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An 8-kilobase *abl* RNA transcript in chronic myelogenous leukemia

(granulocytes/cancer/translocation/oncogene activation/Philadelphia chromosome)

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ABSTRACT **Chronic myelogenous leukemia (CML) is a clonal hematologic malignancy characterized by a reciprocal translocation between chromosomes 9 and 22 [t(9;22)] in >90% of cases. This translocation results in a short chromosome 22, termed the Philadelphia (Ph^1 or $22q^-$) chromosome. Recently, the cellular oncogenes *abl* and *sis* were mapped to human chromosomes 9 and 22, respectively. Moreover, *abl* was shown to be translocated from chromosome 9 to 22 and *sis* from chromosome 22 to 9 in CML patients with t(9;22). These findings raised the possibility that one or both of these oncogenes is activated and directly involved in the development of the disease. We analyzed expression of the *abl* and *sis* oncogenes in leukemic cells from CML patients with t(9;22). We found that *sis* is not expressed but that *abl* is transcribed into an 8-kilobase RNA. This *abl* RNA is also present in two leukemic cell lines (EM2 and K562), which were derived from CML patients and contain the t(9;22). This 8-kilobase RNA is not detected in normal cells, in other human leukemias without t(9;22), or in human cell lines that lack t(9;22). The consistent presence of this *abl* RNA transcript in CML with t(9;22) suggests that it is a consequence of *abl* translocation and that it plays a role in the development of this leukemia.**

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder in which the neoplastic transformation of a stem cell results in the proliferation and accumulation of myeloid cells and their progenitors. Clinically, the disease is divided into two phases, a chronic phase of 3–4 yr duration followed by an acute phase (blast crisis) of 3–6 months (for review, see ref. 1). In some patients, there is a transitional accelerated phase. During the chronic phase, the neoplastic clone represents the majority of the replicating myeloid cells. These cells mature normally and respond to normal myelopoietic regulatory factors. The principal abnormality during the chronic phase appears to be an increase in the stem cell compartment committed to myeloid differentiation. In the acute phase, in contrast, the leukemic cells lose their ability to differentiate and mature normally; response to regulatory factors of myelopoiesis is typically lost.

CML is relatively unusual among human cancers in that a specific chromosome abnormality; the Philadelphia chromosome (Ph^1) ($22q^-$) is present in 90%–95% of patients (2). The Ph^1 chromosome results, in most instances, from a balanced reciprocal translocation between chromosomes 9 and 22 [t(9;22)] (3). The translocation points are very specific, involving bands q34.1 and q11.21 on chromosomes 9 and 22, respectively (3–5). Five to ten percent of the patients have a variant Ph^1 chromosome. Variant Ph^1 chromosomes may be simple or complex. Complex variant translocations typically involve chromosomes 9 and 22 and a third chromosome. Relatively rare (<2%) simple variant translocations have also been reported that appear to involve chromosome 22 and a second chromosome other than 9 (6, 7). Recently, detailed analyses using *in situ* hybridization have shown that chromosome 9 is usually involved in these simple variant translocations, making them genetically equivalent to the complex variant translocations (8). In considering all of these permutations, it is evident that most cases of typical CML involve chromosome 9 as well as chromosome 22.

In typical cases of CML with the t(9;22), there has been considerable controversy as to whether the transfer of genetic information from chromosome 9 to 22 or from chromosome 22 to 9 is the more fundamental abnormality (7, 9–11). Recent data suggest that the translocation of genetic information from chromosome 9 to 22 is the more consistent abnormality (9, 10). This is further supported by data from *in situ* hybridization, indicating variable recipient chromosomes for the translocated fragment of chromosome 22 (12) versus a consistent translocation of the fragment of chromosome 9 to chromosome 22.

Five percent of chronic myeloproliferative disorders resembling CML lack a detectable Ph^1 chromosome, and involvement of chromosome 9 has not been reported. These Ph^1-negative leukemias probably represent a disorder(s) distinct from CML with different histologic and clinical features and briefer survival.

Recently, there has been considerable interest in a group of cellular genes, termed oncogenes, which are the progenitors of the malignant information in the genomes of RNA tumor viruses. Some of these genes have been shown to be activated in certain spontaneous malignancies, either by point mutations or by DNA rearrangements, including translocations (for reviews, see refs. 13 and 14). Approximately 15 oncogenes have been mapped to specific human chromosomes, and several are localized to specific bands (for review, see ref. 5). Chromosomes 9 and 22, which are involved in the formation of the Ph^1 chromosome, carry the oncogenes *abl* and *sis*, respectively (15–17). Recently, *abl* was shown to be translocated from chromosome 9 to chromosome 22 (18), and *sis* was shown to be translocated from chromosome 22 to chromosome 9 in cases of CML with the t(9;22) (19). These findings raised the possibility that *sis* and/or *abl* might be involved in the development of CML. In a preliminary analysis (20), we found that leukemic cells from several CML patients contained an 8-kilobase (kb) *abl* RNA. We now extend this analysis to a large number of patients with a variety of leukemias and to human hematopoietic cell lines with and without the Ph^1 chromosome. In addition, we have examined *sis* expression in these samples to determine its possible involvement in the leukemic process.

MATERIALS AND METHODS

Isolation of RNA. Heparinized peripheral blood (20–50 ml) or bone marrow was collected and allowed to sediment at 1 × *g* for 30–60 min. The buffy coat was removed, centrifuged

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Abbreviations: CML, chronic myelogenous leukemia; AML, acute myeloid leukemia; Ph^1, Philadelphia chromosome; kb, kilobase(s); NaCl/Cit, 0.15 M NaCl/0.015 M Na citrate; Mops, 4-morpholinepropanesulfonic acid.

at 2000 × *g* for 5 min, and the cell pellet was recovered. Cells grown in tissue culture were harvested in the logarithmic phase and pelleted by centrifugation. RNA was extracted by the lithium chloride method (21) and selected twice for poly(A)-containing species on an oligo(dT)-cellulose column. Despite rapid processing of cell preparations from patients, approximately one-half of the samples yielded degraded RNAs that were not suitable for further analysis.

Electrophoresis and Hybridization. Aliquots (10 μg) of twice poly(A)-selected RNA obtained from 3×10^8 to 1×10^9 cells were precipitated with ethyl alcohol and redissolved in 25 μl of a solution containing 50% formamide/7% formaldehyde/4-morpholinepropanesulfonic acid (Mops) buffer (20 mM Mops, pH 7.0/5 mM Na acetate/1 mM EDTA), and heated to 60°C for 5 min. Samples were cooled on ice, and bromophenol blue dye and glycerol were added. The samples were then electrophoresed on a 1.35% agarose (SeaKem Laboratories, Rockland, ME) gel containing Mops buffer and 7% formaldehyde and overlayed with Mops buffer. After electrophoresis at 100 V for 5–7 hr, the gel was equilibrated in 20× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate, pH 6.8) for 45 min and blotted with 10× NaCl/Cit onto nitrocellulose paper (Schleicher & Schüll). After fixation in a vacuum oven at 80°C for 2 hr, the sheet was hybridized to 2.5×10^6 cpm per ml of a specific probe. v-*abl*-specific sequence was cleaved from the pABlsub9 plasmid (22) or from a plasmid containing the genome of Abelson leukemia virus (23) and then nick-translated to a specific activity of 2×10^8 cpm per μg of DNA. v-*sis*-specific probe was prepared by nick-translation of a *Sac* I/*Xba* I 1.0-kilobase-pair DNA fragment cleaved from a pBR322 plasmid containing the integrated genome of simian sarcoma virus (24). After hybridization at 42°C for 48 hr in a solution containing 50% formamide/5× NaCl/Cit/0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/20 mM $NaPO_4$, pH 6.8/tRNA (100 μg/ml), the sheet was washed for 90 min at 50°C in a solution of 0.1× NaCl/Cit/0.1% $NaDodSO_4$, and autoradiographed with screens for 1–10 days at −70°C.

RESULTS

Poly(A)-containing RNA was selected from cellular RNA of normal bone marrow, electrophoresed on agarose gel, blotted to nitrocellulose, and analyzed for *abl* transcripts by hybridization to *abl*-specific radiolabeled probe. This probe was composed of a segment of Abelson leukemia virus genome (v-*abl*) shown to be homologous to mouse *abl* (23). Two major transcripts of 6 and 7 kb were detected (Fig. 1, lane a). These results are in general agreement with previous reports of *abl* transcripts in human hemopoietic cells (26, 27). A diffuse background in the region of 6–9 kb was apparent in this and in several other samples. This could be due to nonspecific hybridization, precursors of *abl* RNA, or degradation products. A faint band of 5 kb, which was occasionally observed, is probably due to nonspecific hybridization with 28S ribosomal RNA. When poly(A)-containing RNA from peripheral blood cells from a CML patient in acute phase was similarly analyzed, we detected a transcript of 8 kb, which appeared to replace most or all of the 7- and 6-kb species (lane b). A similar pattern was seen in bone marrow cells from this patient, obtained and cryopreserved when the patient was in chronic phase (not shown). To verify the identity of the 8-kb species as *abl* RNA, we electrophoresed samples of RNA from a CML patient in chronic phase and analyzed it for hybridization to different portions of the v-*abl* gene. The 8-kb transcript hybridized to the *Hin*cII/*Ava* I segment derived from the 5′ region of v-*abl*, to the *Bgl* II/*Sal* I fragment corresponding to the central section of v-*abl*, and to the *Sal* I/*Hin*dIII DNA derived from the 3′ region of v-*abl* (lanes c–e). Hybridization to the 3′ probe was weak. Since

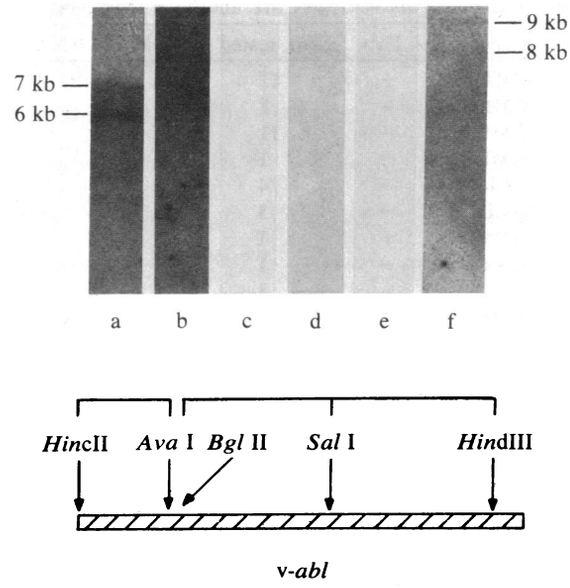

FIG. 1. *abl* RNA in normal bone marrow and in CML. Aliquots (10 μg) of normal bone marrow (lane a) and two CMLs (lanes b and f) were analyzed for *abl* transcripts by hybridization to a v-*abl* probe spanning sequences between the *Hin*cII and *Hin*dIII sites of v-*abl*. RNA aliquots (10 μg) from a third patient with CML were hybridized to the *Hin*cII/*Ava* I, *Bgl* II/*Sal* I/*Hin*dIII radiolabeled v-*abl* fragments (lanes c, d, and e, respectively). At the bottom of the figures is a partial map of v-*abl* DNA based on ref. 25 and the delineation of fragments used as hybridization probes.

the same probe also reacted poorly with the 6- and 7-kb normal *abl* transcripts (not shown), it is likely that the 3′ section of v-*abl* (a mouse gene) has relatively less homology with the corresponding human *abl* sequences. We next examined *abl* RNA of a second CML patient in blast crisis. In this instance, we observed an 8-kb RNA species as well as an additional species of 9 kb (lane f).

To determine the generality of these observations, we analyzed samples from 35 patients with a variety of leukemias and from two patients with normal bone marrow. Representative data are shown in Fig. 2. RNA from patients with CML and the t(9;22) translocation contained the 8-kb *abl* transcript (lanes b, d, f, and h). This species was not apparent in samples from HeLa cells (lane c), in cells from two patients with acute myelogenous leukemia (AML) (lanes a

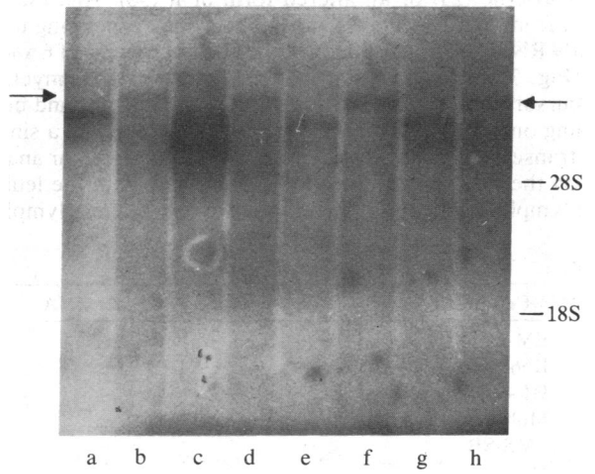

FIG. 2. *abl* RNA in CML, AML, and HeLa cells. Aliquots (10 μg) of RNA from cells of CMLs (lanes b, d, f, and h), AMLs (lanes a and e), Ph^1-negative CML (lane g), and HeLa cells (lane c) were analyzed for *abl* transcripts. Arrows point to 8-kb RNA.

Table 1. The 8-kb *abl* RNA in CML and other leukemias

Diagnosis	Ph[1]	No. tested	8-kb *abl* RNA
CML	+	12	11
CML*	−	1	0
AML	−	11	0
AML	?	1	1
CLL	−	4	0
ALL	−	3	0
AProL	−	1	0
CMoL	−	1	0
AUL	−	1	0
NBM	−	2	0

CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; AProL, acute prolymphocytic leukemia; CMoL, chronic monocytic leukemia; AUL, acute undifferentiated leukemia; NBM, normal bone marrow.
*"Juvenile" CML.

and e), and in cells from a patient with juvenile CML without t(9;22) (lane g). Results of the 37 analyses are summarized in Table 1. The 8-kb *abl* RNA transcript was detected in 11 of 12 patients with CML and the t(9;22) translocation but was not detected in one patient with juvenile CML without t(9;22). The 8-kb *abl* transcript was also detected in 1 of 12 patients with AML. Approximately 5% of individuals with AML have the t(9;22) translocation. Chromosome analyses were not carried out in this patient, so we are unable to determine whether he had the t(9;22). The 8-kb transcript was absent in the remaining 11 patients with AML and in 10 patients with a variety of other leukemias, including chronic lymphocytic leukemia, acute lymphocytic leukemia, prolymphocytic leukemia, chronic monocytic leukemia, and acute undifferentiated leukemia. The 8-kb species was also lacking in cells from two patients with normal bone marrow. A 9-kb *abl* RNA was detected together with the 8-kb species in 2 of 11 samples from patients with CML and t(9;22). In around one-half of the samples containing the 8-kb RNA, we could also see traces or small amounts of the 6- and 7-kb normal abl RNA species (a diffused hybridization background in this region of the gels made the detection of these RNAs more difficult).

Next, we investigated whether human cell lines containing the Ph[1] chromosome synthesized the altered *abl* transcript (Table 2). These included 5 hematopoietic cell lines with and without t(9;22) and 4 nonhematopoietic cell lines without t(9;22). The K562 cell line (28) is an erythroid–myeloid precursor line derived from a patient with CML. K562 has a Ph[1] chromosome (29) or an altered form of it (30). Analysis of RNA from K562 indicated a major band corresponding to 8-kb *abl* RNA and minor bands of the normal species of 6 and 7 kb (Fig. 3, lane a). Similar analyses of the human myeloid precursor line EM-2, derived from a CML patient and containing one or more Ph[1] chromosomes (31), showed a single *abl* transcript of 8 kb (lane b). We carried out a similar analysis on the human cell line SMS-SB derived from the leukemic lymphoblasts of a patient with pre-B-cell acute lymphoblastic leukemia (32). This cell line is Ph[1] negative and was recently shown (27) to contain the normal *abl* transcripts as well as additional species of *abl* RNA. Our analysis (Fig. 3, lane c) demonstrated the normal 6- and 7-kb *abl* transcripts as well as a 6.5-kb *abl* species. Neither 8-kb nor other species (27) of abl RNA were observed. Two other hematopoietic cell lines (HL-60 and Molt-4) which lack the Ph[1] chromosome and 4 Ph[1]-negative nonhematopoietic cell lines demonstrated the normal 6- and 7-kb *abl* transcripts but lacked the 8-kb *abl* transcript. EM-2, K562, SMS-SB, Molt-4, HL-60, and HeLa contain substantially more polyadenylylated *abl* RNA than fresh hematopoietic cells, both normal and leukemic.

Table 2. The 8-kb *abl* RNA in cell lines

Cell line	Ph[1]	8-kb *abl* RNA
EM-2	+	+
K562	+	+
HL-60	−	−
Molt-4	−	−
SMS-SB	−	−
HeLa	−	−
SV80	−	−
Fibroblasts*	−	−

*Two short-term primary cell lines.

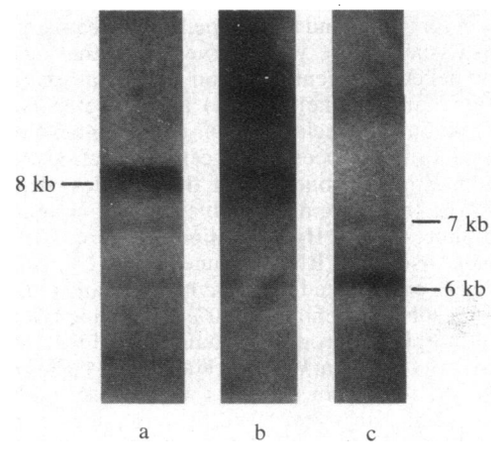

FIG. 3. The 8-kb *abl* RNA in two Ph[1]-positive cell lines. Aliquots (8 μg) of RNA from lines K562 (lane a), EM-2 (lane b), and SMS-SB (Ph[1]-negative line) (lane c) were examined for the presence of 8-kb *abl* RNA.

We next examined transcription of the *sis* gene in CML. RNA samples from four patients with CML and t(9;22), three patients with AML without t(9;22), and HeLa cells were tested by the RNA blot technique for hybridization to a v-*sis* probe composed of sequences of simian sarcoma viral genome homologous to *sis* (24). No discrete species of *sis* RNA could be detected in any of the samples (Fig. 4, lanes a–h). Preparation of RNA from normal rat kidney cells infected with simian sarcoma virus served as a positive control and showed multiple-sized transcripts of v-*sis* (Fig. 4, lane i). "Stripping" of the blot with methylmercury hydroxide followed by hybridization with v-*abl* probe revealed the expected pattern of *abl* transcripts (not shown).

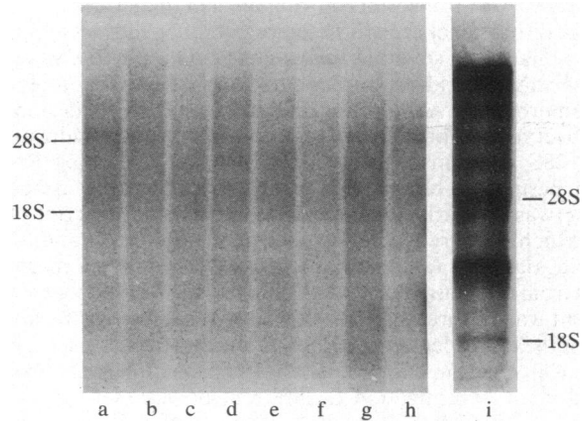

FIG. 4. *sis* RNA in CML, AML, and HeLa cells. Samples of RNA (15 μg) from CMLs (lanes b, d, f, and h), AMLs (lanes a, e, and g), HeLa cells (lane c), and normal rat kidney cells infected with simian sarcoma virus (lane i) were screened for *sis* RNA.

DISCUSSION

The major finding of this work is that an *abl* transcript of 8 kb is found in 11 of 12 patients with CML with the t(9;22) translocation. The 8-kb *abl* transcript was also found in two hematopoietic cell lines containing the Ph^1 chromosome. A single patient with AML also had this transcript, but it is unknown whether his cells had a Ph^1 chromosome. This RNA was not observed unassociated with t(9;22) in cells from 22 leukemia patients, including a case of Ph^1-negative CML, nor was it seen in seven human hematopoietic and nonhematopoietic cell lines that lacked the Ph^1 chromosome. Two samples of normal bone marrow also lacked the 8-kb RNA. The association between the t(9;22) translocation and the presence of the 8-kb *abl* transcript is highly significant ($P < 0.01$).

The strong correlation between the synthesis of the 8-kb transcript and the translocation of *abl* to chromosome 22 (18) suggests a causal association. The 8-kb transcript (Fig. 1) as well as the normal *abl* species (not shown) are homologous to probes from the 5′, central, and 3′ regions of v-*abl*. Therefore, it is likely that the new transcript contains much of the information of normal *abl* RNA. It is possible that the 8-kb *abl* RNA is due to a modified splicing pattern of a normal precursor. However, two lines of evidence suggest a possibility that the extra information in the 8-kb *abl* RNA originates from a region 5′ to the gene. First, in one case of CML, it was shown that the translocation placed the *abl* gene in a position adjacent to the breakpoint, with the 5′ region of the oncogene facing sequences of chromosome 22 (33). Second, the detection in cells from two CML patients of an additional *abl* species of 9 kb might suggest that the 8- and 9-kb *abl* RNAs are related to the 6- and 7-kb normal species, respectively, and that the former were derived by acquisition of the same sequence. Since the 6- and 7-kb human *abl* RNAs [by analogy with the corresponding mouse species (22)] presumably initiate at the same promoter but terminate at different poly(A) signals 1 kilobase pair apart, the 8- and 9-kb RNAs might terminate at the same sites as the normal species but initiate at a transcriptional promoter upstream of the normal promoter. Such a promoter could reside in chromosome 9 sequences or in chromosome 22 information behind the breakpoint (34). The initiation at a different promoter would probably be associated with a modified splicing pattern. Examples of altered transcriptional initiation, coupled to a different splicing pattern, have been described in other systems (35, 36).

In about one-half of the patients with CML with the 8-kb *abl* RNA, we could not detect the normal *abl* transcripts, although the leukemic cells in CML contain one intact chromosome 9. The absence of the 6- and 7-kb *abl* RNA in some patients with CML could be due to a selective rapid degradation of these species compared to the 8-kb transcript and/or the preferred transcription of the translocated *abl*. Another possibility is that transcription of the normal *abl* allele, and not the translocated allele, depends on a cellular factor that is in shortage in the malignant cells.

The formation of the *abl* transcript might be the critical factor in the increased committed myeloid stem cell compartment typical of the chronic phase of CML and/or the loss of differentiative capacity found in the acute phase of CML. The transcript might be translated into an altered protein, perhaps modified at the NH_2 terminus region. This region has been previously shown to be critical for the transforming activity of the v-*abl*-encoded protein (37). Moreover, if the 8-kb *abl* RNA represents a fusion transcript, then it is also possible that it encodes a fused protein.

The absence of *sis* transcripts in leukemic cells from patients with CML indicates that this oncogene is probably not activated by the t(9;22) translocation. These and other data, including the variability of the reciprocal chromosome to which *sis* is translocated, suggest that *sis* does not play a role in CML. This finding is in contrast to the situation in human sarcomas and gliomas, in which *sis* transcripts are readily detected (38). The recent identification of homology between *sis* and the gene coding for platelet-derived growth factor (39, 40), a factor that enhances growth of cells of mesenchymal origin, is compatible with the hypothesis that *sis* activation is involved in the development of sarcomas and gliomas in humans. Whether *sis* plays a role in the development of the myelofibrosis observed in some patients with CML is unknown.

The possibility raised by this study and others that the *abl* gene is directly involved in generation of CML is consistent with the well-documented capacity of Abelson murine leukemia virus, which carries within its genome the viral homologue of mouse cellular *abl*, to transform hemopoietic cells, including lymphocytes, plasma cells, macrophages, and promyelocytes (41–45). Finally, the dramatic differences in properties of the leukemic cells in the chronic and acute phases of CML suggest that although translocation and activation of *abl* may be important in the development of the disease, additional events may underlie the shift from chronic to acute phase.

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