MOLECULAR REARRANGEMENTS OF CHROMOSOME 22 IN CHRONIC MYELOID LEUKEMIA IN A MULTI-ETHNIC MALAYSIAN POPULATION

J J Bosco, J A Dyck

ABSTRACT

Rearrangements in the DNA of chronic myelogenous leukemia patients of Chinese, Malay and Indian origin were detected in the breakpoint cluster region of chromosome 22 using molecular techniques. The DNA of fifty patients was examined using a 1.2 kb DNA probe. Rearrangements were detected in 46/50 patients. Karyotypic data were available in nine patients, all of whom were Philadelphia chromosome positive and exhibited DNA rearrangement. Detection of the Philadelphia translocation by molecular methods, at this institution, where cytogenetics is not routinely performed, confirms its diagnostic value. The rearrangement data obtained in this study is consistent with molecular features of chronic myelogenous leukemia patients of Western countries.

Key Words: Chronic myeloid leukemia, Multi-ethnic, Malaysian

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ABBREVIATIONS

ALL acute lymphoblostic leukemia
CML chronic myelogenous leukemia
Ph1 Philadelphia
kb kilobase pair of DNA
BCR breakpoint cluster region
DNA Deoxyribonucleic Acid

INTRODUCTION

The appearance of the Ph1 translocation in 90-95% of CML patients is a non-random chromosomal aberration (1) and may be considered a true leukemic marker. The net effect of this reciprocal translocation is a smaller 'Ph1' chromosome 22(22q−) and a larger chromosome 9(9q+). Localization of the cellular oncogenes c-abl (2-5) and c-sis (6-8) to the translocated regions of chromosome 9 and 22 respectively, aroused interest in the possibility of their involvement in oncogenesis. Further work (5, 9, 10) demonstrated the consistency of a 5.8 kb breakpoint cluster region (BCR) on chromosome 22 (Figure 1). Rearrangement of the DNA in 17 Ph1-positive CML patients was detected by Groffen (10) using a 1.2 kb molecular probe cloned from the 3' region of the 5.8 kb BCR. The involvement of c-abl in CML became manifest when Canaani and co-workers (11) reported the presence of abnormal c-abl transcripts in CML patients. Shtivelman (12) revealed that the immediate molecular consequence of the Ph1 translocation results in a hybrid gene created by the fusion of chromosome 22 and c-abl sequence. It was discovered that specific splicing of 'BCR' exons to the second c-abl exon produces consistently abnormal CML specific mRNA. A c-abl protein of 210 kD, larger than the normal 145 kD c-abl product, with elevated tyrosine kinase activity was detected in CML cells (13-15).

The availability of DNA probes generated from the BCR of chromosome 22 provides clinical hematologists with a valuable test for the diagnosis of CML. The vast majority of CML patients exhibit the Ph1 translocation which can now be confirmed at the molecular level. Molecular studies of 'simple' variant Ph1 translocations (involving 22 and one non-9 chromosome) revealed the presence of c-abl on chromosome 22q− and the BCR rearrangement (16). The findings of BCR rearrangments in patients diagnosed as Ph1-negative CML indicates the usefulness of this molecular probe as an accurate diagnostic tool (17-21).

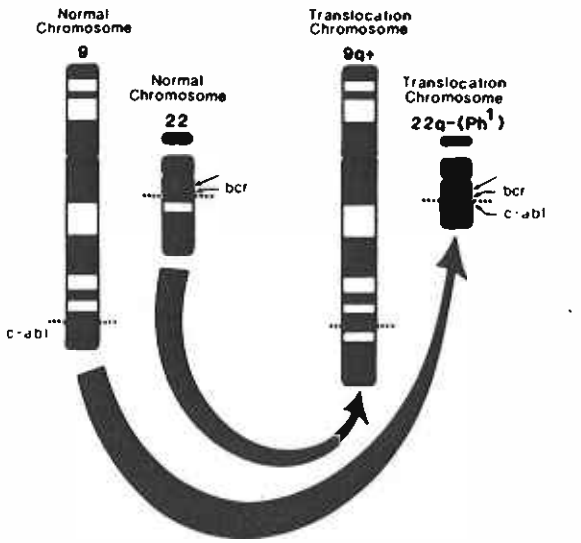

Figure 1: The creation of the Philadelphia chromosome by translocation of the c-abl oncogene from chromosome 9 to bcr at chromosome 22.

**Department of Medicine, Faculty of Medicine
University of Malaya, 59100 Kuala Lumpur
Malaysia**

J J Bosco, Associate Professor and Consultant Hematologist

**Department of Biochemistry/Medicine
University of Malaya**

J A Dyck, Post Graduate Student

Correspondence to: **Dr Bosco**

A recent study by Chan (22) on CML patients of diverse ethnic origin confirmed the presence of BCR rearrangement, using the 5.8 kb probe, in all CML patients positive for the Philadelphia chromosome at the time of sampling. The ethnic groups examined were Oriental (Taiwan), Caucasian (United Kingdom, Malta), Arab (Iraq), West Indian (Trinidad), Indian (India) and Black (South Africa). In Malaysia, cytogenetic analysis is not routinely performed because of difficulties associated with the transportation of fresh sterile samples to a central laboratory. These technical difficulties may account for the fact that only 75% of clinically diagnosed CML patients have a Philadelphia chromosome (23). The aim of this study were i) to examine the DNA of Chinese, Malay and Indian CML patients in West Malaysia to determine if they would demonstrate the same molecular abnormality as reported in the CML patients of Western countries ii) to establish molecular methods as routine diagnostic and research tools at the University Hospital.

MATERIALS AND METHODS

Fifty Malaysian patients of Chinese, Malay and Indian ethnicity were the subjects of this study. The diagnostic criteria for CML included elevated WBC counts, the presence of immature myeloid cells in the peripheral blood, basophilia, a low Leukocyte Alkaline Phosphatase (LAP) score and splenomegaly. The presence of the Philadelphia chromosome was confirmed in the 9 patients for whom evaluable metaphase spreads could be obtained. Peripheral blood obtained by venipuncture was anticoagulated with EDTA. DNA isolation, gel electrophoresis and Southern blotting were performed according to Maniatis (24). In brief, peripheral blood cells were subjected to plasma membrane disruption and the nuclei were isolated and stored at -70°C. The nuclei were subsequently lysed and incubated with a proteolytic enzyme. The nucleic acid was separated from the digested protein by a series of phenol and chloroform extractions. The DNA was precipitated with ethanol, quantitated by spectrophotometry and checked for degradation by electrophoresis in a 0.5% agarose gel. The high molecular weight DNA was then digested with restriction enzymes and the resultant DNA fragments were separated by agarose gel electrophoresis. Treatment of the gel with a sodium hydroxide solution denatured these size fractionated fragments which were then transferred by diffusion of a high concentration salt solution from the gel to a nitrocellulose membrane (Southern Blot). Baking the nitrocellulose blot covalently bound the single stranded DNA to the membrane.

The DNA probe, bcr (Pr-1), was obtained commercially and stored at -20°C. The probe was radioactively labelled with two compounds, ^{32}p dATP and ^{32}p dCTP. Sephadex G50 chromatography was employed to separate the ^{32}p-incorporated DNA from the free label. The specific activity of the probe was determined, based on the measurement of Cerenkov radiation of a dry DNA precipitate. Finally, the probe was rendered single stranded by subjecting it to boiling temperature followed by rapid chilling.

Prior to hybridization of the probe to the nitrocellulose blot, the blot was prehybridized for several hours in order to circumvent non-specific binding of the probe. The hybridization process was allowed to proceed overnight after which, a series of washes of varying salt solutions were carried out. X-ray film was exposed by the washed blots for 48 hours and the resultant autoradiographs were examined for bcr homologous bands. The nick translation and hybridization procedures were those recommended by Oncogene Science Inc. from whom the 1.2 kb 3' BCR probe (Pr-1) was purchased. The restriction enzymes Hind III, Bgl II and Bam HI were purchased from Pharmacia Molecular Biologicals. [α^{32}p] dCTP and [α^{32}p] dATP (at 3000 Ci/mmol) were purchased from Amersham International and air-shipped directly from the U.K. on dry ice and used within 2 weeks of receipt.

RESULTS AND DISCUSSION

46 of 50 CML patients tested revealed the presence of a BCR rearrangement, ie. an additional band to the normal non-rearranged band representing the remaining normal chromosome 22 DNA in the samples tested. Figure 2 depicts a restriction enzyme map of the BCR of normal chromosome 22 sequences and the restriction enzyme fragments generated by Bgl II, Bam HI and Hind III used in this study. On Southern blot analysis, the BCR rearranged CML DNA was found to contain an extra one or two bands in at least one enzyme digest. The appearance of additional abnormal bands is an indication that the Philadelphia breakpoint occurred within the BCR region altering restriction fragment lengths. These extra bands, representing the Ph1 translocation at the molecular level were of a unique size for each patient. Figure 3 demonstrates autoradiographic results of three BCR rearranged CML patients. This figure illustrates the importance of using the three different restruction enzymes as break points detected by one enzyme are not always detected by

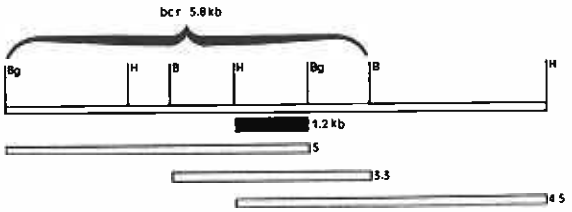

Figure 2: Restriction enzyme map of the bcr of chromosome 22. The 1.2 kb bcr (Pr-1) is represented by the solid bar. The normal non-rearranged chromosome 22 will produce a 5 kb Bgl II fragment, a 4.5 kb Hind III fragment or a 3.3 Bam HI fragment, detectable by this 1.2 kb probe. Restriction enzyme sites : Bg=Bgl II; H=Hind III; B=Bam HI (After Groffen et al, 1984).

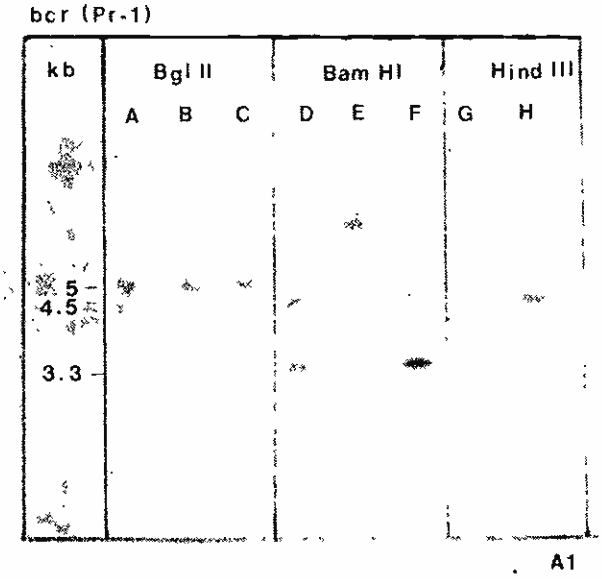

Figure 3: Autoradiographs of Southern blot demonstrating both normal and rearranged fragments of bcr homologous leukemic DNA. The normal bands are marked for size in kb and correspond to those described in Figure 1. Lanes A, D and G contain DNA patinet =17, lanes B, E and H contain DNA from patient =18 and lanes C and F represent patient =20. Restriction enzymes utilized are indicated.

Table 1.
Clinical and biological features of CML patients.

	ID #	Age at onset	Sex	Ethnic Group	Spleno-megaly	Values at time of DNA analysis: WBC X 10^9/liter	plt X 10^9/liter	B=P	MY + ME	n	ba	Clinical status	Ph¹	ber
1	980681	44	M	C	+	268	254	3	31	49	1	b		+
2	854078	37	F	M	+	82	381	1	12	64	0	a		+
3	957596	30	F	C	+	18	1184	0	1	71	8	a		+
4	836061	45	M	I	–	19	866	0	0	45	11	a		+
5	184609	58	M	C	+	9	289	0	6	62	0	a		+
6	840208	20	F	M	+	139	325	3	41	39	7	a		+
7	444970	27	M	M	+	90	71	2	23	88	0	b	+	+
8	637466	46	M	C	+	8	498	0	1	41	9	a		+
9	891058	22	M	C	+	50	314	0	33	55	5	a		+
10	891231	33	M	I	+	8	353	0	6	57	4	a		+
11	990258	11	M	M	+	475	283	16	36	42	4	c		+
12	990289	23	F	C	–	157	660	6	50	36	6	a		+
13	992735	23	F	C	+	24	762	7	5	54	4	a		+
14	899739	17	M	C	+	38	796	11	14	50	11	b	+	+
15	382131	38	M	M	+	33	278	2	9	69	0	a		+
16	424714	56	M	M	+	23	318	0	10	71	1	a		+
17	812294	23	M	M	+	99	251	5	30	53	3	a		+
18	990231	28	M	I	+	11	824	0	2	62	0	b		+
19	869933	35	F	C	+	83	833	08	29	48	6	a		+
20	955197	23	M	I	+	45	128	2	13	63	8	a		+
21	825350	22	F	M	+	57	785	5	13	75	1	b		+
22	836756	52	F	I	+	40	312	1	23	63	2	a		+
23	866640	70	F	C	+	18	756	1	19	37	16	a		+
24	970913	46	M	C	+	10	249	0	0	74	0	a		+
25	597132	46	M	M	+	71	340	19	16	19	43	c	+	+
26	983994	36	M	C	+	51	157	30	11	35	3	d		+
27	480818	42	F	C	+	8	35	33	5	3	48	d		+
28	868925	22	M	C	+	152	17	14	38	30	1	c		+
29	918693	34	F	C	+	147	469	5	25	50	6	a		+
30	738369	50	M	C	+	140	807	21	20	46	6	c	+	+
31	184755	56	M	C	+	219	1235	31	23	38	2	d		+
32	740691	40	M	M	+	129	134	12	40	37	0	c	+	+
33	742429	37	M	C	+	99	2850	4	32	43	7	a		+
34	642520	25	M	I	+	61	815	11	27	43	2	c		+
35	441981	31	M	M	+	97	625	2	12	64	4	a		–
36	899990	30	M	I	+	99	236	16	20	39	4	c		+
37	643392	22	F	C	+	299	1029	17	42	25	6	c		+
38	645407	32	M	M	+	273	1290	6	40	36	5	a	+	+
39	744044	35	M	I	+	165	23	32	47	3	6	d		+
40	860195	23	M	C	+	54	129	0	10	51	31	a		+
41	898220	41	M	M	+	77	358	0	29	65	0	b	+	+
42	517466	57	M	C	–	91	331	2	23	61	3	a	+	+
43	AE17376	15	F	C	+	153	658	55	17	21	1	d		+
44	435523	36	M	M	+	96	486	8	42	41	3	a		+
45	736842	26	M	C	+	49	29	88	1	5	2	d	+	+
46	826927	17	F	C	+	14	393	6	2	84	0	a	–	
47	640137	27	M	C	–	31	290	0	15	49	12	a		–
48	750752	42	F	M	–	63	838	5	14	61	4	a		–
49	652172	14	M	C	+	322	14	45	31	11	0	d		+
50	653122	21	F	C	+	386	921	7	48	25	10	a		+

Legend:

C = Chinese
M = Malay
I = Indian
WBC = white blood cells, plt = platelets
B+P = % blasts + promyelocytes in differential, MY+ME = myelocytes + metamyelocytes in differential
n = % neutrophils in differential, ba = % basophils in differential
a = chronic phase, b = chronic phase (developed BC within 6 mo)
c = accelerated, d = Blast crisis

another. Table 1 summarizes clinical and biological features and results of BCR analyses of the fifty patients examined. In this study, 7 patients were in blast crisis and 8 were in accelerated phase. Of the remaining 35 in chronic phase, 6 developed blast crisis within 6 months. Rearrangement of BCR was detected in each classification on CML and in all three ethnic groups. Of the 9 patients on whom evaluable metaphase spreads were obtained, all 9 did demonstrate both the presence of the Philadelphia chromosome and BCR rearrangement [Patient ID= 7, 14, 25, 30, 32, 38, 41, 42 and 45]. While it cannot be said that all of the remaining 37 BCR positive patients have the Philadelphia chromosome, it is most likely that they do have either a Philadelphia chromosome or an alternative chromosomal aberration such as a complex variant translocation which achieves the same net effect at the molecular level.

The four CML patients who did not demonstrate a BCR rearrangement [Patient ID= 35, 46, 47 and 48] with the 1.2 kb 3' BCR probe may not necessarily be negative for Philadelphia translocation or rearrangement of BCR. If these patients had a breakpoint occur in the 5' region of BCR, followed by a deletion of 3' sequences translocated to 9q+, a rearrangement would not be detected by the 3' probe utilized in this study. Popenoe documented the deletion of 3' BCR sequences in 4 Ph1-positive CML patients(25). Breakpoint analysis of their patients revealed that the deleted region consistently corresponded to sequences 3' of the breakpoint ie; the part of BCR which is translocated to 9q+ while the portion of BCR remaining on 22q−, the Ph1 chromosome, was intact in all CML samples examined.

Normal controls and 10 non-CML hematological disorders (data not shown) demonstrated the presence of the normal restriction enzyme fragments.

Karyotype analysis, a procedure requiring highly trained personnel for the interpretation of giemsa banding patterns, fresh blood or marrow samples, and tissue culture facilities, is not performed routinely at this hospital. While a Ph1-positive karyotype is considered to be the hallmark of CML, the use of BCR technology to detect the Philadelphia translocation is an especially significant contribution to diagnosis where cytogenetic results are not easily obtained.

With the recent availability of the larger 5.8 kb BCR probe the test for BCR rearrangement becomes more accurate in the case where a CML patient has a breakpoint in the 5' part of the breakpoint region with deletion of the 3' region of BCR of chromosome 22. Our data is consistent with the findings of Chan and co-workers (22) that the BCR rearrangement is probably a universal marker for CML, representing an exclusive molecular event associated with this leukemia. Our study also demonstrates that DNA analyses are extremely useful and feasible in a country like Malaysia as non-fresh blood and marrow samples or even non-viable cells can be used for large scale epidemiological studies and clinical diagnosis.

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