Detection of 11q13 Rearrangements in Hematologic Neoplasias by Double-Color Fluorescence In Situ Hybridization

By Lionel J.A. Coignet, Ed Schuuring, Robby E. Kibbelaar, Ton K. Raap, Karin K. Kleverda, Marie-Françoise Bertheas, Joop Wiegant, Geoffrey Beverstock, and Philip M. Kluin

Rearrangements within the chromosome 11q13 region are frequent in hematologic malignancies. 50% to 75% of mantle cell lymphomas (MCLs) carry a translocation t(11;14) (q13;q32). Using Southern blot analysis, a BCL1 breakpoint can be detected in approximately 50% of MCLs. It is not known whether other MCLs harbor also breakpoints at 11q13. Breakpoints in this region not involved in t(11;14), are detected in chronic lymphocytic leukemia and acute myeloid leukemia. To detect and localize breakpoints at 11q13 more accurately, we have developed fluorescence in situ hybridization using two probe sets of differently labeled cosmids, symmetrically localized at either side of the major translocation cluster of BCL1. These probes span a region of 450 to 750 kb. We applied this assay to a series of hematologic malignancies with 11q13 abnormalities identified by classical cytogenetics. All four samples with a t(11;14) (q13;q32) showed dissociation of the differently colored signals in metaphase and interphase cells, thereby indicating a chromosomal break in the region defined by the probe sets. The frequency of abnormal metaphase and interphase cells was comparable with that observed by banding analysis. No dissociation was observed in any of the 13 malignancies with other chromosomal 11q13 abnormalities, indicating that these chromosomal breaks occurred outside the 450- to 750-kb region covered by the probes. One patient showed triplication and one patient showed monoallelic loss of this region. The current data show that double-color fluorescence in situ hybridization is a simple and reliable method for detection of the t(11;14)(q13;q32) in interphase cell nuclei and that it can be used to distinguish this translocation from other 11q13 rearrangements in hematologic malignancies.

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used to study the (11;14)(q13;q32) translocation with FISH. The karyotype as determined by banding showed pseudodiploidy: 42-46 <2n> XX, del(1)(q42), +3, der(3)(3;8)(p13;q12), −8, der(14) (t(11;14)(q13;q32). The karyotype was reconfirmed for the purpose of this study. Peripheral blood lymphocytes from a healthy volunteer were stimulated with PHA (see below) and were cultured with phytohaemagglutinin (PHA; see below) and were used as a normal control. Bone marrow (BM) or peripheral blood (PB) cultures from 16 patients with chromosomal aberrations involving the 11q13 region and from 1 patient with an uncharacterized 11q abnormality were retrieved from the archives of the Laboratory of Hematological Cytogenetics (CHRU, St-Etienne, France) and the Department of Human Genetics (Leiden, The Netherlands). All patients had hematological malignancy. Clinical and cytogenetic data are shown in Table 1. Because of the retrospective nature of the study, no material was left for other molecular investigations such as polymerase chain reaction and Southern blot analysis. From these 16 patients, 3 (cases no. 1 and 2 with MCL and case no. 3 with PCL/MM) had a t(11;14)(q13;q32). Seven patients (cases no. 4-7, 11, 13, and 14) had a translocation with involvement of 11q13 other than t(11;14). Five patients had an interstitial deletion of 11q with involvement of 11q13. One additional patient had a translocation at 11q that could not be characterized (case no. 17).

**Banding analysis.** Cell synchronization, metaphase harvesting, and slide preparation were performed as previously described. Banding analysis was performed directly or after 1 to 5 days of culturing with or without addition of mitogens (12-0-tetradecanoylphorbol-13-acetate [TPA] and interleukin-2 [IL-2]). PB lymphocytes from the healthy volunteer were stimulated with PHA. Karyotypes were analyzed according to the International System of Nomenclature (ISCN-1991).

**FISH analysis.** Five cosmid probes were used for delineation of the 11q13 region surrounding BCL1. These probes, ie, COS6.7, COS3.51, COS3.91, COS6.31, and COSH1.5 probes, have been previously described. The probes were divided into two sets, (1) COS6.7 and COS3.51 and (2) COS3.91, COS6.31, and COSH1.5. The physical order is shown in Fig 1. Together, these probes span a region of minimally 450 and maximally 750 kb. The first set was labeled with digoxigenin and visualized in red (tetramethylrhodamin isothiocyanate [TRITC]), whereas the second set was labeled with biotin and visualized in green (fluorescein isothiocyanate [FITC]). FISH was performed according to standard protocols and as described previously. In brief, the probes were labeled by nick-translation; two cocktails of probes were made, one labeled with biotin and visualized in green (fluorescein isothiocyanate [FITC]), whereas the second set was labeled with digoxigenin and visualized in red (tetramethylrhodamin isothiocyanate [TRITC]), whereas the second set was labeled with biotin and visualized in green (fluorescein isothiocyanate [FITC]). FISH was performed according to standard protocols and as described previously. In brief, the probes were labeled by nick-translation; two cocktails of probes were made, one labeled with biotin and visualized in red (tetramethylrhodamin isothiocyanate [TRITC]), whereas the second set was labeled with digoxigenin and visualized in green (fluorescein isothiocyanate [FITC]).
beled probes were visualized by subsequent incubations with mouse antidigoxigenin, TRITC-conjugated rabbit antimouse antibody, and goat antirabbit TRITC. Cells were counterstained with diaminobenzidine (DAB) and embedded in Vectashield (Vector Laboratories Inc, Burlingame, CA). Hybridization signals were evaluated on a DM Microscope (Leica, Heidelberg, Germany) using a double-band pass-interference filter (Omega Optical Inc, Brattleboro, VT) for simultaneous visualization of FITC and TRITC fluorescence. A 63× 1.4 numeric aperture (NA) and a 100× 1.3 NA objective were used.

Evaluation of FISH signals. Detection of rearrangement was based on analysis of abnormal double-color spot distributions in metaphase and interphase cells. We identified five classes on the basis of hybridization patterns (Fig 2). (1) A normal cell, with respect to the probe sets used, was characterized by two pairs of two colocalizing spots (overlapping or nearly overlapping FITC and TRITC spots; class A). (2) Class B was defined by the presence of one FITC and one TRITC spot widely separated from each other in combination with one colocalizing pair. This pattern is indicative of a monoallelic breakpoint at 11q13 within the 450 to 750-kb region defined by the probe sets. (3) Class C showed three pairs of colocalizing FITC and TRITC spots, indicating duplication of the 11q13 region on one chromosome or on different chromosomes or trisomy 11. (4) Class D showed one colocalizing pair, indicative of either loss of one 11q13 region or monosomy 11. (5) Class E represented uninformative hybridization patterns or nonevaluable cells. If possible, at least 20 metaphase cells and 400 interphase cells of each sample were evaluated (indicated in Table 2). The reproducibility of class A and B was evaluated by repeat hybridizations on the cell line JVM2 and PHA-stimulated control PB lymphocytes. In the patient materials, FISH results in interphase cell nuclei were always compared with the results obtained by FISH and conventional banding analysis of metaphase cells in the same specimen. Several samples showed hybridization failure in some of the available slides. For instance, in patients no. 1, 9, and 13 through 16, hybridization on some slides originally used for banding analysis failed, resulting in none or few (<20) evaluable metaphase cells and sometimes in relatively few evaluable interphase cells (Table 2).

RESULTS

Normal control and JVM2 cell line. PHA-stimulated lymphocytes of control blood from a healthy individual showed a normal karyotype in 20 metaphase cells. Using FISH, colocalization of hybridization signals (class A) was found in all 20 metaphase cells and in 97% of the interphase cells (Fig 3A). Class B (with dissociation of both differently colored signals; see Fig 2 for explanation) was observed in only 1% of interphase cells; 2% of interphase cells showed duplication of a complete set of signals. Banding analysis of the JVM2 cell line known to harbor a rearrangement within the BCL1 locus at 11q13 confirmed the presence of a t(11;14)(q13;q32) in all 20 metaphase cells. FISH pattern B was found in all metaphase cells (n = 20) and in 99% of the interphase cells. Colocalization of both signals in normal interphase cell nuclei and the dissociation of these signals in the JVM2 cell line nuclei indicate that interphase FISH with this probe set can be used to monitor chromosomal breaks within the approximately 450 to 750-kb region surrounding the MTC within the BCL1 locus. To test the feasibility of single cosmid hybridization for detection of breakpoints, we additionally used only the outer cosmids COS6.7 and COSH1.5 spanning a distance of approximately 750 kb. Similar results were obtained for both normal lymphocytes and the JVM2 cell line (Figs 3B and 3C).
DETECTION OF 11q13 REARRANGEMENTS BY FISH

Table 2. Metaphase and Interphase Cytogenetics for Detection of 11q13 Abnormalities

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Banding Data</th>
<th>Hybridization Patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Metaphase (N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A  B  C  D  E</td>
</tr>
<tr>
<td>JVM2</td>
<td>t(11;14)(q13;q32) [20/20]</td>
<td>0  20  0  0  0</td>
</tr>
<tr>
<td>1</td>
<td>t(11;14)(q13;q32) [13/13]</td>
<td>3  8  0  0  0  19</td>
</tr>
<tr>
<td>2</td>
<td>t(11;14)(q13;q32) [11/19]</td>
<td>13  25  0  0  0  38</td>
</tr>
<tr>
<td>3</td>
<td>t(11;14)(q13;q32) [5/25]</td>
<td>47  12  0  0  0  65</td>
</tr>
<tr>
<td></td>
<td>Other 11q13 abnormalities</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>t(10;11)(p13;q13) [30/30]</td>
<td>80  0  0  0  0  90</td>
</tr>
<tr>
<td>5</td>
<td>t(11;11)(p15;q13) [26/26]</td>
<td>0  0  0  0  0  39</td>
</tr>
<tr>
<td>6</td>
<td>t(11;18)(q13;p12) [19/20]</td>
<td>49  0  0  0  0  52</td>
</tr>
<tr>
<td>7</td>
<td>t(10;11)(q13;q13) [4/6]</td>
<td>54  0  0  0  0  54</td>
</tr>
<tr>
<td>8</td>
<td>del(11)(q13;23) [7/17]</td>
<td>38  0  0  0  0  38</td>
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<tr>
<td>9</td>
<td>del(11)(q13q23) [7/50]</td>
<td>11  0  0  0  0  19</td>
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<td>10</td>
<td>dup(11)(q13;23) [20/20]</td>
<td>37  0  0  0  0  37</td>
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<td>11</td>
<td>t(6;7)(p23;q13) [12/16]</td>
<td>20  0  0  0  0  20</td>
</tr>
<tr>
<td>12</td>
<td>del(11)(q13q26) [2/13]</td>
<td>20  0  0  0  0  20</td>
</tr>
<tr>
<td>Other 11q13 abnormalities; FISH on metaphase cells insufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>t(6;11)(q13;13) [4/30]</td>
<td>10  0  0  0  0  10</td>
</tr>
<tr>
<td>14</td>
<td>t(2;11)(q22;q13) [5/32]</td>
<td>0  0  0  0  0  0</td>
</tr>
<tr>
<td>15</td>
<td>del(11)(q13q22) [12/54]</td>
<td>6  0  0  0  0  6</td>
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<tr>
<td>16</td>
<td>del(11)(q13q26) [1/13]</td>
<td>13  0  0  0  0  13</td>
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<tr>
<td>Other 11q abnormalities</td>
<td></td>
<td></td>
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<tr>
<td>17</td>
<td>t(11;7)(q23;7) [11/11]</td>
<td>62  0  0  0  0  62</td>
</tr>
<tr>
<td>Normal control</td>
<td>46 XY [20/20]</td>
<td>20  0  0  0  0  20</td>
</tr>
</tbody>
</table>

In cases no. 9 and 13 through 16, failure of hybridization on the original preparations resulted in relatively few metaphase cells evaluable by FISH and, in cases 13 through 15, also relatively few (198-205) evaluable interphase nuclei. For definitions of A through E, see Fig 2.

Abbreviation: tot, total number of scored cells.

**Patient materials.** To test the feasibility of interphase FISH for the detection of t(11;14)(q13;q32) in patient materials, we analyzed archival specimens from a series of hematologic patients with well-defined cytogenetic chromosome 11q13 abnormalities, using the 2-pooled probe sets (Table 2, Fig 3D). Three patients had a t(11;14)(q13;q32), 13 patients had a break within the 11q13 region other than t(11;14)(q13;q32), and 1 patient had a partially uncharacterized 11q abnormality. Of 3 patients with a t(11;14)(q13;q32), 2 had MCL with BM involvement and 1 patient had PCL/MM. The latter patient had 20% myeloma cells in the blood smear. By banding analysis, the 3 patients showed an abnormal karyotype in 100% (13 of 13), 58% (11 of 19), and 20% (5 of 25) abnormal metaphase cells, respectively. Using FISH on evaluable metaphase cells, we observed a break around the MTC (pattern B) in all 3 cases, ie, 73% (8 of 11), 66% (25 of 38) and 20% (12 of 59) of the cells, respectively. Comparable proportions of abnormal cells were found by interphase FISH; 87%, 79%, and 9% of the evaluable cells showed class B, respectively.

Thirteen patients with various hematologic malignancies (Table 1) had breakpoints at 11q13 other than a t(11;14)(q13;q32). In 9 of 13 cases, ≥2 metaphase cells showed an 11q13 break by banding analysis and 20 or more metaphase cells were evaluable by FISH. These 9 cases (cases no. 4-12) were regarded as informative for comparison of banding and FISH analysis on metaphase cells (Table 2). Class B (with dissociation of hybridization signals) was observed in none of these cases. In case no. 5, three spots with colocalization (class C) were observed in all 39 metaphase cells. Two of three signals were localized on the same chromosome (data not shown). A t(11;11)(p15;q13) was observed in all metaphase cells by banding analysis. This indicates duplication of (a part of) the 11q13 region on this chromosome. The exact configuration of this chromosomal abnormality could not be studied. In case no. 9, del(11)(q13q23) was observed in 7 of 50 metaphase cells by banding analysis. FISH showed the loss of one complete spot in 8 of 19 metaphase cells. This indicates that the whole 450- to 750-kb region flanked by the probes was within the deleted region and that this particular 11q13 breakpoint was centromeric of this region. Interphase analysis showed similar results; none of these 9 cases showed class B in a substantial number of interphase nuclei. In case no. 5, an extra 11q13 signal with colocalization was observed in 84% of cell nuclei, and in case no. 9, 35% of cell nuclei had loss of one complete signal.

In 4 of 13 cases, insufficient numbers of metaphase cells (<20) were available for FISH, mostly because of the above-mentioned failure of hybridization on the original preparations used for banding analysis. In these cases, it could not be excluded that low numbers of metaphase cells had a break within the 11q13 region as defined by the probe sets used. However, interphase analysis of these 4 cases (with 198-563 nuclei evaluated) did not show any significant increase of cell nuclei with class B (Table 2).
Fig 3. (a) FISH with two panels of cosmids for the 11q13 region on metaphase cells of a normal donor. COS6.7 and COS3.51 (centromeric of the MTC) were labeled with TRITC, and COS3.91, COS6.51, and COSH1.5 (telomeric of the MTC) were labeled with FITC. Colocalization results in a yellow signal. Chromosomes are counterstained with DAPI in blue. (b) Interphase FISH with the two outer cosmids, COS6.7 and COSH1.5, on four interphase cells of a normal donor results in colocalization of green and red spots, respectively. (c) FISH with the two outer cosmids, COS6.7 and COSH1.5, on one metaphase cell and one interphase cell of the JVM2 cell line carrying a t(11;14)(q13;q32). COS6.7 (centromeric of the MTC) was labeled with FITC, and COSH1.5 (telomeric of the MTC) was labeled with TRITC. Colocalization results in a yellow signal at one chromosome. Segregation of signals over two different chromosomes giving rise to one green and one red signal is observed for the other allele. A similar pattern is observed in the interphase cell. (d) Interphase FISH with the two panels of cosmids for the 11q13 region on 2 interphase cells of patient no. 2 carrying a t(11;14)(q13;q32). COS6.7 and COS3.51 (centromeric of the MTC) were labeled with TRITC, and COS3.91, COS6.51, and COSH1.5 (telomeric of the MTC) were labeled with FITC. In the lower nucleus, colocalization at one allele results in a yellow signal, and segregation of signals at the other allele gives rise to one green and one red signal. The smaller upper nucleus shows a normal pattern and, therefore, might represent a normal cell.

Patient no. 17 had an incompletely characterized abnormality at 11q. No breakpoint was observed in metaphase and interphase cells by FISH analysis.

DISCUSSION

We have described a novel interphase FISH method for detection of chromosomal breakpoints in a 450- to 750-kb region around the MTC of the BCL1 locus at 11q13. This method was evaluated in a series of leukemias by comparing interphase FISH with metaphase FISH and conventional banding cytogenetics. In the JVM2 cell line and all 3 patients with a documented t(11;14)(q13;q32), a break within this region could be shown in metaphase cells and interphase cell nuclei. The translocation t(11;14)(q13;q32) is specifically
associated with MCL and with few cases of PCL/MM. MCL may present as a chronic B-cell leukemia and may be easily confused with B-CLL. However, the natural course, response to chemotherapy, and prognosis differ between both types of neoplasia, necessitating strict phenotypic characterization. The method may be combined with that described in the current investigation, we aimed at the improvement of the molecular detection of t(11;14) breakpoints in MCL. Apart from pulsed-field gel electrophoresis, which is not generally applicable to patient materials, FISH analysis may be the most elegant way to detect such scattered breakpoints. The feasibility of both metaphase and interphase FISH to detect breakpoints has been shown by several investigators; ie, breakpoints at 8q24 downstream of MYC have been shown by Ried et al and Zeidler et al. These observations have been extended by recently published reports of the detection of breakpoints within the IgH locus at 14q32.45 and for specific detection of t(8;14)(q24;q32) in Burkitt's lymphoma.51 Previous investigations showed the feasibility of FISH to detect unusual rearrangements at 11q13 proximal to the MTC of BCL1 and approximately 500 kb distal of the CCND1 gene. However, these investigations were performed on metaphase cells only.

In the present study, two sets of cosmid probes were selected that covered a region of approximately 450 to 750 kb and almost symmetrically flanked the MTC of the BCL1 locus at 11q13. The FISH procedure might be further simplified, because our results on normal cells and the JVM2 cell line indicated that single cosmids may be sufficient for visualization. The method may be combined with that described by Taniwaki et al43 for breaks at 14q32; thus, simultaneous hybridization with triple-colored probes showing both segregation of 11q13 and 14q32 sequences and colocalization of one 11q13 signal with one 14q32 signal proving a t(11;14)(q13;q32) may soon become feasible.

In the JVM2 cell line and the 3 patients with a t(11;14)(q13;q32), the proportions of abnormal interphase cells and metaphase cells as assessed by FISH analysis were approximately equal to the proportion of abnormal metaphases found by banding cytogenetics. The somewhat higher percentage of abnormal metaphase cells (20%) than interphase cells (8.6%) in patient no. 3 with PCL/MM might be caused by a relatively high percentage of neoplastic cells in mitotic phase of the cell cycle in the blood sample. A similar correlation between all three methods was observed in the single patients with a duplication (case no. 5 with refractory anemia with an excess of blasts) and deletion of 11q13 (case no. 9 with CLL). In the latter patient, loss of one complete signal indicated that the 11q13 breakpoint was more than 250 to 400 kb centromeric of the MTC.

In 9 patients with various hematologic disorders and a breakpoint at 11q13, no abnormalities were detected by FISH in either metaphase or interphase cells, suggesting that these breakpoints were outside the 450- to 750-kb region covered by our probe sets. In 4 additional patients, insufficient numbers of cells were available for metaphase FISH, but the absence of a substantial increase of abnormal interphase nuclei favored the assumption that also in these cases the 11q13 breakpoint was outside the 450- to 750-kb region defined by the probe sets. In 2 patients (no. 12 and 16) very few abnormal metaphase cells were found by banding analysis. It cannot be excluded that abnormal cells were too sporadic, or even nonclonal, to be detected by interphase FISH analysis. In conclusion, we have shown the feasibility of double-color FISH analysis with breakpoint-flanking probes to detect 11q13 rearrangements within a 450- to 750-kb region surrounding the MTC of the BCL1 region. This method is promising because it is relatively simple and fast for cell suspension analysis. Methods are also available to isolate intact cell nuclei from frozen tissues. Furthermore, the exact position of breakpoints may even be more accurately established by application of novel FISH methods to released DNA fibers with a panel of alternately labeled cosmids which is currently being developed in our laboratory.

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Detection of 11q13 rearrangements in hematologic neoplasias by double-color fluorescence in situ hybridization

LJ Coignet, E Schuuring, RE Kibbelaar, TK Raap, KK Kleiverda, MF Bertheas, J Wiegant, G Beverstock and PM Kluin