Development of immunoglobulin variable heavy chain gene consensus probes with conjugated 3’ minor groove binder groups for monitoring minimal residual disease in childhood acute lymphoblastic leukaemia

M Uchiyama, C Maesawa, A Yashima, T Itabashi, T Satoh, M Tarusawa, M Endo, Y Takahashi, S Sasaki, S Tsuchiya, Y Ishida, T Masuda

SHORT REPORT

Aims: To develop immunoglobulin heavy chain variable (VH) gene probes that are shorter and more flexible in position for monitoring minimal residual disease (MRD) in childhood leukaemia (ALL), using minor groove binder (MGB) technology.

Methods: All VH germline sequences registered in the database were aligned and the consensus regions were determined. The reliability of the MGB probes was compared with non-MGB probes in all 24 cases of ALL.

Results: Ten MGB probes (16 to 18 mers) were designed that enabled all the germline sequences on the database to be analysed, whereas the conventional non-MGB probes (21 to 27 mers) did not allow the analysis of four of the VH1 and five of the VH3 germline sequences. The sequencing results in five of the 24 cases of ALL were not matched to the non-MGB probes.

Conclusions: MGB technology allows shorter probes to be designed, enabling MRD to be detected in childhood ALL. This would provide a considerable reduction in cost for a large MRD study.

Real time quantitative polymerase chain reaction (RQ-PCR) is an attractive approach for the quantitative evaluation of minimal residual disease (MRD) in bone marrow and peripheral blood stem cells, monitoring tumour cell reduction in the early response to primary treatment, or predicting disease relapse during long term follow up, because of its high sensitivity, reproducibility, and simplicity.1,2 In childhood acute lymphoblastic leukaemia (ALL), consensus strategies, using a small number of fluorogenically labelled germline probes and an allele specific oligonucleotide (ASO) RQ-PCR method targeting immunoglobulin heavy chain (IgH) gene rearrangements, have been established and used for evaluating the efficacy of various treatment regimens.3 In childhood acute lymphoblastic leukaemia,4–7 minor groove binder (MGB) conjugated DNA probes have been developed and used for5 non-MGB probes vary substantially in length from 14 to 40 mers, depending on the GC content of the amplified DNA fragment, whereas the MGB probes vary from 12 to 20 mers. Therefore, MGB probes show positional flexibility. The aim of our present study was to design MGB probes corresponding to all germlines in each VH family.

Materials and Methods

DNA was isolated from bone marrow samples with a QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) and amplified by PCR to determine the VH sequences, as described previously.5,7 Each PCR product was ligated to pGEM-T Easy Vectors (Promega, Madison, Wisconsin, USA) and transformed into DH5α competent cells (Toyobo, Tokyo, Japan). Thirty subcloned colonies were chosen at random, and plasmid DNA was purified. Sequence analysis was performed by the TIGR (http://www.tigr.org) and GenBank databases to find matched sequences.

RESULTS

Minor groove binder probes show positional flexibility

Recently, minor groove binder (MGB) conjugated DNA probes have been developed and used for5 nuclease PCR assays. These form extremely stable duplexes with single stranded DNA targets, allowing the use of shorter probes for hybridisation based assays.5 Their shorter length resulted in better sequence specificity of the MGB probes and lower fluorescent background staining as a result of a reduction in non-specific probe hybridisation in comparison with ordinary DNA probes (non-MGB probes), and the use of internal non-fluorescent quencher dye instead of ordinary 3’ quencher dye (TAMRA; tetramethylrhodamine). Because of the high melting temperature (Tm) requirements of PCR, non-MGB probes vary substantially in length from 14 to 40 mers, depending on the GC content of the amplified DNA fragment, whereas the MGB probes vary from 12 to 20 mers. Therefore, MGB probes show positional flexibility. The aim of our present study was to design MGB probes corresponding to all germlines in each VH family.

Abbreviations:

ASO RQ-PCR, allele specific oligonucleotide real time quantitative polymerase chain reaction; ALL, acute lymphoblastic leukaemia; IgH, immunoglobulin heavy chain; MRD, minimal residual disease; MGB, minor groove binder; Tm, melting temperature; VH, IgH variable region

REFERENCES

**RESULTS**

Three MGB probes were designed with VH3 rearrangements in the region of sequence homology (fig 1; table 1). These covered all VH3 germline sequences registered in the V BASE database, whereas non-MGB VH3 probes (VH3A and VH3B) by Donovan and colleagues' were not applicable in three of our 15 VH3 cases. In the VH1 family, two MGB probes were designed in the region of sequence homology (fig 1; table 1). Thus, it was possible to analyse all sequences of the VH1 region registered in the V BASE database with either the MGB-VH1A or MGB-VH1B probe. In comparison with unmodified probes (21 to 27 mers), MGB probes were shorter (16 to 18 mers), but had a higher Tm (~70°C).

Because the nominated germline sequences of VH2, VH4, VH5, VH6, and VH7 were almost identical, short regions of sequence identity appropriate for the design of a single probe were found, as with the non-MGB probes designed by Donovan and colleagues' (table 1). All MGB probes were tested in quantitative reactions on serially diluted IgH plasmids from patients, and each gave a characteristic logarithmic amplification plot (data not shown).

We are currently conducting a prospective MRD study to evaluate the practical usefulness of a RQ-PCR assay using our consensus germline probes. The copy number for the target before and after treatment was almost identical between the MGB and non-MGB probes. The study review board permitted us to present MRD data for 18 of 24 patients examined (table 2). One of the patients (number 18 in table 2), whose sequences were matched to the MGB-VH1A probe but not the VH1 non-MGB probe, is shown in fig 2.

**DISCUSSION**

In our study, we designed 10 novel MGB germline probes, and all IgH gene rearrangements registered in the V BASE database could be detected with one of the probes. It was possible to use the probes to assess MRD in patients with four VH1 and five VH3 germlines that could not be evaluated using the non-MGB probes of Donovan et al. Furthermore, the cost of MGB probes is almost equal to that of non-MGB probes, so that the reduction in probe numbers leads to a cost benefit.

Others have recently described an ASO primer approach using consensus JH probes. We tested the ASO primer

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**Table 1** Nucleotide sequences of germline consensus minor groove binder probes designed in our study

<table>
<thead>
<tr>
<th>Probe</th>
<th>Sequences (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGB-VH1A</td>
<td>CGTCCGTACGTCATGAG</td>
</tr>
<tr>
<td>MGB-VH1B</td>
<td>CTACGTCGTACGTGAGC</td>
</tr>
<tr>
<td>MGB-VH2</td>
<td>CCGAGAGGCAGGATGTG</td>
</tr>
<tr>
<td>MGB-VH3A</td>
<td>CTGGAGGTACAGTGATC</td>
</tr>
<tr>
<td>MGB-VH3B</td>
<td>CTTGAGGGAATGAGAC</td>
</tr>
<tr>
<td>MGB-VH3C</td>
<td>CTGGAATGAGGAGACC</td>
</tr>
<tr>
<td>MGB-VH4</td>
<td>CTGCAGTCGGAGAAGAC</td>
</tr>
<tr>
<td>MGB-VH5</td>
<td>CTCGAGCAGGAGAAGCT</td>
</tr>
<tr>
<td>MGB-VH6</td>
<td>CAGTCGAGGAGAAGCTG</td>
</tr>
<tr>
<td>MGB-VH7</td>
<td>GTGTCGAGCAAGAGGTC</td>
</tr>
</tbody>
</table>
approach using a consensus MGB JH probe, and we expect that a far simpler technique would be available for a large scale clinical study.

"It was possible to use the probes to assess minimal residual disease in patients with four VH1 and five VH3 germlines that could not be evaluated using the non-minor groove binder probes of Donovan et al." 

In conclusion, an ASO RQ-PCR assay with MGB probes developed for the detection of MRD in childhood ALL was shown to be a feasible technique for the identification of patients at risk of relapse, because of the shorter length of the probes and their positional flexibility. The assay was easier to use and had better specificity and cost performance than assays using conventional probes.

**ACKNOWLEDGEMENTS**

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Take home messages

- Minor groove binder probe technology allows shorter probes to be designed, which also have more positional flexibility
- These probes enable minimal residual disease to be detected in childhood acute lymphoblastic leukaemia and can therefore identify those patients at risk of relapse
- The assay is easier to use and had better specificity and cost performance than assays using conventional probes

Authors’ affiliations
M Uchiyama, C Maesawa, A Yashima, T Itabashi, T Satoh, T Masuda, Department of Pathology, Iwate Medical University School of Medicine, Uchimaru 19-1, Morioka 020-8505, Japan
M Endo, M Tarusawa, Department of Paediatrics, Iwate Medical University School of Medicine
Y Ishida, Division of Haematology, Third Department of Internal Medicine, Iwate Medical University School of Medicine
Y Takahashi, S Sasaki, Department of Paediatrics, Hirosaki University School of Medicine, 036-8562 Hirosaki, Japan
S Tsuchiya, Department of Paediatric Oncology, Research Institute of Development, Aging and Cancer, Tohoku University, 980-0872 Sendai, Japan

Correspondence to: Assistant Professor C Maesawa, Department of Pathology, Iwate Medical University School of Medicine, Uchimaru 19-1, Morioka 020-8505, Japan; chihaya@iwate-med.ac.jp

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