Molecular Cloning of the Chromosomal Breakpoint of a B-Cell Lymphoma with the t(11;14)(q23;q32) Translocation

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Abstract

The breakpoint of t(11;14)(q23;q32) chromosome translocation in a B-cell lymphoma line, RC-K8, was cloned. Immunoglobulin heavy chain (IGH) constant gene, C\(\gamma\)2 at the 5' end, was involved in this translocation, and the DNA segment juxtaposed to the C\(\gamma\)2 was proved to be derived from chromosome 11 by somatic cell hybrid study. The normal counterpart of chromosome 11 was also isolated. With a DNA probe near the breakpoint of chromosome 11, Southern blot analysis of RC-K8 and 10 other cases with translocation involving the 11q23 region was conducted, but no rearrangement bands have been observed thus far except for RC-K8.

Introduction

Chromosome abnormalities of leukemia and lymphoma have been identified as nonrandom molecular events (1-3). The chromosome 11 band q23(11q23) is of great interest, because this region has been reported to be translocated with a number of different chromosomes such as 1p32, 1q21, 2p21, 4q21, 6q27, 9p21-p22, 10p11-p15, 17q25, and 19p13 in different types of hematopoietic tumors (4). One such translocation, t(11;14)(q23;q32), which was observed in a B-cell lymphoma cell line, RC-K8 (5), is uncommon (1), but cloning of a putative oncogene in chromosome 11q23 may be possible by using immunoglobulin heavy chain (IGH) gene as a probe, similar to that of BCL-2 gene (6-8). Then, it is further possible to elucidate the gene(s) present in the various chromosomes that are juxtaposed to 11q23, if the gene cloned or the one nearby at 11q23 were involved in these translocations. Our previous study with somatic hybrid cells demonstrated that the t(11;14)(q23;q32) breakpoint of the RC-K8 cell line was between C\(\gamma\)\(\gamma\) and C\(\gamma\)2 on chromosome 14q32, and between CD3 genes and ETS-1 on chromosome 11q23 (9). We have now cloned the breakpoint of this translocation. The fragment introduced to 14q32 was demonstrated to be derived from chromosome 11 by somatic cell hybrid study and was tentatively named the rck locus.

Materials and Methods

The RC-K8 cell line carrying the t(11;14)(q23;q32) chromosome translocation was derived from the peritoneal effusion of a patient with B-cell lymphoma; characterization of this cell line was described previously (5, 9). Somatic cell hybrids were made by fusing RC-K8 cells and hypoxanthine-guanine phosphoribosyltransferase-deficient NP1 mouse plasmacytoma cells in the presence of polyethylene glycol. The segregating hybrids were analyzed by Southern blot with probes for T-cell receptor \(\alpha\) chain (14q11), BCL-1(11q13), and ETS-1(11q23) genes (9). A hybrid cell line, 669AA-1, has both the 11q- chromosome and the 14q+ chromosome, but it does not contain an additional chromosome 14.

Results and Discussion

Molecular Cloning of the Breakpoint in t(11;14)(q23;q32). A genomic library of 669AA-1 hybrid cell DNA was constructed in \(\lambda\)EMBL3 phage (Stratagene, La Jolla, CA) and then screened with a 3.5-kilobase BamHI/BamHI fragment of Probe C at 3' of the C\(\gamma\)2 region that does not cross-hybridize to mouse C\(\gamma\) regions (Fig. 1C). Six positive recombinant phage clones were obtained, and the restriction map of these clones was compared with that of the untranslocated IGH in RC-K8 previously obtained (Fig. 1C) (9). The restriction map of the \(\lambda\)CES669AA-1-5 clone is shown in Fig. 1B. C\(\gamma\)3 probe, which is known to cross-hybridize with all human C\(\gamma\) genes (10), hybridized to the 4.3-kilobase HindIII/HindIII fragment, suggesting that the C\(\gamma\)2 gene is in the fragment (Fig. 1B), and it was confirmed by probing with C\(\gamma\)2-specific synthetic oligomer (9). Since the 4.3-kilobase HindIII fragment hybridized with the C\(\gamma\)3 probe is shorter than that of the untranslocated gene (6.6 kilobases), and an extra XhoI site is introduced just centromeric to C\(\gamma\)2, deletion at this region is likely (Fig. 1B). The J\(\gamma\) region is lost from \(\lambda\)CES669AA-1-5 as expected from the Southern blot analysis previously described (9).

Chromosome 11 Region Juxtaposed with the 5' Portion of C\(\gamma\)2. To determine the origin of DNA fragment juxtaposed to the 5' position of the C\(\gamma\)2 region, Southern blot analysis of various human-mouse hybrid cells was carried out (Fig. 2). The 0.5-kilobase HindIII/HindIII fragment of Probe A (Fig. 1B) hybridized to the 2.05-kilobase EcoRI fragment in hybrid cells containing chromosome 11, while the J\(\gamma\) probe detected the 18-kilobase EcoRI fragment in the hybrid cells containing chromosome 14, indicating that the region juxtaposed to chromosome 14 is derived from chromosome 11. Next, the normal chromosome 11q23 region across this breakpoint was cloned from a genomic library of human placenta with Probes A and B (Fig. 1B). The restriction map of one of these clones, \(\lambda\)HP11q23-13 (Fig. 1A), was compared with that of the CES669AA-1-5 containing the breakpoint (Fig. 1B). The telomeric position beyond the breakpoint was demonstrated to have a restriction map identical to that of chromosome 11, while that centromeric to this region is different, suggesting that the breakpoint on chromosome 11 is in a 2.8-kilobase BssHII fragment indicated by an arrow (Fig. 1A). This region...
Fig. 1. Restriction map of the recombinant clone carrying the breakpoint of chromosome 14q+, untranslocated chromosome 14q32, or normal chromosome 11q23. The normal chromosome 11q23 is represented by λHplI2q23-13 (4), and the 14q+ chromosome is represented by ACES669AA-1-5 (B). The restriction map of untranslocated chromosome 14 in RC-K8, which was previously described (8), is illustrated as well (C). Arrowheads, breakpoint. Bold line, chromosome 11 region; thin line, chromosome 14 region. Horizontal arrows, sequenced regions; the 14q+ chromosome is represented by ACES669AA-1-5 (A). The restriction enzyme sites: HinfI, HindIII, EcoRI, BclI, SacI, and XbaI are indicated. Restriction enzyme sites: B, BamHI; H, HindIII; E, EcoRI; B, BstHI; S, SacI; X, XbaI.

is tentatively named the rck locus.

The nucleotide sequence of the 1.3-kilobase HindIII/HindIII fragment containing the breakpoint and of the region around the breakpoint of normal chromosome 11q23 was determined using a Sequenase™ kit (USB, Cleveland, OH) with modifications (Fig. 3) (11). The sequence of chromosome 11 in Fig. 3 is

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human chromosome 11
                          + + + + + + + + + +
                          1 2 3 4 5 6 7 8 9 10

0.0 2.3 9.4 6.6

11, 12, 13
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Fig. 2. Southern blot analysis of human placenta, mouse-human somatic hybrid cells, and mouse BW5147 cells. The filter blotted with EcoRI-digested DNA was hybridized to a 0.5-kilobase HindIII/HindIII fragment (Probe A in Fig. 1B) and Jp probe. The status of human chromosomes 11 and 14 in each hybrid cell line is shown. Probe A hybridizes to the 2.05-kilobase fragment in human placenta (Lane 1) and hybrid cell lines containing chromosome 11 (Lanes 2, 3, 4, 6, and 7). On the other hand, Jp probe hybridizes to the 1.8-kilobase fragment in human placenta and hybrid cells containing chromosome 14 (Lanes 1, 7, 8, and 9). Lane 10, result of mouse BW5147 cells, which are parental cells of the hybrids. The concordance between Probe A positivity and the presence of human chromosome 11 was recognized. Four hybrid cell lines (Lanes 6–9) are from the HMBO series which was previously described (17), and the others (Lanes 2–5) are from the HMNS series which was made between human B-cell leukemia without 14q+ chromosome and mouse NPv in this study. All hybrid cell lines were tested for lactate dehydrogenase A and nucleoside phosphorylase to demonstrate the presence of chromosomes 11 and 14, respectively. Furthermore, Jp and ETS-1 probes were used in Southern blotting to confirm the presence of chromosomes 14 and 11, respectively.

GC rich (57%) and two potential Sp1 binding motifs (GGCGG) (12) existed on chromosome 11 where the breakage occurred in between. Moreover, within several hundred base pairs beyond the breakpoint, there is a region of higher GC content up to 75% containing six potential Sp1 binding motifs (data not shown). These results suggested this region to be a possible promoter region of a candidate protooncogene. Repetitive sequence-free Probe A and Probe B beyond the breakpoint (Fig. 1B), however, did not demonstrate specific signals by conventional Northern blotting. Efforts are now being made to look for a message associated with this translocation.

Since haptamer-spacer-nonamer-like sequence and switch-like repeat sequence (GAGCT and TGGGG) (13) are not present on the 14q+ chromosome, the mechanism of this translocation probably did not involve VDJ joining and isotype switching. Furthermore, Jp probe did not hybridize to DNA from 669AA-1 cells containing both 14q+ and 11q− chromosomes (9), supporting this idea. Thus, it is conceivable that the different recombinational event has occurred in this translocation. It is noted that of nine GAGG tetranucleotides, four of those are present near the breakpoint of chromosomes 11 and 14q+, respectively (Fig. 3). This sequence is reported to be present near the breakpoints of MYC translocation of certain Burkitt’s lymphomas and mouse plasmacytomas (14, 15). This sequence may be the one recognized by either of the enzymes that catalyze immunoglobulin heavy chain switching or some other DNA-cleaving activity and may play a role in defining the position for these breakpoints (14, 15). The Alu sequence is present on or near the breakpoint of chromosome 14 but not on that of chromosome 11, suggesting that the mechanism indicated in the BCR-ABL translocation (16) is not likely to occur in this cell line.

Southern Blot Analysis of Hematopoietic Tumors with Translocations Involving the Chromosome 11q23 Region. Southern blot analysis of RC-K8 cells was carried out with Probe B (Fig. 1B), and rearrangement bands were demonstrated in EcoRI, BclI, SacI, and XbaI digestion, confirming that this region is involved in t(11;14)(q23;q32) translocation of this cell line (Fig. 4). The same probe was used to study whether rearrangements can be detected in leukemia and lymphoma cases with translocations involving chromosome 11q23. The cases studied were three acute undifferentiated leukemias [one t(6;11)(q27;q23), one t(4;11)(q21;q23), and one t(11;12)(q23;q23)], four neonatal acute lymphocytic leukemias [t(11;19)(q23;p13)], two B-cell lymphomas [one t(7;11)(q11;q23) and one t(4;11)(q21;q23)], and one myelodysplastic syndrome [inv (11)(p11;q31)]. No rearranged bands were, however, observed in all these cases,
Molecular Cloning of the Breakpoint in t(11;14) 11q23. We are grateful to C. Ito, K. Nishida, H. Suzuki, and K. Okumura for technical assistance.

References

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