Characterization of IGH Rearrangements in Non-Hodgkin’s B-cell Lymphomas by Fluorescence In Situ Hybridization

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Abstract. Rearrangements involving the IGH gene have been identified in about 50% of non-Hodgkin’s B-cell lymphomas (NHL) and correlated to clinical relevant subgroups. However, the detection rate varied greatly with the technique used. The incidence of IGH rearrangements was analyzed using several fluorescence in situ hybridization (FISH) techniques on metaphases obtained from 57 patients with nodal NHL. An IGH rearrangement was identified in 42 cases (73.7%). A t(14;18)(q32;q21) was found in 17 of the 20 follicular lymphomas (85%) studied and a t(11;14)(q13;q32) in 10 of the 11 mantle cell lymphomas (91%). IGH rearrangements were identified in 12 of the 26 diffuse large B-cell lymphomas (46%), including 5 t(14;18)(q32;q21) and 2 t(3;14)(q27;q32). Conventional cytogenetics was uninformative in several cases. However, the complemented analysis using Multi-FISH and/or chromosomal whole paint enabled the characterization of complex IGH translocations in follicular lymphomas and mantle cell lymphomas and the identification of all the chromosomal partners involved in the IGH rearrangement in diffuse large B-cell lymphomas. This study shows the interest of using metaphase FISH in addition to conventional cytogenetics. Following banding techniques, FISH with the IGH dual color probe could be the first approach in NHL, after which chromosome painting and M-FISH could be used to identify the chromosomal partner involved in the IGH rearrangement.

Keywords: Non-Hodgkin’s lymphoma, FISH, IGH rearrangement, recurrent translocation.

Non-Hodgkin’s lymphomas (NHL) are a heterogeneous group of lymphoproliferative malignancies with few patterns of behavior. Rearrangements involving the immunoglobulin heavy chain gene (IGH) at band 14q32 are observed in 50% of patients with B-cell (B)-NHL (1). These translocations, or more rarely insertions, juxtapose one oncogene of a chromosomal partner close to the IGH promoter, usually in region 14q32. These rearrangements involving IGH are associated with specific subtypes of NHL, such as the t(11;14)(q13;q32) in about 95% of mantle cell lymphomas (ML), the t(14;18)(q32;q21) in 80% of follicular lymphoma (FL) and t(3;14)(q27;q32) in diffuse large B-cell lymphomas (DLBCL) (2-4).

Karyotypes in NHL are usually complex, and banding analysis is usually not sufficient to identify the structural abnormalities. Therefore, most studies have ascertained the incidence of IGH translocations using interphase fluorescent in situ hybridization (FISH). However, this strategy has failed to identify the chromosomal partners (5).

In this study, we used different FISH techniques to detect IGH rearrangements in 57 nodal NHL. This approach allowed us to identify complex chromosomal rearrangements masking specific translocations.

Patients and Methods

Patients. Since 2002, 57 patients diagnosed immunohistochemically and immunophenotypically as having non-Hodgkin’s lymphomas (NHL) of B-cell origin have been referred to the cytogenetic laboratory. Patients with tumors of T-cell lineage were excluded from the present study.

Conventional cytogenetics. Lymph node samples were received at the cytogenetic laboratory in RPMI 1640; they were manually split and crushed with scalpels. The cells were incubated in 5% CO2 at 37°C for one hour. They were then synchronized for 17 hours with FrdU

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Key Words: Non-Hodgkin’s lymphoma, FISH, IGH rearrangement, recurrent translocation.
(10^{-7} \text{ M}) before being released by thymidine (10^{-5} \text{ M}) for 6 hours. Colcemid exposure (15 min) and standard harvesting were performed. The chromosomes were R-banded and the karyotype described according to the ISCN (6). Twenty metaphases were studied for each patient.

**Molecular cytogenetics**

**LSI IGH/CCND1 dual color probe.** The LSI IGH/CCND1 dual color probe is a mixture of the LSI IGH probe labelled with SpectrumGreen and the LSI CCND1 probe labelled with SpectrumOrange (Abbott, Rungis, France). The LSI IGH probe contains two parts hybridizing the sequences on either side of the IGH J region breakpoint (450 Kbp). The LSI CCND1 probe extends from telomeric FGF4 point to beyond the MTC region (350 Kbp). **LSI IGH/BCL2 dual color probe.** The LSI IGH/BCL2 dual color is a mixture of the LSI IGH probe labelled with SpectrumGreen and the LSI BCL2 probe labelled with SpectrumOrange (Abbott). The LSI IGH probe spans 1.5 Mbp and contains sequences of the entire IGH locus. The LSI BCL2 probe covers a 750 kb region, including the entire BCL2 gene. **LSI IGH dual color probe.** This probe is a mixture of two probes: the 900 kb LSI IGHV probe, labelled in SpectrumGreen, covering the entire IGH variable region and the 250 kb 3’ flanking probe, labelled in SpectrumRed, lying completely 3’ to the IGH locus (Abbott).

Hybridization was performed according to the manufacturer’s recommendations. Briefly, before hybridization, DNA slides were immersed in a jar of 2xSSC solution or 2xSSC-0.4% NP40 solution (depending on the probes) and then immediately passed through an ethanol series of increasing concentration (70%, 90%, 100%). The denaturation was performed simultaneously on the DNA slides and the probes for 2 minutes at 75°C. The slides were incubated overnight in a dark, humidified chamber at 37°C. They were washed for 45 seconds in 0.4xSSC/0.3%NP40 at 72°C and 20 seconds in 2xSSC/0.1%NP40 at room temperature and finally counterstained with 4’,6-diamidino-2-phenyl-indole (DAPI). The slides were analyzed using a Zeiss Axioscope Microscope (Zeiss, Le Pecq, France). Subsequent image acquisition was performed using a CCD camera with Isis (significant in situ imaging system) (MetaSystems, Altussheim, Germany).

**Whole-chromosome paint.** Slides hybridized with the IGH probes were dehybridized and rehybridized with whole-chromosome painting (WCP) probes (QiBiogene, Illkirch, France), as previously described (7). The WCP probes were chosen according to the chromosomal rearrangement observed in conventional cytogenetics.

**Table I.** Partner chromosomal band involved in IGH translocations.

<table>
<thead>
<tr>
<th>14q32 chromosomal partner</th>
<th>Follicular lymphomas (n=20)</th>
<th>Mantle cell lymphomas (n=11)</th>
<th>Diffuse large B-cell lymphomas (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11q13</td>
<td>1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>18q21</td>
<td>17</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>3q27</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>8q24</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>9p13</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1p11</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1q21</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

24 color FISH. In several cases, complex rearrangements could not be elucidated by banding techniques. M-FISH using MetaSystems’24 Xcyte kit probe (MetaSystems) containing chromosome-painting probes specific for the 24 different chromosomes was applied. Each paint was labelled with 4 fluorochromes (FITC, Spectra, TexasRed, DEAC) and biotin, respectively, or a unique combination of them. Detection of the biotin-labelled fraction was performed with streptavidin-Cy5 (B-tect).

Briefly, after treatment with proteinase K (200 µg/ml) at 37°C for 10 minutes, the slides were washed in 1xPBS 2 times at room temperature for 5 minutes and placed immediately twice in a 1xPBS + 50 mM MgCl2 solution at room temperature for 5 minutes. They were subsequently immersed in a post-fixative solution (1% formaldehyde in 1xPBS + 50 mM MgCl2) for 10 minutes at room temperature, washed in 1xPBS for 5 minutes and passed through an ethanol series of increasing concentration (70%, 90%, 100%) for 5 minutes. Target DNA was denatured in 70% formamide/2xSSC at 75°C for 3 minutes and then passed through a cold ethanol series and allowed to air dry. The probe mix was denatured at 75°C for 5 minutes, put on ice for 2 minutes and incubated at 37°C for 30 minutes; subsequently 6 µl per hybridization were applied to each slide. After 4 days of hybridization, the slides were washed in 50% formamide/2xSSC twice for 7 min 30 seconds, each at 41°C, and then in 2xSSC twice for 7 min 30 seconds at 41°C and once in 4xSSC containing 0.01% Tween 20 for 3 minutes at room temperature. Biotin-labelled probes were detected with streptavidin-Cy5. Finally, the slides were counterstained with DAPI (8).

**Results**

The 57 NHL included in this study were distributed as 20 follicular lymphomas, 11 mantle cell lymphomas and 26 diffuse large B-cell lymphomas (Table I). Altogether, 42 IGH rearrangements were detected (73.68%).

The translocation t(14,18)(q32;q21) was detected in 17 of the 20 FL cases (85%), including one case in which a complex rearrangement was identified by M-FISH: der(14)(14pter->14q32::18q21::3?p22->3?pter). A t(3;14)(q27;q32) and a t(11;14)(q13;q32) were found in one case each.

A standard t(11;14)(q13;q32) translocation was detected in 8 of the 11 ML cases, including one case in which the 11;14

Conventional cytogenetics was not very useful in the majority of the 26 DLBCL. Indeed, most of the karyotypes were hypo-tetraploid (65-91 chromosomes) and/or contained a large number of structural anomalies and unidentifiable markers. Twelve IGH rearrangements were found (46.2%). A t(14;18)(q32;q21) was identified in 5 cases (19.2%), a t(3;14)(q27;q32) in 2 cases (7.7%) and a t(8;14)(q24;q32) in 2 cases (7.7%). Other chromosomal partners were identified in 3 DLBCL: 9p13, 1p11 and 1q21 (1 case each).

Discussion

Several molecular techniques, such as polymerase chain reaction or Southern blot analysis, can be used to characterize IGH rearrangements. However, due to the dispersed breakpoints around the partner oncogene locus of specific translocations, they can fail to detect IGH rearrangements, therefore leading to a low detection rate (9-11). Compared with these methods, it has been shown that FISH techniques have a greater detection efficiency to investigate the prevalence of IGH rearrangements in NHL (11).

The distribution of the IGH rearrangements found in our study is in agreement with previously published studies that used molecular techniques (12). However, unlike interphase FISH, which is limited by its inability to identify all the chromosomal partners (5), metaphase FISH can detect uncommon rearrangements by combining several techniques (LSI, WCP, M-FISH).

In our study, all the t(14;18)(q32;q21) identified with conventional cytogenetic techniques were confirmed by FISH using the LSI IGH/BCL2 dual color probe. This probe, unlike the other alternative FISH strategies, minimizes the risk of false-positives (13). The t(14;18)(q32;q21) and t(3;14)(q27;q32) were detected in 80% and 5%, respectively, of the 20 follicular lymphomas. A t(14;18)(q32;q21) rate varying between 64% (13, 14) to 100% (15) has been reported using interphase FISH with the same probe, whereas in a study with the same approach as ours, 83% of the FL was found to have a t(14;18)(q32;q21) but no t(3;14)(q27;q32) (16). However, the frequency of t(14;18) in follicular lymphomas has been shown to vary between reports. Segel et al. found, from a literature review, that the rate was lower in Asia (38%), intermediate in Europe (61%) and higher in American studies (71%) (17).

A t(11;14)(q13;q32) was identified in 91% of the mantle cell lymphomas. Previous interphase FISH studies using the same probe were associated with the presence of t(11;14)(q13;q32) in 93% to 100% of MCL (14, 18-20). M-FISH was necessary to identify complex rearrangements in 2 cases.

Furthermore, the best application of metaphase FISH techniques is the analysis of the DLBCL subtype. Indeed, unlike other subtypes associated with a genetic hallmark, no links between DLBCL and any particular chromosomal abnormality have been found. In our study, R-banding cytogenetics identified only 3 t(14;18) whereas the use of the IGH probe enabled us to find 10 cases with IGH rearrangements, for which chromosome partners were identified by whole-chromosome painting probes and M-FISH.

It is commonly accepted that t(14;18)(q32;q21) represents 12 to 35% of DCLBL (21, 22), which, in our study, was detected in 19%. A recent study reported a rate of 57% of t(14;18) in 21 DCLBL, and the authors explained this high rate by the better sensitivity of their technique. However, they used the same approach as ours. These different rates could be explained by the molecular heterogeneity of DLBCL (23). Indeed, two distinct molecular forms have recently been identified on the basis of their immunophenotype (24, 25).

A t(3;14)(q27;q32) was detected in 7.7% of the 26 DLBCL in our study. Oscier et al. reported that the t(3;14) occurred in about 10% of the DCLBL cases (12). Few studies using M-FISH found a t(3;14) in about 14% of DCLBL cases (23, 26). Using the same approach as ours, Fan et al. showed 9.5% of the DCLBL to have a t(3;14) (16). Finally, the frequency of other translocations, such as t(8;14)(q24;q32) and t(9;14)(p13;q32) is in agreement with published studies (11, 12).

This study demonstrated the interest of using a combination of conventional cytogenetics and several FISH techniques to investigate complex karyotypes in lymphoma. Indeed, conventional cytogenetics usually fails to properly identify the numerous markers commonly found in lymphomas. Following conventional cytogenetics, FISH, with the IGH dual color probe, can be the first approach in NHL, after which chromosome painting and M-FISH can be used to identify the chromosomal partner involved in the IGH rearrangement.

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