Brief Definitive Report

bcr REARRANGEMENT WITHOUT JUXTAPOSITION OF *c-abl* IN CHRONIC MYELOCYTIC LEUKEMIA

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Chronic myelocytic leukemia (CML) is associated with the presence of a Philadelphia (Ph^1) chromosome in 95% of cases (1). This cytogenetic hallmark is molecularly characterized by the translocation of the *c-abl* oncogene from chromosome 9 into the breakpoint cluster region (*bcr*) of chromosome 22 (2–5), and the subsequent transcription of a chimeric 8.5 kb *bcr/c-abl* RNA species within leukemic cells (6–8), which represents mRNA for an altered *c-abl* protein with associated tyrosine kinase activity (9). Ph^1-negative CML, however, constitute a heterogeneous group of prognostically distinct disorders (10). Recently (11), my coworkers and I identified a Ph^1-negative CML patient with a *c-abl/bcr* rearrangement; however, other patients lack this recombination (3). Here I report on the first Ph^1-negative CML patient whose leukemic cells exhibit a rearrangement in the *bcr* gene without juxtaposition of *c-abl* sequences.

Materials and Methods

Patients. Patient 1 is a 62-yr-old male in the chronic phase of Ph^1-positive CML. Patient 2 is a 48-yr-old male with splenomegaly; Hb, 107 g/liter; leukocytes, 95×10^9 cells/liter (52% neutrophils, 1% eosinophils, 3% basophiles, 9% myeloblasts, 24% myelocytes, 8% lymphocytes, 3% monocytes); 452×10^9 platelets/liter; bone marrow revealed increased myeloid lines without major maturation disturbance; repeated cytogenetic analysis showed a male karyotype without chromosomal aberrations; this patient is being treated with hydroxyurea and has been in chronic state for 28 mo.

DNA Analysis. Bone marrow DNA (15 μg) obtained from both patients were digested with restriction enzymes (C. F. Boehringer & Soehne, Mannheim, Federal Republic of Germany), electrophoresed on a 0.7% agarose gel, blotted, and hybridized to a 2 kb Bgl II/Hind III 5′ *bcr* probe and a 1.2 kb Hind III/Bgl II 3′ *bcr* probe as described (11). λ-DNA were included as molecular weight standards (not shown). Specific activity of the probes was $1–3.5 \times 10^8$ cpm/μg. After hybridization, filters were washed under high stringency (10% saline citrate, 65°C) and exposed to XAR-2 film (Eastman Kodak Co., Rochester, NY) for up to 3 d at −70°C with DuPont Lightening Plus intensifying screens (DuPont Instruments, Wilmington, DE).

RNA Analysis. RNA was isolated from bone marrow cells according to the LiCl/urea method (12); poly(A) RNA was obtained after two passages over oligo(dT) cellulose; 15 μg of poly(A) RNA was electrophoresed on a 1.2% agarose gel in the presence of formaldehyde (13). After blotting, nitrocellulose filters (Schleicher and Schuell, Dassel, FRG) were first hybridized to a 0.6 kb Eco RI/Bam HI *c-abl* probe washed, and rehybridized to a 2 kb Bgl II/Hind III 5′ *bcr* probe, as described (11). Filters were exposed

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to Kodak XAR-5 film using DuPont Lightning Plus intensifying screens for up to 6 d at −70°C.

In Situ Hybridization. Chromosomes obtained from bone marrow were prepared according to standard techniques. After RNase treatment, chromosomes were denatured in 70% formamide/2× standard saline citrate (SSC) at 70°C for 2 min, rinsed in 2× SSC, and dehydrated. The probe, a 1:1 mixture of human 3′ 1.1 kb Hind III/Eco RI and 5′ 0.6 kb Bam HI *c-abl* plasmids (3), was labeled by nick-translation using [^{3}H]dCTP and [^{3}H]dTTP (New England Nuclear, Boston, MA) to a specific activity of 0.8×10^7 cpm/μg. The probe was denatured for 5 min at 70°C at a concentration of 0.2 μg/ml in 50% formamide/2× SSC/10% dextran sulfate added to the chromosomes, and hybridized for 10 h at 37°C; slides were rinsed in three changes of 50% formamide/2× SSC at 39°C, followed by 6 h washing in 2× SSC at room temperature, exposed to Kodak NTB2 emulsion, for 15 d at 4°C, developed, and stained with quinacrine mustard as described (11).

Results

Southern blot analysis revealed a rearrangement within the *bcr* gene in leukemic cells of a Ph^1-negative CML patient. Hybridization to 5′ *bcr* sequences detects a 5 kb germline band together with a 3.4 kb rearranged fragment (Fig. 1, lane *2*). A Bgl II polymorphism appears to be an unlikely explanation of this result, since digests using different restriction enzymes (Bam HI, Eco RI, and Hind III) demonstrated a similar pattern (not shown). Hybridization to a 1.2 kb Hind III/Bgl II 3′ *bcr* probe also identified an additional autoradiographic band. These results are comparable to the data obtained in Ph^1-positive CML (e.g., Fig. 1, lane *1*).

To investigate a possible involvement of *c-abl* sequences in this genomic alteration, I performed in situ hybridization studies of *c-abl* sequences to metaphase chromosomes of the Ph^1-negative CML patient (Table I). Distribution of silver grains obtained from analysis of 42 metaphases was uniform and at random on all chromosomes except the specific signal ($P < 0.01$) on chromosome 9. Analysis by Poisson distribution, with the number of grains per chromosome adjusted for the relative size of the band in a 400-band idiogram (15), revealed highly significant ($P \ll 10^{-7}$) grain accumulation at band 9q34. Thus, in contrast to all Ph^1-positive CML patients investigated so far (2–4), in situ hybridization studies failed to detect a translocation of *c-abl* sequences in leukemic cells of this patient.

To elucidate in some more detail the consequences of these results I performed

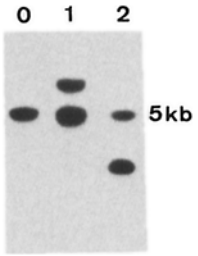

FIGURE 1. Southern blot analysis of *bcr* sequences of human placenta DNA (lane *0*) and bone marrow DNA from two leukemic patients (*lanes 1* and *2*). DNA (15 μg) were digested with Bgl II, electrophoresed on a 0.7% agarose gel, blotted, and hybridized to a Bgl II/Hind III 5′ *bcr* probe that detects a 5 kb normal *bcr* fragment.

TABLE I

Results of In Situ Hybridization to Human c-abl Probes

Chromosome	Grains observed	Grains expected
1	9	17.5
2	20	17.0
3	9	14.1
4	7	13.5
5	15	12.9
6	3	12.2
7	14	11.2
8	8	10.3
9	42*	9.6
10	7	9.4
11	6	9.6
12	10	9.5
13	6	7.5
14	8	7.3
15	9	6.8
16	3	6.3
17	5	6.0
18	7	5.7
19	1	4.4
20	2	4.8
21	5	3.3
22	1	3.5
X	3	10.9
Y	2	3.7

Number of grains expected according to DNA content (14). Analysis by Poisson distribution demonstrate a highly significant grain accumulation at band 9q34. 31 grains on region 9q34.

* 31 grains on region 9q34.

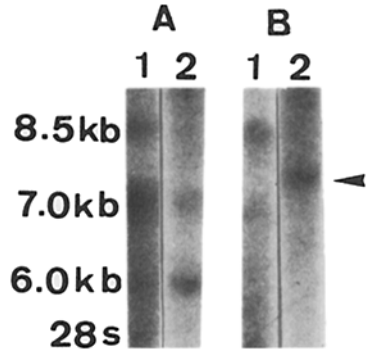

FIGURE 2. Northern blot analysis of poly(A) RNA obtained from patients 1 and 2. The blot was first hybridized to a *c-abl* probe (*A*), washed, and rehybridized to 5′ *bcr* sequences (*B*). Arrow points to a novel 7.3 kb *bcr* RNA species in leukemic cells of patient 2. Molecular weight standards are not shown.

an RNA analysis. Northern blots hybridized to *c-abl* sequences showed 6 and 7 kb normal *abl* RNA species, as well as the fused 8.5 kb transcript in Ph^1-positive CML (Fig. 2*A*, lane *1*); in the Ph^1-negative CML patient, only normal *c-abl* species were visible (Fig. 2*A*, lane *2*). Rehybridization of the filters to 5′ *bcr* sequences, however, led to the detection of a novel 7.3 kb *bcr* transcript in leukemic cells of this individual (Fig. 2*B*, lane *2*) replacing a 6.7 kb normal *bcr* transcript (8); the 8.5 kb chimeric RNA species (7, 8) demonstrated in Ph^1-positive CML (Fig. 2*B*, lane *1*).

Discussion

Molecular analysis of the Ph^1 translocation in CML led to the identification of the *bcr* gene on chromosome 22. A modification of the *c-abl* protein by *N*-terminal substitution of *bcr* sequences appears to be a crucial event in the development of Ph^1-positive CML. Chronic myelocytic leukemia without a Ph^1 chromosome comprises a heterogenous group of related disorders. Recent evidence (3, 11) suggests that a subset of cytogenetic Ph^1-negative CML patients may in fact belong to the molecularly defined entity of Ph^1-positive CML, while leukemic cells of other Ph^1-negative CML patients lack a *c-abl/bcr* rearrangement.

Here I report on yet another type of genomic recombination, i.e., a *bcr* rearrangement without involvement of *c-abl* sequences. Data obtained by both in situ hybridization studies and Northern blotting demonstrate that this oncogene is neither translocated nor abnormally transcribed in the Ph^1-negative CML patient described here. However, a rearrangement within *bcr* leads to a replacement of normal 4.5 and 6.7 kb *bcr* RNA species (7) by a novel 7.3 kb transcript in leukemic cells of this individual.

Chronic myelocytic leukemia is a clonal disease of pluripotent hematopoietic stem cells, and the expression of *bcr* may be closely associated with this cell lineage. However, the normal function of the *bcr*-encoded product is still unknown. The detection of a rearrangement within *bcr* per se is in all likelihood not a sufficient explanation for the development of myeloid leukemia. As for the patient described herein, it may be possible that *bcr* sequences are fused with other, yet unknown sequences, instead of *c-abl*. Molecular cloning and detailed analysis of the novel *bcr* transcript may substantiate this speculation. However, the demonstration of heterogeneity among Ph^1-negative CML cases detected by *bcr* and *c-abl* sequences may finally contribute to a novel subclassification of this poorly defined group of leukemias based on molecular, morphological, and clinical features.

Summary

Southern blot analysis detected a *bcr* gene rearrangement within leukemic cells of a Philadelphia chromosome–negative chronic myelocytic leukemia (CML) patient that led to transcription of a novel 7.3 kb *bcr* RNA species. Participation of the *c-abl* oncogene in this genomic recombination could be ruled out by in situ hybridization studies and Northern blot analysis.

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