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

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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 is not necessary for transformation but that *BCL6* translocation in FL may constitute a subgroup with a higher risk to transform into aggressive lymphoma. (Blood. 2003;102:1443-1448)

Introduction

Follicular lymphoma (FL) is one of the most common types of non-Hodgkin lymphoma (NHL) characterized by a relatively indolent clinical course and prolonged survival.[1] The disease arises from a germinal center (GC)–derived B cell.[2] Patients with FL tend to relapse over time, and they frequently undergo morphologic transformation to aggressive diffuse large B-cell lymphoma (DLBCL).[3-6] This transformation usually is associated with acceleration of the clinical course and is typically the cause of death.[7,8] However, it remains unknown what genes contribute to transformation. Approximately 85% of cases of FL are associated with t(14;18)(q32;q21).[9-11] The chromosomal translocation juxtaposes the *BCL2* gene on chromosome band 18q21 to *IGH* on 14q32,[12] deregulating the expression of the *BCL2* gene product that functions in preventing programmed cell death (apoptosis).[13,14] In morphologic transformation of FL, accumulation of secondary genetic alterations such as nonrandom chromosomal changes,[9,15,16] genetic instability,[17] c-*MYC* oncogene rearrangement,[18,19] changes in the expression profiles of c-*MYC* and genes regulated by c-*MYC*,[20] somatic mutations of the *p53* gene[21-23] and of the translocated *BCL2* gene[24] have been reported. Inactivation of $p16^{INK4A}$ and $p15^{INK4B}$ by deletions, mutations, and hypermethylation has also been demonstrated.[25,26] Those studies suggest that heterogeneous genetic lesions and different molecular mechanisms underlie the transformation process.

Cytogenetic and molecular analyses have demonstrated that alteration of 3q27, *BCL6*, or both is one of the most common genetic abnormalities in B-cell tumors.[27-29] The *BCL6* gene was first identified at the breakpoints on 3q27 involved in t(3;14)(q27;q32) and t(3;22)(q27;q11) translocations in diffuse aggressive large B-cell lymphoma.[30-32] Initial studies suggested that 3q27 translocation or rearrangement of the *BCL6* gene were specifically associated with the DLBCL,[32] but it is observed in 6.4% up to 14.3% of FL.[11,27,33,34] The *BCL6* gene on chromosome 3q27 contains 10 exons and encodes the Bcl-6 protein consisting of 706 amino acids.[30-32] This protein is a transcriptional repressor expressed by GC B cells, and it is necessary for GC formation.[35-37] Although almost all the proto-oncogenes in B-cell NHL such as c-*MYC*, *BCL1*, *BCL2*, *BCL3*, *PAX5* translocate only to the *IG* loci,[38] the 3q27 translocation is unique in that it fuses the *BCL6* gene on 3q27 not only to *IG* genes but also to multiple non-*IG* partners on other chromosomes.[27,32,39,40] To date, more than 20 partner chromosomal loci have been reported.[39] As the result of the translocation in those cases, the structure of the Bcl-6 protein is not affected,[32] but the promoter region of the *BCL6* gene is substituted for that of each partner,[41] respectively, and the rearranged *BCL6* gene is presumed to come under the control of the replaced promoter.[42] In addition to chromosomal translocation, the *BCL6* gene in B-cell neoplasms is also affected by point mutations.[43,44] Furthermore, there is a possibility that the same mechanism is responsible for those 2 processes,[45,46] because the target zones within the *BCL6* gene for rearrangement and for somatic mutation are overlapping. Somatic mutation of the *BCL6* gene was reported to be associated with the

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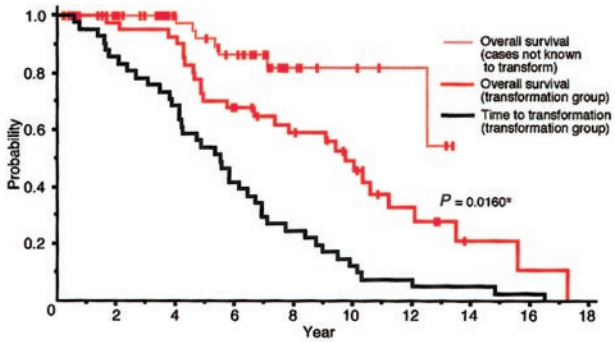

Figure 1. Probability of FL patients. Overall survival of cases not known to transform (n = 64) and transformation group (n = 41), and time to transformation of transformation group (n = 41). *Transformation group versus cases not known to transform.

morphologic transformation of FL.[47] 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.[48]

The aim of this study was to explore the prevalence of *BCL6* gene translocations in FL which eventually transformed to DL-BCL. 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,[49] 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.[20,47,48,50] The remaining 28 specimens were the FL biopsy samples obtained prior to the documented transformation. In addition, FL samples from 64 cases not known to have subsequently transformed were studied. The median time to transformation from time of diagnosis in the 41 transformation cases was 5.5 years (range, 0.6-16.5 years), whereas the median follow-up of the 64 cases not known to have transformed was 5.4 years (range, 0.2-13.4 years) (Figure 1). Materials studied were subjected to immunohistochemical analysis and/or surface immunophenotyping to determine B-cell origin of the lymphoma cells and their clonality through the transformation event. Approval was obtained from the University of Stanford institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki.

LDI-PCR to detect *BCL6* partners

LDI-PCR to detect partners of *BCL6* translocation was carried out as previously described with minor modifications.[40] Briefly, 500 ng genomic DNA was digested with *Bam*HI or *Xba*I and purified by standard methods. The DNA was diluted to a concentration of 1 μg/mL and incubated at 4°C overnight in the presence of *T4* DNA ligase to facilitate intramolecular ligation. The self-ligated circular DNA was used as a template for a nested PCR. PCR primers were modified as the following: BCL6/04, 5′-TTCATACGACCCCAGACATGGAATCACTCTTTAGA-3′; BCL6/08, 5′-CAGCTTGGGACTTTCAGCACCTGGTTTGGGGTCAT-3′; BCL6/09, 5′-TTCGCCAGGGTTCCAATAACACGGCATCATAAAGG-3′; BCL6/36, 5′-CCTGGCAAAGCGGGGGAGTGGGGAGTCGGGTATGG-3′; BCL6/37, 5′-GGGGCCGTTCCTGGTTTCCACTGGGGCAAAGAGAA-3′; BCL6/38, 5′-AGGAACGGCCCCTCCCAACCCTCCCGATGTCCACT-3′; BCL6/39, 5′-AAGACCATACCCGACTCCCCACTCCCCCGCTTTGC-3′; BCL6/44, 5′-GGTGAGGGAGAATGGAAGGCAAAAAGAGGGAAAAA-3′; BCL6/201, 5′-AGACCTTGCACCTTTGATCTCCAGCATTCATAATC-3′; BCL6/202, 5′-ACATTCAAGGGAAGGAAGGGAGGGAGGGAGAGCAT-3′; BCL6/203, 5′-GGTGCACAATTTTCCTCACCATTCATTCAGTTCAA-3′; BCL6/204, 5′-TTGCCAACGTAGGCGGAAGGGGCTTTCTGTTTAGT-3′. The position and orientation of the primers are illustrated in Figure 2. PCR cycling variables as well as the contents of the reaction mixture for LDI-PCR DNA targets were previously described in detail.[40] Aliquots of the PCR products were analyzed by agarose gel electrophoresis and visualized under UV illumination after ethidium bromide (EtBr) staining. In the cases that showed a nongermline band, a nested PCR was performed again using the same self-ligated circular DNA template to confirm whether the PCR band was reproducible.

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).

Sequence analysis

Sequences of the regions of interest were compared with the GenBank databases at the National Center of Biotechnology Information using BLAST, the Human Genome program (http//www.ncbi.nlm.nih.gov/blast/).

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

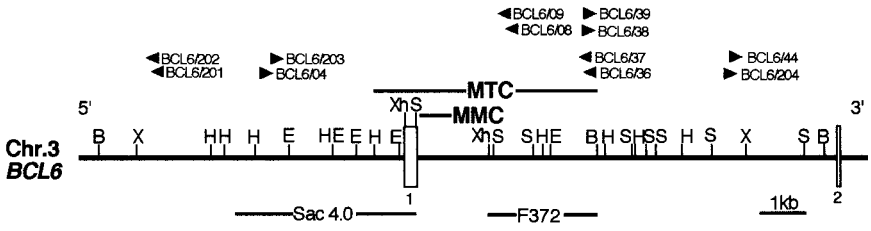

Figure 2. Restriction enzyme maps of the *BCL6*. The major translocation cluster (MTC) of the *BCL6* and the major mutation cluster (MMC) were as determined by Bastard et al[27] and Bernardin et al,[51] respectively. F372[27] and Sac 4.0[32] are probes for Southern blot analysis. Arrowheads are the positions of primers for LDI-PCR. Restriction sites are as follows: E represents, *Eco*RI; B, *Bam*HI; H, *Hind*III; X, *Xba*I; S, *Sac*I; Xh, *Xho*I.

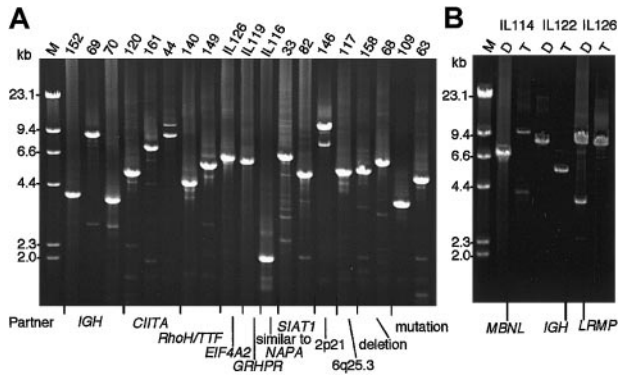

Figure 3. Representative ethidium bromide–stained gel electrophoresis of LDI-PCR of the *BCL6*. (A) The sizes of the LDI-PCR products are unique to each case. (B) A gain or a loss of *partner*/*BCL6* fusion was observed in the paired diagnosis-transformation samples. The LDI-PCR products represent fusions 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. *Hind*III-digested DNA was used as a molecular weight marker. D indicates at the diagnosis; T, at the transformation.

curves were plotted by the method of Kaplan and Meier. The data were analyzed with the Statview statistical software package (Abacus Concepts, Berkeley, CA).

Results

Prevalence of *BCL6* translocation in the transformation cases

This study included 41 cases that experienced morphologic transformation from FL to DLBCL.

To analyze *BCL6* gene translocations, genomic DNA was obtained from the tumor specimens and was subjected to LDI-PCR. As indicated in Figure 2, nested primer pairs in inverse orientation were designed corresponding to sequences 5′ and 3′ of the major translocation cluster (MTC).[27] The LDI-PCR products, therefore, included regions from unknown sequences involved in each translocation, which were flanked by the known *BCL6* sequence. The 3′ primer pairs amplified junctions on the der(3) chromosome, whereas junctional sequences on the partner chromosomes, der(partner), were obtained using the 5′ primer pairs.

Figure 3 shows representative results of EtBr-stained agarose gel electrophoresis of LDI-PCR products. The sizes of the amplified products ranged from 2.0 to 9 kilobase (kb), and they were unique to each case. The nested primer pairs can potentially amplify both the translocated *BCL6* gene and the untranslocated germ line *BCL6* sequences. Because each of the PCR products competes for the primers, the shorter size translocated *BCL6* allele is preferentially amplified; therefore, the PCR products from the translocated allele and the germ line are not both visible on EtBr-stained agarose gel.

Nongermline-altered PCR products corresponding to either or both the der(3) and the der(partner) were detected in the samples from 18 (43.9%) of the 41 patients known to have transformed. Two of these cases had a point mutation leading to the generation of a new restriction site resulting in nongermline-altered PCR product and did not harbor *BCL6* translocation. Therefore, a total of 16 (39.0%) cases of translocation were detected among the 41 patients known to have transformed.

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 *Xba*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 *(MHC2TA*, *CIITA)* gene on 16p13, eukaryotic translation initiation factor 4A, isoform 2 *(EIF4A2)* gene on 3q27.3, Ras homolog gene family, member H gene *(ARHH*, *RhoH/TTF)* 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*/*TTF*, and *U50HG*, have been reported from our and/or other laboratories, and those of the other 4 cases, *MBNL*, *GRHPR*, *LRMP*, and LOC92 656 (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

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

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.

Table 2. Comparison of *BCL6* translocation in FL

	Our study				
	Transformation	Not known to transform	US 1994 (Lo Coco et al[34])	France 1994 (Bastard et al[27])	US 1995 (Otsuki et al[11])
BCL6 translocation*	16/41†	9/64†	2/31	11/80	6/42
Ratio (%)	39.0	14.1	6.4	13.8	14.3
P value	.0048‡		.0020‡	.0025‡	.0134‡
Methodology	LDI-PCR	LDI-PCR	Southern blot (Sac 4.0§)	Southern blot (F372§)	Southern blot (F372§)

**BCL6* translocation means nongermline-altered PCR amplification by LDI-PCR or rearrangement by Southern blot.
†Two cases, which had a point mutation leading to the generation of a restriction site, were excluded from positive cases.
‡*P* value of transformation cases versus cases not known to transform, US 1994, France 1994, and US 1995, respectively.
§Sac 4.0 or F372 was used to detect *BCL6* rearrangement as a probe for Southern blot (Figure 2).

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

	BCL6 partner		*BCL2* breakpoint*	
Case	At diagnosis	At transformation	At diagnosis	At transformation
IL105	A deletion	A deletion	A 5′mcr	A 5′mcr
IL114	A *MBNL*	G	A mcr	A mcr
IL115	G	G	A Far 3′-MBR	A Far 3′-MBR
IL116	A similar to *NAPA*	A similar to *NAPA*	A150-bp MBR	A 150-bp MBR
IL117	G	G	A 150-bp MBR	A 150-bp MBR
IL119	A *GRHPR*, 6q16.1	A *GRHPR*, 6q16.1	A mcr	A mcr
IL120	G	G	A 150-bp MBR	A 150-bp MBR
IL121	G	G	A Far 3′-MBR	A Far 3′-MBR
IL122	A 3q28	A 3q28, *IGH*	A 150-bp MBR	A 150-bp MBR
IL123	G	G	A mcr	A mcr
IL124	A *CIITA*	A *CIITA*	A 3′-MBR	A 3′-MBR
IL125	A *U50HG*	G	ND	ND
IL126	A *EIF4A2, LRMP*	A *EIF4A2*	ND	ND

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. Southern blot analysis commonly uses F372 and Sac 4.0 probes that usually detect DNA fragments derived from der(3) and der(partner) chromosomes, respectively. None of the previous studies used both probes. However, our results using LDI-PCR demonstrated that in some cases only one of the der(3) or der(partner) could be detected. One of the explanations for this observation is the location of the restriction enzymes sequences. Because the *BCL6* gene can be fused to multiple partners, the size of the DNA fragment involving the breakpoint, which is cut by a restriction enzyme, is variable. Therefore, usage of only one probe could fail to detect some of the translocations. 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 might underlie minor prevalence discrepancies and could 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.[53] 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 DLBCL did not find a different prevalence compared with random FL cases.[11] 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.[40,54] It is also possible that distinct partner genes may differently affect *BCL6* expression and function.

What role does *BCL6* translocation play in FL? Bastard et al[27] reported no difference in prognosis (overall survival) between *BCL6* rearrangement-positive FL cases and the negative cases[27]; 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.[55] 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.

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