Breakpoint cluster region (BCR) gene rearrangement studies in chronic myeloid and acute lymphoblastic leukemias

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Summary

Deoxyribonucleic acid (DNA) of twenty chronic myeloid leukemia (CML) and thirty acute lymphoblastic leukemia (ALL) patients were analysed by Southern hybridization. The DNA was digested with *Bgl*II and hybridized with a 4.5-kilobase (kb) *phl*/bcr-3 DNA probe. All the 20 CML patients showed gene rearrangement within a 5.8-kb segment (the major breakpoint cluster region, M-bcr) of the breakpoint cluster region (*bcr*) gene of chromosome 22, indicating the presence of the Philadelphia chromosome. M-bcr rearrangement at the *bcr* gene of chromosome twenty-two was not detected in all the thirty ALL patients (nine adults and twenty-one children) and two normal controls.

Key words: Major breakpoint cluster region (M-bcr), Chronic myeloid leukemia, Acute lymphoblastic leukemia

Introduction

Cytogenetic studies reveal that 90-95% of chronic myeloid leukemia (CML) patients have a 'deleted' chromosome 22, called the Philadelphia (Ph) chromosome. The Ph chromosome is due to the standard reciprocal translocation between chromosomes 9 and 22, t(9;22) (q34;q11) in about 90% of the cases[1]. At the molecular level, as a result of the Ph translocation, a segment of the Abelson (*abl*) proto-oncogene on chromosome 9q34 becomes joined to a segment of the breakpoint cluster region (*bcr*) gene on chromosome 22q11. The breakpoints of chromosome 9 seems to occur in a 200-kilobasepair intron 5' of *abl* exon 2[2]. The breakpoints on chromosome 22 are clustered within a 5.8-kb region (major breakpoint cluster region, M-bcr) of the *bcr* gene of chromosome 22[3]. The *bcr-abl* chimeric gene on the Ph chromosome results in the translation of a protein of 210 kD (kilodalton), p210 $^{bcr\text{-}abl}$, with enhanced tyrosine kinase activity when compared to the normal p145 ^{abl}[4].

The Ph chromosome is found not only in CML. It occurs with a frequency of about 20% in adult ALL[5], 5% in childhood ALL[6] and less than 1% in acute myeloid leukemia (AML)[7]. Molecular analysis of Ph-positive (Ph+) ALL reveals two types of rearrangements: Ph+ bcr-positive (bcr+) and Ph+ bcr-negative (bcr-). The former has breakpoints within the M-bcr of the *bcr* gene of chromosome 22 and is indistinguishable from Ph+ CML. The latter has breakpoints 5' upstream from the M-bcr, i.e., in the

first intron of the *bcr* gene (the minor breakpoint cluster region, m-bcr)[8]. The *bcr-abl* chimeric gene in Ph+ bcr- individuals results in the translation of a protein, $p190^{bcr\text{-}abl}$ with a more potent growth promoting effect than $p210^{bcr\text{-}abl}$[9]. There have been suggestions that Ph+ bcr+ adult ALL represents the blastic phase of CML not clinically apparent or undiagnosed, whereas Ph+ bcr- ALL reflects *de novo* acute leukemia[10].

In an earlier study to detect the presence of M-bcr rearrangement at the *bcr* gene of chromosome 22 in Malaysian CML patients, Dyck and Bosco (1989)[11] used a 1.2-kb 3'bcr probe to hybridize with DNA digested with two restriction enzymes, *Bgl*II and *Hin*dIII. Here, we report the use of one restriction enzyme, *Bgl*II, and a larger probe, the 4.5-kb *phl*/bcr-3 DNA probe to detect M-bcr rearrangement at the *bcr* gene of chromosome 22 in twenty CML and thirty ALL patients in Malaysia.

Materials and Methods

Bone marrow (BM) or peripheral blood (PB) specimens or both were obtained at random from 20 CML and 30 ALL patients referred to the University Hospital, Kuala Lumpur, from July 1988 to October 1989. The diagnosis of CML and ALL was based on cytochemistry and morphological studies by the hematologists at the hospital.

The isolation of cellular DNA, digestion with *Bgl*II, agarose (0.8%) gel electrophoresis, Southern transfer, nick translation, hybridization, and autoradiography were performed according to Maniatis *et al.* 1982.[12] After hybridization the DNA bound nylon membranes were subjected to high stringency wash at 65°C.

A 4.5-kb *phl*/bcr-3 DNA probe (13), which spans the entire 5.8-kb segment of the M-bcr of the *bcr* gene of chromosome 22 with the exception of an internal 1.6-kb *Hin*dIII fragment found to contain repetitive sequences, was labelled with α^{32}P deoxyadenosine triphosphate by nick translation to a specific activity of 1.0×10^9 cpm/ug. The probe was from Oncogene Science Inc., U.S.A.

Results and Discussion

Figure 1 shows a typical result of an autoradiograph obtained after hybridization with *Bgl*II digested DNA from CML and ALL patients, and normal controls. The normal control (lane 7) yields three fragments, 4.8, 2.3, and 1.1 kb, detectable by the *phl*/bcr-3 probe.

As a consequence of the Ph translocation at the M-bcr two novel junctions are generated. Because only one chromosome 22 is affected, DNA from Ph+ CML patients would yield an additional one or two DNA fragments that will hybridize with the *phl*/bcr-3 probe (13,14) as shown in Figure 1, lanes 1 to 6,8,and 9. The arrows indicate the positions of the rearranged DNA fragments generated by the Ph translocation. The three germ line fragments (4.8, 2.3, 1.1 kb) representing the normal chromosome 22 are present in every case. CML patients at lanes 8 and 9 have two novel bands probably corresponding to 22q- and 9q+ fragments.

By digestion with one restriction enzyme and hybridization with a 4.5-kb *phl*/bcr-3 DNA probe, all the 20 CML patients were found to have M-bcr rearrangement at the *bcr* gene of chromosome 22, indicating the presence of the Ph chromosome in the patients.

All the 30 ALL patients (twenty-one children and nine adults), as exemplified by two in lanes 10 and 11, Figure 1, yielded three normal germ line fragments, indicating the absence of gene rearrangement at the M-bcr. The age range of the twenty-one children (less than 15 years) was 2 to 14, and that of nine adults (15 years and above) was 18 to 70. Ph+ bcr+ ALL have been detected with the *phl*/bcr-3 DNA

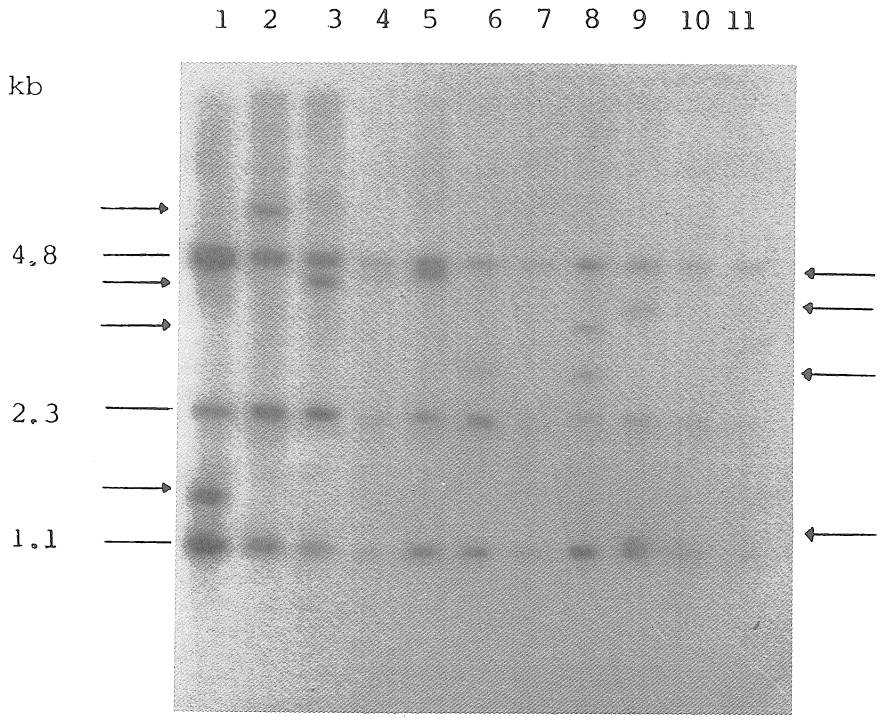

Fig. 1 : Autoradiograph of *bcr* gene rearrangement studies by hybridization with *phl*/bcr-3 DNA probe. Sources of DNA: lanes 1 to 6, 8 and 9, CML patients; lane 7, normal control; lanes 10 and 11, ALL patients. Positions of normal germ line bands (sizes in kb) are indicated by the numbers 4.8, 2.3 and 1.1. Arrows indicate the positions of the rearranged DNA fragments from CML patients.

probe by other researchers[13]. The absence of bcr rearrangement in the nine adult and twenty-one childhood ALL in this study could be due to the small sample size, since the frequencies of Ph+ bcr+ adult and childhood ALL have been estimated to be 10% and 0.5%, respectively[15].

The *phl*/bcr-3 DNA probe is useful for the identification of Ph+ CML as well as Ph+ bcr+ ALL especially where cytogenetic results are not easily obtained. Cytogenetic studies cannot differentiate Ph+ bcr+ ALL from Ph+ bcr- ALL. Unlike cytogenetic studies, DNA analysis could be performed on non-fresh blood and bone marrow samples, or even non viable cells.

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