Localization by Chromosome Microdissection of a Recurrent Breakpoint Region on Chromosome 6 in Human B-Cell Lymphoma


Deletion of the long arm of chromosome 6 (6q) is one of the most common chromosomal alterations in human B-cell lymphomas. Conventional cytogenetic banding analysis and loss-of-heterozygosity (LOH) studies have detected several common regions of deletion ranging across the entire long arm (6q), with no defined recurrent breakpoint yet identified. We describe here a strategy combining chromosome microdissection and fluorescence in situ hybridization (Micro-FISH) to determine a minimal region of deletion along chromosome 6. Seven clinical cases and one cell line of follicular lymphoma containing a t(14;18) and one case of diffuse lymphoma, also with a t(14;18), were used for this study. All nine cases had previously defined abnormalities of chromosome 6 determined by cytogenetic analysis. The results of chromosome dissection were unexpected and in contrast to the suggestion of disparate breakpoints by conventional chromosome banding. Specifically, Micro-FISH analysis provided evidence for a common breakpoint at 6q11 in seven of nine cases. After Micro-FISH analysis, all of the presumed simple deletions of chromosome 6 were carefully reanalyzed and shown to actually represent either nonreciprocal translocations (three cases), interstitial deletions (five cases), or isochromosome (one case). The recurrent proximal breakpoint (6q11) was detected in seven of nine cases, with the minimal region of deletion encompassing 6q11 to 6q21. By analogy to other tumor systems, the identification of recurring breakpoints within 6q11 may suggest that a gene(s) important to the genesis or progression of follicular lymphoma can be localized to this band region.

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HUMAN TUMORS HAVE been shown to progress through a multistep process of genetic abnormalities, which frequently involves recurring chromosomal change mediating alterations in the expression of oncogenes or tumor suppressor genes. A reciprocal chromosome translocation, t(14;18)(q32;q21), has been detected in approximately 85% of cases of follicular lymphoma studied by cytogenetic or molecular techniques. This translocation results in the juxtaposition of the oncogene, BCL-2, at 18q21 with the Ig heavy-chain locus on 14q32.4 Cytogenetic studies have also shown that deletion of the long arm of chromosome 6 (6q) is one of the most common chromosomal changes in all non-Hodgkin’s lymphomas, including follicular lymphoma. Deletion of 6q is thought to be associated with a clinically aggressive form of follicular mixed small- and large-cell histologic type. Clinically, patients with an abnormal chromosome 6 also have a higher frequency of immunoblastic lymphoma. Finally, nonrandom rearrangement of 6q is also commonly observed in other hematopoietic malignancies, including acute lymphoblastic leukemia, and in many solid tumors, including malignant melanoma, ovarian carcinoma, and breast carcinoma. Evidence for the biological importance of chromosome 6 alterations in malignancy has been provided by the introduction into tumor cells of a normal human chromosome 6 after microcell transfer and the resulting suppression of either the tumorigenic phenotype or the metastatic potential. Accordingly, identification of a recurrent site of chromosome breakage in follicular lymphoma may be of considerable importance in localizing a gene(s) involved in malignancies with chromosome 6 alterations.

In lymphomas and lymphoblastic leukemias, several commonly deleted band regions along 6q have already been reported, including (1) two regions of minimal molecular deletion (RMDs) termed RMD1 (localized to 6q25-27) and RMD2 (localized to 6q21-23) determined by loss of heterozygosity (LOH), and (2) two common regions of deletion at 6q23.1-27 and 6q14-q21 suggested in acute lymphoblastic leukemia by fluorescence in situ hybridization (FISH) of yeast artificial chromosome clones mapped to 6q. Unfortunately, despite these significant recent gains in our knowledge of the breakpoints on 6q, most involve a series of chromosomal bands. One significant technical problem that continues to limit this area of research is our inability to exactly characterize cytogenetic breakpoints by conventional G-banding analysis.

We have recently developed an approach (termed Micro-FISH) that combines chromosome microdissection and FISH to rapidly cytologically define the exact limits of breakpoints involved in chromosome deletions. In this report, nine cases of B-cell lymphoma with identified chromosome 6 abnormalities have been studied by microdissection. Using this method, all presumed chromosomal deletions involving 6q were shown to represent nonreciprocal translocations (three cases), interstitial deletions (five cases), or isochromosome (one case). In addition to the identification of a RMD encompassing 6q11 to 6q21, a recurrent proximal breakpoint at 6q11 has been identified.

MATERIALS AND METHODS

Cytogenetic analysis. Cytogenetic analysis was performed on blood, bone marrow, and lymph node specimens as previously described. Metaphases were G-banded, and abnormal copies of chromosome 6 from the follicular lymphoma cell line, SU-DHL-4, and eight B-cell lymphoma biopsy specimens were analyzed by chromosome microdissection. Tissue specimens were obtained from biopsies of peripheral blood (93C280), bone marrow (91C037), and
recurrent breakpoint in B-cell lymphoma

Table 1. Cytogenetic Alterations From Eight Patients With B-Cell Lymphoma

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Original Karyotype Interpretation</th>
<th>6q Interpretation by Micro-FISH</th>
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<tbody>
<tr>
<td>91C037</td>
<td>Clone 1: 47,XY,add(4)(q27),del(6)(q15),der(8)(q17)(p11;41),11(11)(q22), +13,14(t;14;18)(q32;q21),-17,-mar(7)(q6)2,6,4,XY(1)</td>
<td>del(6)(q5.6)(p13;q11)</td>
</tr>
<tr>
<td>91C313</td>
<td>Clone 1: 80-88,XY,Y+,X(del)(1)(q11),der(1)(q13),-6,del(6)(q21q27),del(6)(q23)t(14;18)(q32;q21),inc(p15)46,XY(3)</td>
<td>del(6)(q10)q11(p11)</td>
</tr>
<tr>
<td>92C147</td>
<td>Clone 1: 46,XY,add(1)(p36),del(6)(q15),t(14;18)(q32;q21)(15)46,XY(2)</td>
<td>del(6)(q11q21)(q22q25)</td>
</tr>
<tr>
<td>92C326</td>
<td>Clone 1: 46,XX,del(6)(q13p26)t(14;18)(q32;q21)10(46,XX(4)</td>
<td>del(6)(q11q22)</td>
</tr>
<tr>
<td>92C390</td>
<td>Clone 1: 50.X,del(6)(q26)-t(6;14)(q21;11),add(7)(q36)der(7)(t;7;7;13)(q11;7;p22)(q36;q13),-14,add(15)</td>
<td>del(6)(q12q22)</td>
</tr>
<tr>
<td>93C280</td>
<td>Clone 1: 44-45.Y,add(X)(p22),del(2)(q13q41),del(3)(p12p25),7d4(4;17)(p16;p11),der(5)(t;1;5)</td>
<td>del(6)(q11q22)</td>
</tr>
<tr>
<td></td>
<td>(q21;14),add(6)(q13),add(8)(q22),add(9)(q13),add(10)(q24),add(11)(q25),der(13)(q12q32),der(14)(t;12;14;18)(q24;1;32;q21),der(18)(t;12;14;18)(q24;1;32;q21)</td>
<td></td>
</tr>
<tr>
<td>94C016</td>
<td>Clone 1: 95-100,XXXXX+,Xx2,add(1)(p11)x2,3,4x2+del(6)(q21)x2,7,7del(7)(q32p36),8x2, +add(8)(p,11),11,12x2,+14;14(q32;21)x2,15x2,-16,-17,-18,+20,+21,2mers(c8p8/46,XX(12)</td>
<td>del(6)(q11q22)</td>
</tr>
<tr>
<td>94C180</td>
<td>Clone 1: 48.X,Y,del(1)(p35p36),t(3;22)(q27;q21),+5,-6,del(6)(q13q25)x2,7,14;18(q32;q21)</td>
<td>del(6)(q11q24)</td>
</tr>
<tr>
<td></td>
<td>(15)46,XY(6)</td>
<td>iso(6)(q11)</td>
</tr>
</tbody>
</table>

Table 1. Cytogenetic Alterations From Eight Patients With B-Cell Lymphoma

lymph node (91C313, 92C147, 92C326, 92C390, 94C016, and 94C180). Mitotic cells were dropped onto clean cover slips (22 x 60 mm) and stored at 37°C for 2 to 3 days. Standard G-banding with trypsin-Giemsa was performed before microdissection.

Microdissection and topoisomerase I treatment. The procedure for chromosome microdissection was performed essentially as described previously. Briefly, two to five copies of target rearranged chromosome 6 were dissected with glass microneedles controlled by a Narishige micromanipulator (Narishige USA Inc. Sea Cliff, NY) attached to an inverted microscope. The dissected chromosome fragments were transferred to a 5-μL collection drop containing 40 mmol/L Tris hydrochloride, pH 7.5, 20 mmol/L MgCl2, 50 mmol/L NaCl, 200 μmol/L of each dNTP, 0.1 U topoisomerase I, and 5 pmol universal primer [UNG1] CCGACTCGAGNNNNNNNT-GTGG. After the desired number of dissected DNA fragments was collected, the collection drop was covered with a drop of mineral oil, incubated at 37°C for 30 minutes, and denatured at 96°C for 10 minutes.

Amplification of dissected DNA. An initial eight cycles of polymerase chain reaction (PCR) denaturation at 94°C for 1 minute, annealing at 30°C for 2 minutes, and extension at 37°C for 2 minutes) were performed by adding approximately 0.3 U T7 DNA polymerase (Sequenase version 2.0; USB/Amersham Life Sciences, Inc, ARLington, HIL) at each cycle (Sequenase 13 U/μL was diluted 1:8 in enzyme dilution buffer US Biochemical, and 0.2 μL was added to 5 μL reaction mixture). Following this preamplification step, a conventional PCR reaction catalyzed by Taq DNA polymerase was performed in the same tube. PCR reaction mix consisted of each dNTP, 10 mmol/L Tris hydrochloride, pH 8.4, 2 mmol/L MgCl2, 50 mmol/L KCl, 0.1 mg/mL gelatin, 200 μmol/L of each dNTP, 50 pmol UN1 primer, and 2 U Taq DNA polymerase (Perkin-Elmer/Cetus). The reaction was heated to 95°C for 3 minutes followed by 35 cycles of 1 minute at 94°C, 1 minute at 56°C, 2 minutes at 72°C, and a 5-minute final extension at 72°C.

FISH. Amplified microdissected DNA (2 μL) was labeled with Biotin-16-dUTP (Boehringer Mannheim Biochemical, Indianapolis, IN) in a secondary PCR reaction identical to that already described except for addition of 20 μmol/L Biotin-16-dUTP. The reaction was continued for 15 to 20 cycles of 1 minute at 94°C, 1 minute at 56°C, and 2 minutes at 72°C, with a 5-minute final extension at 72°C. The PCR products were then purified with a Centricon 100 (Amicon, Beverly, MA) filter and used for FISH. Hybridization of the FISH probes followed our procedure described previously, which is based on the procedure of Pinkel et al. A

RESULTS

Cytogenetic analysis. Cytogenetic analysis was performed on all cases. Table 1 summarizes the karyotype alterations interpreted by conventional G-banding analysis of the clinical samples. Six of eight primary tumor cases contained an abnormal chromosome 6 with an apparent terminal deletion of 6q (Fig 1). The breakpoints determined by banding analysis were between bands 6q15 and 6q22 (Table 1). The remaining two primary tumor cases each contained rearranged chromosome 6 with undefined addition of material on 6q at 6q11 and 6q13. The lymphoma cell line, SU-DHL-4, contained an isochromosome of the short arm of chromosome 6. Examples of the abnormal copies of chromosome 6 chosen for microdissection are illustrated in Fig 1.
Fig 2. Detection of a complex interstitial deletion involving chromosome 6 in a primary lymphoma (92C147). (A) Representative normal G-banded lymphocyte metaphase. (B) Metaphase identical to (A) after FISH with a probe generated from the microdissected DNA recognizing a complex interstitial deletion [der(6)del(q11q21)del(6)(q22q25)].

Micro-FISH. To identify the chromosomal composition of additional material on 6q and to precisely determine the breakpoints of 6q deletions, microdissection of the entire abnormal chromosome 6 was performed in all nine cases. Briefly, two to five copies of the entire rearranged chromosome 6 in each case were microdissected, and DNA was directly amplified by PCR and labeled with Biotin-16-dUTP in a secondary PCR reaction. The labeled probe was then used for FISH to normal peripheral blood lymphocyte metaphases. Sequential G-banding and FISH were performed using the probes generated from the microdissected chromosome 6 samples, allowing definition of breakpoints. The results were unexpected compared with those suggested by G-banding analysis. The chromosomal composition and

Fig 3. Detection of a cryptic chromosome translocation of chromosome 6 in case 91C037. (A) Representative normal G-banded lymphocyte metaphase. (B) Same metaphase hybridized with a micro-FISH probe recognizing a nonreciprocal translocation [der(6)t(5;6)(p13;q11)].
consistent region of minimal deletion observed in all nine cases encompassed 6q11 to 6q21.

**DISCUSSION**

Loss of genetic material on the long arm of chromosome 6 is commonly detected in human B-cell lymphoma by both cytogenetic and LOH analyses. These and other studies strongly support the notion that a tumor suppressor gene(s) involved in lymphoma biology is localized to 6q. To date, a wide distribution of breakpoints along 6q have been reported in non-Hodgkin’s lymphomas (including follicular lymphomas). Several common regions of deletion have been reported in non-Hodgkin’s lymphomas, including 6q21-q23 and 6q25-q27,\(^6\) 6q21-6q25,\(^5,7\) 6q14-21, and 6q23-27.\(^8\) Although the banding quality obtained from cases of follicular lymphoma is often acceptable, it remains difficult to interpret the banding pattern subsequent to a deletion. This may in part reflect the repetitive nondistinctive banding pattern of 6q. The fact that other investigators have not appreciated the proximal nature of these deletions supports this notion. As a consequence, no recurrent breakpoint information has been available to facilitate positional cloning studies in this disease.

In this study, two important and novel findings have been made regarding chromosome 6 rearrangements in follicular lymphoma. First, it was striking that all cases of 6q rearrangement defined by cytogenetic analysis as terminal deletions were, in fact, demonstrated to be either interstitial deletions or nonreciprocal translocations. The extent of the deletion of the long arm of chromosome 6 among each case varied considerably in this study. Four cases lost the entire 6q, with the remaining five cases showing partial loss of the long arm of chromosome 6 (Fig 4). The common RMD was determined to be 6q11-6q21. Comparison of our results to those previously derived from cytogenetic and LOH studies suggests that the RMD in this disease may be more proximal than previously recognized. This results from previous chromosomal breakpoint assignments made on the basis of G-banding, and the relative lack of tested proximal polymorphic markers along 6q.

Second, we have identified a recurrent proximal breakpoint on 6q in human follicular lymphomas at 6q11. In this study, seven of nine cases demonstrated a rearranged chromosome 6 with the proximal breakpoint at 6q11. Four cases of interstitial deletion had the proximal breakpoint at 6q11, whereas in two cases of nonreciprocal translocations and one case of isochromosome 6p, the breakpoint occurred in 6q11. This raises the possibility that the recurrent breakage at 6q11 could represent either a primary chromosomal change interrupting a gene of importance to the biology of follicular lymphoma, or merely a region of “fragility” that is broken frequently because of some structurally unique feature. To clarify this question, studies are currently under way in our laboratory using region-specific microclone libraries to facilitate positional cloning of this breakpoint.

**REFERENCES**

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