No Evidence for Genomic Imprinting of the Human BCR Gene

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Chronic myeloid leukemias and 5% to 20% of acute lymphoid leukemias are characterized by the Philadelphia chromosome, a reciprocal chromosomal translocation, t(9;22)(q34;q11), generating BCR-ABL and ABL-BCR fusion genes. Cytogenetic studies have recently shown a preferential involvement of the paternally derived chromosome 9 and the maternally derived chromosome 22 in this translocation, indicating that imprinting might be involved in the formation or selection of the translocation. In this study, we have identified a BamHI polymorphism in the coding region of BCR exon 1, allowing us to investigate whether both BCR alleles are transcribed. By using a reverse transcriptase-polymerase chain reaction assay, we show that both BCR alleles are expressed in the peripheral blood cells of normal individuals.

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RESULTS

Previous unpublished experiments aimed at isolating BCR cDNAs had shown a BamHI polymorphism in BCR exon 1. To assess the frequency of this polymorphism and to identify individuals heterozygous for this polymorphism, DNAs were isolated from 30 unrelated individuals. Hybridization to BamHI-digested DNA identified a two-allele polymorphism (A1, 6.8 kb; A2, 1.0 kb; Fig 1A). The polymorphism is caused by a C to A transversion within the BamHI restriction

RT-PCR. Of the 30 individuals studied by Southern blotting, fresh peripheral blood, which allowed the extraction of intact RNA, was available from 2 individuals heterozygous for the polymorphic BamHI restriction enzyme site, and from 3 individuals homozygous for the presence of the BamHI site. RNA extraction and RT-PCR using 3 to 5 μg of RNA were essentially as described. Primers used were from BCR exon 1 and 3 with the following sequences: BEX1 5’ GAGGGTTCTCCGGGTAAGGCC 3’ and BEX3R 5’ CCAATTTCCTCATCTCCAAGCC 3’. The cDNA was amplified for 35 cycles with denaturation at 98°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes. The PCR product of 1,125 bp was purified by phenol/chloroform/isoamylalcohol extraction and ethanol-precipitated. Half of the product was further digested with BamHI to investigate whether a BamHI site was present. All samples were purified and digested using identical procedures to avoid misinterpretation caused by incomplete digestion. Undigested and digested products were loaded next to each other and electrophoresed through a 1.5% agarose gel.

MATERIALS AND METHODS

Polymorphism study. A total of 30 unrelated individuals were studied for the presence of a BamHI polymorphism in BCR exon 1. DNA was isolated from peripheral blood using standard procedures. Ten micrometers of DNA was digested with BamHI (BRL, Gaithersburg, MD), size-separated using a 0.7% agarose gel, transferred to nylon membranes (Magna NT; Micron Separations Inc, Westborough, MA), and hybridized to an 0.6-kb Xho I/Bgl II probe corresponding to a part of BCR exon 1. Hybridization and washing conditions were as previously described.

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site at nucleotide position 1017. In addition to the two-allele polymorphism, a \textit{BamHI} fragment of 5.8 kb is observed in individuals homozygous or heterozygous for the \textit{BamHI} restriction site. This is caused by the presence of an inverted repeat within the \textit{BCR} gene. The probe detects this repeat and hybridizes to \textit{BCR} sequences immediately 5' and 3' of the polymorphic restriction site (Fig 1B). Thus, in individuals lacking the \textit{BamHI} site, a doublet of 6.8 kb (A1) is observed. Individuals heterozygous for the presence of the \textit{BamHI} site show bands of 6.8 kb (A1) and 1.0 kb (A2), whereas individuals with two alleles containing the \textit{BamHI} site show a single band of 1.0 kb (A2) with double intensity, reflecting contributions of the two alleles, in addition to the 5.8-kb fragment. The allele frequencies (n = 30) found for A1 and A2 were 0.30 and 0.70, with an observed heterozygosity frequency of 0.40. Mendelian inheritance was observed in 7 two-generation families.

To analyze whether both \textit{BCR} alleles are expressed in the peripheral blood of normal individuals, RT-PCR was used to amplify the region surrounding the \textit{BamHI} polymorphism. Primers were from exon 1 and exon 3; exon 1 is located at a distance of approximately 70 kb from exon 3 on a genomic level. \textit{BamHI} digestion of the 1,125-bp RT-PCR product showed an expected size reduction of 121 bp in individuals homozygous for the \textit{BamHI} restriction site, whereas heterozygous individuals showed two bands of 1,125 and 1,004 bp, respectively (Fig 2). The latter finding is consistent with the amplification of both \textit{BCR} alleles in the RT-PCR, thus indicating that both alleles are expressed and that the \textit{BCR} gene is not subjected to genomic imprinting. The RT-PCR assay used in this study is a qualitative method. Thus, the relative differences in band intensity in Fig 2, lanes 4B and 5B, should not be interpreted as differences in expression of the two alleles. Moreover, heteroduplex formation of the amplified RT-PCR products in individuals heterozygous for the \textit{BamHI} site is likely to render a fraction of the RT-PCR products refractory to \textit{BamHI} digestion.

**DISCUSSION**

Genomic imprinting, or parental allele-specific expression of genes, most likely plays an important role in the manifestation of a variety of genetic diseases, including disorders such as Prader-Willi and Angelman syndromes. Also, allele-specific loss of heterozygosity has been described in several tumors, eg, Wilms' tumor, retinoblastoma, and rhabdomyosarcoma, providing indirect evidence that imprinting is involved in tumor development (reviewed in Feinberg). The recent finding that the t(9;22) in CML shows a preferential involvement of the maternal chromosome 22 and of the paternal chromosome 9 is the first study to implicate that parental imprinting mechanisms might be involved in reciprocal chromosomal translocations. By studying polymorphisms of the C-band–positive heterochromatin in chromosome 9 and the nucleolus organizing region in chromosome 22, it was shown that in 11 informative cases the chromosome 9 involved in the translocation was of paternal origin, whereas chromosome 22 was derived from the maternal copy. Different mechanisms may account for this observa-
tion, but the most straightforward explanation, as also suggested\textsuperscript{9,10} would be that the BCR and ABL genes are imprinted. Assuming that BCR is normally only expressed on the maternal chromosome 22 and the ABL gene on the paternal chromosome 9, a BCR-ABL gene would only be expressed after translocation of ABL to the maternal but not the paternal BCR gene. Even if the t(9;22) were to occur randomly, cells containing the translocation of the maternal BCR might have a selective advantage caused by an active BCR-ABL gene. To explain the preferential involvement of the paternal chromosome 9, one could assume that the ABL gene is expressed only on the paternal chromosome 9. This would lead to expression of an ABL-BCR fusion gene, and subsequent selection of these cells only if the paternal ABL is translocated. However, this implies that an ABL-BCR messenger is necessary for the leukemogenic process, something that is contradicted by the finding that BCR-ABL in itself can induce leukemia in transgenic mice\textsuperscript{20,21} and upon retroviral transduction into mouse bone marrow cells followed by bone marrow transplantation.\textsuperscript{22,23} Alternatively, the selection of paternal chromosome 9 and of maternal chromosome 22 in the t(9;22) might occur if only fusion of normally expressed BCR and ABL genes, ie, the maternal BCR gene and paternal ABL gene, would result in the expression of a BCR-ABL gene.

To the best of our knowledge, no polymorphisms in the coding regions of the BCR and/or ABL genes have been reported previously, which would enable one to investigate whether these genes are normally expressed in a monallelic or biallelic fashion. In this study, we describe a BanHI polymorphism in the first BCR exon and, by using an RT-PCR assay, we have been able to show that the BCR gene is expressed on both alleles in the peripheral blood of normal individuals. Therefore, mechanisms other than parental-specific gene expression of the BCR gene are most probably responsible for the preferential involvement of the maternal chromosome 22 and the paternal chromosome 9 in the t(9;22). One such possible mechanism is parental-specific DNA methylation of certain chromosomal regions, making one of the two homologous chromosomes more susceptible to rearrangement.\textsuperscript{9}

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