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Association Between the Philadelphia Chromosome and a Unique *abl* Transcript*

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A. Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder in which the neoplastic transformation of a stem cell results in the proliferation and accumulation of granulocytes and their progenitors. The disease, which accounts in western countries for 20%–25% of all leukemias, is divided clinically into a chronic phase of 3–4 years duration followed by a terminal acute phase of 3–6 months. During the chronic phase, the neoplastic clone is already established and represents the majority of replicating cells. The cells mature normally and the principal abnormality during this phase appears to be an increase in the stem cell compartment committed to granulopoiesis. In contrast, during the acute phase, cells from the leukemic clone lose their ability to differentiate and mature normally [1]. Perhaps the hallmark of CML is that a specific chromosomal abnormality, the Ph^1 (Philadelphia) chromosome is present in over 90% of cases [2]. The Ph^1 chromosome, also termed $22q^-$, results in most instances from a balanced reciprocal translocation between chromosomes 22 and 9 with very specific breakpoints [3–5]. Recently, the oncogenes *abl* and *sis* were mapped to chromosomes 9 and 22, respectively [6–8]. Moreover, *abl* was shown to reside on the translocated segment of chromosome 9 [9] and *sis* on the corresponding portion of chromosome 22 [10]. Finally, in one case of CML, the translocation breakpoint was localized to the 5′ region of the *abl* gene [11]. We asked whether one or both of these oncogenes is activated and altered in its expression because of the translocation. To answer this we used the RNA transfer technique (Northern blotting) to analyze transcription of *abl* and *sis* in leukemic cells from the peripheral blood or bone marrow of CML patients.

B. Results

We 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 Northern technique for hybridization to a v-*sis* probe composed of sequences of simian sarcoma viral genome homologous to *sis*. No discret species of *sis* RNA could be detected in any of the samples (Fig. 1, lanes *a–h*). Preparation of RNA from normal rat kidney (NRK) cells infected with simian sarcoma virus served as a positive control and showed multiple size transcripts of v-*sis* (Fig. 1, lane *i*).

We next analyzed expression of the *abl* oncogene in samples from 13 CML patients, 22 patients with other leukemias, and 2 normal bone marrows. Representative data are shown in Fig. 2. Human cells contain two major *abl* transcripts of 6 and 7 kilobases, as well as a few other minor species [12–14] and the non-CML samples

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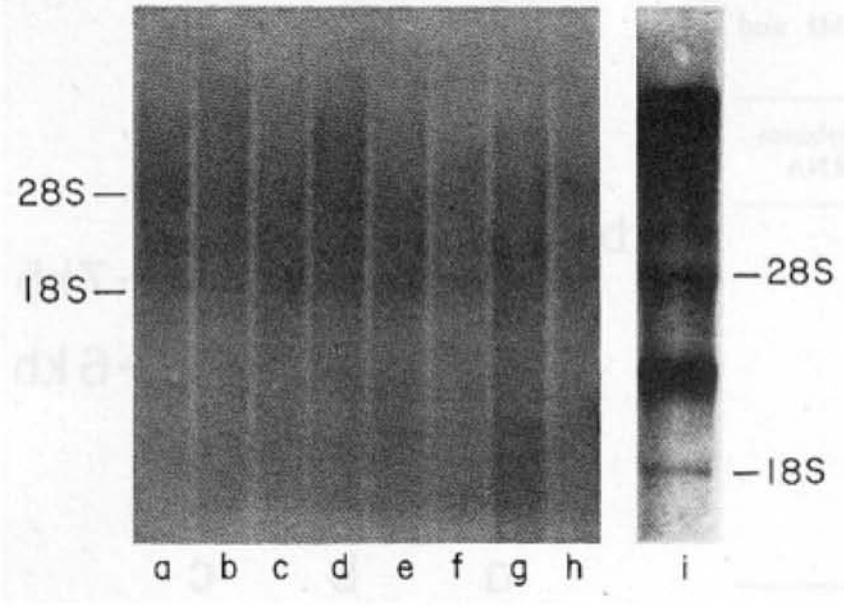

Fig. 1. Absence of *sis* RNA in CML, AML, and HeLa cells; 15-μg samples of RNA from CML (*lanes b, d, f, h*), AML (*lanes a, e, g*), HeLa cells (*lane c*), and NRK cells infected with simian sarcoma virus (*lane i*) were screened for *sis* RNA. Data from [20]

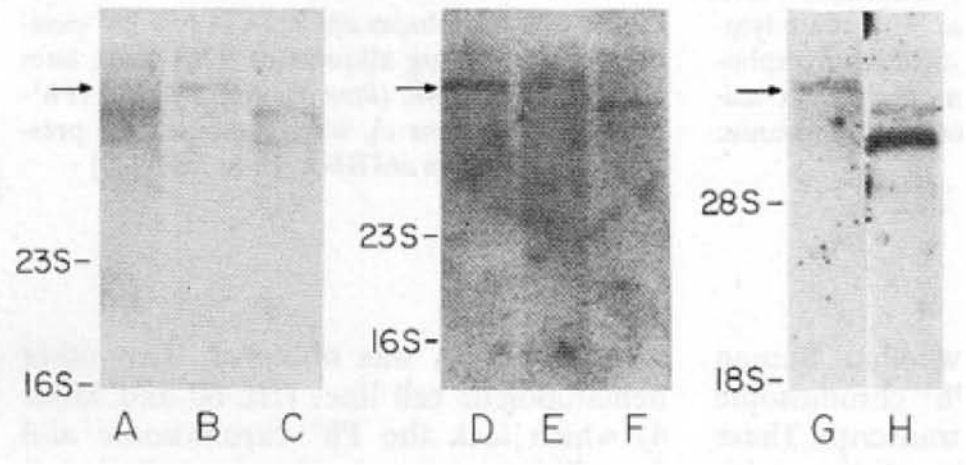

Fig. 2. Gel electrophoresis of *abl* RNA from normal bone marrow, HeLa cells, CML, and AML. *Lane A* normal bone marrow; *lanes B, E* bone marrow from CML patients in chronic phase; *lanes C, F* peripheral blood of AML patients; *lane D* peripheral blood of CML patient in chronic phase; *lane G* peripheral blood of CML patient in blast crisis; *lane H* HeLa cells. Bacterial 23 and 16 *S* ribosomal RNA and human 28 and 18 *S* ribosomal RNA were used as molecular weight standards. *Arrows* correspond to new 8 kilobases *abl* RNA species. Samples of 10 μg RNA were analyzed in *A–G* and a sample of 3 μg was analyzed in *H*. Data from [28]

we analyzed showed the major two RNA (Fig. 2, lanes *A, C, F, H*). CML patients with the Ph[1] chromosome and the 9;22 translocation showed a new *abl* RNA species of 8 kilobases (Fig. 2, lanes *B, D, E, G*). This transcript either replaced the 6 and 7 kilobases species or appeared with them, it was present in samples obtained during both the chronic and acute phases of the disease.

Results of the 37 samples analyzed are summarized in Table 1. The 8 kilobases *abl* RNA transcript was detected in 11 of 12 patients with CML and the t(9;22) translocation, but not in one patient with juvenile CML without t(9;22). The 8 kilobases *abl* transcript was also detected in 1 of 12 patients with AML. Approximately 5% of individuals with AML have the t(9;22) translocation. Unfortunately, chromosome analysis was not performed in this patient so we are unable to determine if he had the t(9;22). The 8 kilobases 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 kilobases species was also lacking in cells from two normal bone marrows. A novel 9 kilobases *abl* RNA was detected together with the 8 kilobases species in 2 of 11 samples from patients with CML and t(9;22).

Table 1. The 8 kilobases *abl* RNA in CML and other leukemias (data from [20])

Diagnosis[a]	Ph^1	Number tested	8 kilobases *abl* RNA
CML	+	12	11
CML[b]	–	1	0
AML	–	11	0
AML	?	1	1
CLL	–	4	0
ALL	–	3	0
AProL	–	1	0
CMoL	–	1	0
AUL	–	1	0
Normal BM	–	2	0

[a] CML chronic myelogenous leukemia; AML acute myelogenous leukemia; ALL acute lymphoblastic leukemia; AProL acute prolymphocytic leukemia; CMoL chronic monocytic leukemia; AUL acute undifferentiated leukemia; BM bone marrow

[b] "Juvenile" CML

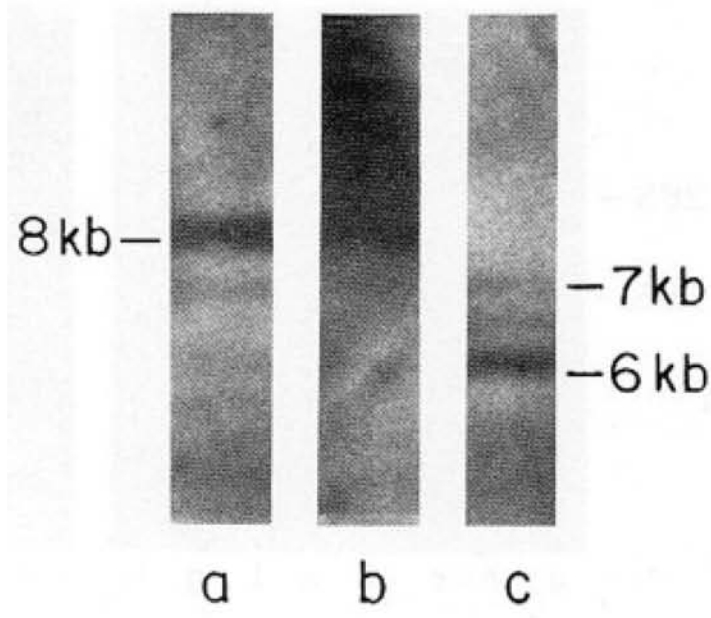

Fig. 3. The 8 kilobases *abl* RNA in two Ph^1-positive cell lines; 8-μg aliquots of RNA from lines K562 (*lane a*), EM2 (*lane b*), and SMS-SB (Ph^1-negative line) (*lane c*), were examined for presence of 8 kilobases *abl* RNA. Data from [20]

Next, we investigated whether human cell lines containing the Ph^1 chromosome synthesized the altered *abl* transcript. These included five hematopoietic cell lines with and without t(9;22) and four nonhematopoietic cell lines without t(9;22). The K562 cell line [15] is an erythroid–myeloid precursor line derived from a patient with CML. K562 has a Ph^1 chromosome [16] or an altered form of it [17]. Analysis of RNA from K562 indicated a major band corresponding to 8 kilobases *abl* RNA and minor bands of the normal species of 6 and 7 kilobases (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 [18] showed a single *abl* transcript of 8 kilobases (Fig. 3, lane *b*). We performed 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 [19]. This cell line is Ph^1 negative and was recently shown [13] 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 kilobases *abl* transcripts as well as additional 6.5 kilobases *abl* species. No 8 kilobases *abl* RNA was observed. Two other hematopoietic cell lines (HL-60 and Molt-4) which lack the Ph^1 chromosome and four Ph^1 negative nonhematopoietic cell lines demonstrated the normal 6 and 7 kilobases *abl* transcripts, but lacked the novel 8 kilobases *abl* transcript. EM-2, K562, Molt-4, HL-60, and HeLa cells contain substantially more polyadenylated *abl* RNA than fresh hematopoietic cells, both normal and leukemic.

C. Discussion

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. The important finding of this work is that a new *abl* transcript of 8 kilobases is found in 11 of 12 patients with CML with the t(9;22) translocation. The 8 kilobases *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 novel RNA was not observed in cells from 22 leukemias unassociated with t(9;22), including a case of Ph^1 negative CML, nor in seven human hematopoietic and nonhematopoietic cell lines which lacked the Ph^1 chromosome. Two samples of normal bone marrow also lacked to 8 kilobases RNA. The association between the t(9;22) translocation and presence of the 8 kilobases *abl* transcript is highly significant.

The strong correlation between the synthesis of the new 8 kilobases transcript and the translocation of *abl* to chromosome 22 suggests a causal association. The new transcript, as well as the normal *abl* species, are homologous to probes from the 5′, central, and 3′ regions of v-*abl* [20]. Therefore, it is likely that the new transcript contains much of the information of normal *abl* RNA. It is possible that the new *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 kilobases *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 into a position adjacent to the breakpoint, with the 5′ region of the oncogene facing sequences of chromosome 22 [11]. Second, the detection in cells from two CML patients of an additional new *abl* species of 9 kilobases might suggest that the 8 and 9 kilobases *abl* RNA are related to the 6 and 7 kilobases normal species, respectively, and that the former were derived by acquisition of the same sequence. Since the 6 and 7 kilobases human *abl* RNA (by analogy with the corresponding mouse species [14]) presumably initiate at the same promoter, but terminate at different poly (A) signals 1000 base pairs apart, the 8 and 9 kilobases RNA might terminate at the same sites as the normal species, but initiate at a new transcriptional promoter upstream of the normal promoter. Such a new promoter could reside in chromosome 9 sequences or in chromosome 22 information behind the breakpoint [21]. The initiation at a new promoter would be probably associated with a modified splicing pattern.

The formation of the new *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 [22]. Moreover, if the 8 kilobases *abl* RNA represents a fusion transcript, then it is also possible that it encodes a novel fused protein. Finally, 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 homolog of mouse cellular *abl,* to transform hematopoietic cells, including lymphocytes, plasma cells, macrophages, and promyelocytes [23–27].

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