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# Are *ABL* and *BCR* Imprinted?

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## INTRODUCTION

An increasing number of clinical observations and genetic experiments have shown that some parts of the genome behave differently depending on whether they are of paternal or maternal origin. This phenomenon is known as "genomic imprinting" and has been defined as "a reversible process whereby a gamete-specific modification in the parental generation can sometimes lead to functional differences between maternal and paternal genomes in diploid cells of the offspring" [1]. The accumulating evidence for its important role in cancer predisposition syndromes as well as for the pathogenesis of certain types of sporadic tumors prompted us to investigate whether imprinting may also be instrumental in selecting particular parental chromosome regions involved in balanced rearrangements, such as the leukemia-specific translocation t(9;22) [2]. Several reports have analyzed the expression, the methylation and the replication patterns of the two genes, ABL and BCR, on chromosomes 9 and 22, respectively, which are affected by this translocation [3-6]. Although not directly comparable, the results of these studies seem to invalidate our cytogenetic observations. We therefore review this controversial issue and provide some possible explanations for the contradictory results.

## Parental origin of affected chromosomes and genes

In our original work, we utilized the unique banding pattern polymorphisms of chromosomes 9 and 22 to analyze the parental origin of the translocated homologues. The results of this study indicated a preferential involvement of the paternal chromosome 9 and the maternal chromosome 22 [2]. The parental origin of the translocated chromosome 9 has only been determined in one additional case, in which the duplication of a cytogenetically normal, but *BCR/ABL*-rearranged chromosome 9 with somatic recombinations facilitated the proof of its paternal origin [7]. The parental origin of the rearranged *ABL* allele in patients with chronic myeloid leukemia (CML) and a t(9;22) was recently demonstrated with the help of a polymorphic marker in exon 11 of the *ABL*

coding sequence [6]. This polymorphism occurs in CML patients in the same frequency as in the normal population (8.5%). Thus, out of 135 CML patients, 19 were found to be heterozygous. It was shown that in a group of 11 informative patients, the paternal and the maternal *ABL* gene participated equally in the translocation [6]. Litz and Copenhaver [4] used rare P*vu*II and M*ae*II restriction site polymorphisms located within the major breakpoint cluster region (M-*BCR*) on chromosome 22 and found that the rearranged gene sequence was of paternal origin in the only three informative patients with CML.

The obvious discrepancy between the cytogenetic and the molecular genetic results was attributed to the possible, but yet unproven, occurrence of homologous mitotic recombinations which might have taken place between the centromere and the respective gene rearrangement. However, such somatic homologous recombinations seem to be extremely rare. At the most, such exchanges could perhaps account for rare deviations in the nonrandom pattern predicted by the cytogenetic analysis, but they are certainly not sufficient to explain the random involvement established with molecular genetic means.

Perhaps a more likely explanation relates to the selection criteria of the patients studied. Our cytogenetic analyses were performed on 15 patients who were, depending on the availability of the parents, selected randomly. The molecular genetic analyses, on the other hand, had to rely on the additional presence of rare restriction site polymorphisms. Despite the belief that these restriction site polymorphisms should not affect the translocation event, there nevertheless remains a remote possibilitiy that this selection criterion could introduce a bias. A similar situation exists in patients with Beckwith-Wiedemann syndrome (BWS) and an A*va*II restriction site polymorphism which is located in the ninth exon of the insulin-like growth factor 2 gene (IGF2) [8]. BWS patients without uniparental disomy had allele frequencies similar to those of normal controls, whereas this particular *Ava*II allele was significantly more common in patients with uniparental disomy of the relevant region. It is therefore conceivable that particular allelic variants influence the levels of transcription and as such may alter their mutational vulnerability, since it has been shown that transcription enhances the likelihood of recombination [8, 9]. In this context it should also be pointed out that in comparison with healthy controls, expression of the normal *ABL* allele was either significantly stronger or weaker in the CML patients [10]. However, whether this difference was in any way related to the parental origin of the translocated alleles has not been demonstrated.

Finally, the translocation breakpoints are scattered over a wide range, particularly within the *ABL* gene. Nevertheless, there are strong indications for their nonrandomness [11]. Therefore, it cannot yet be completely excluded that the locations of these breakpoints may differ depending on whether they occur on the paternal or maternal chromosome.

## Expression

Monoallelic expression is considered to be one of the crucial hallmarks of an imprinted gene [1, 12]. Using the above-described polymorphism, Melo et al. [10] have therefore also analyzed the expression status of *ABL* in healthy controls, but found no evidence for monoallelic expression in the peripheral blood or individual CFU-GM colonies. Riggins et al. [13] have exploited a highly polymorphic CGG repeat in the 5' untranslated region

of the BCRgene. They have also noted biallelic expression in both a complex mixture of whole blood cells as well as fibroblast colonies which were derived from single cells [13]. In a similar approach Fioretos et al. [5] used a transcribed *Bam*HI polymorphism in the first *BCR* exon to demonstrate that both alleles are expressed in the peripheral blood of two normal individuals.

However, are these studies really sufficient to exclude the possibility that *ABL* and *BCR* are nevertheless expressed monoallelicly in specific instances? Repression and/or expression of imprinted alleles is not an all or nothing effect [14, 15]. Particularly with regard to the t(9;22), it has been shown that a considerable proportion of colonies derived from Ph-positive cells do not contain detectable levels of *BCR/ABL* hybrid mRNA [16, 17]. Imprinted genes may also switch between mono – or biallelic expression in a very time – and tissue-specific manner [1, 12] Moreover, the phenomenon of imprinting may also depend on the genetic background and it has been shown that monoallelic expression can be inherited as a polymorphic trait [15]. Owing to the promoter-specific transcription, imprinting and lack of imprinting can even occur both within a single gene and in a single tissue [18]. For example, parent-specific monoallelic transcription of the IGF2 gene occurs exclusively from the promoters P2, P3 and P4, whereas recruitment of the promoter P1 is responsible for biallelic expression [18]. It is therefore intriguing that in the mouse, two major *ABL* mRNAs are found, the types I and IV which correspond to the types 1a and 1b in the human [19, 20]. They derive from two different 5 exons, which are initiated from two separate promoters, and a set of common exons. Interestingly, the levels of expression of the mouse type IV mRNA remain constant among tissues, whereas the type I mRNA varies in abundance [19]. Considering the rather simple RT-PCR approaches applied for the expression assays, it can definitely not be completely excluded that such specific and subtle imprinting mechanisms are operative in the transcription regulation of the *ABL* and *BCR* genes.

## Methylation

In the normal *ABL* allele, the promoter of exon 1a (Pa) is nested within the transcriptional unit of the promoter of exon 1b (Pb) [21]. This situation remains analogous in the rearranged *BCR/ABL* hybrid gene in most cases of CML [21]. Therefore, one might expect the type 1a normal mRNA to be transcribed from the *BCR/ABL* hybrid gene. Nevertheless, 1a transcripts are only found in cell lines which also contain a normal *ABL* allele [21]. The apparent inactivation of the nested Pa is associated with progressive allele-specific de novo methylation, a so far unique and extraordinary situation which, however, is in line with the concept of imprinting [21]. Zion et al. [21] have therefore proposed that masked imprinting of *ABL* may become apparent only after chromosomal translocation.

## Replication

*ABL* and *BCR* replicate asynchronously, a feature which is also regarded as highly specific for imprinted genes [22]. Moreover, expression status and relative timing of replica-

tion of individual genes is considered to be strictly correlated [23]. We are currently developing a method which should allow us to determine the parental origin of the early – and late – replicating allele of any asynchronous replicating gene sequence. This method is based on the fact that human triploid fetuses and partial hydatidiform moles are composed of either two maternal and one paternal or two paternal and one maternal haploid chromosome sets. Preliminary experiments with probes from the *ABL* and *BCR* gene regions not only confirmed that these two genes replicate asynchronously, but also that they display a reciprocal replication pattern.

Finally, another intriguing observation suggests that other loci located in the vicinity of *ABL*, such as the genes for the ABO blood group, may be subjected to imprinting effects in hematological malignancies [24].

## CONCLUSIONS

Given all these arguments, we believe that there remains enough evidence to warrant further investigation of this currently rather controversially discussed topic. The issue of imprinting has evolved into an increasingly complex one, which has to take into account the parental origin of chromosomes or genes, but also their allele-specific methylation pattern, expression status and replication behavior. Before a final decision can be made about the possible imprinting status of *ABL* and *BCR*, more data need be collected. In particular, the parental origin of the translocated and nontranslocated chromosomes of unselected patients, the expression status of the two alleles in various tissues during different stages of development, both in pure cell populations and in single cells, and a possible promoter-specific monoallelic transcription have to be analyzed. Moreover, the expression of *normal* blood cells from leukemic patients should be analyzed separately, since they may perhaps reveal expression/replication/methylation patterns which might differ from those observed in both healthy individuals and/or leukemic cells.

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