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Oncogenetic Aspects of Chronic Myelocytic Leukemia

C. R. Bartram [1], A. de Klein, and G. Grosveld [2]

A. Introduction

Over the last several years, evidence has been accumulating that cellular oncogenes are involved in carcinogenesis, whatever its proximal cause. These genes constitute a functionally heterogeneous group that may cooperate with one another during tumorigenesis. The localization of oncogenes near breakpoints involved in specific chromosomal aberrations occurring in various neoplasms emphasizes their possible role in the development of those tumors [1, 4, 12, 15].

The cytogenetic hallmark of chronic myelocytic leukemia (CML) is the Philadelphia chromosome (Ph^1) in leukemic cells of 94% of cases. Recently, we demonstrated the consistent, reciprocal translocation of the human c-*abl* oncogene from chromosome 9 to the Ph^1 chromosome in standard (t (9; 22)), complex, variant, and masked cytogenetic subtypes of Ph^1-positive CML [2, 6, 10, unpublished work].

Moreover we have molecularly cloned sequences from chromosome 9 and 22 involved in the Ph^1 translocation, immediately adjacent to the chromosomal breakpoints [7, 9]. The sites of these breakpoints are individual in different patients and distributed over a relatively large region on chromosome 9, 5′ of human v-*abl* sequences. The breakpoints on chromosome 22, however, are clustered within a very limited region of yet unknown function [7], termed the breakpoint cluster region (*bcr*).

1 Universitätskinderklinik Ulm, Prittwitzstraße 43, 7900 Ulm, FRG
2 Department of Cell Biology and Genetics, Erasmus University Rotterdam

B. Results

DNA samples isolated from peripheral blood of nine Ph^1-positive CML patients were subjected to Southern blot analysis and hybridized to a *bcr*-specific Hind III/ Bgl II probe [7]. As is evident from Fig. 1, this probe detects, apart from a 4.8 kilobases fragment hybridizing to normal chromosome 22 sequences, variable rearranged fragments in Ph^1-positive patients. Since abnormalities were not seen in fibroblasts from Ph^1-positive CML patients (e.g., lane *J**), leukemic cells of Ph^1-negative patients (e.g., *K*) or in DNA isolated from other neoplasms, including acute myelocytic or myelomonocytic leukemia, lymphoma, glioblastoma, melanoma, and teratocarcinoma [7], we believe these rearrangements to be highly specific for leukemic cells in Ph^1-positive CML.

To investigate the possibility that the breakpoint cluster region exhibits additional rearrangements during progression of the chronic to the final acute state of CML, we studied DNA from leukemic cells of two Ph^1-positive CML patients in chronic phase and blast crises, respectively (Fig. 2). Obviously, there occur no further rearrangements within this limited region associated with blast crisis; this result is in agreement with the observation of specific, additional, secondary chromosomal aberrations during that terminal state of disease in the majority of patients.

Strong support for the hypothesis that an activation of the c-*abl* oncogene represents an important step during the development of Ph^1-positive CML results from the de-

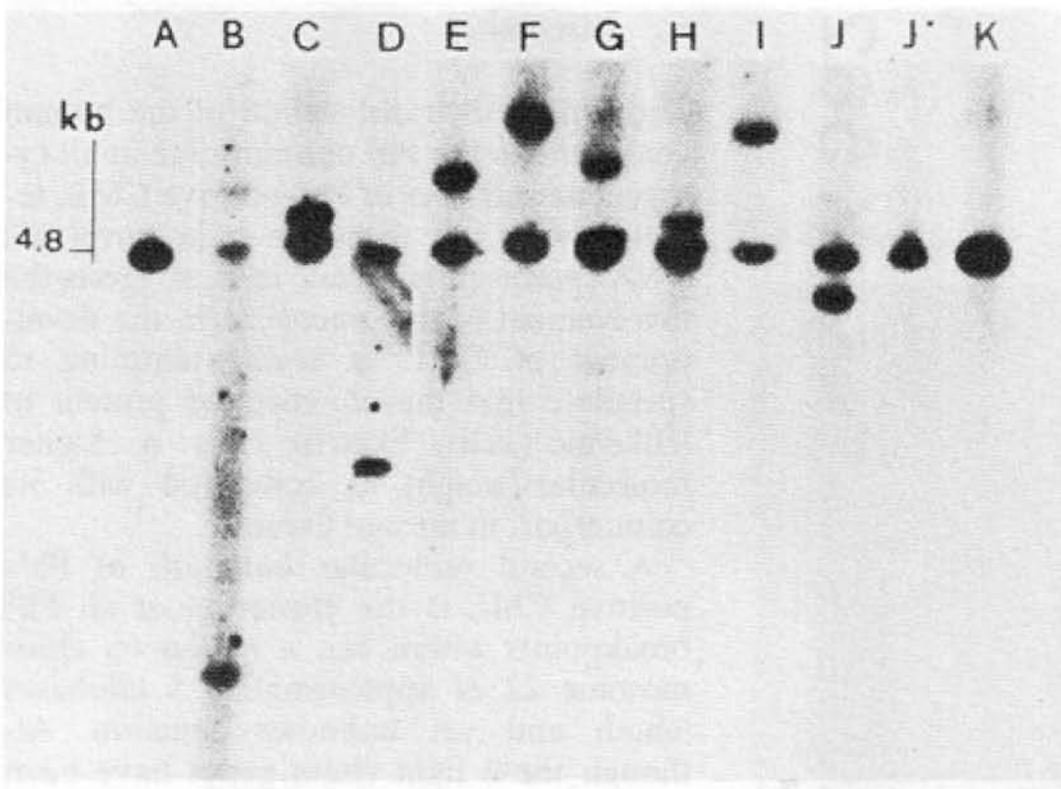

Fig. 1. Analysis of DNA samples from CML patients. DNA was isolated from human placenta (control, *lane A*), peripheral blood cells of Ph[1]-positive CML patients (*B–J*) and a Ph[1]-negative CML patient (*K*) as well as from fibroblasts of patient *J* (*J**); 10 μg DNA was digested with BGl II, electrophoresed on agarose gel, blotted, and hybridized to a *bcr*-specific 1.0 kilobases Hind III/Bgl II probe from chromosome 22 [7]. This probe detects abnormal Bgl II fragments in Ph[1]-positive CML patients in addition to a 4.8 kilobases fragment representing normal, unrearranged chromosome 22 sequences. Patient B apparently has a breakpoint in a region encompassing the probe; therefore the 1.0 kilobases Hind III/ Bgl II probe detects both the 22q- and 9q+ breakpoint fragments

tection of a new *abl* RNA transcript in leukemic cells (Fig. 3). HeLa and normal human hemopoietic cells (data not shown) contain major *abl* transcripts of 6.0 and 7.0 kilobases, as demonstrated by us and others [5, 14]. However, in CML cell line K562 and leukemic cells of Ph[1]-positive CML patients obtained during both chronic and acute phase (data not shown), a novel *abl* RNA species of 8.5 kilobases is apparent; we thus confirm similar results recently published by Canaani et al. [5]. It appears to be of particular importance that exactly the same novel RNA transcript is identified using *bcr*-specific probes instead of c-*abl* fragments. On the other hand,

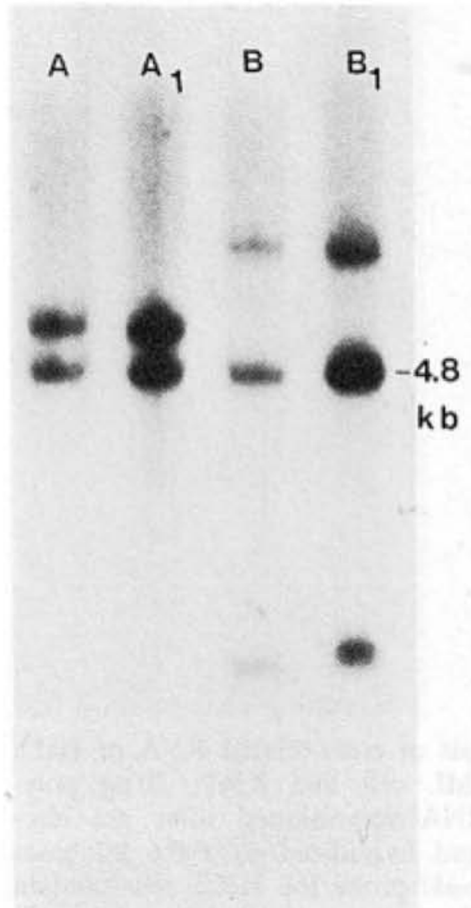

Fig. 2. Analysis of DNA samples isolated from peripheral blood cells of two CML patients during chronic phase (*lanes A, B*) and final blast crisis (A_1, B_1), respectively. DNA was digested, blotted, and hybridized as described in Fig. 1

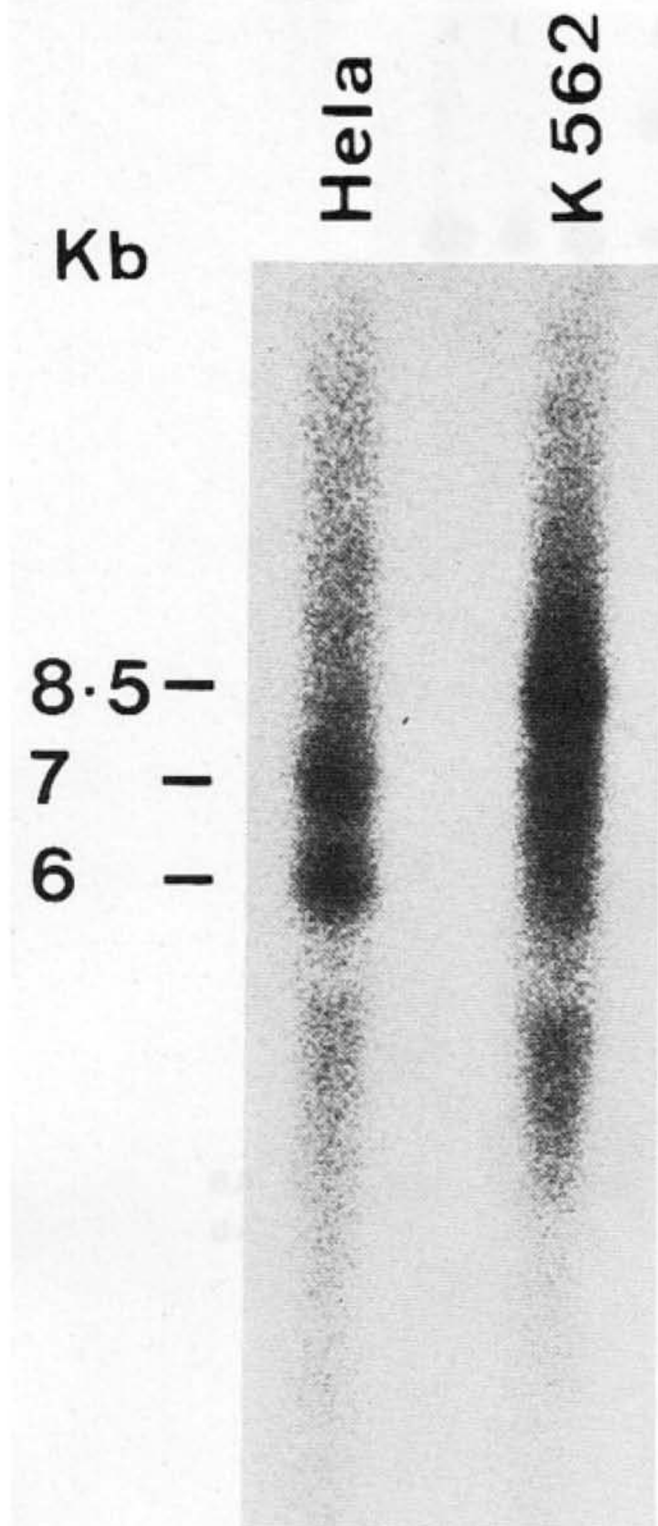

Fig. 3. Analysis of c-*abl*-related RNA in HeLa cells and CML cell line K562; 20 μg poly-A-selected RNA was blotted after gel electrophoresis and hybridized to a 0.6 kilobases BamH I 5′ c-*abl* probe [6]. HeLa cells contain two major *abl* transcripts of 6 and 7 kilobases; in contrast, K562 exhibits a new *abl* RNA species of 8.5 kilobases

there is no significant increase in *abl* expression in leukemic cells, with the known exception of cell line K562 containing amplified c-*abl* sequences [9].

C. Discussion

The consistent translocation of the human c-*abl* gene to the Ph[1] chromosome in all cytogenetic subtypes of Ph[1]-positive CML, together with the detection of a novel *abl* RNA species in leukemic cells, suggests the involvement of this oncogene in the development of CML; it seems tempting to speculate that the *abl*-encoded protein in leukemic cells likewise has a higher molecular weight as compared with its counterpart in normal tissues.

A second molecular hallmark of Ph[1]-positive CML is the clustering of all Ph[1] breakpoints within *bcr*, a region on chromosome 22 of approximately 5 kilobases length and yet unknown function. Although the λ light chain genes have been localized to the same band 22q11, no cross-homology was observed between *bcr* and λ clones. The detection of a new 8.5 kilobases RNA species in leukemic cells by c-*abl* and *bcr* probes further supports the view that sequences residing on both chromosomes 9 and 22, may be associated with Ph[1]-positive CML. However, mechanisms responsible for the recombination of the two gene transcripts have still to be elucidated and one has particularly to take into account that the breakpoints on chromosome 9 are distributed over a relatively large region of several hundred kilobases.

Apparently, additional genes are essential for the generation of Ph[1]-positive CML, as suggested by DNA transfection assays [11, 13]. The c-*sis* oncogene, however, appears to be an unlikely candidate (Fig. 4). This gene is localized on region 22q12.3-q13.1, far away from the breakpoint region, and segregates with the translocated part of chromosome 22 to different chromosomes in Ph[1]-positive patients [3]. Moreover, *sis*-related transcripts have not been detected in CML cells or other hematopoietic malignancies.

The minority (6%) of CML patients without a Ph[1] chromosome exhibit neither c-*abl* translocation, novel *abl* transcripts, nor rearrangements within *bcr* on chromosome 22. Therefore, this disease appears to be a distinct subclass of myelocytic leukemia with an altogether different origin; this view is supported by clinical observations,

Fig. 4. Ph[1]-positive CML is molecularly characterized by translocation of c-*abl* sequences from chromosome 9 to the Ph[1]-chromosome and by a limited region on chromosome 22, termed *bcr,* that contains the Ph[1] breakpoints. However, c-*sis* is localized far away from the breakpoint region 22q11 and seems not to be important for the generation of CML

including correlations which reveal a poorer prognosis.

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