Consistent Disruption of the \textit{AML1} Gene Occurs within a Single Intron in the t(8;21) Chromosomal Translocation\textsuperscript{1}

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Abstract

The \textit{AML1} gene on chromosome 21 was rearranged by the t(8;21) chromosomal translocation in acute myeloid leukemia (AML). Southern blot analysis of 21 AML patients with t(8;21), including three with complex translocations, t(8;V;21), demonstrated that all the breakpoints occurred at random within a single intron between two coding exons of \textit{AML1}. Clustering of the breakpoints in the restricted intron suggests the formation of a unique fusion gene between the \textit{AML1} gene and a presumable counterpart gene on chromosome 8. Nucleotide sequencing of the breakpoint region revealed that the translocation event was accompanied by deletion of a short stretch of nucleotides.

Introduction

Nonrandom chromosome abnormalities associated with particular subtypes of human leukemia and lymphoma are considered to be involved in neoplastic transformation and tumor progression (1, 2). The t(8;21)(q22;q22) translocation is one of the most frequent chromosome abnormalities in AML,\textsuperscript{3} especially in the M2 subtype (French-American-British classification) (3, 4). Leukemic cells with t(8;21) are characterized by granulocytic maturation and a high frequency of Auer rods (5).

Recently we have mapped the translocation breakpoints on chromosome 21 (6) with one of the \textit{NotI} linking clones specific for chromosome 21 (7) and isolated a novel gene, \textit{AML1}, which located at the breakpoint region (8). We have also shown that the breakpoints in three patients occurred within a single intron of 25 kilobases between two coding exons of \textit{AML1} (8). To determine whether the disruption of the \textit{AML1} gene consistently occur within the specific intron should provide information as to how the \textit{AML1} gene is involved in leukemogenesis as well as a useful tool for the diagnosis of this disease.

In this paper, we performed detailed mapping of the breakpoints in 21 AML patients with t(8;21) including three with complex translocations and an AML cell line with t(8;21) by Southern blot analysis using \textit{AML1} cDNA and multiple intronic probes. In addition, we cloned and sequenced the breakpoint region of one patient.

Materials and Methods

DNA Probes. An \textit{AML1} cDNA probe C6E6H2 was prepared as reported previously (8). Genomic DNA probes were derived from \textit{A}D11 and \textit{A}D13 genomic clones (8) by digestion with appropriate restriction enzyme(s). In particular, the D11X1, D11X2, D11X3, and D11X4 probes (see Fig. 3) are 6.5-, 4.8-, 4.0-, and 2.2-kilobase \textit{XbaI} fragments of the \textit{A}D11 clone, respectively.

Southern Blot Analysis. High molecular weight DNAs were prepared from leukemic cells. DNA (5 \mu g) was digested with appropriate restriction enzymes, fractionated by conventional or field inversion gel electrophoresis with a Bio-Rad apparatus (CHEF MAPPER), and transferred to a Hybond-N membrane (Amersham, United Kingdom). Hybridization with random primer-labeled probe was carried out in 6 x SSC, 10% dextran sulfate, 1% SDS, 1 x Denhardt’s solution, and 50% formamide at 42\degree C with or without human placental DNA (100 \mu g/ml). The final washing was in 0.1 x SSC, 0.1% SDS at 65\degree C. Autoradiography was performed using a bioimage analyzer, Fujix BAS 2000 (Fuji, Japan).

Genomic Library Construction and Screening. Leukemic cell DNA from patient KH was digested with \textit{BamH}I and cloned into \textit{A}DASH II phage vector (Stratagene). The library was screened with the C6E6H2 and D11X1 probes to isolate the clones containing the breakpoint region of the der(8), der(21), and normal chromosome 21. The chromosome 8-specific portion of the clone derived from the der(21) was used as a probe to isolate the clone containing the corresponding region of normal chromosome 8. The inserts of phage clones containing the breakpoint region were digested with EcoRI, subcloned into pBluescript II KS (+) (Stratagene) and sequenced.

DNA Sequencing. DNA sequencing was performed by the dyeoxy chain termination method, using a Sequenase version 2.0 DNA sequencing kit (United States Biochemical) with [\alpha-\textsuperscript{35}S]dCTP or an A.L.F. DNA Sequencer (Pharmacia, Sweden) with fluorescent labeled primers. When needed, nested deletions were created using Exo/Mung deletion kit (Stratagene).

Results and Discussion

To determine whether rearrangements constantly occurred in the single specific intron of the \textit{AML1} gene, we analyzed the breakpoints in 21 AML patients with t(8;21) including three with complex t(8;V;21) translocations, t(8;4;21), (8;12;21), and t(8;20;21) (9), and an AML cell line with t(8;21), Kasumi-1 (10). Fig. 1 shows Southern blot analysis on \textit{BamH}I-digested DNAs with the \textit{AML1} cDNA probe C6E6H2. In 18 of 22 cases (21 patients and Kasumi-1), abnormal rearranged bands were detected in addition to germline bands of 11 and 19 kilobases. No rearrangements in 4 cases (patients HMO, FO, MU, and KK) detected with the C6E6H2 probe may be due to comigration of the rearranged bands with the germline ones. The 5' and 3' segments of the exonic probe C6E6H2 were both located close to the external ends of the region covered by the 11 and 19-kilobase \textit{BamH}I fragments. On the other hand, the intronic probe D11X2, which originated from the internal boundary

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\textsuperscript{3} The abbreviations used are: AML, acute myeloid leukemia; cDNA, complementary DNA.
region of both *BamHI* fragments, revealed rearranged bands in 18 cases (data not shown) including three (patients FO, MU, and KK) of four cases mentioned above. Thus, using the C6E6H2 and D11X2 probes in *BamHI* digestion, *AML1* rearrangements were detected in 21 of 22 cases. In 15 cases (patients KH, TR, IMK, IK, IT, NT, AS, KT, HMK, KU, HU, IMS, KM, SN, and MK), both C6E6H2 and D11X2 detected rearranged bands of different sizes, which were derived from the der(8) and der(21) chromosomes, indicating that the breakpoints occurred within the same intron. Patient MK was first diagnosed as having t(8;20) by cytogenetic analysis. However, after detection of the *AML1* gene rearrangement, reexamination clarified that patient MK had the complex translocation t(8;20;21).

Subsequently, detailed mapping of the breakpoints in 22 cases was performed by Southern blot analysis using several restriction enzymes and multiple genomic probes derived from λD11 and λD13 genomic clones. Representative Southern blots are shown in Fig. 2. In patient FO, the D11X1 probe detected two rearranged *EcoRI* bands (1.8 and 6.0 kilobases) in addition to the germline *EcoRI* band of 7.3 kilobases (Fig. 2a), indicating that the breakpoint on chromosome 21 occurred in the 7.3-kilobase *EcoRI* fragment. In patient HMO, the D11X3 and D11X4 probes detected two rearranged *HindIII* bands of 3.5 and 6.2 kilobases that derived from the der(8) and der(21) chromosomes, respectively (Fig. 2b). Therefore, the breakpoint in patient HMO occurred in the region indicated in Fig. 3. The locations of the breakpoints in 25 cases including those in three patients reported previously (8) are summarized in Fig. 3. The rearrangements were identified in more than 2 digests with different restriction enzymes in most cases (22 of 25); thus the abnormal bands were probably not due to restriction fragment length polymorphisms. In three cases (patients MU, NT, and HMO), the rearrangements were analyzed with a single enzyme: *BamHI* for patients MU and NT; *HindIII* for patient HMO. However, thus far as examined, no restriction fragment length polymorphism sites for *BamHI* and *HindIII* have been detected in this region. The results shows that all breakpoints occurred within the same 25-kilobase intron of the *AML1* gene with no specific localization of breaks; therefore the existence of a translocation hot spot seems unlikely. Considering the clustering of breakpoints in the single specific intron, the *AML1* probes used here should be useful for the diagnosis and monitoring of this type of leukemia.

We cloned and sequenced the breakpoint regions of the der(8) and der(21) chromosomes as well as the corresponding germline regions from patient KH (Fig. 4). Comparison of the rearranged sequences with that for germline showed that the small deletions of 18 bases of chromosome 21 and 5 bases of chromosome 8 were accompanied by chromosomal breakage and rejoining. Chromosomal breakage occurred in the regions between the arrowheads on both chromosomes shown in Fig. 4.
CONSISTENT DISRUPTION OF AML1

Fig. 3. Locations of the t(8;21) translocation breakpoints and restriction map of the AML1 locus with relation to the AML1 cDNA. Patients SM, NS, and CS were previously reported as patients 1, 2, and 3, respectively (5). XD 11 and XD 13 are overlapping genomic clones (5). Boxes on the genomic map represent exons and shaded parts in boxes correspond to the AML1 cDNA probe C6E6H2. Box in the cDNA represents the open reading frame. Lines above the map indicate the regions in which the breakpoints occurred. The left end of the region for patients KT and IT is the SpeI site. Bold horizontal lines, genomic probes. Restriction fragment length polymorphism sites are shown in parentheses. B, BamHI; E, EcoRI; H, HindIII; S, Smal; P, PstI; X, XbaI; bp, base pairs; kb, kilobases.

Fig. 4. Nucleotide sequences at the breakpoint region in patient KH. Bold lines, homologous sequence on chromosome (ch.) 8 and chromosome 21. Arrows, inverted homologous region. Dots and dashed lines, repeated sequences on chromosome 21. Chromosomal breakage occurred in the regions between the arrowheads on both normal chromosomes. Asterisks, nucleotide polymorphism.

Some small homologous regions and direct repeats were noticed around the recombination site; however, no tandem repeats as often seen in chromosomal translocations were found (11–13). The AML1 gene has a region (amino acid residues 60–177), including an ATP- or GTP-binding site motif, of homology with the Drosophila segmentation gene runt, which encodes a potential transcriptional regulator (14, 15). Interestingly, the AML1 protein is disrupted at the COOH-terminal end of this homologous region by the t(8;21) translocation. Cytogenetic studies of complex translocations indicate that the der(8) chromosome is the critical constant rearrangement. Therefore, considering the orientation of the AML1 gene on chromosome 21 and the conservation of breakpoints in the standard t(8;21) and complex translocations within the single specific intron of AML1, a chimeric gene on the der(8) chromosome between the 5' part of the AML1 gene and a presumable counterpart gene on chromosome 8 may play an important role in the pathogenesis of AML.

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