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Persistence of CML Despite Deletion of Rearranged *bcr/c-abl* Sequences

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A. Introduction

Chronic myelocytic leukemia (CML) is clinically divided into a chronic phase lasting for about 4 years, followed by an acute phase (blast crisis) of a few months' duration [5]. The cytogenetic hallmark of 95% of CML cases is the Philadelphia (Ph) chromosome, resulting from a reciprocal translocation between chromosomes 9 and 22 [21, 22] that places the *c-abl* oncogene into the breakpoint cluster region (*bcr*) on chromosome 22 [7, 11]; this area is part of a gene of yet unknown function [17, 24]. An involvement of *c-abl* and *bcr* sequences in the development of Ph-positive CML has been deduced from (a) the consistent rearrangement of both genes in all cytogenetic subtypes of this leukemia [1, 7, 14, 15], (b) the concurrent detection of a novel 8.5-kb hybrid *bcr/abl* RNA transcript [6, 10, 15], and (c) the expression of an altered *c-abl* protein that differs from its normal counterpart in having a higher associated tyrosine kinase activity [18, 19].

B. Material and Methods

I. Patient

A 49-year-old man developed blast crisis 45 months after diagnosis of Ph-positive CML. Blast cells were of the T-cell phenotype and genotype (Tβ gene rearrangement) and showed a second Ph chromosome as an additional chromosomal aberration. Both Ph chromosomes in blast crisis were similar in size to the single pH chromosome in the chronic state. Combination chemotherapy achieved clinical remission; the patient has been in the chronic state for 14 months.

II. DNA Analysis

Bone marrow DNA (15 µg) was digested with appropriate enzymes, electrophoresed, blotted, and hybridized to 2-kb 5′*bcr*, 1.2-kb 3′*bcr*, and 1-kb 5′8E *bcr* cDNA probes as described elsewhere [2, 12, 25].

III. RNA Analysis

RNA was isolated from bone marrow cells as described by de Klein et al. [8]; 10 µg of poly-(A)-RNA was electrophoresed in the presence of formaldehyde, blotted, and hybridized to 0.6-kb *c-abl* and 2-kb 5′*bcr* probes as previously described [3].

IV. In Situ Hybridization

Chromosomes obtained from bone marrow were prepared according to standard techniques and treated for in situ hybridization as described [1]. The tritiated probe was a 1:1 mixture of human 1.1-kb 3′ and 0.6-kb 5′*c-abl* plasmids [1]. After exposure for 12 days, slides were developed and stained with quinacrine mustard.

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C. Results

A recombination within the *bcr* gene of this patient was established by Southern blot analysis of acute phase cells to a 3′*bcr* probe (Fig. 1, lane b). However, in contrast to all Ph-positive CML patients investigated to date, 5′*bcr* sequences detected only a 5-kb germline fragment for this man (Fig. 1, lane a). Hybridization of different digests of blast-cell DNAs to a cDNA probe covering most 5′*bcr* sequences known thus far [12]

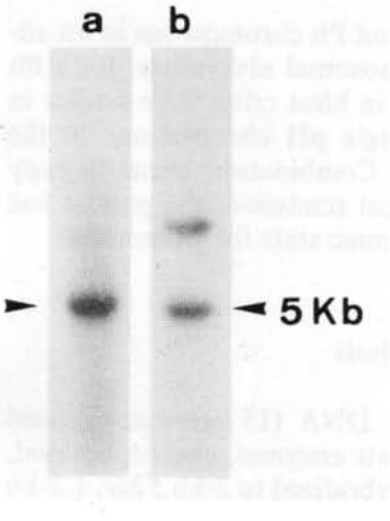

Fig. 1. Southern blot analysis of 15 μg DNA obtained from blast crisis. Bgl II digests were hybridized to a 5′*bcr* probe (*lane a*) and to a 3′*bcr* probe (*lane b*) that detect 5-kb germline bands. Note the rearranged 3′*bcr* fragment in lane b

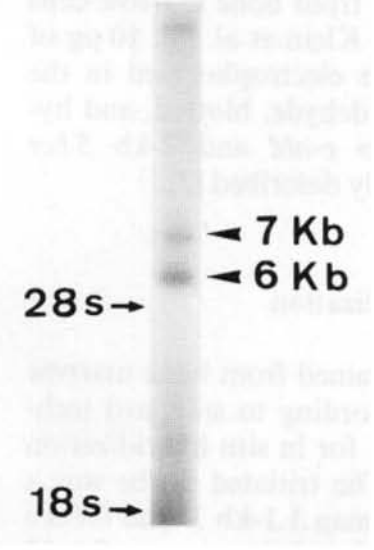

Fig. 2. Northern blot analysis of 10 μg poly-(A)-RNA obtained from blast crisis cells and hybridized to *c-abl* sequences

Table 1. Results of in situ hybridization to *c-abl* probes

Chromosome	Chronic phase		Blast crisis	
	Observed[a]	Grains Expected[b]	Observed	Grains Expected
1	7	9.0	24	18.1
2	12	8.8	14	17.7
3	4	7.3	12	14.6
4	9	7.0	7	14.0
5	6	6.7	11	13.4
6	2	6.3	6	12.6
7	5	5.8	10	11.6
8	2	5.3	8	6.5
9	13	2.5	34	5.0
9q+	5	3.0	7	5.7
10	2	4.9	8	9.7
11	3	5.0	6	10.0
12	0	4.9	11	9.8
13	6	3.9	4	7.8
14	1	3.7	5	7.5
15	2	3.5	2	7.1
16	3	3.2	9	6.5
17	5	3.1	8	6.2
18	0	2.9	5	5.9
19	1	2.3	5	4.5
20	0	2.5	0	5.0
21	0	1.7	1	3.4
22	3	0.9	0	1.8
Ph	9	0.5	4[c]	2.2[c]
X	4	2.8	7	5.6
Y	0	1.0	3	1.9

[a] Grains were counted on complete, well-spread metaphases.
[b] Number of grains expected according to DNA content [26].
[c] On two Ph chromosomes.

likewise failed to detect rearranged fragments (not shown). These results suggested a deletion of rearranged 5′*bcr* sequences on the Ph's in blast crisis. Since cell samples from the chronic state of this patient were not available for Southern blot analysis, we performed in situ hybridization studies of the *c-abl* oncogene to metaphases obtained from both phases to investigate a possible concurrent deletion of *abl* sequences.

Distribution of silver grains was uniform and random on 21 chromosomal spreads obtained from the chronic state, except for specific signals ($P<0.01$) on chromosomes 9 and 22q− (Table 1). This result demon-

strated the expected translocation of *c-abl* sequences to the Ph chromosome. However, 49 metaphases from blast-crisis cells demonstrated a significant ($P<0.01$) grain accumulation only on chromosome 9 (Table 1), and thus established a deletion of *c-abl* sequences from the blast-phase Ph chromosomes.

Northern blot analyses were in agreement with these data. Hybridization to *c-abl* and *bcr* sequences exhibited normal 6-kb and 7-kb *c-abl* (Fig. 2), as well as 4.5-kb and 7-kb *bcr* transcripts (not shown) respectively. Neither probe detected the hybrid 8.5-kb *bcr/abl* RNA species usually observed in Ph-positive CML.

D. Discussion

Various data suggest a multistep pathogenesis of CML, with the development of the cytogenetically visible Ph chromosome as well as the molecularly detectable *bcr/c-abl* rearrangement being a second event in this process [9, 13, 16, 20]. Transition from the chronic to the acute phase of Ph-positive CML would represent a third step, characterized by marked differences in the biology of leukemic cells and additional chromosomal aberrations [23]. The detection of identically rearranged *bcr* fragments as well as of comparable levels of the 8.5-kb *abl/bcr* transcript in blast cells and chronic-state cells of the same patient suggest that genes other than *c-abl* and *bcr* induce this terminal shift of biological properties within leukemic cells [4, 10].

However, the data presented here may indicate a possible modulating effect on the clinical course of this leukemic phase. In this respect it seems to be noteworthy that the patient has survived the acute phase for more than 14 months already in remarkably good condition. A deletion of the rearranged *abl/bcr* sequences, i.e., the withdrawal of the second step on the way to CML blast crisis, may result in the manifestation of a leukemic state different from the more aggressive blast crisis usually observed in CML.

On the other hand, the results of the present study suggest that once a leukemic cell has entered blast crisis, rearranged *abl/bcr* sequences are no longer essential for the maintenance of a leukemic state. In this respect it would be of interest to investigate in vitro the effect of antisense RNA or monoclonal antibodies directed against altered *bcr/abl* sequences in chronic-phase cells of Ph-positive CML patients. While the reported case appears to be a unique in vivo model, it can address only the respective roles of these genes in blast crisis.

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