Interphase and Metaphase Detection of the Breakpoint of 14q32 Translocations in B-Cell Malignancies by Double-Color Fluorescence
In Situ Hybridization

By Masafumi Taniwaki, Kazuhiro Nishida, Yutaka Ueda, Shinichi Misawa, Masami Nagai, Shinichi Tagawa, Tamosu Yamagami, Haruo Sugiyama, Masafumi Abe, Shiro Fukuhara, and Kei Kashima

The breakpoint of 14q32 translocations found in B-cell malignancies was delineated specifically in both metaphase spreads and interphase nuclei by double-color fluorescence in situ hybridization (FISH) using bacteriophage clones containing the human immunoglobulin gamma chain gene locus (Iγγ) and a cosmide clone, CY24-68, containing Vγ segments. CY24-68 is more telomeric than Iγγ, separated by approximately 1 megabase (Mb). FISH studies were performed on four patients with non-Hodgkin’s lymphoma (NHL), one with acute lymphoblastic leukemia (ALL), and four cell lines derived from ALL, NHL, and plasmacytoma (PCM) were studied. Immunophenotype was studied in cell suspensions using a panel of monoclonal antibodies and monospecific antisera against heavy and light chains. G-banding analysis showed 14q32 translocations in all cases except for one; t(8;14)(q24.1;q32.3) was found in a patient with NHL and in ALL cell line HS-1, t(14;18)(q32.3;q21.3) in two patients with NHL, t(3;14)(q27;q32.3) in a patient with NHL, t(1;14)(q13;q32.3) in a patient with PCL and in ALL cell line HBL-2, and complex 14q32 translocation involving chromosome 1, 8, and 14 in PCM cell line FR4.

CHROMOSOME 14q32 translocation is a nonrandom structural abnormality in B-cell malignancies with major phenotype, occurring at a frequency of 40% to 60% in non-Hodgkin’s lymphoma (NHL),1-4 approximately 10% in chronic lymphocytic leukemia (CLL),5-7 and 30% to 60% in multiple myeloma (MM) with abnormal karyotype.5-7 Certain subtypes of 14q32 translocations have been associated with distinct histologic or clinical subsets of the disease; for example, t(8;14) has been commonly associated with Burkitt’s lymphoma/leukemia, t(14;18) with follicular lymphoma, t(11;14) with intermediate lymphoma or CCL, and possibly t(6;14) with MM.7 These specific translocations can be discerned by routine G-/Q- and high-resolution banding techniques. In addition, the recent advance of fluorescence in situ hybridization (FISH) using yeast artificial chromosome (YAC) clones has provided a new approach to detect cryptic translocation of the immunoglobulin heavy chain (IγH) gene locus at each partner site in 14q32 translocations.8 However, even this FISH approach has not been successfully applied to identify the breakpoint of 14q32 translocations. Moreover, cytogenetic studies have not been uniformly successful on tumor samples from B-cell malignancies, particularly from CLL and MM because of the difficulty of obtaining mitotic cells.

To identify the breakpoint of 14q32 translocations in both metaphase spreads and interphase nuclei, we performed double-color FISH with bacteriophage clones containing the human Ig gamma (Iγγ) gene and a cosmid clone containing variable region (VγH) gene segments of the IgH locus.9 Using this technique, 14q32 translocations were clearly detected as the separate signals of these two loci, or those of Iγγ genes in individual tumor cells.

MATERIALS AND METHODS

Patients and cell lines. One patient with acute lymphoblastic leukemia (ALL), and four with NHL, one with plasma cell leukemia (PCL), and three cell lines derived from ALL, NHL, and plasmacytoma (PCM) were studied. Immunophenotype was studied in cell suspensions using a panel of monoclonal antibodies and monospecific antisera against heavy and light chains. G-banding analysis showed 14q32 translocations in all cases except for one; t(8;14)(q24.1;q32.3) was found in a patient with NHL and in ALL cell line HS-1, t(14;18)(q32.3;q21.3) in two patients with NHL, t(3;14)(q27;q32.3) in a patient with NHL, t(1;14)(q13;q32.3) in a patient with PCL and in ALL cell line HBL-2, and complex 14q32 translocation involving chromosome 1, 8, and 14 in PCM cell line FR4.

Chromosome preparation. For high-resolution mapping, prometaphase chromosomes were prepared from phytohemagglutinin (PHA)-stimulated normal lymphocyte cultures synchronized with methotrexate and folinic acid release with concomitant addition of 5-bromodeoxyuridine. Metaphase spreads of tumor cells were prepared either from short-term cultures of lymph node suspensions or bone marrow cells or cell suspensions fixed in methanol/acetic acid (3:1) and stored at −20°C. GTG-banded metaphases were arranged ac-

From the Third Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto; First Department of Internal Medicine, Kaga Medical School, Kaga; Department of Hematology and Oncology and Second Department of Internal Medicine, Osaka University, Osaka; Department of Pathology, Fukushima Medical School, Fukushima; and First Department of Internal Medicine, Kansai Medical School, Osaka, Japan.


Supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education (No. 05151048) and in part by a Grant-in-Aid from the Ministry of Health and Welfare, Japan.

Address reprint requests to Masafumi Taniwaki, MD, Third Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kawaiamachi-Hirokoji, Kamiyogo-ku, Kyoto 602, Japan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1995 by The American Society of Hematology.
probes, CY24-68 and Igy, are indicated under the genomic map of IgH gene locus. CY24-68 probe normal nuclei with the single signal of CY24-68 resulting from the deletion of VH segments caused by VDJ and class switch recombination.

cording to the recommendations of ISCN (1991). Control samples for interphase FISH consisted of PHA-stimulated peripheral blood lymphocytes (PBL) obtained from healthy donors and lymph node cells from a patient with lymphadenitis, all with normal chromosome status.

DNA probes. Three bacteriophage clones containing the gamma constant region of the IgH locus, Igγ-15, Igγ-2, and Igγ-10, each approximately 10 kb in size, a cosmide clone CY24-68 containing seven Vκ segments (Fig 1A), and YAC clone Y12 were kindly provided by Dr Fumihiro Matsuda (Center for Molecular Biology and Genetics, Kyoto University, Kyoto, Japan). CY24-68 is approximately 40 kb in size, subcloned from a YAC clone Y24, and is ~1 Mb telomeric from Igy. Y12 was assigned to the band 15q11 in our laboratory. The plasmid libraries from sorted human chromosome 11 and 14 were a generous gift from Dr Joe W. Gray (University of California, San Francisco).

Double-color fluorescence in situ hybridization (FISH). Labeling of the DNA probes and chromosomal in situ suppression hybridization were performed as described previously. Briefly, bacteriophage DNA, Alu-PCR-amplified Y12, and chromosome-specific library DNA were labeled by standard nick translation using biotin-16-dUTP (Boehringer, Mannheim, Germany), and cosmide DNA was labeled using digoxigenin-11-dUTP (Boehringer). Three Igy-gene clones, Igy4-2, Igy4-1S, and Igy-1O, showed specific signal on chromosome 14q32.33. Regardless of such an insert DNA as small as 10 kb in size, the signal of these clones was as intense as that of alphoid DNA probes. The signal intensity did not differ between clones, possibly because of the striking homology (more than 90%) among the human Igy chain genes, indicating cross-hybridization to the target DNA. We used pooled bacteriophage DNA as a probe for FISH to cover the whole Igy chain locus, although a single phage clone was sufficient to show the Igy locus under the present in situ hybridization conditions. CY24-68 containing Vκ segments was also assigned to band 14q32.33. Double-color FISH showed that the physical distance between Igy and CY24-68 was measured in G1 interphase nuclei obtained from a patient with lymphadenitis.10

RESULTS

Three Igy-gene clones, Igy4-15, Igy4-2, and Igy4-10, showed specific signal on chromosome 14q32.33. Regardless of such an insert DNA as small as 10 kb in size, the signal of these clones was as intense as that of alphoid DNA probes. The signal intensity did not differ between clones, possibly because of the striking homology (more than 90%) among the human Igy chain genes, indicating cross-hybridization to the target DNA. We used pooled bacteriophage DNA as a probe for FISH to cover the whole Igy chain locus, although a single phage clone was sufficient to show the Igy locus under the present in situ hybridization conditions. CY24-68 containing Vκ segments was also assigned to band 14q32.33. Double-color FISH showed that the physical distance between Igy and CY24-68 was 0.76 ± 0.51 µm (mean ± SD) in lymph node cells with normal karyotype, confirming the genomic distance of ~1 Mb between them.

Table 1 shows the summary of double-color FISH studies on metaphase and interphase cells obtained from three normal control samples and from nine cases of various 14q32 translocations. No significant difference in hybridization efficiency was observed between freshly prepared samples and archival cell suspensions. In each patient with t(8;14), t(14;18), and t(3;14), green signal of Igy was observed at the cytogenetic breakpoint on the der(14), 14q32.33, and red signal of CY24-68 at respective partner site (8q24.1, 18q21.3, and 3q27). Figure 2 shows the representative metaphase spreads from patients no. 1 and no. 2 by double-color FISH with CY24-68 and Igy.

In the ALL cell line HS-1, FISH with CY24-68 and Igy showed a colocalized red and green signal on the normal chromosome 14, whereas separated green signals of Igy were detected at 8q24.1 on the der(8) and at 14q32.33 on
the der(14); no red signal of CY24-68 was observed on the der(8) (Fig 3A). As already described in the previous FISH studies, the cell line FR4 showed a complex 14q32 translocation involving both homologues of chromosome 1 (1p13 and 1q21), 8q24, and 14q32. Using double-color FISH, the present study showed only the green signal of IgY at the breakpoint 8q24.1 on dic(8)t(1;8). As shown in Fig 3C, colocalized green and red signals were noted at 1q21 on der(1)t(1;14) and the terminus of the long arm of der(1)t(1;7), suggesting that der(1)t(1;7) might be derived from der(1)t(1;14) by insertion of the short arm of chromosome 7.

Double-color FISH studies of the cell line HBL-2 clearly identified t(11;14) by showing red signal (IgY) on der(11) and green signal (CY24-68) on der(14) (Fig 3B). This green signal was extremely intense and occupied the characteristic pale band at the breakpoint region 14q32.33 on the der(14), which is faintly painted with chromosome 14-specific library DNA. HBL-2 exhibited two different 14q+ chromosomes on the same metaphase spreads (Fig 4). No signal was observed on the 14q+ chromosome derived from t(14;15) (q32.3;q15), suggesting that the breakpoint is more centromeric than the IgY locus on the der(14)t(14;15). Y12 specific to 15q11 labeled the paracentromeric region of the small acrocentric chromosome, possibly the der(15)t(14;15). In addition, the colocalization of green and red signals was noted on both add(8)(p23) and add(11)(q23) (Figs 3 and 4).

The split signal of green and red spots was shown in interphase nuclei, indicating 14q32 translocation. For quantitative analysis, four categories were defined to classify each nucleus according to hybridization pattern, ie, normal (N), split (S), tumor (T), and indeterminate (ID) hybridization pattern (Fig 1B). Nuclei with two colocalized signals of green and red spots were classified as the N pattern. Based on the interphase distance between IgY and CY24-68, the S pattern was defined as green and red spots separated by more than 2 μm. The T pattern was defined as abnormal hybridization patterns expected from metaphase FISH characteristic to each cell line, as shown in Fig 3D-F. Interphase nuclei, which were difficult to evaluate or classify into these categories, were included under the category of ID. These are the result of not only incomplete hybridization or heavy
Fig 2. Double-color FISH of patient no. 1 with ALL carrying t(8;14) (A and C) and of patient no. 2 with NHL carrying t(14;18) (B and D). Arrows indicate the split signals of IgH gene locus on both metaphase cells and interphase nuclei; the green and red signals are originated from Igγ and CY24-68, respectively. DAPI pictures are shown on the right side of the corresponding FISH metaphases.

Fig 3. Metaphase and interphase FISH of three cell lines. DAPI pictures are shown on the right side of the corresponding FISH metaphases. (A and D) HS-1 cell line. Arrows indicate the green signals of Igγ split in both metaphase and interphase cells. (B and E) HBL-2 cell line. An arrow indicates the intense and multiple signals of Igγ on der(14)t(11;14), showing the amplification of Igγ gene locus. The arrowhead in (B) indicates the red signal of CY24-68 on der(11)t(11;14)add(11)(p15). (C and F) FR4 cell line. Small and large arrows indicate colocalized Igγ and CY24-68 signals (green and red spots) on der(11)t(11;14) and der(11)t(11;7), respectively. In interphase nuclei, multiple colocalized Igγ and CY24-68 were also noted. Inset: The arrowhead indicates the signal of Igγ on dic(8)t(1;8), suggesting that the Igγ gene locus is involved in the complex 14q32 translocation in this cell line. (A) and (F) show double exposures of signals together with DAPI.
the signal of the YAC clone Y12 on 15q11.

INTERPHASE DETECTION

of FISH signals in HBL-2 cell line. From left to right, the normal chromosome 8, add(8)(q23), add(11)(q23), der(11)(t(11;14)(q13;q32.3) add(11)(p15), der(14)(t(11;14), der(14)(t(14;15)(iq52.1;q15), add(15) (p11), and der(15)(t(14;15) are shown. Open circles and closed squares represent the signals of Igy and V_H genes, respectively. A large open circle indicates the amplification of Igy gene locus. Asterisks indicate the signals of Igy and VH genes, respectively. A large open circle indicates the amplification of Igy gene locus. Asterisks indicate the signals of Igy and VH genes, respectively.

The intense signal of Igy in the cell line HBL-2 indicates amplification of Igy gene locus at the breakpoint 14q32.33 on der(14)(t(11;14). It is possible to deduce that the resultant chimeric DNA between Igy and DNA sequence at 11q13 is amplified in this region, as amplification units have been shown to encompass various positions of 11q13 in a wide range of human carcinomas. Amplifics were estimated to range from 700 kb to 4.5 Mb in size. It will be of interest to determine in the future whether a similar phenomenon occurs in clinical samples to clarify the relevance of Igy amplification to the development and progression of B-cell malignancies carrying t(11;14).

The present technique provided a rapid diagnostic procedure to detect the established subsets of 14q32 translocations, ie, t(8;14), t(14;18), t(3;14), and t(11;14) in interphase nuclei by identifying split signals of Igy and CY24-68 (Table 1 and Fig 2). Interphase FISH is also useful in identifying tumor patterns specific to each cell line, as expected from metaphase FISH. A similar approach using multicolor FISH has been described to show split signals of c-myc oncogene in JI Burkitt’s lymphoma cell line carrying a t(2;8)(p13;q24) translocation.24 Interphase FISH with region-specific probes is based on distinct territories of each chromosome and chromosomal segment in interphase nuclei, as well as with whole chromosome painting probes.25 In the range of DNA distance within 1 Mb, interphase distance is linearly correlated with DNA distance.26 Because we defined split signal as green and red signals separated by more than 2 µm in interphase nuclei, it is possible to miss some positive cells containing the Igy signal less than 2 µm distant from the CY24-68 and outside the territories of chromosome 14 (false-negatives). It is also possible using interphase FISH to falsely diagnose cells as positive. Hence, a more specific approach should be combined with the current study to show the established 14q32 translocations using double-color FISH with Igy and DNA sequences located at partner sites; for example, a combination of Igy and c-myc for the detection of t(8;14), and Igy and BCL2 for t(14;18) (Taniwaki et al, manuscript in preparation). Such an approach has been applied successfully to show BCR-ABL fusion in chronic myelogenous leukemia.27,28

In conclusion, double-color FISH using bacteriophage containing Igy genes and a cosmide clone containing V_H segments provides a powerful tool for the rapid detection of breakpoints of 14q32 translocations in interphase nuclei, thereby allowing unambiguous diagnosis of 14q32 translocation-positive B-cell malignancies.

DISCUSSION

By using double-color FISH with Igy genes and a cosmide clone containing VH segments (CY24-68), we showed the breakpoint of IgH locus involved in 14q32 translocations in both metaphase spreads and interphase nuclei. In addition, the amplification of Igy was identified at the breakpoint region on der(14)(t(11;14) in the cell line HBL-2 derived from B-cell NHL. As expected, in each patient carrying t(8;14), t(14;18), and t(3;14), FISH with Igy and CY24-68 clearly showed green signal (Igy) on the der(14) and red (CY24-68) on each partner chromosome. The results provide a rapid and unequivocal cytogenetic diagnosis even on late metaphase spreads with poor morphology. Furthermore, FISH studies on the cell lines raveled out complicated structural abnormalities by detecting, not only cryptic translocation, but also amplification or deletion of the Ig heavy chain gene locus.

In two cell lines each carrying a t(8;14) or its complex form, the signal of Igy was observed at the breakpoint region 8q24.1. As shown in Table 1 and Fig 3A, split green signals of Igy on both der(14) and der(8) were observed in HS-1, no signal of CY24-68 was detected on der(8). These results indicate that the breakpoint of der(14) was within the Igy locus and that the DNA sequences representative of CY24-68 underwent deletion, presumably due to VDJ recombination. Similarly, green signals of Igy were found both at 8q24.1 on dic(8)(t;18) and at 1q21 on der(1)(t(1;14), suggesting that cytogenetic breakages occurred in the DNA sequence centromeric to the Igy locus and subsequently within it. As reported previously, in a Burkitt-type t(8;14) and its variant forms t(2;8) and t(8;22), the breakpoints within the immunoglobulin gene locus can occur in regions including V_H and J_H segments and switch regions for C_H, C_G or C_A.17,16

The switch regions are also involved in other 14q32 translocations; C_J in t(9;14)(p13;q32.3)19 and t(14;19)(q32.3; q13.1),20 and C_L in t(2;14)(p13;q32).21 Our FISH results obtained from each case of t(8;14) and its complex variant were consistent with previous documentations of breakpoints on derivative chromosome 14 in t(8;14).17,18

The high incidence of the ID pattern in control samples (45% to 20%) despite sufficient hybridization. According to this criteria, it was shown that the percentage of the S pattern in tumor samples of t(8;14), t(14;18), t(3;14), and t(11;14) was significantly higher than that in normal controls (45% to 74% v 4% to 5%). The majority of nuclei from the three cell lines exhibited T patterns (89% to 98%).

Fig 4. Partial G-banded karyotype and schematic representation of FISH signals in HBL-2 cell line. From left to right, the normal chromosome 8, add(8)(q23), add(11)(q23), der(11)(t(11;14)(q13;q32.3) add(11)(p15), der(14)(t(11;14), der(14)(t(14;15)(iq52.1;q15), add(15) (p11), and der(15)(t(14;15) are shown. Open circles and closed squares represent the signals of Igy and V_H genes, respectively. A large open circle indicates the amplification of Igy gene locus. Asterisks indicate the signals of Igy and VH genes, respectively.
ACKNOWLEDGMENT

We are grateful to Dr Fumihiko Matsuda and Dr Tasuku Honjo (Kyoto University, Kyoto) for providing Igγ2,15, Igγ6,2, and Igγ-10, CY24,58, and Y12, and to Dr Joe W. Gray (University of California, San Francisco) for providing the plasmid library from sorted human chromosome 14. We are also grateful to Dr Haruki Wakasa (Department of Pathology, Fukushima Medical School) for providing the HBL-2 cell line.

REFERENCES
11. Tagawa S, Doi S, Taniwaki M, Abe T, Kanayama Y, Nogima J, Matsubara K, Kitanis T, Amayase-produce plasmacytoma cell lines, AD3 and FR4, with det(14)(q32.3;14) and dic(3)(1;19) established from ascites, Leukemia 6:100, 1990

From www.bloodjournal.org by guest on October 3, 2017. For personal use only.
Interphase and metaphase detection of the breakpoint of 14q32 translocations in B-cell malignancies by double-color fluorescence in situ hybridization

M Taniwaki, K Nishida, Y Ueda, S Misawa, M Nagai, S Tagawa, T Yamagami, H Sugiyama, M Abe and S Fukuhara