Increased Expression of a Novel *c-abl*–Related RNA in K562 Cells

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The *c-abl* locus is translocated from chromosome 9 to chromosome 22 in chronic myelogenous leukemia (CML), creating the Philadelphia chromosome (22q-, Ph[1]), one of the most consistent chromosomal abnormalities found in human hematologic malignancy.[1,2] The K562 cell line is a human cell line originally derived from a patient with CML.[3] We have isolated cloned human *c-abl* probes to analyze the organization and expression of *abl* genes in patients with CML and in K562 cells. With these probes, we confirm the amplification of *abl* genes in K562 cells.[4–6] In addition, we demonstrate the presence of increased amounts of a novel RNA species hybridizing to a *c-abl* probe in K562 cells. This same large RNA species is present in addition to two normal transcripts in the leukemic cells of patients with CML. These results provide evidence that the *c-abl* locus is abnormally expressed in CML.

THERE IS GENERAL agreement on the importance of viral oncogenes (*v-onc*) in causing malignancy.[7] It is less obvious how cellular oncogenes (*c-onc*, or proto-oncogenes) are active in tumorigenesis, but several lines of evidence point toward their involvement. The recurrent localization of *c-onc* at the site of chromosomal abnormalities characteristic of specific malignancies implies a direct involvement of the *c-onc* sequences.[8] In Burkitt's lymphoma and murine plasmacytoma, the *c-myc* locus undergoes translocation, rearrangement, and alteration of RNA transcription.[9] Amplification of sequences related to *c-myc* has been found in fresh tumor tissue as well as cell lines from neuroblastoma.[10,11] Single nucleotide changes have conferred the ability to transform NIH3T3 cells upon members of the *ras* family.[12] Increased expression of a *c-onc* following adjacent integration of a retroviral long terminal repeat has been reported in a number of different tumors.[13,14]

One of the earliest described human chromosomal abnormalities associated with a malignancy is the Ph[1] chromosome in CML.[8] Originally considered to be a movement of material from chromosome 22 to chromosome 9, it has since been shown to be a reciprocal translocation that involves two oncogenes. The *c-abl* locus moves from chromosome 9 to chromosome 22, while the *c-sis* locus makes the reverse move.[1,15] The chromosome 9 fragment containing *c-abl* appears always to join chromosome 22 in a specific 4- to 6-kilobase (kb) region.[16] By analyzing the occasional complex translocations (involving more than two chromosomes) that occur in patients with CML, Rowley concluded that the movement of material from 9 to 22 was the consistent finding in CML.[17] This would suggest that the *abl* locus is important in the disease process.

Amplification and rearrangement of the *abl* locus in K562 cells has recently been reported.[4] In addition, an *abl*-specific mRNA 8 kb in size has also recently been described.[18] Both of these studies used *v-abl* probes with limited homology to *c-abl* sequences. We report here studies of *c-abl* gene organization and expression using cloned *c-abl* probes we have isolated. We find that the amplification of the *c-abl* locus extends at least 11.4 kb 3′ to the *v-abl* homologous region. We also find a reproducible 8- to 9-kb mRNA species present in large amounts in K562 cells, and in lesser amounts in CML patients using this specific *c-abl* probe.

MATERIALS AND METHODS

Heparinized peripheral blood samples were obtained and buffy coats were removed after centrifugation at 1,800 rpm for 20 minutes at 4 °C. RNA was prepared by a modification of the Auffray and Rougeon procedure.[19] The buffy coat was dissolved in 3 mol/L LiCl/6 mol/L urea/0.05% sodium dodecyl sulfate/50 mmol/L Na acetate, mixed at room temperature for five to ten minutes, and then homogenized in a Polytron (Brinkman Instruments, Westbury, NY) after the addition of 0.5 mL of Antifoam A (Sigma, St Louis). The homogenate was stored overnight at 4 °C and then collected by centrifugation at 10 K rpm for 30 minutes at 2 °C. The pellet was resuspended in 3 mol/L LiCl/6 mol/L urea/50 mmol/L Na acetate, and then centrifuged for 15 minutes at 10 K rpm. This pellet was resuspended in 5 mL 100 mmol/L Tris pH8/5 mmol/L EDTA/0.2% sodium dodecyl sulfate (SDS), and then extracted with 5 mL chloroform ($CHCl_3$)/isoamyl alcohol/phenol (48:2:50). The extraction was centrifuged at 7 K rpm for ten minutes, after which the aqueous phase was added to a separate tube containing 5 mL $CHCl_3$. The interface was reextracted with 5 mL 100 mmol/L Tris pH 9, the aqueous phases were combined and extracted with $CHCl_3$, and the aqueous phase was then precipitated overnight. Oligo dT cellulose was used to enrich for polyadenylate-rich RNA as described.[20] DNA was processed as described.[21] To isolate nucleic acid from K562 cells, the washed cells were pelleted, resuspended in 20 mmol/L Tris pH 7.5/10 mmol/L EDTA/1% Sarkosyl, and processed as described.[22] Southern and Northern blots were performed as described.[23]

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RESULTS

To obtain a *c-abl* clone with which to study the structure and expression of the human *c-abl* locus, we screened a normal human library with a *v-abl* probe at low hybridization stringency.[24,25] This produced a series of clones that cover the regions homologous to *v-abl* (Fig 1). Using the initial group of *c-abl* clones, it was possible to "walk" in both 5′ and 3′ directions along the chromosome. Using the resulting probes with Southern blots of normal human DNA and K562 cell DNA, we found that the *abl* locus was amplified three- to fivefold in K562 cells (Fig 2). The unique human *c-abl* probes also revealed that the unit of amplification in K562 extended at least 11 kb in the 3′ direction from the *v-abl* homologous region. High-stringency hybridization with the cloned human *c-abl* probes has not indicated any DNA rearrangement within the *v-abl* homologous region or the first 11 kb located 3′ to this region. A 4.2-kb fragment in a *Pvu*II digest of K562 DNA that was not present in normal DNA has recently been reported.[4] The reported band hybridizes intensely to a *v-abl* probe in conditions of low stringency and appears to be amplified to the same extent as the other *v-abl* homologous bands in K562 cell DNA.[4] We can identify this band in *Pvu*II digests of K562, using the *v-abl* probe. However, we also find this band in normal control human DNA samples and do not find this band amplified in K562 cells (Fig 3).

The human *c-abl* probes have also been used to examine RNA from K562 cells and CML patients. Dot blots have shown an increased intensity of hybridization in K562 compared with other cell lines and with normal human WBCs (data not shown). When Northern blots are hybridized to a *c-abl* probe, the K562 RNA shows three bands measuring approximately 6, 7, and 9 kb (Fig 4). The 4-kb band seen in some lanes is probably an artifact since it appears intermittently, and comigrates with the 28S ribosomal RNA band (as in lane B, Fig 4). The band measuring 9 kb is much more intense in K562 RNA than the other bands.

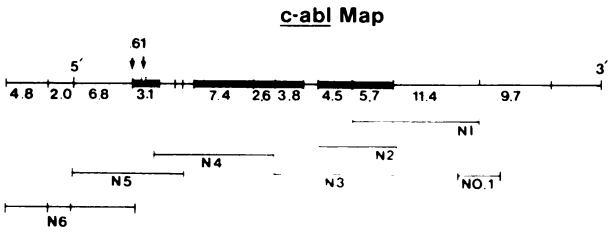

Fig 1. Restriction map of the human *abl* locus. Vertical lines indicate *Eco*RI sites. Numerals indicate approximate size in kilobases. ▽, *Bam*HI site. Vertical arrows indicate the *Eco*RI–*Bam*HI fragment used as probe in Fig 3. Clone N-0.1 is a *Hind*III fragment.[26] Clones N-1 to N-6 are *Eco*RI fragments. The darker black line indicates the fragments that hybridize to *v-abl*.

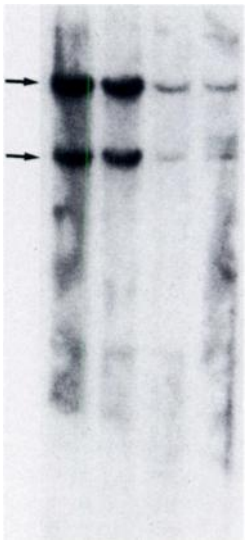

Fig 2. Southern blot using clone N1 (Fig 1) as probe. Each lane contains 15 μg of *Eco*RI-digested DNA. Lane A, uninduced K562 cells; lane B, K562 cells induced with hemin; lanes C and D, two different patients with CML. The upper band is approximately 11 kb, the lower band is 5.7 kb.

RNA from one patient with chronic lymphocytic leukemia (CLL) and from one patient with hairy cell leukemia showed three bands of equal intensity migrating with the 4-, 6-, and 7-kb K562 bands, but did not show the 9-kb band (data not shown). We have examined the RNA of seven patients with CML and consistently see the 6-, 7-, and 9-kb bands in high-stringency hybridizations with a cloned human *c-abl* probe (Fig 4). We have not seen a CML patient with the 9-kb RNA band who does not also have the 6-kb and 7-kb bands, a situation reported by others.[18] The

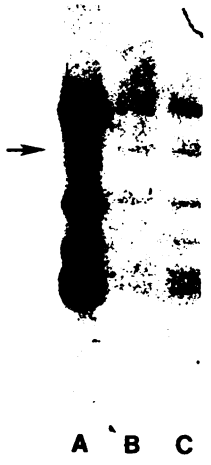

Fig 3. Southern blot of *Pvu*II-digested human DNA, probed with *v-abl*. Lane A, K562 cells; lanes B and C, normal human DNA. Arrow indicates the 4.2-kb band mentioned in text.

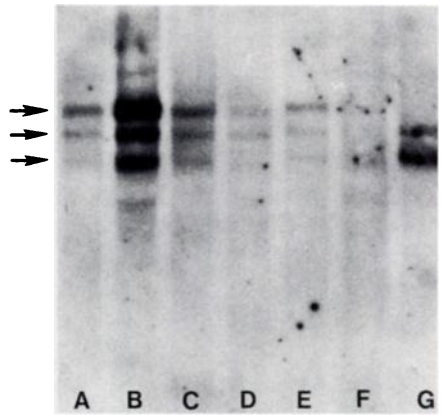

Fig 4. Northern blot of poly A-selected total cellular RNA. Each lane contains 20 μg of pA-selected RNA run on a 2.2 mmol/L formaldehyde gel[23] and blotted to nitrocellulose. The probe is a nick-translated *Eco*RI–*Bam*I fragment shown in Fig 1. The arrows indicate bands of approximately 9, 7, and 6 kb. Lanes A, C–E, patients with CML; lane B, K562; lane G, HSB2. Sample in lane F was inadequate.

HSB2 human T cell leukemia cell line shows only 6-kb and 7-kb *abl* RNA bands, similar to our non-CML samples.

DISCUSSION

Studies with cloned human *c-abl* probes used at high stringency on Southern and Northern blots define more precisely the structure and expression of the *abl* locus in human hematopoietic cells. Our results confirm the previously reported amplification of *c-abl* genes in K562 cells,[4] and demonstrate that it extends at least 11 kb 3′ to the *v-abl* homologous region. We have not observed the reported rearrangement of the *v-abl* homologous region in K562 cells.[4] There are at least two possible explanations for this discrepancy. K562 undergoes karyotypic alteration in culture. One possibility is that the 4.2-kb DNA fragment is part of the amplified unit in some K562 cells, but not in our line. Another possibility is that the fragment is from another locus, possibly an *abl* pseudogene. The initial description of the *c-abl* locus describes an *Eco*RI band approximately 4 kb in size that hybridized to a *v-abl* probe but did not seem to be included in the *c-abl* locus.[26] If this band represents a different but *abl*-related locus, it will be most interesting to determine why this *abl*-related locus is amplified in some but not all K562 cells.

We also report a novel RNA species hybridizing to *c-abl* at high stringency both in K562 cells and in patients with CML. The finding of this new and more intensely hybridizing RNA species provides additional evidence that the *abl* gene is not only amplified but also abnormally expressed in K562 cells. In our hands, the *v-abl* probe has not been adequate to detect *abl*-related RNA sequences consistently and easily on Northern blots. The *c-abl* probe easily detects two normal *abl* transcripts, measuring approximately 6 and 7 kb, in addition to the novel 9-kb band in CML and K562 cells. A previous report using a *v-abl* probe in a low-stringency hybridization did not find the 6- and 7-kb *abl* transcripts in all the CML patients examined.[18] Our *c-abl* probe clearly demonstrates that the 6- and 7-kb *abl* transcripts are present in CML along with the new 9-kb transcript. Furthermore, in K562 cells, there is increased expression of all three transcripts, in comparison to the patient samples.

The DNA sequences responsible for the novel 9-kb *abl* transcript must still be elucidated. The new 9-kb *abl* transcript may be the product of the rearranged locus, including *abl* sequences. The presence of this new transcript, along with the recent report that the *c-abl* locus translocates into a specific small region of chromosome 22 to create the Ph[1] chromosome, suggests that the *abl* locus fulfills some central function in the pathogenesis of CML. Further investigation should reveal precisely how the new transcript relates to the rearranged *abl* locus in CML and K562 cells. We do not yet know whether this 9-kb *abl* transcript is always associated with a Ph[1] chromosome, although to date the association is strong. Alternatively, the 9-kb RNA could result from aberrant transcription of the normal locus. If the 9-kb transcript and the specific region for the *c-abl* translocation are always present with the Ph[1] chromosome, we will not only have acquired new diagnostic aids, but also further insights into the normal and abnormal biology of *c-abl*.

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