A rapid communication

Molecular Cloning of Translocation t(1;14)(q21;q32) Defines a Novel Gene (BCL9) at Chromosome 1q21


Abnormalities of chromosome 1q21 are common in B-cell malignancies and have been associated with a poor response to therapy. The nature of the involved gene(s) on chromosome 1q21 remains unknown. A cell line (CEMO-1) has recently been established from a patient with precursor-B-cell acute lymphoblastic leukemia (ALL), which exhibited a t(3;14)(q21;q32). To identify the gene involved in this translocation, we have cloned both rearranged IGHI alleles using long-distance inverse polymerase chain reaction (LDI-PCR). Two IGHI fragments were amplified from CEMO-1 DNA and sequenced. One allele showed novel sequences upstream of J H5 with no homology to either IGH or any other sequences on the databases.Using a single-copy XhoI fragment immediately 5’ of J H5, PAC clones were isolated and mapped to chromosome 1q21 on normal metaphases by fluorescence in situ hybridization (FISH), confirming that this allele represented the t(1;14)(q21;q32) breakpoint. Sequence analysis of the 1q21 XhoI fragment showed identity with an expressed sequence tag (EST), and this probe was therefore used to probe Northern blots. Two transscripts of 6.3 kb and 4.2 kb expressed at low level in mRNA from all tissues were detected; a third transcript of 1.6 kb was expressed only in thymus, spleen, and small intestine. Full-length BCL9 cDNA clones were obtained from a normal human fetal brain cDNA library supplemented by 5’ and 3’ RACE. Sequence analysis predicted a protein of 1394 amino acids containing 18% proline, 11% glycine, 11% serine, and 6% methionine, but no recognizable protein motifs or significant homologies to any other known proteins. The CEMO-1 1q21 breakpoint fell within the 3’ UTR of the BCL9 gene. Low-level expression of BCL9 was detected in Epstein-Barr virus-transformed normal B cells by Northern blot; in contrast, abundant BCL9 expression was observed in CEMO-1, indicating that deregulated expression of this gene was one pathological consequence of the translocation. Screening of a panel of 39 B-cell malignancies with 1q abnormalities by Southern blot showed one additional case with a breakpoint in the 3’ UTR of BCL9, indicating that this was a recurrent breakpoint. FISH analysis using an 850-kb YAC spanning BCL9 identified a further case with t(1;22)(q21;q11) causing juxtaposition of BCL9 to the IGH locus. Other breakpoints were heterogeneous, falling both centromeric (10 cases) and telomeric (10 cases) of the BCL9 gene. These data suggest that BCL9 may be the target of translocation in some B-cell malignancies with abnormalities in 1q21 and that deregulated BCL9 expression may be important in their pathogenesis.

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which, after the MYC translocations, is the second most common cytogenetic abnormality found in BLs, occurring in greater than 30% of cases. However, no oncogene involved in lymphoid malignancy has yet been identified within this region. A new pre-B acute lymphoblastic leukemia (ALL) cell line, CEMO-1 (elg7+/slg+ /CD19+/CD20+ /CD22+/CD34+), was recently established. Both the original patient material and cell line initially exhibited t(1;14)(q21;q32) as the sole cytogenetic abnormality, although with continued growth in vitro the cell line developed the additional translocation, t(9;9)(p24;q32).

We report here the molecular cloning using LDLI-PCR of the t(1;14)(q21;q32) in the CEMO-1 cell line, in which IGHIJ had become juxtaposed to novel sequences on 1q21, and the isolation of a new gene (BCL9) of unknown functions. We have also shown that translocation breakpoints in some B-NHL cases with 1q21 breakpoints were located in close proximity to BCL9. In addition, we have found evidence of deregulated overexpression of BCL9 in the CEMO-1 cell line, suggesting that overexpression of BCL9 may be of pathogenic significance in a proportion of B-cell malignancies with breakpoints at 1q21.

### MATERIALS AND METHODS

**Cell lines, cell culture, and cytogenetics.** The CEMO-1 cell line was derived from the peripheral blood of a previously healthy 30-year-old man with precursor B-cell ALL. He achieved complete remission after intensive induction therapy, but sustained a bone marrow relapse 3 months later and died of progressive disease. Cytogenetic analysis of the original patient material and the cell line initially showed the t(1;14)(q21;q32) as the sole cytogenetic abnormality, although with continued growth in vitro the cell line developed a further t(9;9)(p24;q32).

**DNA sequencing.** Automated sequencing was performed using an ABI PRISM automated sequencer and AmpliTaq polymerase, FS (Perkin Elmer, Foster City, CA). Sequences were checked against the Genbank and EMBL databases using BLAST and FASTA programs made available through the Human Genome Mapping Project Resource Centre (HGMP-RC; Hinxton Hall, Cambridge, UK). Larger clones were sequenced by primer walking or by subcloning into pBluescript (Stratagene, La Jolla, CA).

**Isolation of PAC and YAC clones and fluorescence in situ hybridization (FISH).** PAC and YAC clones containing BCL9 sequences were isolated by radioactive screening using BCL9 cDNA probes from gridded PAC and YAC libraries provided by the HGMP-RC. DNA was extracted from PAC and YAC clones according to standard techniques. FISH was performed on interphase nuclei of peripheral blood (Phibroernger Mannheim, Mannheim, Germany). Competition hybridization by preannealing with a 100- to 200-fold excess of Cot-1 DNA (GIBCO-BRL) was performed before overnight hybridization on slides with previously denatured DNA. Probes were visualized by subsequent incubations with streptavidin-fluorescein isothiocyanate (FITC), biotinylated goat antistreptavidin, and streptavidin-FITC (VECTOR Laboratories, Burlingame, CA). Cells and chromosomes were counterstained with diamidino-phenylindole (DAPI) and embedded in Vectashield (VECTOR Laboratories, Burlingame, CA). Digital images were captured using a Photometrics cooled-coupled device (CCD) camera mounted on a Zeiss Axiosplan fluorescence microscope equipped with selective filters for fluorescein and DAPI and controlled by a Quadra 950 Macintosh computer using SmartCapture software (Digital Scientific, Cambridge, UK).

**Northern blot analysis.** A commercial multiple tissue Northern blot with 2 µg poly(A+) RNA from a variety of tissues (Clontech, Palo Alto, CA) was screened with a 665-bp Xho1 fragment single-copy probe (probe X665) was performed at 68°C for 90 minutes using Rapid-Hyb buffer (Amersham). The filters were washed with 2× SSC, 0.05% sodium dodecyl sulfate (SDS) at room temperature for 30 minutes and with 0.1× SSC, 0.1% SDS at 65°C for 30 minutes. Filters were autoradiographed at −80°C for up to 7 days.

**Isolation of cDNA clones.** cDNA clones were isolated by screening an oligo (dT)-primed normal human fetal brain cDNA ZAP library (Stratagene) with probe X665. Positive phase clones were excised from the ZAP phage as pBluescript SK+ plasmids, subcloned, and sequenced. Rapid amplification of cDNA ends (RACE) was performed using Marathon Ready skeletal muscle cDNA CDNA (Clontech) and Advantage cDNA PCR kit (Clontech). PCR primers were sense 5’-CTTCTCAGGGCATTGATGTGTC-3’ (nucleotide 4866) and 5’-CATGATGGGACCCAAAGAACACATC-3’ (nucleotide 4897) for 3’ RACE and antisense
RESULTS

Molecular cloning of a t(1;14) breakpoint in CEMO-1. Southern blot analysis of HindIII-digested DNA from CEMO-1 using the JH probe showed two rearranged bands of 5.2 and 5.8 kb. These rearrangements were amplified by long-distance inverse PCR, resulting in two products of 3.7 and 4.3 kb (Fig 1). Two cases showed an apparent variant translocation t(1;22)(q21;q11): direct involvement of IgH was shown in 2 cases by FISH using cosmids spanning the IgH locus (I.W., unpublished observations). One of these cases exhibited a breakpoint within the BCL9 YAC, whereas the breakpoint was telomeric in 1 case and centromeric in another. The final case was untested due to insufficient material. One case with no cytogenetic abnormality of 1q21 and no abnormality of YAC 882B3 showed rearrangement with the probe X665 on southern blot (Fig 6): this was a case of mantle cell lymphoma. Also, 1 case with t(1;19)(q21;p13) showed amplification of the BCL9 YAC. Otherwise, none of the other cases appeared to involve the BCL9 locus. These cases included 2 with t(1;3)(q21;q27) in which both breakpoints were telomeric of BCL9, 2 with t(1;12)(q21;q13) and 6 cases with dup(1)(q21qter) with a variable distal breakpoint cytogenetically: other partner chromosomes were unique. 1q21 breakpoints in these cases were heterogeneous, falling both centromeric and telomeric of the BCL9 gene.

Abbreviations: BCP-ALL, B-cell precursor ALL; FL, follicular lymphoma; MCL, mantle cell lymphoma; R, rearranged; G, germline; NT, not tested; +, overexpression; --, no overexpression.

Table 1. Analysis of BCL9 in Cases With 1q21 Breakpoints

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Cytogenetic 1q21 Abnormality</th>
<th>Histology</th>
<th>Southern Blot (X665)</th>
<th>BCL9 Overexpression</th>
<th>Location of YAC 882B3 Signals</th>
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<tbody>
<tr>
<td>Patient material</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>der(14)t(1;14)(q21;q32)</td>
<td>B-NHL</td>
<td>NT</td>
<td>NT</td>
<td>1, der(1), der(22) -- split</td>
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<tr>
<td>2</td>
<td>t(1;22)(q21;q11)</td>
<td>B-NHL/FL</td>
<td>G</td>
<td>NT</td>
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<tr>
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<td>t(1;22)(q21;q11)</td>
<td>B-NHL/DLCL</td>
<td>G</td>
<td>NT</td>
<td></td>
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<tr>
<td>4</td>
<td>t(1;22)(q21;q11)</td>
<td>B-NHL/FL</td>
<td>NT</td>
<td>NT</td>
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<td>B-NHL</td>
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<td>NT</td>
<td>1, der(22)</td>
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<td>B-NHL/MCL</td>
<td>R</td>
<td>NT</td>
<td>1, der(1)</td>
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<td>7</td>
<td>add(1), der(19)t(1;19)(q21;p13)</td>
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<td>NT</td>
<td>add(1) amplified + der(19)</td>
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<td>NT</td>
<td>1, der(1)</td>
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<td>9</td>
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<td>NT</td>
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<td>NT</td>
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<td>11</td>
<td>t(1;5)(q21p14)</td>
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<td>G</td>
<td>NT</td>
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<td>NT</td>
<td></td>
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<tr>
<td>21</td>
<td>add(1);q12)</td>
<td>B-NHL</td>
<td>NT</td>
<td>NT</td>
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<tr>
<td>22</td>
<td>dup(1p)(q21q24)</td>
<td>ALL</td>
<td>G</td>
<td>NT</td>
<td>1, duplicated on dup(1)</td>
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<td>Cell lines</td>
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<tr>
<td>UOCB-1</td>
<td>der(12)t(1;12)(q21;q13)</td>
<td>BCP-ALL</td>
<td>NT</td>
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<td>NT</td>
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<tr>
<td>AS283A</td>
<td>der(1)t(1;4)(q21;q35)</td>
<td>Burkitt's</td>
<td>NT</td>
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<td>NT</td>
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<tr>
<td>BL 32</td>
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<td>dup(1p)(q21q25)</td>
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<tr>
<td>BL 103</td>
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<td>BL 104</td>
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<tr>
<td>BL 115</td>
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<td>Burkitt's</td>
<td>G</td>
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<td>BL 136</td>
<td>der(1p)pter-q21: 1q21-q36: 1q21-q36: 1q21-qter)</td>
<td>Burkitt's</td>
<td>NT</td>
<td>--</td>
<td>NT</td>
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<tr>
<td>Namalwa E6</td>
<td>dup(1)(p)(q21q32)</td>
<td>Burkitt's</td>
<td>NT</td>
<td>--</td>
<td>NT</td>
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</tbody>
</table>

Twenty-two cases of fresh patient material and 3 malignant B-cell lines derived from various disease subtypes were studied by either Southern blot using the 3' BCL9 probe X665 and/or the nonchimeric BCL9 YAC 882B3: cell lines were additionally studied by Northern blot. One case showed a der(14)t(1;14)(q21;q32) similar to that seen in CEMO-1: FISH with the BCL9 YAC showed a telomeric 1q21 breakpoint. Four cases showed an apparent variant translocation t(1;22)(q21;q11): direct involvement of IgH was shown in 2 cases by FISH using cosmids spanning the IgH locus (I.W., unpublished observations). One of these cases exhibited a breakpoint within the BCL9 YAC, whereas the breakpoint was telomeric in 1 case and centromeric in another: the final case was untested due to insufficient material. One case with no cytogenetic abnormality of 1q21 and no abnormality of YAC 882B3 showed rearrangement with the probe X665 on southern blot (Fig 6): this was a case of mantle cell lymphoma. Also, 1 case with t(1;19)(q21;p13) showed amplification of the BCL9 YAC. Otherwise, none of the other cases appeared to involve the BCL9 locus. These cases included 2 with t(1;3)(q21;q27) in which both breakpoints were telomeric of BCL9, 2 with t(1;12)(q21;q13) and 6 cases with dup(1)(q21qter) with a variable distal breakpoint cytogenetically: other partner chromosomes were unique. 1q21 breakpoints in these cases were heterogeneous, falling both centromeric and telomeric of the BCL9 gene.

Abbreviations: BCP-ALL, B-cell precursor ALL; FL, follicular lymphoma; MCL, mantle cell lymphoma; R, rearranged; G, germline; NT, not tested; +, overexpression; --, no overexpression.
copy 665-bp \textit{Xho} I fragment (probe X665) containing this region of identity was used to screen a gridded PAC library, resulting in the isolation of two PAC clones that were mapped back to chromosome 1q21 on normal metaphases by FISH (Fig 2A), confirming that this rearrangement represented the t(1;14) translocation breakpoint. In addition, the probe X665 gave a rearranged signal on Southern blot that comigrated with the 5.8-kb rearranged \textit{JH} HindIII fragment (data not shown).

**Isolation of BCL9 cDNA clones.** Because of its identity with an EST, probe X665 was hybridized to multiple tissue Northern blots containing 2 µg poly(A\(^+\)) RNA from a variety of different tissues. Two low-level transcripts were detected in all

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**Fig 1.** (A) LDI-PCR products from \textit{Hind}III-digested CEMO-1 DNA. Bands of 3.7 and 4.3 kb were amplified, corresponding to the rearrangements seen on Southern blot. Five microliters of PCR product was analyzed on a 0.8% agarose stained with ethidium bromide. (B) DNA sequence analysis of translocation breakpoint from 4.3-kb LDI-PCR product (clone CH4.3). DNA sequence is compared with germline \textit{IGH} sequence and \textit{BCL9} cDNA sequence. Shaded region denotes \textit{JH5} sequence. (C) Restriction map of LDI-PCR clone CH4.3. The breakpoint in CEMO-1 is indicated by an arrow. Probe X665 is indicated by heavy horizontal bar. This probe was used to screen PAC library and for Northern and Southern analysis. \textit{Bg}, \textit{Bgl} II; \textit{H}, \textit{Hind}III; \textit{P}, \textit{Pst} I; \textit{X}, \textit{Xho} I.

**Fig 2.** FISH results for \textit{BCL9} probes. (A) Localization of PAC containing \textit{BCL9} sequences to chromosome 1q21 on normal metaphases. (B) Rearrangement of CEPH YAC 882B3 in case no. 2 with t(1;22)(q21;q11), showing hybridization signals at the breakpoints of both der(1) and der(22) chromosomes. (C) Metaphase of case no. 7 showing YAC 882B3 hybridization signals on normal 1 and der(19) chromosomes. Amplification of hybridization signal on the add(1) is shown by a solid arrow. Note the extra unidentified material between centromere and YAC signal.
tissues, a major transcript of 6.3 kb and a less prominent 4.2-kb species, whereas a third transcript of 1.6 kb was detected only in spleen, thymus, and, most strongly, in small intestine (Fig 3).

Probe X665 was therefore used to screen a normal fetal brain cDNA library and two overlapping clones covering a region of 1.5 kb were cloned and sequenced. In addition, primers were designed within the region of identity with the EST for 3' RACE. Amplification in the 3' direction yielded a major product of 1.6 kb that was cloned and sequenced. To clone the 5' end of the gene, 5' RACE was performed. In the first stage, a major product of 2.1 kb was amplified, cloned, and sequenced. Primers were designed from this sequence and a further round of 5' RACE was performed, resulting in a product of 1.3 kb. The full-length BCL9 sequence was found to be 6,268 bp, with no further regions of nucleic acid homology on the databases other than identity with some ESTs. This sequence (accession no. Y13620) is shown together with the predicted amino acid translation in Fig 4. Sequence analysis showed a long open reading frame of 4.1 kb with termination codons in all three reading frames 5' of it. The first ATG codon of this ORF was at nucleotide 740; it was unclear whether this represented the site of translation initiation because it did not adhere to the Kozak consensus sequence. The second ATG codon, at nucleotides 818, showed a closer resemblance to a potential translation initiation site.

Analysis of the BCL9 predicted protein of 1,394 amino acids showed that it was rich in proline (18%), glycine (11%), serine (11%), and methionine (6%) residues. Several pentapeptide repeats (SPSGN, SSTPL, PSSTP, QGMMG, and PGPVG) were identified. A potential nuclear localization signal (KRRK) was identified at residues 243-246, but there were no other recognizable protein domains. There was also no homology with other proteins on the databases, although one region showed amino acid homology (90% homology >30 amino acids) to an EST cloned from a Drosophila embryo cDNA library. The sequences of the shorter transcripts of 4.2 and 1.6 kb have not yet been determined.

The translocation t(1;14) in CEMO-1 results in overexpression of BCL9. Sequence analysis showed that the t(1;14) breakpoint in CEMO-1 fell within the 3' UTR of the BCL9 gene: in this the translocation was comparable to those involving IGHI and the major breakpoint region of BCL2, where there is no disruption of the ORF. To assess whether the juxtaposition of BCL9 to IGHI resulted in its deregulation, expression of BCL9 was determined in CEMO-1 and also in other malignant and nonmalignant B-cell lines. At least 50-fold overexpression of BCL9, as calculated by densitometric analysis of hybridization bands, was detected in CEMO-1 as compared with ASLCL, an Epstein-Barr virus (EBV)-transformed normal B-lymphoblastoid cell line (Fig 5). Both a truncated BCL9 transcript of 6.3 kb and the 4.2-kb transcript were overexpressed, but not the 1.6-kb transcript. However, no such overexpression was detected in other cell lines with and without abnormalities of 1q21, including 2 precursor B-cell ALL cell lines, 10 BL cell lines, 2 mantle cell lymphoma cell lines, and 1 plasma cell leukemia cell line (Karpas 620). These data are shown in Table 1.

Rearrangements of BCL9 in other cases with abnormalities of 1q21. To assess the extent of involvement of BCL9 in other cases of B-cell malignancy with abnormalities of 1q21 but in which no RNA was available, rearrangements of BCL9 were sought in a panel of 39 cases with 1q21 abnormalities. Conventional DNA blot using the 3' probe X665 detected additional rearrangements in multiple digests in case no. 6 (Table 1 and Fig 6). Southern blot detected two additional bands in Bgl II and Xba I digests, showing that the breakpoint fell within the X665 probe itself. These data indicated that the 3' UTR within the BCL9 gene may contain a breakpoint cluster region. However, in this case, although the G-band pattern of one copy of chromosome 1q was abnormal, no cytogenetic abnormality of 1q21 could be detected by either regular cytogenetics or by FISH using a BCL9 YAC. Furthermore, hybridization with a chromosome 1 paint showed that all of the abnormal chromosome material telomeric to BCL9 was derived from chromosome 1. These data therefore indicate that these rearrangements probably represented an internal chromosome 1q21 deletion or insertion. Molecular cloning of the rearrangements is being undertaken.

We then analyzed 17 cases and 3 cell lines by FISH using the nonchimeric CEPH YAC 882B3, which contains the BCL9 gene, as a probe, to assess the extent of involvement of BCL9 in cases in which breakpoints fell outside the range of conventional DNA blot (Table 1). Four cases and 1 cell line exhibited reciprocal translocations involving 1q21. Case no. 2, a case of follicular lymphoma with the translocation t(14;18) exhibiting the additional translocation t(1;22)(q21;q11) involving the IGH locus at 22q11 (I.W., unpublished observations), showed rearrangement involving the genomic region of 850 kb covered by this YAC (Fig 2B). An additional case (case no. 7) that was initially described as having add(1)(q42) and der(19)t(1;19)(q21; p13) showed amplification of the YAC signal on the der(1) chromosome with apparently normal signals on both the normal 1 and the der(19) chromosomes. The abnormal derivative 1

Fig 3. Northern analysis of BCL9 expression in a variety of normal human tissues. A multiple tissue Northern blot was hybridized with the BCL9 probe X665. Reprobing with a GAPDH probe showed equal RNA loading in all samples (data not shown).
Fig 4. (Cont’d).
chromosome, carrying the amplified signal, also showed the presence of additional material between the centromere of chromosome 1 and the hybridization signal, suggesting the presence of an insertion within the long arm of chromosome 1 (Fig 2C).

Results in the other cases showed a heterogeneity of breakpoints at 1q21, breaks occurring both telomeric and centromeric of \textit{BCL9} with equal frequency (10 each). However, 12 cases exhibited unbalanced abnormalities of chromosome 1; therefore, breakpoints falling within the YAC may not have been readily detectable. Additional experiments with YAC clones flanking \textit{BCL9} are currently being performed to assess the location of breakpoints in these cases.

**DISCUSSION**

In B-cell malignancy, a number of recurrent translocations involving the \textit{IG} loci have been characterized. However, a
number of other recurrent cytogenetic abnormalities that only rarely involve the IG loci have also been identified, some of which are of prognostic significance. Among these are breakpoints involving 1q21-q23 and 1p32-36, which are associated with a poor prognosis and appear to exhibit promiscuity in the translocation partners with which they rearrange.16 In this, both loci are comparable to 3q27, the location of the BCL6 gene, which has been shown to be translocated to a large number of loci other than the IG loci, eg, the t(3;4)(q27;p13) involving the TTF gene at 4p1335 and the t(3;11)(q27;q23) involving the BOB1 gene at 11q23.26

Using a PCR-based method for cloning IGHJ rearrangements,14 we have identified a novel gene, BCL9, at chromosome 1q21 through the cloning of a translocation t(1;14)(q21;q32) in a precursor B-cell ALL cell line. This gene coded for a large proline-rich protein that contained a potential nuclear localization signal but no other significant motifs and no homology with known proteins. One region of BCL9 showed homology over a region of 30 amino acids to an EST cloned from a Drosophila cDNA library. The significance of this homology remains to be determined: further sequencing of the full-length Drosophila cDNA clone is currently being undertaken. We have shown that one consequence of the t(1;14)(q21;q32) in the CEMO-1 cell line was the overexpression of BCL9. This gene is normally expressed at very low levels in B cells, and the pathological consequences of such overexpression remain to be determined. Expression of a 1.6-kb transcript was seen in spleen, thymus, and small intestine, suggesting that this isoform may be differentially involved in cell proliferation. Interestingly, this isoform was not seen in CEMO-1.

We have also demonstrated that breaks within the region around BCL9 were found in some cases of B-NHL with abnormalities of 1q21. In one case, the breakpoint was within the 3’ UTR of BCL9, similar to that found in CEMO-1, indicating that this may be a recurrent breakpoint. Furthermore, FISH analysis using an 850-kb YAC indicated that, in 1 of 17 other cases with abnormalities of 1q21, the breakpoint fell close to the BCL9 gene. This case exhibited the translocation t(1;22)(q21;q11) involving the IGα locus, which would appear to be a variant of the t(1;14)(q21;q32), similar to the variant translocation t(8;22)(q24;q11) involving MYC and IGα. By analogy with rearrangements of BCL1, BCL2, MYC, or PAX-5 loci in other cases of B-NHL in which deregulation by IG transcriptional enhancers can occur at distances of several hundred kilobases,4,12,27-29 it is feasible that BCL9 expression could be deregulated at this distance by regulatory elements within the IG loci. In case no. 6, both conventional cytogenetics and FISH failed to show any abnormality of the BCL9 locus, suggesting the presence of a submicroscopic chromosomal rearrangement. In case no. 7, there was clear evidence of amplification of the YAC hybridization signal on an abnormal 1q chromosome. Whether these abnormalities resulted in increased BCL9 expression could not be determined due to a lack of suitable material.

However, it is clear that not all rearrangements of 1q21 in B-cell malignancy involve BCL9. We failed to find evidence of gene rearrangement and/or BCL9 overexpression in 13 cell lines of various subtypes of B-cell malignancy and in 19 of 22 cases of ALL/B-NHL with abnormalities of 1q21. FISH analysis showed a surprising heterogeneity of 1q21 breakpoints: these fell both centromeric and telomeric of BCL9 with equal frequency, although in many of these cases the translocations were unbalanced and therefore rearrangement of the YAC signal would not have been clearly seen, and RNA was not available for analysis of BCL9 expression.

Abnormalities of the long arm of chromosome 1, and in particular the 1q21 region, are seen in a variety of preneoplastic and neoplastic diseases, including both hematopoietic malignancies and solid tumors,30-32 and in some cases occur as an early event. Further work will be required to determine what role the BCL9 gene has to play in these other diseases and to identify other genes of pathological importance in B-cell malignancy.

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