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Rearrangement of *bcl*-2 Is Detectable in Hodgkin's Disease by Polymerase Chain Reaction

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The authors examined the occurrence of the t(14;18) chromosomal translocation in 44 cases of Hodgkin's disease (HD) using the polymerase chain reaction and Southern blot hybridization with non-radioactive oligonucleotide probes. DNAs were extracted from unfixed, fresh-frozen and formalin-fixed, paraffin-embedded biopsy specimens. Southern blot hybridization of the amplification product showed that, of 44 HD DNAs, three had a detectable t(14;18) breakpoint at the mbr (major breakpoint region), while none had a detectable t(14;18) breakpoint at the mcr (minor cluster region). Of the three cases positive for a t(14;18) breakpoint at the mbr, two were of lymphocyte predominance type, and the other was of mixed cellularity type.

Key words: Hodgkin's disease — t(14;18) translocation — *bcl*-2 — PCR — Non-radioactive hybridization

Chromosomal translocations that activate the *bcl*-2 oncogene appear to have a definite role in the pathogenesis of lymphoid malignancies.[1,2] The t(14;18)(q32;q21) translocation is found in 85% to 90% of follicular lymphomas and 30% of diffuse large cell lymphomas in Europe and America,[1,3–6] and approximately 30% to 44% of Japanese follicular lymphomas.[7,8]

The t(14;18) translocation joins *bcl*-2 at 18q21 with the immunoglobulin heavy chain (IgH) locus at 14q32[9] and generates the *bcl*-2-IgH fusion gene. This results in the deregulation of *bcl*-2 gene expression, leading to high levels of *bcl*-2 mRNA, and overproduction of the bcl-2 protein.[10–17] This *bcl*-2 gene overexpression delays the onset of programmed cell death, prolongs the survival of cells, and may play a role in malignant changes.[18–20] Approximately 60% of the breakpoints on chromosome 18 are clustered at the 150 bp major breakpoint region (mbr), located in the 3′ untranslated region of the *bcl*-2 gene, while 25% of the breakpoints are clustered at the minor cluster region (mcr) located 30 kb downstream from the mbr.[10,13,21,22] Breakpoints on chromosome 14 occur within or near the IgH joining region (J_H), where the D-J joining normally occurs during heavy chain rearrangement. The clustering of breakpoints on both chromosomes 14 and 18 enabled the use of polymerase chain reaction (PCR) to detect the *bcl*-2-IgH(J_H) fusion gene by using a *bcl*-2 specific chromosome 18 primer together with another primer, a consensus sequence which is able to bind to any of the six J subunits.[23,24]

In Hodgkin's disease (HD), the occurrence of the t(14;18) translocation remains controversial. Conflicting reports have appeared on the occurrence of t(14;18) in HD in Western countries. The t(14;18) translocation was first observed in an HD patient using a cytogenetic technique,[25] although it was not confirmed.[26] Recently, with the use of PCR, some investigators have detected t(14;18) at the mbr in 9% to 32% of specimens diagnosed as HD.[27–29] In these studies, mcr assays were not performed. In other laboratories, the t(14;18) translocation in HD was not detected with the use of PCR.[30–32] Moreover, to the authors' knowledge, no study has been reported from Japan on the occurrence of t(14;18) translocation in a number of HD cases. The objective of this study was to examine 44 Japanese HD patients for evidence of t(14;18) at the mbr and mcr through the use of PCR and hybridization, in the hope of resolving the reported discrepancies.

MATERIALS AND METHODS

Materials DNAs were extracted from 21 unfixed, fresh-frozen and 23 formalin-fixed, paraffin-embedded lymph node specimens that were histologically diagnosed as HD. Histological subtypes consisted of 15 mixed cellularity type (MC), 5 nodular sclerosis type (NS), and 15 lymphocyte predominance type (LP). The subtypes of the remaining 9 cases were unknown.

Control DNAs As a positive control, DNAs were extracted from two follicular lymphomas bearing a t(14;18) mbr breakpoint, a lymphoma cell line SU-DHL-6[14] bear-

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ing a t(14;18) mbr breakpoint, and a t(14;18) mcr breakpoint-bearing follicular lymphoma.

PCR For detection of the t(14;18) breakpoint at the mbr and mcr, 1 μg of purified DNA from each sample was subjected to 50 cycles of amplification, using a Gene Amp kit (Perkin-Elmer/Cetus, Norwalk, CT) (95°C, 2 min; 55°C, 2 min; 72°C, 3 min) in a 100 μl volume containing 2.5 units of *Taq* DNA polymerase enzyme, 0.2 m*M* dNTPs, and 1.0 μ*M* each of the appropriate primers. The primers used for amplification of the mbr[1] were 5′-TTAGAGAGTTGCTTTACGTG-3′ (complimentary to the negative strand segment, 5′ of the mbr[15,23]) and 5′-ACCTGAGGAGACGGTGACCAGGGT-3′ (a consensus sequence that recognizes all six J regions[13]). The primers for amplification of the mcr were 5′-GACTCCTTTACGTGCTGGTACC-3′ (complimentary to the negative strand segment, 5′ of the mcr) and the 3′-anti-sense J_H consensus sequence, 5′-ACCTGAGGAGACGGTGACC-3′. These primers and two oligonucleotide probes described below were synthesized using a Cyclon Plus DNA Synthesizer (Milligen/Bioresearch, Tokyo).

The PCR products (10 μl) were electrophoresed in a 3% Nusieve GTG (FMC, USA) agarose gel, stained with ethidium bromide and blotted onto positively charged nylon filters, Hybond-N+ (Amersham Japan Inc., Tokyo). The filters were then hybridized to the digoxigenin-tailed oligonucleotide probe[33] as described earlier,[8] and washed under a high stringency condition. The hybridized DNAs were detected using a Dig-ELISA (enzyme-linked immunosorbent assay) kit (Boehringer Mannheim GmbH, Germany). The sequence of the mbr-specific oligonucleotide probe was 5′-GCCTGTTTCAACACAGACCC-3′,[23] and that of the mcr-specific probe was 5′-GATGGCTTTGCTGAGAGGTAT-3′.[24]

RESULTS AND DISCUSSION

A titration experiment was performed. One μg of DNA from SU-DHL-6 was diluted serially with the DNA from a human normal lymph node, then subjected to PCR amplification of the t(14;18) breakpoint at the mbr. The resulting PCR products were analyzed by Southern blot hybridization with the nonradioactively labeled oligomer probe and ELISA. The result showed that our detection system produced unequivocal signals at sensitivity of at least 10^{-3} dilution (data not shown). This dilution is comparable to the frequency of Reed-Sternberg (R-S) cells and variants in HD. The R-S cells typically constitute a small minority, approximately 0.1–1% of the HD tumor.[34] The titration experiment showed that the sensitivity of our detection system was adequate for the molecular detection of a t(14;18)-bearing cell at a frequency comparable to that of R-S cells and their variants in HD. The results of HD analysis are summarized in Table I.

Detection of *bcl*-2(mbr)/J_H Specimens from 3 out of 44 HD cases were found to contain a *bcl*-2(mbr)/J_H se-

Table I. PCR Amplification of *bcl*-2/J_H in Hodgkin's Disease

Histological subtype	No. of cases	No. of positive cases for *bcl*-2(mbr)/J_H	No. of positive cases for *bcl*-2(mcr)/J_H
Mixed cellularity	15	1	0
Lymphocyte predominance	15	2	0
Nodular sclerosis	5	0	0
Lymphocyte depletion	0	0	0
Not verified	9	0	0
Total	44	3	0

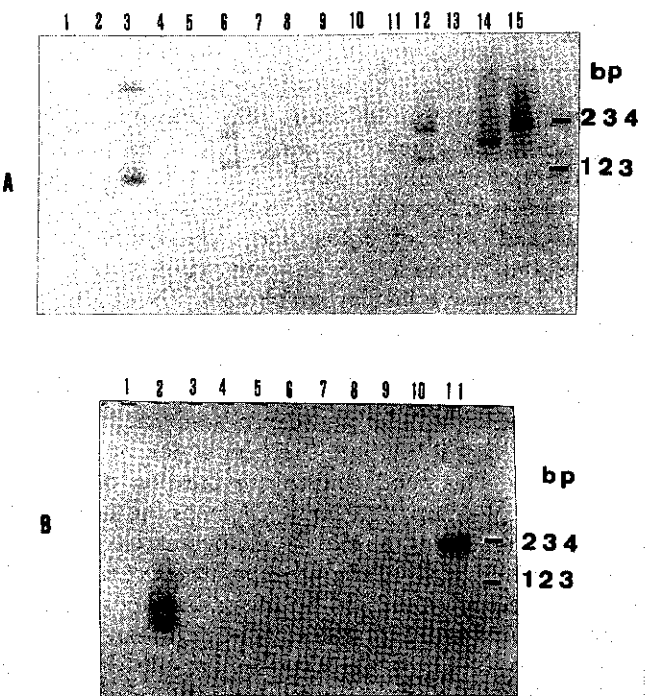

Fig. 1. Southern blot hybridization of PCR-amplified *bcl*-2/J_H sequences using digoxigenin-labeled oligomer probe for the t(14;18) mbr. A. HD DNAs were extracted from formalin-fixed, paraffin-embedded specimens. Lanes 3 and 14 are positive controls of 2 follicular lymphomas carrying t(14;18) mbr. Lane 15 is SU-DHL-6. Lanes 1, 2, 12, and 13 are the LP type of HD. Lanes 4, 5, 8, and 10 are the nodular sclerosis type of HD. Lanes 6, 7, 9, and 11 are the mixed cellularity type of HD. B. HD DNAs were extracted from unfixed, fresh-frozen specimens. Lane 11 is the positive control, SU-DHL-6. Lanes 1, 3–7, and 10 are of the mixed cellularity type. Lanes 2, 8, and 9 are of the lymphocyte predominance type.

quence amplified by PCR and detected by Southern blot hybridization using a Dig-ELISA kit. The distribution of histologic subtypes among these 3 cases was one MC and two LPs. Of the 23 HD DNAs extracted from formalin-fixed, paraffin-embedded specimens, one MC HD and one LP HD had an approximately 230 bp *bcl*-2(mbr)/J_H rearranged band, as shown in lanes 6 and 12 of Fig. 1A, respectively. Similarly, one LP HD case out of 21 HD DNAs extracted from unfixed, fresh-frozen specimens had an approximately 120 bp *bcl*-2(mbr)/J_H rearranged band (Fig. 1B, lane 2). The faster-migrating bands seen in these 3 HD cases carrying a t(14;18) mbr breakpoint may represent the single-stranded *bcl*-2(mbr)/J_H sequence, which has a different electrophoretic mobility from double-stranded *bcl*-2(mbr)/J_H. We carefully examined for the second time the histology of these t(14;18)-positive cases (1 MC and 2 LPs), and found no evidence of composite lymphoma. Further, one (Fig. 1B, lane 2) of the 2 LPs was nodular lymphocyte-predominance Hodgkin's disease. As no case of lymphocyte depletion type (LD) of HD, and few cases of NS, were available in our laboratory, our observations on LD and NS are inconclusive. However, other laboratories have reported the occurrence of the (14;18) translocation in LD and NS.[27–29] Therefore, it seems unlikely that the t(14;18) translocation corresponds to a specific histological subtype of HD. Our results thus support the view that t(14;18) translocation can be found in HD. Whether the t(14;18)-carrying cell population is the neoplastic component or the reactive component of HD needs to be studied further. However, since no t(14;18) translocation was detected previously in 20 T-cell lymphomas[8] and lymph nodes of tonsillitis contain cellular infiltrates similar to the benign component of HD, the cells with t(14;18) in HD may be a neoplastic component such as R-S cells, rather than a reactive component of HD. To identify precisely the t(14;18)-carrying cells in HD, further studies such as *in situ* hybridization, flow cytometry, and so on should be performed.

Detection of *bcl*-2(mcr)/J_H Studies were made on 44 cases of HD together with a positive control (one follicular lymphoma case carrying a *bcl*-2(mcr)/J_H rearranged sequence) to seek evidence of a t(14;18) mcr breakpoint in the same way as described above. Only the positive control gave a detectable *bcl*-2(mcr)/J_H rearranged band (data not shown).

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