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Rearrangement of *bcr* and *c-abl* Sequences in Ph-positive Acute Leukemias and Ph-negative CML – an Update

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A. Introduction

The molecular hallmark of the Philadelphia (Ph) translocation in CML is a rearrangement between the *c-abl* oncogene and a gene provisionally called *bcr* [7, 11, 13, 19]. As a consequence of this genomic recombination on the Ph chromosome, CML cells transcribe a chimeric 8.5-kb RNA species, consisting of both 5'*bcr* and *c-abl* sequences [6, 10, 20], that is translated into a p 210 *abl* protein [15, 16]. As yet the normal cellular functions of *c-abl* and *bcr* have not been characterized. However, *c-abl* belongs to a family of genes coding for proteins with associated tyrosine kinase activity; it is tempting to speculate that the *bcr* moiety of the hybrid *bcr/abl* molecule has altered the structure of the *abl* protein and thus changed its tyrosine kinase activity. Recently, we extended the analyses of *c-abl* and *bcr* sequences to Ph-negative CML and Ph-positive acute leukemias. The results are summarized below.

B. Ph-negative CML

About 5% of all CML patients exhibit no Ph chromosome in leukemic cells. While Ph-negative CML is associated with a generally less favorable course, it is widely accepted that this entity constitutes a heterogeneous group of prognostically distinct disorders [21]. In our studies we included only cases that met stringent criteria of CML [5]. As listed in Table 1, these patients usually lack *c-abl* and *bcr* rearrangements in Southern blot and in situ hybridization studies. However, in seven cases an involvement of both genes was established. Among these seven patients, two exhibited an involvement of chromosome region 9q34 on the cytogenetic level, i.e., t(8;9) and t(9;12) and this may represent masked complex Ph translocations.

Table 1. Ph-negative CML ($n = 31$ [a])

I	No *bcr* rearrangement, no *c-abl* translocation	23
II	*bcr/c-abl* rearrangement	7
III	*bcr* rearrangement, no *c-abl* translocation	1

[a] Data of some patients have been published elsewhere [1–5, 7, 11, 13].

The other cases showed no chromosomal abnormality, even when high-resolution banding techniques were used. Yet Southern blots revealed a *bcr* rearrangement, and in situ hybridization studies demonstrated a *c-abl* translocation toward chromosome 22. In one patient, Northern blot analysis exhibited the 8.5-kb *abl/bcr* transcript. Similar results have recently been reported by Morris et al. [17]. Leukemic cells of only one Ph-negative CML patient showed a *bcr* rearrangement without juxtaposition of *c-abl* sequences [3]. In leukemic cells of this patient Northern blots revealed a novel 7.3-kb *bcr* transcript.

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C. Ph-positive ALL and AML

Initially considered specific for CML, the Ph chromosome has been described in other hematopoietic neoplasias; in adult ALL the Ph translocation is the most frequent detectable chromosomal aberration, with an incidence of about 20% (van den Berghe, this volume). The question of whether Ph-positive acute leukemias represent distinct clinical entities or comprise CML patients initially diagnosed in the acute phase is still a matter of controversy [14]. Since Ph chromosomes in CML and acute leukemias are indistinguishable cytogenetically, we applied molecular approaches to further elucidate this problem.

Ten Ph-positive ALL patients exhibited a *bcr/c-abl* rearrangement comparable to Ph-positive CML (Table 2); moreover, Northern blots showed the 8.5-kb *abl/bcr* transcript in two of these ten cases that could be investigated. It may be sensible to assume that those patients are suffering from CML blast crisis. Three other Ph-positive ALLs likewise revealed a *bcr* rearrangement, but 3′*bcr* sequences, usually transferred to chromosome 9q+, have been deleted. The biological meaning of this observation remains obscure, but similar deletions have never been detected in Ph-positive CML.

Despite the presence of a Ph chromosome, a third group of ALL patients showed no *bcr* rearrangement (Table 2). Similar results have recently been reported by other investigators [9, 18]. In situ hybridization studies exhibited a *c-abl* translocation in three of our patients. The possibility remains that at least in some of the patients *c-abl* translocated to 5′ sequences of the *bcr* gene mapping outside the CML-specific cluster region. Since the entire *bcr* gene has recently been cloned (Mes-Masson et al., this volume), this problem can now be investigated directly. However, the observation that Northern blots of one of our ALL patients and of an ALL cell line [8, 9] detect normal-size *bcr* and *abl* transcripts argues against this interpretation.

Thus far we have analyzed four Ph-positive AML patients (Table 2). As in Ph-positive ALL, two cases exhibited *abl/bcr* rearrangements and thus may be regarded as in CML blast crisis. In situ hybridization studies of one variant Ph-positive AML patient lacking a *bcr* recombination showed *c-abl* sequences exclusively on chromosome 9 [12]. This case may be an example of yet another leukemic subgroup comprising cytogenetically defined "Ph-like" leukemias that exhibit no alteration of either *c-abl* or *bcr* sequences.

D. Discussion

These data, although still preliminary, emphasize the possible value of *c-abl* and *bcr* sequences in the subclassification of heterogeneous leukemic entities as Ph-negative CML or Ph-positive acute leukemias. However, these differences on the molecular level cannot readily be correlated with specific clinical, morphological, or immunological features. Thus, in contrast to a recent report based on five cases [17], we detected no significant distinctions in the clinical course of our 31 Ph-negative CML patients. The same holds true for Ph-positive ALL. While nine cases of childhood Ph-positive ALL investigated by us and others [8, 9, 18] exhibited no *bcr* rearrangement, the demonstration of a similar genomic configuration in four adult

Table 2. Ph-positive ALL and AML

Molecular hallmark		Ph-pos ALL ($n=23$[a])	Ph-pos AML ($n=4$[a])
I	*c-abl/bcr* rearrangement as in Ph-positive CML	10	2
II	Rearrangement of 5′*bcr* and *c-abl*, deletion of 3′*bcr*	3	–
III	No *bcr* rearrangement	10	2

[a] Data of some patients have been published elsewhere [8, 12].

cases [8] at least rules out a restriction of this molecular pattern to pediatric patients. Nevertheless, investigation of more cases with longer follow-up may finally unravel the possible clinical importance of molecular differences among these heterogeneous leukemic entities and supplement our rather incomplete understanding of what overall biological consequences are triggered by such genomic rearrangements as those discussed above.

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