Molecular Heterogeneity of Adult Philadelphia Chromosome-positive Acute Lymphoblastic Leukemia

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ABSTRACT

The (9;22) translocation which produces the Philadelphia (Ph') chromosome activates the abl oncogene from chromosome 9 by recombination with the bcr gene from chromosome 22. This fusion gene is transcribed into a new 8.5-kilobase chimeric mRNA which is translated into a novel Mr 210,000 fusion protein which has a protein tyrosine kinase activity that is greatly increased in comparison to the activity of the normal abl protein. Studies from this laboratory and others have shown that virtually all patients with chronic myelogenous leukemia have this new bcr/abl fusion gene. In contrast to these findings in chronic myelogenous leukemia, a small number of patients with Ph' (+) acute lymphoblastic leukemia (ALL) have been studied and found to lack the bcr/abl fusion gene (bcr(−)), but to have a new activation of abl, by recombination with an as yet undetermined region on chromosome 22. In this study, nine adults with Ph' (+)-ALL have been examined for evidence of a bcr/abl fusion gene. Of the nine patients, five have a bcr/abl recombination, whereas the remaining four patients do not. In contrast, the children studied to date have all been bcr(−). These data suggest that adults with Ph' (+)-ALL are a more heterogeneous group on a molecular level than are children, and that further studies will be required to determine the spectrum of molecular defects in patients with Ph' (+)-ALL, and the relationship of these various molecular defects to the clinical disease state of the individuals.

INTRODUCTION

The Philadelphia chromosome was first described as a shortened chromosome 22, and is found in approximately 90% of patients with CML (1–3). The t(9;22) which creates the Ph' chromosome moves the abl oncogene from its normal location on chromosome 9 to chromosome 22, where it is combined with a gene named bcr on the Ph' chromosome (Fig. 1, top) (4–6). This generates a new fusion gene, the bcr/abl gene, which is transcribed as a novel 8.5-kilobase mRNA, that is in turn translated into a unique fusion protein with a molecular weight of 210,000 (p210) (7–13).

The translocation breakpoints on chromosome 9 are spread over a region greater than 100 kilobases, while the chromosome 22 breakpoints are located within a short 5.8-kilobase region named the breakpoint cluster region (providing the label "bcr" for the 5.8-kilobase region, and "bcr" for the larger gene on chromosome 22, where it is included in the bcr/abl fusion gene (Fig. 1, bottom) (4, 5, 14)). The mRNA transcript of the bcr/abl fusion gene contains a splice from either exon 2 or exon 3 of the bcr region to the "common" exon of the abl gene (15, 16). This exon is located just 5' to the region of the abl gene which codes for the protein tyrosine kinase activity. The transforming activity of the abl oncogene is associated with its activity as a protein tyrosine kinase, and the tyrosine kinase activity of the bcr/abl fusion protein is greatly increased in comparison to the activity of the normal abl protein (12, 17).

The Ph' chromosome containing the bcr/abl fusion gene is strongly implicated in the pathogenesis of CML. This hypothesis has been strengthened by the discovery of the bcr/abl fusion gene in many Ph' negative CML patients (18–21). Although these patients lack a Ph' chromosome in a karyotype, they do have a recombinant bcr/abl gene when examined on a molecular level. The translocation in these patients has to be more complicated than the usual t(9;22) which generates a Ph' chromosome. The presence of a bcr/abl fusion gene may be a more specific marker for CML than the presence of a Ph' chromosome.

The Ph' chromosome is also present in approximately 5% of children and 20–30% of adults who are first diagnosed as having ALL (22). Some of these Ph' (+)-ALL patients may have had a previously undetected chronic phase of CML and then been diagnosed after progressing into a lymphoid blast crisis (23–25). Data from two recent reports prompted us to investigate our patient population. One report noted that 3 of 5 Ph' (+)-ALL patients lacked a rearrangement within the bcr [bcr(−)], and that an examination of the RNA from one of the patients failed to show an 8.5-kilobase abl-related mRNA (the size of the bcr/abl fusion gene transcript) (26). Another report examined 6 Ph' (+)-ALL patients and found evidence of abl activation different from the bcr/abl fusion gene in all of the patients, including the presence of a new Mr 185,000 abl-related protein tyrosine kinase (27). These findings led us to examine a group of our adult patients to determine whether adult Ph' (+)-ALL patients were different from CML patients at a molecular level.

MATERIALS AND METHODS

The clinical characteristics of the patients are listed in Table 1. Peripheral blood samples anticoagulated with EDTA were obtained from each patient, centrifuged at 1800 rpm for 20 min, the plasma was removed, the RBC were lysed with ammonium chloride, and DNA was prepared by methods previously described (28). Southern blotting and restriction mapping makes it possible to determine whether the chromosome 22 translocation breakpoint lies within the bcr, as illustrated in Fig. 2. Each patient was examined with the 4 probes shown in Fig. 1, bottom, and at least two different restriction enzymes for each probe (see Ref. 30). If a probe detects only the germ line restriction fragment with page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Fig. 1. Top, restriction map of chromosome 22 at the location of the bcr gene. Arrows, borders of the 5.8-kilobase breakpoint cluster region (bcr). Bottom, detailed restriction map of the bcr. Line A, restriction map of the 5.8-kilobase bcr. The small solid black rectangles indicate the exons of the bcr gene within the bcr. In Line B, the genomic probes used in restriction mapping are indicated, numbered 1–4. The zones chosen for the localization of the patient breakpoints are shown in Line C.

The doublet in Lane A indicates that there is a rearrangement of the genomic DNA within that fragment. This is illustrated in Fig. 2, where the restriction enzyme BglII was used in combination with probe 3 (the HindIII-EcoRI fragment from the 3' end of the bcr). The single band in the central portion of the gel, which is shared by all the lanes, is the normal 4.8-kilobase BglII restriction band. Since we have previously noted that deletion of 3' bcr sequence can artifactually produce a single germ-line fragment with 3' bcr probes even though a rearrangement is present, each patient was examined with 4 probes and multiple restriction enzymes for each probe (detailed restriction data for the patients are available on request). By digesting the patients' DNA with multiple restriction enzymes, and using multiple probes on Southern blots, it is possible to localize the breakpoints to one of the bcr zones shown in Fig. 1, bottom.

The blot in Fig. 2 includes patients with ALL and no Ph' chromosome, patients with Ph'(+) ALL, patients with CML, and one patient with a leukemoid reaction (as identified in the legend for Fig. 2). Patients with ALL and no Ph' chromosome are shown in Lanes b, c, and f, where only the germ-line 4.8-kilobase band is seen, indicating that there are no rearrangements within the bcr. Two of the Ph'(+) ALL patients who are bcr(−) are included in this gel: the patient in Lane a (patient 5 in Table 1) and in Lane n (patient 6 in Table 1) do not have bcr rearrangements. The patient in Lane f had a leukemoid reaction, and shows only the germ-line restriction band. In Lane e only one band is visible, but this patient with CML had additional restriction bands indicating a bcr rearrangement when examined with other restriction enzymes and probes.

Table 1 Clinical characteristics of patients studied

<table>
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<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC (/mm³)</th>
<th>% of blasts</th>
<th>Peripheral blood</th>
<th>Marrow</th>
<th>CALLA*</th>
<th>Karyotype*</th>
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<td>NA</td>
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<td>F</td>
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<td>75</td>
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<td>88</td>
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* All patients were 46,XX or 46,XY (as appropriate) unless otherwise noted.
* CALLA, common acute lymphocytic leukemia antigen; NA, not available.

The Ph'(+) ALL patient in Lane f (patient 4 in Table 1) has an extra restriction band, indicating that a rearrangement within the bcr was present. The CML patients seen in Lanes d, h, i, k, m, o, and p all show abnormal bands in addition to the normal 4.8-kilobase BglII band, indicating that they have rearrangements within the bcr.

Of the 9 Ph's'(+) ALL patients studied, 5 patients had breakpoints within the bcr, as listed in Table 2. The other 4 patients showed only germ-line restriction fragments with all four of the bcr probes and multiple restriction enzymes, indicating that they did not have any rearrangements within the bcr. Since the 5' extent of the bcr gene remains unknown, it is possible that...
these patients have a bcr/abl fusion gene in which the translocation breakpoint is located 5' to the bcr region, but within the bcr gene, or that the translocation has fused the abl gene to a different gene located closer to the centromere than bcr.

**DISCUSSION**

The data presented here indicate that adults with ALL who are Ph+ positive are a heterogeneous group on a molecular level. Some of the patients have generated a bcr/abl fusion gene which is structurally similar to the fusion gene arising in CML. Another group of patients is without such a CML-type bcr/abl fusion gene. The characteristics, both clinical and molecular, of the non-CML type remain unclear. This series has shown that adults with ALL, as well as children, can fall into the non-CML type Ph+ chromosome group. The published results from other investigators are summarized in Table 3. This includes a group of 22 adults and 9 children. Of this group, the patients who were bcr(-) and whose RNA or protein were studied did not have either the 8.5-kilobase bcr/abl mRNA or the p210 bcr/abl fusion protein. Further studies will be necessary to determine whether these patients consistently generate an activated abl oncogene, and if so, whether the mechanism of activation is similar in the different cases.

Clinical studies have suggested that Ph+(+)ALL carries a worse prognosis than Ph-negative ALL (31, 32). However, when adults with Ph+(+)ALL are compared with lymphoid blast crisis of CML, the clinical characteristics, including cellular phenotype, response to treatment, and survival are comparable. Some Ph+(+)ALL patients possess a bcr/abl fusion gene structurally similar to that found in CML. This suggests that certain aspects of the disease process may be the same in both groups. The steps in leukemogenesis which precede the formation of the bcr/abl gene may be different in these two entities even though both groups share a bcr/abl gene and phenotypic similarities. It may also be significant that the bcr breakpoints of the Ph+(+)ALL patients are spread throughout the bcr, in contrast to the clustering of lymphoid and myeloid CML blast crisis breakpoints at the 3' end of the bcr.

The group of adult Ph+(+)ALL patients without a bcr rearrangement are of special interest and raise additional issues. In at least some of the non-CML-type (bcr(-)) Ph+(+)ALL patients, the abl gene is activated in a manner different from the bcr/abl fusion gene. An initial recent report found that in 4 patients with bcr(-) Ph+(+)ALL there was a novel new abl-related RNA transcript 7.5 kilobases long and a new abl-related protein with a molecular weight of 190,000 (27). This new abl-related protein has a markedly increased protein tyrosine kinase activity in comparison to the normal abl protein. Subsequent reports (see Table 3) have suggested that children with Ph+(+)ALL are consistently bcr(-), whereas adults are heterogeneous (33–35). Our results suggest that almost one-half of adults with Ph+(+)ALL lack a rearrangement within the bcr. Precisely what fraction of bcr(-) Ph+(+)ALL patients have a new abl-related protein, whether it is the same in all of the patients, and how its protein tyrosine kinase activity compares to the bcr/abl p210 remain to be determined.

Detailed information about the molecular heterogeneity of patients with ALL raises new questions about the leukemic process which are important at both the clinical and molecular genetic levels. Further detailed examination of the defect in these patients will provide new insights into leukemogenesis, and may lead to more rational management of the patients.

### REFERENCES


27. Clark, S. S., MaLaughlin, J., Crist, W. M., Champlin, R., and Witte, O. N. Unique forms of the abl tyrosine kinase distinguish Ph'-positive CML from Ph'-positive ALL. Science (Wash, DC), 235: 85-88, 1987.


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