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 (15/33, 45%) 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.

NON-HODGKIN'S LYMPHOMA (NHL), the most frequent tumor occurring in patients between the ages of 20 and 40, includes several distinct clinico-pathologic subtypes, among which diffuse lymphoma with a large cell component (DLLC) is the most clinically relevant in terms of morbidity and mortality.[1] DLLC include intermediate-grade lymphomas with pure diffuse large (DLCL), or mixed small and large cell (MX-D) histology, as well as high-grade immunoblastic (IMB) lymphoma. These tumors can occur "de novo," accounting for 30% to 40% of initial NHL diagnosis and, in addition, can represent the final "transformation" stage of follicular lymphomas (FL), small lymphocytic lymphoma, and chronic lymphocytic leukemia. Considered together, "de novo" and "posttransformation" DLLC account for up to 80% of NHL mortality.[1]

During the past decade, abnormalities involving proto-oncogenes and tumor suppressor genes have been identified in association with distinct NHL subtypes.[2] These genetic lesions represent important steps in lymphomagenesis as well as tumor-specific markers that have been exploited for diagnostic and prognostic purposes.[3,4] Examples include alterations of the *MYC* oncogene in Burkitt's lymphoma (BL), and of the *BCL2* and *BCL1* oncogenes in FL and mantle-cell NHL, respectively. With respect to DLLC, several molecular alterations have been detected at variable frequency, but none has been specifically or consistently associated with the disease.[2]

We and others have recently cloned the chromosomal junctions of cytogenetic translocations involving band 3q27,[5-7] which are common in NHL.[8,9] A candidate proto-oncogene *BCL6* was identified at 3q27 that is structurally altered as a consequence of the translocations.[7,10] The *BCL6* gene codes for a zinc-finger protein sharing homologies with several transcription factors.[7,10] In multiple NHLs, rearrangements of *BCL6* truncate the gene in its 5′ noncoding region, presumably leading to deregulated protein expression.[10] In this study, we report the frequency and disease-specificity of *BCL6* rearrangements among the principal categories of lymphoproliferative disease, including different NHL subtypes, acute and chronic lymphoid leukemias, and multiple myeloma.

MATERIALS AND METHODS

Samples of lymphnode biopsies, bone marrow (BM) aspirates, and peripheral blood were collected by standard diagnostic procedures during the course of routine clinical evaluation in the Division of Surgical Pathology, Department of Pathology, Columbia University. In all instances, the specimens were collected before specific antitumor treatment. Diagnoses were based on the results of histopathologic, immunophenotypic, and immunogenotypic analysis.[11] In all cases, the fraction of malignant cells in the pathologic specimen was at least 70% as determined by cytofluorimetric or immunohistochemical analysis of cell-surface markers or antigen receptor (Ig heavy chain and T-cell receptor β chain) gene rearrangement analysis.[11]

Genomic DNA was prepared from diagnostic specimens by cell lysis, proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. For Southern blot analysis, 6 μg of DNA was digested with the appropriate restriction endonuclease, electrophoresed in a 0.8% agarose gel, denatured, neutralized, and transferred to Duralose filters (Stratagene, La Jolla, CA). Filters were then hybridized with the *BCL6*-specific Sac 4.0 probe[10] that had been ^{32}P-labeled by the random priming technique. After hybridization, filters were washed in 0.2× SSC (1× SSC = 0.15 mol/L NaCl + 0.015

From the Divisions of Oncology and Surgical Pathology, the Department of Pathology, College of Physicians & Surgeons, Columbia University, New York, NY; the Divisione di Ematologia, Universita' di Torino, Italy; and the Cell Biology and Genetic Program and the Departments of Pathology and Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

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Address reprint requests to Riccardo Dalla-Favera, MD, Division of Oncology, Department of Pathology, College of Physicians & Surgeons, Columbia University, 630 W 168th St, New York, NY 10032.

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Table 1. Rearrangements of the *BCL6* Gene in Lymphoid Tumors

Tumor	Histotype	Rearranged/Tested	%
NHL			
Low grade:	SL	0/10	0
	SCC-F	2*/18	11
	MX-F	0/13	0
Intermediate grade:	MX-D	1/10	10
	DLCL	15/33	45
	SCC-D	0/2	0
High grade:	IMB	0/2	0
	SNCL	0/22	0
Others:	CTCL	0/15	0
ALL			
B-lineage:		0/34	0
T-lineage:		0/11	0
CLL			
B-lineage:		0/41	0
T-lineage:		0/10	0
MM		0/23	0

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.

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.[11]

Comparisons of histologic subsets with or without *BCL6* rearrangement were made using the method of inferences from proportions.[12]

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)[10] and restriction enzymes (*Bam*HI 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).[10] This region was previously shown to contain the cluster of breakpoints detected in NHL.[10] 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.

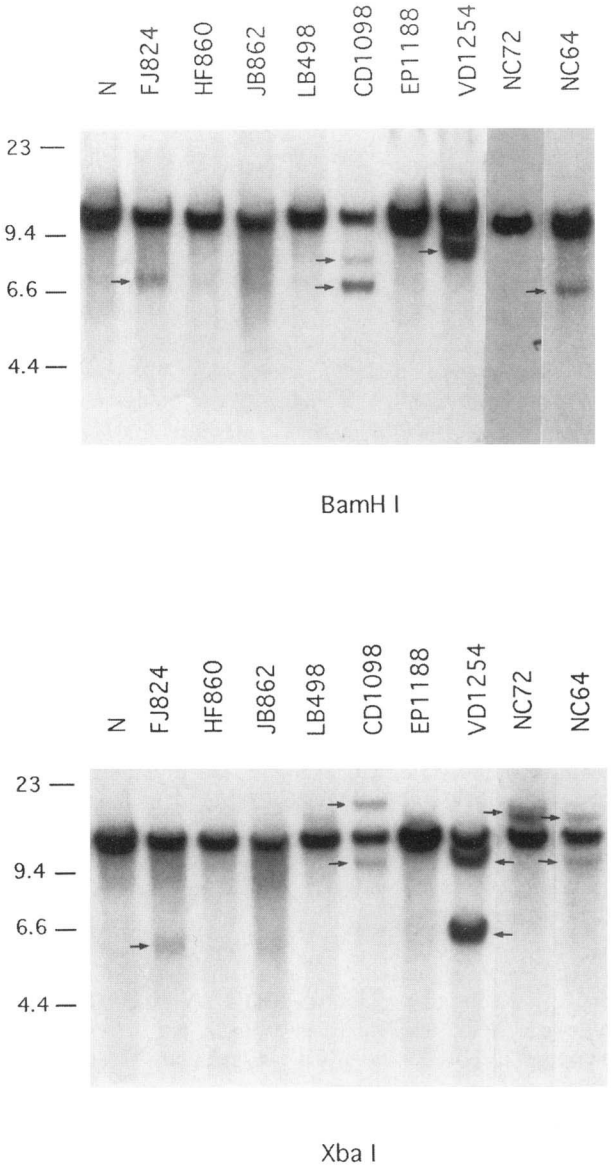

Fig 1. Rearrangements of the *BCL6* gene in DLCL. Genomic DNA extracted from tumor biopsies of DLCL cases and from normal lymphocytes (lane N) was digested with the indicated restriction enzymes and analysed by Southern blot hybridization using the Sac 4.0 probe.[10] Abnormal restriction fragments are indicated by the arrows. Note that the intensity of hybridization to the rearranged bands varies depending on the abundance of the clonal population as well as the position of the breakpoint. For a detailed restriction map of the *BCL6* locus and for data on the precise mapping of the breakpoint in selected cases, see ref 10.

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),[8,9] 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 κ and λ light chains, and B-cell–associated antigens CD19, CD20, and CD22.[11] 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.[13] 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 DLCL.

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.[14]

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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