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

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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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A CHARACTERISTIC molecular rearrangement between BCR and ABL genes occurs in almost all adults with Philadelphia (Ph')-chromosome positive chronic myelogenous leukemia (CML). Here, most of ABL is joined to a truncated BCR. The break in ABL typically occurs in the long first intron between alternative exons Ia and Ib. The break in BCR typically occurs in a 5.8-kb major breakpoint cluster region (Mbcr) in the middle of the gene. Most often, the break is between Mbcr exons 2 and 3 or 4. The rearranged BCR-ABL gene is transcribed into a chimeric 8-kb mRNA and translated into a chimeric 210-kd BCR-ABL protein with increased tyrosine kinase activity. In this process, ABL exon Ia is spliced out of the chimeric mRNA resulting in Mbcr exons 2 or 3 being joined to ABL exon II.

Leukemia cells from about one half of adults with Ph'-chromosome positive acute lymphoblastic leukemia (ALL) have a BCR-ABL rearrangement similar to adults with CML. The others have a different rearrangement whereby BCR exon 1 is joined to ABL exon II. Most children with Ph'-chromosome positive ALL have this rearrangement. However, few children have a break in the Mbcr region. The BCR 1-ABL II rearrangement occurs only rarely in adults with Ph'-chromosome positive CML.

Ph'-chromosome positive CML is uncommon in children. We wondered whether children with this disease have the same BCR and ABL rearrangement characteristic of adult Ph'-chromosome positive CML or whether they resemble children with Ph'-chromosome positive ALL.

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 \times 10^9/L and 644 \times 10^9/L (median 128 \times 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 indicated this fact.

DNA isolation and Southern blotting. DNA was phenol-chloroform extracted, ethanol precipitated, and dissolved in TE by standard techniques. Fifteen micrograms of DNA was digested with BglII Bethesda Research Laboratories (BRL), (BRL, Gaithersburg, MD) (eight subjects) or a combination of HindIII (BRL) and BamHI (USB, Cleveland, OH) according to the manufacturer's instructions. After digestion, samples were electrophoresed on 0.75% agarose gels and alkaline-transferred overnight to Zeta-Probe membranes (Bio-Rad, Richmond, CA). A 1.2-kb genomic DNA probe from the Mbcr region (BCR-1; Oncogene Sciences, Manhasset, NY) was labeled with \textsuperscript{32}P using the random primer labeling kit (BRL). Membranes were prehybridized overnight at 65°C in 5X SSPE (saline sodium phosphate, EDTA), 1% sodium dodecyl sulfate (SDS), and 0.1 mg/mL salmon-sperm DNA. Labeled probe, 1 \times 10^6 cpm, was added and hybridization performed at 65°C overnight. Membranes were washed twice in 2X SSPE and 0.5% SDS and once in 0.5X SSPE and 0.5% SDS for 45 minutes each. Controls included DNA from two subjects with CML and one with Ph'-chromosome positive ALL.

RNA isolation. Total cellular RNA was isolated as described. Cells were lysed in 5 mol/L guanidine monothiocyanate, 10 mmol/L EDTA, 50 mmol/L Tris pH 7.5, and 8% β-mercaptoethanol. RNA was precipitated with 4 mol/L LiCl, incubated overnight at 4°C, and pelleted by centrifugation at 11,000g for 90 minutes. Pellets were resuspended in 3 mol/L LiCl and recentrifuged for 1 hour. Pellets were dissolved by vortexing in 0.1% SDS, 1 mmol/L EDTA, and 10 mmol/L Tris pH 7.5. To facilitate resuspension, pellets were frozen in solubilization buffer and

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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.

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 Mbcr 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 Mbcr 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 Mbcr 2-ABL II primers were electrophoresed on separate 3% agarose gels and alkaline-transferred overnight to Zeta-Probe membranes. Probes were labeled with °P 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, 10° cpmlmL, 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. Mbcr 2-ABL II junction specific probe was hybridized to the Mbcr 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 Mbcr 3-ABL II junction specific probe.

RESULTS AND DISCUSSION

The eight children studied by Southern blotting had BCR rearrangements. The breakpoint location was between Mbcr 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 Mbcr exon 2 to ABL exon II. In one, Mbcr exon 3 was joined to ABL exon II. In the final child, both BCR-ABL junctions were detected indicating alternative splicing (Figs 1 and 2). No child had BCR 1 joined to ABL II.

BCR-ABL rearrangements are probably important in the pathogenesis of Ph'-chromosome positive CML and ALL. It is not clear whether differences between these two diseases result from different rearrangements (BCR 1-ABL II vs Mbcr 2 or 3-ABL II). BCR 1-ABL II is more efficient in transforming cells in vitro than Mbcr 2 or 3-ABL II.20,21 Also, the former preferentially causes acute leukemia in transgenic mice,22 while the latter causes different malignan-
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.26,27 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,27,28 but others found no difference.29,30 In our prior review of children with CML we found no evidence that they have a briefer chronic phase than adults.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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REFERENCES

8. Bartram CR: Rearrangement of the c-ab1 and bcr genes in Ph-negative CML and Ph-positive acute leukemias. Leukemia 2:63, 1988
24. Elefanty AG, Hiriharan IK, Cory S: bcr-ab1, the hallmark of chronic myeloid leukemia in man, induces multiple haemopoietic neoplasms in mice. EMBO J 9:1069, 1990


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