Direct Sequence Analysis of the t(14;18) Chromosomal Translocation in Hodgkin’s Disease

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There have been conflicting reports about the occurrence of the t(14;18) chromosomal translocation in Hodgkin’s disease. A polymerase chain reaction analysis of biopsy specimens from 21 patients with Hodgkin’s disease (HD) has shown the presence of the t(14;18) translocation in four cases (20%). All four patients had nodular sclerosing HD. Direct sequencing of the amplified 14q+ junctions established that the BCL-2 (major breakpoint region) sequence was fused to an Ig joining region (J6 in all four cases). Different breakpoints were observed in each case but were similar in nature to the breakpoints described in follicular lymphoma. The exact nature and cell of origin in HD remains obscure, although the presence of the t(14;18) translocation may reflect either a B-cell origin in these cases or associated lymphoid hyperplasia.

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NONRANDOM CHROMOSOME rearrangements occur frequently in lymphoid malignancies. The most frequent translocation in B-cell non-Hodgkin’s lymphoma (NHL) is the t(14;18)(q32;q21) translocation found in 85% to 90% of follicular lymphomas, 30% diffuse large cell lymphomas, and occasionally other histologic types of NHL. Details of the t(14;18) translocation, including sequence data at the major breakpoint region (mbr) and minor cluster region (mcr), are well described. The involvement of the J6 joining region on chromosome 14, and the breakpoint on chromosome 18 involves no substantial loss or insertions into the BCL-2 gene sequence. During the translocation, short random nucleotide sequences called ‘N’ regions appear to be inserted between the two breakpoints reminiscent of normal VD and DJ recombination under the influence of the recombinase system. There is also evidence that some breaks occur within the diversity regions (Dn) on chromosome 14. It has been suggested that the mechanism of such translocations and others involving the Ig heavy chain locus on chromosome 14 is a result of errors in Ig heavy chain (IgH) rearrangement controlled by recombinase enzyme. This is supported by the presence of the N regions and the presence of somatic point mutations within the translocated IgH joining regions. The involvement of the BCL-2 gene on chromosome segment 18q21 and its translocation to the IgH chain region results in deregulation of BCL-2 gene expression. This expression is normally down regulated as the pre-B cell matures to the B cell concomitantly with V(D)J recombination under the influence of recombinase enzyme. The translocated BCL-2 allele and its protein products, having escaped normal control mechanisms, are overexpressed and may play a role in malignant change.

The observation of the t(14;18) translocation in Hodgkin’s disease (HD) was first made using cytogenetic techniques. Eighteen abnormal metaphases were found in 49 tumor samples, one of which showed the t(14;18)(q32;q21). Other investigators were unable to confirm this and more sensitive methods of detection, such as Southern blot analysis and polymerase chain reaction (PCR), failed to demonstrate the presence of t(14;18) in HD. In 1990, the t(14;18) chromosome translocation was detected using PCR methods in 17 of 53 (32%) HD tumors. However, more recent studies have not been able to demonstrate the translocation in HD.

In this report, PCR techniques were used to study the t(14;18) translocation in 21 histologically confirmed cases of HD. Sequence analysis was used to confirm the identity of the amplified products and to compare the breakpoints with those found in follicular lymphomas.

MATERIALS AND METHODS

Patient material. Twenty-one paraform-embedded biopsies from patients with histologically proven HD (19 nodular sclerosing, one mixed cellularity, and one lymphocyte predominant) were selected at random and snap-frozen samples, taken at the same biopsy, were available in five of these cases. Original sections were reviewed to confirm the histology. The paraffin and snap-frozen material was sectioned at separate laboratories using a new cryostat blade and standard precautions to avoid possible cross contamination. The cut sections were placed into autoclaved eppendorf tubes and taken to another laboratory that had not previously worked with any lymphoma material for the DNA extraction and PCR. A final section was stained in each case and examined histologically to exclude the possibility of composite lymphoma.

PCR. DNA was extracted as previously described from paraform-embedded biopsy material. Digestion buffer with 0.5 mg/mL of proteinase K was added to 20 sections cut 4 μm thick and left at 37°C for 5 days. Fresh-frozen tissue was cut 10 μm thick and processed as described. All steps were performed using new autoclaved tubes. DNA obtained from the t(14;18) lymphoma cell line DOHH2 was used as a positive control. Enzymatic amplification was performed by the PCR using Taq polymerase and Hybaid thermal reactor (Hybaid Ltd, Teddington, UK). The final reaction volume of 100 μL included 0.1 μL to 1 μL of tumor DNA, oligonucleotide primers (0.5 μmol each), 2.5 U of Taq (Promega, Madison, WI), 10X Taq buffer (Promega) and nucleotide triphosphates (dNTP) at 200 μmol/L each. Primers used were at the mbr junction, BCL (5’-CTTAAAGGATGTTCTTTACCT-3’) and the consensus primer, JH1 (5’-ACCTGAGGAGCCGTGCAC-3’). After an initial denaturation step of 10 minutes at 95°C, 40 cycles of annealing at 57°C for 30 seconds, extension at 72°C for 1 minute, and denaturing at 95°C for 30 seconds were performed with a final extension at 72°C for 10 minutes. The amplified products (8 μL)
were analyzed by electrophoresis on 2% agarose gel and staining with ethidium bromide.

**Direct sequence analysis.** Direct nucleotide sequencing by the dideoxy chain termination method was performed on the PCR products as previously described. DNA was purified from the reaction mix using a Sephadex G50 column (Pharmacia, Uppsala, Sweden) or PrimeErase Quik push columns (Stratagene, La Jolla, CA) followed by ethanol precipitation, freeze drying, and resuspension in TE buffer. The reaction was performed using BCL-2 internal oligonucleotide BC3 (5'-CACAGACCCACCCAAGGCC-3') as sequencing primer as well as BCI primer with the modified T7 DNA polymerase (Sequenase version 2.0). In patient D, sequence could not be derived using BC3 from the PCR product. BCI gave partial sequence of the N region and J6. To confirm this, a new clone-specific oligonucleotide was synthesized in sequence to the opposite direction. The primers were kinase labeled with 3P and the reactions performed according to the Sequenase protocol.

**RESULTS**

The t(14;18) translocation was detected by PCR in four of the 21 cases of HD examined. Histology was confirmed and composite lymphoma excluded in all cases both before and after sectioning for DNA extraction. The clinical details of the patients are shown in Table 1. The breakpoints involved the mbr on chromosome 18 as the mcr was not investigated in this study. The amplified products were of predictable size, ie, 130 to 210 bp (Fig 1). No sample gave a fragment similar to that obtained from the control cell line at 230 bp. Performing the PCR with 40 amplification cycles was necessary to allow detection of the products by ethidium bromide staining. In patient B, snap-frozen material was available and gave the same size PCR product as the paraffin-embedded material.

Direct sequence analyses of these PCR products across the t(14;18) translocation on the 14q+ derivative chromosome are shown in Table 2. In all cases, a fusion was shown between a BCL-2 (mbr) sequence and Ig heavy chain region exons. In patient D, the breakpoint determined was unusually close to the BCI primer, thus explaining the problems found during the sequence analysis. The J6 segment was involved in all four cases and the breakpoints are within the cluster regions of previously demonstrated breaks in the mbr for follicular lymphomas. As a further precaution, the results obtained were compared with previously amplified and sequenced breakpoints from follicular lymphoma and found to differ. Recently, similar results showing clustering within the mbr have been described for low-grade centroblastic-centrocytic lymphoma (cb-cc) and other subtypes based on the Kiel classification.

Table 3 shows part of the BCL-2 (mbr) sequence detailing the primers used and the sites of the breakpoints. The N regions of patients A and B were less than 10 bases, similar in size to those seen in follicular lymphoma and with normal VDJ rearrangement. The N regions in patients C and D are considerably larger and may include a previously unknown part of DNA sequence as reported in follicular lymphoma. In patient C, an eight nucleotide sequence is identical with a sequence in the previously described DNA segment D2. As in follicular lymphoma, somatic mutations in the Jm sequences were detected and are underlined, while none were seen in the BCL-2 sequence.

**DISCUSSION**

This study has established that the amplification products obtained from the HD biopsies are the result of the t(14;18) translocation. Because all the sequences showed different breakpoints, there is no possibility that they all derive by cross contamination from one of the samples or the control cell line. This also was verified by obtaining the same result for patient B from two separate sources of material. These results confirm and extend the previous observations of Stetler-Stevenson et al (32%). The total incidence of the t(14;18) in HD might be higher because the mcr breaks were not investigated. However, there are studies that have failed to detect the translocation in HD using a variety of techniques such as cytogenetics, Southern blot analysis, and PCR. This may be explained in part by our lack of knowledge of which cell type bears the translocation in HD. Also, the four positives described are from the 19 cases of nodular sclerosing subtype. Thus, studies in which the other subtypes of HD have been investigated, in particular lymphocyte predominant HD, have not demonstrated t(14;18) using both Southern blot and PCR analysis. Cytogenetic studies in HD are scarce because of the technical difficulties in obtaining malignant cells; nevertheless, cytogenetic abnormalities identified in HD have shown that the most frequent involves chromosome 14q in 35% of cases.
Table 2. Sequences of the Chromosome 14q+ Junction in Four Cases of HD

<table>
<thead>
<tr>
<th>Patient</th>
<th>BCL-2</th>
<th>J6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>TCCTTGCAGGGAAGGAGGCCCTAGGCCTAGGCCTCTGCGGGGGCcccctggcTACTACTACCCGGTATGGGACTTGGGGCCAAGGGA CC</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>GGTCTTCCTGAAATGgagtggggA CTACTACTACCCGGTATGGGACTTGGGGCCAAGGGAG C</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>BCL-2</td>
<td>J6</td>
</tr>
<tr>
<td></td>
<td>CCTGAATGCGACTGCCACTAACACACAGACCGACACCGACCACCCACACACACTACTCCGGAT</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>BCL-2</td>
<td>J6</td>
</tr>
<tr>
<td></td>
<td>CTTCAGTGCCTGTgaggtagcaatgamgaatgtA CCGGACTGGGACCATGGGACCCCAACCCGGCAACACCAACACACACTACTCCGGAT</td>
<td></td>
</tr>
</tbody>
</table>

BCL-2 sequence is shown in upper case; joining region (J6) sequence is in upper case italics. Differences between the J6 sequence and the germline are underlined. The N regions are in lower case. The possible DH3 sequence in the intervening sequence of patient C is underlined.

Table 3. The mbr Sequence indicating the Translocation Breakpoints

| 2954 | CCTTACGAGAGTTGGTTCCTAGGAGCCTGCGGTTTTACAAACACAGCCACCCAGACCGCCCTCTGGCC |
|  | " | " |
|  |  |  |
|  | B | C |
|  | DoHH2 |  |

The part of the mbr sequence showing BC1 and BC3 primers (underlined). Arrows indicate the precise translocation breakpoints of the four cases described and of the cell line DoHH2. These are in the subclustering areas previously seen in follicular/cb-cc lymphomas.
In conclusion, the (t(14;18)) translocation may be detected in 20% to 32% of the cases of HD. Sequence analysis, previously unreported, shows breakpoints comparable to those of follicular lymphoma, suggesting that the mechanism is similar. This may give further indications as to the nature of HD, although the cells bearing the translocation remain unidentified.

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