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Review

Pathogenetic role of *BCL6* translocation in B-cell non-Hodgkin's lymphoma

H. Ohno

Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Present address: Department of Medicine, Takeda General Hospital, Kyoto, Japan

Summary. Chromosomal translocation affecting the 3q27 band, where the *BCL6* gene is localized, is one of the most common genetic abnormalities in non-Hodgkin's lymphoma of B-cell type (B-NHL). The translocation occurs within the major translocation cluster (MTC) of *BCL6*, and as the result of translocation either one of the three immunoglobulin (*Ig*) genes or a heterogeneous non-Ig gene is juxtaposed to the coding regions of *BCL6*. On the other hand, somatic hypermutation involves the *BCL6* gene of not only B-NHL but also B-cells from normal individuals. The mutations are clustered within a region of the MTC, suggesting that a common molecular mechanism is operating for the two genetic lesions of *BCL6*. The Bcl-6 protein is a transcriptional repressor that is an important regulator of lymphoid development and function. The protein is preferentially expressed in germinal center (GC) B-cells of normal lymphoid tissues as well as in a variety of B-NHL subtypes derived from GC B-cells irrespective of whether the *BCL6* is rearranged. Although there is no consensus on the effect of *BCL6* translocation on the clinical outcome of B-NHL, many studies coincide in showing that a high-level of *BCL6* expression at either or both the mRNA and protein levels is a favorable prognostic marker of diffuse large B-cell lymphoma. *In vitro* evidence suggests that non-*Ig*/*BCL6* translocation transiently enhances the level of Bcl-6 expression, which may perturb a molecular network that controls the differentiation of GC B-cells to Ig-secreting plasma cells, thereby predisposing the B-cells to neoplastic transformation.

Key words: *BCL6* gene, immunoglobulin (*Ig*) gene, non-*Ig*/*BCL6* translocation, somatic hypermutation, diffuse large B-cell lymphoma

Offprint requests to: Hitoshi Ohno, MD, Department of Medicine, Takeda General Hospital, 28-1 Ishida-Moriminami-cho, Fushimi-ku, Kyoto 601-1495, Japan. Fax: +81(JAPAN)-75-571-8877. e-mail: hohno@k2.dion.ne.jp

Introduction

Chromosomal translocation and rearrangement of a series of oncogenes are observed in a large proportion of non-Hodgkin's lymphoma of B-cell type (B-NHL) (Willis and Dyer, 2000). 3q27 translocation affecting the site of the *BCL6* gene has been identified by cytogenetic analysis and/or Southern blot analysis using a probe for the major translocation cluster (MTC) of *BCL6* (Offit et al., 1989, 1994; Bastard et al., 1992, 1994; Ohno and Fukuhara, 1997). An early study demonstrated a specific association of *BCL6* translocation with diffuse large B-cell lymphoma (DLBCL) (Ye et al., 1993). However, later studies showed that other B-NHL subtypes carry such abnormalities (Bastard et al., 1994; Muramatsu et al., 1996; Ohno and Fukuhara, 1997). The approximate rates of *BCL6* translocation in the two major B-NHL subtypes are 5% to 15% in follicular lymphoma (FL), and 20% to 40% in DLBCL (Ohno and Fukuhara, 1997; Willis and Dyer, 2000). *BCL6* translocation is unique in that it can involve not only one of the three immunoglobulin (*Ig*) genes but also another non-*Ig* partner gene (Ye et al., 1993; Bastard et al., 1994; Akasaka et al., 1997; Ohno and Fukuhara, 1997). As the result of non-*Ig*/*BCL6* translocations, *BCL6* comes under the control of diverse promoter activities, leading to deregulated expression of the *BCL6* gene product (Chen et al., 1998).

On the other hand, the *BCL6* gene is altered by somatic hypermutation (SHM), which preferentially affects the first exon-intron boundary region of the gene (major mutation cluster, MMC) (Migliazza et al., 1995; Pasqualucci et al., 1998; Shen et al., 1998). Since the MTC and MMC overlap, a common molecular mechanism for both aberrations has been suggested (Kuppers and Dalla-Favera, 2001). In contrast to the translocation, however, SHM is observed not only in B-NHL cells but also in B-cells from normal individual (Pasqualucci et al., 1998; Shen et al., 1998). Point mutations within particular domains of the MTC/MMC were recently shown to significantly affect the level of *BCL6* expression (Artiga et al., 2002; Wang et al., 2002;

Pasqualucci et al., 2003), whereas other mutations are apparently functionally irrelevant.

In this review, I first summarize the genetic abnormalities of *BCL6* in B-NHL. Secondly, I discuss the effects of *BCL6* translocations as well as the expression levels of mRNA/protein of this gene on the treatment outcome of DLBCL. I finally propose a model for the role of *BCL6* translocation in the development of B-NHL based upon the *in vitro* and *in vivo* evidence about the deregulated expression of *BCL6*.

Chromosomal translocation affecting the 3q27 chromosomal band and isolation of the *BCL6* gene

Although abnormalities of the long arm of chromosome 3 in NHL were described in the past, earlier studies suggested that these abnormalities were secondary cytogenetic abnormalities (Konishi et al., 1990). In 1989, t(3;22)(q27;q11) was first reported in 8 cases of DLBCL (Offit et al., 1989), and t(3;14)(q27;q32) and t(2;3)(p11;q27) were subsequently identified in terms of the association with the three *Ig* gene loci (Bastard et al., 1992). On the other hand, more than 20 non-*Ig* partner loci have been cytogenetically identified (Ye et al., 1993; Bastard et al., 1994; Akasaka et al., 1997; Ohno and Fukuhara, 1997; Yonetani et al., 1998). The overall incidence of 3q27 abnormalities determined by conventional cytogenetic analysis is estimated to be 16% in all types of NHL (Bastard et al., 1994) and 23% in DLBCL (Offit et al., 1994). These incidences, however, are probably underestimated, as this terminal alteration is sometimes difficult to detect cytogenetically. Indeed, 3q27 was the most common reciprocal partner of 14q+ translocation detected by fluorescence *in situ* hybridization analysis (Ueda et al., 1996). At present, many investigators agree that 3q27 translocation is one of the most common translocations in B-NHL.

By analogy with other B-NHL-associated translocations, the cytogenetic observation of 3q27 translocations suggested the presence of a potential oncogene on this chromosomal site. Four independent groups performed molecular cloning of the junctional areas of t(3;14)(q27;q32) and t(3;22)(q27;q11) by using probes for the *Ig* genes, leading to the isolation of *BCL6* on 3q27 (Baron et al., 1993; Kerckaert et al., 1993; Ye et al., 1993; Miki et al., 1994b). The *BCL6* gene spans 26 kb and contains 10 exons; exon 1b was included in a cDNA clone isolated by Miki et al. (1994b). The ATG signal for initiation of protein synthesis is within exon 3 and is followed by an open reading frame, which encodes the Bcl-6 protein consisting of 706 amino acids. The Bcl-6 protein is a transcriptional repressor containing six 'Krüppel' Cys_2-His_2 zinc finger motifs at the C-terminus, through which the protein binds DNA with a particular sequence (Dent et al., 2002; Albagli-Curiel, 2003). The N-terminal region contains a conserved protein-protein interaction motif, the POZ domain, which plays a role not only in homodimerization (Dhordain et al., 1995) but also in heterodimerization. The POZ domain can bind to co-repressors NCoR, SMART and BCoR (Dhordain et al., 1997; Huynh and Bardwell, 1998; Huynh et al., 2000), which are able to recruit the histone deacetylase protein complex (Dhordain et al., 1998; Lemercier et al., 2002), thereby rendering Bcl-6 able to exert repressive activity. A central domain having multiple potential phosphorylation sites (Moriyama et al., 1997; Niu et al., 1998) can bind to mSIN3A and is involved in transcriptional repression independently of the POZ domain (Albagli-Curiel, 2003). Another feature of Bcl-6 is that it interacts with other transcription factors and influences their transcriptional activities (Okabe et al., 1998; Dhordain et al., 2000; Dent et al., 2002).

Targeted inactivation of *BCL6* in the mouse germline has provided evidence that the gene product is an important regulator of lymphoid development and function (Dent et al., 1997; Fukuda et al., 1997; Ye et al., 1997). The *BCL6*$^{-/-}$ mice show normal lymphoid organ development, including primary follicles in the spleen, and normal numbers and distributions of B- and T-cells. When the mice are immunized with T-cell-independent antigens, they produce primary antibodies at comparable levels to wild-type control mice. In contrast, the *BCL6*$^{-/-}$ mice are severely impaired with respect to generating secondary IgG antibodies against T-cell-dependent antigens, and their spleens lack germinal center (GC) formation. This defect is intrinsic to B-cells, since T-cells from wild-type mice do not restore GC formation (Fukuda et al., 1997). Another phenotype of the *BCL6*$^{-/-}$ mice is inflammatory response in multiple organs. The involved tissues show infiltration of eosinophils and IgG1-/IgE-bearing B-cells as well as increased production of interleukin 4 (IL4), IL5 and IL13, indicating hyper-response of the T-helper cell type 2 (T_H2) subset (Dent et al., 1997; Ye et al., 1997).

Bcl-6 expression in normal lymphoid tissues and lymphoid neoplasms

Immunohistochemical studies of Bcl-6 expression in various tissues have been extensively performed using polyclonal or monoclonal antibodies raised against recombinant Bcl-6 protein (Cattoretti et al., 1995; Flenghi et al., 1995, 1996; Onizuka et al., 1995; Falini and Mason, 2002). In normal lymphoid tissues, Bcl-6 is preferentially expressed in GC B-cells in secondary follicles, and is localized to the nucleus in a diffuse or microgranular pattern (Cattoretti et al., 1995; Flenghi et al., 1995; Onizuka et al., 1995). Expression of Bcl-6 is also observed in a few $CD4^+$ T-cells, both in the intrafollicular and interfollicular areas, and in a small population of perifollicular $CD30^+$ large lymphoid cells (Cattoretti et al., 1995; Flenghi et al., 1995). Other components of the GC, including plasma cells, macrophages and follicular dendritic cells, are negative for Bcl-6.

Bcl-6 is positive in GC-type B-cell tumors, which is

in 100% of FL and 71% to 93% of DLBCL, irrespective of whether the *BCL6* gene is rearranged (Flenghi et al., 1995; Onizuka et al., 1995; Flenghi et al., 1996; Skinnider et al., 1999; King et al., 2000; Colomo et al., 2003). Primary mediastinal large B-cell lymphoma (PMLBCL), which is a distinctive subcategory of DLBCL, is positive for Bcl-6 with lower frequency (47%) than other types of DLBCL (Pileri et al., 2003). Burkitt's lymphoma and DLBCL with centroblastic features that develop in patients with acquired immunodeficiency syndrome (AIDS) are positive for Bcl-6, whereas the expression is negative in DLBCL showing immunoblastic morphology (Carbone, 2003). H&L cells of nodular lymphocyte predominance Hodgkin lymphoma (HL) express this protein at a high level, in clear contrast with classical HL, in which only a small percentage of Reed-Sternberg cells are stained by the anti-Bcl-6 antibody (Falini et al., 1996). On the other hand, B-cell precursor leukemia, mantle cell lymphoma and marginal zone lymphoma consistently lack expression of Bcl-6 (Flenghi et al., 1996). Thus, Bcl-6 is a valuable marker of GC B-cell origin (Falini and Mason, 2002); the combination of Bcl-6 with CD10, MUM1/IRF4 and CD138/syndecan-1 defines the subtypes of DLBCL in parallel with the stage of differentiation of GC B-cells (Colomo et al., 2003). Non-B-cell tumors expressing Bcl-6 include T-cell lymphoblastic lymphoma and anaplastic large cell lymphoma (40%-45%) (Carbone et al., 1997; Kerl et al., 2001). The latter finding suggests that $CD30^+$ ALCL may represent the neoplastic transformation of extrafollicular lymphoid cells that coexpress CD30 and Bcl-6 (Carbone et al., 1997).

Molecular anatomy of chromosomal translocations involving the site of *BCL6*

Chromosomal translocation involving the 3q27 chromosomal band occurs within the MTC, which is a highly conserved 4.0-kb region of *BCL6* that spans the promoter, the non-coding exon 1 and the 5' region of intron 1 (Ye et al., 1993; Bastard et al., 1994; Offit et al., 1994; Bernardin et al., 1997; Ohno and Fukuhara, 1997). In the majority of cases, breakpoints are localized immediately 3' of exon 1 (hyper-cluster) (Akasaka et al., 2000a). The most common type of *BCL6* translocation is t(3;14)(q27;q32), involving the *Ig* heavy chain gene (*IgH*) as the partner (Bastard et al., 1992; Akasaka et al., 2000a). The breakpoints on IgH are invariably within the switch (*S*) regions; S_γ is the most frequently affected (Akasaka et al., 2000a). On the der(3)t(3;14)(q27;q32), the *IgH* upstream sequences are juxtaposed to the *BCL6* in the same transcriptional orientation, whereas the 5'-*BCL6* sequences are fused to downstream sequences of *IgH* on the reciprocal der(14)t(3;14)(q27;q32). As the result of t(3;14)(q27;q32), *BCL6* expression is initiated from the *IgH* germline transcript promoters (I_μ and I_γ), which are followed by the *BCL6* coding sequences (Ye et al., 1995).

Two 'variant' translocations, t(3;22)(q27;q11) involving the λ-light chain gene (*IgL*λ) and t(2;3)(p12:q27) involving the κ-light chain gene (*IgL*κ), lead to juxtaposition of the 3' sequences of *IgL*s to *BCL6* in divergent orientation (Miki et al., 1994a; Suzuki et al., 1994; Akasaka et al., 2000a). The breakpoints within the two *IgL*s are variable, including 5' of variable (*V*) genes, 5' of V_l/J_l complex (*J*, joining), at a point between V_l2-1, which is the most 3' *V* gene of IgL_1, and the J_l1 segment, and between the J_λ and C_λ segments (Akasaka et al., 2000a).

To obtain the DNA sequence of the non-Ig partner, two molecular approaches have been applied, i.e. 5'-rapid amplification of cDNA ends (Dallery et al., 1995; Galiegue-Zouitina et al., 1996) and long-distance inverse polymerase chain reaction (LDI-PCR) (Akasaka et al., 2000a; Akasaka et al., 2003). In the latter method, PCR primers are designed for the *BCL6* sequences in the divergent orientation and PCR amplification targets circular DNA that is prepared by self-ligation of restriction enzyme-digested genomic DNA; the PCR product contains an unknown segment that is flanked by the known *BCL6* sequences.

Table 1 lists non-Ig partners that have been identified using these two PCR-based approaches (Dallery et al., 1995; Galiegue-Zouitina et al., 1996, 1999; Akasaka et al., 1997, 2000a; Yoshida et al., 1999; Hosokawa et al., 2000; Tanaka et al., 2000; Xu et al., 2000; Chen et al., 2001; Ueda et al., 2002a; Akasaka et al., 2003). In our series of B-NHL with *BCL6* rearrangements, 52% of cases had *Ig*s as partners, while 40% were determined to have non-*Ig* partners (Akasaka et al., 2000a). The non-*Ig* partner genes reported to date are too diverse to find common properties. However, these non-*Ig* partners are not random but instead have been recurrently identified, indicating that tumor cells carrying a fusion of *BCL6* with a particular gene are likely to gain a growth advantage over normal cells. The common molecular features of non-*Ig*/*BCL6* translocations include 1) that the gene fusion occurs in the same transcriptional orientation, 2) that the breakpoint on the partner gene is localized in close proximity to its promoter sequence, and 3) that the complete region of the promoter is fused upstream to the coding region of *BCL6* on the der(3) chromosome. As a result of the translocation, many types of regulatory sequences on each partner locus substitute for the 5' untranslated region of *BCL6*, and the rearranged *BCL6* thereby comes under the control of the replaced promoter activity (promoter substitution) (Ye et al., 1995; Chen et al., 1998). In this regard, it should be noted that partner genes are transcriptionally activated by a variety of stimuli, including cell-cycle control (*H4*), changes in the physical environment (*HSP89α* and *HSP90β*), and response to cytokines (*PIM1* and *CIITA*) (Akasaka et al., 2000a). As GC B-cells proliferate rapidly in response to antigen stimulation, it is likely that the *BCL6* gene affected by the translocation is inappropriately expressed during B-cell proliferation.

SHM of *BCL6* in B-cell tumors

SHM of the 5' non-coding region of *BCL6* was initially recognized on B-NHL-derived *BCL6* alleles (Migliazza et al., 1995), while later studies showed that a large proportion of memory B-cells isolated from normal individuals as well as GC B-cells from a reactive tonsil carry SHM within the MMC (Pasqualucci et al., 1998; Shen et al., 1998). These mutations are often multiple and frequently biallelic, and in B-NHL their occurrence is independent of *BCL6* translocation. SHM of *BCL6* occurs in the spectrum of GC/post-GC type B-cell tumors, including FL, lymphoplasmacytoid lymphoma, MALT lymphoma, DLBCL, Burkitt's lymphoma, AIDS-related lymphomas and multiple myeloma (Gaidano et al., 1997; Pasqualucci et al., 1998; Peng et al., 1999; Capello et al., 2000). Intraclonal heterogeneity indicative of ongoing mutation is observed in cases of FL and DLBCL (Lossos and Levy, 2000b), and mutations are accumulated in association with the process of FL transformation to DLBCL (Lossos and Levy, 2000a). In contrast, the mutations are absent or rare in B-cell precursor leukemia and mantle cell lymphoma (Pasqualucci et al., 1998; Capello et al., 2000). The frequency of the mutation ranges from 47% in FL to 73% in DLBCL (Migliazza et al., 1995). Although a significantly lower frequency was reported in PMLBCL (Capello et al., 2000), another series showed comparable frequency with that in DLBCL (Pileri et al., 2003). At present, it is considered that SHM of *BCL6* is acquired at the time of transit through the GC, and therefore is a marker for GC/post-GC type tumors (Capello et al., 2000; Pasqualucci et al., 2000). The presence of *BCL6* SHM in a fraction of hairy cell leukemia and chronic lymphocytic leukemia of B-cell type suggests that a fraction of these two diseases is of GC B-cell origin (Capello et al., 2000; Pasqualucci et al., 2000). In the same line, micro-manipulated Reed-Sternberg cells from classical HL as well as HL-derived cell lines with the B-cell genotype harbor mutated *BCL6* genes (Seitz et al., 2001).

The majority of *BCL6* SHM, in addition to deletions of up to 8 base pairs of nucleotides, are clustered within a 5' region of intron 1 (GenBank accession number AF191831) (Lossos and Levy, 2000b). The frequency of mutations per base pair in FL and DLBCL ranges from $1.3x10^{-3}$ to $1.3x10^{-2}$ (Lossos and Levy, 2000b). This MMC region starts around 640 bp 3' of the TATA box of *BCL6* and contains 11 RGYW motifs, where R = purine, Y = pyrimidine and W = A or T. Single nucleotide substitutions are more common than deletions and insertions, and nucleotide transitions occur more frequently than expected. It is apparent that these characteristics are shared with those of the SHM of

Table 1. Non-*Ig* partner genes of *BCL6* translocation.

GENE SYMBOL HUGO (Alias)	GENE PRODUCT	CHROMOSOMAL LOCUS[1]
MBNL1 (*KIAA0428*)	Muscleblind-like protein (Triplet-expansion RNA-binding protein)	3q25/3q25.1
TFRC	transferrin receptor (p90, CD71)	3q26.2-qter/3q29
SIAT1 (*CD75*)	sialyltransferase 1 (beta-galactoside alpha-2,6-sialytransferase)	3q27-q28/3q27.3
EIF4A2	eukaryotic translation initiation factor 4A, isoform 2	3q28/3q27.3
ARHH (*RhoH, TTF*)	Rho-related GTP-binding protein RhoH (GTP-binding protein TTF)	4p13/4p14
HSPCB (*HSP90ß*)	heat shock 90kDa protein 1, beta	6p12/6p21.1
PIM1	pim-1 oncogene product	6p21.2
SFRS3 (*SRp20*)	Splicing factor, arginine/serine-rich 3 (Pre-mRNA splicing factor SRP20)	6p21/6p21.31
HIST1H4I (*H4/m*)	H4 histone family, member M	6p21.33
U50HG[2]	small nucleolar RNA	6q15
ZNFN1A1 (*IKAROS*)	Ikaros (zinc finger protein)	7p13-p11.1
GRHPR (*GLXR*)	glyoxylate reductase/hydroxypyruvate reductase	9q12/9p13.2
POU2AF1 (*BOB1, OBF-1*)	POU domain class 2, associating factor 1 (B-cell-specific coactivator OBF-1) (OCT binding factor 1) (BOB-1) (OCA-B)	11q23.1
LRMP (*JAW1*)	lymphoid-restricted membrane protein	12p12.1
NACA	nascent-polypeptide-associated complex alpha polypeptide	12q23-q24.1/12q13.3
LCP1	L-plastin (Lymphocyte cytosolic protein 1) (LCP-1) (LC64P)	13q14.3/ 13q14.13
HSPCA (*HSP90α*)	heat shock 90kDa protein 1, alpha	14q32.33/14q32.31
IL21R	interleukin-21 receptor	16p11/16p12.1
MHC2TA (*CIITA*)	MHC class II transactivator CIITA	16p13/16p13.13

[1]: LocusLink and/or Ensembl cytogenetic band. [2]: not included in the HUGO gene nomenclature database. References: Dallery et al., 1995; Galiegue-Zouitina et al., 1996; Akasaka et al., 1997; Galiegue-Zouitina et al., 1999; Yoshida et al., 1999; Akasaka et al., 2000a; Hosokawa et al., 2000; Tanaka et al., 2000; Xu et al., 2000; Chen et al., 2001; Ueda et al., 2002a; Akasaka et al., 2003.

variable (*V*) region genes of *Ig* (Peng et al., 1999), although the frequency of *BCL6* mutations is significantly lower than that of *IgV* mutations in both normal B-cells and DLBCL cells (Pasqualucci et al., 1998). It is generally accepted that the *Ig*-SHM machinery also targets *BCL6* (Stamatopoulos et al., 2000).

The role of *BCL6* SHM in the pathogenesis of B-NHL as well as in normal B-cell development remains to be determined. Since the MMC contains a negative regulatory region (IS) of *BCL6* (Kikuchi et al., 2000), mutations can affect the binding of regulatory elements, thereby deregulating the level of *BCL6* expression. Compilation of the published findings about *BCL6* SHM indicated that mutations are not uniformly distributed within the 790 bp of the MMC, but rather that there are some hot spots: some mutations appear in more than one patient (recurrent mutation) and some nucleotide positions are substituted by different bases in several cases (recurrently mutated position) (Lossos and Levy, 2000b; Artiga et al., 2002). It is possible that these positions are mutational hot spots, potentially playing a role in the pathogenesis of B-NHL. Indeed, DLBCL carrying mutations within the 423 to 443 hot spot express increased levels of Bcl-6 protein, as shown by immunohistochemical analysis (Artiga et al., 2002).

Although there is no clear difference in the distribution patterns of mutations within the MMC between normal and neoplastic B-cells (Pasqualucci et al., 1998), some mutations within exon 1 were recently reported to be specifically restricted to DLBCL (Wang et al., 2002; Pasqualucci et al., 2003). Transcription of *BCL6* is negatively self-regulated by means of the interaction of two tandemly aligned Bcl-6 binding sites within exon 1 and the Bcl-6 protein itself, which is a potent transcriptional repressor. Wang et al. (2002) and Pasqualucci et al. (2003) found a total of four mutations within the binding sites from DLBCL cases and performed structure-function analyses to determine the effect of these mutations. They constructed mutated allele/reporter gene plasmids and transfected them into *BCL6*-permissive cells. The results showed significant overexpression of the reporter gene that was fused with the mutated sequences, suggesting that the mutations abolish the binding of Bcl-6 transcriptional repressor, thereby promoting transcriptional activity. Their study suggests that activating mutations within particular domains of *BCL6* may play a role in the pathogenesis of a portion of cases of DLBCL.

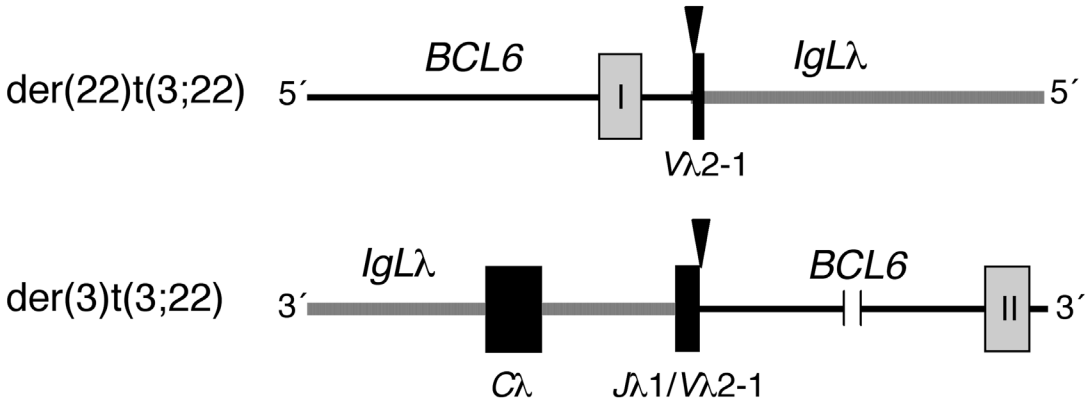
Molecular mechanism of the development of *BCL6* translocation

It is generally accepted that chromosomal translocations in mature B-cell tumors develop in association with a series of *Ig* remodeling processes (Willis and Dyer, 2000; Kuppers and Dalla-Favera, 2001). These include *V/(D)/J* recombination (D, diversity), SHM and isotype class switching recombination (CSR); the first of which occurs in the bone marrow, while the latter two occur sequentially in the GC of secondary lymphoid organs. In *V/(D)/J* recombination, the RAG1/RAG2 recombinases cleave DNA at the signal sequences flanking the relevant segments, whereas in CSR DNA is cleaved within the repetitive *S* regions located upstream of the two constant (*C*) genes. Since many translocations involving *Ig*s are targeted to the *J* segments and the *S* regions, DNA double-strand breaks (DSBs) created by these two remodeling processes most likely predispose these regions to the development of translocations (Kuppers and Dalla-Favera, 2001). On the other hand, there is evidence that SHM is also accompanied by DSBs. Two studies using ligation-mediated PCR showed the presence of DSBs in the IgV_H and IgV_λ genes of Ramos Burkitt's lymphoma cells as well as the targeted V_HB1-8 gene of GC B-cells isolated from the V_HB1-8 IgH knock-in mouse strains (Bross et al., 2000; Papavasiliou and Schatz, 2000). Both studies showed that the positions of DSBs were preferentially associated with the RGYW motif, and that the generation of DSBs was coupled with the transcription of *Ig* and dependent on the Ig enhancer, suggesting that DNA DSBs are involved in the process of SHM. It is possible that error-prone DNA polymerases, such as polymerase ζ, are involved in the introduction of mutations in proximity to DNA breaks during the repair pathway (Zan et al., 2001), although both homologous recombination and non-homologous end-joining models have been proposed (Bross et al., 2000; Papavasiliou and Schatz, 2000).

As demonstrated above, t(3;14)(q27;q32) invariably occurs within the *S* regions of *IgH* and the MTC/MMC of *BCL6* (Akasaka et al., 2000a). Thus, the combination of CSR- and SHM-associated DSBs is presumably responsible for the development of t(3;14)(q27;q32). Although CSR and SHM target distinct segments of *Ig*, they share the molecular mechanism for cleavage and rejoining of DNA; a common nicking endonuclease, which is generated by RNA-editing activity of AID, recognizes and cleaves the stem-loop structure of DNA (Honjo et al., 2002). It is therefore suggested that CSR and SHM, the latter of which targets not only *Ig* but also *BCL6*, can occur simultaneously in a single GC B-cell. Since t(3;14)(q27;q32) results in reciprocal exchange of materials between the two chromosomes, the two regions involved in the translocation may be physically juxtaposed at a specific stage of B-cell development (Roix et al., 2003).

In contrast, CSR-associated DSBs cannot account for the 'variant' *IgL/BCL6* translocations. We and others cloned *IgL/BCL6* fusion areas and found that breakpoints of IgL are localized within rearranged *V* genes or distributed over large intronic sequences (Miki et al., 1994a; Suzuki et al., 1994; Akasaka et al., 2000a). Figure 1 shows an example of IgL_λ/*BCL6* translocation which is the molecular equivalent t(3;22)(q27;q11). The translocation occurred reciprocally with breakpoints within a V_λ gene associated with the $J_1$1 segment and at a

A



B

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der(22)t(3;22)(q27;q11)

                                                                                                    
#1230  GGGGGCGGCCGGAGCAGAGAGGACGAGACAGTGCTTGGGGGGTGATTCGGGCTAGTCTGGGGGCTGTCTGGCCCCAGACC
GL     ---------------------------------------------------------------------------------
                                                  BCL6
       GCGGAGAGGACGCGCGCTCGCGCTCTCGCTCTTTCTACTGCTGCTTGCGTACGGCTTGTGATCTCTCTGGATTCGTGCGG
       ----------------*GT---------------G-----------------------------------------
                                                                                                    
       CTGTGTTTTTTCCCTCTTTTCTGACTGGCCTGACTTCTGCTGATACCAGCAAGCATATTTATCCCCCAATTTATCTCCAG
       -------------------GG----------G-----------------------------------------------
        Vλ2-1
       AGCAGGTGATGCTGGCTGTCTGTCCTGGGGACACGGACACTGAGGGTGGCTGAGTCAGCTCATAGGAGGCCACGGATCCT
       ------------------------------------------------------------------C--------

       ACAGGAGAGAAAACGGGAGAGAAAAGGC
       ----------------------------


der(3)t(3;22)(q27;q11)
                                                  Jλ1
#1230  AGACAGGCTGGGAAAGGTTGAGAGCCACTTTCCTAGGACGGTGACCTTGGTCCCAGTTCCGAAGACCCTCAGTGCTGCTG
GL     ----------------------------A-----------------------------------   -----------
                                                                 Vλ2-1
       TCCCACGCCTGACAGTAATATTCAGCCTCATCCATAGCCTGGGTCCCGCTGATGGTCAGAGTGGCTGTGTTCCCAGAGTT
       -------------------G-----------------------------------------------------------
                                                                                                    
       GGAGCCAGAGAATCGCTCAGGGATCCCTGAGGGCCGCTTGTTATCTTGATAGATGACCAACACAGCTCGCTTGCAAACTG
       ----------------------------------------C------------------G-------------------
                                                  BCL6
       CTTTCCTTGC*CCGTCGCTCCTGGCCTCCGCTTCCCTCCCTCCTCTTTTTCCTCGCCTCTTCTTCCTTCCCTTCCCCTGT
       ----------T-------------*--------------------------------------------------------

       CCTTCTGGGTAACTCCGGGAGGCAAAAA
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Fig. 1. Molecular anatomy of a t(3;22)(q27;q11) breakpoint (case number 1230). **A.** Schematic diagram of the t(3;22)(q27;q11) translocation. The *Vl* and J_λ segments of IgL_λ, and the relevant regions of *BCL6* are illustrated. Vertical arrowheads indicate the junctional points. **B.** Nucleotide sequences of the *BCL6*/IgL_λ and IgL_λ/*BCL6* junctional areas on the der(22)t(3;22) and der(3)t(3;22) chromosomes, respectively. The junctional points are indicated by vertical arrowheads. The sequences are aligned with the germ-line (GL) sequences of the MTC/MMC region of *BCL6* (GenBank accession number AF191831) (Lossos and Levy, 2000b), and the corresponding V_λ and J_λ segments of IgL_λ. Dashes show nucleotide identities. Deletion of T at position 520 (underlined) is polymorphic (Artiga et al., 2002). The breakpoint hyper-cluster (Akasaka et al., 2000a) is shaded in gray.

point of the breakpoint hyper-cluster of *BCL6* (Akasaka et al., 2000a). Comparison of the *Vλ* gene with its germline sequences registered in the database revealed multiple nucleotide substitutions, indicating that the IgL_{λ} gene had been exposed to the GC reaction. On the other hand, *BCL6* carried 3 mutations as well as 2 insertions within the MMC. These data suggest that *IgL*/*BCL6* translocations develop in close association with the SHM process. Alternatively, multiple mutations of both *IgL* and *BCL6* may facilitate the exchange of chromosomal materials.

In addition to *BCL6*, several non-*Ig* genes are potentially affected by the *Ig*-SHM machinery. The *CD95* (*FAS*) gene, which is specifically expressed in GC B-cells, is the second example shown to acquire SHM in the normal GC reaction (Muschen et al., 2000). About 15% of GC and memory B-cells carry mutations downstream of the promoter of *CD95*. However, translocation involving *CD95* has not been described. On the other hand, mutations of four proto-oncogenes, i.e. *PIM1*, *c-MYC*, *ARHH* (originally designated as *TTF*) and *PAX5*, are not detectable in normal GC B-cells but rather are restricted to DLBCL, suggesting an aberrant SHM activity in DLBCL (Pasqualucci et al., 2001). Since *PIM1* and *ARHH* are included among the non-*Ig* partners of *BCL6* translocation, and since the regions involved in the mutation and translocation overlap, SHM is likely to play a central role in generating non-*Ig*/*BCL6* translocations (Kuppers and Dalla-Favera, 2001; Pasqualucci et al., 2001). Along the same line, we cloned and sequenced 5 *H4*/*BCL6* junctional areas, and found 8 (*BCL6*) and 4 (*H4*) single nucleotide substitutions in each gene within the areas involved in the translocation (Kurata et al., 2002). Since the *H4* mutation was restricted to B-NHL carrying t(3;6)(q27;p21), it remains to be determined whether *H4* is mutated independently of the translocation. Nevertheless, the SHM machinery most likely targets *H4* to generate DNA DSBs, predisposing this region to the development of translocations with *BCL6*.

Deregulated expression of *BCL6* resulting from non-*Ig*/*BCL6* translocation

Non-*Ig*/*BCL6* translocation replaces the normal transcription regulatory sequences of *BCL6* with those of a variety of partner genes (Chen et al., 1998; Akasaka et al., 2000a). The translocation leads to the formation of chimeric transcripts composed of the 5' sequences of the partner followed by the open reading frame of *BCL6*. The reciprocal mRNA containing information of *BCL6* and the partner, in this order, can be detected by RT-PCR (Kaneita et al., 2001). It was recently shown that *BCL6* transcription predominantly occurs from the translocated allele irrespective of the partner gene (Lossos et al., 2003). Thus, expression of *BCL6* as well as that of Bcl-6 protein in B-NHL cells carrying non-*Ig*/*BCL6* translocations is most likely under the control of the replaced promoter activity.

We showed that t(3;6)(q27;p21) results in fusion of *BCL6* with a particular histone *H4* gene on 6p21 (Akasaka et al., 1997; Kurata et al., 2002). The *H4* gene is composed of a single exon followed by a terminal palindrome. Transcriptional control of the histone *H4* gene is mediated by two multipartite proximal promoter elements (Sites I and II), the activity of which is augmented by two distal domains (Sites III and IV) (Stein et al., 1992). The Site II-equivalent of the *H4* gene contains consensus binding sites for HiNF-M, HiNF-D and HiNF-P transcription factors (van Wijnen et al., 1996). These Site II binding proteins, in addition to other co-regulatory molecules, contribute to enhancing the *H4* gene transcription at the G1/S phase transition (Ramsey-Ewing et al., 1994; van Wijnen et al., 1996). On the other hand, the terminal palindrome sequence initially contributes to cleavage of the primary *H4* gene transcript and mediates mRNA destabilization at the end of the S phase (Dominski and Marzluff, 1999). The sequences approximately 16 bp further downstream of the palindrome are also essential and through base-pairing interactions mediate the U7 snRNP-dependent processing of the mRNA 3' end. We cloned 5 *H4*/*BCL6* fusion genes and found that the breakpoints on *H4* were distributed within the 3' half of the *H4* protein coding region or in the vicinity of the palindrome (Akasaka et al., 1997; Kurata et al., 2002). Therefore, the mechanism of 3' end formation of H4 is perturbed and the resulting fusion mRNAs with *BCL6* are predicted to be processed like normal polyadenylated mRNAs. Indeed, sequencing analysis of *H4*/*BCL6* fusion mRNA from lymphoma cells carrying t(3;6) revealed that the *H4* sequences were contiguous with the intron sequences of *BCL6*, which were then followed by the *BCL6* exon 2 sequences (Kurata et al., 2002). It appears then that the deregulation of Bcl-6 protein expression is facilitated by 'capturing' sequences that support cell-cycle control of *H4* gene transcription during the cell cycle, while simultaneously inactivating the regulatory sequences required for post-transcriptional control of *H4* gene expression.

To determine the level of Bcl-6 protein expression directed by the *H4*/*BCL6* fusion gene as compared with germ-line *BCL6*, we constructed expression plasmids that mimicked the structure of t(3;6)(q27;p21) (Kurata et al., 2002). Transient transfection of the plasmid into COS-7 cells resulted in transcription of *H4*/*BCL6* fusion mRNA that had an identical structure to that of mRNA from clinical materials with t(3;6)(q27;p21). Comparison of the levels of Bcl-6 expression revealed that *H4*/*BCL6*-transfected cells produced markedly higher levels of Bcl-6 than cells transfected with a plasmid carrying *BCL6 d*riven by its normal promoter. We next stained the COS-7 cells by indirect immunofluorescence using a polyclonal antibody against Bcl-6 and showed that *H4*/*BCL6*-transfected cells displayed bright nuclear staining with a characteristic granular pattern. Introduction of a series of deletion mutants that lacked the Site II sequences led to reduction of the Bcl-6

expression to the basal level. These findings indicate that *H4*/*BCL6* gene fusion leads to enhanced Bcl-6 expression, which is promoted by the *H4* regulatory elements. Replacement of *H4* with other non-Ig partners, such as the genes for interleukin-21 receptor (*IL21R*) or nascent-polypeptide-associated complex alpha polypeptide (*NACA*), also caused high levels of expression of Bcl-6 in the transfected cell nuclei (Ueda et al., 2002a,b).

Although the composition of granules labeled by anti-Bcl-6 antibodies is not yet fully understood, the granules have been shown to contain SMRT and N-CoR co-repressors (Dhordain et al., 1997; Huynh and Bardwell, 1998). Comparison of gene expression profiles by a cDNA array analysis between *H4*/*BCL6*-transfected cells and the cells transfected with *BCL6* driven by the normal promoter revealed that 224 (19%) out of 1,175 genes were > 1.5-fold under-expressed in the former cells as compared with the latter reference cells (Kurata et al., 2002). This observation is in agreement with the fact that Bcl-6 acts as a transcriptional repressor. It is therefore suggested that the large granular structures observed in the nuclei of *H4*/*BCL6*-transfected cells represent multi-subunit repressor complexes.

Clinical implications of *BCL6* translocation and mRNA/protein expression of *BCL6* in B-NHL

The correlation between *BCL6* translocation and clinical features of B-NHL has been the subject of controversy. An earlier study demonstrated that *BCL6* rearrangement determined by Southern blot analysis occurred more frequently in extranodal DLBCL than in node-based disease and was correlated with a favorable clinical outcome (Offit et al., 1994). A study of Hong Kong Chinese patients revealed that DLBCL of the stomach showed *BCL6* rearrangement more frequently than nodal DLBCL, and that patients with a rearranged *BCL6* appeared to have better survival (Liang et al., 1997). However, other studies have failed to find a statistically significant impact of *BCL6* translocation on clinical outcome (Bastard et al., 1994; Pescarmona et al., 1997; Kramer et al., 1998; Skinnider et al., 1999). In a larger series of 156 patients with *de novo* DLBCL, in which association of *BCL6* rearrangement with extranodal disease was confirmed, disease-free survival (DFS) and overall survival (OS) were not influenced by *BCL6* rearrangement (Kramer et al., 1998). We surveyed a total of 203 cases of DLBCL by Southern blotting analysis using the MTC probe of *BCL6* and found that 43 (21.2%) had rearrangement of the gene (Akasaka et al., 2000b,c). A comparison between the *BCL6* rearrangement-positive and -negative groups revealed no statistically significant difference between the two groups with respect to widely used prognostic variables. However, OS of patients with *BCL6* rearrangement was significantly worse than that of rearrangement-negative patients (P = 0.0413) (Akasaka et al., 2000c). At present, there is no consensus on the effect of *BCL6* translocation on the clinical outcome of B-NHL.

We studied whether the diverse partner loci of *BCL6* translocation influence clinical behaviors and/or treatment outcome of DLBCL (Akasaka et al., 2000c; Ueda et al., 2002b). Of 43 cases of DLBCL, in which LD-/LDI-PCR was used to determine each partner locus, 26 cases had *Ig*s as partners, while 15 cases involved non-*Ig* partners. The remaining 2 had deletion of a >1-kb segment encompassing the non-coding exon 1 of *BCL6*. Although there were no significant differences in pretreatment clinical features between the 26 patients associated with *Ig* partners and the 17 patients with non-*Ig* partners, including 2 cases with a deletion, OS of the non-*Ig* group was inferior to that of the *Ig* group (P = 0.0400). Fourteen patients of the non-*Ig* group died within 2 years, while 5 patients of the *Ig* group are currently enjoying long-term DFS. The estimated 2-year OS of the *Ig* and non-*Ig* groups was 58.1% and 17.6% (P = 0.003), respectively. Although the total number of patients analyzed was quite small, our study suggested that non-*Ig*/*BCL6* fusion is an indicator of poor prognosis in DLBCL, and additional studies of larger patient populations are warranted.

BCL6 translocation sometimes coexists with other B-NHL-specific translocations and/or gene rearrangements (Horsman et al., 1995). We applied LD-/LDI-PCR assays to clinical materials of B-NHL and found that a total of 15 cases carried concurrent rearrangements of either 2 or 3 of the c-*MYC*, *BCL2* and *BCL6* genes (manuscript in preparation). Of 9 cases having *BCL2* rearrangement with 3' breakpoints as well as *BCL6* translocation either with *Ig* or non-*Ig* partners, 7 cases had FL and showed indolent clinical behavior, even though the initial treatments failed to achieve a complete clinical response. One patient carrying rearrangement of the 3 genes showed an intermediate-grade DLBCL histopathology and has enjoyed long-term DFS. Although multiple translocations may occur at the time of transformation from low- to high-grade disease (Macpherson et al., 1999; Willis and Dyer, 2000), our study suggested that dual/triple rearrangements do not necessarily exhibit synergistic effects on the malignant phenotype of B-NHL.

A cDNA microarray analysis revealed that DLBCL patients with the GC B-cell-like pattern of gene expression show significantly better OS than those with the activated B-cell-like expression profile (Alizadeh et al., 2000). *BCL6* is a representative gene of the GC B-cell signature and has been shown to be a predictor of favorable treatment outcome (Rosenwald et al., 2002). This observation was confirmed by a real-time quantitative PCR study, in which high *BCL6* mRNA expression alone could predict better survival in DLBCL (Lossos et al., 2001). In accordance with these studies at the mRNA level, the GC B-cell phenotype (defined by CD10 and *Bcl-6* protein expression as shown by immunohistochemistry) is associated with a significantly longer survival of primary nodal DLBCL (Barrans et al.,

2002), although another series failed to confirm the survival advantage of GC-CD10$^+$ DLBCL (Colomo et al., 2003). Mutations within the 423 to 443 hot spot, which led to a high Bcl-6 protein level, are also associated with a better clinical outcome (Artiga et al., 2002). Thus, most independent studies agree in showing that a high level of *BCL6* expression at either or both the mRNA and protein levels is a favorable prognostic marker of DLBCL.

On the other hand, many studies of DLBCL have demonstrated that the presence of *BCL6* translocation does not necessarily lead to an increased level of Bcl-6 protein expression (Cattoretti et al., 1995; Flenghi et al., 1995; Onizuka et al., 1995; Flenghi et al., 1996; Skinnider et al., 1999). To address the issue of the effects of the *BCL6* translocation on *BCL6* mRNA expression levels, we measured *BCL6* mRNA levels of DLBCL specimens that carried or did not carry *BCL6* translocation by using real-time quantitative PCR (Ueda et al., 2002c). The amount of *BCL6* mRNA of the test materials was divided by that of the endogenous reference, the glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*), and the *BCL6-GAPDH* value was further normalized by that of Raji cells. A comparative study showed that the *BCL6* mRNA levels of DLBCL lacking *BCL6* translocation were higher than those of DLBCL with *BCL6* translocation, and that the difference was significant ($P = 0.003$). Thus, high *BCL6* mRNA levels need not be the consequence of *BCL6* translocation, providing an explanation for why the presence of *BCL6* translocation is not associated with improved clinical outcome of DLBCL.

To link our finding that DLBCLs with non-*Ig*/*BCL6* translocation show worse prognosis than DLBCLs with *Ig*/*BCL6* translocation to the levels of *BCL6* mRNA expression, we compared the levels of *BCL6* mRNA between the two groups (Ueda et al., 2002c). The results showed that the *BCL6* mRNA levels of the *Ig*/*BCL6* group were significantly higher than those of the non-*Ig*/*BCL6* group ($P = 0.0003$). This observation suggests that high versus low *BCL6* mRNA expression and *Ig* versus non-*Ig* partner of *BCL6* translocation are concordant prognostic indicators of DLBCL. Additional studies are needed to determine whether DLBCL with *Ig*/*BCL6* translocation and moderate levels of *BCL6* mRNA belong to the GC B-cell-like DLBCL subset defined by the microarray analysis.

A model for the pathogenetic role of *BCL6* translocations

It has been proposed that chromosomal translocations in B-NHL disrupt normal B-cell homeostasis in three ways, i.e. by enhancing cell growth and proliferation, by preventing cell death and by blocking terminal differentiation (Shaffer et al., 2002b). Many studies have addressed the relationship of Bcl-6 to apoptosis (Albagli et al., 1999; Yamochi et al., 1999; Zhang et al., 2001; Albagli-Curiel, 2003). Whether expression of Bcl-6 induces or inhibits apoptosis appears to be dependent upon the experimental setting. Enforced expression of Bcl-6 in established cell lines including NIH3T3, U2OS osteosarcoma cells, HeLa and CV1, induces apoptosis, accompanied by down-regulation of apoptosis regulators Bcl-2 and Bcl-xl. These cells are arrested either at the S or G2/M phase, indicating that Bcl-6 impairs cell-cycle progression. In accordance with these in vitro experiments, immunohistochemical analysis of DLBCL revealed a significant correlation between increased apoptotic index and the Bcl-6$^+$/CD10$^+$ phenotype (Bai et al., 2003). It has been proposed that high-level expression of Bcl-6 may kill the cell, in which this protein is not naturally expressed (Dent et al., 2002). This proposal may be supported by the fact that no transgenic mouse strain carrying conventional promoter-driven *BCL6* has yet been established.

In contrast, the protective role of Bcl-6 against apoptosis has been demonstrated in established cells as well as in primary lymphoid cells. Loss-of-function of Bcl-6 by expression of a dominant-negative form of Bcl-6 in Raji cells leads to increased cell death and cell-cycle arrest at the G1 phase (Shaffer et al., 2000). When human primary tonsillar B-cells are infected by retrovirus to induce expression of a high level of Bcl-6, the cells show a markedly extended lifespan in culture as compared with the mock-infected controls through a mechanism mediated by up-regulation of *CCND1* (Shvarts et al., 2002). The programmed cell death-2 (*PCDC2*) gene was isolated as a target of Bcl-6 repression, and its expression pattern in human tonsils was mutually exclusive with that of Bcl-6 (Baron et al., 2002). These data suggest that the physiological role of Bcl-6 in GC B-cells may be the inhibition of apoptosis, permitting these cells to generate and amplify an immune response (Baron et al., 2002).

A cDNA microarray analysis along with loss-of-function experiments revealed a set of genes that are negatively regulated by Bcl-6. These target genes of Bcl-6 repression are involved in B-cell activation, B-cell differentiation, inflammation and cell-cycle control (Shaffer et al., 2000). The *BLIMP1* (B-lymphocyte-induced maturation protein 1) gene, which is a representative target, plays a key role in the differentiation of B-cells to plasma cells by turning off the entire mature B-cell gene expression program (Shaffer et al., 2002a). On the other hand, Blimp-1 represses c-*MYC* expression, leading to cell-cycle arrest in G1 (Lin et al., 1997; Shaffer et al., 2002a). It is therefore proposed that these molecules form a circuit that controls the differentiation of GC B-cells to terminally differentiated Ig-secreting plasma cells (Shaffer et al., 2002b). Altogether, it is conceivable that inappropriate expression of *BCL6* due to chromosomal translocation may perturb this molecular network, resulting in a block of terminal differentiation, prevention of apoptosis and continued proliferation, thereby predisposing the B-cells to the neoplastic

transformation (Lossos et al., 2003).

We showed that the introduction of expression plasmids that mimicked the non-*Ig*/*BCL6* fusion genes into transformed cells led to high-level Bcl-6 protein expression in transfected cell nuclei (Kurata et al., 2002; Ueda et al., 2002a,b). The gene expression profiles of the infected cells were significantly altered in accordance with the fact that Bcl-6 acts as a transcriptional repressor (Kurata et al., 2002). When the plasmids lacked the promoter sequences of non-*Ig* partners, the expression levels of Bcl-6 were dramatically reduced, providing direct evidence that the expression of *BCL6* is regulated by the juxtaposed non-*Ig* promoter. Furthermore, non-*Ig*/*BCL6* fusion mRNA may be subjected to a distinct degradation process compared with that of non-*Ig* or *BCL6*.

In contrast, the *BCL6* mRNA levels of B-NHL cells carrying a non-*Ig*/*BCL6* fusion gene were unexpectedly low (Kurata et al., 2002; Ueda et al., 2002a,c). A DLBCL cell line, YM, with an *IL21R*/*BCL6* fusion gene resulting from t(3;16)(q27;p11) expressed only moderate levels of *BCL6* mRNA despite active transcription of the *IL21R* gene (Ueda et al., 2002a; Ueda et al., 2002c). Since *BCL6* mRNA is transcribed from the translocated allele (Lossos et al., 2003), there may be a mechanism that represses the transcriptional activity of the non-*Ig*/*BCL6* fusion gene in B-NHL cells. These observations suggest that persistent high-level *BCL6* mRNA and protein expression may not be required to maintain the malignant phenotype of B-NHL with non-*Ig*/*BCL6*. It is presumed that the *BCL6* gene of a B-cell carrying a non-*Ig*/*BCL6* translocation is over-expressed in the GC-microenvironment, thereby triggering neoplastic transformation. However, such transcriptional activation would be transient and, once the neoplastic B-cell gains a growth advantage over normal cells, the expression would be down-regulated (Fig. 2). In contrast, B-NHL with *Ig*/*BCL6* translocation may show a persistent expression of Bcl-6 at higher levels, corresponding to a feature of GC B-cell-like DLBCL. Further studies are needed to elucidate the mechanistic details of the transcriptional control of *BCL6* on the translocated allele in B-NHL cells.

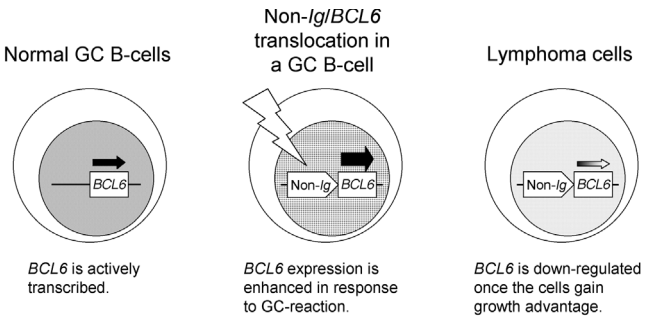

Fig. 2. A model of deregulated expression of Bcl-6 leading to the development of B-NHL. Bcl-6 is normally expressed at high levels in GC B-cells. When a GC B-cell acquires a non-Ig/*BCL6* translocation, transcription of *BCL6* mRNA from the translocated allele is further enhanced under the GC micro-environment. Once the neoplastic B-cells gain growth advantage over normal cells, the overexpression of Bcl-6 may be down-regulated by an unknown mechanism.

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References

Akasaka H., Akasaka T., Kurata M., Ueda C., Shimizu A., Uchiyama T. and Ohno H. (2000a). Molecular anatomy of *BCL6* translocations revealed by long-distance polymerase chain reaction-based assays. Cancer Res. 60, 2335-2341.

Akasaka T., Akasaka H., Ueda C., Yonetani N., Maesako Y., Shimizu A., Yamabe H., Fukuhara S., Uchiyama T. and Ohno H. (2000b). Molecular and clinical features of non-Burkitt's, diffuse large-cell lymphoma of B-cell type associated with the c-*MYC*/immunoglobulin heavy-chain fusion gene. J. Clin. Oncol. 18, 510-518.

Akasaka T., Lossos I.S. and Levy R. (2003). *BCL6* gene translocation in follicular lymphoma: a harbinger of eventual transformation to diffuse aggressive lymphoma. Blood 102, 1443-1448.

Akasaka T., Miura I., Takahashi N., Akasaka H., Yonetani N., Ohno H., Fukuhara S. and Okuma M. (1997). A recurring translocation, t(3;6)(q27;p21), in non-Hodgkin's lymphoma results in replacement of the 5' regulatory region of *BCL6* with a novel *H4 histone* gene. Cancer Res. 57, 7-12.

Akasaka T., Ueda C., Kurata M., Akasaka H., Yamabe H., Uchiyama T. and Ohno H. (2000c). Nonimmunoglobulin (non-*Ig*)/*BCL6* gene fusion in diffuse large B-cell lymphoma results in worse prognosis than *Ig/BCL6*. Blood 96, 2907-2909.

Albagli O., Lantoine D., Quief S., Quignon F., Englert C., Kerckaert J. P., Montarras D., Pinset C. and Lindon C. (1999). Overexpressed BCL6 (LAZ3) oncoprotein triggers apoptosis, delays S phase progression and associates with replication foci. Oncogene 18, 5063-5075.

Albagli-Curiel O. (2003). Ambivalent role of BCL6 in cell survival and transformation. Oncogene 22, 507-516.

Alizadeh A.A., Eisen M.B., Davis R.E., Ma C., Lossos I.S., Rosenwald A., Boldrick J.C., Sabet H., Tran T., Yu X., Powell J.I., Yang L., Marti G.E., Moore T., Hudson J. Jr., Lu L., Lewis D.B., Tibshirani R., Sherlock G., Chan W.C., Greiner T.C., Weisenburger D.D., Armitage J.O., Warnke R., Levy R., Wilson W., Grever M.R., Byrd J.C., Botstein D., Brown P.O. and Staudt L.M. (2000). Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403, 503-511.

Artiga M.J., Saez A.I., Romero C., Sanchez-Beato M., Mateo M.S., Navas C., Mollejo M. and Piris M. A. (2002). A short mutational hot spot in the first intron of BCL-6 is associated with increased BCL-6 expression and with longer overall survival in large B-cell lymphomas. Am. J. Pathol. 160, 1371-1380.

Bai M., Agnantis N.J., Skyrlas A., Tsanou E., Kamina S., Galani V. and Kanavaros P. (2003). Increased expression of the BCL6 and CD10 proteins is associated with increased apoptosis and proliferation in

diffuse large B-cell lymphomas. Mod. Pathol. 16, 471-480.

Baron B.W., Anastasi J., Thirman M.J., Furukawa Y., Fears S., Kim D.C., Simone F., Birkenbach M., Montag A., Sadhu A., Zeleznik-Le N. and McKeithan T.W. (2002). The human programmed cell death-2 (*PDCD2*) gene is a target of BCL6 repression: implications for a role of BCL6 in the down-regulation of apoptosis. Proc. Natl. Acad. Sci. USA 99, 2860-2865.

Baron B.W., Nucifora G., McCabe N., Espinosa R.… 3rd, Le Beau M.M. and McKeithan T.W. (1993). Identification of the gene associated with the recurring chromosomal translocations t(3;14)(q27;q32) and t(3;22)(q27;q11) in B-cell lymphomas. Proc. Natl. Acad. Sci. USA 90, 5262-5266.

Barrans S.L., Carter I., Owen R.G., Davies F.E., Patmore R.D., Haynes A.P., Morgan G.J. and Jack A.S. (2002). Germinal center phenotype and bcl-2 expression combined with the International Prognostic Index improves patient risk stratification in diffuse large B-cell lymphoma. Blood 99, 1136-1143.

Bastard C., Tilly H., Lenormand B., Bigorgne C., Boulet D., Kunlin A., Monconduit M. and Piguet H. (1992). Translocations involving band 3q27 and Ig gene regions in non-Hodgkin's lymphoma. Blood 79, 2527-2531.

Bastard C., Deweindt C., Kerckaert J. P., Lenormand B., Rossi A., Pezzella F., Fruchart C., Duval C., Monconduit M. and Tilly H. (1994). *LAZ3* rearrangements in non-Hodgkin's lymphoma: correlation with histology, immunophenotype, karyotype, and clinical outcome in 217 patients. Blood 83, 2423-2427.

Bernardin F., Collyn-d'Hooghe M., Quief S., Bastard C., Leprince D. and Kerckaert J.P. (1997). Small deletions occur in highly conserved regions of the *LAZ3/BCL6* major translocation cluster in one case of non-Hodgkin's lymphoma without 3q27 translocation. Oncogene 14, 849-855.

Bross L., Fukita Y., McBlane F., Demolliere C., Rajewsky K. and Jacobs H. (2000). DNA double-strand breaks in immunoglobulin genes undergoing somatic hypermutation. Immunity 13, 589-597.

Capello D., Vitolo U., Pasqualucci L., Quattrone S., Migliaretti G., Fassone L., Ariatti C., Vivenza D., Gloghini A., Pastore C., Lanza C., Nomdedeu J., Botto B., Freilone R., Buonaiuto D., Zagonel V., Gallo E., Palestro G., Saglio G., Dalla-Favera R., Carbone A. and Gaidano G. (2000). Distribution and pattern of *BCL*-6 mutations throughout the spectrum of B-cell neoplasia. Blood 95, 651-659.

Carbone A. (2003). Emerging pathways in the development of AIDS-related lymphomas. Lancet Oncol. 4, 22-29.

Carbone A., Gloghini A., Gaidano G., Dalla-Favera R. and Falini B. (1997). *BCL*-6 protein expression in human peripheral T-cell neoplasms is restricted to CD30+ anaplastic large-cell lymphomas. Blood 90, 2445-2450.

Cattoretti G., Chang C. C., Cechova K., Zhang J., Ye B. H., Falini B., Louie D. C., Offit K., Chaganti R. S. and Dalla-Favera R. (1995). BCL-6 protein is expressed in germinal-center B cells. Blood 86, 45-53.

Chen W., Iida S., Louie D. C., Dalla-Favera R. and Chaganti R.S. (1998). Heterologous promoters fused to *BCL6* by chromosomal translocations affecting band 3q27 cause its deregulated expression during B-cell differentiation. Blood 91, 603-607.

Chen W., Itoyama T. and Chaganti R. S. (2001). Splicing factor *SRP20* is a novel partner of *BCL6* in a t(3;6)(q27;p21) translocation in transformed follicular lymphoma. Genes Chromosomes Cancer 32, 281-284.

Colomo L., Lopez-Guillermo A., Perales M., Rives S., Martinez A., Bosch F., Colomer D., Falini B., Montserrat E. and Campo E. (2003). Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. Blood 101, 78-84.

Dallery E., Galiegue-Zouitina S., Collyn-d'Hooghe M., Quief S., Denis C., Hildebrand M. P., Lantoine D., Deweindt C., Tilly H. Bastard C. and Kerckaert J.-P. (1995). *TTF*, a gene encoding a novel small G protein, fuses to the lymphoma-associated *LAZ3* gene by t(3;4) chromosomal translocation. Oncogene 10, 2171-2178.

Dent A.L., Shaffer A.L., Yu X., Allman D. and Staudt L.M. (1997). Control of inflammation, cytokine expression, and germinal center formation by BCL-6. Science 276, 589-592.

Dent A.L., Vasanwala F.H. and Toney L.M. (2002). Regulation of gene expression by the proto-oncogene BCL-6. Crit. Rev. Oncol. Hematol. 41, 1-9.

Dhordain P., Albagli O., Ansieau S., Koken M.H., Deweindt C., Quief S., Lantoine D., Leutz A., Kerckaert J.P. and Leprince D. (1995). The BTB/POZ domain targets the LAZ3/BCL6 oncoprotein to nuclear dots and mediates homomerisation *in vivo*. Oncogene 11, 2689-2697.

Dhordain P., Albagli O., Honore N., Guidez F., Lantoine D., Schmid M., The H. D., Zelent A. and Koken M.H. (2000). Colocalization and heteromerization between the two human oncogene POZ/zinc finger proteins, LAZ3 (BCL6) and PLZF. Oncogene 19, 6240-6250.

Dhordain P., Albagli O., Lin R.J., Ansieau S., Quief S., Leutz A., Kerckaert J.P., Evans R.M. and Leprince D. (1997). Corepressor SMRT binds the BTB/POZ repressing domain of the LAZ3/BCL6 oncoprotein. Proc. Natl. Acad. Sci. USA 94, 10762-10767.

Dhordain P., Lin R. J., Quief S., Lantoine D., Kerckaert J.P., Evans R.M. and Albagli O. (1998). The LAZ3(BCL-6) oncoprotein recruits a SMRT/mSIN3A/histone deacetylase containing complex to mediate transcriptional repression. Nucleic Acids Res. 26, 4645-4651.

Dominski Z. and Marzluff W.F. (1999). Formation of the 3' end of histone mRNA. Gene 239, 1-14.

Falini B. and Mason D.Y. (2002). Proteins encoded by genes involved in chromosomal alterations in lymphoma and leukemia: clinical value of their detection by immunocytochemistry. Blood 99, 409-426.

Falini B., Bigerna B., Pasqualucci L., Fizzotti M., Martelli M.F., Pileri S., Pinto A., Carbone A., Venturi S., Pacini R., Cattoretti G., Pescarmona E., Lo Coco F., Pelicci P.G., Anagnastopoulos I., Dalla-Favera R. and Flenghi L. (1996). Distinctive expression pattern of the BCL-6 protein in nodular lymphocyte predominance Hodgkin's disease. Blood 87, 465-471.

Flenghi L., Bigerna B., Fizzotti M., Venturi S., Pasqualucci L., Pileri S., Ye B.H., Gambacorta M., Pacini R., Baroni C.D., Pescarmona E., Anagnostopoulos I., Stein H., Asdrubali G., Martelli M. F., Pelicci P. G., Dalla-Favera R. and Falini B. (1996). Monoclonal antibodies PG-B6a and PG-B6p recognize, respectively, a highly conserved and a formol-resistant epitope on the human BCL-6 protein amino-terminal region. Am. J. Pathol. 148, 1543-1555.

Flenghi L., Ye B.H., Fizzotti M., Bigerna B., Cattoretti G., Venturi S., Pacini R., Pileri S., Lo Coco F., Pescarmona E., Pelicci P.-G., Dalla-Favera R. and Falini B. (1995). A specific monoclonal antibody (PG-B6) detects expression of the BCL-6 protein in germinal center B cells. Am. J. Pathol. 147, 405-411.

Fukuda T., Yoshida T., Okada S., Hatano M., Miki T., Ishibashi K., Okabe S., Koseki H., Hirosawa S., Taniguchi M., Miyasaka N. and Tokuhisa T. (1997). Disruption of the *Bcl6* gene results in an impaired germinal center formation. J. Exp. Med. 186, 439-448.

Gaidano G., Carbone A., Pastore C., Capello D., Migliazza A., Gloghini A., Roncella S., Ferrarini M., Saglio G. and Dalla-Favera R. (1997). Frequent mutation of the 5' noncoding region of the *BCL*-6 gene in acquired immunodeficiency syndrome-related non-Hodgkin's lymphomas. Blood 89, 3755-3762.

Galiegue-Zouitina S., Quief S., Hildebrand M.P., Denis C., Detourmignies L., Lai J.L. and Kerckaert J.P. (1999). Nonrandom fusion of *L-plastin*(*LCP1*) and *LAZ3*(*BCL6*) genes by t(3;13)(q27;q14) chromosome translocation in two cases of B-cell non-Hodgkin lymphoma. Genes Chromosomes Cancer 26, 97-105.

Galiegue-Zouitina S., Quief S., Hildebrand M.P., Denis C., Lecocq G., Collyn-d'Hooghe M., Bastard C., Yuille M., Dyer M. J. and Kerckaert J.P. (1996). The B cell transcriptional coactivator *BOB1*/*OBF1* gene fuses to the *LAZ3*/*BCL6* gene by t(3;11)(q27;q23.1) chromosomal translocation in a B cell leukemia line (Karpas 231). Leukemia 10, 579-587.

Honjo T., Kinoshita K. and Muramatsu M. (2002). Molecular mechanism of class switch recombination: linkage with somatic hypermutation. Annu. Rev. Immunol. 20, 165-196.

Horsman D.E., McNeil B.K., Anderson M., Shenkier T. and Gascoyne R.D. (1995). Frequent association of t(3;14) or variant with other lymphoma-specific translocations. Br. J. Haematol. 89, 569-575.

Hosokawa Y., Maeda Y., Ichinohasama R., Miura I., Taniwaki M. and Seto M. (2000). The *Ikaros* gene, a central regulator of lymphoid differentiation, fuses to the *BCL6* gene as a result of t(3;7)(q27;p12) translocation in a patient with diffuse large B-cell lymphoma. Blood 95, 2719-2721.

Huynh K.D. and Bardwell V.J. (1998). The BCL-6 POZ domain and other POZ domains interact with the co-repressors N-CoR and SMRT. Oncogene 17, 2473-2484.

Huynh K.D., Fischle W., Verdin E. and Bardwell V.J. (2000). BCoR, a novel corepressor involved in BCL-6 repression. Genes Dev. 14, 1810-1823.

Kaneita Y., Yoshida S., Ishiguro N., Sawada U., Horie T., Mori S. and Moriyama M. (2001). Detection of reciprocal fusion 5'-*BCL6*/partner-3' transcripts in lymphomas exhibiting reciprocal *BCL6* translocations. Br. J. Haematol. 113, 803-806.

Kerckaert J. P., Deweindt C., Tilly H., Quief S., Lecocq G. and Bastard C. (1993). *LAZ3*, a novel zinc-finger encoding gene, is disrupted by recurring chromosome 3q27 translocations in human lymphomas. Nat. Genet. 5, 66-70.

Kerl K., Vonlanthen R., Nagy M., Bolzonello N.J., Gindre P., Hurwitz N., Gudat F., Nador R.G. and Borisch B. (2001). Alterations on the 5' noncoding region of the BCL-6 gene are not correlated with BCL-6 protein expression in T cell non-Hodgkin lymphomas. Lab. Invest. 81, 1693-1702.

Kikuchi M., Miki T., Kumagai T., Fukuda T., Kamiyama R., Miyasaka N. and Hirosawa S. (2000). Identification of negative regulatory regions within the first exon and intron of the BCL6 gene. Oncogene 19, 4941-4945.

King B.E., Chen C., Locker J., Kant J., Okuyama K., Falini B. and Swerdlow S.H. (2000). Immunophenotypic and genotypic markers of follicular center cell neoplasia in diffuse large B-cell lymphomas. Mod. Pathol. 13, 1219-1231.

Konishi H., Sakurai M., Nakao H., Maseki N., Kaneko Y., Yagiri Y., Notohara K. and Frizzera G. (1990). Chromosome abnormalities in malignant lymphoma in patients from Kurashiki: histological and immunophenotypic correlations. Cancer Res. 50, 2698-2703.

Kramer M. H., Hermans J., Wijburg E., Philippo K., Geelen E., van Krieken J. H., de Jong D., Maartense E., Schuuring E. and Kluin P. M. (1998). Clinical relevance of BCL2, BCL6, and MYC rearrangements in diffuse large B-cell lymphoma. Blood 92, 3152-3162.

Kuppers R. and Dalla-Favera R. (2001). Mechanisms of chromosomal translocations in B cell lymphomas. Oncogene 20, 5580-5594.

Kurata M., Maesako Y., Ueda C., Nishikori M., Akasaka T., Uchiyama T. and Ohno H. (2002). Characterization of t(3;6)(q27;p21) breakpoints in B-cell non-Hodgkin's lymphoma and construction of the histone H4/*BCL6* fusion gene, leading to altered expression of Bcl-6. Cancer Res. 62, 6224-6230.

Lemercier C., Brocard M.P., Puvion-Dutilleul F., Kao H.Y., Albagli O. and Khochbin S. (2002). Class II histone deacetylases are directly recruited by BCL6 transcriptional repressor. J. Biol. Chem. 277, 22045-22052.

Liang R., Chan W.P., Kwong Y.L., Xu W.S., Srivastava G. and Ho F.C. (1997). High incidence of BCL-6 gene rearrangement in diffuse large B-cell lymphoma of primary gastric origin. Cancer Genet. Cytogenet. 97, 114-118.

Lin Y., Wong K. and Calame K. (1997). Repression of *c-myc* transcription by Blimp-1, an inducer of terminal B cell differentiation. Science 276, 596-599.

Lossos I.S. and Levy R. (2000a). Higher-grade transformation of follicle center lymphoma is associated with somatic mutation of the 5' noncoding regulatory region of the *BCL-6* gene. Blood 96, 635-639.

Lossos I.S. and Levy R. (2000b). Mutation analysis of the 5' noncoding regulatory region of the BCL-6 gene in non-Hodgkin lymphoma: evidence for recurrent mutations and intraclonal heterogeneity. Blood 95, 1400-1405.

Lossos I.S., Akasaka T., Martinez-Climent J.A., Siebert R. and Levy R. (2003). The *BCL6* gene in B-cell lymphomas with 3q27 translocations is expressed mainly from the rearranged allele irrespective of the partner gene. Leukemia 17, 1390-1397.

Lossos I.S., Jones C. D., Warnke R., Natkunam Y., Kaizer H., Zehnder J.L., Tibshirani R. and Levy R. (2001). Expression of a single gene, *BCL-6*, strongly predicts survival in patients with diffuse large B-cell lymphoma. Blood 98, 945-951.

Macpherson N., Lesack D., Klasa R., Horsman D., Connors J.M., Barnett M. and Gascoyne R.D. (1999). Small noncleaved, non-Burkitt's (Burkit-Like) lymphoma: cytogenetics predict outcome and reflect clinical presentation. J. Clin. Oncol. 17, 1558-1567.

Migliazza A., Martinotti S., Chen W., Fusco C., Ye B. H., Knowles D. M., Offit K., Chaganti R.S. and Dalla-Favera R. (1995). Frequent somatic hypermutation of the 5' noncoding region of the *BCL6* gene in B-cell lymphoma. Proc. Natl. Acad. Sci. USA 92, 12520-12524.

Miki T., Kawamata N., Arai A., Ohashi K., Nakamura Y., Kato A., Hirosawa S. and Aoki N. (1994a). Molecular cloning of the breakpoint for 3q27 translocation in B-cell lymphomas and leukemias. Blood 83, 217-222.

Miki T., Kawamata N., Hirosawa S. and Aoki N. (1994b). Gene involved in the 3q27 translocation associated with B-cell lymphoma, *BCL5*, encodes a Kruppel-like zinc-finger protein. Blood 83, 26-32.

Moriyama M., Yamochi T., Semba K., Akiyama T. and Mori S. (1997). BCL-6 is phosphorylated at multiple sites in its serine- and proline-clustered region by mitogen-activated protein kinase (MAPK) *in vivo*. Oncogene 14, 2465-2474.

Muramatsu M., Akasaka T., Kadowaki N., Ohno H., Yamabe H., Edamura S., Dor S., Mori T., Okuma M. and Fukuhara S. (1996). Rearrangement of the *BCL6* gene in B-cell lymphoid neoplasms:

comparison with lymphomas associated with BCL2 rearrangement. Br. J. Haematol. 93, 911-920.

Muschen M., Re D., Jungnickel B., Diehl V., Rajewsky K. and Kuppers R. (2000). Somatic mutation of the CD95 gene in human B cells as a side-effect of the germinal center reaction. J. Exp. Med. 192, 1833-1840.

Niu H., Ye B. H. and Dalla-Favera R. (1998). Antigen receptor signaling induces MAP kinase-mediated phosphorylation and degradation of the BCL-6 transcription factor. Genes Dev. 12, 1953-1961.

Offit K., Jhanwar S., Ebrahim S.A., Filippa D., Clarkson B.D. and Chaganti R.S. (1989). t(3;22)(q27;q11): a novel translocation associated with diffuse non-Hodgkin's lymphoma. Blood 74, 1876-1879.

Offit K., Lo Coco F., Louie D.C., Parsa N.Z., Leung D., Portlock C., Ye B.H., Lista F., Filippa D.A., Rosenbaum A., Ladanyi M., Jhanwar S., Dalla-Favara R. and Chaganti R. S.K. (1994). Rearrangement of the *bcl*-6 gene as a prognostic marker in diffuse large-cell lymphoma. N. Engl. J. Med. 331, 74-80.

Ohno H. and Fukuhara S. (1997). Significance of rearrangement of the *BCL6* gene in B-cell lymphoid neoplasms. Leuk. Lymphoma 27, 53-63.

Okabe S., Fukuda T., Ishibashi K., Kojima S., Okada S., Hatano M., Ebara M., Saisho H. and Tokuhisa T. (1998). BAZF, a novel Bcl6 homolog, functions as a transcriptional repressor. Mol. Cell. Biol. 18, 4235-4244.

Onizuka T., Moriyama M., Yamochi T., Kuroda T., Kazama A., Kanazawa N., Sato K., Kato T., Ota H. and Mori S. (1995). *BCL-6* gene product, a 92- to 98-kD nuclear phosphoprotein, is highly expressed in germinal center B cells and their neoplastic counterparts. Blood 86, 28-37.

Papavasiliou F.N. and Schatz D.G. (2000). Cell-cycle-regulated DNA double-stranded breaks in somatic hypermutation of immunoglobulin genes. Nature 408, 216-221.

Pasqualucci L., Migliazza A., Basso K., Houldsworth J., Chaganti R. S. and Dalla-Favera R. (2003). Mutations of the *BCL6* proto-oncogene disrupt its negative autoregulation in diffuse large B-cell lymphoma. Blood 101, 2914-2923.

Pasqualucci L., Migliazza A., Fracchiolla N., William C., Neri A., Baldini L., Chaganti R.S., Klein U., Kuppers R., Rajewsky K. and Dalla-Favera R. (1998). BCL-6 mutations in normal germinal center B cells: evidence of somatic hypermutation acting outside Ig loci. Proc. Natl. Acad. Sci. USA. 95, 11816-11821.

Pasqualucci L., Neri A., Baldini L., Dalla-Favera R. and Migliazza A. (2000). BCL-6 mutations are associated with immunoglobulin variable heavy chain mutations in B-cell chronic lymphocytic leukemia. Cancer Res. 60, 5644-5648.

Pasqualucci L., Neumeister P., Goossens T., Nanjangud G., Chaganti R. S., Kuppers R. and Dalla-Favera R. (2001). Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature 412, 341-346.

Peng H.Z., Du M.Q., Koulis A., Aiello A., Dogan A., Pan L.X. and Isaacson P.G. (1999). Nonimmunoglobulin gene hypermutation in germinal center B cells. Blood 93, 2167-2172.

Pescarmona E., De Sanctis V., Pistilli A., Pacchiarotti A., Martelli M., Guglielmi C., Mandelli F., Baroni C.D. and Le Coco F. (1997). Pathogenetic and clinical implications of Bcl-6 and Bcl-2 gene configuration in nodal diffuse large B-cell lymphomas. J. Pathol. 183, 281-286.

Pileri S. A., Gaidano G., Zinzani P. L., Falini B., Gaulard P., Zucca E., Pieri F., Berra E., Sabattini E., Ascani S., Piccioli M., Johnson P. W., Giardini R., Pescarmona E., Novero D., Piccaluga P. P., Marafioti T., Alonso M. A. and Cavalli F. (2003). Primary mediastinal B-cell lymphoma: high frequency of BCL-6 mutations and consistent expression of the transcription factors OCT-2, BOB.1, and PU.1 in the absence of immunoglobulins. Am. J. Pathol. 162, 243-253.

Ramsey-Ewing A., Van Wijnen A.J., Stein G.S. and Stein J.L. (1994). Delineation of a human histone H4 cell cycle element *in vivo*: the master switch for H4 gene transcription. Proc. Natl. Acad. Sci. USA 91, 4475-4479.

Roix J.J., McQueen P.G., Munson P.J., Parada L.A. and Misteli T. (2003). Spatial proximity of translocation-prone gene loci in human lymphomas. Nat. Genet. 34, 287-291.

Rosenwald A., Wright G., Chan W.C., Connors J.M., Campo E., Fisher R.I., Gascoyne R.D., Muller-Hermelink H.K., Smeland E.B., Giltnane J.M., Hurt E.M., Zhao H., Averett L., Yang L., Wilson W.H., Jaffe E.S., Simon R., Klausner R.D., Powell J., Duffey P.L., Longo D.L., Greiner T.C., Weisenburger D.D., Sanger W.G., Dave B.J., Lynch J.C., Vose J., Armitage J.O., Montserrat E., Lopez-Guillermo A., Grogan T.M., Miller T.P., LeBlanc M., Ott G., Kvaloy S., Delabie J., Holte H., Krajci P., Stokke T. and Staudt L.M. (2002). The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N. Engl. J. Med. 346, 1937-1947.

Seitz V., Hummel M., Anagnostopoulos I. and Stein H. (2001). Analysis of *BCL-6* mutations in classic Hodgkin disease of the B- and T-cell type. Blood 97, 2401-2405.

Shaffer A.L., Lin K.I., Kuo T.C., Yu X., Hurt E.M., Rosenwald A., Giltnane J.M., Yang L., Zhao H., Calame K. and Staudt L.M. (2002a). Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. Immunity 17, 51-62.

Shaffer A.L., Rosenwald A. and Staudt L.M. (2002b). Lymphoid malignancies: the dark side of B-cell differentiation. Nat. Rev. Immunol. 2, 920-932.

Shaffer A.L., Yu X., He Y., Boldrick J., Chan E.P. and Staudt L.M. (2000). BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. Immunity 13, 199-212.

Shen H. M., Peters A., Baron B., Zhu X. and Storb U. (1998). Mutation of *BCL-6* gene in normal B cells by the process of somatic hypermutation of Ig genes. Science 280, 1750-1752.

Shvarts A., Brummelkamp T.R., Scheeren F., Koh E., Daley G.Q., Spits H. and Bernards R. (2002). A senescence rescue screen identifies *BCL6* as an inhibitor of anti-proliferative $p19^{ARF}$-p53 signaling. Genes Dev. 16, 681-686.

Skinnider B.F., Horsman D.F., Dupuis B. and Gascoyne R.D. (1999). Bcl-6 and Bcl-2 protein expression in diffuse large B-cell lymphoma and follicular lymphoma: correlation with 3q27 and 18q21 chromosomal abnormalities. Hum. Pathol. 30, 803-808.

Stamatopoulos K., Kosmas C., Belessi C., Stavroyianni N., Kyriazopoulos P. and Papadaki T. (2000). Molecular insights into the immunopathogenesis of follicular lymphoma. Immunol. Today 21, 298-305.

Stein G.S., Stein J.L., van Wijnen A.J. and Lian J.B. (1992). Regulation of histone gene expression. Curr. Opin. Cell Biol. 4, 166-173.

Suzuki K., Miki T., Kawamata N., Hirosawa S., Yoshizawa K., Kiyosawa K. and Aoki N. (1994). Variant translocation of the *BCL6* gene to immunoglobulin κ light chain gene in B-cell lymphoma. Jpn. J.

Cancer Res. 85, 911-917.

Tanaka R., Satoh H., Moriyama M., Satoh K., Morishita Y., Yoshida S., Watanabe T., Nakamura Y. and Mori S. (2000). Intronic U50 small-nucleolar-RNA (snoRNA) host gene of no protein-coding potential is mapped at the chromosome breakpoint t(3;6)(q27;q15) of human B-cell lymphoma. Genes Cells 5, 277-287.

Ueda C., Akasaka T., Kurata M., Maesako Y., Nishikori M., Ichinohasama R., Imada K., Uchiyama T. and Ohno H. (2002a). The gene for interleukin-21 receptor is the partner of *BCL6* in t(3;16)(q27;p11), which is recurrently observed in diffuse large B-cell lymphoma. Oncogene 21, 368-376.

Ueda C., Akasaka T. and Ohno H. (2002b). Non-immunoglobulin/*BCL6* gene fusion in diffuse large B-cell lymphoma: prognostic implications. Leuk. Lymphoma 43, 1375-1381.

Ueda C., Uchiyama T. and Ohno H. (2002c). Immunoglobulin (*Ig*)/*BCL6* versus non-*Ig*/*BCL6* gene fusion in diffuse large B-cell lymphoma corresponds to a high- versus low-level expression of *BCL6* mRNA. Blood 99, 2624-2625.

Ueda Y., Matsuda F., Misawa S. and Taniwaki M. (1996). Tumor-specific rearrangements of the immunoglobulin heavy-chain gene in B-cell non-Hodgkin's lymphoma detected by in situ hybridization. Blood 87, 292-298.

van Wijnen A.J., van Gurp M.F., de Ridder M.C., Tufarelli C., Last T.J., Birnbaum M., Vaughan P.S., Giordano A., Krek W., Neufeld E.J., Stein J.L. and Stein G.S. (1996). CDP/*cut* is the DNA-binding subunit of histone gene transcription factor HiNF-D: a mechanism for gene regulation at the G1/S phase cell cycle transition point independent of transcription factor E2F. Proc. Natl. Acad. Sci. USA. 93, 11516-11521.

Wang X., Li Z., Naganuma A. and Ye B.H. (2002). Negative autoregulation of BCL-6 is bypassed by genetic alterations in diffuse large B cell lymphomas. Proc. Natl. Acad. Sci. USA. 99, 15018-15023.

Willis TG. and Dyer M.J. (2000). The role of immunoglobulin translocations in the pathogenesis of B-cell malignancies. Blood 96, 808-822.

Xu W.S., Liang R.H. and Srivastava G. (2000). Identification and characterization of *BCL6* translocation partner genes in primary gastric high-grade B-cell lymphoma: heat shock protein 89 alpha is a novel fusion partner gene of *BCL6*. Genes Chromosomes Cancer 27, 69-75.

Yamochi T., Kaneita Y., Akiyama T., Mori S. and Moriyama M. (1999). Adenovirus-mediated high expression of BCL-6 in CV-1 cells induces apoptotic cell death accompanied by down-regulation of BCL-2 and BCL-X_L. Oncogene 18, 487-494.

Ye B. H., Cattoretti G., Shen Q., Zhang J., Hawe N., de Waard R., Leung C., Nouri-Shirazi M., Orazi A., Chaganti R. S., Rothman P., Stall A. M., Pandolfi P.P. and Dalla-Favera R. (1997). The *BCL-6* proto-oncogene controls germinal-centre formation and Th2-type inflammation. Nat. Genet. 16, 161-170.

Ye B.H., Chaganti S., Chang C. C., Niu H., Corradini P., Chaganti R. S. and Dalla-Favera R. (1995). Chromosomal translocations cause deregulated *BCL6* expression by promoter substitution in B cell lymphoma. EMBO J. 14, 6209-6217.

Ye B.H., Lista F., Lo Coco F., Knowles D. M., Offit K., Chaganti R.S. and Dalla-Favera R. (1993). Alterations of a zinc finger-encoding gene, *BCL-6*, in diffuse large-cell lymphoma. Science 262, 747-750.

Yonetani N., Akasaka T., Akasaka H., Ohno H., Okuma M., Miura I., Takahashi N., Miyanishi S., Okumura A., Muramatsu M. and Fukuhara S. (1998). Molecular features of a new human lymphoma cell line carrying both *BCL2* and *BCL6* gene rearrangements. Oncogene 17, 971-979.

Yoshida S., Kaneita Y., Aoki Y., Seto M., Mori S. and Moriyama M. (1999). Identification of heterologous translocation partner genes fused to the *BCL6* gene in diffuse large B-cell lymphomas: 5'-RACE and LA - PCR analyses of biopsy samples. Oncogene 18, 7994-7999.

Zan H., Komori A., Li Z., Cerutti A., Schaffer A., Flajnik M.F., Diaz M. and Casali P. (2001). The translesion DNA polymerase ζ plays a major role in Ig and *bcl-6* somatic hypermutation. Immunity 14, 643-653.

Zhang H., Okada S., Hatano M., Okabe S. and Tokuhisa T. (2001). A new functional domain of Bcl6 family that recruits histone deacetylases. Biochim. Biophys. Acta 1540, 188-200.

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