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Influence of BCR/ABL fusion proteins on the course of Ph leukemias

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The hallmark of chronic myeloid leukemia (CML) and a subset of acute lymphoblastic leukemia (ALL) is the presence of the Philadelphia chromosome as a result of the t(9;22) translocation. This gene rearrangement results in the production of a novel oncoprotein, BCR/ABL, a constitutively active tyrosine kinase. There is compelling evidence that the malignant transformation by BCR/ABL is critically dependent on its Abl tyrosine kinase activity. Also the bcr part of the hybrid gene takes part in realization of the malignant phenotype. We supposed that additional mutations accumulate in this region of the *BCR/ABL* oncogene during the development of the malignant blast crisis in CML patients. In ALL patients having p210 fusion protein the mutations were supposed to be preexisting.

Sequencing of PCR product of the *BCR/ABL* gene (Dbl, PH region) showed that along with single-nucleotide substitutions other mutations, mostly deletions, had occurred. In an ALL patient a deletion of the 5th exon was detected. The size of the deletions varied from 36 to 220 amino acids. For one case of blast crisis of CML changes in the character of actin organization were observed. Taking into account the functional role of these domains in the cell an etiological role of such mutations on the disease phenotype and leukemia progression is plausible.

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Abbreviations: ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; CNL, chonic neutrophilic leukemia; DH, Dbl homology; GEF, guanine nucleotide exchange factor; PTK, protein tyrosine kinase.

More than 90% of cases of chronic myeloid leukemia (CML) and 10–25% of cases of acute lymphoblastic leukemia (ALL) are characterized by a reciprocal translocation between chromosomes 9 and 22 (Clark *et al.*, 1988; Cortes *et al.*, 1995). As a result, a *BCR/ABL* hybrid gene is formed on the derivative Philadelphia chromosome (Ph). Depending on the location of the breakpoint in *BCR*, three types of fusion protein can be formed, all of which exhibit deregulated protein tyrosine kinase (PTK) activity compared to normal ABL (Konopka *et al.*, 1984; Quackenbush *et al.*, 1997). As a result, there is excessive tyrosine phosphorylation of many intracellular proteins including the BCR/ABL protein itself (Laneuville, 1995; Liu *et al.*, 1993).

Several forms of the *BCR/ABL* oncogene responsible for the pathogenesis of Philadelphia chromosomes positive (Ph^+) human leukemias are generated by this translocation. The breakpoint locations in the *BCR* gene and the specific parts of *BCR* that are left intact determine the forms of the *BCR/ABL* oncogene. The main forms of BCR/ABL are as follows: P210, which is found in most cases of CML and in 50% of cases of ALL, P185, which is found in ALL and P230, found in chronic neutrophilic leukemia (CNL) (Pane *et al.*, 2000; Martinelli *et al.*, 2002).

The differences between the p210 and p185 proteins are associated with additional Dbl homology (DH) and PH domains (exons 3–12 of the *BCR/ABL* gene) which are present only in p210. The Dbl family proteins catalyses guanine nucleotide exchange on the Rho family of small GTPases. Members of this family control cell progression, transcription and actin cytoskeletal arrangement. DH functions together with the PH domain (membrane targeting module).

CML is a biphasic disease with an initial chronic phase during which the disorder is easily controlled. However, chronic-phase CML is followed by a terminal blastic phase that resembles acute leukemia and is usually refractory to treatment. Transformation of the chronic phase to blast crisis is accompanied by secondary cytogenetic changes in approximately 85% of cases (Gribble *et al.*, 1999). However, the genetic events responsible for the transformation of CML are poorly understood. We supposed that additional mutations accumulate in the Dbl, PH region of *BCR/ABL* oncogene during the development of the malignant blast crisis in CML patients. In ALL patients, having the p210 fusion protein, we suppose that these mutations were already preexistent.

MATERIALS AND METHODS

Patient samples and cell culture. Peripheral blood cells from patients with a referring diagnosis of CML were obtained for molecular studies as part of diagnosis workup. The Ph-positive cell line K562 and the Ph-negative cell line U937 were cultured in the presence of 10% fetal calf serum in RPMI 1640 medium in a humidified atmosphere with 5% CO_2 at 37°C.

RT-PCR, DNA amplification, cloning and sequencing. Total RNA was extracted from white blood cells as well as from the control cell lines by the method of Chomczynski and Sacchi (1987). The Ph-chromosome was detected as described (Kawasaki *et al.*, 1988). For amplification of the Dbl homology region bp 1955–2810 of *BCR/ABL* cDNA were: 30 s of denaturation at 94°C, 30 s annealing at 56°C and 90 s of extension at 72°C (30 cycles). The following primers were used: ext1 dbl (5′-GGCTGCCCTACATTGATGACTCGC-3′) and extr1 dbl (5′-GATGTTGGGCACTGCCTCCAGTTC-3’) for the first round and ext dbl (5′-AAGCTTGCCCTGGAGTCCACTAAAG-3′) and extr dbl (5′-GAATTCTGCCTCCAGTTCATCCAC-3′), for the second round. PCR products were gel separated, excised and after purification were cloned in pUC19 and sequenced using the T7-sequencing TM Kit (Amersham).

Fluorescence microscopy. Cells were plated onto cover slips and grown overnight before preparing them for immunofluorescence. Cells were fixed in paraformaldehyde, washed with phosphate-buffered saline (PBS) and permeabilized with 0.1% Triton X-100. FITC-labeled phalloidin (Sigma-Aldrich, U.S.A.) was applied to the cells as previously described (Wulf *et al.*, 1997.)

RESULTS AND DISCUSSION

The medical diagnoses "Ph-positive CML" and "Ph-positive ALL" were confirmed by nested RT-PCR. Polymorphonuclear cells of peripheric blood from patients C. and K. (CML, blast crisis), patients F. and Y. (ALL), normal donors A. and B., cell lines K562 (CML, erythrocyte blast crisis), U937 (Ph-negative promonocytic leukemia) were used for fluorescence microscopy. Comparative analysis of actin distribution in polymorphonuclear leukocytes of the different donors made it possible to distinguish the following types of cell staining: 1) diffuse distribution (normal donors) (Fig. 1, D); 2) paramembrane actin distribution in the cells of patients C., F., Y., cell line K562 and U937 (Fig. 1, AB); 3) formation of amorphous cytoplasm accumulation, "dot-like structures" in one case of patient K. (CML, blast crisis) (Fig. 1, C). The BCR/ABL

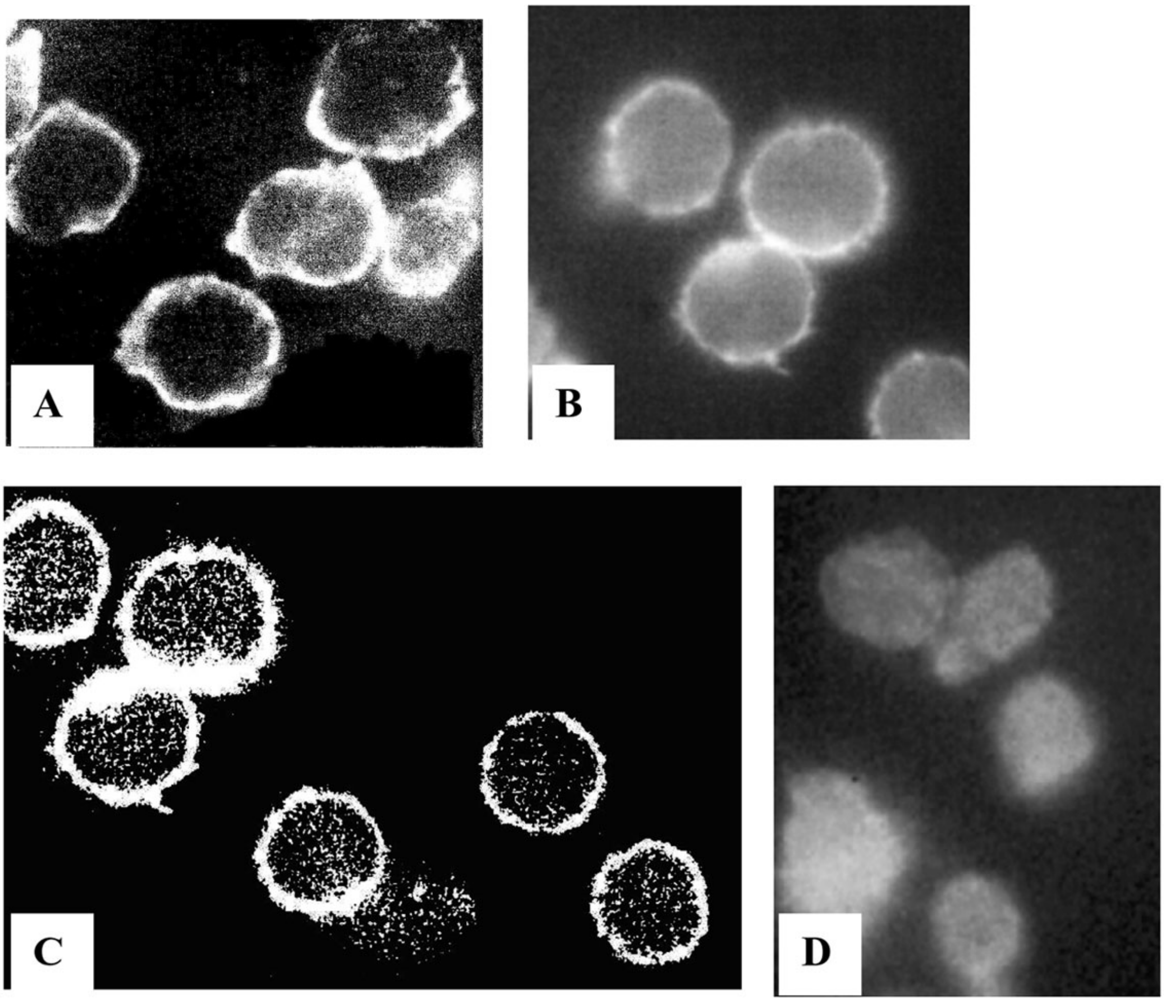

Figure 1. Comparative analysis of actin distribution in cells of patients with Ph-positive leukemias, cells lines K562 (Ph-positive) and U 937 (Ph-negative).

A, B, paracortical actin distribution which is peculiar to cells from lines K562, U937 and to cells of patients C. (CML, blast crisis), Y. (ALL), F. (ALL); C, "dot-like" structures, which were detected in patient K. (CML, blast crisis); D, diffuse actin distribution in cells of normal donors.

protein is a polyfunctional protein composed of several domains with diverse properties (Gishizky *et al.*, 1996; Butturini *et al.*, 1996). This functional diversity of the BCR/ABL protein enables its participation in different signalling pathways. The DH domain of is presented in p210 BCR/ABL but not in p185 BCR/ABL. This domain encodes a guanine nucleotide exchange factor activity specific for Rho GTPases which modulate the cell actin structure (Chuang *et al.*, 1995). The modulatory effect of the Dbl domain on actin structure may underlay the different transforming properties of the two types of BCR/ABL fusion proteins. We supposed here that the observed dot-like distribution of actin correlated with some mutation, which could arise in the Dbl-homology part of the *BCR/ABL* gene. Indeed, sequencing of the *BCR/ABL* amplification product from patient K. confirmed the presence of mutational changes in the Dbl homology region in positions 2127 (replacement T→C) and 2449 (replacement C→A) of *BCR/ABL* cDNA that corresponded to substitution in position 547 (Phe→Leu) and 654 (Thr→Lys) of the protein molecule. Therefore the mutations were likely to influence GEF function of the Dbl domain and, as a consequence, change the BCR/ABL transforming potential leading to progression in CML. The data obtained may shed light on the nature of CML blast crisis development and can be used for early detection of CML tumor progression as well as for elaboration of more effective treatment protocols.

Table 1. Analysis of Dbl domain of BCR/ABL protein from clones obtained from blood samples of patients with CML and ALL.

The form of disease	Clone number	The deleted domain, amino-acid residues
Case 1 (ALL)	CL2	514–733 (220)
	CL6	585–620 (36)
	115	556–683 (129)
Case 2 (CML)	2K10	558–712 (155)
	2K19-1	517–573 (57) + 581–734 (154)
	2K19-2	493–660 (168)
	2K23	493–660 (168)
Case 3 (CML)	2L1	582–729 (148)

During electrophoretic analysis in some samples together with the full-length amplification products shorter fragments were detected. Alteration of PCR conditions (i.e. increase in temperature of primer annealing, decrease of elongation time) did not significantly affect the pattern of the PCR products. For further analysis the PCR products were cloned in pUC19 vector and sequenced. Analysis of those clones revealed that the changes in length are caused by deletions in the Dbl

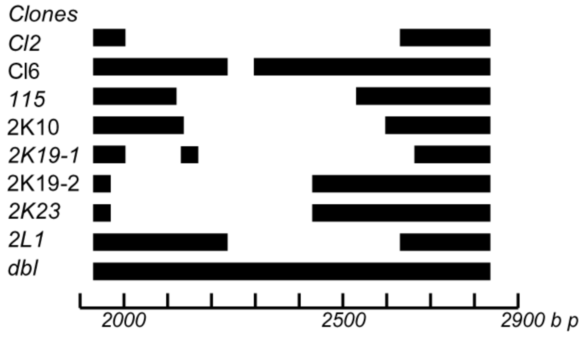

Figure 2. Localization of deletions in Dbl domain of *BCR/ABL* gene.

Numbers of clones are on the left. Numbering of nucleotides is according to human bcr protein mRNA 5′ end (Genbank, HUMBCRD, Accession: M24603).

domain. These deletions did not affect the reading frame. Localization of the deletions in comparison with the structure of full-length Dbl domain of the *BCR/ABL* gene is presented in the Fig. 2. The respective deletion-dependent alterations in the p210 BCR/ABL protein are shown in the Table 1.

It is well known that in 50–60% of CML patients simultaneous expression of p210 *BCR/ABL* and p185 *BCR/ABL* genes is observed (Saglio *et al.*, 1996; Lichty *et al.*, 1998). Our study found novel mutations in the Dbl region of p210 encoding transcripts of CML patients. It may be suggested that the different functional forms of the fusion proteins studied affect the factors determining cell morphology and this may influence the course of the disease. The results obtained show that a mutational change occurred in the Dbl region of the *BCR/ABL* gene giving rise to a p210 protein whose properties could be similar to those of p185. Long term monitoring of patients with such mutations as well as increasing the number of such cases may contribute to our understanding of progression in this disease and aid in patients management.

REFERENCES

Butturini A, Arlinghaus RB, Gale RP. (1996) *Leukemia Res.*; **20**: 523–9.

Chomczynski P, Sacchi N. (1987) *Anal Biochem.*; **162**: 156–9.

Chuang TH, Xu X, Kaartinen V, Heisterkamp N. (1995) *Proc Natl Acad Sci USA.*; **92**: 10282–6.

Clark SS, McLaughlin J, Timmons M, Pendergast AM, Ben Neriah Y, Dow LW, Crist W, Rovera G, Smith SD, Witte ON. (1988) *Science.*; **239**: 775–7.

Cortes JE, Talpaz M, Beran M, O'Brien SM, Rios MB, Stass S, Kantarjian HM. (1995) *Cancer.*; **75**: 464–70.

Gishizky ML. (1996) *Cytokines Mol Ther.*; **2**: 251–61.

Gribble SM, Sinclair PB, Grace C, Green AR, Nacheva EP. (1999) *Cancer Genet Cytogenet.*; **111**: 7–17.

Kawasaki SS, Coyone MY. (1988) *Proc Natl Acad Sci USA.*; **85**: 5698–2.

Konopka JB, Watanabe SM, Witte ON. (1984) *Cell.*; **37**: 1035–2.

Laneuville P. (1995) Abl tyrosine protein kinase. *Semin Immunol.*; **7**: 255-66.

Lichty BD, Keating A, Callum J, Yee K, Croxford R, Corpus G, Nwachukwu B, Kim P, Guo J, Kamel-Reid S. (1998) *Br J Haematol.*; **103**: 711–5.

Liu J, Campbell M, Guo JQ, Lu D, Xian YM, Anderson BS, Arlinghaus RB. (1993) *Oncogene.*; **8**: 101–9.

Martinelli G, Amabile M, Giannini B, Terragna C, Ottaviani E, Soverini S, Saglio G, Rosti G, Baccarani M. (2002) *Haematologica.*; **87**: 688–4.

Pane F, Frigeri F, Sindona M, Luciano L, Ferrara F, Cimono R. (1995) *Blood.*; **88**: 2410–4.

Quackenbush RC, Reuther GW, Pendergast AM. (1997) *Blood.*; **90**: 247a.

Saglio G, Pane F, Gottardi E, Frigeri F, Buonaiuto MR, Guerrasio A, de Micheli D, Parziale A, Fornaci MN, Martinelli G, Salvatore F. (1996) *Blood.*; **87**: 1075–80.

Wulf E, Deboben A, Bautz A, Faulstich H, Wieland Th. (1979) *Proc Natl Acad Sci USA.*; **76**: 4498–2.