Common Region of ALL-1 Gene Disrupted in Epipodophyllotoxin-related Secondary Acute Myeloid Leukemia

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Abstract

Translocations at chromosomal band 11q23 characterize most de novo acute lymphoblastic leukemias (ALL) of infants, acute myeloid leukemias (AML) of infants and young children, and secondary AMLs following epipodophyllotoxin exposure. The chromosomal breakpoints at 11q23 have been cloned from isolated cases of de novo ALL and AML. Using an 859-base pair BamHI fragment of human ALL-1 complementary DNA that recognizes the genomic breakpoint region for de novo ALL and AML, we investigated two cases of secondary AML that followed epipodophyllotoxin-treated primary B-lineage ALL. In the first case, the translocation occurred between chromosomes 9 and 11 and the breakpoint at 11q23 localized to the same 9-kilobase region of the ALL-1 gene that is disrupted in most of the de novo leukemias. In the second case the translocation was between chromosomes 11 and 19. The breakpoint occurred outside of the ALL-1 breakpoint cluster region.

Introduction

Two distinct forms of secondary AML2 occur as late effects of cancer treatment. One form is a complication of therapy with alkylating agents and is characterized by myelodysplasia, a mean latency period of 5 to 7 years, and complete or partial monosomy of chromosomes 5 or 7 (1). The other form of secondary AML is associated with the epipodophyllotoxins, teniposide and etoposide (2–5). It is characterized by a relatively shorter latency period, French-American-British classification M4 or M5 morphology, and translocations involving chromosomal band 11q23. The most common 11q23 translocation in epipodophyllotoxin-related secondary AML is the t(9;11)(p21;q23) (6) but variant reciprocal partner chromosomes including 1, 2, 16, 17, and 19 have been observed (7).

Chromosomal translocations at 11q23 also are involved in de novo pediatric ALL and AML. The most common of the de novo translocations at 11q23 is the t(4;11)(q21;q23), found in a majority of the ALLs in infants less than 1 year old (8–10). Variant reciprocal translocations nonrandomly fuse chromosomal band 11q23 with partner chromosomes including 1, 6, 9, 10, or 19 and occur in lymphoblastic, myelomonocytic, or monocytic (French-American-British classification M4 and M5) leukemia in infants and young children (6, 11–15).

The chromosomal breakpoints at 11q23 recently have been cloned from isolated cases of de novo ALL and AML (15–19). The gene on 11q23 encompassing the breakpoints has been called ALL-1, MLL, or Htrx1 (15–19). The majority of the breakpoints identified by Southern blot are located within a 9-kilobase BamHI fragment of genomic DNA that spans exons 5–11 (17, 18, 20–22). Occasional breakpoints at chromosomal band 11q23 located outside of this region may involve either another site in ALL-1 or the recently identified, more telomeric rck gene (13, 23).

During 1986–1992, 235 children with ALL were treated on a Dallas-Fort Worth protocol that included etoposide during consolidation and maintenance. Ten cases of secondary AML have been reported at intervals of 23–68 months from diagnosis of the ALL (7). The secondary AML marrow specimens from two of these patients were available for molecular analysis. In the present study we map the translocation breakpoint at 11q23 in one secondary AML to the same 9-kilobase BamHI fragment of the ALL-1 gene commonly disrupted in de novo acute leukemia.

Materials and Methods

The diagnoses of ALL and AML were made by morphological and immunohistochemical examination of the marrow and by fluorescence-activated cell sorter analysis with standard monoclonal antibodies (7). Mononuclear cells frozen in liquid nitrogen were obtained from the Pediatric Oncology Group Leukemia Bank. Informed consent was obtained for the performance of molecular analyses. Genomic DNA and total cellular RNA were isolated using 4 M guanidine isothiocyanate–5.7 M CsCl gradients (24, 25). Five µg of genomic DNA were digested to completion with 15 units of BamHI (Bethesda Research Laboratories, Bethesda, MD), size fractionated on 0.8% agarose gels, and transferred to nitrocellulose using standard methodology. The filters were hybridized with an 859-base pair BamHI fragment of human ALL-1 complementary DNA that was radiolabeled with [32P]dCTP by the nick translation method. The 859-base pair BamHI fragment of ALL-1 complementary DNA spans exons 5–11, the region of the ALL-1 gene breakpoint cluster for de novo ALL and AML (18).

Results

The first case of secondary AML occurred 31 months after the diagnosis of the primary B-lineage ALL in a 3.6-year-old male, was of M5a morphology, and showed a (9;11)(p22;q23) karyotype (7). Southern analysis of BamHI-digested genomic DNA with the B859 probe showed both the normal allele and two additional bands consistent with both derivative chromosomes that had resulted from the translocation (Fig. 1). The second case of etoposide-related secondary AML, also morphologically M5a, occurred 16 months after ALL and showed a t(11;19)(q23;p13). The primary B-lineage leukemia was diagnosed at age 2.4 years. In this case the translocation breakpoint at 11q23 was not located within the ALL-1 breakpoint cluster region.

Discussion

We have demonstrated by Southern blot analysis that the location of a breakpoint on 11q23 in an epipodophyllotoxin-associated secondary t(9;11) AML falls within the same breakpoint cluster region of the ALL-1 gene as the breakpoints in de novo AML and ALL. De novo pediatric AMLs of the M4 and M5 morphological subtypes that occur during infancy have been associated with maternal marijuana and ethanol use and the exposure of either parent to pesticides (26–28). Many infant AMLs have translocations at 11q23, suggesting that
The finding of a common breakpoint cluster region in epipodophyllotoxin exposure to environmental toxins may play some role in pathogenesis. The mechanism of cytotoxicity of the epipodophyllotoxins involves the inhibition of topoisomerase II. Topoisomerase II is a nuclear enzyme that catalyzes and regulates both DNA cleavage and ligation in multiple cellular processes including DNA replication, transcription, and recombination (29, 30). Treatment with epipodophyllotoxins causes DNA breakage that is related to the formation of a stable region also has been reported in one case of de novo AML of infancy (13). That etoposide-related secondary AMLs may not be limited to AML, inasmuch as one case of ALL with a t(4:11) has been described after treatment of an osteosarcoma with etoposide (33). Thus, although this group of leukemias with 11q23 breakpoints may share common etiological and cytogenetic features, they do display phenotypic, clinical, and molecular heterogeneity. Cloning of the translocation junction sequences in epipodophyllotoxin-associated AMLs with the t(9;11) and other 11q23 variant translocations may explain the basis for the heterogeneity.

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References


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