Detection of 14q32 Translocations in B-Cell Malignancies by In Situ Hybridization With Yeast Artificial Chromosome Clones Containing the Human IgH Gene Locus

By Masafumi Taniwaki, Fumihiko Matsuda, Anna Jauch, Kazuhiro Nishida, Teruyuki Takashima, Shinichi Tagawa, Haruo Sugiyama, Shinichi Misawa, Tatsuo Abe, and Kei Kashima

Partner sites of 14q32 translocations found in B-cell malignancies were detected by fluorescence in situ hybridization (FISH) using yeast artificial chromosome (YAC) clones. Y20 and Y6, containing the human Ig heavy chain (IgH) gene locus. Y20 spans a 160-kb upstream and 40-kb downstream region of the JH segments on chromosome band 14q32.33. Y6 is 300-kb upstream of Y20, and spans a further 320-kb telomeric region. The human DNA sequences amplified by Alu polymerase chain reaction of the YAC clones were used as probes for FISH to study six patients with non-Hodgkin’s lymphoma (NHL), one patient with acute lymphoblastic leukemia, and one cell line FR4 established from a plasmacytoma. Three telomeric YAC clones each specific for 3q, 8q, and 18q were also used to further characterize 14q32 translocations. The IgH YACs were successfully applied to detect cytogenetically invisible subtelomeric translocation of the IgH gene locus to each partner site in t(14;18), t(8;14), and t(14;19), and to identify t(3;14) (q27;q32.33) in three patients with 14q32 translocation of unknown origin. Furthermore, complex translocations involving more than three chromosomes were detected in an NHL patient with t(8;14) and t(13;12), and in the FR4 with der(14)(t;8;14), der(8)dic(1;8), and del(1)(q21). The technique would be a useful tool in elucidating the mechanisms of a 14q32 translocation in B-cell malignancies.

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PARTNER SITES of 14q+ chromosomes have been of particular interest in B-cell malignancies, because specific 14q32 translocations have been often associated with distinct histologic or clinical subsets of the diseases, and because the molecular characterization of their breakpoints has led to the identification of genes deregulated by chromosome rearrangements. As already established, t(8;14) has been commonly associated with Burkitt lymphoma, t(14;18) with follicular lymphoma, and t(11;14) with intermediate lymphocytic lymphoma or chronic lymphocytic leukemia. Moreover, the recent delineation of new recurring 14q32 translocations, eg, t(3;14)(q27;q32) and t(9;14)(p13;q32), has further resolved the cytogenetic and clinical subsets of non-Hodgkin’s lymphoma (NHL). These specific translocations can be discerned by routine cytogenetic analysis. In addition, since high-resolution banding has been in common use, it accordingly allowed us to define subtle structural abnormalities in many cases of NHL. However, even this technique is not uniformly successful in unambiguously identifying the source of extra material on the 14q+ chromosome, because most patients had a complex karyotype with multiple structural abnormalities. This often reflects the limited resolution inherent in cytogenetic techniques, as known from the estimation that average high-resolution bands contain approximately 5 Mb DNA; it is difficult to distinguish between two high-resolution bands that are similar in size and in Giemsa staining property.

To overcome this limitation and therefore to identify the partner chromosomes of 14q32 translocations of unknown origin, we have performed fluorescence in situ hybridization (FISH) studies applying yeast artificial chromosome (YAC) clones. It has been shown in recent years that YAC clones, which span several hundred kilobases within a chromosome region of interest, have provided an ideal tool for in situ detection of the specific chromosomal translocations of leukemic cells. In the present study, using two YAC clones containing the human Ig heavy chain (IgH) locus, we detected the partner sites of 14q32 translocations in seven patients and in one cell line with B-cell malignancies through mapping the translocated 14q32.33 material on metaphase spreads of tumor cells.

MATERIALS AND METHODS

Patients and cell line. Six patients with NHL, one patient with acute lymphoblastic leukemia (ALL), and one cell line FR4 derived from a patient with plasmacytoma were subjected to the present study. Immunologic phenotype was studied in cell suspensions using a panel of monoclonal antibodies and monospecific antisera against heavy and light chains. The clinical and cytogenetic features are given in Table 1. All cases showed a variety of 14q32 translocations. In three patients with NHL, the origin of added material on 14q+ chromosomes could not be defined by GTG-banding. Translocations t(8;14), t(14;18), and t(14;19) were found in each case of NHL with the histologic subtype of small noncleaved, diffuse large, and intermediate lymphocytic lymphoma. A t(8;14) was also found in a patient with ALL-L3. The current karyotype of the FR4 cell line was near pentaploidy, containing characteristic chromosomal abnormalities, two each of der(14)(t;8;14) and del(1)(q21), and one of der(8)dic(1;8).

Metaphase chromosome preparation. For high-resolution mapping, prometaphase chromosomes were prepared from phytohemagglutinin (PHA)-stimulated normal lymphocyte cultures synchronized with methotrexate treatment and folinic acid release with concomitant addition of 5-bromodeoxyuridine (BrdU). Metaphase spreads of tumor cells were prepared either from short-term cultures of lymph cells.
Table 1. Clinical and Cytogenetic Findings in Seven Patients and One Cell Line Carrying 14q32 Translocation

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/Age (yr)</th>
<th>Disease Phenotype</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/64</td>
<td>ML, DL</td>
<td>48-47,XX,del(7)(q7),add(12)(q7),add(14)(q32),add(19)(p7),ino</td>
</tr>
<tr>
<td>2</td>
<td>M/64</td>
<td>ML, DL</td>
<td>45,X,-y,add(14)(q32.3);60-61qY,del(3p13),add(3q11.2),+14,+add(14)(q32.3),-18,+3-4mar</td>
</tr>
<tr>
<td>3</td>
<td>F/70</td>
<td>DSC, DSC</td>
<td>89-91,XX,del(11)(p38.1)(l)2,1(2)(10),-6,-7,-9,-10,add(14)(q32.3),der(16)(8;16);1(p11.2;p11.2),+1-3mar</td>
</tr>
<tr>
<td>4</td>
<td>F/50</td>
<td>ML, DL</td>
<td>47,XX,+12,14(14)(q32.3),+48-49,1dem,der(18)(14)(14),+1-2mar</td>
</tr>
<tr>
<td>5</td>
<td>M/69 ILL</td>
<td></td>
<td>48,XY,1(2)(q27;q11.2),t(14;19)(q32.3;q13.1)</td>
</tr>
<tr>
<td>6</td>
<td>F/69 M/69</td>
<td></td>
<td>46,XX,1(1)(q13p12.3q13.5),t(13q27p13.1),t(14q14q24.3);3q13(13q32q34)</td>
</tr>
<tr>
<td>7</td>
<td>M/66 ALL, L3</td>
<td>NA</td>
<td>46,XX,1(14)(q24.1q23.3)</td>
</tr>
<tr>
<td>8*</td>
<td>M/69</td>
<td>PCM, FR4 cell line</td>
<td>103-117,X,-Xx2,-Yx3,del(11q21)x2,del(11q17);1(q11);11p12x2,11q10x2,-2x2,-3x3,add(3q11),-4x2,-5xx2,del(5q12),12(2q23),i(6q10)x2,-7,add(7)x2,22x2,-8x2,der(8)dic(1;8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(p13;q24.1),-9x4,-10x3,-11,-12,-13x4,13(11q11p11)x2,-14x4,14(14q14.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(q24.1q32)x2,-15x4,der(15)(9;15)(q13p11)x2,-16x3,-17x2,+21i(21q10)x6,+10-15mar</td>
</tr>
</tbody>
</table>

Abbreviations: ML, malignant lymphoma; DL, diffuse large; DSC, diffuse small cleaved; ILL, intermediate lymphocytic lymphoma; PCM, plasmacytoma; NA, not available.

* Previously described by Tagawa et al.28

RESULTS

Ah-PCR products of Y20 and Y6 showed a number of bands of 0.3 to 4 kb in length (Fig 1). The size and intensity of individual bands were found to be specific to each YAC clone. Signals of Y20 were observed on both chromatids of chromosome 14, band 14q32.33 and on chromosome 15, band 15q11. Y6 showed strong signal on 14q32.33. Table 2 shows the summary of CISS hybridization analyses using Y20, Y6, and three other telomeric YAC clones. The intensity of the hybridization signal of IGH YAC clones is expected to be affected by the location of V segments to be involved in V(DJ) recombination. On the normal chromosome 14, Y20 showed no or considerably weak signal in every cases except patient no. 3; in contrast, Y6 demonstrated strong signal in five cases (patients no. 1 through 3, 6, and 7). Even using Y6, very weak signal was observed on the normal chromosome 14 in two NHL patients, each with t(14;18) and t(14;19), and no signal was observed in the FR4 cell line. FISH analysis with Y20 identified the partner sites of 14q32 translocations in seven of eight cases, whereas Y6 detected those in all. However, split signals expected to be on both the der(14) and the translocation partner were not observed in all cases, but signal was visible only on the partner chromosomes. In all three patients with 14q32 translocation of unknown origins, the signal of both Y20 and Y6 was observed on the terminus of 3q, although that of Y20 extremely weak in patient no. 3. Figure 2 shows FISH of Y6 to a metaphase spread from patient no. 3. Further characterization using a 13q9-specific YAC clone (HTY3206) showed the definitive transposition of terminal portion of 3q to the der(14). Moreover, the homologs of chromosome 3 were decorated from the p to the q terminus with 0.2 μg/mL of propidium iodide and mounted in antifade solutions. For high-resolution mapping of Y20 and Y6 to normal metaphases, R-bands were obtained by fluorescence-phototaxis technique after hybridization and detection of specific signals.21 Results were analyzed on a conventional fluorescence microscope Olympus BH2-RFC (Olympus, Tokyo, Japan) and photographed on Fuji D1600 (Fuji Film, Tokyo, Japan) or Scotch CHROME 1000 (Scotch, Italy) film.
Fig 1. Agarose gel (1.2%) of Alu-PCR-amplified YAC clone DNA. Lane 1, Y20 using CL1; lane 2, Y20 using CL2; lane 3, Y6 using CL1; lane 4, Y6 using CL2. Molecular sizes estimated from the relative migration distances of λ/HindIII marker are given to the right of the pictures of gel.

by a painting probe. Taking the G-banding pattern of der(3) into account, these results, together, unambiguously indicate that the translocation partner derives from the chromosomal material 3q27-ter. Figure 3A and B show the representative metaphase spreads from patient no. 2 by FISH with Y20.

Signal of the IgH YACs on metaphase cells from patient no. 4 was observed on the derivative chromosome 18, on both arms of the marker chromosome, and occasionally on the normal chromosome 14, indicating that the marker chromosome (mar) originated from the isochromosome of der(18) (Fig 3C). Further characterization using a 18q23-specific YAC clone (HTY3045) defined a reciprocal t(14;18) translocation (Fig 3D). Similarly, typical t(8;14) found in patient no. 7 with ALL-L3 was also confirmed to be a reciprocal translocation by FISH with both IgH YACs and a 8q24.3-specific YAC clone (HTY3048) (Fig 3E and F). In patient no. 5, the signal of Y20 was seen on the der(19) (Fig 3G).

G-banding analysis of patient no. 6 showed the characteristic abnormalities of t(3;12)(q27;q13.1), t(8;14)(q24.1;q32.3), and t(1;3;2)(p13.3;q12;q35), as shown in Fig 4. The signal of Y20 was found on the q arm of der(3)(3;12) (Fig 5A), although not on the normal chromosome 14, whereas Y6 detected both the normal and translocated chromosome band 14q32.33. The signal of a 3q29-specific YAC clone was observed on the p arm of der(12)t(3;12) and the q arm of derivative chromosome 2 [der(2)t(1;3;2)], the distal part of which was painted by the chromosome-3-specific library DNA (Fig 5C and D). As expected, further characterization using a 8q24.3-specific YAC clone showed signals on the der(14) and the normal chromosome 8 (Fig 5B). These results suggest that chromosomes 3, 8, 12, and 14 are involved in a complex translocation, although we could not perform the study using DNA probe specific for 12p13 to define translocated material on the der(8).

As shown in Fig 6, the FR4 cell line contained two each of der(14)(8;14) and der(19)q21), and one der(8)dic(1;8) (p13;q24.1). Both Y20 and Y6 showed hybridization signals on the del(1)s at their breakpoint 1q21 (Fig 3H). Signals are not observed on the normal chromosome 14,der(14)s or on the der(15)s. The signals of a 8q24.3-specific YAC clone were seen on the der(14)s and the normal chromosome 8, as expected from the G-banding pattern. The chromosome 14-specific DNA library showed two der(14)s that have an unpainted additional end of the q arm (data not shown). However, the two del(1)(q21)s were not painted.

**DISCUSSION**

Y20 containing the sequence that spans both the upstream 160-kb and the downstream 40-kb region of the JH segments was assigned to band 14q32.33 and 15q11 on R-banded prometaphase chromosomes. Chromosome band 15q11 was expected to show signal because Y20 contained D segments,15 translocated human D gene segments have been mapped on 15q11-q12.23 The most telomeric IgH YAC clone Y6 was assigned to band 14q32.33. No signal on 16q11, in the present study, confirms that Y20 and Y6 contain no VH-F region-homolog, which was located on chromosome 16.24 By using FISH with the IgH YAC clones, we have detected the partner sites of 14q32 translocation on metaphase.

**Table 2. Characterization of 14q32 Translocations by FISH With YAC Clones Containing the Human Ig Heavy Chain Locus**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Disease</th>
<th>Y20*</th>
<th>Y6</th>
<th>Telomeric YACst</th>
<th>GTG</th>
<th>14q32 Translocations</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ML, DL</td>
<td>3qter</td>
<td>14,3qter</td>
<td>HGY3206:3, der(14)</td>
<td>14q+</td>
<td>t(3;14)(q27;q32.33)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ML, DL</td>
<td>3qter</td>
<td>14,3qter</td>
<td>HGY3206:3, der(14)</td>
<td>14q+</td>
<td>t(3;14)(q27;q32.33)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ML, DSC</td>
<td>14x2</td>
<td>14x2, 3qterx2</td>
<td>HGY3206:3, der(14)</td>
<td>14q+</td>
<td>t(3;14)(q27;q32.33)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ML, DSC</td>
<td>der(18)</td>
<td>der(18)</td>
<td>HGY3048:18, der(14)</td>
<td>t(14;18), mars</td>
<td>t(14;18)(q23.23;q21), der(18)t(14;18), ider(18)t(14;18)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ILL</td>
<td>der(19)</td>
<td>der(19)</td>
<td>HGY3045:18, der(14)</td>
<td>t(14;19)</td>
<td>t(14;19)(q23.23;ql3.1)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>ML, DSC</td>
<td>3qter</td>
<td>14,3qter</td>
<td>HGY3206:3, der(22)</td>
<td>t(8;14)</td>
<td>t(3;12;8;14)(q27;ql3.1;q24;ql3.23)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>ALL, L3</td>
<td>der(8)</td>
<td>14, der(8)</td>
<td>HGY3048:8, der(14)</td>
<td>t(8;12)</td>
<td>t(8;14)(q24;q32.33)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>PCM, FR4</td>
<td>del(11)x2</td>
<td>del(11)x2</td>
<td>HGY3048:8, der(14)</td>
<td>del(14)(8;14)</td>
<td>der(14)(8;14)(q24.1;q32.33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cell line</td>
<td></td>
<td></td>
<td></td>
<td>del(1)(q21)</td>
<td>del(1)(q21)(14)(q21)(q32.33)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: GTG, G-bands by trypsin using Giemsa.

* Signals were also observed on chromosome 15, band 15q11.
† HGY3206, HGY3048, and HGY3045 were mapped to the respective bands, 3q29, 8q24.3, and 18q23.
DETECTION OF 14q32 TRANSLOCATIONS BY FISH

Fig 2. FISH of Alu-PCR-amplified Y6 to lymphoma cells of patient no. 3 (A). The same metaphase is counterstained with DAPI (B). Arrows and arrowheads indicate der(3)s and normal chromosomes 14, respectively.

Fig 5. FISH to lymphoma cells of patient no. 6 carrying a complex translocation involving chromosomes 3, 8, 12, and 14. DAPI pictures corresponding to each FISH illustration are given in the bottom photographs. Arrows indicates the translocated YACs: (A) Y20 on the q arm of der(3)t(3;12); (B) 8q24.3-specific YAC on the 14q- chromosome; and (C) 3q29-specific YAC on the der(12)t(3;12). The signal of 3q29-specific YAC was also observed on the derivative chromosome 2 [der(2)t(1;3;2)], the distal part of which was painted by the chromosome 3-specific library (arrowheads in [D]).

cells from seven patients and one cell line with B-cell malignancies. However, the der(14) was not labeled at all when using Y20 that spans the breakpoint in the majority of 14q32 translocations. It seems likely that the sequences downstream of the JH segments contained in Y20 are deleted, as has been reported in B-cell malignancies of various histologic or clinical subsets. It has also recently been reported that a submicroscopic interstitial deletion of the der(14) within
the region defined by the \(S_\mu\) on the 5' side and the C, on the 3' end, extending from 100 to 300 kb DNA, occurred in approximately 75% of cases of follicular lymphoma with t(14;18). Indeed, our FISH study could not demonstrate the signal of Y20 on der(14) in patient no. 4 carrying t(14;18). Alternatively, the constant region can not be effectively amplified by Alu-PCR. On the other hand, no signal or a considerably weak signal of Y20 on the normal chromosome 14, as observed in seven of eight cases, and that on the translocation partner in patient no. 3 presumably resulted from significant DNA deletion caused by V(D)J and class switch recombination of the corresponding allele of IgH genes.

In the established subsets of 14q32 translocations, t(8;14), t(14;18), and t(14;19), Y20 and Y6 clearly detected the translocation partners, indicating that these YACs are valuable as a diagnostic aid. Additionally, FISH provided the direct evidence of reciprocal translocation in each case through visualization of IgH locus on the translocation partners. Likewise, the isochromosome of der(18), which has been previously noted in t(14;18) as well as in the duplication,\(^{27,28}\) was identified in patient no. 4. FISH analysis showed the amplification of translocated IgH locus on der(18). Amakawa et al\(^{29}\) found that the 3' sequences of BCL-2 gene were truncated within the major breakpoint cluster region and retained on the der(18) amplified in two lymphoma cell lines through the duplication of a preexisting der(18). Together with these findings, our results indicate that the resultant chimeric DNA between IgH and 3' BCL-2 amplified on der(18).

All three cases of a 14q+ chromosome with unknown added material, which was similar in size, were diagnosed as having t(3;14)(q27;q32.33) by FISH. Since we previously described two other B-cell NHL patients with t(3;14),\(^{30}\) five patients have been encountered in our own series of 90 patients with NHL, suggesting that the t(3;14) is among the most common translocations in NHL. There are differences in the incidence rates of these specific 14q32 translocations, which may be partly caused by geographic or racial differences. For example, t(14;18) was significantly less frequent in Japanese NHL cases compared with those of western countries;\(^{31}\) the incidence of t(11;14) was higher in USSR compared with an American series.\(^{32}\) The t(3;14) may be the most recurrent 14q32 translocation in Japanese NHL cases because, compared with western countries, more than 40% of patients suffered from diffuse lymphomas, which are correlated with this translocation. This study has documented 15 cases of t(3;14) in a series of French 319 patients;\(^{3}\) whereas no cases were noted in the large series of 278 American patients with clonal chromosome abnormalities.\(^{3}\) However, in the latter study, a variant form t(3;22)(27;q11) was described in eight patients. Thus, it remains to be re-

solved whether there are geographic differences in incidence rates of t(3;14) and its variants.

Because the cytogenetic appearance of this telomeric reciprocal translocation makes it difficult to recognize, banding studies of lymphomas can sometimes result in an indeterminate classification of 14q32 translocations. By using the standard culture technique, the origin of extra chromosomal material on 14q+ chromosome was not established in 28% to 65% of previously reported cases.\(^{27,27,28}\) Moreover, even high-resolution banding techniques are not uniformly successful in our laboratory in defining added material on 14q+ chromosome.\(^{3}\) In this regard, IgH YAC clones, particularly Y6, promise a new approach in determining the partner site of 14q32 translocations of unknown origins. The translocation, in which one of the two reciprocal translocation products can be seen by G-banding and the other cannot be detected, is not uncommon in tumor cytogenetics. The transposition of the IgH gene locus could be detected neither by conventional cytogenetic methods nor by FISH with chromosome 14-specific library DNA. Such a painting probe has delineated complex translocations,\(^{34}\) but has been insufficient to detect extremely small translocations that may occur at telomeres. In contrast, FISH with YAC clones is so sensitive that the technique supplements conventional cytogenetic techniques to define cryptic chromosomal translocation events that are
undetectable cytogenetically. However, further characterization of the cryptic translocations of the IgH locus provides an additional strategy for the identification of partner chromosomes of 14q32 translocations and their complex variants, and thereby the clinically relevant classification of these translocations may be delineated. Moreover, the detailed molecular analysis will be focused on particular DNA joining sequences between chromosome 14 and translocation partners.

ACKNOWLEDGMENT

We are grateful to Helen Donis-Keller (Washington University, St Louis, MO) for providing the telomeric YAC clones and to Joe W. Gray for providing the plasmid libraries from sorted human chromosomes 3 and 14. We thank Thomas Cremer (Institut für Humangenetik and Anthropologie) for critical reading of the manuscript.

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Fig 6. Partial karyotype of the FR4 cell line. (A) G-banding. The del(1)(q21), der(8)t(1;8), and der(14)t(8;14) are shown. (B) Q-banding with actinomycin D and DAPI staining. Arrowheads indicate the secondary constriction region of chromosome 1.
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