

## SHORT REPORT

# 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/LJH/VLJH 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/VLJH and in 14 of the remaining cases with FR1c/JH/VLJH. Most of the cases detectable with FR2a/JH/VLJH 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/VLJH primer set only.

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

Immunoglobulin 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.

"Recent studies have shown that the detection rate of IgH rearrangement is closely related to the cell origin of malignant lymphomas"

FR2a primers are commonly used as the forward primer in IgH amplification, and the addition of an FR1c primer

**Table 1** Nucleotide sequences of the primers

Primers	Sequences (5'-3')
FR2a	TGG(A/G)TCCG(C/A)CAG(G/C)C(T/C)(T/C)CNGG
FR1c	AGGTGCAGCTG(G/C)(T/A)G(G/C)AGTC(T/G/A)GG
LJH	TGAGGAGACGGTGACC
VLJH	GTGACCAGGGTNCCTTGCCCCAG

FR, framework.

increases the number of IgH positive cases.<sup>1</sup> 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.<sup>2-5</sup> 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 DH5 $\alpha$  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

**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

Forward primer		VH usage				Total
		VH1	VH3	VH4	VH7	
FR2a (+) and FR2a (-) and FR2a (+) or	FR1c (-) FR1c (+) FR1c (+)	1 5 6	36 5 41	8 3 11	0 1 1	45 14 59/83 (71.1%)

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

primer set. The detection rate was 59 of 83 (71.1%) (table 2). Table 2 shows the VH usage of each amplicon. The cases amplified exclusively with the FR1c/JH/VLJH primer set and not with the FR2a/JH/VLJH primer set were derived from four of the seven human VH gene families, whereas most of the cases amplified exclusively with the FR2a/JH/VLJH primer set were derived from the VH3 and VH4 families. In the VH1 family, five of six cases were amplified only with the FR1c/JH/VLJH primer set.

## DISCUSSION

A monoclonal B cell population is characterised by amplified DNA of a single size, whereas a polyclonal B cell population is characterised by amplified DNA with a range of sizes. The use of consensus primers is suitable for PCR in B-NHLs because of its simplicity and flexibility. Consensus primers for FR1 or FR2 and the LJH/VLJH region have been used to amplify complementarity determining region 2 and the FR3 region. Each region is unique to each IgH rearrangement, and is used to generate either diagnostic oligonucleotide probes or PCR primers that do not cross hybridise with the sequences of normal B cells. Using such probes, minimal residual disease can be successfully detected in patients with B cell malignancies.

In our study, PCR amplification of the IgH gene rearrangement was performed with two sets of primers, and the use of the FR1c/LJH/VLJH primer set in sequence analyses has never been described previously. Use of both the FR2a/LJH/VLJH and FR1c/LJH/VLJH primer sets increased the detection rate, and the results were comparable to those reported in series based on a large number of B cell malignancies. In B-NHLs, various frequencies of somatic hypermutations make it difficult to detect IgH gene rearrangement, and degenerate primers are more suitable than family specific primers, unlike the situation for acute lymphoblastic leukaemia and B cell lymphomas derived from naïve lymphocytes. From this viewpoint, the two primer sets used here are suitable for routine use in B-NHLs.

We found that each primer set showed close VH family specificity. The FR1c/LJH/VLJH primer amplified the VH1, VH3, VH4, and VH7 families, whereas the FR2a primer only amplified the VH1, VH3, and VH4 families. We aligned the germline sequences registered in the V BASE database and found that the sequence of the FR2a primer had one or more base substitutions from each germline consensus sequence in the VH1, VH2, VH5, VH6, and VH7 families, respectively.

"The FR1c/LJH/VLJH primer amplified the VH1, VH3, VH4, and VH7 families, whereas the FR2a primer only amplified the VH1, VH3, and VH4 families"

In particular, VH1, VH3, and VH4 were the main families encountered in B-NHLs.<sup>6</sup> The combined use of two primer sets (FR2a/LJH/VLJH and FR1c/LJH/VLJH) allowed not only high detection of B cell clonality, but also non-biased VH family specificity, which would be of great help in understanding the pathology of B cell malignancies. In fact, there is evidence for deviation from this random involvement of VH families in certain tumours; the most studied group of these tumours is

## 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

chronic lymphocytic leukaemia, in which biased expression of genes from the VH5 and VH6 families, accompanied by under-representation of VH1 and VH2, has been reported.<sup>7,8</sup>

Our results indicate that further studies should be undertaken, particularly in specific lymphoma subtypes.

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