**SHORT REPORT**

Efficacy of screening the intermediate cluster region of the bcl2 gene in follicular lymphomas by PCR

P J Batstone, J R Goodlad

**Background:** The t(14;18) translocation is a common finding in nodal follicular B cell lymphomas and diffuse large B cell lymphomas, and results in the overexpression of the antiapoptotic bcl2 protein. This chromosome rearrangement can be detected by the polymerase chain reaction (PCR), with most breakpoints found in the bcl2 gene occurring within either the major breakpoint region (mbr) or the minor cluster region (mcr). However, recent investigations have revealed several breakpoints between these two regions, which cluster 19 kb 3' of mbr in the "intermediate cluster region" (icr).

**Aims/Methods:** To analyse a series of 57 B cell follicular lymphomas known to carry the t(14;18) by PCR with primers directed against all three cluster regions to determine the efficacy of screening the icr site.

**Results:** Twenty six samples had an mbr rearrangement, four an mcr rearrangement, and three an icr rearrangement.

**Conclusions:** These results suggest that screening for icr is at least as efficacious as screening for mcr rearrangements.

**METHODS**

A series of 57 B cell follicular lymphomas cytogenetically known to harbour the t(14;18) translocation (55 cases) or rearrangement of 14q32 (one case) or 18q21 (one case) were screened using our own standard PCR protocols to determine the efficacy of analysing the icr. Crude DNA extracts were produced by proteinase K digestion of fresh lymph node tissue. PCR amplification was carried out using a "hot start" technique and primer pairs comprising a consensus primer to IgH and one of three different primers homologous to sequences in the mbr, mcr, or icr regions of bcl2 (table 1). Each reaction volume of 25 μl contained 1 × PCR buffer IV, 0.4 U Thermoprime+ Taq polymerase, 0.15mM of each nucleotide, 1.5mM MgCl2 (all ABgene, Epsom, Surrey, UK), 10pM of each primer, and 1 μl of extracted DNA. An initial five minute denaturation step at 98°C was added. Thirty cycles were performed (denaturation at 94°C for one minute, annealing and extension at 60°C for 1.5 minutes), followed by a final extension step at 72°C for seven minutes. Positive and negative controls were included in each PCR assay; the positive control comprised a nodal follicular lymphoma with known t(14;18). The final products were visualised by means of ultraviolet illumination of ethidium bromide stained polyacrylamide gels.

**RESULTS**

Of the 57 samples analysed, 33 yielded a single monoclonal band indicative of a t(14;18) translocation. Of these, 26 exhibited rearrangement of the bcl2 major breakpoint region. A further four samples were shown to have a rearrangement of the bcl2 mcr and three samples a rearrangement involving the bcl2 icr (fig 1). The two cases where no overt t(14;18) was present, but which contained rearrangements of 14q32 or 18q21, were monoclonal for an icr and mbr rearrangement, respectively. Mcr and icr PCR products were confirmed by sequence analysis (Cytomyx, Cambridge, UK).

**Table 1**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
<th>Product size range (bp)</th>
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<tbody>
<tr>
<td>LbH</td>
<td>TGA GGA GAC GGT GAC C</td>
<td>80–300</td>
</tr>
<tr>
<td>mbr</td>
<td>GAG TGT TAC TGT GGC TG</td>
<td>500–700</td>
</tr>
<tr>
<td>mcr</td>
<td>CGC TGT ACT CCT TTA GCT GC</td>
<td>75–300</td>
</tr>
<tr>
<td>si-cr</td>
<td>TCG TCT CTA GTA AGT GAG GCT</td>
<td></td>
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</tbody>
</table>

LbH, consensus primer to the immunoglobulin heavy chain gene joining regions; mbr, major breakpoint region primer; mcr, minor cluster region primer; si-cr, intermediate cluster region primer.

Abbreviations: icr, intermediate cluster region; IgH, immunoglobulin heavy chain gene; JH, joining region; mbr, major breakpoint region; mcr, minor cluster region; PCR, polymerase chain reaction.
positive cases (11 of 109) as 5’mcr positive cases (five of 109), which is in complete contrast to the findings of Albinger-Hegyi et al.5 It should also be borne in mind that the total number of s-icr/mcr/5’mcr positive cases in all these reports remains small, and therefore further studies using these and other primer sequences will be required to determine the optimum combination of primers for detecting gene recombinations in the icr. For those cases that remain negative by PCR, it may be useful to use fluorescence in situ hybridisation techniques to maximise the detection of Bcl2/ IgH recombinations in the diagnostic setting.

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