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Review Article

Pathogenetic and Clinical Implications of Non-Immunoglobulin ; *BCL6* Translocations in B-Cell Non-Hodgkin's Lymphoma

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Chromosomal translocations affecting band 3q27, where *BCL6* gene is located, are among the most common genetic abnormalities in non-Hodgkin's lymphoma of B-cell type (B-NHL). The *BCL6* gene encodes a BTB/ POZ zinc finger transcription factor, which exerts repressive activity by recruiting corepressor molecules. The 3q27/*BCL6* translocation is unique in that it can involve not only immunoglobulin (*Ig*) genes but also non-*Ig* chromosomal loci as a partner. To date, around 20 non-*Ig* partner genes have been identified. As a result of non-*Ig* ; *BCL6* translocations, many types of regulatory sequences of each partner gene substitute for the 5′ untranslated region of *BCL6*, and the rearranged *BCL6* comes under the control of the replaced promoter. The introduction of non-*Ig* ; *BCL6* constructs into transformed cells led to high-level Bcl-6 protein expression in the nucleus, while *BCL6* mRNA levels in clinical materials of diffuse large B-cell lymphoma (DLBCL) with non-*Ig* ; *BCL6* translocations were unexpectedly low. A comparative study suggested that non-*Ig* ; *BCL6* translocation and a low level of *BCL6* mRNA expression are concordant indicators of a poor clinical outcome in cases of DLBCL. The coexistence of a non-*Ig* ; *BCL6* translocation with t(14 ; 18)(q32 ; q21) in a single clone did not significantly affect the clinical features of follicular lymphoma. The pathogenetic and clinical implications of non-*Ig* ; *BCL6* translocations in B-NHL subtypes may not be identical to those of *Ig* ; *BCL6*. 〔*J Clin Exp Hematopathol 46(2) : 43-53, 2006*〕

Keywords: non-Hodgkin's lymphoma of B-cell type, *BCL6* gene, non-immunoglobulin gene, prognostic marker, concurrent translocations

INTRODUCTION

Chromosomal translocations and rearrangements of oncogenes located at breakpoints are observed in a large proportion of patients with non-Hodgkin's lymphoma of B-cell type (B-NHL). These genetic aberrations, in general, involve immunoglobulin (*Ig*) gene loci as a partner[1]. As a result of the translocation, the oncogene is transcriptionally deregulated under the influence of the juxtaposed *Ig* regulatory sequences. t(3 ; 14)(q27 ; q32) and its variants t(2 ; 3)(p11 ; q27) and t(3 ; 22)(q27 ; q11), involving *Ig* heavy chain (*IgH*), κ light chain (*IgL*κ) and λ light chain (*IgL*λ) genes, respectively, were first reported to be detected in 6.3% of cytogenetically analyzed B-NHLs[2]. By analogy with previously well-characterized translocations, an oncogene was expected to be located at 3q27, and three groups independently cloned *BCL6* (B-cell CLL/ lymphoma 6) gene on this particular chromosomal band[3-5]. A group at Columbia University headed by Dr. Dalla-Favera very recently engineered transgenic mouse strains harboring a *BCL6* gene driven by the *IgH* Im promoter, mimicking t(3 ; 14)(q27 ; q32), using the knock-in strategy[6]. The mice at between 15 and 20 months of age developed B-cell tumors showing features of diffuse large B-cell lymphoma (DLBCL) at a frequency of 36% to 62%, confirming clearly the oncogenetic role of *Ig* ; *BCL6* translocations in the pathogenesis of B-NHL.

In contrast to other B-NHL-specific translocations, 3q27/*BCL6* translocation is unique in that it can involve not only *Ig* gene loci but also non-immunoglobulin (non-*Ig*) chromosomal loci as a partner[7]. Molecular cloning of these translocations revealed that a gene is located at each partner locus and rearranged with *BCL6* so that the normal regulatory sequences of *BCL6* are replaced with those of the partner gene[7, 8]. Some of these translocations are not random but recurrent, indicating that non-*Ig* ; *BCL6* translocations as well as *Ig* ; *BCL6* play a pathogenetic role in B-NHL. In this review, I first describe the molecular anatomy of non-*Ig* ; *BCL6* translocations. Second, I present our experiments to show to what

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extent non-*Ig* ; *BCL6* translocations affect the level of Bcl-6 protein expression. Finally, I discuss the clinical implications of this unique type of translocation in subtypes of B-NHL.

THE *BCL6* GENE AND ITS PRODUCT

The *BCL6* gene spans 24, 310 bases and contains 11 exons, generating 4 types of splicing patterns (http : //www.genecards.org/cgi-bin/carddisp?BCL6). The ATG signal for the initiation of protein synthesis is within exon 3 and is followed by an open reading frame. The 3q27/*BCL6* translocation occurs within the major translocation cluster (MTC) spanning the non-coding exon 1 and intron 1 ; in a majority of cases, breakpoints are located immediately 3′ of exon 1[3,5,9]. The translocation, therefore, does not interrupt the protein-coding region of *BCL6*.

The Bcl-6 protein, consisting of 706 amino acids with a calculated molecular weight of 78,846 Da, is a sequence-specific transcription factor that can repress transcription from promoters containing its DNA-binding site (Fig. 1)[10]. The C-terminal region of Bcl-6 contains six C_2-H_2 type zinc fingers, each separated by a conserved stretch of seven amino acids. The two cysteine and two histidine residues coordinate a zinc ion and fold the domain into a finger-like projection that can

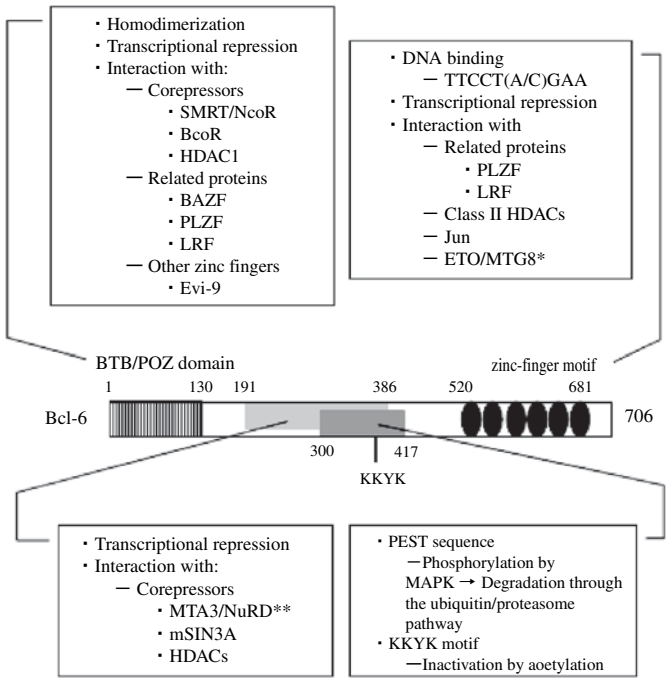

Fig. 1. Functional domains of the Bcl-6 protein[10]. The domains are involved in protein-protein interaction, transcriptional repression of target genes, post-translational modification and DNA binding. * ETO/ MTG8 binds to the fourth zinc finger[61]. ** MTA3/ NuRD interacts with the region spanning aa 141 to 507[62]. PEST, where P = proline, E = glutamic acid, S = serine and T = threonine ; KKYK, where K = lyine and Y = tyrosine.

interact with DNA.

The BTB/POZ domain (broad-complex, tramtrack, and bric-a-brac/pox virus and zinc finger) at the N-terminus is a conserved 120-amino acid motif, which is found in 5 to 10% of zinc finger proteins[11]. The primary function of the BTB/POZ domain appears to be the mediation of protein-protein interactions. It has been shown that the repressive effect of Bcl-6 on the target gene is exerted via the recruitment of SMRT (silencing mediator of retinoid and thyroid receptor), NCoR (nuclear receptor corepressor) and BCoR (BCL6 corepressor) corepressors[12-15]. Crystallographic analysis of the BTB/POZ domain revealed that it forms a butterfly-shaped homodimer to generate a 'lateral grove' motif that interfaces with a 17-residue sequence (BBD motif, Bcl-6 binding domain) of SMRT[12,15]. A fusion protein that contains the HIV-TAT protein transduction domain and the BBD sequence can penetrate the cell membrane and bind to Bcl-6, thereby blocking the BTB/POZ-mediated recruitment of SMRT[16].

The middle portion of Bcl-6 contains a second domain required for the repressive transcriptional activity (Fig. 1). Bereschchenko *et al*.[17] showed that the KKYK motif within the PEST sequence is targeted by p300-mediated acetylation. This post-translational modification disrupts the ability of Bcl-6 to recruit histone deacetylase (HDAC), thereby hindering its capacity to repress transcription.

Targeted inactivation of *BCL6* in the mouse germline leads to impaired production of secondary IgG antibody against T-cell-dependent antigens and the spleen of the $BCL6^{-/-}$ mouse lacks germinal center (GC) formation[18-20]. A cDNA array analysis along with loss-of-function experiments revealed a set of genes that are negatively regulated by Bcl-6[21]. The *BLIMP1* (B-lymphocyte-induced maturation protein 1) gene, which is a representative target gene, plays a key role in the differentiation of B-cells into plasma cells by turning off the entire mature B-cell gene expression program[22,23]. It is presumed that *BCL6* is the master gene for the generation by B-cells of a GC, acting to modulate the transcription of genes involved in cell proliferation, differentiation and apoptosis.

MOLECULAR ANATOMY OF NON-*IG* ; *BCL6* TRANSLOCATIONS

To determine the partner of the *BCL6* translocation, two polymerase chain reaction (PCR)-based approaches have been applied, i.e. 5′-rapid amplification of cDNA ends (5′-RACE) and long-distance inverse (LDI-) PCR (Fig. 2)[9,24,25]. Both methods isolate sequences adjacent to the junction, a database search of which leads to identification of the partner gene.

Table 1 lists non-*Ig* partners that have been identified to date[9, 24-35]. These include the genes for a transcription factor, serine/threonine-protein kinase, cytokine receptor, Ras small

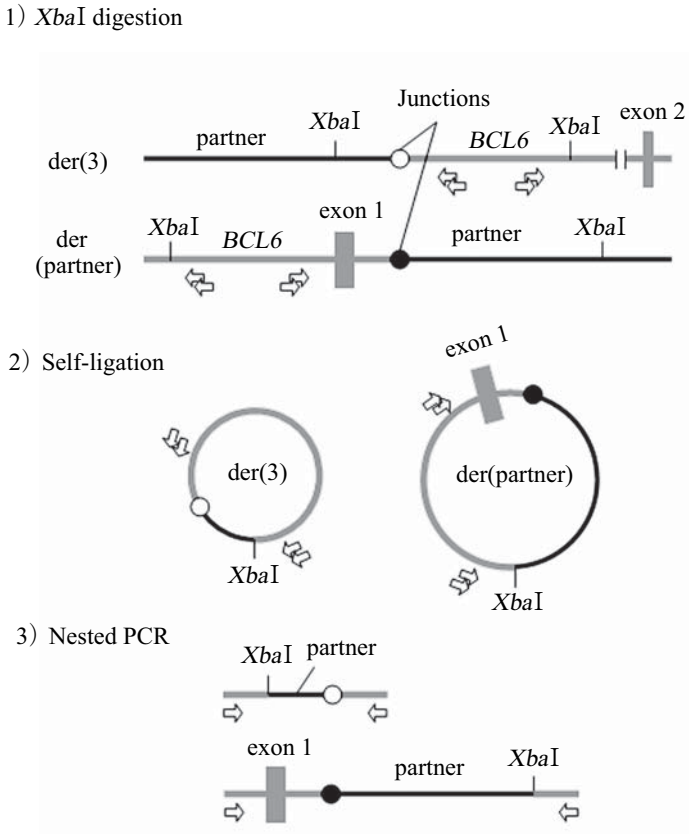

Fig. 2. Schematic diagram of the long-distance inverse PCR assay used to detect non-*Ig* ; *BCL6* translocations[9]. PCR primers (open arrows) were designed for the *BCL6* sequences in the divergent orientation. Genomic DNA from tumor tissues was digested with *Xba*I, and self-ligated at a low DNA concentration. These circular DNAs are targeted by nested PCR amplification. The PCR product contains a fragment of an unknown partner that is flanked by the known *BCL6* sequences. The fragment beyond the artificial *Xba*I site is the sequence of the partner.

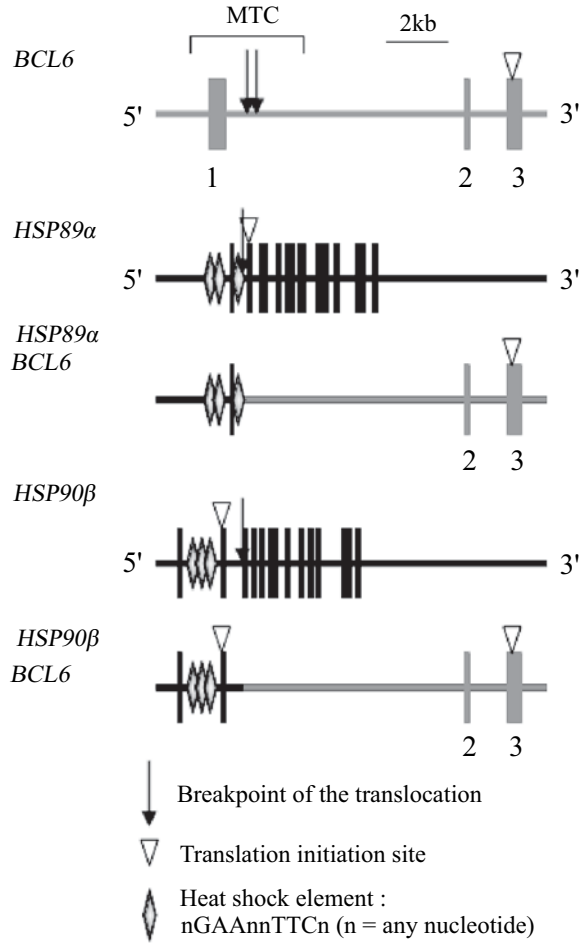

Fig. 3. Molecular anatomy of *BCL6* translocations involving two heat shock protein (*HSP*) genes. The breakpoints on the *BCL6* gene were within the MTC region, while those on the *HSP* genes were either 5′ or 3′ of the translation initiation sites. Transcriptional control of *HSP* genes is mediated by three tandem copies of heat shock element (HSE). In response to heat shock, the heat shock factors as a trimer bind to these HSEs[38]. As the result of translocation, the complete set of the HSEs is fused upstream of *BCL6*. Therefore, the expression of *BCL6* apparently comes under the control of the heat shock factors that bind to the HSEs

GTPase, heat shock proteins and so on. In spite of this marked diversity of protein products, there are common features in the molecular anatomy of non-*Ig* ; *BCL6* translocations. First, the gene fusion occurs in the same transcriptional orientation ; second, the breakpoint on the partner gene is located in close proximity to the promoter sequence ; and third, the complete sequence of the promoter is fused upstream of the coding region of *BCL6* on the der(3) chromosome (Fig. 3). As the result of translocation, many types of regulatory sequences of each partner gene substitute for the 5′ untranslated region of *BCL6*, and the rearranged *BCL6* comes under the control of the replaced promoter (promoter substitution[36]). The translocated allele generates chimeric transcripts composed of 5′ sequences of the partner followed by the open reading frame of *BCL6*, which is readily detectable by reverse transcriptase-mediated PCR[33,37].

Although the transcriptional control of these non-*Ig* genes has not been fully determined, it should be noted that some genes are transcriptionally activated by a variety of stimuli, including cell cycle control (*H4*), changes in the physical environment (*HSP89α* and *HAP90β*)[38], and response to cytokines (*PIM1* and *CIITA*). Considering that GC B-cells, in which the *BCL6* translocation is presumed to occur, proliferate rapidly in response to antigen, it is likely that a *BCL6* gene affected by the translocation is inappropriately expressed during B-cell proliferation.

There is evidence to suggest that the somatic hypermuta-

tion (SHM) machinery of the *Ig* gene is involved in the development of non-*Ig* ; *BCL6* translocations. SHM of *BCL6* occurs in a large proportion of memory B-cells isolated from normal individuals, in GC-B-cells from reactive tonsils, and across the spectrum of GC/post-GC type B-cell tumors[39-41]. The mutations are clustered within the first exon-intron boundary of the gene, which overlaps with the MTC[42], suggesting that SHM and translocations involving *BCL6* are mediated by common molecular mechanisms. On the other hand, *PIM1* and *RHOH*, both of which are non-*Ig* partners (Table 1), are mutated in DLBCL and the regions involved in the mutation match those in the translocation[43]. These observations suggest that the SHM machinery, which generates double-stranded breaks of DNA, targets both the *BCL6* and non-*Ig* partners of GC-B-cells, thereby predisposing these genes to exchanges of genetic material.

DEREGULATED EXPRESSION OF BCL-6 PROTEIN BY HISTONE *H4*; *BCL6* TRANSLOCATION

We showed that t(3 ; 6)(q27 ; p21) results in the fusion of *BCL6* with a particular histone *H4* gene (HUGO nomenclature : *HIST1H4I*) on 6p21[27]. 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)[44]. The Site II-equivalent of the *H4* gene contains consensus binding sites for the transcription factors HiNF-M, HiNF-D and HiNF-P. 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[44]. 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 S phase[45]. The sequence approximately 16 bp downstream of the palindrome is also essential and through base-pairing interactions mediates the U7 snRNP-dependent processing of the mRNA 3′ end[45].

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[37]. 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. 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

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

Gene symbol : HUGO (Alias)	Gene product	Chromosomal locus[1]	References
MBNL1 (*KIAA0428*)	Muscleblind-like protein (Triplet-expansion RNA-binding protein)	3q25/ 3q25.1	34
TFRC	Transferrin receptor (p90, CD71)	3q26.2-qter/ 3q29	9, 28
ST6GAL1 (*CD75*)	Sialyltransferase 1 (beta-galactoside alpha-2, 6-sialytransferase)	3q27-q28/ 3q27.3	34
EIF4A2	Eukaryotic translation initiation factor 4A, isoform 2	3q28/ 3q27.3	28
RHOH (*RhoH*, *TTF*)	Rho-related GTP-binding protein RhoH (GTP-binding protein TTF)	4p13/ 4p14	24, 43
HSPCB (*HSP90β*)	Heat shock 90kDa protein 1, beta	6p12/ 6p21.1	9
PIM1	Pim-1 oncogene product	6p21.2	9, 28, 43
SFRS3 (*SRp20*)	Splicing factor, arginine/serine-rich 3 (Pre-mRNA splicing factor SRP20)	6p21/ 6p21.31	32
HIST1H4I (*H4/m*)	H4 histone family, member M	6p21.33	27, 37
U50HG[2]	Small nucleolar RNA	6q15	30
ZNFN1A1 (*IKAROS*)	Ikaros (zinc finger protein)	7p13-p11.1	9, 29
GRHPR (*GLXR*)	Glyoxylate reductase/hydroxypyruvate reductase	9q12/ 9p13.2	34
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	25
LRMP (*JAW1*)	Lymphoid-restricted membrane protein	12p12.1	34
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	12p13.31	35
NACA	Nascent-polypeptide-associated complex alpha polypeptide	12q23-q24.1/ 12q13.3	9
LCP1	L-plastin (Lymphocyte cytosolic protein 1) (LCP-1) (LC64P)	13q14.3/ 13q14.13	26
HSPCA (*HSP90α*)	Heat shock 90kDa protein 1, alpha	14q32.33/ 14q32.31	9, 31
IL21R	Interleukin-21 receptor	16p11/ 16p12.1	33
CIITA	MHC class II transactivator	16p13/ 16p13.13	9, 28

[1]LocusLink and/or Ensembl cytogenetic band

[2]not included in the HUGO gene nomenclature database

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 the germ-line *BCL6*, we constructed expression plasmids that mimicked the structure of t(3 ; 6)(q27 ; p21) (Fig. 4A)[37]. Transient transfection of the plasmids into COS-7 cells resulted in transcription of *H4* ; *BCL6* fusion mRNA that had the same structure as 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 more Bcl-6 than cells transfected with a plasmid carrying *BCL6* driven by its normal promoter (Fig. 4B). We next subjected the COS-7 cells to indirect immunofluorescence microscopy using a polyclonal antibody against Bcl-6 and found that *H4* ; *BCL6*-transfected cells displayed bright nuclear staining with a characteristic granular pattern (Fig. 4C) ; the granules have been shown to contain SMRT and N-CoR co-repressors[13]. The introduction of a series of deletion mutants that lacked the Site II sequences led to a reduction in the expression of Bcl-6 to the basal level (Fig. 4C). These findings indicate that *H4* ; *BCL6* gene fusion leads to enhanced Bcl-6 protein expression, which is promoted by the *H4* regu-

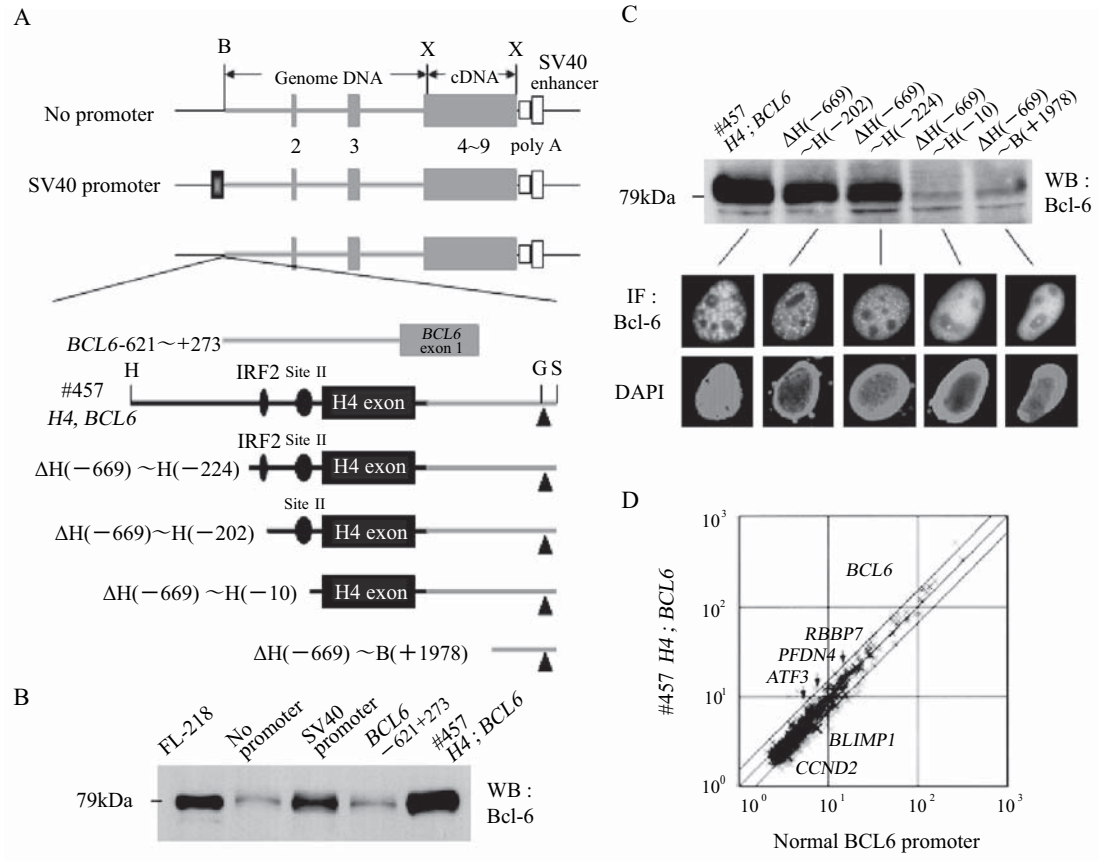

Fig. 4. Construction of *H4* ; *BCL6* fusion gene mimicking t(3 ; 6)(q27 ; p21) and the effect of transfection into COS-7 cells[37]. (A) Diagram of the Bcl-6-expression plasmids driven by the SV40 promoter, normal *BCL6* promoter, and *H4* ; *BCL6* fusion gene of case no. 457. Transcription initiated from the *H4* ; *BCL6* fusion gene was contiguous with the *BCL6* exon2 at the cryptic 5' splice-donor site (▲). A series of deletion mutants of the last fragments was generated by digestion with exonuclease III and mung bean nuclease. IRF2, interferon regulatory factor 2 ; B, *Bam*HI ; X, *Xba*I ; H, *Hin*dIII ; G, *Bgl*II ; and S, *Sac*I. (B) Western blot analysis of COS-7 transfectants for Bcl-6 expression. FL-218 is a follicular lymphoma cell line. (C) Effect of the promoters of *H4* upon the level of Bcl-6 expression. The COS-7 transfectants were subjected to Western blot analysis and indirect immunofluorescence microscopy. (D) Scatter plot analysis comparing the expression profile of *H4* ; *BCL6*-transfected cells (Y axis) to that of normal *BCL6* promoter-transfected cells (X axis). Alteration of the levels of expression was determined by the Atlas Human Array (Clontech) and ArrayGauge software (Fuji Photo Film). Up- or down-regulation by > 1.5-fold, indicated by two diagonal lines, was considered significant.

latory elements.

A comparison of gene expression profiles using a cDNA array analysis between the *H4* ; *BCL6*- transfected cells and the cells transfected with *BCL6* driven by the normal promoter revealed that 224 (19%) of 1,175 genes were > 1. 5 fold under- expressed in the former cells as compared with the latter reference cells (Fig. 4D). The down-regulated genes included *BLIMP1* and cyclin D2 (*CCND2*), both of which are primary target genes negatively regulated by Bcl-6[21]. These experiments provided clear evidence that the *H4* ; *BCL6* translocation leads to high- level Bcl- 6 expression, thereby repressing the transcription of the target genes.

PROGNOSTIC SIGNIFICANCE OF NON-*IG* ; *BCL6* TRANSLOCATIONS IN DLBCL

The correlation between *BCL6* translocations and clinical features of DLBCL has been a subject of controversy. An earlier study showed that *BCL6* rearrangements determined by Southern blotting occurred more frequently in extranodal DLBCL than in node- based diseases and correlated with a favorable clinical outcome[46]. However, later studies failed to find a statistically significant impact of *BCL6* translocations on the clinical outcome of DLBCL[47- 50].

We studied whether the partner in *BCL6* translocations influences the clinical behavior and/ or treatment outcome of DLBCL[47, 51]. Of 43 DLBCL patients having *BCL6* abnormalities, 26 had *Ig* ; *BCL6* translocation, while 15 had non-*Ig* ; *BCL6*. The remaining 2 had a deletion of a > 1 kb segment encompassing exon 1 of *BCL6*. Although there were no significant differences in pretreatment clinical features between the 26 patients with *Ig* ; *BCL6* translocation and the 17 patients with non- *Ig* ; *BCL6*, including the 2 with a deletion, overall survival (OS) of the non-*Ig* ; *BCL6* group was inferior to that of the *Ig* ; *BCL6* group (Fig. 5A). Fourteen patients of the non-*Ig* ; *BCL6* group died within 2 years, while 5 patients of the *Ig* ; *BCL6* group have been disease- free for some time. The estimated 2-year OS of the *Ig* ; *BCL6* and non- *Ig* ; *BCL6* 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* translocation is an indicator of poor prognosis in DLBCL, and additional studies of larger patient populations are warranted.

A cDNA microarray analysis revealed that DLBCL patients with the GC B-cell-like (GCB) pattern of gene expression have a significantly better OS than those with the activated B-cell-like (ABC) expression profile[52]. *BCL6* is a representative gene of the GCB- type signature and high- level expression of *BCL6* at both the mRNA and protein levels has been shown to be a predictor of a favorable treatment outcome in cases of DLBCL[53, 54]. To address the relationship between our finding that DLBCL with non-*Ig* ; *BCL6* translocation has a worse prognosis than DLBCL with *Ig* ; *BCL6*

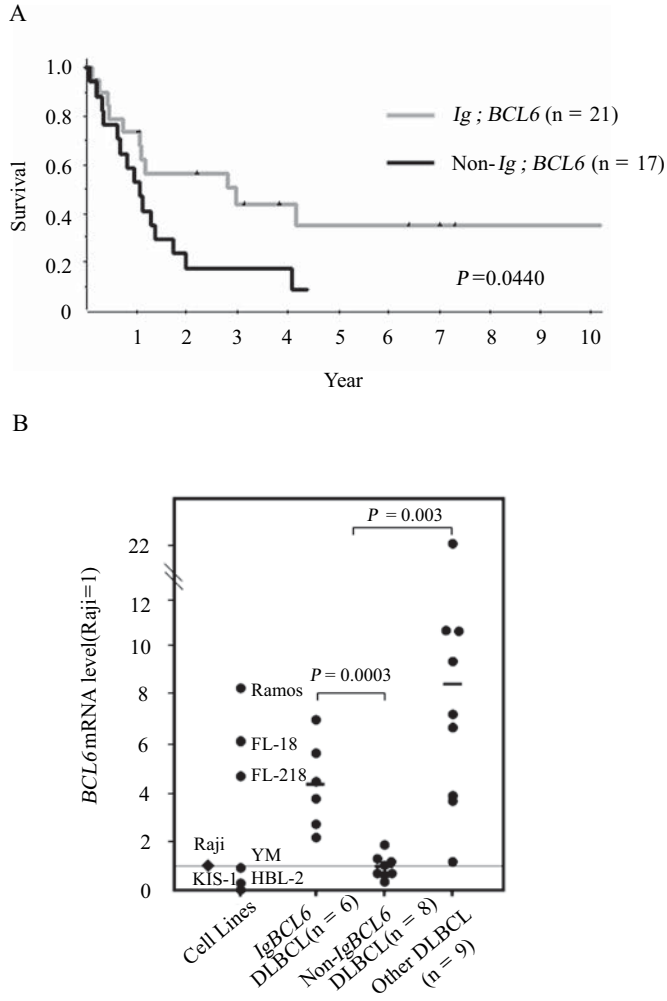

Fig. 5. Prognostic impact of non-*Ig* ; *BCL6* translocation on DLBCL, and *BCL6* mRNA levels in DLBCL subgroups stratified by *BCL6* translocation[47, 51, 60]. (A) Overall survival curves of DLBCL patients with *Ig* ; *BCL6* or non-*Ig* ; *BCL6* translocation. The latter group included two patients having a deletion within the MTC region. (B) *BCL6* mRNA levels of B- NHL cell lines, clinical materials of DLBCL patients with *Ig* ; *BCL6* or non-*Ig* ; *BCL6* translocation, and those lacking *BCL6* translocation. The levels of the last group were significantly higher than those of the former two DLBCL groups. Ramos, a Burkitt lymphoma cell line ; FL- 18 and FL- 218, follicular lymphoma cell lines ; YM, HBL- 2 and KIS- 1, DLBCL cell lines.

and the fact that the level of *BCL6* expression is related to clinical outcome, we compared the levels of *BCL6* mRNA between the two groups. The amount of mRNA measured by real-time quantitative PCR was divided by that of the *GAPDH* gene, and the *BCL6*- *GAPDH* value was normalized to that of Raji cells. A comparative study showed that the *BCL6* mRNA levels of the *Ig* ; *BCL6* group (n = 6 ; range, 2.7- 7.0 ; mean, 4.3) were significantly higher than those of the

non-*Ig* ; *BCL6* group (n = 8 ; range, 0.4-1.9 ; mean, 1.0)[51], and the level of the latter group was below the threshold (= 1.3) for a poor prognosis determined by Lossos *et al*[54] (Fig. 5B). This observation suggests that high versus low-level *BCL6* mRNA expression and an *Ig* versus non-*Ig* partner of *BCL6* translocation are concordant prognostic indicators of DLBCL. Further study is required to determine whether *Ig* ; *BCL6* and non-*Ig* ; *BCL6* translocations correspond to GCB and ABC-type DLBCL, respectively.

REARRANGEMENTS OF *BCL6* CONCURRENT WITH THOSE OF C-*MYC* AND/OR BCL2 IN B-NHL

Since the 3q27 translocation is the sole cytogenetic abnormality in some cases of B-NHL, the translocation and/or *BCL6* rearrangements may be primarily involved in the development of B-NHL. However, 3q27/*BCL6* translocation sometimes coexists with other translocations in a single clone[55, 56], including t(8 ; 14)(q24 ; q32) and t(14 ; 18)(q32 ; q21) involving c-*MYC* and *BCL2*, respectively, making the role of the 3q27/*BCL6* translocation in the pathogenesis of B-NHL unclear.

We conducted a series of LD/LDI-PCR assays, which readily detect rearrangements involving c-*MYC*, *BCL2* and *BCL6*[9, 57, 58], using clinical materials of B-NHL, and found that a total of 14 patients carried a rearrangement of *BCL6* concurrently with rearrangements of c-*MYC* and/or *BCL2* ; 10 patients had both *BCL2* and *BCL6* rearrangements, 3 had both c-*MYC* and *BCL6* rearrangements, and the remaining patient had rearrangements of all 3 genes (Table 2). The constant genes affected by c-*MYC* ; *IgH*, which is the molecular equivalent of t(8 ; 14)(q2 ; q32), were Cγ. The *BCL2* genes in 10 patients were rearranged at the 3′, i.e. from the major breakpoint cluster region (MBR) through to the minor cluster region (mcr), while 1 patient had a 5′ breakpoint[59]. *BCL6* translocations involved *Ig* genes as partners in 5 patients, and non-*Ig* genes as partners in 8 patients. The remaining patient had a deletion of a 1.2-kb segment within the MTC region of *BCL6*[9].

Seven of the 10 patients with both *BCL2* and *BCL6* rearrangements had follicular lymphoma (FL), while all of the

Table 2. Clinical and molecular features of B-NHL patients concurrently carrying rearrangements of *BCL6* and of c-*MYC* and/or *BCL2*

Case #	Age/ Sex	WHO[1]	Stage/ PS	Involved organs[2]	LDH (IU)	Immunophenotype		Treatment[3]	Outcome[4]	Survival (days)[5]	Rearrangement		
						CD10	sIg				c-*MYC* Constant gene	*BCL2* Breakpoint[6]	*BCL6* Partner gene[7]
464	74/F	FL	IV/1	LN, BM	265	−	μ/λ	CHOP, other	PR	2822+	−	150 bp-MBR	*RhoH/TTF*
514	43/F	FL	IV/1	LN, BM	208	−	μ/κ	CHOP, radiation	CR-R-CR-R-CR	5146+	−	150 bp-MBR	*IgL*κ
652	37/M	FL	III/1	LN	370	−	μ/κ	LSG9	CR-R-CR	3814+	−	150 bp-MBR	*RhoH/TTF*
733	53/M	FL	II/0	LN	236	+	α/λ	VEPA, radiation, other	PR-R-PR-R-PD	1157	−	150 bp-MBR	deletion
834	54/M	FL	IV/1	LN, BM, spleen, stomach	372	+	μ/λ	VEPA, other	PR	2344+	−	3′-MBR	*HSP90*β
1034	39/F	FL	IV/0	LN, BM	342	+	$\mu\delta/\lambda$	CHOP, radiation, other	PR	1860+	−	150 bp-MBR	*CIITA*
1035	70/M	FL	IV/0	LN, BM, stomach	302	+	$\mu\delta/\lambda$	CHOP, radiation, other	PR	1458+	−	150 bp-MBR	3q25-27
422	69/F	DLBCL	I/2	Ileum	412	−	−/κ	CHOP	CR	4034+	Cγ	−	*IgH*
742	61/M	DLBCL	IV/1	LN, spleen, tonsils	1825	−	$\mu\delta/\kappa$	CHOP, other	PR-R-PD	397	−	5′-*BCL2*	*IL21R*
854	86/M	DLBCL	I/1	LN	549	−	ND	Radiation, VEPA	PR-R-PD	229	Cγ	−	*HNRPC*
893	66/M	DLBCL	IV/2	LN, stomach, pleural effusion	1530	+	δ/κ	CHOP	PR-R-PD	164	Cγ	−	*IgH*
1230	49/M	DLBCL	III/1	LN	195	+	−/−	CHOP, radiation, other	PR	1045+	Cγ	mcr	*IgL*λ
1262	53/M	DLBCL	II/0	LN, tonsil	180	+	$\alpha\gamma/\kappa$	CHOP, radiation, other	CR-R-PD	451	−	150 bp-MBR	*IgH*
1263	54/F	DLBCL	IV/2	LN, BM, skin, bones	310	−	$\mu\delta/\lambda$	CHOP, other	PR	187+	−	mcr	*MEF2C*

[1] : FL, follicular lymphoma ; DLBCL, diffuse large B-cell lymphoma. [2] : LN, lymph node ; BM, bone marrow. [3] : VEPA and CHOP, vincristine, cyclophosphamide, doxorubicin, prednisolone ; LSG9, multi-agent combination chemotherapy designed by the Japan Lymphoma Study Group. [4] : CR, complete response ; PR, partial response ; R, relapse ; PD, progressive disease. [5] : Vital status was followed through January 2004. [6] : The *BCL2* breakpoints were as defined previously[58]. [7] : *HNPRC*, heterogeneous nuclear ribonucleoprotein C (C1/C2) on 14q11.1 ; *MEF2C*, myocyte enhancer factor 2C, MADS box transcription enhancer factor 2, polypeptide C on 5q14

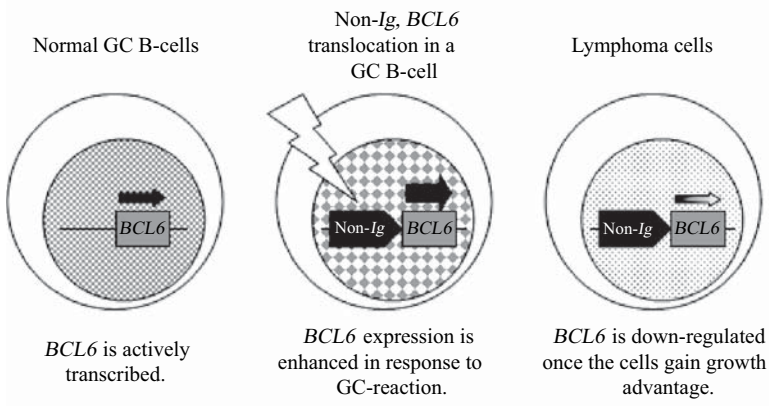

Fig. 6. A model of deregulated expression of Bcl-6 leading to the development of DLBCL. Bcl-6 is normally expressed at high levels in GC B-cells. When a GC B-cell acquires a non-*Ig* ; *BCL6* translocation, transcription of *BCL6* mRNA from the translocated allele is further enhanced under the GC micro-environment.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

4 patients with c-*MYC* rearrangement had DLBCL. In particular, of 7 FL patients, 5 had a non-*Ig* ; *BCL6* translocation ; they had advanced-stage disease at presentation and 3 were positive for CD10 expression. All but one patient with FL showed indolent clinical behavior, although initial treatment with a doxorubicin-containing regimen failed to achieve a complete clinical response. Thus, in contrast to the general consensus that multiple translocations can occur at the time of transformation from low- to high-grade disease, the coexistence of a non-*Ig* ; *BCL6* translocation with a *BCL2* rearrangement did not significantly affect the clinical features of FL defined by t(14 ; 18)(q32 ; q21). In contrast, the pretreatment parameters as well as treatment outcome of DLBCL patients varied. Thus, a dual/triple rearrangement does not necessarily result in synergic effects on the malignant phenotype of B-NHL subtypes.

CONCLUSION

We showed that the introduction of expression plasmids mimicking the non-*Ig* ; *BCL6* fusion genes into transformed cells led to high-level Bcl-6 protein expression in the nucleus[33, 37, 60]. In contrast, the *BCL6* mRNA levels of DLBCL cells carrying a non-*Ig* ; *BCL6* translocation were unexpectedly low[37, 51]. 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 a 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. 6). We next showed that the coexistence of a non-*Ig* ; *BCL6* translocation with other translocations does not necessarily have a significant impact on the clinical features of B-NHL subtypes. Thus, there may be a hierarchical order to the rearrangement of oncogenes that ultimately determines the characteristics of B-NHL. Our findings suggested that the pathogenetic and clinical implications of non-*Ig* ; *BCL6* translocations in B-NHL may not be identical to those of *Ig* ; *BCL6*.

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