Localization of Breakpoints by Polymerase Chain Reactions in Burkitt’s Lymphoma With 8;14 Translocations

By Bruce Shiramizu and Ian Magrath

Translocations involving chromosomes 8 and 14 in Burkitt’s lymphoma (BL) often involve the switch μ (Su) region on chromosome 14, which contains multiple repeats. This has enabled us to use the polymerase chain reaction (PCR) to detect breakpoints that involve this region on chromosome 14 and the c-myc gene on chromosome 8. Using pairs of flanking primers, each pair including one annealing to repeat sequences within the switch region and one of three primers from the c-myc region (first intron, 3’, or 5’ flanking sequence of the first exon of c-myc), we have been able to amplify DNA fragments containing the corresponding breakpoint regions from chromosome 14 in both cell lines and biopsied tumor samples. The definitive demonstration of sequences from both chromosomes in these fragments permitted the confirmation of the presence of a translocation. Because of the sensitivity of PCR, we were able to localize breakpoints in samples containing as few as 1 neoplastic cell in 10^6 cells. PCR provides a valuable tool for the detection of 8;14 chromosomal translocations, which should prove to be of value in diagnosis and molecular epidemiologic studies, as well as providing a means of detecting minimal disease.

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MATERIALS AND METHODS

Samples and DNA preparation. The majority of the cell lines and tumor samples were obtained from the tumors of patients treated in the National Cancer Institute Pediatric Branch (Table 1). Details of the derivation and characteristics of the cell lines have been previously reported.1 DNA from these cell lines and tumors was extracted as previously described.2

Restriction endonuclease digestion and Southern blotting. DNA was digested with restriction endonucleases: HindIII, PstI, PvuII, and BamHI using protocols provided by the supplier (Bethesda Research Laboratory [BRL], MD). Southern blots were prepared, hybridized, and washed as described.3 The following probes were used: Cμ (EcoRI-EcoRI)3; third exon c-myc (ClaI–EcoRI); first exon c-myc (PvuII-PvuII)2; Jμ (BamHI–HindIII); Su (SacI–SacI).2 Probes were labeled with 32P (New England Nuclear, Dupont, Wilmington, DE) using a nick-translation kit (BRL).

Restriction maps of the c-myc and immunoglobulin (Ig) heavy chain loci and examples of the possible 8;14 translocation products are shown in Fig 1. Tumors or cell lines with breakpoints far 5' of the HindIII site have germline configuration of the c-myc gene when DNA is cut with this enzyme. Breakpoints within the HindIII fragment, which result in a rearrangement, can be further localized by determining whether the PstI and PvuII fragments are intact. Lack of comigration of the first and third c-myc exons and rearrangements within the 5' PstI fragment, but not within the 5' PvuII fragment using a first exon probe, indicate that the breakpoint is in the first intron, and rearrangements demonstrated with both
Table 1. Summary of Breakpoint Locations of Samples Subjected to PCR

<table>
<thead>
<tr>
<th>Sample</th>
<th>Translocation</th>
<th>Chromosome 8 Breakpoint*</th>
<th>Chromosome 14 Breakpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>JD38</td>
<td>8;14</td>
<td>1st Intron</td>
<td>Su†</td>
</tr>
<tr>
<td>SET</td>
<td>8;14</td>
<td>1st Intron</td>
<td>Su</td>
</tr>
<tr>
<td>ST486</td>
<td>8;14</td>
<td>1st Intron</td>
<td>Su</td>
</tr>
<tr>
<td>JLPI19</td>
<td>8;14</td>
<td>Immed 5'</td>
<td>Su</td>
</tr>
<tr>
<td>MC116</td>
<td>8;14</td>
<td>Immed 5'</td>
<td>Su</td>
</tr>
<tr>
<td>RAMOS</td>
<td>8;14</td>
<td>Immed 5'</td>
<td>Su</td>
</tr>
<tr>
<td>HO2208</td>
<td>8;14</td>
<td>1st Exon</td>
<td>Su</td>
</tr>
<tr>
<td>HO2218</td>
<td>8;14</td>
<td>1st Exon</td>
<td>Su</td>
</tr>
<tr>
<td>CA46</td>
<td>8;14</td>
<td>1st Intron</td>
<td>Sw‡</td>
</tr>
<tr>
<td>Raji</td>
<td>8;14</td>
<td>Immed 5'</td>
<td>Sr§</td>
</tr>
<tr>
<td>AGB76</td>
<td>8;14</td>
<td>Far 5'</td>
<td>JhⅠ</td>
</tr>
<tr>
<td>Daudi</td>
<td>8;14</td>
<td>Far 5'</td>
<td>DⅣ§</td>
</tr>
<tr>
<td>DW6</td>
<td>8;14</td>
<td>1st Intron</td>
<td>JhⅠ</td>
</tr>
<tr>
<td>EW36</td>
<td>8;14</td>
<td>Far 5'</td>
<td>NSμ-NSⅡ,NSμ-NSⅠ#</td>
</tr>
<tr>
<td>P3HR1</td>
<td>8;14</td>
<td>Far 5'</td>
<td>NSμ</td>
</tr>
<tr>
<td>PA682</td>
<td>8;22</td>
<td>3'</td>
<td>λ-Ⅴ**</td>
</tr>
<tr>
<td>KK124</td>
<td>8;22</td>
<td>3'</td>
<td>λ-Ⅴ†</td>
</tr>
<tr>
<td>MOLT</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Abbreviation: Immed, immediate.
*Location with respect to c-myc.
†Switch-µ loci.
‡Switch-Ⅷ loci.
§Switch-γ loci.
Ⅰ-Heavy loci.
D-Heavy.
#Non-Sμ; non-Jc.
**λ-Variable loci.
††λ-Constant loci.

PstI and PvuII confirm a first exon breakpoint. The location of the breakpoint on chromosome 14 was determined by similar analyses using probes from the Ig locus.

PCR. Oligonucleotide primers and probes, based on published sequence information, were synthesized by Synthecell (Rockville, MD) and on an Applied Biosystems (Foster City, CA) DNA synthesizer. The sequences of the primers used are shown in Fig 2. DNA was subjected to PCR with slight modifications from a previously described procedure. Amplification with Taq (Thermus aquaticus) polymerase took place in 100-μL reaction mixtures. The samples were heated to 95°C for 2 minutes, cooled to room temperature before adding 2 U of polymerase, and subjected to 25 to 30 cycles of PCR as described. An aliquot of the PCR reaction was processed and transferred to a nylon membrane, hybridized to appropriate probes, and autoradiographed as previously described.

Sensitivity experiments. DNA was extracted from cell suspensions containing various proportions of ST486 cells (in which there is a breakpoint within the first intron of c-myc) and Daudi cells (with a far 5' breakpoint not detectable by any of the oligomer pairs used for PCR) ranging from 1:10² to 1:10⁶. The most dilute cell mixtures were prepared by transferring a specific number of ST486 cells, visualized microscopically, to tubes containing appropriate numbers of Daudi cells. Subsequently, the DNA was subjected to PCR as described and aliquots of the reaction product processed as above.

RESULTS

The breakpoint locations, as detected by Southern blotting, are summarized in Table 1. Intron breakpoints have been further confirmed by the demonstration that c-myc RNA transcripts derived from such tumors hybridize with a third exon c-myc probe, but not with a first exon c-myc probe, demonstrating absence of the first exon from the c-myc transcripts. The breakpoints in ST486, CA46, and Raji have been previously cloned and sequenced. In Raji and MC116, c-myc transcripts contain a first exon, and S1 protection analysis confirms initiation from at least one of the major c-myc promoters, P1 and P2.

First intron breakpoints. Using the primers for Sμ and first intron c-myc regions, we were able to amplify a fragment in three tumors: ST486, JD38, and SET (Fig 2). In each case the amplified fragments hybridized with Sμ and first intron c-myc probes. To further confirm the amplification of the targeted sequences, oligomer probes encompassing part of the DNA that is expected to be amplified were end-labeled and hybridized to the same samples. Similar results were obtained (data not shown). The ability of this primer pair to specifically amplify fragments from tumors containing switch/intron breakpoints was substantiated by showing that amplified fragments were not obtained in PCR performed in 12 additional tumors containing chromosomal breakpoints in different regions of c-myc, including the first exon and 5' flanking sequences. These oligomers also failed to amplify fragments in the cell lines Raji and CA46, which also have first intron breakpoints but involve S-γ and Scx, respectively, rather than Sμ (Fig 2). Thus, the switch oligomer appears to be specific to Sμ sequences and permits differentiation between breakpoints in different heavy chain switch regions.

Immediate 5' first exon c-myc 5' breakpoints. When primers derived from Sμ and a region 5' of the first exon of c-myc were used, amplification of a DNA fragment was observed in MC116, JLP119, and Ramos (Fig 2), but not in cell lines with breakpoints within the intron, first exon, or far 5' of c-myc. Similarly, using a primer from the 5' region of the first intron region, close to the first exon, we were able to identify fragments from DNA obtained from two samples in which the chromosome 8 breakpoint is within the first exon of c-myc (Fig 2), but not from cell lines with intron, immediate 5', or far 5' breakpoints. In each case, the amplified fragments were shown to contain sequences from both chromosomes 8 and 14 by hybridization with specific probes (Fig 2).

These results are consistent with the data from the Southern blot analysis as well as sequencing data previously published.

Size of the amplified fragment. Of interest was the finding that the size of the amplified fragments differed by approximately 10 to 50 nucleotides, regardless of the c-myc breakpoint. In view of the fact that the Sμ primer can hybridize to multiple sites within the Scx region (which may be responsible for the relatively broad bands seen on the blots), it seems probable that these differences in fragment size are primarily due to differences in the precise location of the breakpoint within the c-myc region. Analysis of larger numbers of tumors could permit the identification of preferential chromosome 8 breakpoint locations in subgroups of tumors. This would have relevance to the mechanism of deregulation of c-myc.

Sensitivity of PCR. The presence of a chromosomal translocation provides a specific marker for BL. Therefore,
PCR is a potentially valuable tool for the detection of minimal residual disease, or tumor at sites that appear microscopically normal. DNA was extracted from cell suspensions containing various mixtures of ST486 (which contains an intron breakpoint), and Daudi cells (with a breakpoint not detectable with the oligomer pairs), subjected to 30 to 50 cycles of PCR, and hybridized with the Sp probe. In several experiments in which DNA was extracted from cell mixtures containing a single ST486 cell per million Daudi cells, and aliquoted such that each reaction mixture was calculated to contain the DNA from a single ST486 cell or even less, a clear signal was obtained, indicating that PCR is capable of detecting a single DNA fragment containing the breakpoint region derived from chromosome 14 (Fig 3).
PCR IN BURKITT'S LYMPHOMA

A

B

C

Fig 2. Southern hybridization of amplification products obtained with the three pairs of oligomers. In each case, the blots were hybridized with probes derived from both chromosomes involved in the translocation. Diagrams of the structure of the derivative chromosome 14 for each of the three 8;14 breakpoints analyzed are shown in the lower part of the figure along with the primers and oligonucleotide probes used (see results for further information). (A) First intron breakpoint, present in cell lines/tumor JD38, SET, and ST486. CA48 and AG876 are examples of control cell lines. (B) Immediate 5' flanking/Sμ breakpoint present in cell lines JLP119, MC118, and Ramos. Raji and Daudi are examples of control cell lines. (C) First exon breakpoint present in the tumors HO2208 and HO2218. DW6 and PA682 are examples of control cell lines.

Fig 3. Sensitivity of the PCR. DNA extracted from purified ST486 and Daudi cells was amplified with intron/Sμ oligomer pair, which will amplify a fragment in ST486 but not in Daudi cells. DNA from ST486 cells, Daudi cells, and a mixture of 1 ST486 cell per million Daudi cells was subjected to 30 cycles of amplification, electrophoresed, and transferred to filters before hybridization with the Sμ probe (see Materials and Methods for details).

Conjunction with the Sμ oligomer, could theoretically result in amplification of a fragment when the breakpoint is in the first exon. In this case, the use of suitable probes would avoid the assignment of the breakpoint location to the wrong region.

While the oligomer pairs described here are only capable of detecting 8;14 translocations when these involve the Sμ region of chromosome 14, the use of oligomers that bind to the consensus sequences at the 3' end of J regions (or V regions), as has been used in PCR reactions designed to detect 14;18 translocations, should enable 8;14 translocations with breakpoints on chromosome 14 that are upstream of the switch region to be detected.

Presently available techniques for detecting chromosomal and molecular abnormalities, which can be valuable adjuncts to diagnosis and the detection of minimal residual disease, include cytogenetic analysis, flow cytometry, and analysis of gene rearrangements via Southern hybridization. Cytogenetic analysis does not provide identification of the breakpoint locations at a genetic level, and is relatively insensitive. Flow cytometry requires a larger number of cells for analysis and suffers from the disadvantage that, with the exception of anti-idiotypic antibodies, no truly tumor-specific marker exists. Until now, the most precise means of identifying breakpoint locations has been molecular cloning, but this method is too cumbersome for routine clinical use or for the analysis of a large number of tumors. In addition, this method is not suitable for the detection of tumor cells admixed with normal cells. Southern blotting is more practical, and with this technique, a clone of cells representing as little as 1% of the total cell population can be detected, making it suitable for following patients serially. However, Southern blotting may not provide much greater sensitivity than microscopy. Because of its very great sensitivity and simplicity, PCR could provide a valuable means of detecting chromosomal translocations and localizing the breakpoints to specific genetic regions. PCR can also be performed on fixed tissue, providing an additional advantage. In the case of 8;14 translocations, each oligomer pair defines the breakpoint regions on both chromosomes 8 and 14. If localization at the nucleotide level is required, this could readily be achieved by sequencing the amplified fragments. Furthermore, the presence of small numbers of tumor cells in apparently normal tissue, either at the time of diagnosis or
subsequent to therapy, can be determined with precision by PCR detection of a specific chromosomal translocation (an additional degree of specificity is provided by the size of the amplified fragment). In this regard, since we were able to detect a breakpoint region in a single cell (others have also reported successful amplification by PCR applied to a single cell\textsuperscript{23}), sensitivity is limited by the size of the sample and the potential for loss of sequences during DNA extraction rather than by the PCR itself. To exclude the presence of a translocation in a tissue sample, DNA from the entire sample would need to be analyzed.

PCR is likely to become applicable to an increasing number of situations relevant to the diagnosis and management of cancer patients.

REFERENCES


2. Yang JQ, Remmers EF, Marcu KB: The first exon of the c-myc proto-oncogene contains a novel positive control element. EMBO J 5:3553, 1986


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