

SHORT REPORT

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

P J Batstone, J R Goodlad

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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 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.

The t(14;18)(q32;q21) translocation is a common finding in nodal B cell lymphomas of follicular morphology and to a lesser degree in diffuse large B cell lymphomas. At the molecular level, the *bcl2* gene on chromosome 18 is brought adjacent to one of the immunoglobulin heavy chain gene (IgH) joining regions (JH) on chromosome 14. This results in *bcl2* coming under the control of the IgH E μ enhancer, and overexpression of the antiapoptotic *bcl2* protein ensues. The subsequent suppression of cell death is thought to enable the accumulation of other genetic lesions, which in turn leads to uncontrolled cell proliferation and progression to lymphoma.

"The t(14;18)(q32;q21) translocation is a common finding in nodal B cell lymphomas of follicular morphology and to a lesser degree in diffuse large B cell lymphomas"

PCR analysis of the t(14;18) translocation is possible using primers directed towards the IgH JH and the *bcl2* gene. Most chromosome 18 breakpoints occur in a cluster region 150 bp long in exon 3 of the *bcl2* gene, termed the major breakpoint region (mbr).¹ A second minor cluster region (mcr), approximately 500 bp in length, is located 30 kb downstream.² Recently, there have been several reports using long distance and inverse polymerase chain reaction (PCR) that have revealed additional breakpoints occurring between these two cluster regions.^{3,4} This region has now been sequenced and primers designed to detect t(14;18) rearrangements with breakpoints contained therein.⁵ It too contains a 200 bp cluster region 19 kb 3' of mbr, called the "intermediate cluster region" (icr).

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 JH and one of three different primers homologous to sequences in the mbr,¹ mcr,² or icr⁵ regions of *bcl2* (table 1). Each reaction volume of 25 μ l contained 1 \times 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 μ l of extracted DNA. After an initial five minute denaturation step at 98°C the enzyme 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 Polymerase chain reaction primers

Primer	Sequence (5'→3')	Product size range (bp)
JH	TGA GGA GAC GGT GAC C	
mbr	GAG TTG TAC GTG GCC TG	80–300
mcr	CGC TTG ACT CCT TTA CGT GC	500–700
s-icr	TCG TTC TCA GTA AGT GAG AGT GC	75–300

JH, consensus primer to the immunoglobulin heavy chain gene joining regions; mbr, major breakpoint region primer; mcr, minor cluster region primer; s-icr, 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

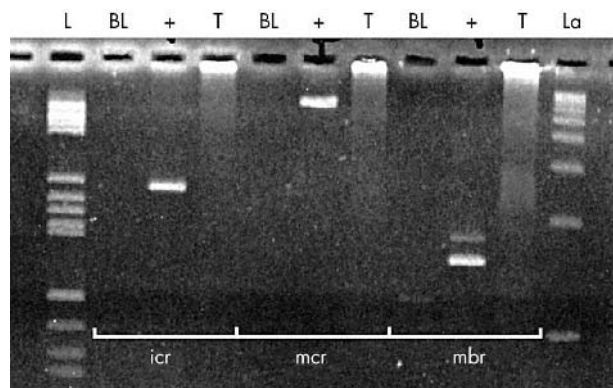


Figure 1 Polymerase chain reaction gel illustrating positive control samples (+) for major breakpoint region (mbr), minor cluster region (mcr), and intermediate cluster region (icr) bcl2 rearrangements and a negative test (T) sample. BL, negative control lanes; L and La, DNA size ladders (pBR322/haeIII and 100 bp ladder, respectively).

DISCUSSION

The results of our small study suggest that screening of the intermediate region between mbr and mcr of the bcl2 gene can detect several t(14;18) rearrangements that currently go unnoticed with protocols involving primers directed only towards the mbr and mcr. We found three rearrangements within the intermediate region, a similar number to that seen with the use of mcr primers. Finding positive PCR products in samples with rearrangements of 14q32 and 18q21 suggests that cryptic or complex t(14;18) rearrangements are present in these cases.

“Our results indicate that screening of the intermediate cluster region with just one primer set is at least as efficacious as screening for minor cluster region rearrangements”

Because we have a mixed referral pattern of both fresh and paraffin wax embedded tissue samples we confined ourselves to our standard PCR technique supplemented with the s-icr primer, which has been designed specifically by Albinger-Hegyí *et al* to allow analysis of the low molecular weight DNA fragments that formalin fixed tissue yield.⁵ Although we did not find three times as many icr as mcr breakpoints, as reported by Albinger-Hegyí and colleagues⁵ using a combination of PCR techniques and primers, our results indicate that screening of the icr with just one primer set is at least as efficacious as screening for mcr rearrangements. If resources were available, more detailed analysis of the region between mbr and mcr but outside of icr would yield an even greater number of (14;18) translocations.

The recently described BIOMED 2 primer sets published by van-Dongen *et al* also contain a primer (5' mcr) covering the intermediate region.⁶ However, direct comparison between their findings, those of Albinger-Hegyí *et al*,⁵ and our results is complicated by the fact that different primer sequences and techniques are used. Nonetheless, it is interesting to note that Van-Dongen and colleagues⁶ found twice as many mcr

Take home messages

- We analysed 57 B cell follicular lymphomas known to carry the t(14;18) by the polymerase chain reaction (PCR) with primers directed against the major breakpoint region (mbr), the minor cluster region (mcr), and the more recently identified “intermediate cluster region” (icr)
- These rearrangements result in the overexpression of the anti-apoptotic bcl2 protein
- Twenty six samples had an mbr rearrangement, four an mcr rearrangement, and three an icr rearrangement, suggesting that screening for icr is at least as efficacious as screening for mcr rearrangements

positive cases (11 of 109) as 5'mcr positive cases (five of 109), which is in complete contrast to the findings of Albinger-Hegyí *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 rearrangements in the diagnostic setting.

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