BCR-ABL Rearrangements in Children With Philadelphia Chromosome-Positive Chronic Myelogenous Leukemia

By Igor Auer, Anna Butturini, and Robert Peter Gale

Leukemia cells from adults with Philadelphia (Ph')-chromosome positive chronic myelogenous leukemia (CML) have a characteristic molecular rearrangement between the BCR and ABL genes whereby major breakpoint cluster region (Mbcr) exons 2 or 3 are joined to ABL exon II. Ph'-chromosome positive CML is uncommon in children and it is unknown whether these children have similar rearrangements. We studied 17 children with Ph'-chromosome positive CML. Five were studied for Mbcr rearrangement using Southern blotting, nine for the presence of chimeric BCR-ABL mRNA using reverse transcription and polymerase chain reaction, and three for both. All eight children studied by Southern blotting had BCR rearrangement. Of 12 children in whom BCR-ABL mRNA was studied, 10 had Mbcr exon 2 joined to ABL exon II, one had Mbcr exon 3 joined to ABL II, and one had both Mbcr-ABL junctions. These data indicate a similarity to adult CML. However, mRNA processing in children may preferentially splice Mbcr exon 2 to ABL exon II. No child had Mbcr exon 1 joined to ABL exon II, the rearrangement typical of childhood Ph'-chromosome positive acute lymphoblastic leukemia.

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MATERIALS AND METHODS

Subjects. Seventeen children with Ph'-chromosome positive CML in chronic phase aged 4 to 16 years at diagnosis were studied. Ten were boys and seven were girls. Median age at diagnosis was 7 years. White blood cell count at diagnosis was between 33 x 10^9/L and 644 x 10^9/L (median 128 x 10^9/L). Nucleated cells were isolated from blood or bone marrow by standard techniques and stored in liquid nitrogen. Approval was obtained from the Institutional Review Board for these studies. Informed consent was obtained from the parents of all subjects with BCR-ABL mRNA was studied, 10 had Mbcr exon 2 joined to ABL exon II, one had Mbcr exon 3 joined to ABL II, and one had both Mbcr-ABL junctions. These data indicate a similarity to adult CML. However, mRNA processing in children may preferentially splice Mbcr exon 2 to ABL exon II. No child had Mbcr exon 1 joined to ABL exon II, the rearrangement typical of childhood Ph'-chromosome positive acute lymphoblastic leukemia.

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*Mbcr exons 2, 3, and 4 are equivalent to BCR exons 13, 14, and 15, respectively.

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vortexed while thawing. Freezing and thawing were repeated until pellets were completely dissolved. RNA was obtained by phenol-chloroform extraction and precipitation in ethanol. Samples were dissolved in deionized sterile water and stored in liquid nitrogen.

Reverse transcription. Moloney murine leukemia virus-reverse transcriptase (BRL) was used. ABL antisense primer, 20 pmol, and 20 U of RNAsin (Promega, Madison, WI) were added and reverse transcription performed under recommended conditions for 1 hour in a total volume of 20 μL. Negative and positive controls were included in each experiment. RNAs isolated from cell lines with Mbcr 3-ABL II and BCR 1-ABL II junctions were used as positive controls.

Polymerase chain reaction (PCR). Primers and probes were kindly provided by Dr E. Canaani (Weizmann Institute of Science, Rehovot, Israel). Sequence data are reported. Ten microliters of the reverse transcription reaction mixture and 2.5 U of Taq DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT) were used for each PCR. Two amplifications were performed with each sample: in one, primers specific for BCR 1 and ABL II exons were used; in the second, primers for Mbc 2 and ABL II exons were used. In the former, only cDNA fragments with BCR 1 joined to ABL II are amplified, resulting in a 190-bp product. In the latter, cDNA fragments with Mbc exons 2 or 3 joined to ABL II are amplified; the resulting products are 244 and 319 bp, respectively. PCR was performed in a Perkin-Elmer PCR-machine for 30 cycles: denaturation at 95°C for 1 minute, annealing at 55°C for 30 seconds, and elongation at 72°C for 90 seconds.

Samples amplified with BCR 1-ABL II and Mbc 2-ABL II primers were electrophoresed on separate 3% agarose gels and alkaline-transferred overnight to Zeta-Probe membranes. Probes were labeled with 32P using T-4 kinase (BRL). Membranes were prehybridized for 3 hours at 55°C in 5X SSPE, 1% SDS, and 0.1 mg/mL salmon-sperm DNA. Probe, 106 cpm/mL, was added and hybridization was performed overnight at 5°C below the dissociation temperature. Membranes were washed twice for 10 minutes in 2X SSPE and 1% SDS at room temperature and once in 5X SSPE, 1% SDS at 65°C before exposure.

BCR 1-ABL II junction specific probe was hybridized to the BCR 1-ABL II membrane. Mbc 2-ABL II junction specific probe was hybridized to the Mbc 2-ABL II membrane. After film exposure the membrane was stripped by washing twice in 0.2X SSC and 0.1% SDS at 95°C for 15 minutes and rehybridized with the Mbc 3-ABL II junction specific probe.

RESULTS AND DISCUSSION

The eight children studied by Southern blotting had BCR rearrangements. The breakpoint location was between Mbc exons 2 and 3 in four children where it was mapped. In 10 of 12 children studied by PCR, the chimeric BCR-ABL mRNA was spliced to join Mbc exon 2 to ABL exon II. In one, Mbc exon 3 was joined to ABL exon II. In the final child, both BCR-ABL junctions were detected indicating alternative RNA splicing (Figs 1 and 2). No child had BCR 1 joined to ABL II.

BCR-ABL rearrangements are probably important in the pathogenesis of Ph1-chromosome positive CML and ALL. It is not clear whether differences between these two diseases result from different rearrangements (BCR 1-ABL II vs Mbc 2 or 3-ABL II). BCR 1-ABL II is more efficient in transforming cells in vitro than Mbc 2 or 3-ABL II. Also, the former preferentially causes acute leukemia in transgenic mice, while the latter causes different malignan-

![Fig 1. Two representative samples hybridized with the Mbc 2-ABL II and Mbc 3-ABL II probes. The 319-bp band corresponds to a junction between Mbc exon 2 and ABL exon II, and the 244-bp band corresponds to a junction between Mbc exon 3 and ABL exon II.](image)

![Fig 2. Amplification of BCR-ABL cDNA by PCR. Bands of 319 and 244 bp in lane 1 correspond to mRNA with Mbc 2-ABL II and Mbc 3-ABL II junctions, indicating alternative RNA splicing. This subject had an Mbc 3-ABL II junction on Southern blotting of DNA.](image)
in children with Ph'-chromosome positive CML, Mbcr 2 or 3 are joined to ABL II. These data indicate that in children, Ph'-chromosome positive ALL and CML are distinct. Also, molecular analysis distinguishes between children with Ph'-chromosome positive ALL and those with lymphoid acute phase of CML.

In approximately one third of adults with Ph'-chromosome positive CML, Mbcr 2 is joined to ABL II. In the other two thirds, Mbcr 3 is joined to ABL II.\textsuperscript{20-23} Therefore, it is interesting that almost all children with CML had the Mbcr 2-ABL II junction. Whether adult cases of CML with different BCR breakpoint locations differ clinically is controversial. Some studies suggest a longer duration of chronic phase in persons with Mbcr 2-ABL II junctions than in those with Mbcr 3-ABL II junctions,\textsuperscript{23,26} but others found no difference.\textsuperscript{21,24} In our prior review of children with CML we found no evidence that they have a briefer chronic phase than adults.\textsuperscript{24}

In conclusion, we found that children with Ph'-chromosome positive CML have a different BCR-ABL rearrangement than those children with Ph'-chromosome positive ALL. This rearrangement is similar to that found in adults with CML. These data suggest a different pathogenesis of these two diseases. Why a predominance of children with CML had a chimeric BCR-ABL mRNA in which Mbcr exon 2 is spliced to ABL exon II is unclear.

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