Potential Topoisomerase II DNA-binding Sites at the Breakpoints of a t(9;11) Chromosome Translocation in Acute Myeloid Leukemia

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Abstract

We have examined a t(9;11)(p22;q23) chromosome translocation in an acute myeloid leukemia of an infant. The breakpoints on the two chromosomes occurred within introns of the involved genes: AF-9 on chromosome 9, and ALL-1 on chromosome 11. Sequence analysis identified heptamers flanking the breakpoints on both chromosomes 9 and 11, suggesting that the V-D-J recombinase was involved in the translocation. The presence of an N-region between the two chromosomes supports the hypothesis that a mistake in V-D-J joining was involved in the genesis of the translocation and indicates that terminal deoxynucleotidyl transferase was expressed in the cells from which this acute myeloid leukemia originated. In addition, potential topoisomerase II DNA-binding sites were found near the breakpoints of both chromosomes, suggesting the involvement of altered topoisomerase II activity in this translocation. Altered topoisomerase II activity in the presence of an active V-D-J recombinase may be a pathogenic mechanism of acute myeloid leukemia with rearrangements at 11q23.

Introduction

Approximately 70% of ALLs in infants, 60% of AMLs in infants, 90% of monoblastic AML in young children, and 6% of AML and ALL in older children and adults have abnormalities of chromosomal band 11q23 (1-4). More than 50% of the cases of topoisomerase II inhibitor-associated secondary AML also have abnormalities of chromosomal band 11q23 (5, 6). Translocations involving chromosome 11 at band q23 and at least 13 different reciprocal partners have been described (4, 7).

The most common 11q23 translocation in both de novo AML during infancy and secondary AML is the t(9;11)(p21;q23) (8-10). Most 11q23 breakpoints occur within a discrete 8-kilobase cluster region of the ALL-1 gene (11-14). The human ALL-1 gene has regions of homology with the Drosophila Trithorax gene; i.e., the positive regulator of homeobox genes within the Antennapedia and Bithorax complexes and regulates thoracic body segmentation (15, 16). The presence of zinc finger motifs in the ALL-1 product suggests that the gene encodes a DNA-binding transcription factor protein (15, 16), but the role of ALL-1 in hematopoiesis and leukemogenesis is unknown (17). The sequence of the AF-9 gene also suggests that the AF-9 protein is localized in the nucleus (18).

Secondary AMLs that are characterized by translocations involving chromosomal band 11q23 are associated with chemotherapeutic use of inhibitors of topoisomerase II, most commonly the epipodophyllotoxins, etoposide and teniposide (10, 19, 20). Secondary AML also occurs after therapy with the intercalating topoisomerase II inhibitors dictamycin and anthracyclines (21).

The V-D-J recombinase has been implicated in several translocations where one or even both genes are neither immunoglobulin (Ig) nor TCR loci yet contain appropriate recombinase-like recognition signals (22-24). Heptamer and nonamer-like V-D-J recombinase recognition sequences are present within few base pairs of the breakpoints on both chromosomes 4 and 11 in ALLs with the t(4;11) chromosomal translocation (25). In lymphoid leukemias, malignant transformation may occur during developmental stages when the V-D-J recombinase is active and immune receptor genes are rearranging. At that time, the ALL-1 gene heptamer- and nonamer-like sequences may be potential substrates for the V-D-J recombinase (25).

The fact that 10–20% of acute myeloid leukemias carry Ig or TCR gene rearrangements suggests that V-D-J recombinase activity may be present in the precursor cells of the myeloid leukemias (26). However, it is possible that different lineages of 11q23 leukemias may involve different pathogenic mechanisms. Topoisomerase II has been implicated in illegitimate DNA recombination (27, 28), and because of the association of topoisomerase II inhibitors with secondary leukemias with rearrangements at 11q23, we investigated whether topoisomerase II DNA binding sites may play a role in translocations affecting region 11q23 in AMLs by characterizing the sequence of the genomic breakpoints of a t(9;11) chromosome translocation in a de novo AML.

Materials and Methods

Informed consent was obtained and genomic DNA was prepared by standard methodology from the primary leukemic cells of an infant male. Cells were of French-American-British M5 morphology. Southern blot analysis with the B859 probe was used to localize the 11q23 translocation breakpoint. This 859-base pair BamHI fragment of ALL-1 cDNA spans exons 5-11 (15), the region of the ALL-1 gene breakpoint cluster for de novo ALL. The region from 8-kilobase breakpoints to 11q23 was sequenced by the Walkat method using a primer set derived from the sequence data. This primer set was used to make a unique PCR product by PCR with template DNA isolated from the leukemia cells. The PCR product was cloned into the pBluescript II KS+ vector. The sequences of the fragments were determined by the dideoxynucleotide chain termination method.

Results

We have examined the leukemic cells of patient 704, a male infant carrying a t(9;11)(p22;q23) chromosome translocation for rearrangements of the ALL-1 gene by using an ALL-1 cDNA (B859) probe that allows the detection of breakpoints within the ALL-1 8-kilobase breakpoint cluster region (11, 15). Southern blot analysis showed the presence of the normal ALL-1 allele and of both derivative chromosomes...
that resulted from the translocation (Fig. 1). A partial Sau3A genomic library was constructed in the EMBL3 λ phage vector and screened with the B859 ALL-1 probe. One positive clone (704-10A) contained the translocation breakpoint. The breakpoint in the ALL-1 gene on chromosome 11q23 was mapped to the intron between exons 6 and 7 (Fig. 2). Southern blot analysis with genomic probes derived from clone 704-10A of DNAs from somatic cell hybrids containing chromosomes 9 or 11 as the only human material, confirmed that the translocation was a t(9;11) (data not shown). Furthermore, hybridization of the λ clone 704-10A with AF-9 cDNA probe (18) demonstrated that the breakpoint on chromosome 9 in patient 704 occurred in the AF-9 gene. The breakpoint region was subcloned into the Bluescript vector and sequenced. About 3 kilobases of the sequence surrounding the der(11) breakpoint were determined. On the basis of the DNA sequence, the breakpoint in the AF-9 gene on chromosome 9p22 was localized to an intron.

Heptamer-like sequences were identified at the breakpoints on both chromosome 9 and chromosome 11 (Fig. 3). This finding suggests that the t(9;11) chromosome translocation in AML also may be catalyzed by the recombinase involved in Ig and TCR gene rearrangement, similarly to the t(4;11) chromosome translocation in ALL. Extra nucleotides, resembling an N-region, were present at the joining site between the two chromosomes. This observation indicates that the enzyme terminal deoxynucleotidyl transferase was active at the time the translocation took place. Thus, the presence of potential heptamer and nonamer signal sequences at the breakpoints and the presence of an N-region at the joining site suggest that the t(9;11)(p22;q23) chromosome translocation occurred by V-D-J recombination. In addition, sequence analysis indicated the presence of consensus topoisomerase II DNA-binding sites in both the ALL-1 and AF-9 loci in close proximity to the breakpoints (Fig. 3). Since both strands of DNA are involved in cleavage by the topoisomerase II dimer, a relationship exists between the strength of cleavage and the match of both strands to the consensus sequence. According to this criterion, the sequences in proximity to the translocation breakpoint that show homology to the consensus are categorized as “strong cleavage sites” (Fig. 4) (30). These sites are the strongest among other potential sites found in the 3 kilobases of sequence analyzed.

Discussion

Analysis of the sequences at the regions of breakpoints in chromosome translocations can reveal enzymatic mechanisms involved in DNA breakage and nonhomologous recombination. We previously proposed that aberrant V-D-J recombination is involved in chromosomal translocations in B- and T-cell malignancies (22, 23). While the V-D-J recombinase has been implicated in several translocations juxtaposing Ig or TCR loci to cellular oncogenes, the analysis of t(4;11) chromosome translocations suggested recombinase involvement also in translocations between non-Ig, non-TCR genes (25). The heptamer- and nonamer-like sequences at the translocation breakpoints of ALLs with rearrangements at 11q23 are potential substrates for the V-D-J recombinase. The present work suggests V-D-J recombinase involvement not only in de novo ALL but also in de novo AML with a t(9;11) chromosome translocation. The orientation of the heptamer-like sequences with their 5' end at the boundary of the breakpoints, as shown on the two normal chromosome sequences (Fig. 3), and the presence of an N-region at the junction between ALL-1 and AF-9 genes suggests that the der(11) chromosome represents the “coding junction” of a V-D-J-like recombination (24).

Since secondary leukemias are frequently induced by inhibitors of topoisomerase II, we investigated whether topoisomerase II DNA binding sites may be present in proximity to this t(9;11) breakpoint. Indeed, in the DNA of patient 704 we identified two regions of homology with topoisomerase II binding sites adjacent to the translocation breakpoints. These results suggest that aberrant topoisomerase II activity may be involved in the pathogenesis of myeloid leukemias with translocations at 11q23.

Topoisomerase II catalyzes and regulates double stranded DNA cleavage and religation during replication, condensation, mitotic segregation or strand passage, transcription, and recombination (31, 32). The cleavage and religation reactions enable topoisomerase to par-

Fig. 1. Southern blot analysis of leukemic cells from patient 704. Genomic DNAs were digested with the restriction enzyme BamHI and hybridized with an ALL-1 cDNA probe (B859). DNAs from the RS4:11 cell line, which carries a t(4;11) chromosome translocation, and from human placenta were used as controls.

Fig. 2. Schematic representation of the der(11) chromosome of patient 704. Exons are represented by empty (AF-9) or full (ALL-1) boxes. B, BamHI; RI, EcoRI; Bg, BglII; H, HindIII. ALL-1 exons are from 5 to 8 and the two AF-9 exons correspond to nucleotides 1627–1698 and 1699–1770 of the published cDNA sequence (18).
As Heptamer-like sequences, ~und on the AF-9 gene and the ~-globin gene (34) are shown. R, purine; Y, pyrimidine; N, any base; K, G or T; M, A or C. The breakpoint fMls between ALL are imperfect. Therefore, enzymes other than the V-D-J recom-binase may be necessary for 11q23 translocations (24). The inserted 43-base pair duplicated and inverted sequence as well as the deletion of bases at the (4;11) breakpoints of the cell line RS4:11, and the additional bases inserted in the region of the MV4:11 breakpoint suggest complex patterns of DNA breakage and repair (25). Conceiv-ably, disrupted topoisomerase II activity; the V-D-J recombinase system including RAG-1, RAG-2, terminal deoxynucleotidyl transferase and/or other enzymes all may be involved (24, 39).

Pesticide exposure, maternal use of marijuana, and maternal alcohol consumption are independent risk factors for monoblastic AML (40–42). The finding of a common breakpoint cluster region in secondary leukemias and leukemias associated with the other toxins suggests that the ALL-1 genomic region is vulnerable to chemical injury (43, 44). The finding of topoisomerase II sites at a breakpoint in a de novo AML suggests that 11q23 leukemias with different etiologies may have a common pathogenetic mechanism. The AML case described in this work, even though it might not represent a general rule for de novo AMLs, may instead reflect a more general situation for secondary leukemias. In order to elucidate this point, it will be necessary to characterize the genomic breakpoints of translocations involving region 11q23 in secondary leukemias.

Acknowledgments

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References


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