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

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ABSTRACT

A recurrent translocation, t(3;6)(q27;p21), in non-Hodgkin’s lymphoma results in fusion of BCL6 with a particular histone H4 gene on 6p21. We cloned five H4/BCL6 junctions from both der(3) and der(6) chromosomes. The breakpoints on H4 were distributed within the single exon or close to the terminal palindrome, and those on BCL6 were localized within or close to the translocation hypercluster. Deletions or duplications of variable numbers of nucleotides were identified at the junctions. A total of eight single nucleotide alterations were introduced into the translocation/mutation cluster of BCL6, whereas four single nucleotide substitutions were identified within a 360-bp region of H4. Thus, the somatic hypermutation mechanism is likely to target H4, resulting in a predisposition to the development of translocation with BCL6. Lymphoma cells carrying H4/BCL6 produced fusion transcripts containing both H4 and BCL6 messages; however, the cells expressed only moderate levels of BCL6 mRNA. We constructed expression plasmids that mimicked the H4/BCL6 fusion gene and transiently introduced them into COS-7 cells. H4/BCL6-transfected cells expressed markedly higher levels of Bcl-6 protein than cells transfected with a plasmid carrying BCL6 driven by its normal promoter and displayed bright nuclear staining with a characteristic punctate pattern with an anti-Bcl-6 antibody. Deletion analyses revealed that the high-level Bcl-6 expression was promoted by the H4 regulatory sequences. The levels of expression of activating transcription factor 3, prefoldin 4, and retinoblastoma-binding protein 7 significantly increased in accordance with that of BCL6, suggesting that Bcl-6 may act as a transcriptional activator. Our study suggested that t(3;6)(q27;p21) leads to BCL6 overexpression; however, the high-level BCL6 expression may not be required to maintain the malignant phenotype of lymphoma cells.

INTRODUCTION

A 3q27 translocation affecting the BCL6 gene has been identified by cytogenetic studies and/or Southern blot analysis using a probe for the major translocation cluster of BCL6 (1, 2). The studies of B-NHL,4 have shown that BCL6 translocation occurs in 5–15% of FLs and 20–40% of DLBCLs (2, 3). One characteristic feature of BCL6 translocation is that it can involve not only the three Ig genes but also diverse non-Ig genes as partners. To date, a total of 15 recurrent non-Ig partner genes have been identified (3–7). As a result of non-Ig/BCL6 translocation, the regulatory sequences of the various partner genes substitute for the 5’ untranslated region of BCL6, and the rearranged BCL6 is presumed to be under the control of the replaced promoter activity. On the other hand, somatic mutations of BCL6 have been described in a large proportion of memory B cells isolated from normal individuals and GC B cells from reactive tonsils as well as GC/post-GC type B-NHL (8, 9). The majority of the mutations are clustered immediately 3’ of exon 1 in a region that has been referred to as the MMC (10). Because the major translocation cluster and MMC overlap, possible linkage between the translocation and mutation has been suggested (6, 11).

The histone H4 gene (accession no. AB000905) was first cloned as the partner of BCL6 in a t(3;6)(q27;p21) translocation (12) and was assigned as member M of the histone H4 gene family, which is clustered on 6p21. Because the expression of H4 is tightly regulated by cell cycle control (13, 14), H4/BCL6 gene fusion in B cells most likely leads to inappropriate expression of BCL6 in response to antigenic stimuli in the GC. However, no data are currently available about the extent to which the gene fusion affects the level of BCL6 expression. Another unresolved issue is the molecular mechanism underlying formation of the H4/BCL6 translocation. BCL6 translocation involving the immunoglobulin heavy chain gene (IgH) as the partner invariably occurs within the switch region of IgH (6), suggesting that IgH/BCL6 translocation is closely associated with the isotype class switch mechanism. In contrast, the somatic hypermutation mechanism that targets not only IgH but also several non-Ig oncogenes, including BCL6, may play a role in the formation of non-Ig/BCL6 translocation (11). In this study, to explore the mechanism of development of t(3;6), we cloned and sequenced 5 H4/BCL6 fusion genes. We next produced a construct that mimicked the H4/BCL6 fusion gene and introduced it into transformed cell lines to determine to what extent the gene fusion affects the level of BCL6 expression. The transfected cells expressed a high level of Bcl-6 protein, providing evidence that t(3;6) leads to quantitative alteration of BCL6 expression.

MATERIALS AND METHODS

Patients. We studied here five cases of B-NHL that carried an H4/BCL6 fusion gene. Two had a t(3;6)(q27;p21), as shown by cytogenetic analysis, whereas the partners of BCL6 in the remaining three were determined by the long-distance inverse PCR method (6). Histopathological findings showed that the cases consisted of follicular mixed small cleaved and large cell lymphoma (FMIX, case no. 457), follicular large cell lymphoma (FLAR, case no. 739), and DLBCL (case nos. 229, 764, and 897). Other lymphoma cases were selected from a collection in our laboratory.

PCR, Southern Blot Hybridization, Cloning, and Nucleotide Sequencing. Long-distance inverse PCR to clone DNA fragments of non-Ig partners was described in detail previously (6). PCR amplification of H4/BCL6 and the reciprocal BCL6/H4 fragments was performed using appropriate primer sets under standard PCR conditions. Total cellular RNA was extracted from cryopreserved tumor tissues using an RNeasy kit (Qiagen K.K., Tokyo, Japan). For RT-PCR, randomly primed cDNA was synthesized from 1 μg of total cellular RNA and subjected to PCR amplification using an automated thermal cycler (GeneAmp PCR System 2400; Applied Biosystems, Foster City, CA). Ali-
quot of the PCR products were analyzed by ethidium bromide-stained gel electrophoresis and transferred onto nylon membranes. The membranes were hybridized with probes labeled with \( ^{32}P \)dCTP. PCR products with A-overhangs were ligated into a plasmid vector with T-overhangs at the cloning site (TA cloning; Invitrogen, San Diego, CA). Nucleotide sequencing of the insert was performed with a BigDye Terminator Cycle Sequencing kit (Applied Biosystems), and the sequencing reactions were resolved using an automated sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems).

**Real-Time Quantitative RT-PCR of BCL6 mRNA.** Real-time PCR analysis based on the TaqMan methodology was performed using an ABI Prism 7700 Sequence Detection System (Applied Biosystems). The sequences of oligonucleotide primers and a fluorogenic probe for BCL6 were as described (15). cDNA was synthesized from 1 μg of total cellular RNA isolated from clinical specimens using random primers. The DNA standard template containing the BCL6 cDNA sequence was generated by cloning into pGEM-T-EASY vector (Promega Corp., Madison, WI). The \( C_T \) (threshold cycle) parameter was defined as the fractional cycle number at which the fluorescence generated by cleavage of the probe passed a preset threshold. The standard curve, in which \( C_T \) decreased in linear proportion to the log of the template copy number, was established by using serially diluted pGEM-T-BCL6 plasmid DNA. The \( C_T \) values of test materials were plotted on the standard curve, and the corresponding copy number was calculated using the Sequence Detector version 1.6 software (Applied Biosystems). All assays were performed in triplicate.

**Transfection.** COS-7 and 293T cells were grown in 35-mm 6-well plates in DMEM (BioWhittaker, Walkersville, MD) supplemented with 10% fetal bovine serum and penicillin-streptomycin. Twenty-four h after cells were plated at 2 × 10^5 cells/well, they were transfected with 4 μg of plasmid by using LipofectAMINE 2000 reagents (Life Technologies, Inc.) or by the calcium phosphate method (CalcPhos™ Mammalian Transfection Kit; Clontech, Palo Alto, CA).

**Luciferase Reporter Assay.** The DNA fragments of interest were cloned into a firefly luciferase reporter plasmid, pGL3-Basic Vector (Promega). The pGL3 plasmid constructs were assayed in COS-7 cells to measure promoter-driven luciferase expression. At 80–90% confluence, cells were cotransfected with 1.0 μg of plasmid DNA plus 0.01 μg of pRL-TK DNA (Promega) using LipofectAMINE 2000 in serum-free DMEM for 24 h. Cells were lysed, and firefly and Renilla luciferase activities were measured using the Dual Luciferase Assay System (Promega) in a luminometer (Lumat LB 9507; Berthold Technologies, Bad Wildbad, Germany).

**Construction of Bcl-6 Expression Plasmids.** The cDNA clone of BCL6 was provided by Dr. T. Miki (Tokyo Medical and Dental University, Tokyo, Japan). The sequences of interest were ligated into pGL3-Enhancer (Promega) carrying SV40 enhancer or pGL3-Control (Promega) carrying both SV40 promoter and enhancer, and the luciferase gene was replaced by the BCL6 cDNA fragment. The cloning was verified by DNA sequencing.

**Western Blotting.** Cells were lysed with 1/10 loading buffer containing a protease inhibitor mixture. The total cell lysates were loaded onto 8% SDS acrylamide gels and electrotransferred onto Immobilon polyvinylidene difluoride membranes. The membranes were probed with antibodies specific for BCL6 and actin. The blots were incubated with appropriate secondary antibodies conjugated with horseradish peroxidase, and the protein bands were visualized using the Enhanced Chemiluminescence Western Blotting Detection System (Amersham Pharmacia Biotech).

Fig. 1. Nucleotide sequences of H4 (A) and BCL6 (B) genes within the areas involved in t(3;6)(q27;p21) translocation. The germ-line sequences of H4 are as registered in the database (accession no. AB000905), whereas those of BCL6 are based upon our own study. Numbers indicating the nucleotide positions refer to the A of the ATG initiation codon of H4 (12) or the 5’ boundary of BCL6 exon 1 determined by Ohashi et al. (17). Dashes show nucleotide identity. Breakpoints on der(3) and der(6) are indicated by open and closed triangles, respectively, and the intervening sequences were deleted or duplicated (indicated by –). The coding sequences of H4 are in boldface letters. The boxes within H4 indicate the RGYW ([A/G/G/C/T/G/A/T]) consensus motif and its inverse complement WRCY ([A/T/G/C/G/A/T]). The terminal palindrome of H4 (12) and the breakpoint hypercluster of BCL6 (6) are underlined.
oride transfer membranes (Millipore, Bedford, MA). The membranes were incubated with polyclonal rabbit anti-Bcl-6 antiserum against amino acids 687–706 of human Bcl-6 (Santa Cruz Biotechnology, Santa Cruz, CA). After extensive washing, the blots were incubated with horseradish peroxidase-conjugated secondary antiserum followed by enhanced chemiluminescence reaction (Amersham Pharmacia Biotech, Piscataway, NJ).

**Indirect Immunofluorescence.** COS-7 cells were washed with PBS and fixed with 3.0% paraformaldehyde in PBS. The fixed cells were treated with 0.1% Triton X-100 and cold methanol. The cells were then permeabilized with 0.05% PBT solution (0.05% Tween 20 in PBS containing 0.1% FCS) and blocked. After three washes with 0.05% PBT, the specimens were incubated with primary rabbit antibody against Bcl-6. They were washed three times with 0.05% PBT and incubated with secondary antirabbit antibody conjugated to Alexa 488 (Molecular Probes, Eugene, OR). After rinsing in PBS, the DNA was stained with 4',6-diamidino-2-phenylindole (Vysis, Downers Grove, IL).

**RESULTS**

**Somatic Mutations of Histone H4 and BCL6 Genes within the Regions Involved in t(3;6)(q27;p21).** We showed previously that the breakpoints in H4 of the five cases studied here were distributed within the single exon or close to the terminal palindromes, and those in BCL6 were localized in cases 229, 739, 764, and 897 or within ~0.5-kb downstream (case 457) of the translocation hypercluster (6, 12). We designed appropriate primer sets to amplify both H4/BCL6 and the reciprocal BCL6/H4 fusion sequences and studied whether somatic mutations were introduced into not only the BCL6 but also the H4 gene within these particular areas. Fig. 1 shows the nucleotide sequences of the five cases compared with the germ-line sequences of each gene. In case 229, sequences on der(6) were not amplified. At the H4/BCL6 and BCL6/H4 junctions, deletions of 11–23 nucleotides and duplications of 1–49 nucleotides of both genes were identified. In terms of somatic mutation of BCL6, case 457 carried a total of six single nucleotide alterations within the MMC. In the remaining four cases, one nucleotide substitution and one single nucleotide deletion were identified (Fig. 1B).

On the other hand, we found four single nucleotide substitutions within a 360-bp region of H4 (Fig. 1A); the mutation frequency was calculated as 0.28 × 10^-7/bp. To determine whether these mutations were of somatic origin and whether mutations are detectable in GC/post-GC stage lymphomas independently of t(3;6), we amplified and sequenced the H4 from 40 specimens, including lymphoblasticoid cell lines, FL and DLBCL cells that lacked H4/BCL6 fusion. The results showed that mutations were not detected in DNA from these specimens, indicating that the observed mutations were not attributable to polymorphisms. However, our study failed to provide conclusive evidence that somatic mutation of H4 can develop independently of the translocation. Another question is why t(3;6) does not involve H4 genes clustered in 6p21 except for this particular H4 gene. We sequenced another H4 gene (member H, accession no. X60487) of lymphoma specimens that included the current t(3;6) cases; no mutations were detectable in the member H H4 gene (data not shown). The mechanism for the restriction of the involvement of this particular member M H4 gene remains to be determined.

**Histone H4/BCL6 Fusion Transcripts Detected by RT-PCR.** To detect fusion mRNA containing both H4 and BCL6 messages, we designed a forward primer for the H4 exon and a reverse primer for BCL6 exon 3 (Fig. 2A). Total RNA extracted from cryopreserved tumor cells of cases 457 and 739 were subjected to RT-PCR analysis. As shown in Fig. 2B, amplified products of 640 and 620 bp, respectively, were obtained from these samples. Sequencing analysis of the PCR products revealed that the sequences of H4 were contiguous with the intron sequences of BCL6, which were then followed by the BCL6 exon 2 sequences (Fig. 2C). The two cryptic 5' splice junctions within the BCL6 intron 1 6/9 matched the consensus sequence (Fig. 2A).

**Level of BCL6 mRNA Expression in H4/BCL6-carrying Lymphoma Cells.** To determine whether t(3:6) affects the level of expression of BCL6, we measured BCL6 transcripts in lymphoma cells carrying t(3:6) using real-time quantitative RT-PCR (15). The BCL6 mRNA level of test materials was divided by that of the endogenous reference, GAPDH mRNA, and the BCL6-GAPDH ratios were further normalized with the corresponding ratio of Raji cells (15, 16). The values of cases 457 (FMIX), 739 (FLAR), and 764 (DLBCL) were 4.2, 0.7, and 1.3, whereas the mean values of primary tumors of FL (n = 12) and DLBCL (n = 21) were 7.7 (range, 0.7–22.6; median, 6.95) and 5.1 (range, 0.7–22.3; median, 3.8), respectively. We next performed RT-PCR using primer sets for BCL6 exon 1 (forward) and exon 4 (reverse) to determine whether the observed BCL6 mRNA of the three cases represented transcription not from germ-line BCL6 but from the H4/BCL6 allele. The results showed that none of these
mRNA preparations contained measurable amounts of normal BCL6 transcripts. These data suggest that H4/BCL6 fusion does not significantly increase the level of BCL6 expression in lymphoma cells but rather leads to lower levels than the mean values of the corresponding B-NHL subtypes.

**Construction of the H4/BCL6 Fusion Gene and Transient Expression of Bcl-6 in COS-7 Cells.** To estimate the promoter activity of the H4/BCL6 fusion gene compared with that of germ-line BCL6, we isolated the HindIII/BglII fragments of the H4/BCL6 fusion regions from cases 229 and 457 and cloned them into pGL3-Basic vector (Fig. 3A). The −1355/+273 and −621/+273 fragments of BCL6 were reported previously to carry essential promoter elements of the gene (17). Each construct was transiently transfected into COS-7 cells, and the luciferase activity was normalized by the cotransfected Renilla luciferase activity. As shown in Fig. 3B, the four promoters showed comparable levels of luciferase activity, which was expressed relative to that of the pGL3-Basic control.

We next constructed expression plasmids that mimicked the H4/BCL6 fusion gene and investigated whether the H4/BCL6 fusion affected the expression level of the BCL6 gene itself. Fig. 4A shows a schematic representation of the construct; the H4/BCL6 fusion sequences including the cryptic splice-donor site were prepared from case 457 DNA; the 5′ portion of the BCL6 cDNA sequence contained BCL6 exons 2 and 3, as well as their flanking sequences. Introduction of the plasmid into COS-7 cells resulted in transcription of H4/BCL6 fusion mRNA that had identical component regions to those of mRNA from 457 lymphoma cells (data not shown). This may be of functional
significance, because inclusion of intron sequences of BCL6 may have substantial impact on the stability of the fusion transcripts. In control constructs, the SV40 promoter or normal BCL6 regulatory region was placed at the position of the H4/BCL6 fusion sequences (Fig. 4A).

These plasmids were transiently introduced into COS-7 cells, and the whole-cell lysates of the cells were subjected to Western blot analysis. Blotting with anti-Bcl-6 monoclonal antibody showed a 4.3-fold larger amount of BCL6 in the whole-cell lysates of the transfected cells. As shown in Fig. 4C, H4/BCL6-transfected cells displayed bright nuclear staining with a characteristic punctate pattern. These findings clearly indicate that the H4/BCL6 gene fusion leads to enhanced Bcl-6 expression in transiently transfected COS-7 cells.

To confirm that the H4 regulatory sequences promote the high level expression of Bcl-6, we generated a series of deletion mutants that lacked the IRF-2 binding site or both the IRF-2 binding site and the Site II element; the latter region has been shown to be essential for transcriptional control of H4 (Fig. 4A; Ref. 14). Western blot analysis showed that the level of Bcl-6 expression was dramatically reduced to the basal level in the Site II-deleted transfectant (Fig. 4C). It is noteworthy that the sizes of the granules labeled by the anti-Bcl-6 antibody were in good accord with the amount of Bcl-6 protein detected by Western blot analysis, whereas the number of granules was not significantly affected (Fig. 4C).

**Atlas Array Analysis of H4/BCL6-transfected Cells.** We next introduced the Bcl-6 expression plasmids into human embryonic kidney fibroblast cell line 293T and found that the H4/BCL6 fusion construct also led to higher levels of Bcl-6 expression than the normal BCL6 promoter in human cells (data not shown). Because Bcl-6 is a transcriptional regulator, it seemed possible that the expression of target genes of Bcl-6 might be influenced in H4/BCL6-transfected cells. To test this hypothesis, we studied alterations of the gene expression profile of H4/BCL6-transfected 293T cells as compared with that of normal BCL6 promoter-transfected cells. 32P-labeled cDNA prepared from the transfectants were hybridized with the Atlas Human 1.2 Array, and the hybridization data were analyzed using the ArrayGauge software. Comparison of the PSL values representing the BCL6 mRNA level revealed that the H4/BCL6-transfected cells produced a 4.3-fold larger amount of BCL6 mRNA than the normal BCL6 promoter-transfected cells.

A scatter plot analysis (Fig. 5A), in which the data of normal BCL6 and those of H4/BCL6 were displayed on the X axis and Y axis, respectively, revealed that the scores of 224 of 1175 genes were below the 1.5-fold line, indicating that these genes were underexpressed in the H4/BCL6-transfected cells as compared with the normal BCL6-transfected cells. This observation is in agreement with the fact that Bcl-6 acts as a transcriptional repressor (18); indeed, the B-lymphocyte-induced maturation protein 1 (BLIMP1) and cyclin D2 (CCND2) genes, both of which have been shown to be primary target genes negatively regulated by Bcl-6 (19), fell below the line (Fig. 5A). In contrast, we found that the expression of 12 genes was increased >1.5-fold in the H4/BCL6-transfected cells (Fig. 5A). To confirm that the up-regulation of these genes paralleled the level of BCL6 expression, we compared the mRNA levels among the two transfectants and parental 293T cells by RT-PCR analysis. The results showed that, in these three types of cells, the mRNA levels of activating transcription factor 3 (ATF3), prefoldin 4 (PFDN4), and retinoblastoma-binding protein 7 (RBP7) were in concordance with those of BCL6 (Fig. 5B). In contrast, previously recognized target genes, including BLIMP1, CCND2, CD44, chemokine (C-X-C motif) receptor 4 (CXCR4), IFN-stimulated transcription factor 3, gamma (ISGF3G), and signal transducer and activator of transcription 1 (STAT1) were negatively regulated as reported previously (19). The PCR primers for each gene were as described in the information from Clontech.

**Discussion**

**Molecular Basis for the Development of H4/BCL6 Translocation.** Many studies have shown that the BCL6 in B-cell tumors is affected by two somatic events, i.e., chromosomal translocation and somatic mutation (6, 8, 9, 11). The majority of BCL6 translocations as
well as somatic mutations are clustered immediately 3’ of the non-coding exon 1, indicating that this region includes sequences susceptible to these two genetic alterations. We identified the ~0.2-kb translocation hypercluster, where 19 (36%) of 52 breakpoints cloned were localized (6). On the other hand, ~35% of somatic mutations from a compilation of published lymphoma cases occurred within the hypercluster, and some of the positions of somatic mutation matched those of the translocations (20). Thus, it is likely that the two processes are mediated by the same molecular mechanism.

Because somatic hypermutation of BCL6 shares many features with that of the variable (V) region of Ig, the Ig-somatic hypermutation machinery is likely to target BCL6 (8). However, the mechanism of somatic hypermutation is still unknown. Two studies using ligatlon-mediated PCR showed the presence of DSBs in the IgVH4 and IgVH6 genes of Ramos Burkitt’s lymphoma cells as well as the targeted V(1)B1–8 gene of GC-B-cells isolated from the V(1)B1–8 IgH knock-in mouse strains (21, 22). 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 implicated in the process of somatic hypermutation.

In the present study, to elucidate whether the hypermutation mechanism is also functioning in the non-Ig partner gene and involved in the development of translocation, we cloned and sequenced the H4 gene affected by the t(3;6) translocation. The remarkable findings included that the positions of breakpoints and somatic mutations were both distributed from 200 to 460 bp downstream of the H4 promoter, in agreement with the distribution pattern of IgV mutations and BCL6 mutations/translocations. It is of further interest that some of the positions were within or close to the terminal palindromic sequence, which can be targeted by the somatic hypermutation (23). The deletions or duplications of variable numbers of nucleotides at the breakpoints may be comparable with those observed in IgV genes (24). On the other hand, we found a total of 11 RGYW/WRCY motifs within the 360-bp region affected by the mutation/translocation (Fig. 1A); however, there was no apparent association between these motifs and mutation/translocation. These observations suggest that the somatic hypermutation machinery most likely targets H4, predisposing this region to the development of translocation with BCL6, although not all features described in the IgV hypermutation were not identified.

Deregulated Expression of BCL6 Resulting from H4/BCL6 Gene Fusion. Transcriptional control of the histone H4 gene is mediated by two multipartite proximal promoter elements (Sites I and II), the activity of which is assisted by two distal domains (Sites III and IV; Ref. 13). The Site II equivalent of the H4 gene studied here contains consensus binding sites for IRF-2/HnIF-M, CDP-cut/ HnIF-D, and HnIF-P transcription factors (25, 26). These Site II binding proteins, in addition to other coregulatory molecules, contribute to enhance the H4 gene transcription at the G1-S-phase transition (14, 25, 26). In the present study, we introduced a Bcl-6 expression plasmid that mimicked the H4/BCL6 fusion gene into transformed cells and found high levels of Bcl-6 expression in transfected cell nuclei. When the plasmid lacked the Site II sequence, the expression levels were dramatically reduced. Thus, the expression of BCL6 on the t(3;6) allele is likely to be regulated by the juxtaposed H4 promoter.

It should be noted that four breakpoints (cases 229, 457, 764, and 897) of H4 eliminate the terminal palindrome sequence, which is critical for 3’ end processing, and the fifth (case 739) eliminates the U7 snRNP sequence ~16-bp downstream of the palindrome. Therefore, the mechanism of 3’ end formation is perturbed, and all of the resulting H4/BCL6 fusion mRNAs are predicted to be processed like normal polyadenylated mRNAs. It appears then that the deregulation of Bcl-6 protein expression is facilitated by “capturing” sequence that support cell cycle control of histone gene transcription during cell cycle while simultaneously inactivating the regulatory sequences required for posttranscriptional control of histone gene expression.

In contrast, lymphoma cells carrying an H4/BCL6 fusion gene only expressed moderate levels of BCL6 mRNA. Immunohistochemical analysis of lymphoma tissues has revealed that the level of Bcl-6 protein expression is not related to the presence or absence of BCL6 gene rearrangement (27). We recently found that BCL6 mRNA levels in non-Ig/BCL6 DLBCLs were significantly lower than those in Ig/BCL6 DLBCLs (16). These observations suggest that persistent high-level BCL6 mRNA and protein expression may not be required to maintain the malignant phenotype of lymphoma cells with non-Ig/BCL6. It is presumed that the BCL6 gene of a B-cell carrying a non-Ig/BCL6 translocation is overexpressed in the GC microenvironment, thereby triggering malignant transformation. However, such transcriptional activation would be transient, and once the B-cell gains a growth advantage over normal cells, the expression would be down-regulated. Additional studies are needed to elucidate the mechanistic details of the transcriptional control of BCL6 in lymphoma cells.

t(3;6)(q27;p21) Can Alter Gene Expression Profiles. The Bcl-6 protein is a sequence-specific transcriptional repressor that contains two identified functional domains, i.e., the COOH-terminal zinc finger motifs and the NH2-terminal POZ domain (18). The protein is distributed in the nucleus in a diffuse microgranular fashion, and overexpression of Bcl-6 by transient transfection into NIH3T3, COS, and HeLa cell lines leads to enlargement of the granules, producing punctate structures (28). Although the composition of the granules is not yet fully understood, the granules have been shown to include SMRT and N-CoR corepressors (29). In the present study, we showed that the nuclei of H4/BCL6-transfected cells contained large granular structures labeled by anti-Bcl-6 antibody, and many genes were down-regulated in response to the Bcl-6 overexpression, suggesting that these structures may represent multisubunit repressor complexes.

On the other hand, the discordance between the promoter activity of the H4/BCL6 fusion gene measured by the luciferase assay and the level of Bcl-6 expression driven by the H4 promoter raised the possibility that Bcl-6 itself and/or its downstream proteins can enhance the activity of the H4 promoter, leading to the higher level of Bcl-6 expression. We performed a computer-assisted search for the binding sequences of Bcl-6 (18) in the H4 promoter region; however, no matching sequences were identified. Thus, Bcl-6 may indirectly affect the H4 promoter activity through Site II factors. A cDNA array analysis showed that a set of genes, including transcriptional factors ATF3 and PFDN4, was up-regulated in response to the transient induction of Bcl-6 expression. It remains to be determined whether these factors can bind to the Site II or act as coregulatory molecules and whether a positive feedback loop is responsible for the high level expression of Bcl-6. Of course, other genes that are not contained in the Atlas array may be involved in this circuit, and Bcl-6 may also control induction of Bcl-6 expression. It remains to be determined whether these factors can bind to the Site II or act as coregulatory molecules and whether a positive feedback loop is responsible for the high level expression of Bcl-6. Of course, other genes that are not contained in the Atlas array may be involved in this circuit, and Bcl-6 may also control induction of Bcl-6 expression.

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