The BCL11 gene family: involvement of BCL11A in lymphoid malignancies

Ed Satterwhite, Takashi Sonoki, Tony G. Willis, Lana Harder, Rachael Nowak, Emma L. Arriola, Hui Liu, Helen P. Price, Stefan Gesk, Doris Steinemann, Brigitte Schlegelberger, David G. Oscier, Reiner Siebert, Philip W. Tucker, and Martin J. S. Dyer

Many malignancies of mature B cells are characterized by chromosomal translocations involving the immunoglobulin heavy chain (IGH) locus on chromosome 14q32.3 and result in deregulated expression of the translocated oncogene. t(2;14)(p13;q32.3) is a rare event in B-cell malignancies. In contrast, gains and amplifications of the same region of chromosome 2p13 have been reported in 20% of extranodal B-cell non-Hodgkin lymphomas (B-NHL), in follicular and mediastinal B-NHL, and in Hodgkin disease (HD). It has been suggested that REL, an NF-κB gene family member, mapping within the amplified region, is the pathologic target. However, by molecular cloning of t(2;14)(p13;q32.3) from cases of aggressive B-cell chronic lymphocytic leukemia (CLL)/immunocytoma, this study has shown clustered breakpoints on chromosome 2p13 immediately upstream of a CpG island located about 300 kb telomeric of REL. This CpG island was associated with a Krüppel zinc finger gene (BCL11A), which is normally expressed at high levels only in fetal brain and in germinal center B-cells. There were 3 major RNA isoforms of BCL11A, differing in the number of carboxy-terminal zinc fingers. All 3 RNA isoforms were deregulated as a consequence of t(2;14)(p13;q32.3). BCL11A was highly conserved, being 95% identical to mouse, chicken, and Xenopus homologues. BCL11A was also highly homologous to another gene (BCL11B) on chromosome 14q32.1. BCL11A coamplified with REL in B-NHL cases and HD lymphoma cell lines with gains and amplifications of 2p13, suggesting that BCL11A may be involved in lymphoid malignancies through either chromosomal translocation or amplification.

Introduction

Many subtypes of malignancy are associated with specific chromosomal translocations, which play a pivotal role in the pathogenesis of disease. In the leukemias and lymphomas of mature B-cells, these frequently involve the immunoglobulin (IG) loci and result in deregulated expression of the translocated oncogene, due, in part, to the presence of potent B cell–specific transcriptional enhancers within the IG loci. All the common IG translocations have been cloned. Paradigms include the deregulation of cyclin D1 by t(11;14)(q13;q32.3), found in all cases of mantle cell lymphoma; BCL2 by t(14;18)(q32.3;q21.3), found in 80% of follicular lymphoma; and MYC by t(8;14)(q24.1;q32.3) and variant translocations in all cases of Burkitt lymphoma.

On the basis of cytogenetics alone, several rare, but nonetheless recurrent IG translocations remain to be cloned, principally in aggressive large-cell B-NHL; their molecular cloning continues to allow the isolation of novel dominant oncogenes and to define new pathogenic mechanisms. Chromosomal translocation t(2;14)(p13;q32.3) is one example and has been reported in a variety of B-cell malignancies ranging from B-cell precursor acute lymphoblastic leukemia to myeloma. This translocation is frequently the sole cytogenetic abnormality within the neoplastic clone (Watson et al.), Geisler et al., Sonoki et al., and http://cgap.nci.nih.gov/Chromosomes/Mitelman). We report here the recurrent involvement and deregulated expression of a Krüppel zinc finger gene, BCL11A, in 4 cases of B-cell malignancy with t(2;14)(p13;q32.3).
progressive disease in October 1995. Cyto genetics of this case showed 46,XX,t(2;14)(p13;q32)(14)46,ident,t(3;6)(p21q25),del(11)(q22q23)(2)/
46,ident.add(5)(p23)[2] indicating that t(2;14)(p13;q32) was the primary
cytogenetic abnormality. Patient 4 presented in February 1986 with
generalized lymphadenopathy, hepatosplenomegaly, and a white blood cell
count of 38.3 × 10^9/L with 84% lymphocytes. The clinical diagnosis of
CLL was established. However, a lymph node biopsy was consistent with a
lymphoplasmacytic immunocytoma according to the Kiel classification
with increased proliferation activity and monoclonal IgM kappa expression.
Cytogenetic analysis of both the lymph node specimen and of the peripheral
blood showed the karyotype: 46,XY,t(2;14)(p13;q32),t(18;21)(p11;q21). In
February 1992, the patient presented with clinical progression including
lymphadenopathy, B-symptoms, and a WBC of 76.5 × 10^9/L with 85% lymphocytes. Histopathology of a repeat lymph node biopsy again revealed
lymphoplasmacytic immunocytoma with increased proliferation activity.
Chromosome analyses showed the tumor cells to contain the karyotype:
46,XY,t(2;14)(p13;q32),t(18;21)(p11;q21)(6)/46,ident,t(13;15)[q12–13; q21][3]. The patient died in 1999 due to progressive disease.

B-NHL and HD cell lines

The Wien 133 B-NHL cell line used in this study has been described.19 The
NAB-2 Burkitt lymphoma cell line13 was kindly provided by Dr N Popescu,
(National Institutes on Health [NIH], Bethesda, MD). The Raji B-NHL and
the HD cell lines as well as the HEK293 human fibroblast cell line were
obtained from the German Collection of Microorganisms and Cell Cultures
(DSMZ), Braunschweig, Germany (Drexler14 and http://www.dsmz.de) or
from Dr Stefan Joos, German Cancer Research Center (DKFZ), Heidelberg, Germany.

Long-distance inverse polymerase chain reaction

Long-distance inverse polymerase chain reaction (LDI-PCR) for rearrange-
ments involving the IGHS segments was performed as previously de-
scribed.15 To amplify translocations involving the IGHS regions, primers
were designed to amplify rearrangements of 5′ switch μ (Sμ). DNA was
template DNA, 0.5 mM CTP, 0.5 mM GTP, 0.5 mM ATP, 0.005 mM UTP,
incubated with 1 hour in a 20-μL reaction containing 1 μg
digested to completion with
DNA was

Radiation hybrid mapping

Radiation hybrid (RH) mapping was performed with DNA primers 669
(5′-AAATGGAGAGGCCACAGGCAAGGTTT-3′) and 670 (5′-CTCGAGAAGA-
GGGAGGAG-3′) as well as 673 (5′TTCCCTAACAGCACCACCACACAT-3′) and
674 (5′-GCAGGGCCGCTTGTATCTC-3′) derived from the breakpoint
sequence of the cloned LDI-PCR fragment by means of the
Stanford G3 Panel (Research Genetics, Huntsville, AL). Map localization
was calculated on the Stanford Human Genome Center RH server
(http://www.shgc.stanford.edu).

Southern and Northern blotting

Southern and Northern blotting were carried out as described previ-
ously.1,16,17 EH3.0p was a 940-bp fragment from clone EH3.0.16,3′Sy was
a PCR product as previously described.18 The cDNA probe for BCL11A was
digested to completion with HindIII and ligated at low concentration
followed by nested PCR using the following primers: SAE 5′-GGACTCAGA
TGGGCAAAAC-TGAC-

Fluorescence in-situ hybridization

Bacterial artificial chromosome (BAC) and cosmid DNA and Alu-PCR
products from yeast artificial chromosome (YAC) clones were labeled with
biotin or digoxigenin using a random prime labeling kit (Life Technologies,
Gaithersburg, MD) and fluorescence in-situ hybridization (FISH) was
washed with 2× SSPE at 60°C.

In-situ hybridization studies

The 5′ 341 bp of BCL11A common to all 3 RNA isoforms was cloned into
cDNA cloning was performed in pcRII (Invitrogen, San Diego, CA), then amplified from this vector by PCR
using M13 forward and reverse primers. A probe was generated from the
PCR product by incubation for 1 hour in a 20-μL reaction containing 1 μg
template DNA, 0.5 mM CTP, 0.5 mM GTP, 0.5 mM ATP, 0.005 mM UTP,

5′-TAATGAGAGGCCACAGGCAAGGTTT-3′, SAI 5′-GAAAATTAACCGAGT-

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molecular cloning of \( t(2;14)(p13;q32.3) \) translocation breakpoints

Four cases with \( t(2;14)(p13;q32.3) \) were studied; 3 cases exhibited \( t(2;14)(p13;q32.3) \) as the sole or primary cytogenetic abnormality. Cloning of 2 cases of pediatric CLL was performed using conventional bacteriophage cloning and has been reported previously. In both cases the \( IGH \) breaks fell within the \( IGS \) region. The third case, of leukemic transformation of immunoblastic lymphoma (patient 4), which exhibited \( t(18;22)(p11;q11) \) in addition to \( t(2;14)(p13;q32.3) \), was studied using LDI-PCR methods. Two rearranged \( IGH \) alleles were observed on Southern blotting (data not shown); both were amplified, cloned, and sequenced. Neither contained the translocation breakpoint (T.S. et al, unpublished data, December 1999).

These data therefore raised the possibility that the \( IGH \) break might fall within the switch \( (IGHS) \) regions. There are 9 \( IGH \) regions. To clone all possible \( IGHS \) translocations using LDI-PCR would require a large number of primer pairs. In an attempt to simplify the process we devised an LDI-PCR method designed to amplify the other derivative chromosome. Briefly, in cases of \( IGHS \) translocations where both derivative chromosomes are retained, so long as the translocation occurred after regular \( IG \) class-switching, it should be possible to LDI-PCR amplify from the translocated \( 5'S\mu \) sequence onto the other derivative chromosome, since the \( 5'S\mu \) sequence should remain intact. The strategy for cloning such translocations from \( 5'S\mu \) is depicted schematically in Figure 1A.

PCR primers were designed to this region and in an attempt to amplify the der(2;2)(2;14)(p13;q32.3) from patient 4. High-molecular-weight DNA was digested with \( \text{HindIII} \) and ligated overnight to promote circularization of DNA fragments after restriction endonuclease inactivation. Using \( 5'S\mu \) primers, a product of 1.6 kb was obtained, cloned, and sequenced (Figure 1B). Sequence analysis showed loss of homology with \( IG \) sequences beyond \( S\mu \) and identity to a partially sequenced BAC clone RP11-440P05. Comparison of the derived sequence showed that this break fell in close proximity to those previously described for the 2 cases of pediatric CLL (Figure 1C). To confirm that no artifacts had been introduced during the LDI-PCR, cloning, and sequencing, Southern blot with probes spanning the \( IGH \) locus and a single-copy probe from the translocation breakpoint showed comigration of rearranged \( IGH \) and 2p13 fragments (data not shown).

To confirm that the novel sequence was genuinely derived from chromosome 2p13, RH mapping was performed. According to RH mapping, the breakpoint sequence was closely linked (0 cRs; LOD 6.88) to marker SHGC-21466 (AFM126y1, D2S2160). This marker is located 84.0 cM from top of chromosome 2 according to the genetic map (http://carbon.wi.mit.edu:8000), which refers approximately to 2p12 to 16 in the cytogenetic map (http://www.ncbi.nlm.nih.gov/genemap99). In addition, BAC clones containing the breakpoint were mapped to chromosome 2p13 on metaphase spreads from healthy individuals and from patients with \( t(2;14)(p13;q32) \) by FISH. Results for a single BAC clone are shown in Figure 2A. In metaphase and interphase preparations from patient 4, BAC clones were shown to span the translocation breakpoint (Figure 2B). In patient 3, an adult with rapidly progressive and chemotherapy-resistant CLL in which \( t(2;14)(p13;q32.3) \) was the primary cytogenetic abnormality, it was not possible to amplify the translocation breakpoint using the same LDI-PCR method. However, this case also showed split of the FISH probe for the \( BCL11A \) locus as well as rearrangement and comigration of the 2p13 probe on Southern blot, indicating that the 2p13 breakpoint also fell within the same cluster region (Figure 2C.D).

**Identification of \( BCL11A \)**

Comparison of the breakpoint sequences from the 3 cloned cases showed that all were clustered immediately centromeric to a CpG island associated with the 5’ end of a gene. Due to the direct involvement of this gene in 4 cases of B-cell malignancy with \( t(2;14)(p13;q32.3) \), we termed this gene \( BCL11A \) (B-cell lymphoma/leukemia 11A). An ideogram of the structure of the translocation and the \( BCL11A \) transcripts is shown in Figure 3A. In B-NHL, \( IG \) chromosomal translocation breakpoints frequently fall in the vicinity of CpG islands. CpG islands are associated with genes, which are often expressed in a tissue-specific fashion. To identify the associated gene, a combination of extensive cDNA library screening using derived genomic probes was used; probing a fetal brain cDNA library with genomic probes adjacent to the CpG island allowed the identification of the 5’ end of the gene. Additionally, genomic and EST DNA database searching (http://www.ncbi.nlm.nih.gov/dbEST/), Northern blotting, and 3’ reverse transcriptase (RT)–PCR methods were used to define the full-length transcripts. A longest transcript of 5941 bp was identified, containing an open-reading frame of 835 amino acids, with a predicted molecular weight of 91.3 kd. The predicted amino acid sequence is shown in Figure 3B.

Most of the \( BCL11A \) gene sequence was contained within BAC RP11-158I21. Comparison of the cDNA and genomic sequences showed the presence of 5 exons. The structure of the translocation was a “head-to-head” arrangement, with the breakpoints falling centromeric to the first exon (Figure 3A). In the 2 cases with \( t(2;14)(p13;q32.3) \) of pediatric CLL in which there was adequate material, Northern blotting showed overexpression of this gene in comparison with other normal and malignant lymphoid tissues (Figure 4B and data not shown). Neither of these 2 cases showed \( REL \) overexpression on Northern blot. Given the clear overexpression of \( BCL11A \) in cases with \( t(2;14)(p13;q32) \) and the close physical association of the gene to the translocation breakpoints, we now consider the 2.85-kb transcript originally reported to be involved in this translocation to have been artificial; this transcript does not appear to represent any recognized RNA isoform of \( BCL11A \).

**BCL11A RNA and protein isoforms**

To determine the patterns of expression of \( BCL11A \), Northern blotting, in-situ hybridization, and database searching were performed. Northern blotting showed low-level or undetectable \( BCL11A \) RNA expression in most adult tissues. Among adult tissues, highest levels of expression were seen in normal lymph node, thymus, and bone marrow, although levels of expression were low (Figure 4A); the predominant, if not exclusive \( BCL11A \) RNA isoform was the 5.8-kb transcript. Some developmental
stages of fetal brain showed levels comparable to those seen in leukemias with t(2;14)(p13;q32.3), also Figure 4B and data not shown). BCL11A is represented by the Unigene cluster Hs.130881 (http://www.ncbi.nlm.nih.gov/UniGene/index.html). Among the 114 EST clones represented by this cluster, 32% were derived from germinal center B-cell or CLL cDNA libraries, 16% from fetal brain, and 16% from fetal and adult lung. EST clones corresponding to the 3' UTR of the gene were included on the "lymphochip"22; analysis of these data showed that BCL11A RNA is expressed in germinal center B cells and is down-regulated in response to anti-Ig stimulation (http://lmp.p3.nih.gov/lymphoma). To confirm expression of BCL11A within the germinal center, in-situ hybridization using antisense RNA probes was performed (Figure 4C). Staining with the antisense probe was seen only within the germinal center and not in the adjacent mantle zone, indicating tightly regulated expression of BCL11A during B-cell development.

In both normal fetal brain and malignant B cells with t(2;14)(p13;q32.3), 3 major BCL11A transcripts of 5.8 kb, 3.8 kb, and 1.5 kb were observed. In the 2 pediatric CLL cases with t(2;14)(p13;q32) that were studied, there was overexpression of all 3 isoforms.
Figure 4. BCL11A RNA expression in fetal, adult, and malignant tissues. (A) Low-level expression of BCL11A RNA in a range of normal adult lymphoid tissues and fetal liver (Clontech Immune System MTN blot; 2 μg poly[A−] mRNA per lane; exposure time, 10 days). A low-level BCL11A transcript of 5.8 kb was seen, with highest levels present in normal lymph node. (B) High-level BCL11A expression was seen only in fetal brain (CNS d117) and in patient AS with t(2;14)(p13;q32.3). Predominant BCL11A transcripts of 5.8 kb, 3.5 kb, and 1.5 kb were seen in cases with t(2;14)(p13;q32.3) and in fetal brain. Otherwise, note low-level expression of BCL11A in malignant B-cell lines (NAB-2 and Granta 452) and in human embryonic kidney fibroblast cell line, HEK-293. Loading control denoted by reprobing of same filters with glyceraldehyde phosphate dehydrogenase (GAPDH) probe shown below. (C) BCL11A mRNA accumulates in germinal center (GC) but not follicular mantle (FM) B cells. Antisense (i) and sense (ii) 35S-labeled riboprobes were hybridized to frozen sections of normal human tonsils, or a serial section (iii) was weakly stained with Giemsa to visualize the boundary of the high cellularity FM area. GC cells but not mantle cells exhibited BCL11A expression.

Figure 5. Sequence comparisons of BCL11A, Evi9, and BCL11B. (A) Comparison of BCL11A previously isolated mouse Evi9 isoforms, and BCL11B. C2H2 zinc fingers are denoted by shaded boxes, the acidic region is denoted by a hatched box. The positions of the first and last amino acids of C2 H2 zinc fingers are shown underneath. Note lack of last 3 zinc fingers in the longest mouse Evi9 isoform and close similarities in the overall structure of BCL11A and BCL11B. (B) BCL11A isoforms. Proteins of 835 (BCL11AXL, accession no. AJ404611), 773 (BCL11AX, accession no. AJ404612), and 243 (accession no. AJ404613) amino acids resulting from the common alternative splices. Dots represent identical amino acid residues. RT-PCR analysis, but were less frequent (data not shown). We termed the 5.8 kb, 3.8 kb, and 1.5 kb RNA isoforms BCL11AXL, BCL11AX, and BCL11A, respectively. The overall structure and the predicted amino acid sequences of the splice variants BCL11AXL and BCL11AX, representing the 3.8-kb and 1.5-kb transcripts are shown in Figure 5A-B. Normal B-cell populations expressed the 5.8-kb BCL11AXL isoform preferentially, whereas the BCL11AX isoform was expressed preferentially in derived B-cell malignant cell lines (Figure 4A,B and Figure 7). The possible significance of this observation is not clear.

BCL11AXL contained 6 Krüppel C2H2 zinc fingers as well as a proline-rich domain between zinc fingers 1 and 2 and an acidic domain between 3 and 4 containing a run of 21 consecutive acidic residues. The zinc fingers showed homology to each other. Zinc fingers 1 and 6 were different from 2, 3, 4, and 5 in that they had 4 amino acids separating the 2 zinc-binding histidines, whereas 2, 3, 4, and 5 had 3 amino acids. The internal zinc fingers (2, 3, 4, and 5) were arranged in pairs, each pair being separated by a canonical “linker” sequence; these pairs were nearly duplicated, with 37 of 49 amino acids being identical. The alternative isoforms showed alterations in the carboxy-terminus and thus in the terminal zinc fingers (Figure 5A,B).

Conservation of BCL11A and identification of a homologue (BCL11B) on chromosome 14q32.1

BCL11A showed a high level of conservation across a wide range of species. BCL11A is the human homologue of mouse Evi9, being 94% identical at nucleotide levels, and 98% identical at protein levels. Evi9 was isolated in a search for murine leukemia genes using proviral integration. The same mouse gene has been isolated as an interacting partner (CTIP-1) of the orphan nuclear receptor COUP-TF2. Like BCL11A, 3 common isoforms Evi9/CTIP-1 were identified. However, the mouse and human isoforms did not correspond exactly. The intermediate splice form reported for Evi9 has not been seen in humans (Figure 5A). Rat, chicken, Xenopus, and zebrafish BCL11A homologues have also been identified (data not shown).

Apart from being evolutionarily conserved, database analysis showed a human homologue of BCL11A mapping to chromosome 14q32.1. This gene (BCL11B) was 67% identical to BCL11A at the nucleotide level and 61% identical overall at the protein level. BCL11B, like BCL11A, contained 6 C2H2 zinc fingers and proline-rich and acidic regions with 95% identity in the zinc finger domains (Figure 6). Like BCL11A, BCL11B showed a high level of conservation across a wide range of species. BCL11A is the human homologue of mouse Evi9, being 94% identical at nucleotide levels, and 98% identical at protein levels. Evi9 was isolated in a search for murine leukemia genes using proviral integration. The same mouse gene has been isolated as an interacting partner (CTIP-1) of the orphan nuclear receptor COUP-TF2. Like BCL11A, 3 common isoforms Evi9/CTIP-1 were identified. However, the mouse and human isoforms did not correspond exactly. The intermediate splice form reported for Evi9 has not been seen in humans (Figure 5A). Rat, chicken, Xenopus, and zebrafish BCL11A homologues have also been identified (data not shown).
preferentially in malignant T-cell lines derived from patients with adult T-cell leukemia/lymphoma; the possible pathologic significance of this observation is not clear. **BCL11B** was not expressed at detectable levels by Northern blot in any malignant B-cell lines examined (data not shown).

**Involvement of BCL11A in B-NHL and HD cell lines with abnormalities of chromosome 2p13**

Both **BCL11A** and **BCL11B** map to regions of recurrent cytogenetic abnormality in lymphoid malignancies. Amplifications and gains of 2p13 have been commonly detected, not only in various subtypes of B-NHL, including 20% of aggressive extra-nodal and mediastinal B-NHL but also in 50% of primary cases of HD.26-31 **REL** was amplified in all these B-NHL subtypes, although the pathologic consequences remain to be determined. In the BAC contig of the region, **BCL11A** mapped closely telomeric (about 300kb) of **REL** (HFPCctg13617; http://genome.wustl.edu, NCBI homo sapiens chromosome 2 working draft sequence segment NT_005399), and was shown to be coamplified with **REL** by FISH in primary material from a panel of patients with B-NHL (Figure 7A and data not shown). Unfortunately, there was no suitable material available from any of these cases for RNA analysis. Gains of chromosome 2p13 have recently been described in up to 50% of primary cases of HD.30 In line with these observations, supernumerary copies of the **BCL11A** locus, including high-level amplifications, were detected in primary HD disease cases (Martin-Subero et al, unpublished data, May 2001). Moreover, of 6 HD cell lines examined, L428 and KM-H2 exhibited overexpression of **BCL11A** by Northern blot (Figure 7B). All 3 isoforms were expressed co-ordinately in KM-H2, whereas L428 showed high-level expression of only **BCL11A**L. In contrast, the 4 other HD and other B-NHL cell lines exhibited low-level expression of all 3 **BCL11A** isoforms (Figure 7B and data not shown).

**Discussion**

The molecular cloning of **IG** translocation breakpoints allows the identification of genes that play an important role in the genesis of normal and malignant B-cells. Chromosomal translocation...
t(2;14)(p13;q32.3) occurs as the sole cytogenetic abnormality in a rare but clinically aggressive subset of CLL/immunocytoma, suggesting that deregulated expression of BCL11A may play a major and primary role in the pathogenesis of this disease. Whether similar deregulated expression occurs by other mechanisms in other cases of CLL lacking the translocation is currently under investigation. However, the identification of BCL11A is of interest for several additional reasons. First, BCL11A is the homologue of the murine gene Ev9 that was isolated in a search for dominant transforming oncogenes using retroviral insertional mutagenesis. This gene was found to be deregulated in 2 of 205 myeloid leukemias induced in the BX2H mouse strain following proviral integration within the first intron of Ev9.23,24 These data strongly implicate Ev9 as a dominant oncogene. Some derived leukemias lacking Ev9 proviral integration nevertheless showed high-level Ev9 expression (Figure 1C and Nakamura et al24) suggesting that other mechanisms may drive deregulated expression of Ev9. It was subsequently shown that 2 of the 3 Ev9 isoforms (Ev9a and Ev9c) but not the intermediate form Ev9b, were capable of inducing in vitro anchorage independence of murine NIH3T3 fibroblasts.24 While this manuscript was in preparation, the same group reported the isolation of some isoforms of the human homologue via homology with the mouse gene and showed that human Ev9/ BCL11A was expressed in CD34+ myeloid precursors and down-regulated in retinoic acid–induced differentiation of HL-60 cells.32 Second, some but not all isoforms of murine Ev9 were capable of interacting directly with BCL6.23,24 We have confirmed these observations with BCL11A and have shown additionally that BCL11A is a DNA sequence–specific transcriptional repressor (H.L. et al, unpublished data). Finally, BCL11A is also of interest as it may be another target gene for amplifications and gains of chromosome 2p13 in B-cell malignancies and HD. The true frequency of these amplifications is not clear as they may occur in the absence of changes detectable by comparative genomic hybridization (CGH), but they have been detected in B-NHL of various histologic subtypes as well as HD. Taken with the data from the t(2;14)(p13;q32.3) translocation, the close linkage of BCL11A with REL on chromosome 2p13 and the coamplification of the 2 genes, suggest that deregulated expression of BCL11A may have a role in the pathogenesis of divergent subtypes of aggressive human B-NHL and HD. Studies on the relative expression of BCL11A and REL in primary cases of B-cell malignancy and HD are currently being undertaken, but it is difficult to demonstrate this point directly in many cases as RNA is often not available. Detection of BCL11A expression in HD may be of particular value, since there is currently a lack of genetic markers for this disease.33 BCL6 expression in HD defines a distinct subset of disease.34 The possible diagnostic and prognostic significance of BCL11A overexpression in both HD and B-NHL, and the possible correlation with BCL6 expression, await the development of antibodies suitable for use in paraffin sections. Whether BCL11B is targeted in lymphoid malignancies such as adult T-cell leukemia, where translocations and amplifications of 14q32.1, which do not involve the TCL1 gene complex and which lie about 4 megabases (Mb) centromeric of BCL11B, are currently under investigation.35

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