Molecular Heterogeneity of Adult Philadelphia Chromeosome-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 novel 8.5-kilobase chimeric mRNA which is translated into a novel M, 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 which includes the 5.8-kilobase region; see 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 is strongly supported 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 M, 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 to localize the chromosome 22 translocation breakpoint was performed by the usual methods, as previously described in detail (29, 30).

All patients were terminal deoxynucleotidyl transferase positive, and in those who were assessed, all reacted with the J5 monoclonal antibody (common acute lymphocytic leukemia antigen positive).

RESULTS

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 a particular restriction enzyme, then there has not been any
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.

rearrangement of the genomic DNA within that fragment. This is illustrated in Fig. 2, where the restriction enzyme Bglll was used in combination with probe 3 (the HindIII-EcoRII 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 Bglll 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 d, 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.

Fig. 2. Gel 1042: Southern blot of patients with CML, ALL, and also a normal patient. DNA was digested with the restriction enzyme Bglll (Bg), and hybridized to probe 3 (see Fig. 1, bottom). The band in the center of the gel which is present in all of the lanes is the normal 4.8-kilobase Bglll fragment which continues to be present in the normal allele. Additional probes and restriction enzymes were used to examine all the patients listed in Tables 1 and 2 (29, 30). The diagnoses of the patients are as follows: a, Ph'(+)-ALL (patient 5 in Table 1); b, c, ALL; d, e, CML; f, leukemoid reaction; g, h, i, CML; j, Ph'(+)-ALL (patient 4 in Table 1); k, CML, l, ALL; m, Ph'(+)-ALL (patient 6 in Table 1); o, p, CML. The shadows in Lane n above the 4.8-kilobase germ-line Bgl III fragment, and in Lane o above the rearranged junction fragment band are artifacts which were not present on repeated examinations. The presence of an additional abnormal band indicates the occurrence of a translocation within the region examined by this probe and the restriction enzyme Bglll.

The Ph'(+)-ALL patient in Lane j (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, k, i, m, o, and p all show abnormal bands in addition to the normal 4.8-kilobase Bglll band, indicating that they have rearrangements within the bcr.

Of the 9 Phs'(+)-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

### Table 1 Clinical characteristics of patients studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC (/mm³)</th>
<th>Peripheral blood</th>
<th>Marrow</th>
<th>Karyotype</th>
<th>CALLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>M</td>
<td>263,000</td>
<td>88</td>
<td>89</td>
<td>5 of 6 metaphases = t(9;22), 1 of 6 = t(9;22) + marker</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>F</td>
<td>60,000</td>
<td>85</td>
<td>56</td>
<td>(t(9;22))</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>F</td>
<td>104,000</td>
<td>75</td>
<td>65</td>
<td>(t(9;22))</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>F</td>
<td>44,000</td>
<td>84</td>
<td>75</td>
<td>(t(9;22))</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>F</td>
<td>121,000</td>
<td>97</td>
<td>59</td>
<td>(t(9;22),9p−)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>M</td>
<td>50,000</td>
<td>80</td>
<td>71</td>
<td>64% = (t(9;22), 36% = normal</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>M</td>
<td>27,000</td>
<td>88</td>
<td>79</td>
<td>(t(9;22),5q−)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>F</td>
<td>70,000</td>
<td>80</td>
<td>90</td>
<td>(t(9;22),−7)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>45</td>
<td>M</td>
<td>65,000</td>
<td>85</td>
<td>88</td>
<td>(t(9;22))</td>
<td></td>
</tr>
</tbody>
</table>

* All patients were 46,XX or 46,XY (as appropriate) unless otherwise noted.
* CALLA, common acute lymphocytic leukemia antigen; NA, not available.
these patients have a $bcr/abl$ fusion gene in which the translocation breakpoint is located 5' to the $ber$ region, but within the $ber$ gene, or that the translocation has fused the $abl$ gene to a different gene located closer to the centromere than $ber$.

**DISCUSSION**

The data presented here indicate that adults with ALL who are Ph1 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 Ph1 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 $ber(-)$ 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 Ph1(+)ALL carries a worse prognosis than Ph-negative ALL (31, 32). However, when adults with Ph1(+)ALL are compared with lymphoid blast crisis of CML, the clinical characteristics, including cellular phenotype, response to treatment, and survival are comparable. Some Ph1(+)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 Ph1(+)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 Ph1(+)ALL patients without a $bcr$ rearrangement are of special interest and raise additional issues. In at least some of the non-CML-type $bcr(-)$ Ph1(+)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 $bcr(-)$ Ph1(+)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 Ph1(+)ALL are consistently $bcr(-)$, whereas adults are heterogeneous

### Table 2 Breakpoint locations of Ph1(+)-ALL patients

<table>
<thead>
<tr>
<th>Zone</th>
<th>Patient*</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>1, 4, 9</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The other four patients, numbers, 2, 5, 6, and 7, did not have any rearrangements within the $bcr$ region, based on finding only germ-line restriction fragments on Southern blots with the four probes shown in Fig. 1, and multiple restriction enzymes.

### Table 3 Summary of published data on Ph1(+)-ALL

The patient totals do not tally with each other because the studies did not examine each patient for the presence of abnormal mRNA, protein, and also restriction fragments, but rather only some of these characteristics in each patient. The following conventions are used in this table: $Age$ is defined as adult (A) = age greater than or equal to 18 years, child (Ch) = age less than 18 years; $ber(+)$/– = with or without a breakpoint within the $ber$ region, respectively; $p185(+)$/– = with or without an abnormal $abl$-related protein measuring approximately 185,000, respectively; Abnormal mRNA +/– = with or without an abnormal $abl$-related mRNA, respectively.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age</th>
<th>$bcr(+)$</th>
<th>$bcr(-)$</th>
<th>$p185$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erikson et al. (26)</td>
<td>A</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clark et al. (27)</td>
<td>Ch</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DeKlein et al. (33)</td>
<td>A</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Kurzrock et al. (34)</td>
<td>Ch</td>
<td>3</td>
<td>1 (6.5 kilobases)</td>
<td></td>
</tr>
<tr>
<td>Chan et al. (35)</td>
<td>Ch</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>A</td>
<td>10</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ch</td>
<td>0</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

Including the present study A 15 12 2

### References

MOLECULAR HETEROGENEITY


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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