CONCISE REPORT

bcr-abl RNA in Patients With Chronic Myelogenous Leukemia

By E. Shitivelman, R.P. Gale, O. Dreazen, A. Berrebi, R. Zaizov, I. Kubonishi, I. Miyoshi, and E. Canaani

The major consequence of the formation of the Philadelphia (Ph') chromosome characteristic of leukemia cells of patients with chronic myelogenous leukemia (CML) is fusion of c-abl and bcr genes. Using a sensitive RNase protection technique, we analyzed mRNA from a large number of CML patients. In most, we identified one or both species of bcr-abl chimeric transcripts. These two mRNAs vary in the specific bcr exon joined to abl exon II and are translated into slightly different proteins. The amounts of the fused mRNAs within leukemia cells vary considerably between individuals and do not correlate with the phase of the disease.

CHRONIC MYELOGENOUS LEUKEMIA (CML) is characterized by two major phases. The chronic phase lasts an average of 3 years and is characterized primarily by accumulation of granulocytes and their precursors in the bone marrow and peripheral blood. This is followed by the acute phase (blast crisis) in which the leukemia cells fail to differentiate and to respond to regulatory factors of myelopoiesis. In some patients there is an intermediate accelerated phase.

A specific chromosomal abnormality, the Philadelphia chromosome (Ph'), is present in 90% to 95% of CML patients. This aberration results from a reciprocal translocation between chromosomes 9 and 22 designated t(9;22). Recent molecular studies implicate the abl oncogene and the bcr gene in CML. As a result of the translocation, abl is moved from chromosome 9 into the bcr gene on chromosome 22. The fused bcr-abl gene is transcribed into a large chimeric RNA which presumably initiates at the bcr promoter and is composed of bcr sequences 5' of the translocation point on chromosome 22 and sequences of chromosome 9 including abl, located downstream of the translocation point. The primary transcript is spliced into a mature bcr-abl mRNA of 8 kb. This mRNA is translated into a 210-kd protein unique to CML cells, and possessing enhanced tyrosine kinase activity compared to the normal abl protein.

In a previous study we reported that in the 8-kb RNAs of two CML cell lines (K562 and EM2) the junction point is composed of abl exon II and bcr exon "3" sequences. We now sought to characterize the RNA junction in CML patients. In addition, we inquire whether the transition from the chronic to the acute phase of the disease involves increased transcription of the fused bcr-abl gene.

MATERIALS AND METHODS

Leukemia cells were isolated from the buffy coat of peripheral blood. RNA was prepared using the urea/lithium chloride method. Polyadenylated messenger RNA was isolated by purification on oligo(dT)-cellulose columns. RNase protection analysis for the alternative bcr-abl junctions has been described. RNA probes were synthesized by standard techniques.

RESULTS

For qualitative and quantitative analyses of bcr-abl RNA junctions in CML patients, we used the RNase protection technique. For probes we used two different junctions derived from cDNA clones K-28 and L-6. In the K-28 clone abl exon II is joined to bcr exon "3"; in L-6, abl exon II is linked to bcr exon "2" (enumeration of bcr exons is according to ref 16). The positions of the relevant abl and bcr exons on the Philadelphia chromosome are shown in Fig 1, and the structure of the two probes is shown in Fig 2. RNAs of CML patients were annealed to each of the two probes and following RNase digestion were electrophoretically analyzed. Examples of these analyses are shown in Fig 2. For example, RNA from patient S-15 (Fig 2A, lane 1) revealed the full-length 450-b K-28 probe. This indicates that within the 8-kb RNA of this patient, abl exon II is linked to bcr exon "3". The additional bands apparent in the autoradiogram result from protection of the 278-b abl segment within the probe by normal abl RNA, and from protection of the 172-b bcr DNA in the probe by normal bcr RNA. Hybridization of the L-6 probe to RNA from patient 1380 (Fig 2B, lane 1) resulted in protection of the 280-b probe. This implies that the bcr-abl RNA junction of this patient is composed of abl exon II and bcr exon "2" sequences. Bands of 250 and 255 b seen in this experiment represent protection of segments of the probe by normal abl RNAs containing alternative first exons.

Results of analyses of samples from 21 CML patients are shown in Table 1. RNAs of 19 patients contained one or both RNA junctions. In two patients with Ph' chromosome we could not detect either junction. Seven patients had only the K-28 junction, and four only the L-6 linkage. Surprisingly, eight patients showed both junctions.

We next questioned whether the progression of the disease...
alternative splicing involving skipping of bcr

1-6 RNA junction

at the top is formed by a mechanism of abi
evernented according to ref 15. Open boxes represent splicing. Hatched boxes represent bcr

depends on the chromosomal breakpoint and on alternative point

tion of the 1-6 junction by RNAs from patients 1 380. 1 1 76, 9033. 1158.

gel. L-6 junction was not detected in these patients. (B) Protec-
site was shifted 20 nucleotides to the 5' because of a small deletion detected

in these patients. Ch and bI designate patients in chronic respectively). The last two

of the K-28 junction by RNAs from patients 5-1 5. 5-10, 1266.

9037. 1158.

RNA junctions transcribed from the chimeric bcr-abl gene formed on the Ph'-chromosome. In two patients with the

breakpoint is between these individuals may have been below detection. Alterna-
tion point on chromosome 22. Thus, if the breakpoints is between bcr exons "3" and "4," abl exon II will be linked to bcr exon "3." If the breakpoint occurs between bcr exons "2" and "3," the RNA junction point will be derived from abl exon II and bcr exon "2" (Fig 1). The

DISCUSSION

In this study we demonstrate that RNA from 19 of 21 CML patients contain one or both of two alternative bcr-abl RNA junctions transcribed from the chimeric bcr-abl gene formed on the Ph'-chromosome. In two patients with the Ph'-chromosome, neither junction was detected. Levels in these individuals may have been below detection. Alternatively, these individuals may have fused RNAs in which different abl and bcr exons constitute the junction point. The resolution of which bcr exon will constitute the RNA junction point is most simply determined by the position of the t(9;22) translocation point on chromosome 22. Thus, if the breakpoint is between bcr exons "3" and "4," abl exon II will be linked to bcr exon "3." If the breakpoint occurs between bcr exons "2" and "3," the RNA junction point will be derived from abl exon II and bcr exon "2" (Fig 1). The

Table 1. Qualitative and Quantitative Analysis of bcr-abl RNA in CML Patients

<table>
<thead>
<tr>
<th>UPN</th>
<th>Ph</th>
<th>Phase</th>
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<th>L-6 Junction</th>
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*Relative amount of fused bcr-abl RNA ranked in a scale of 1 to 10 by signal intensity.

†No signal detected.

Fig 1. Formation of RNAs with a particular bcr-abl junction point depends on the chromosomal breakpoint and on alternative splicing. Hatched boxes represent bcr exons and are arbitrarily enumerated according to ref 15. Open boxes represent abl exons. The L-6 RNA junction at the top is formed by a mechanism of alternative splicing involving skipping of bcr exons "3".

Fig 2. bcr-abl RNA junctions in CML patients. (A) Protection of the K-28 junction by RNAs from patients S-15, S-10, 9011, 1266, 9037, 1158, and 1158 (lanes 1, 2, 3, 4, 5, 6, and 7, respectively). The last two samples were analyzed on a separate gel. L-6 junction was not detected in these patients. (B) Protection of the L-6 junction by RNAs from patients 1380, 1176, 9033, and 9011 (lanes 1, 2, 3, and 4, respectively). K-28 junction was not detected in these patients. Ch and bI designate patients in chronic phase or blast crisis, respectively. *EcoRi is a linker site. *HindIII site was shifted 20 nucleotides to the 5' because of a small deletion in L-8.
REFERENCES


transcript. On the basis of these data, we conclude that transition from chronic to acute phase of CML is not the result of increased expression of the bcr-abl gene. Our studies do not exclude qualitative and quantitative alterations in the fused protein during phase transition. However, it is likely that other genetic changes, such as those associated with the additional chromosomal alterations typical of CML in acute phase, underlie the phase transition of the disease.

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