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Somatic Mutation of the 5′ Noncoding Region of the *BCL-6* Gene Is Associated with Intraclonal Diversity and Clonal Selection in Histological Transformation of Follicular Lymphoma

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Follicular lymphoma (FL) is a B cell non-Hodgkin's lymphoma (NHL) that frequently displays a t(14;18) translocation. Clonal evolution and histological transformation of FL is frequently associated with the accumulation of secondary genetic alterations. It has been demonstrated that the *BCL-6* gene can be altered by chromosomal rearrangements and by mutations clustering in its 5′ noncoding region in a significant fraction of FL and diffuse large cell lymphoma (DLCL). To elucidate the role of the *BCL-6* gene alterations in the histological transformation and clonal progression of FL, we analyzed serial biopsy specimens from 12 patients with FL. Two cases of FL showed no histological alteration in the second biopsy, and 10 cases of FL showed morphological transformation to DLCL in the second biopsy. Southern blot analysis was used to detect rearrangement of the *BCL-6* gene, polymerase chain reaction-single strand conformation polymorphism and sequence analysis were performed for identification of mutations in the 5′ noncoding region of the *BCL-6* gene, and immunohistochemical analysis was applied to reveal the *BCL-6* protein expression. No *BCL-6* gene rearrangement was detected in any of the samples, but a total of 58 mutations were found in the 5′ noncoding region of the *BCL-6* gene in seven cases. In five cases, both the FL and the clonally related FL or DLCL, and in two cases only the DLCL samples were mutated. The mutations were identical in multiple biopsy specimens of FL that did not show morphological transformation. In six patients where FL cells underwent morphological transformation, considerable intraclonal sequence heterogeneity was observed, indicating an ongoing type of somatic mutation. Based on the pattern of shared and nonshared mutations, the genealogical relationship of neoplastic clones could be established. In all of these cases, the histological transformation of FL was associated with the emergence of a subpopulation marked by new sites of mutations in the *BCL-6* 5′ noncoding sequences. In three of these six cases, the histological transformation is also associated with the reduced expression of the *BCL-6* protein. These findings demonstrate that mutation of the 5′ noncoding region of the *BCL-6* gene developed in the clonal evolution of FL, and at different time points in the lymphoma evolution different clonotypes dominate. ***(Am J Pathol 2000, 156:1017–1024)***

Follicular lymphoma (FL) is a B cell non-Hodgkin's lymphoma (NHL) that is believed to originate from germinal center (GC) cells.[1] Generally, FL starts as a relatively indolent malignancy; however, it has a tendency to transform into high-grade aggressive diffuse large cell lymphoma (DLCL) during the later stage of the disease.[2–4] Approximately 85% of FLs are associated with the t(14;18) translocation, which juxtaposes the *BCL-2* oncogene with the immunoglobulin (Ig) heavy chain (H) gene.[5,6] In the clonal evolution and histological transformation of the FL, the neoplastic cells retain the t(14;18) translocation and frequently acquire additional genetic abnormalities at cytogenetic[7–11] and molecular levels.[12–16]

The *BCL-6* gene is located on chromosome 3q27 and encodes a POX/Zinc finger protein, which functions as a sequence-specific DNA-binding transcription repressor.[17] The *BCL-6* protein is expressed in B cells within GC and is involved in the control of GC formation and T-cell-dependent antigen responses.[17,18] Several lines of evidence suggest that structural alterations of the regulatory region of the *BCL-6* gene are involved in lymphomagenesis. Chromosomal rearrangements affecting

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Table 1. Summary of the *BCL-6* Gene Rearrangement, PCR-SSCP Analysis of the *BCL-6* 5′ Noncoding Regions and Expression of the BCL-6 Protein in 12 Cases of Paired FL and Subsequent FL or DLCL Samples

Case	Sample	Date of biopsy	Histology	*BCL-6* rearrangement		*BCL-6* 5′ Noncoding region				*BCL-6* expression (%)
				*Xba*I	*BamH*I	E1.10	E1.11	E1.12	E1.13	
1	A	1991	FL-II	G	G	WT	M	M	WT	100
	B	1992	DLCL	G	G	WT	M	WT	WT	100
2	A	1986	FL-II	G	G	WT	M	WT	WT	100
	B	1992	DLCL	G	G	WT	M	WT	WT	100
3	A	1989	FL-III	G	G	WT	M	WT	M	100
	B	1990	DLCL	G	G	WT	M	WT	M	100
4	A	1988	FL-III	G	G	WT	WT	WT	WT	100
	B	1989	DLCL	G	G	WT	WT	WT	WT	100
5	A	1985	FL-I	G	G	WT	M	WT	WT	100
	B	1988	DLCL	G	G	WT	M	WT	WT	20
6	A	1987	FL-I	G	G	WT	WT	WT	WT	100
	B	1988	DLCL	G	G	WT	M	M	WT	10
7	A	1983	FL-I	G	G	WT	WT	WT	WT	100
	B	1990	DLCL	G	G	M	M	WT	WT	10
8	A	1984	FL-I	G	G	WT	WT	WT	WT	100
	B	1986	DLCL	G	G	WT	WT	WT	WT	100
9	A	1989	FL-II	G	G	WT	WT	WT	WT	100
	B	1990	DLCL	G	G	WT	WT	WT	WT	100
10	A	1986	FL-I	G	G	WT	WT	WT	WT	ND
	B	1987	DLCL	G	G	WT	WT	WT	WT	ND
11	A	1998	FL-I	G	G	WT	M	WT	WT	100
	B	1999	FL-I	G	G	WT	M	WT	WT	100
12	A	1997	FL-II	G	G	WT	WT	WT	WT	100
	B	1999	FL-II	G	G	WT	WT	WT	WT	100

A, first biopsy sample; B, second biopsy sample; FL-I, follicular lymphoma—cytological grade I; FL-II, follicular lymphoma—cytological grade II; FL-III, follicular lymphoma—cytological grade III; DLCL, diffuse large cell lymphoma; G, germline configuration; M, mutations identified in PCR-SSCP fragments; ND, not detected; WT, wild-type.

the *BCL-6* gene cluster within a 4-kb region spanning the promoter and first noncoding exon are associated with ~40% of DLCL and ~10% of FL.[19–22] Recent studies also indicate that the *BCL-6* gene may be altered by somatic point mutations clustering within the 5′ noncoding regions of the gene in malignancies, which display a GC phenotype and hypermutated Ig variable (V) genes.[23] Approximately 70% of DLCL, 45% of FL, 58% of AIDS-related NHL, 39% of Burkitt's lymphoma, and 44% of posttransplant lymphoproliferative disorders carry *BCL-6* genes mutated in the 5′ noncoding region.[24–27] These mutations are of somatic origin, frequently biallelic, and found in cases displaying either normal or rearranged *BCL-6* alleles, indicating their independence of chromosomal translocation.[24,26]

To further characterize the nature of *BCL-6* gene mutations in FL and to gain insight into the role of the *BCL-6* gene in lymphoma progression, we have performed longitudinal analysis of the *BCL-6* gene organization in sequential biopsy specimens from patients with FL that showed ho histological alterations in subsequent biopsy specimens or that underwent morphological transformation to DLCL. Our results indicate ongoing somatic mutations of the *BCL-6* 5′ noncoding sequences in the majority of FLs and DLCLs, and provide evidence that histological transformation of the FL may be associated with the emergence of subclones marked by divergent *BCL-6* clonotypes.

Materials and Methods

Pathological Samples and DNA Extraction

Sequential biopsy samples of 12 patients with FL observed at Stanford University Medical Center and University Medical School of Pécs were selected for this study based on the availability of frozen tissue for the molecular analyses. Diagnoses were based on histopathological, immunophenotypic, and immunogenotypic analyses.[15] All lymphoma samples were classified according to the Revised European-American Lymphoma Classification proposed by the International Lymphoma Study Group.[1] The histology of the first lymph node biopsy in six patients (Cases 5–8, 10, and 11) was FL, provisional cytologic grade I, in four patients (Cases 1, 2, 9, and 12) was FL, provisional cytological grade II, and in two patients (Cases 3 and 4) was FL, provisional cytological grade III. In two cases (Cases 11 and 12), the histology of the second biopsy was identical with the first biopsy. In Cases1–10, histology of the second biopsy was classified as DLCL (Table 1). All samples included in this study displayed monoclonal IgH gene rearrangement and t(14;18) translocation. In each case, the subsequent biopsy samples showed identical Ig heavy chain gene rearrangements and identical breakpoint sequences of the t(14;18) translocation, indicating common clonal origin of the tumor samples of the first and the second biopsy. Genomic

DNAs were extracted from cryopreserved tissue samples using the salting out technique.[28]

Southern Blot Analysis of BCL-6 Gene Rearrangement

The presence of *BCL-6* gene rearrangement was analyzed by the 4.0-kb Sac fragment *BCL-6* probe.[19] Five-microgram aliquots of genomic DNA were digested with the *Bam*HI or *Xba*I restriction endonucleases according to the manufacturer's instructions (Boehringer Mannheim GmbH, Mannheim, Germany), electrophoresed in 0.8 or 1% agarose gels, denatured with alkali, neutralized, and transferred to nitrocellulose filters (Schleicher & Schuell, Keene, NH). The filters were hybridized in 50% formamide/3× standard citrate (SSC) buffer at 37°C to DNA probes that had been ^{32}P-labeled using the Random Primed DNA Labeling Kit (Boehringer Mannheim) according to the manufacturer's instructions. The filters were washed in 0.2 × SSC/0.5% sodium dodecyl sulfate at 60°C for 2 hours and then autoradiographed at −70°C for 16 to 48 hours, as described before.[29]

Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP) Analysis of the 5′ Noncoding Region of the BCL-6 Gene

PCR-SSCP analysis of *BCL-6* 5′ noncoding regions was performed on four partially overlapping PCR fragments (E1.10, E.1.11, E1.12, and E1.13) spanning 998 bp located downstream of the first *BCL-6* noncoding exon.[24,26] The selection of this region for the mutational analysis of *BLC-6* was based on evidence that ~45% of FLs and ~70% of *de novo* DLCLs carry somatic mutations in these sequences.[24,26]

DNA Sequencing

PCR products encompassing fragments E1.10, E1.11, E1.12, and E1.13 were cloned into the pCR vector using the TA Cloning Kit (Invitrogen Corp., San Diego, CA). In samples subjected to DNA sequencing, six independent subclones were analyzed. DNA sequencing was performed directly from a small-scale plasmid preparation using the Sequenase version 2.0 (United States Biochemical, Cleveland, OH) system according to the manufacturer's instructions. DNA sequences were analyzed using the MacVector version 4.5 (Eastern Kodak Co., New Haven, CT) software and the GenBank data base.

Analysis of the Sequence Polymorphism of the 5′ Noncoding Region of the BCL-6 Gene

To analyze whether sequence alterations of the 5′ noncoding region of the *BCL-6* gene shared by the FL and DLCL samples of Cases 3 and 5 are associated with somatic mutation or sequence polymorphism, normal tissue samples of these two patients were amplified by the primers specific for the E1.13 and E1.11 fragments, respectively. The PCR products were cloned, sequenced, and compared with the corresponding *BCL-6* 5′ noncoding sequences of the tumor samples.

Immunohistochemistry

The expression of the *BCL-6* protein was detected by using the mAb PG-B6 directed against the amino-terminal portion of the human *BCL-6* gene product.[30] Immunostaining for *BCL-6* was performed on frozen sections by the alkaline phosphatase-anti-alkaline phosphatase method.[31]

Results

Analysis of BCL-6 *Gene Rearrangement*

Genomic DNAs from paired samples of FL and subsequent FL or DLCL were digested with *Bam*HI and *Xba*I restriction endonucleases and hybridized with radiolabeled *BCL-6* probe. None of the FL or transformed DLCL samples of the 12 patients displayed *BCL-6* gene rearrangement in either the *Bam*HI- or *Xba*I-digested DNAs (Table 1).

PCR-SSCP Analysis of the 5′ Noncoding Sequences of the BCL-6 Gene

In each case, the samples of the first and second biopsies were evaluated in parallel. The 24 samples from the 12 patients displayed a total of 16 PCR-SSCP variants. Representative examples of the PCR-SSCP analyses are shown in Figure 1. In five cases (Cases 4, 8–10, and 12), neither first nor second biopsy samples showed altered migration patterns. In two cases (Cases 6 and 7) only the second sample, and in five cases (Cases 1–3, 5, and 11) both the first and subsequent samples, showed altered electrophoretic migration patterns. In those cases, where both the first and the second biopsy samples showed altered PCR-SSCP migration, the patterns were identical in one case (Case 11) and different in six cases (Cases 1–3 and 5–7).

Nucleotide Sequence Analysis of the BCL-6 5′ Noncoding Sequences

To confirm and characterize the mutations affecting the *BCL-6* 5′ noncoding region, we cloned and sequenced those pairs of PCR products in which any of the samples showed altered migration pattern in the PCR-SSCP analysis. In these cases, DNAs were PCR-amplified under the same conditions as described for the SSCP, but the radioactive nucleotide was omitted. In each sample, six independent bacterial isolates were analyzed and compared with germline sequences of the *BCL-6* 5′ noncoding region. For each tumor sample studied, PCR fragments that appeared abnormal by

Table 2. Distributions of Mutations within the *BCL-6* 5′ Noncoding Sequences in Seven Cases of Paired FL and Subsequent FL or DLCL Samples

		Case 1		Case 2		Case 3	
Fragment	Allele	A	B	A	B	A	B
E1.10	a	–	–	–	–	–	–
E1.11	a	–	523 (G → A)	531 (G → C)	–	504 (T → C)*	–
		–	554 (G → T)	–	551 (G → C)	–	522 (A → G)*
		586 (C → G)*	–			–	533 (T → G)*
		602 (G → A)*	–			–	544 (A → C)*
						–	553 (A → C)*
						–	577 (C → G)*
						–	580 (T → C)*
						–	581 (T → C)*
						583 (C → G)*	–
						–	585 (G → A)*
						–	588 (G → A)*
						–	590 (T → C)*
						593 (T → G)*	–
						–	596 (C → T)*
						–	599 (A → G)*
						–	604 (T → G)*
						–	634 (T → G)*
						636 (T → C)*	–
	b	–	–	–	–	–	–
E1.12	a	839 (T → C)	–	–	–	–	–
E1.13	a	–	–	–	–	–	946 (T → C)*
						–	973 (T → C)*
						976 (C → T)*	976 (C → T)*
						1054 (T → C)*	–
						–	1109 (C → G)*
						–	1116 (C → T)*
						1137 (G → C)*	–

A, first biopsy sample; B, second biopsy sample.
*Mutation is associated with intraclonal divergence.
–, not mutated.

SSCP analysis were found to contain mutations. The mutations found are shown in Table 2. In 14 samples of the seven cases analyzed, a total of 58 alterations were detected. The mutations observed included single basepair substitutions and a 31-bp stretch deletion. All mutations found were distributed in one or two clusters of clonally related *BCL-6* 5′ noncoding sequences, indicating mono- or biallelic distribution of the mutations. In three samples (5B, 6B, and 7B), both alleles carried mutations. In two cases (Cases 6 and 7) only the DLCL samples, and in five cases (Cases 1–3, 5, and 11) both the FL and subsequent FL or DLCL samples were mutated. In six samples (1A, 3A, 3B, 5A, 6B, and 7B), the nucleotide sequences showed evidence of intraclonal heterogeneity, ie, clones of a given sample differed from the others in one or more nucleotides. To distinguish intraclonal heterogeneity from possible *Taq* error, we considered sequence heterogeneity as intraclonal divergence provided nucleic acid differences are found in more than one clones. Sequences with intraclonal heterogeneity are demonstrated in Figure 2. In those four cases (Cases 1–3 and 5) where both FL and DLCL samples were mutated, mutations were different in the subsequent biopsy samples; however, in Cases 3 and 5 a single shared mutation was detected. From the pattern of shared and unique mutations, assuming that shared mutations represent single events and not independent mutations, genealogical trees of the evolution of tumor cells could be constructed. A representative example of a genealogical tree is shown in Figure 3.

Analysis of the Sequence Polymorphism of the 5′ Noncoding Region of the BCL-6 Gene

In Cases 3 and 5, shared mutations of the 5′ noncoding region of the *BCL-6* gene were detected in FL and subsequent DLCL samples at positions 976 (C → T) and 551 (G → C), respectively. To determine whether shared mutations are associated with sequence polymorphism or somatic mutation, we have analyzed the *BCL-6* 5′ noncoding region sequences in normal tissue samples of these two patients. The sequence analysis of the normal tissue samples revealed no alterations in the *BCL-6* 5′ noncoding sequences. Thus, these findings are consistent with our presumption that nucleotide sequence alterations shared by FL and DLCL samples in Cases 3 and 5 arose by somatic mutation.

Table 2. Continued

Case 5		Case 6		Case 7		Case 11	
A	B	A	B	A	B	A	B
–	–	–	–	–	242 (C → G)	–	–
				–	285 (A → G)		
				–	259 (T → C)		
				–	285 (T → A)		
551 (G → C)*	551 (G → C)	–	586 (C → G)	–	552 (G → A)*	481 (A → C)	481 (A → C)
613 (C → G)*	–	–	588 (G → A)	–	577 (C → G)*		
614 (T → A)*	–	–	602–633 (del)	–	604 (T → C)*		
624 (T → C)*	–			–	638 (T → C)*		
635 (T → C)*	–						
–	481 (A → T)	–	626 (C → A)*	–	556 (G → C)		
–	482 (G → A)	–	637 (T → C)*				
–	–	–	772 (G → T)	–	–	–	–
–	–	–	–	–	–	–	–

Expression of BCL-6 Gene

Expression of the *BCL-6* gene was detected by immunoreactivity of tumor cells by anti-BCL-6 mAb. The staining pattern on frozen tissue sections was nuclear. The percentage of the *BCL-6*-positive cells in FL and subsequent FL or DLCL samples is summarized in Table 1. In all of the FL and in six of the DLCL samples, 100% of the tumor cells expressed the *BCL-6* protein. In three transformed DLCL samples (Cases 5–7), the number of the *BCL-6*-positive cells reduced to 10 to 20%.

Discussion

We have analyzed sequential biopsy samples from 12 patients with FL that showed no histological transformation in subsequent biopsy specimens or underwent histological transformation to DLCL for structural alterations of the *BCL-6* gene. We found no rearrangement of the *BCL-6* gene in any of the samples, but multiple mutations of the 5′ noncoding sequences of the *BCL-6* gene were detected in seven cases. In those cases where FL underwent histological transformation, these mutations generated considerable intraclonal divergence of the neoplastic clones in both FL and transformed DLCL. The histological transformation of FL was associated with the emergence of new subpopulations representing divergent clonotypes of the *BCL-6* 5′ noncoding sequences. These findings suggest that genetic diversity of the 5′ noncoding sequences of the *BCL-6* gene is generated in the FL or DLCL clones and histological transformation is associated with clonotypic shift of the original FL clone.

Somatic mutation of the 5′ noncoding region of the *BCL-6* gene has been described in B cell NHLs of the GC/post-GC origin.[23–25,32] These mutations are always found in a 3.5-kb region spanning the first noncoding exon and clustering in the 5′ region of the first intron, which may occur in the absence of physical linkage to antigen receptor loci. In the present study, we have revealed further characteristics of these mutations showing that mutation of the 5′ noncoding region of the *BCL-6* gene is associated with considerable intraclonal heterogeneity in FL and transformed DLCL cells. This sequence heterogeneity within the tumor clones indicates that the tumor cells are still under the influence of the mutation mechanism after neoplastic transformation. Although intraclonal heterogeneity of the *BCL-6* 5′ noncoding sequences has not been reported in NHLs, several lines of evidence suggested such a possibility. In the majority of FLs, the PCR-SSCP analysis of the *BCL-6* 5′ noncoding sequences showed multiple variant fragments, suggesting subclonal variations of the neoplastic clone.[24] Furthermore, Migliazza et al[24] found additional mutations in subcloned products, which were interpreted as a possi-

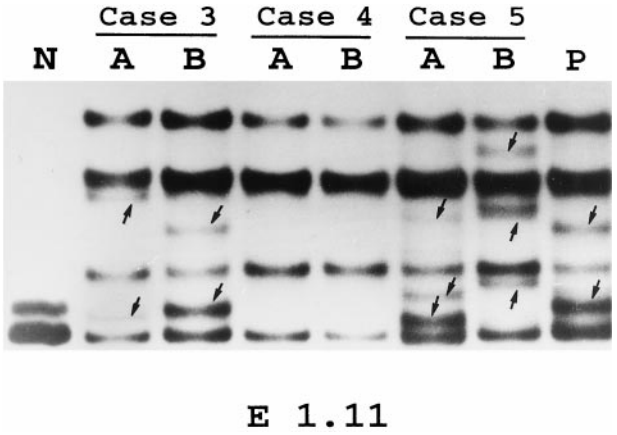

Figure 1. PCR-SSCP analysis of the 5′ noncoding regions of *BCL-6* gene in paired serial biopsy specimens of FL (**A**) and transformed DLCL (**B**). Representative results obtained for PCR-amplified fragments from the E1.11 regions of *BCL-6* gene in Cases 3–5 are shown. A positive control (**P**), represented by a tumor sample known to harbor *BCL-6* mutations, as well as sample that was not denatured before electrophoresis (**N**) are shown. **Arrows** identify abnormally migrating bands.

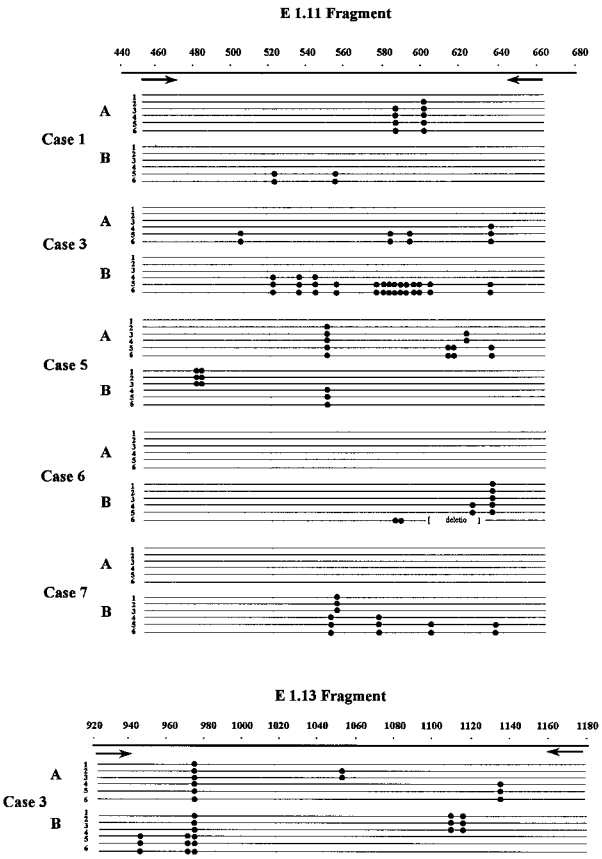

Figure 2. Schematic representation of the *BCL-6* 5′ noncoding region diversification and sequence intraclonal heterogeneity in FL (**A**) and transformed DLCL (**B**) samples. Each sequence is represented as a horizontal line. The first nucleotide of the *BCL-6* cDNA is designated as position +1. The **arrows** represent the positions of the primers. Within each sequence, nucleotide differences from the *BCL-6* cDNA sequences are indicated by **dots**. The type and exact positions of the mutations are specified in Table 2.

ble consequence of *Taq* polymerase error. Although some of the nucleotide substitutions found in our study may be attributed to the infidelity of *Taq* polymerase, this explanation is unlikely to account for more than a few of the mutations. The ongoing mutation rate of approximately 0.3% in the *BCL-6* 5′ noncoding region of NHLs analyzed in this study is an order of magnitude greater than our *Taq* error rate of 0.03%, which was calculated from the analysis of unmutated *BCL-6* 5′ noncoding sequences in our laboratory. In addition to the high frequency, many of the uncommon mutations were shared by different clones of a given *BCL-6* 5′ noncoding sequence, which also indicates that they are most likely of lymphoma cell origin.

The follow-up sequence analysis of the 5′ noncoding region of the *BCL-6* gene revealed considerable differences between sequences of FL and subsequent DLCL samples. These nucleotide differences ranged from two to 18 nucleotides. Because there were identical Ig gene rearrangements and identical t(14;18) breakpoints of the subsequent biopsy samples, mutated *BCL-6* 5′ noncoding sequences derived from a single neoplastic B cell clone in each case. The common clonal origin of the FL and DLCL cells is further supported by the findings that in two cases, single shared mutations were detected in the subsequent biopsy samples. Any nucleotide alterations not shared by both FL and DLCL cells necessarily accumulated during clonal evolution or histological transformation of the neoplastic cells. The extent and nature of *BCL-6* mutations detected in this study is most similar to the hypermutation mechanism of IgV genes detected in the clonal evolution and histological transformation of FL.[33] This observation is also supported by our previous findings. The IgH gene mutational analysis performed in Case 5 showed similar type of intraclonal heterogeneity and clonal selection in the histological transformation of FL as *BCL-6* 5′ noncoding sequences in this study.[34]

The functional significance of the intraclonal divergence of the *BCL-6* 5′ noncoding sequences and the pathogenic role of the intensive clonal selection of the mutated sequences in histological transformation of FL are unknown. Although functional characterization of *BCL-6* is still in its early stages, it is thought that the *BCL-6* 5′ noncoding region contains important regulatory elements. Initial studies on tumor-derived *BCL-6* alleles indicate that few mutations can significantly deregulate *BCL-6* expression, whereas others are apparently functionally irrelevant or associated with silent alleles.[35] In three cases with FL that underwent histological transformation, the 100% of *BCL-6* immunoreactivity of the FL cells reduced to 10 to 20% reactivity in the transformed DLCL sample. Although this finding suggest that the development of additional mutations in the *BCL-6* 5′ noncoding region may deregulate the expression of the *BCL-6* gene, further functional analysis of the *BCL-6* gene is required to demonstrate whether mutations detected in the DLCL samples cause biological and/or functional alterations of the tumor cells.

The intensive clonal selection of the mutated *BLC-6* 5′ noncoding sequences in histological transformation of FL suggests that they may have been selected based on their functional role. As a result of ongoing somatic mutation, the tumor cell population becomes heteroge-

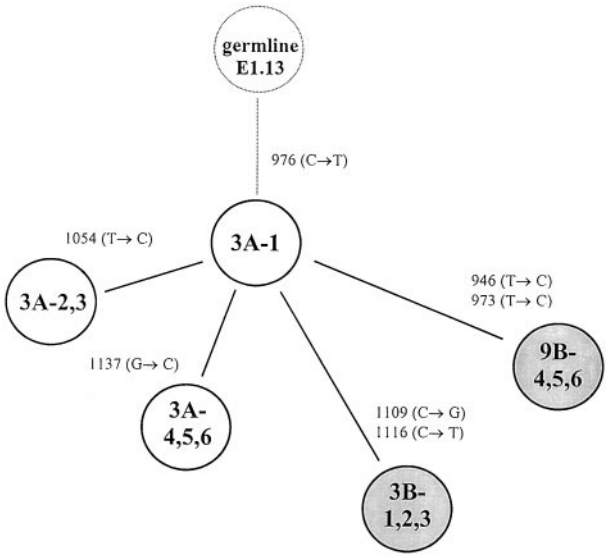

Figure 3. Probable clonal relationship of FL and transformed DLCL clones in Case 3. The genealogical tree is constructed from the pattern of shared and unique mutations of E1.13 PCR fragment of the *BCL-6* 5′ noncoding sequences (Table 2 and Figure 2), assuming that shared mutations represent single events. The FL clones (**A**) are drawn as **open circles** and DLCL clones (**B**) are drawn as **shaded circles**. The number of mutations that separate each branch is given.

neous, and a mutational variant having selective growth advantage compared to those of parental clones gives rise to the DLCL cell population. Functional selection of the neoplastic clones in clonal evolution and histological transformation of FLs has also been suggested by the mutational analysis of IgV_H genes.[34,36–38] The pattern and distribution of these mutations suggest that antigen selection may play an important role in the clonal evolution and histological transformation of FL. Although the nature of antigens that appear to function through the Ig receptor expressed by NHLs is not known, self-antigens have been considered as potential candidates.[36] In this regard, it would be of particular interest to know the type of selective force that influences the *BCL-6* gene in clonal selection and histological transformation of FL.

Ongoing somatic mutation of the *BCL-6* 5′ noncoding sequences and clonal selection of these mutations have also been detected in normal GC B cells.[32,39] The pattern of mutations is not clearly different in normal and malignant B cells, which suggests that mutations may not have a pathological effect. Furthermore, in our study ongoing mutations have been detected in the majority of neoplastic clones both before and after transformation of FL. These results may indicate that mutations of the *BCL-6* 5′ noncoding sequences represent genetic instability of the neoplastic cells. Because the majority of FLs, which eventually will transform to DLCL, shows mutations in the *BCL-6* 5′ noncoding region, it is possible that these mutations are simply a reflection of an increased genetic instability, which, in turn, may result in alterations of other oncogenes or tumor suppressor genes responsible for histological transformation of FL.

In conclusion, this study provides the first evidence that somatic mutation of the 5′ noncoding sequences of the *BCL-6* gene may occur after neoplastic transformation of FL and DLCL, and demonstrates that histological transformation of FL is associated with clonal selection of the mutated clones. However, it remains to be determined whether these mutations are responsible for histological transformation of FLs or are simply a reflection of increased genetic instability.

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