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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^1) 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.

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THE PHILADELPHIA chromosome (Ph^1) in chronic myelogenous leukemia (CML) is the first described human chromosomal abnormality consistently associated with a malignancy.[1] The Ph^1 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^1), 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 *Bgl*II–*Bgl*II fragment. This region was then extended to 6 kb, but there were still some patients whose breakpoints were not seen.[6] We now report studies of the Ph^1 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^1 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 *Hind*III–*Eco*RI probe (Fig 2). Comparison with the known restriction sites on chromosome 9[5] 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 22[6] (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^1 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 *Bgl*II–*Bam*HI fragment initially identified as containing the Ph^1 breakpoint. The restriction data for T.H. are consistent with a breakpoint either 5′or 3′ to the *Bgl*II–*Bam*HI fragment, but not within it.

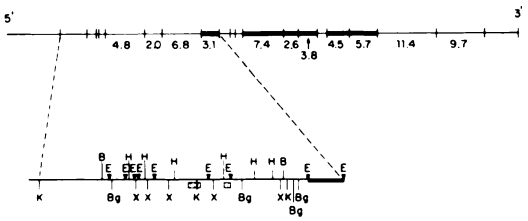

Fig 1. Restriction map of the *v-abl* homologous region on chromosome 9. The vertical marks on the upper line indicate *Eco*RI sites. The thick black segments are the *Eco*RI fragments that hybridize to a *v-abl* probe. The enlarged area shows the DNA fragments used as probes on Southern blots. The more 5′ probe is an *Sst*I fragment; the more 3′ probe is a *Hind*III/*Eco*RI fragment. E, *Eco*RI; H, *Hind*III; B, *Bam*HI; Bg, *Bgl*II; K, *Kpn*I; X, *Xba*I.

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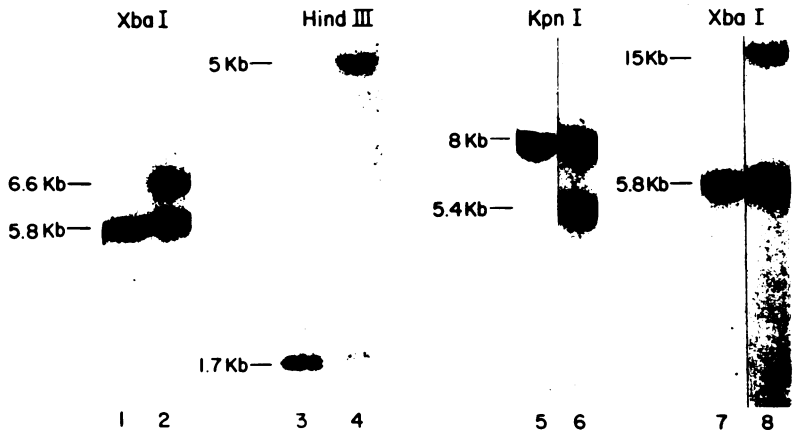

Fig 2. Southern blots of DNA from normal controls and two CML patients, F.P. and T.H. The *Hind*III/*Eco*RI fragment located 5′ to *c-abl* in Fig 1 was used as probe. Lanes 2 and 4 contain DNA from patient F.P., digested with the restriction enzymes *Xba*I and *Hind*III, respectively. Lanes 6 and 8 contain DNA from patient T.H., digested with *Kpn*I and *Xba*I, 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 *Xba*I band measuring 6.6 kb in addition to the normal 5.8-kb band. In lane 4, there is a new 5-kb *Hind*III band in addition to the normal 1.7-kb band. Patient T.H.: Lane 6 shows a new 6-kb *Kpn*I band in addition to the normal 5.4-kb band. Lane 8 shows a new 15-kb *Xba*I band in addition to the normal 5.8-kb band.

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.[5] 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.

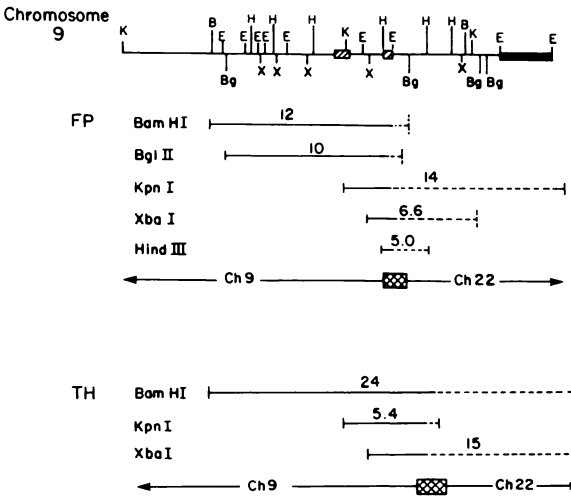

Fig 3. Summary of restriction data for CML patients F.P. and T.H. The top line repeats the restriction map 5′ to *c-abl*, as shown in Fig 1. Patient F.P.: Abnormal restriction fragments were identified using the restriction enzymes *Bam*HI, *Bgl*II, *Kpn*I, *Xba*I, and *Hind*III. The abnormal fragments 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 *Hind*III fragment. Patient T.H.: Abnormal fragments were identified with the enzymes *Bam*HI, *Kpn*I, and *Xba*I; 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.

Originally, the breakpoints on chromosome 22 were reported to be localized to a very small region.[6] 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*

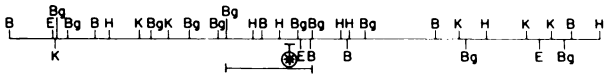

Fig 4. Restriction map of the region on chromosome 22 previously described as containing the Ph[1] chromosome breakpoints. The *Bgl*II/*Bam*HI fragment includes the previously published breakpoints, and the breakpoint for patient F.P. (•). The breakpoint for patient T.H. could be either 5′ or 3′ to this fragment.

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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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