Molecular evidence for the clonal origin of blast crisis in chronic myeloid leukaemia

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Summary  Cytogenetic and enzymatic studies have shown that chronic myeloid leukaemia (CML) represents the clonal proliferation of a pluripotent stem cell. The Philadelphia chromosome (Ph') is the characteristic karyotypic abnormality seen in this disease, although the exact role of this clonal marker in the pathogenesis of CML is uncertain. At a molecular level, the Ph' has recently been shown to represent the translocation of \( c-abl \) to a limited region (breakpoint cluster region, \( bcr \)) on chromosome 22. We have used probes for the \( bcr \) gene to obtain molecular evidence for the clonal origin of blast crisis in 2 patient with CML. In both cases, the first with myeloid and the second with lymphoid blast crisis, there was rearrangement of the \( bcr \) gene. The patterns of rearrangement varied between patients but were identical when comparing acute and chronic phases within the same individual. As the Ph' translocation is thought to represent a random recombination event, these data not only provide further evidence for the clonal origin of blast crisis in CML, but also suggest that in the second patient this translocation event had already occurred at the level of the pluripotent stem cell.

Chronic myeloid leukaemia (CML) represents the clonal proliferation of a pluripotent stem cell capable of differentiation into several haemopoietic cell types (Greaves et al., 1979). These include the granulocytic, monocytic, erythroid, platelet, and lymphoid cell lineages (Fialkow et al., 1977). Two lines of evidence support this statement. Firstly, in patients heterozygous for the X-linked isoenzyme, glucose-6-phosphate dehydrogenase (G6PD), involved tumour cells of varying lineages expressed a single, common G6PD allele (Fialkow et al., 1977). Secondly, in over 95% of cases, cytogenetic studies have identified the Philadelphia chromosome (Ph') – a consistent karyotypic abnormality seen in both the chronic and acute phases of this disease (Rowley & Testa, 1983). Fialkow has suggested that this cytogenetic marker is a secondary event occurring in an already transformed haemopoietic stem cell (Fialkow et al., 1981), although more recent evidence suggests that the Philadelphia abnormality may be critical in the pathogenesis of CML (Adams, 1985).

Using banding techniques, Rowley (1973) first demonstrated that the Philadelphia chromosome resulted from a reciprocal translocation between chromosomes 9 and 22, i.e. t(9;22). However recently, molecular studies have determined that the critical genetic event underlying this karyotypic abnormality is the translocation (to chromosome 22) of the Abelson proto-oncogene \( c-abl \), normally located on chromosome 9 (de Klein et al., 1982). The breakpoint region (\( bcr \)) on chromosome 22 has been cloned from one CML cell line and subsequent analysis of 17 patients with CML has revealed that in each case, the breakpoints were clustered within a 5.8 kilobase (kb) – pair segment (Groffen et al., 1984).

We have used a probe for the \( bcr \) gene to confirm the clonal origin of blast crisis in two patients with CML. In addition, the results of these molecular studies suggest that the fusion of \( c-abl \) to the \( bcr \) gene – the molecular equivalent of the Philadelphia chromosome, has already occurred at the level of the pluripotent stem cell.

Patients and methods

Patients

The first patient was a 20 year old female with CML first diagnosed in July 1983. She remained in chronic phase for approximately 9 months but while being considered for bone marrow transplantation in April 1984, was noted to be in blast crisis. She was treated with high dose Ara-C but sustained only a partial response. Marrow samples harvested during the chronic and acute phase were available for study.

The second patient was a 39 year old female who was first diagnosed as having CML in 1976. She was treated intermittently with Busulphan until April 1984, when she developed lymphoid blast crisis. Following remission induction, an allogeneic bone marrow transplant was performed in July 1984, but blast crisis recurred in March 1985. Samples of this patient’s marrow obtained in chronic phase prior to transplantation and during

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the second episode of blast crisis were available for analysis. Both patients were Ph'-positive throughout the entire course of their disease and exhibited no additional cytogenetic abnormalities.

Methods

Sources of DNA DNA was prepared from peripheral blood or bone marrow cells of patients with CML either in chronic phase or during blast crisis. DNA prepared from the bone marrow of normal donors was used as a control.

Isolation of DNA DNA was purified as previously described (Gusella et al., 1980). In brief, cells were washed twice in PBS, resuspended in TNE (10 mM Tris/100 mM NaCl/1 mM EDTA pH 8.0) and added to an equal volume of TNE containing proteinase K (400 µg ml⁻¹) and 1% NaDod SO₄. The suspension was incubated at 37°C for 4–16 h. The DNA was then extracted once with phenol/chloroform/isomylalcohol and several times with chloroform/isomylalcohol. The DNA was then precipitated with isopropanol and resuspended in 10 mM Tris/1 mM EDTA, pH 7.5. The concentration of DNA was determined by spectrophotometry; the A₂₆₀/A₂₈₀ ratio was 1.8–2.0.

Southern blot analysis Genomic DNA was cut with one of several restriction enzymes including EcoRI, Bgl II, or Hind III. Digested DNA was separated on 0.8% agarose gels and transferred (Southern 1975) to nylon membranes. The filters were hybridized in 0.75 M NaCl/0.075 M sodium citrate, pH 7.0/0.1% NaDod SO₄/0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinyl-pyrrolidone/50% formamide/10% dextran sulphate containing sonicated salmon sperm (200 µg ml⁻¹) and radioactive probe (5 × 10⁶ cpm ml⁻¹). Hybridization was at 42°C for 24 h. Filters were then washed thoroughly to high stringency and autoradiographed for varying times at −70°C with the aid of an intensifying screen.

Probes Several probes were used in these experiments. A 1.2 kb human bcr restriction fragment (see Figure 1 for restriction map) was subcloned into a pSP vector construct immediately downstream of the bacteriophage SP6 promoter (Oncogene Science, Mineola, NY, USA). This probe recognizes the 'break point cluster region' which is a 5.8 kb in length region on chromosome 22. The Jₐ probe was a 3 kb cloned Eco R1 – Hind III restriction fragment that recognizes the J and adjoining intervening sequence regions of the immunoglobulin heavy chain gene. The probes were radiolabelled by nick translation with (α-³²P)d CTP (Rigby et al., 1977) to a specific activity of 10⁶ cpm µg⁻¹ DNA.

Surface marker studies A panel of fluorescent monoclonal antibodies was used to study the cell-surface phenotype of leukaemic cells obtained during the acute phase of CML in each patient.

Results

A panel of fluorescent monoclonal antibodies was used to determine the surface phenotype of leukaemic blasts harvested during the acute phase of each patient’s illness. The results of this analysis are shown in Table I. In patient no. 1, blasts made up 64% of the cells examined by light microscopy. However the majority of blasts examined did not react significantly with any of the antibodies tested. OKM-1, MO2, and MY-906 recognize myeloid differentiation antigens but these markers were only identified on a small (15%) subpopulation of cells, defined mainly by OKM-1. In this patient, lymphoid antigens such as cALLa, Tdt, cIgM were virtually undetectable. In the second patient, blasts represented 88% of the total cell population and in this case, the cells were predominantly lymphoid in type. Nearly all cells were Tdt and cALLa (recognized by J5) positive and a majority (69%) expressed the Ia antigen (Table I). Tdt may be present on both T and B lymphocytes (Bollum, 1979) although Ia and cALLa expression correspond to early stages of B cell differentiation in which immunoglobulin heavy chain genes have been rearranged (Korsmeyer et al., 1983). Marker studies were not performed on the bone marrow.

![Figure 1](image)

Figure 1  Restriction map of the cloned region in which occurs is illustrated. The 1.2 kb probe is indicated by the hatched box. B = BAM H1; Bg = Bgl II; E = EcorR1; H = Hind III; K = KpnI.
samples obtained during chronic phase. However, at these times, blasts constituted less than 10% of the total cell population (data not shown).

**Immunoglobulin gene rearrangement**

The results of the immunoglobulin gene rearrangement studies are consistent with the marker studies shown in Table I. Paired DNA samples representing the chronic phase and blast crisis in each patient were analyzed using Southern blotting techniques. For these experiments, DNA was cut with Eco R1 and probed with Jh (Figure 2). In the first patient, no evidence of immunoglobulin heavy chain gene rearrangement was detected. Both DNA samples (Figure 2, Lane B, C) displayed a germ line configuration identical to the normal control (Figure 2, Lane A). In the second patient a comparison of DNA patterns revealed that immunoglobulin heavy chain genes had retained their germ line structure during the chronic phase of CML (Figure 2, Lane D), but had rearranged during blast crisis (Figure 2, Lane E). This latter observation not only confirms the lymphoid nature of blast crisis in this patient but assigns the blasts to the B cell lineage (Korsmeyer et al., 1983). A faint germ line band visible in the acute phase DNA sample (Figure 2, Lane E) probably represents residual myeloid cells.

**BCR rearrangement**

Paired DNA samples from each patient were cut with Eco R1, Hind III or Bgl II and probed with a 1.2 kb bcr restriction fragment. In both patients the bcr gene had rearranged, although the patterns of rearrangement differed considerably. In Figure 3, in which DNA was cut with EcoR1, patient no. 1 appeared to have a germ line band in both lanes (Lanes B & C). However, when the appropriate DNA samples were digested with Hind III, a new band (Figure 4, Lanes A & B) representing rearrangement of the bcr gene was observed. This band was the same size in both the acute and chronic phase DNA samples. In this case, translocation of c-abl to chromosome 22 had not involved the R1 sites upstream of the Hind III restriction sites. In the second patient, a new band has appeared in both the acute and chronic phase DNA samples (Figure 3, Lanes D & E) and as with the first patient these appeared to be of an identical size. When probing these filters with the bcr probe, a germ line band is visible in each lane.

**Table I** Cell-surface phenotype of blast crises in two patients with CML

| Antibody | Patient no. 1 | Patient no. 2 |
|----------|---------------|---------------|
| OKM-1    | 15            |               |
| MO2      | 2             | <1            |
| MY-906   | 10            | 0             |
| J5 (cALLa) | 3       | 84            |
| HLA-Dr (Ia) | 5       | 69            |
| Tdt      | 0             | 88            |
| clgM     | 0             | 0             |

*The antibodies listed are a representative list of the 36 different antibodies that constitute the entire panel. OKM1, MO2 and MY906 recognize myeloid-associated antigens and J5, HLA-Dr, Tdt and clgM, lymphoid antigens. Numbers represent the percentage of fluorescent cells in a field of 200. cALLa, Tdt and clgM represent the common acute lymphoblastic leukaemia, terminal deoxy-nucleotidyl transferase and cytoplasmic IgM antigens respectively.
corresponding to the normal unrearranged bcr gene present on the uninvolved chromosome 22.

Discussion

This report describes two patients with Philadelphia positive CML terminating in blast crisis. In the first case (patient no. 1) the leukaemic blasts did not express lymphoid or myeloid antigens (Table I) and the immunoglobulin heavy chain genes (Figure 2) were not rearranged. By contrast in the second patient, as well as having rearranged their heavy chain genes, the leukaemic blasts expressed several lymphoid antigens normally only present during the earliest stages of B-cell differentiation (Table I). Thus, these patients have developed myeloid (based on morphologic rather than phenotypic criteria) and lymphoid blast crises respectively. Similar findings were reported by Bakhshi et al. (1983) who examined 18 cases of CML. Eight of the nine episodes of lymphoid crisis had heavy chain rearrangement whereas during the chronic myeloid,
myeloid blast and erythroid phases of CML, cells
were shown to have germ line immunoglobulin
genes.

Both patients described in this report had a
Philadelphia chromosome, the molecular equivalent
of which is thought to be the translocation of c-abl
to chromosome 22 with an associated structural
reorganization of the bcr gene (Heisterkamp et al.,
1985). Rearrangement of this gene (bcr) was
documented in both patients (Figures 3 & 4). We
have also observed varying patterns of rearrange-
ment of the bcr gene in three other patients with
lymphoid blast crisis (data not shown). At the
molecular level, the Ph' translocation is felt to
represent a random recombination event limited to
a 5.8 kb region of chromosome 22 (Heisterkamp et
al., 1985). This conclusion was based on the absence
of sequence homology between different breakpoint
regions of individual patients with CML. A detailed
restriction enzyme analysis of 17 patients with
proven or probable (based on molecular studies)
Ph'-positive CML strongly supports this model of
random recombination (Groffen et al., 1984). In
that report, the majority of patients studied had
different patterns of bcr gene rearrangement.
Similarly in our own study, restriction enzyme
patterns varied between patients, but were identical
when comparing the chronic myeloid and blastic
phases within the one individual. Based on the
proposed model, the last observation suggests that
a common cell of origin gave rise to both the
chronic myeloid and blastic phases of CML in each
case.

In the second patient with lymphoid blast crisis
(Table I and Figure 2), the finding of identical bcr
restriction enzyme patterns (Figure 3, Lanes D &
E), not only suggests that the chronic myeloid and
blastic phases of CML were derived from a single
progenitor cell, but also that genetic recombination
has already occurred at the level of the pluripotent
stem cell. This concept extends the model proposed
by Fialkow et al. (1981). Based on the development
of clonally-derived (implied by isoenzyme studies)
Ph'-negative lymphoid cell lines from a patient with
Ph'-positive CML, Fialkow suggested that the Ph'
abnormality occurred as a secondary event in
descendants of haemopoietic stem cell precursors.
The molecular studies described herein do not
preclude this hypothesis but based on a model of
random recombination, the data presented suggest
that recombination of the bcr gene – the molecular
equivalent of the Ph' translocation, has already
occurred in stem cells capable of myeloid or
lymphoid differentiation. Evidence of the Ph'
abnormality at the level of the pluripotent stem cell,
in conjunction with recent data suggesting that the
fusion of the bcr and abl genes in CML results in
the production of a novel 8.7 kb mRNA (Shivellman
et al., 1985), implies that this marker may be critical in the pathogenesis of CML.

However these results do not prove that the 9:22
translocation occurred during the initial trans-
formation event. It is also of interest that since the
same bcr-c-abl rearrangement is seen in both the
acute and chronic phases in the same patient,
progression into blast crisis does not appear to
depend on further molecular alteration at the bcr-c-
abl locus. Further molecular studies are underway
to define the exact role of the bcr and c-abl
oncogenes in the biology of CML.

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