CONCISE REPORT

Variable Breakpoints on the Philadelphia Chromosome in Chronic Myelogenous Leukemia

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The abl oncogene is translocated from chromosome 9 to 22 in the creation of the Philadelphia (Ph') chromosome. This article describes new translocation breakpoints identified in two patients with chronic myelogenous leukemia using Southern blotting and cloned human DNA probes from chromosome 9. The translocation breakpoints on chromosome 9 in both of these patients lie closer to the human cellular abl (c-abl) gene, and the chromosome 22 breakpoints are distributed more widely than previously reported. These data help to define more clearly the chromosomal span of the breakpoints and indicate that some translocations include very little chromosome 9 sequence located 5' to the c-abl gene.

The Philadelphia chromosome (Ph') in chronic myelogenous leukemia (CML) is the first described human chromosomal abnormality consistently associated with a malignancy.1 The Ph' chromosome results from a reciprocal translocation between chromosomes 9 and 22.2,3 During this translocation, the abl oncogene moves from chromosome 9 to the 22q- chromosome (Ph'), and the sis oncogene moves from chromosome 22 to the 9q+ chromosome.4 The sis oncogene is not close to the translocation breakpoint, and there is no clear evidence implicating it in the pathogenesis of CML. The possibility that the translocation of the abl oncogene has a role in the development of CML has focused attention on the molecular details of this genomic rearrangement.

To date, studies have shown that the region homologous to the viral abl (v-abl) gene is adjacent to the chromosome 9 breakpoint.5 Though the entire c-abl locus has not been mapped, the term c-abl will be used in this article to denote the v-abl homologous region. The location of the breakpoint on chromosome 9 has been reported to be quite variable; the closest breakpoint previously reported is ~15 kilobases (kb) 5' to the end of the v-abl homologous region, while most of the breakpoints are apparently >40 kb from the c-abl locus.5,6

The reported breakpoints on chromosome 22 occur within a much more limited region. The initial report suggested that the breakpoints were located within a 5-kb BglII-BgllII fragment. This region was then extended to 6 kb, but there were still some patients whose breakpoints were not seen.5 We now report studies of the Ph' breakpoints in CML patients which demonstrate that their locations are more variable than previously noted, but which suggest a minimum amount of translocated c-abl gene needed to form a Ph' chromosome.

MATERIALS AND METHODS

Techniques of Southern blotting, preparation of recombinant human DNA libraries, and isolation of cloned fragments of human DNA are as described in previous publications from this laboratory.7

RESULTS

Probes from the region 5' to c-abl were used to screen Southern blots of DNA from 15 CML patients (Fig 1). Using several different enzymes, two patients had abnormal fragments detected by the HindIII–EcoRI probe (Fig 2). Comparison with the known restriction sites on chromosome 9 indicates the presence of a new sequence at the 3' end of the fragments. The 5' part of each abnormal fragment is from chromosome 9 (Fig 3). The 3' part of each fragment matches the published restriction map of chromosome 226 (Fig 4). One of the abnormal fragments has been cloned, and the 3' part identifies chromosome 22 fragments on Southern blots. Therefore, these abnormal fragments are the junction fragments from the 9q+ chromosomes (the reciprocal member of the translocation creating the Ph' chromosome).

When compared to the known restriction sites, the breakpoints of these translocations on chromosome 9 are 4 and 6 kb 5' to the c-abl locus, respectively. Similarly, by aligning the new fragments with the chromosome 22 restriction map, it is possible to predict the approximate breakpoint on chromosome 22. The breakpoint for the patient F.P. falls within the BglII–BamHI fragment initially identified as containing the Ph' breakpoint. The restriction data for T.H. are consistent with a breakpoint either 5' or 3' to the BglII–BamHI fragment, but not within it.

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DISCUSSION

The precise way in which cellular oncogenes participate in tumorigenesis remains undefined. The studies described here provide more evidence that the abl oncogene is activated in the translocation which creates the Ph\(^1\) chromosome. Defining the consistent molecular alterations in c-abl may help clarify the precise role of c-abl in malignant transformation. One of the most important questions regarding the translocation is: What sequences from chromosomes 9 and 22 are required on the Ph\(^1\) chromosome in CML? Prior to this report, the closest known chromosome 9 breakpoint was 15 kb 5' to the v-abl homologous region.\(^2\) The results presented here show that in fact as little as 4 kb of chromosome 9 sequence located 5' to the v-abl homologous region can be associated with CML. This implies that the translocation of c-abl sequence, which is homologous to v-abl, is sufficient for a role in CML.

Originally, the breakpoints on chromosome 22 were reported to be localized to a very small region.\(^3\) The restriction mapping data presented here suggest that the chromosome 22 breakpoints are more heterogeneous than previously recognized. The data from patient T.H. determined to date suggest that the region on chromosome 22 affected by the translocation may be larger than 6 kb. It will be necessary to analyze a considerably larger number of patients before concluding how large a variation there is in the location of chromosome 22 breakpoints.

Further analyses of the breakpoints on chromosomes 9 and 22 should provide more structural information about the permutations of the abl gene in CML. These structural alterations are particularly exciting in light of the new larger abl transcript present in CML.\(^7,9\) and of the abnormal new protein which is precipitated by v-abl antibodies and has a tyrosine kinase activity missing in the normal c-abl protein.\(^10\) The involved regions on the 22q- chromosome may be extremely important since they may be involved in the generation of the new larger c-abl RNA transcript, and may be translated into components of the new larger protein. The manner in which a relatively homogeneous sized novel c-abl containing RNA is consistently generated despite the great variations in the breakpoints in different CML patients remains to be elucidated. The pathophysiology of the abl

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**Fig 1.** Southern blots of DNA from normal controls and two CML patients, F.P. and T.H. The HindIII/EcoRI fragment located 5' to c-abl in Fig 1 was used as probe. Lanes 2 and 4 contain DNA from patient F.P., digested with the restriction enzymes XbaI and HindIII, respectively. Lanes 6 and 8 contain DNA from patient T.H., digested with KpnI and XbaI, respectively. Lanes 1, 3, 5, and 7 contain normal control DNA digested with the same enzyme as the adjacent patient sample. Patient F.P.: In lane 2, there is a new XbaI band measuring 6.8 kb in addition to the normal 5.8-kb band. In lane 4, there is a new 5-kb HindIII band in addition to the normal 5.8-kb band. Patient T.H.: Lane 6 shows a new 6-kb KpnI band in addition to the normal 5.4-kb band. Lane 8 shows a new 15-kb XbaI band in addition to the normal 5.8-kb band.

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**Fig 2.** Summary of restriction data for CML patients F.P. and T.H. The top line repeats the restriction map 5' to c-abl, as shown in Fig 1. Patient F.P.: Abnormal restriction fragments were identified using the restriction enzymes BamHI, BglII, KpnI, XbaI, and HindIII. The abnormal fragments are diagrammed to the right of the enzyme names. The numbers indicate the size (in kb) of the abnormal fragments. The dashed line indicates the portion of the fragments that does not conform to the restriction map of chromosome 9. The approximate location of the translocation breakpoints between chromosomes 9 and 22 is indicated by the crosshatched box on the line below the HindIII fragment. Patient T.H.: Abnormal fragments were identified with the enzymes BamHI, KpnI, and XbaI; sizes (in kb) for the abnormal fragments are indicated. The dashed line indicates the part of the fragment not from chromosome 9. The approximate location of the chromosome 9–22 translocation breakpoint is indicated by the crosshatched box on the lower line.

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**Fig 3.** Southern blots of DNA from normal controls and two CML patients, F.P. and T.H. The HindIII/EcoRI fragment located 5' to c-abl in Fig 1 was used as probe. Lanes 2 and 4 contain DNA from patient F.P., digested with the restriction enzymes XbaI and HindIII, respectively. Lanes 6 and 8 contain DNA from patient T.H., digested with KpnI and XbaI, respectively. Lanes 1, 3, 5, and 7 contain normal control DNA digested with the same enzyme as the adjacent patient sample. Patient F.P.: In lane 2, there is a new XbaI band measuring 6.8 kb in addition to the normal 5.8-kb band. In lane 4, there is a new 5-kb HindIII band in addition to the normal 5.8-kb band. Patient T.H.: Lane 6 shows a new 6-kb KpnI band in addition to the normal 5.4-kb band. Lane 8 shows a new 15-kb XbaI band in addition to the normal 5.8-kb band.

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**Fig 4.** Southern blots of DNA from normal controls and two CML patients, F.P. and T.H. The HindIII/EcoRI fragment located 5' to c-abl in Fig 1 was used as probe. Lanes 2 and 4 contain DNA from patient F.P., digested with the restriction enzymes XbaI and HindIII, respectively. Lanes 6 and 8 contain DNA from patient T.H., digested with KpnI and XbaI, respectively. Lanes 1, 3, 5, and 7 contain normal control DNA digested with the same enzyme as the adjacent patient sample. Patient F.P.: In lane 2, there is a new XbaI band measuring 6.8 kb in addition to the normal 5.8-kb band. In lane 4, there is a new 5-kb HindIII band in addition to the normal 5.8-kb band. Patient T.H.: Lane 6 shows a new 6-kb KpnI band in addition to the normal 5.4-kb band. Lane 8 shows a new 15-kb XbaI band in addition to the normal 5.8-kb band.
gene in CML may provide us with further insights into the development of malignancies, and also into the normal control of cell growth and development.

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REFERENCES
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