Consensus primers for detecting monoclonal immunoglobulin heavy chain rearrangement in B cell lymphomas

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Aims: To demonstrate the usefulness of polymerase chain reaction (PCR) methodology with both the FR2A/LJH/VLJH and the FR1c/UH/VUJH primer sets for detecting monoclonal immunoglobulin heavy chain (IgH) gene rearrangement in B cell non-Hodgkin lymphomas (B-NHLs).

Methods: Eighty-three patients with B-NHL were enrolled in this study. DNA was isolated from paraffin wax embedded sections and amplified by PCR to determine the sequences of the rearranged IgH gene. Each PCR product was subcloned. Cycle sequences and sequence analyses were done to determine the clone specific IgH variable region (VH) sequences.

Results: Clonal IgH gene rearrangements were detected in 45 cases with FR2a/JH/VUH and in 14 of the remaining cases with FR1c/JH/VUH. Most of the cases detectable with FR2a/JH/VUH were derived from VH3 and VH4 families. Five of six cases in the VH1 family and one in the VH7 family were amplified with the FR1c/JH/VUH primer set only.

Conclusion: The detection rate of IgH rearrangement in B-NHLs can be increased by using both FR2A/LJH/VLJH and FR1c/UH/VUH, and these two primer sets are suitable for routine PCR methodology. Moreover, each primer set appears to be closely related to VH family specificity.

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mmunoglobulin heavy chain (IgH) gene rearrangement studies have been used successfully to investigate the clonality and cell lineage of various B cell lymphoid malignancies. Primers complementary to the consensus regions have been developed from a limited number of IgH sequences, and oligonucleotides complementary to the framework 1 (FR1) or FR2 region and the JH segment are frequently used for detecting B lymphoid clonality in the routine laboratory setting. Recent studies have shown that the detection rate of IgH rearrangement is closely related to the cell origin of malignant lymphomas. Tumours derived from naïve lymphocytes, also designated pregerminal centre (pre-GC) naïve B cells, express unmutated IgH variable region (VH) genes and show a high detection rate of clonal IgH gene rearrangement. The other group of tumours derived from memory B cells, generated in the GC and characterised by cells bearing somatically hypermutated VH genes (GC and post-GC memory B cells), give a lower rate of clonality.

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FR2a primers are commonly used as the forward primer in IgH amplification, and the addition of an FR1c primer increases the number of IgH positive cases.1 We examined 83 cases of B cell non-Hodgkin lymphoma (B-NHL) and assessed the relation between sequences detected exclusively by an FR2a/JH primer set and those detected exclusively by an FR1c/JH primer set, and found that the results showed close VH family specificity.

METHODS

Our subjects comprised 83 patients diagnosed as having B-NHL at the department of pathology, Iwate Medical University School of Medicine, Japan, between 1997 and 2002. DNA was isolated from formalin fixed, paraffin wax embedded tissue sections. Seminested polymerase chain reaction (PCR) was performed according to the method of Ramasamy et al using the primer sets FR1c/JH/VLJH and FR2a/JH/VLJH (table 1) and the PCR conditions described previously.24 PCR was initially performed with the FR2a/JH/VLJH primer set, after which those cases not detected with this primer set were subjected to PCR with the FR1c/JH/VLJH primer set. The PCR products were ligated to pGEM-T easy vectors (Promega, Madison, Wisconsin, USA) and used to transform DH5α competent cells (Toyobo, Tokyo, Japan). We selected 40 subcloned colonies at random and purified the plasmid DNAs using a PI-200 DNA automatic isolation system (Kurabo, Osaka, Japan). Cycle sequencing was performed using a BigDye terminator cycle sequencing FS ready reaction kit (Applied Biosystems, Foster City, California, USA) and an ABI PRISM 3100 DNA sequencer (Applied Biosystems). The nucleotide sequences of each PCR product were aligned with the closest germline sequence derived from the V BASE database (http://www.mrc-cpe.cam.ac.uk/DNAPlot.php).

RESULTS

Among the 83 B-NHLs, clonal IgH gene rearrangements were detected in 45 cases with the FR2a/JH/VLJH primer set, and 14 of the remaining cases were amplified with the FR1c/JH/VLJH

Table 1 Nucleotide sequences of the primers

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences (5′-3′)</th>
</tr>
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<tbody>
<tr>
<td>FR2a</td>
<td>TGG(A/G)TCCG(C/A)CAG(G/C)CT/CT/CJNCNG</td>
</tr>
<tr>
<td>FR1c</td>
<td>AGTG(C/A)CAGT(G/C)CT/CT/CJNCNG</td>
</tr>
<tr>
<td>UH</td>
<td>TGAGGAGACGCTGACC</td>
</tr>
<tr>
<td>VLJH</td>
<td>GTGACCAAGGTCTTGGCC</td>
</tr>
</tbody>
</table>

Abbreviations: B-NHL, B cell non-Hodgkin lymphoma; FR, framework; GC, germinal centre; IgH, immunoglobulin heavy chain; PCR, polymerase chain reaction; VH, immunoglobulin heavy chain variable region
Table 2: VH gene usages using each forward primer in 83 B cell non-Hodgkin lymphomas

<table>
<thead>
<tr>
<th>Forward primer</th>
<th>VH1</th>
<th>VH3</th>
<th>VH4</th>
<th>VH7</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR2a (+) and</td>
<td>FR1c (-)</td>
<td>1</td>
<td>36</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>FR2a (-) and</td>
<td>FR1c (+)</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>FR2a (+) or</td>
<td>FR1c (+)</td>
<td>6</td>
<td>41</td>
<td>11</td>
<td>59/83 (71.1%)</td>
</tr>
</tbody>
</table>

FR, framework; VH, immunoglobulin heavy chain variable region.

Take home messages

- Clonal immunoglobulin heavy chain (IgH) gene rearrangements were detected in 45 of 83 patients with B cell non-Hodgkin lymphoma (B-NHL) using the FR2a/JH/VLJH primer set and in 14 of the remaining cases with FR1c/JH/VLJH.
- Thus, the detection rate of IgH rearrangement in B-NHLs can be increased by using both FR2a/JH/VLJH and FR1c/JH/VLJH, and these two primer sets are suitable for routine polymerase chain reaction methodology.
- Each primer set appears to be closely related to variable region family specificity.

REFERENCES