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 majority involve 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 in 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 paraffin-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 left at 4°C. After an initial denaturation step of 10 minutes at 95°C, 40 cycles of PCR were 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 μg DNA, oligonucleotide primers (0.5 μM each), 2.5 U of Taq (Promega, Madison, WI), 10X Taq buffer (Promega) and nucleotide triphosphates (dNTP) at 200 μM/L each. Primers used were at the mbr junction, BCL1 (5’-CTTGAAGATTTCCATTCG-3’) and the consensus primer, JH1 (5’-ACCTGAGGAGCGTACG-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'-CACAGACCCACCCA-GAGCCC-3') as sequencing primer as well as BC1 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. BC1 gave partial sequence of the N region and J6. To confirm this, a new clone-specific oligonucleotide was synthesized to sequence in the opposite direction. The primers were kinase labeled with 32P 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 the case of patient B, snap-frozen material was available and gave the same size PCR product as the paraffin-embedded material.

Direct sequence analysis 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 BC1 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 involving chromosome 14q in 35% of cases, 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 D9 sequence as reported in follicular lymphoma. In patient C, an eight nucleotide segment is identical with a sequence in the previously described D9 segment D12. As in follicular lymphoma, somatic mutations in the JH 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 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.
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>TCCTTCCGGGGGCCcccctggcTACTACTACTACGGTA TGGACGTCTGGGGCCAAGGGAGC</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>GGTCTTCCTGAAATGGagtggggA CTACTACTACGGTA TGGACGTCTGGGGCCAAGGGAGC</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>BCL-2</td>
<td>J6</td>
</tr>
<tr>
<td></td>
<td>CTTGAATGCGAGTGggcactacaaaccacagaagcacggacacgccaacacaACTACTACGGTA TGGACGTCTGGGGCCAAGGGAGC</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>CTlTACGTGGCCTGTgaggtagcaatgamgaatgtA CCCGGACTGGACCATGlTCCGA</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.

with the commonest breakpoint occurring at 14q32.34 The more sensitive PCR method may also have limitations because optimizing the conditions is essential and even the number of tubes in the automated cycler can influence results.35 There may be geographic variation, as seen in follicular lymphoma, but this is speculation at the present time.

These data show the similarity of the breakpoints to those seen in follicular lymphoma with the presence of N regions, subclustering in the mbr, and somatic mutations in Ig region. The four cases with the t(14;18) were all nodular sclerosing HD, thus avoiding any potential controversy regarding the histologic diagnosis of HD as opposed to NHL. The clonal malignant cell in HD remains unknown and, indeed, it may differ between tumors.37,38

The number of Reed-Sternberg cells and their variants differed in the four positive biopsies, but, nevertheless, gave similar intensity after ethidium bromide staining of the PCR products on the agarose gel. Although methods to increase the population of RS cells have been successfully used in other investigations, it was still not possible to conclude the cell of origin with certainty.39 The consequence of the translocation, and hence the function of the BCL-2 gene, is open to speculation. It was initially thought that the BCL-2 gene rearrangement is seen exclusively in B-cell tumors.40,41 The finding of the translocation in HD39 raises doubt about this, because it is by no means clear that the Reed-Sternberg cell is of B-cell origin.42,43 However, the translocation may be present in any of the cells within the biopsy specimen, ie, lymphocytes, histiocytes, cosinophils, immunoblasts, plasma cells, and Reed-Sternberg cells or equivalents, the latter constituting less than 1% of the total cell population, as DNA extraction from the tissue samples incorporates DNA from all of them.

B-cell lymphomas with t(14;18) translocation have increased BCL-2 expression and, consequently, increased BCL-2 protein production. Overexpression of the BCL-2 gene after deregulation allows a proliferation advantage to the cells bearing the translocation as well as influencing cell survival.42,44 The oncogenic potential of BCL-245 raises speculation about the development of HD through a further secondary oncogene-mediated event, as shown in transgenic mouse experiments.46,47 The potential role of the Epstein-Barr virus (EBV) in HD is intriguing, particularly with the demonstration of EBV particles in Reed-Sternberg cells with in situ hybridization.48,49 Tsujimoto50 has demonstrated that the overexpression of the BCL-2 gene product results in the growth enhancement of EBV-immortalized B cells. EBV is generally considered to be B-lymphotrophic, although the virus may rarely infect T cells.

It is possible that the t(14;18) translocation in HD has no direct bearing on malignant change. Indeed, reports have suggested the presence of t(14;18) translocation in nonmalignant tissue such as lymphoid hyperplasia.51,52 However, it is also apparent that the BCL-2 gene, either in t(14;18) translocation or variants, can be found in other malignancies such as salivary gland lymphomas,53 primary intestinal lymphoma,54 chronic lymphocytic leukemia,55 and acute lymphoblastic leukemia.56

Table 3. The mbr Sequence Indicating the Translocation Breakpoints

| 2954 | CTTTTAGAGAGTTGCTTTAGTGCGGCTGTTTTCAACACAGAGCCACCAGAGGCCCTCTCGCC |
| B1C | \( \uparrow \) D \( \uparrow \) BC3 |
| CTCCTCCGGGGGGGCTTTCTCATGGCTCCTTCTGAGGTTCTCTTCTGAAATGCACTGGTGTC | \( \uparrow \) A \( \uparrow \) B \( \uparrow \) C |
| TTACGGCTCACCAAAGAAAGCDDAAACCCTGTGTATGAACCAAGCCTCCGGGGGCC | \( \uparrow \) 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 DoHH2.26 These are in the subclustering areas previously seen in follicular/cb-cc lymphomas.28,29
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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REFERENCES

6. Cleary ML, Sklar J: Nucleotide sequence of a t(14;18) chromosomal translocation breakpoint in follicular lymphoma and demonstration of a breakpoint cluster region near a transcriptionally active locus on chromosome 18. Proc Natl Acad Sci USA 82:7439, 1985


56. Thangavelu M, Olopade O, Beckman E, Vardiman JW, Larson RA, McKeithan TW, Le Beau MM, Rowley JD: Clinical, morphologic and cytogenetic characteristics of patients with lymphoid malignancies. Characterised by both t(14;18)(q32;q21) and t(8;14)(q24;q32) or t(8;22)(q24;q11). Genes Chromosomes Cancer 2:147, 1990
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