Identification of Novel Cryptic Translocations Involving \textit{IGH} in B-Cell Non-Hodgkin’s Lymphomas

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ABSTRACT

Chromosomal rearrangements involving the immunoglobulin heavy chain gene (\textit{IGH}) at 14q32 are observed in ~50% of patients with B-cell Non-Hodgkin’s lymphoma (NHL). The 5’ end of the \textit{IGH} gene is located within 8 kb of the telomeric repeats of 14q. Translocations involving the \textit{IGH} locus and the telomeric band of a partner chromosome are difficult to identify, because most terminal bands of human chromosomes appear pale by conventional G-banding techniques. To determine whether there are cryptic translocations involving the \textit{IGH} locus, we used dual-color fluorescence in situ hybridization (FISH) of 5’ and 3’ \textit{IGH} genomic clones containing the variable sequences, or the J<sub>H</sub> and the 5’ constant regions, respectively. We examined cells from 51 patients with B-cell NHL who had a normal karyotype (3 patients), clonal abnormalities not involving 14q32 (35 patients), or alterations of 14q32 other than recurring translocations, i.e., add(14)(q32) (13 patients). FISH detected 17 \textit{IGH} translocations in 16 of 51 (31%) cases. Of the 13 cases with add(14)(q32), FISH identified the partner chromosome in 9 cases (69%); 3q27, 6 cases; 2p13, 19p13.3, and 18q21.3, 1 case each). Six of thirty-eight (16%) patients without visible alterations of 14q32 and 2 of 13 (15%) patients with an abnormality of one chromosome 14 had masked (5 patients) or cryptic \textit{IGH} translocations (3 patients), involving 3q27 (3 patients), 5p15.3 (2 patients), 19p13.3 (3 patients), or 14q32 (1 patient; 1 patient had two rearrangements). We identified two novel, recurring, cryptic translocations: t(5;14)(p15.3;q32) (2 patients) and t(14;19)(q32;p13.3) (3 patients). In summary, FISH permitted the detection of cryptic or masked \textit{IGH} rearrangements in ~20% of lymphoma cases without visible rearrangements of 14q32 analyzed retrospectively.

INTRODUCTION

A number of recurring chromosomal abnormalities correlate with clinical, morphological, and immunophenotypic features in NHL.\textsuperscript{3} The detection of these abnormalities can be helpful in establishing or confirming the correct diagnosis, in selecting therapy, and in providing prognostic information (1, 2). Chromosomal rearrangements involving the immunoglobulin heavy chain gene (\textit{IGH}) gene at 14q32 are observed in up to 50% of B-cell lymphomas (3, 4). In particular, t(8;14)(q24.1q32) is associated with Burkitt’s lymphoma, t(11;14)(q13;q32) is associated with MCL, t(14;18)(q32;q21.3) is observed in a high proportion of follicular lymphomas (70–90%), and t(3;14)(q27;q32) is associated with DLBCLs (1–6). The \textit{IGH} locus is adjacent to the telomere of 14q (the 5’ end of 14q is 8 kb from the telomeric repeats; Ref. 7). This location complicates the detection of translocations involving the terminal bands of partner chromosomes by cytogenetic methods, because most of the terminal bands of human chromosomes, including 14q32, are G-negative (pale) with conventional G-banding techniques. Of interest is that the telomeric bands of human chromosomes have a high gene content (8).

We hypothesized that the frequency of 14q32 alterations in B-cell NHL may be underestimated using conventional cytogenetic analysis. The aims of our study were to identify cryptic rearrangements of \textit{IGH} in NHL and to identify new translocation partner chromosomes, by analyzing samples from B-cell NHL patients without abnormalities of 14q32, or with rearrangements involving an unknown partner chromosome. Because translocation breakpoints within the \textit{IGH} locus occur most frequently within the D or J segments, our strategy was to use dual-color FISH of two \textit{IGH} probes; the first probe (IGH 3’) contains the \textit{J<sub>H</sub>} region and the most 5’ constant regions (B158A2; Fig. 1A, in red), whereas the second probe (IGH 5’) contains \textit{V<sub>H</sub>} sequences (B200D12; Fig. 1A, in green). \textit{IGH}-specific translocation breakpoints typically occur in the sequences between these probes, resulting in the relocation of the \textit{IGH} 5’ sequences (green probe) to the partner chromosome. Of note is that rarer 3’ \textit{IGH} translocations occurring centromeric to \textit{C<sub>el</sub>}, e.g., those involving \textit{So<sub>2</sub>}, will also be detected because both probes will be relocated to the partner chromosome. The discovery of novel genes involved in recurring translocations would be important in identifying new prognostic factors and in developing new therapies based on underlying molecular alterations.

PATIENTS AND METHODS

Patient Population. We examined samples from 51 patients who were diagnosed and treated at the University of Chicago Medical Center or who were treated at other metropolitan Chicago hospitals and who had specimens referred to our cytogenetics laboratory between 1989 and 1999. All specimens were obtained after informed consent. The population comprised B-cell NHL samples with a normal karyotype by conventional cytogenetic analysis with G-banding (3 patients), samples with clonal abnormalities not involving 14q32 (35 patients), or with alterations of 14q32 other than recurring translocations, i.e., add(14)(q32) (13 patients). We included all patients for whom stored material (cells processed for cytogenetic analysis) was available. In most instances, lymph node samples were examined; however, bone marrow samples containing lymphoma were studied for several patients if clonal abnormalities were detected by cytogenetic analysis.

Diagnosis and Disease Classification. The diagnosis and classification of NHL was based on morphological and immunophenotypic studies of lymph node biopsy specimens, peripheral blood smears, bone marrow aspirates, and biopsy specimens obtained before therapy, according to the Revised European American Lymphoma (REAL) classification (Table 1; Ref. 9). The immunophenotype was determined by standard flow cytometric analysis or immunohistochemical studies with a variety of commercially available antibodies.

Cytogenetic Analysis. Cytogenetic analysis was performed with a trypsin-Giemsa banding technique on lymph node or tumor biopsy specimens, peripheral blood samples, and bone marrow aspirates, and biopsy specimens obtained before therapy, according to the Revised European American Lymphoma (REAL) classification (Table 1; Ref. 9). The immunophenotype was determined by standard flow cytometric analysis or immunohistochemical studies with a variety of commercially available antibodies.

Probes. The location of the \textit{IGH} probes used for FISH is shown in Fig. 1A. We used 5’ and 3’ \textit{IGH} BAC clones. B158A2 (kindly provided by H.

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\footnotetext[3]{The abbreviations used are: NHL, non-Hodgkin’s lymphoma; DLBCL, diffuse large B cell lymphoma; FISH, fluorescence in situ hybridization; BAC, bacterial artificial chromosome; SLL, small lymphocytic lymphoma; CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; MCL, mantle cell lymphoma; \textit{IGH}, immunoglobulin heavy chain locus.
The results of FISH analysis are described in Tables 1–2 and are illustrated in Fig. 1. We detected 17 IGH translocations in 16 of 51 (31%) patients (1 patient had two different IGH translocations involving both chromosome 14 homologues). With the exception of patient 1 (described below), the translocation breakpoints within IGH occurred between the two probes, and the telomeric probe containing the variable segments was translocated to the partner chromosome. The partner chromosome band involved most often was 3q27 (10 cases). Interestingly, we detected four cases with translocations involving novel partner chromosomes, previously unknown in B-cell NHL, 5p15.3 (2 patients) and 19p13.3 (3 patients). Note that one patient had

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**Table 1 Clinical and cytogenetic features of patients with NHL**

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Diagnosis</th>
<th>Age: mean (range)</th>
<th>Clinical features</th>
</tr>
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<tr>
<td>M/F</td>
<td>DLBCL</td>
<td>30/21</td>
<td>M/F</td>
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<tr>
<td></td>
<td>Burkitt-like</td>
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<td></td>
<td>FL</td>
<td>6</td>
<td></td>
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<tr>
<td></td>
<td>Grade I (1 pt), II (2 pts), III (3 pts)</td>
<td>59 (3–90)</td>
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<tr>
<td></td>
<td>SLL/CLL</td>
<td>6</td>
<td></td>
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<tr>
<td></td>
<td>MZL</td>
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<td>LPL</td>
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<td>Normal karyotype</td>
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<td></td>
</tr>
<tr>
<td>Other abnormalities</td>
<td>35</td>
<td>(7, 20%)</td>
<td></td>
</tr>
</tbody>
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*LPL: lymphoplasmacytic lymphoma; MZL, marginal zone lymphoma; pts, patients.*

*The number and percentage of patients with IGH translocations detected by FISH are indicated in parentheses.*
both translocations (Table 2; Fig. 1, C and D). A deletion of the 14q subtelomeric probe, containing variable sequences (B200D12), was noted in an additional 5 patients who had clonal abnormalities but cytogenetically normal chromosome 14 homologues. This finding is most likely the result of nonproductive IGH rearrangements; however, we cannot formally exclude the possibility that unbalanced translocations resulted in the relocation of material from another chromosome to 14q32, with subsequent loss of 5’ IGH sequences.

Patients with add(14)(q32)

Of the 13 patients examined with add(14)(q32), 9 (69%) had a translocation involving IGH, whereas the remaining 4 patients showed a normal configuration of the two probes. In the latter 4 patients, the presence of other chromosomal abnormalities representative of the abnormal clone verified that the metaphase cells analyzed were derived from the malignant clone. The observation of a normal configuration of the IGH probes indicates that the translocation breakpoint did not involve the most common IGH breakpoint regions. However, we cannot rule out the possibility that the breaks occurred in the most 5’ IGH sequences. In 1 patient (no. 1), two novel, cryptic translocations were detected involving both chromosome 14 homologues, and 5p15.3 or 19p13.3. The partner chromosome most frequently involved was 3q27 (6 cases); 2p13 and 18q21.3 were each involved in 1 patient.

Detection of Cryptic Translocations Involving IGH in Patients without Abnormalities of 14q32

Six of 38 patients (16%) who did not have visible alterations of 14q32 detectable by standard cytogenetic analysis had a masked or a cryptic IGH translocation. In a cryptic translocation, both partner chromosomes appear normal by cytogenetic analysis, whereas in a masked translocation, structurally rearranged chromosomes are identified, but the actual involvement of one or more chromosomes is unrecognized, i.e., the rearrangement is more complex than can be appreciated by cytogenetic analysis. Three patients (nos. 8, 35, and 42) had a masked, complex rearrangement involving 3q27, whereas 3 patients had a cryptic IGH translocation involving 5p13.3 (1 patient) or 19p13.3 (2 patients). To verify that the rearrangement involved 19p, rather than 19q as in the recurring t(14;19)(q32;q13.3), we used a series of cosmids probes from 19p for FISH analysis. This analysis revealed that 19p sequences were translocated to 14q32 (data not shown).

FISH Analysis of t(5;14)

In patient 1, the results of FISH analysis revealed a masked, three-way translocation involving 1q, 14q, and 5p [t(1:14:5)(q21:q32; p15.3)], as well as a cryptic t(14;19)(q32;p13.3) (Fig. 1D). By cytogenetic analysis, the rearrangement of 14q was interpreted to be t(1:14)(q21:q32); however, FISH revealed that the IGH variable sequences were located on 5p. The involvement of chromosome 5 was evaluated by FISH of a subtelomeric probe for 5p (P24H17, kindly provided by D. H. Ledbetter). This probe hybridized to the long arm of the der(1)t(1;14;5), revealing a three-way translocation in which 1q21–qter is translocated to 14q32, 14q32–qter is translocated to 5p, and 5p15–pter is translocated to 1q. Several recurring 1:14 translocations in NHL have been cloned and found to involve the MCL9, MUC1, IRT1A1, or IRT2A genes at q21. Whether the oncogenic potential is contributed by der(14), which contains 1q21–qter and 3’ IGH sequences, or the der(5) containing 1q43–qter including the 5’ IGH sequences, is unknown. In other studies, we have determined that the breakpoints on 5p in patients 1 and 2 occur within a 50-kb interval, raising the possibility that a single gene on 5p may be involved.

In the cryptic t(14;19) in this patient, the 5’ IGH sequences, including the B200D12 probe, were translocated to 19p. The breakpoint within IGH appeared to occur within sequences encompassed by the 3’ IGH probe (B158A2), because red signal was observed on both 14q and 19p.

In patient 2, the 5’ IGH probe was present on the end of 5p, whereas the 3’ IGH probe remained on der(14). The reciprocal nature of the translocation was confirmed by hybridization of the 5p subtelomeric probe, which hybridized to der(14).

Clinical and Morphological Features of Patients with Novel Translocations

t(5;14)(p15.3;q32) or variant t(5;14)

Patient 1. A 74-year-old white male was diagnosed in 1983 with a SLL based on a biopsy of a nasopharyngeal mass. CLL was diagnosed in 1991. The phenotype was CD19+, CD20+, CD21+, CD5+, IgG κ+.. In March 1994, a biopsy of an enlarged lymph node noted in the
cervical region showed a DLBCL, which was felt to be a possible Richter transformation. Abdominal and pelvic computed tomography revealed enlarged cardiophrenic lymph nodes and increased periaortie and perisophageal nodes. The malignant large cells had lost expression of CD5. The patient received a 4-day course of ESHAP (etoposide, high dose cytarabine, cisplatin, and methylprednisolone) but expired from sepsis 1 month later. Cytogenetic analysis revealed an abnormal mosaic karyotype with a t(1;14) and add(14)(q32). FISH analysis resulted in the refinement of the abnormalities of 14q32. The revised karyotype was 47,XY,+12,t(14;19)(q32:p13.3)[1]/47,ident,[t(1;14;5)q22:q22;p15.3:q1]/49,ident, +X,t(1;14;5)t(2;12)(p12;p13),+18[9]/46,XY[8].

**Patient 2.** A 64-year-old white female with a history of Crohn’s disease was admitted in June 1997 to the University of Chicago Hospitals because of fever and loss of appetite, suspicious for endocarditis or an abdominal abscess secondary to residual Crohn’s disease. The patient presented with a large right and posterior cervical lymphoadenopathy; the biopsy revealed a de novo CD5+ DLBCL. The patient was treated with CHOP (cyclophosphamide, Adriamycin, vincristine, and prednisone) for one cycle, but her condition did not improve. She was readmitted in July, when a biopsy revealed infiltration of the liver by the DLBCL. She was then treated with a course of DHAP (dexamethasone, high-dose cytarabine, and cisplatin) but expired from sepsis 1 month later.

**DISCUSSION**

By using FISH analysis of metaphase cells, we detected 17 IGH translocations in 16 of 51 (31%) patients with a diagnosis of B-cell NHL. In our study, 16% of all NHL patients (6 of 38) with a normal chromosome 14 by standard cytogenetic analysis had a cryptic or masked translocation of IGH. In this group, the partner chromosome band involved most often (3 cases) was 3q27; 2 of these patients had DLBCL, and 1 had FL. In addition, we identified two novel, recurring, cryptic translocations: t(5;14)(p15.3;q32) (2 patients) and t(14;19)(q32;p13.3) (3 patients). Patients 3 and 4 with t(14;19) had SLL/CLL (CD5+ or a de novo CD5+, respectively. Of interest, patient 2 with t(5;14) had a de novo CD5+ DLBCL, and patient 1 with both the t(14;19) and a variant t(5;14)(t(1;14;5)) had a DLBCL, associated with CLL (CD5+).

The incidence of 14q32 translocations is ~50% in B-cell NHL in the United States, whereas a lower frequency has been reported in other countries (3, 5, 6). To ascertain the incidence of IGH translocations in Japan, Taniwaki and colleagues (11, 12) analyzed samples from patients with B-cell NHL, using a probe for part of the constant regions (Cy1 and Cy3) and a yeast artificial chromosome (Y6) spanning the telomeric part of IGH. They identified IGH rearrangements in 29 of 70 (41%) patients examined. This strategy permitted them to detect rearrangements in interphase cells, thus enabling them to analyze poor-quality samples or samples without a sufficient number of metaphase cells. Nonetheless, the analysis of interphase cells is limited by the inability to identify the partner chromosome. Our study does not allow us to determine the frequency of IGH rearrangements, because we analyzed a selected population of patients, mainly those presenting without 14q32 rearrangements by standard cytogenetic analysis. Our goal was to search for cryptic translocations and novel partners of IGH; thus, we elected to analyze only metaphase cells.

FISH can identify subtle translocations that are difficult to identify by cytogenetic analysis. In our study, t(3;14) was identified in 10 cases, 6 of which were interpreted as add(14q32) by conventional cytogenetic analysis. A number of these cases were ascertained before the first description of t(3;14) as a recurring abnormality in DLBCL; in other cases, the poor quality of the metaphase cells contributed to our failure to identify t(3;14). The BCL6 gene on 3q27 has been shown to be rearranged in 30–40% of DLBCLs and in 10% of follicular lymphoma (13–15). In 70% of the cases, somatic point mutations occur in the 5′ noncoding region, independent of the translocation (16). BCL6 encodes a Mr 96,000 nuclear protein that functions as a transcriptional repressor (17–20). Gene inactivation studies have shown that BCL6 plays a critical role in the activation and proliferation of B cells within the germinal center (19, 20).

In summary, our studies reveal the presence of masked and cryptic IGH translocations in NHL. The identification of new and different subsets of lymphoma, directly correlated with alterations of specific genes, will be important in the future to predict prognosis and to select therapy based on underlying molecular alterations.

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**REFERENCES**


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