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Molecular Cloning of 19p13 Breakpoint Region in Infantile Leukemia with t(11;19)(q23;p13) Translocation

Shinsuke Iida,[1,2] Masao Seto,[1,6] Kazuhito Yamamoto,[1] Hirokazu Komatsu,[1,2] Yukihiro Akao,[1,3] Shinpei Nakazawa,[4] Yutaka Ariyoshi,[2] Toshitada Takahashi[5] and Ryuzo Ueda[1]

The Laboratories of [1]Chemotherapy and [5]Immunology, Aichi Cancer Center Research Institute, Department of [2]Hematology and Chemotherapy, Aichi Cancer Center Hospital, Kanokoden 1-1, Chikusa-ku, Nagoya 464, [3]Department of Anatomy, Osaka Medical College, Daigaku-cho 2-7, Takatsuki 569 and [4]Department of Pediatrics, Yamanashi Medical College, Tamaho-machi 1110, Yamanashi 409-38

We studied the breakpoint regions involved in t(11;19)(q23;p13) translocation associated with infantile leukemias. Southern blot analysis with the partial cDNA clone for the *MLL* gene at 11q23 which we had isolated previously detected gene rearrangements in all three cell lines and three leukemia samples from the patients with t(11;19) translocation, indicating that these breakpoints were clustered within the 8.5 kb *Bam*HI germline fragment detected by the probe. To study the breakpoint region, a genomic library of one of the cell lines, KOCL-33, was made. We have isolated the der(19) allele containing the breakpoint as well as the germline alleles at 19p13 and 11q23. Using the genomic probes on chromosome 19 near the breakpoint, Southern blot analysis was performed. The breakpoints at 19p13 of the two other cell lines and the three leukemia samples were not located within 36 kilobases of the KOCL-33 breakpoint, although pulsed-field gel electrophoresis showed that the breakpoints of all three cell lines were on the same *Nru*I fragment of 230 kilobases. These results showed that the breakpoints at 19p13 were not clustered like those at 11q23 in t(11;19) translocation.

Key words: Infantile leukemia — t(11;19)(q23;p13) — *MLL/ALL-1* — Translocation

Chromosomal translocation is one of the most important events in oncogenesis of hematopoietic malignancies. Chromosome 11 band q23 (11q23) has been recognized as the target region of the translocations in various types of leukemia, lymphoma, and myelodysplastic syndrome.[1] The 11q23 region is characteristic in that it involves various chromosomes as the partner chromosomes for the reciprocal translocations, such as 1p32, 4q21, 9p22, 10p15, 14q32, 17q25, and 19p13.[2–5] Recent studies by us and others disclosed at least two rearrangement-associated genes at 11q23. One is *RCK/p54* gene encoding a 472- or 483-amino-acid polypeptide (depending on the ATG initiation codons) of the translation initiation factor/helicase family, isolated from the breakpoint region of t(11;14)(q23;q32) of the B-cell lymphoma cell line, RC-K8.[6–8] Another gene is *MLL/ALL-1*, encoding a protein with limited homology to the *Drosophila trithorax* gene, cloned from yeast artificial chromosome (YAC), yB22B2, containing *CD3D/G* gene.[9–14]

The *MLL/ALL-1* gene has been demonstrated to be involved in t(4;11)(q21;q23) and t(11;19)(q23;p13) translocations frequently found in infantile leukemia with the specific feature of biphenotypic leukemia.[7, 10, 12–16] Since our previous study with a partial *MLL/ALL-1* cDNA also revealed chimeric mRNA in leukemic cell lines with t(4;11)(q21;q23) and t(11;19)(q23;p13), it was speculated that *MLL/ALL-1* gene together with unknown genes located on the recipient chromosome of 11q23 plays an important role in oncogenesis during either the fetal or the neonatal period and in producing the clinical characteristics of infantile leukemia.[16] In this study, we focused on t(11;19) and analyzed the breakpoints of leukemias with this translocation.

MATERIALS AND METHODS

Leukemia samples and cell lines Leukemia cells of three patients were obtained from bone marrow aspirates after informed consent had been obtained from the patients and/or their parents. Both patient 1 (8 months old) and patient 2 (3 months old) were female and diagnosed as having acute lymphoblastic leukemia (ALL)-L1 with 46, XX, t(11;19)(q23;p13). Patient 3 (60 years old) was female and diagnosed as having ALL-L2 with 47, XXX, t(11;19)(q23;p13). Infantile leukemia cell lines with t(11;19), KOCL-33, KOCL-44, and KOPN-1, were previously described.[4, 16]

Somatic cell hybrids GM10115 human-Chinese hamster somatic cell hybrid containing human chromosomes 19 was obtained through the NIGMS human mutant cell repository (Coriel Institute for Medical Research,

[6] To whom correspondence and reprint requests should be addressed.

Camden, NJ). A9(Neo-11)-1, a human-mouse somatic cell hybrid containing a human chromosome 11, was kindly provided by Dr. M. Oshimura.[16]

Southern blot analysis and pulsed-field gel electrophoresis (PFGE) analysis Southern blot analysis and PFGE analysis using the CHEF DR II system (Bio-Rad, Richmond, CA) were performed as described previously.[4, 6, 17] cDNA probes mapped at 19p13 used in this study were as follows: *Lyl-1* probe (1.1 kb), *LDL-R* (human LDL receptor) probe (5.3 kb), *Tyk-2* probe (4.0 kb), and *E2A* probe (0.3 kb) were provided by Dr. M. L. Cleary, Dr. D. W. Russel, Dr. J. J. Krolewski, and Dr. C. Murre, respectively. *INSR* (insulin receptor) probe (4.1 kb) and *C3* (complement component) probe (4.2 kb) were obtained through American Type Culture Collection (ATCC).

Genomic library High-molecular-weight DNAs of KOCL-33 line and human placenta were partially digested with *Sau*3AI and size-fractionated by using low-melting-point agarose gel. DNA ranging from 9 kb to 23 kb was purified and ligated in the *Bam*HI site of λ dash II phage vector (Stratagene, La Jolla, CA). After packaging, 6×10^5 recombinant clones of the KOCL-33 and 2×10^5 recombinant clones of the human placenta were screened with probe x, a 0.9 kb *Bam*HI fragment of MLL-b cDNA (Fig. 1),[16] and probe P derived from der(19) clone, λK33-6 (Fig. 2a), respectively. Positive clones were subcloned into pBluescript vectors and analyzed.

DNA sequencing Nucleotide sequence was determined by the dideoxy chain termination method using a Sequenase kit (United States Biochemicals, Cleveland, OH).

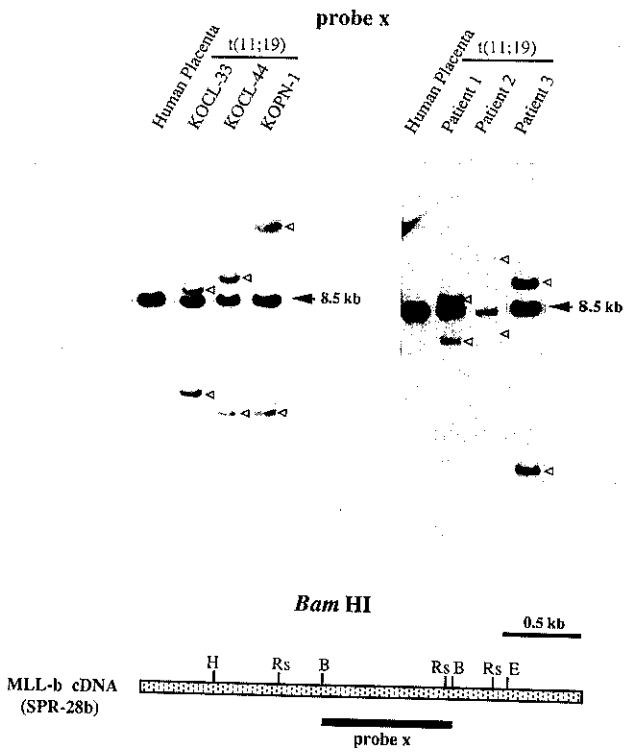

Fig. 1. Southern blot analysis of cell lines and leukemia samples with t(11;19)(q23;p13) with 0.9 kb *Bam*HI fragment of MLL-b cDNA (probe x). DNAs were digested with *Bam*HI, blotted, and hybridized with probe x shown below. Detection of two rearranged bands (indicated by open arrowheads) in all the cell lines and leukemia samples argues that the breakpoints occurred within the 8.5 kb germline band (marked by an arrow on the right) detected by the probe. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Rs, *Rsa*I.

RESULTS

Rearrangement of *MLL* gene at 11q23 in t(11;19) leukemia cell lines and samples DNAs from three cases of ALL and three cell lines with t(11;19) were studied by Southern blot analysis with probe x of MLL-b cDNA (Fig. 1).[16] Two rearranged bands were detected in each of the samples tested here (Fig. 1), indicating that all of the breakpoints at 11q23 were clustered within the 8.5 kb germline fragment detected by probe x.

Isolation of breakpoint region of KOCL-33 cell line with t(11;19) The genomic library of KOCL-33 was screened with probe x, and three clones, λK33-1, λK33-5, and λK33-6, were isolated. Two of them, λK33-1, and λK33-5, were considered to be the germline allele of 11q23, because they overlapped with λHp23-13, which was previously cloned from a human placental genomic library with probe b of 6n clone derived from the YAC clone, yB22B2 (Fig. 2a).[7, 16] The composite map covering 30 kb at the 11q23 breakpoint region is shown in Fig. 2a. In contrast, the λK33-6 clone was considered to be the rearranged allele of derivative 19, because the restriction map at the centromeric side of the clone was different from that of λK33-1. The chromosomal origin of the centromeric side of this clone was confirmed by somatic cell hybrid analysis with a 2.1 kb *Bam*HI fragment (probe P, Fig. 2a) as a probe. As shown in Fig. 3a, probe P showed positive signals in the GM10449 hybrid cell containing a human chromosome 19, being consistent with derivation of the clone from the derivative 19 allele (Fig. 3a).

We next attempted to isolate the germline 19p13 region by screening the human placental genomic library with probe P. The clone, λHp-5, was isolated and restriction enzyme sites were determined as shown in Fig. 2a. Reiterated sequence-free probe Q (0.9 kb *Bam*HI/*Eco*RI fragment of λHp-5) was isolated and confirmed to be of chromosome 19 origin by somatic cell hybrid analysis

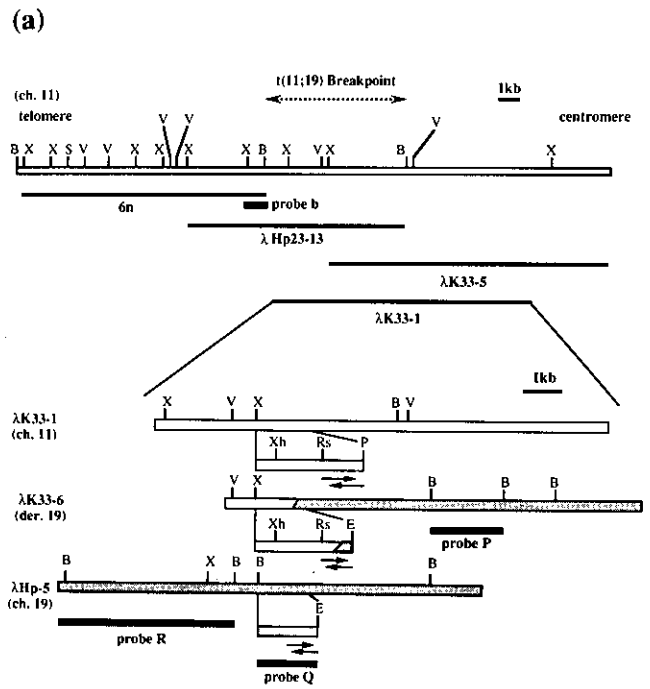

Fig. 2. Restriction map of breakpoint regions at 11q23 and 19p13 and molecular cloning of the breakpoint of KOCL-33 cell line with t(11;19)(q23;p13). (a) Restriction map of clones from 11q23, der(19), and 19p13. The phage clone, 6n, and λHp23-13 were described previously.[7,13] λK33-1 and λK33-5 are two phage clones isolated from a KOCL-33 genomic library with probe x. Breakpoints clustered within the 8.5 kb *Bam*HI fragment are indicated by a dashed line with arrowheads. λK33-6 represents the der(19) allele. λHp-5, which was cloned from a human placental genomic library with probe P of λK33-6, represents the normal chromosome 19 allele. The hatched region depicts the portion derived from chromosome 19. Arrows under the subcloned fragment indicate the region sequenced. Probes utilized in this study are also indicated. B, *Bam*HI; X, *Xba*I; V, *Eco*RV; S, *Sal*I; Xh, *Xho*I; Rs, *Rsa*I; P, *Pst*I; E, *Eco*RI. (b) Comparison of nucleotide sequence in the breakpoint regions. The homologous region is indicated by asterisks. The arrows depict the breakpoints at 11q23 and 19p13, respectively.

(data not shown). The 0.6 kb *Rsa*I/*Pst*I fragment of λK33-1, 0.35 kb *Rsa*I/*Eco*RI fragment of λK33-6, and 0.9 kb *Bam*HI/*Eco*RI fragment of λHp-5 (probe Q) were subcloned into pBluescript for sequence analysis. The resulting nucleotide sequence revealed that the 11q23 and 19p13 sequences were joined to each other with addition of one A on derivative 19 (Fig. 2b). No heptamer-like signal for the immunoglobulin gene rearrangement was found near the breakpoint regions at 11q23 and 19p13. Probe Q containing the breakpoint region of KOCL-33 could not detect any transcripts in Northern analysis (data not shown).

Breakpoints at 19p13 in leukemia cell lines and samples with t(11;19) We then investigated the breakpoints of the cell lines and three leukemia samples with t(11;19) by Southern blot analysis using probes Q and R (5.8 kb *Bam*HI fragment) near the breakpoint of the KOCL-33 at 19p13 (Fig. 2a). No rearrangement bands, however, were detected with *Bam*HI, *Eco*RV, or *Xba*I digestion except for KOCL-33 (Fig. 3b and data not shown for probe R). Since the longest germline bands detected by probe Q and probe R were 20 kb and 16 kb in size by *Xba*I digestion, the breakpoints of these leukemias other than KOCL-33 were beyond the 36 kb region detected by these probes.

Next, PFGE analysis was conducted to examine the breakpoints of the three cell lines, since the leukemia cells of the patients were not available for this analysis. As shown in Fig. 4, the rearrangement bands of 195 kb in KOCL-33, 210 kb in KOCL-44, and 300 kb in KOPN-1, were detected by *Nru*I digestion (Fig. 4), indicating that the breakpoints on chromosome 19 of these three cell lines are within the 230 kb *Nru*I fragment. Importantly, each rearranged band was found to comigrate with one of the two rearranged bands detected by probe x of MLL cDNA in each cell line (data not shown). When *Tyk*-2, *E2A*, *INSR*, *Lyl-1*, *LDL-R*, and *C3* were used as probes for the PFGE blots, none of them showed comigration with the rearranged band detected by breakpoint region probes in *Not*I, *Sac*II and *Nru*I digestion (data not shown).

DISCUSSION

In this study, we showed that the breakpoints at 11q23 of three cell lines and three leukemia samples with t(11;19) were clustered in the same 8.5 kb *Bam*HI fragment, in accordance with other findings.[11,12,14,15] We also showed here that the breakpoints at 19p13 were not clustered in a narrow region like those at 11q23, since the conventional Southern blot analysis covering a region as long as 36 kb containing the breakpoint of KOCL-33 did not detect rearrangements in these cell lines or in leukemia samples except for KOCL-33. By PFGE analysis, the

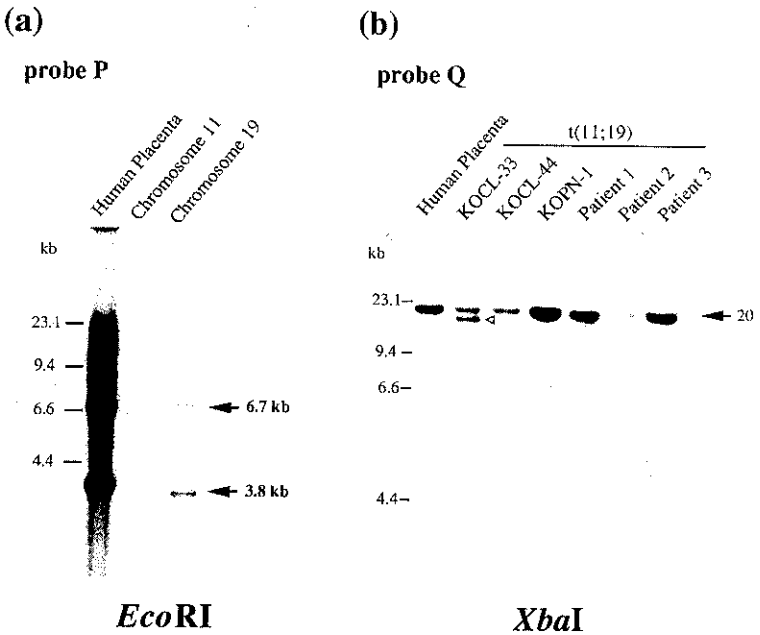

Fig. 3. Chromosomal assignment and Southern blot analysis of cell lines and leukemia samples with t(11;19) using probes at the 19p13 breakpoint region of KOCL-33. (a) Assignment of probe P from λK33-6 (Fig. 2a) to chromosome 19 by Southern blot analysis using somatic cell hybrids. Ten μg of *Eco*RI-digested DNAs of human placenta and somatic cell hybrids containing a single human chromosome, 11 or 19, were applied. The blot was hybridized with probe P. Arrows indicate the two germline bands which are due to the internal *Eco*RI site in the probe. (b) Southern blot analysis with probe Q (see Fig. 2a). Ten μg of *Xba*I-digested DNA of cells was applied. The blots were hybridized with 0.9 kb *Bam*HI/*Eco*RI fragment, probe Q. The rearrangement band (open arrowhead) except for KOCL-33 cell line is not recognized. The size of the germline bands is shown on the right (in kb). The size markers are shown on the left of each panel.

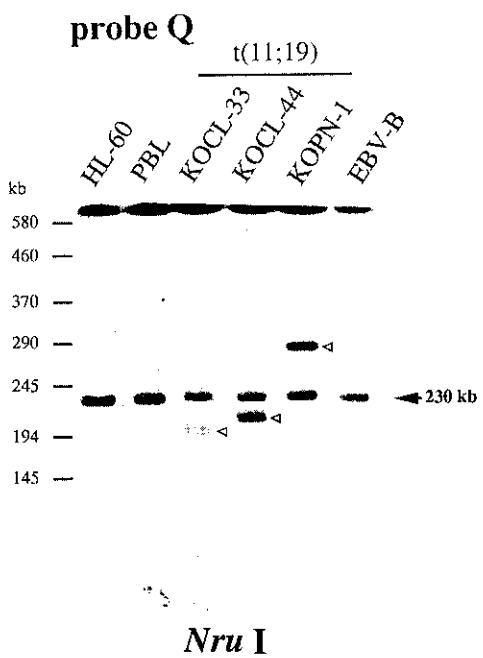

Fig. 4. PFGE analysis of KOCL-33, KOCL-44 and KOPN-1 cell lines with probe Q derived from 19p13. Cell plugs prepared from the three cell lines with t(11;19) and from HL-60 (myeloid cell line), PBL (peripheral blood lymphocyte), and EBV-B (Epstein-Barr virus-transformed B cell line) were digested with *Nru*I and analyzed by PFGE. The blot was hybridized with probe Q derived from 19p13. The size of the germline is given on the right. Rearranged bands are shown by open arrowheads.

breakpoints of three cell lines were demonstrated to be on the same 230 kb *Nru*I fragment. This may indicate that different introns of the same gene at 19p13 are involved in t(11;19) translocation. Alternatively, a different gene may be involved, as suggested by Mitani *et al.*, who demonstrated the heterogeneity of the breakpoints at 19p13 by *in situ* hybridization analysis.[18] We have shown that the size of truncated mRNA of *MLL* transcripts in t(11;19) cell lines is not identical, i.e., it is 10 kb for KOCL-33 and 44, but 9.2 kb for KOPN-1.[16] It was also demonstrated that the truncation of *MLL* transcripts occurs in fusion transcripts between *MLL* and genes on the partner chromosomes.[16] A recent report by Tkachuk *et al.* predicted that a chimeric protein would result from the fusion mRNA between *ENL* gene at 19p13 and *HRX* gene, which is identical to *MLL/ALL-1* gene.[13]

Several groups of investigators have found *MLL/ALL-1* transcripts with a size of more than 10 kb and truncated *MLL/ALL-1* transcripts in t(4;11) and t(11;19) cell lines.[7, 10, 12–14, 16, 19] We have cloned a partial cDNA of *MLL/ALL-1* gene which detected 15 kb, 14 kb, 12 kb, 9.7 kb, and 5 kb transcripts, together with chimeric mRNAs in cell lines with t(4:11) and in those with t(11;19).[16] Both 12.5 kb and 11 kb signals were specific to four cell lines with t(4;11), which suggests that they are truncated in the same or adjacent introns of both the *MLL/ALL-1* and the gene on chromosome 4 (designated *LTG4*, for leukemia translocation gene on chromosome 4), producing the same size of chimeric mRNAs in Northern blot analysis.[16] Indeed, a recent report by Gu

et al. has demonstrated the clustering of the breakpoints within 7–8 kb on chromosome 4 in leukemia cells with t(4;11).[20] On the other hand, in the cell lines with t(11;19), either a 10 kb signal in KOCL-33 and KOCL-44 or a 9.2 kb signal in KOPN-1 was recognized.[16] This difference suggests heterogeneity of chimeric transcripts expressed in leukemia cells with t(11;19), and is consistent with the data presented here. Thus, it is important to determine whether the same gene at 19p13 is involved in this translocation. Our preliminary data with cDNA clones for the gene on chromosome 19 (designated as *LTG19*, for leukemia translocation gene on chromosome 19) which seems to be identical to the *ENL* gene reported by Tkachuk *et al.*,[13] showed that the breakpoint of KOPN-1 is located at a different site in the same gene from those of KOCL-33 and KOCL-44. In this regard, it should be noted that the cDNA probe of the *ENL* gene could not necessarily detect the rearrangement bands in all cell lines or leukemia samples with t(11;19), indicating the heterogeneity of breakpoints at the 19p13 region.[13] It is now important to characterize the truncated mRNA with different sizes found in t(11;19) cell lines to study the possible role of *LTG19/ENL* in malignant transformation, together with *MLL/ALL-1/HRX* gene at 11q23.

The presence of *Alu* homologous regions and heptamer-spacer-nonamer-like sequence has been suggested to be associated with the translocation for t(4;11) and t(9;11).[11,20] In *BCL-2* translocation, the breakpoints of the immunoglobulin heavy chain and light chain genes consistently involve the heptamer-spacer-nonamer recombination signals, but many of the breakpoints at *BCL-2* gene do not.[21–23] These findings argue that the breakpoints of the genes at the partner chromosomes might arise via a different mechanism other than breakage by the recombinase for the immunoglobulin genes. Indeed, in the present study, no such heptamer-spacer-nonamer-like sequence or repetitive sequence was found near the breakpoint regions we sequenced. Although the mechanism of the translocation for t(11;19) remains to be studied, such a difference might result in heterogeneity of the breakpoints for t(11;19) translocation. Further analysis of several other cell lines or leukemia samples should allow us to solve this interesting problem.

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