>
<td>A EIF4A2, LRMP</td>
<td>A EIF4A2</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

A indicates nongermline-altered PCR amplification; G, germline-derived PCR amplification; bp, base pair; and ND, not detected.

*The classification of BCL2 breakpoint was described previously.52
The prevalence of the BCL6 translocation in FL specimens that subsequently underwent transformation to DLBCL was significantly higher than the previously reported prevalence in unselected FL biopsies (Table 2). This discrepancy could have been caused by different detection methods used in these studies or could suggest that FL tumors harboring BCL6 translocations represent a distinct FL subgroup that is prone to transformation.

Most of the previous reports investigating prevalence of BCL6 rearrangement in FL used Southern blot methodology, whereas we have used LDI-PCR. Southern blot analysis might fail to detect BCL6 rearrangement in some cases. Moreover, large deletions in the BCL6 gene, as was found in the IL105 case, in which a 4.8-kb segment involving MTC and encompassing the whole region of F372 probe was deleted, would also not be detected by the Southern blot analysis. However, these explanations for the observed discrepancy may not account for the marked difference in the prevalence of the BCL6 translocations as was observed in this study.

An alternative explanation for the observed discrepancy might be the sensitivity of the LD-PCR–based assay. The sensitivity of the LD-PCR–based assay is comparable to the sensitivity of standard-size PCR. Therefore, LDI-PCR could detect not only the main clone but also minor subclones, whereas the sensitivity limit of the Southern blot is 5%, significantly lower than our method. To address this possibility we have analyzed the prevalence of the BCL6 translocation by LDI-PCR in a randomly selected group of FL cases not known to have transformed. The prevalence of the BCL6 translocation in this group of FL cases was 13.0% and was not different from the previously reported prevalence detected by Southern blot in similar groups of FL cases (Table 2). Therefore, higher sensitivity of the LDI-PCR compared with Southern blot could not explain the observed high prevalence of the BCL6 translocations in FL that underwent transformation to DLBCL.

Because the methodologic differences could not explain the observed high prevalence of the BCL6 translocation in patients undergoing higher-grade transformation, it is possible that FL with such translocation indeed represents a distinct subgroup of FL. A previous study of BCL6 translocation in posttransformation DL-BCL did not find a different prevalence compared with random FL cases. However, the studied cases were analyzed at the posttransformation stage, and because BCL6 translocation can be lost during transformation, this study might have underestimated BCL6 translocation prevalence in FL cases that subsequently transform. It should be noticed that in addition to the higher prevalence of the BCL6 translocation in the transformation group, we also observed that partner genes of the BCL6 translocations may have been different between this group and the control group of the 69 FL samples (Table 1). The partner genes involved in the translocation with BCL6 are transcriptionally activated by a variety of stimuli that could affect the clinical behavior. It is also possible that distinct partner genes may differentially affect BCL6 expression and function.

What role does BCL6 translocation play in FL? Bastard et al reported no difference in prognosis (overall survival) between BCL6 rearrangement-positive FL cases and the negative cases; however, the number of cases in their study was very small. They very recently reported that FL with BCL6 rearrangement and without t(14;18) constitutes a subgroup with distinct pathologic, molecular, and clinical characteristics. Our FL cases carrying BCL6 translocation had BCL2 translocation in more than half of the cases (Table 3 and T.A., R.L., unpublished data, December 2002). Thus our cases may represent a different subgroup from theirs. It is generally accepted that morphologic transformation is a grave event leading to poor response to therapy and early death. However, if it is confirmed that FL with BCL6 translocation is destined to early transformation, such a group could be selected for a distinct strategy of therapy at the time of initial diagnosis.

The observation of BCL6 translocation loss during transformation suggests that BCL6 deregulation itself is not necessary for the transformation process. Because the same mechanism has been suggested responsible for both BCL6 mutations and BCL6 translocations, it is possible that BCL6 translocations reflect an active mutational machinery in the tumor, a marker of genomic instability which affects other genes that are more directly related to the transformation process.

In conclusion, our study suggests that FL with BCL6 translocation constitutes a FL subgroup, which may be prone to subsequent early transformation. Further studies, involving larger series of FL patients whose subsequent transformation propensity is known, will be required to confirm our findings.

Acknowledgment

R.L. is an American Cancer Society Clinical Research Professor.

References


