The RCK Gene Associated with t(11;14) Translocation Is Distinct from the MLL/ALL-1 Gene with t(4;11) and t(11;19) Translocations

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Abstract

We previously demonstrated that the 11q23 breakpoint region, designated the RCK locus, of the RC-K B-lymphoma cell line with t(11;14)(q23;p13) is centromeric to PBGD, while breakpoints of infantile leukemia cell lines with t(11;19)(q23;p13) are detectable by pulsed-field gel electrophoresis with the CD3D probe. In the present study, using a probe within 1.0 kilobase of the t(11;14) breakpoint, we isolated a partial complementary DNA clone for the putative RCK gene, which detects a 7.5-kilobase mRNA. Sequence analysis predicted a novel protein of 472 amino acids which demonstrated sequence homology to a translation initiation factor/helicase family. We also isolated a phage clone from the CD3D/G yeast artificial chromosome clone (yB22B2) which detects 11- and 12-kilobase mRNAs, most likely for the MLL/ALL-1 gene and also a phage clone from the CD3 YAC clone, containing the PBGD gene on 11q23 in order to see the relationship between RCK and MLL/ALL-1.

Materials and Methods

Cell Lines and YAC. Karpas 422, RS4;11, KOCL-45, KOCL-48, and KOCL-58 cell lines have t(4;11) translocations, while KOPN-1, KOCL-33, and KOCL-44 cell lines have t(11;19) translocations. RC-K has a t(11;14) translocation. The culture and characterization of these lines have been described (5). The YAC clone, yB22B2, containing CD3 (7) was generously provided by Dr. M. O. Diaz at Chicago University and Dr. P. Domer at Washington University on request to Dr. M. Olson at Washington University.

Isolation of a Partial cDNA Clone for RCK. Recombinant clones (6×10⁶) of a cDNA library prepared from a human small cell lung cancer cell line, SCLC-SA, in Agt10 vector (8) were screened with the conserved BstHII/BstHII 0.6-kilobase fragment (probe c) which is within 1.0 kilobase of the t(11;14) breakpoint (Fig. 1A). Hybridization was performed in a buffer containing 40% formamide, 100 μg/ml of salmon sperm DNA, 10% dextran sulfate, and 5× standard sodium-citrate buffer (1× concentration is 0.15 M NaCl-0.015 M sodium citrate) at 42°C. The filters were washed in a final stringency of 0.1× standard saline-citrate-0.1% sodium dodecyl sulfate at 56°C. Positive phage plaques were purified by standard procedure (8) and used for further analysis.

Construction of Genomic Library from a YAC Clone yB22B2. A genomic library was constructed using the λDASH II vector (Stratagene, La Jolla, CA). High-molecular-weight DNA extracted from YAC clone was digested with BamHI and size fractionated on low melting point agarose, and DNA ranging from 9 to 23 kilobases was purified. After ligation into λDASH II and packaging, 3×10⁶ PFGE, we studied the long range mapping from CD3 genes to the PBGD gene on 11q23 in order to see the relationship between RCK and MLL/ALL-1.

Introduction

A wide variety of hematopoietic malignancies, including acute leukemia, myeloproliferative disorder, and malignant lymphoma, are associated with chromosomal translocations at chromosome 11 band q23 (11q23), although the donor chromosomes are variable. Considering the variety of the cytogenetic events, it would be interesting to know whether the same gene is associated with some or all of these translocations. A candidate gene, designated mixed lineage leukemia (MLL) or acute lymphocytic leukemia-1 (ALL-1), has been reported by two groups of investigators (1, 2) to be associated with t(4;11)-(q21;q23) and t(11;19)(q23;p13) translocations. In this study, we cloned the breakpoint of t(11;14)(q23;p13) in B-cell lymphoma, the RC-K cell line, and named the locus RCK (3, 4). By PFGE, RCK was shown to be centromeric to PBGD, while the breakpoints of t(11;19)(q23;p13) were detected by the CD3D gene probe which is centromeric to RCK (5, 6). In this study, we isolated a partial cDNA for the putative RCK gene and also a phage clone from the CD3 YAC clone, which probably detects transcripts of the MLL/ALL-1 gene. By

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3 The abbreviations used are: PFGE, pulsed-field gel electrophoresis; cDNA, complementary DNA; YAC, yeast artificial chromosome.
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B

Fig. 2. Primary structure of RCK cDNA with the deduced amino acid sequence and in situ hybridization of RCK cDNA to R banding metaphase peripheral lymphocyte. (A) Nucleotide sequence of RCK cDNA shown with the deduced amino acid sequence. The 39-base pairs at the 5' end found in conserved region c (Fig. IA) is double underlined. Wavy line, an in-frame stop codon. Two possible initiation codons are single underlined. The second initiation codon, ATG, is in good agreement with Kozak's rule (11) and the deduced amino acid is shown under the nucleotide sequence. Motif I (AxAxxGTGK) and motif II (LDEAD) of the translation initiation factor/helicase family (12, 13) are marked by dotted underlines. (B) RCK cDNA clone, SA-30 (1.8-kilobase fragment at 5' end of cDNA), used as a probe for lymphocyte. (A) Nucleotide sequence of RCK cDNA shown with the deduced amino acid sequence. The 39-base pairs at the 5' end found in conserved region c (Fig. IA) is double underlined. Wavy line, an in-frame stop codon. Two possible initiation codons are single underlined. The second initiation codon, ATG, is in good agreement with Kozak's rule (11) and the deduced amino acid is shown under the nucleotide sequence. Motif I (AxAxxGTGK) and motif II (LDEAD) of the translation initiation factor/helicase family (12, 13) are marked by dotted underlines. (B) RCK cDNA clone, SA-30 (1.8-kilobase fragment at 5' end of cDNA), used as a probe for in situ hybridization. White arrows, positive fluorescence at chromosome 11 band q23.
Results and Discussion

**RCK Gene Is Associated with t(11;14)(q23;q32) Chromosome Translocation.** The nucleotide sequence across the breakpoint on 11q23 of the (11;14) translocation in RC-K8 cells is GC rich (69%) and 9 potential SP-1 binding sequences (GGGGCGG) existed, suggesting that this is a promoter region of a housekeeping gene. Probe c (BssHII 0.6-kilobase fragment (Fig. 1A)), containing the region conserved in various species, was used to screen a cDNA library from a small cell lung cancer cell line, SCLC-SA. Five overlapping cDNA clones with the same restriction map at the 5' end were obtained. Repeat cDNA walking resulted in overlapping clones covering 4.2 kilobases (Fig. 2A).

Sequence analysis of the cDNA demonstrated that the initiation codon at the 340 nucleotide surrounded by Kozak's consensus sequence (11) was followed by a long open reading frame encoding a 472-amino acid protein with a relative molecular mass of 53,216 Da (Fig. 2A). A homology search of the entire RCK amino acid sequence revealed a sequence similar to that of mouse elF-4A (38.4% identity in 407 amino acids) or Droso phila ME31B (70.0% identity in 426 amino acids) of a translation initiation factor/helicase family (12, 13). In this regard, it is noted that one translation initiation factor, elF-4E, exhibits transforming activity in NIH3T3 cells when it is overexpressed by retroviral expression construct (14). Comparisons of the nucleotide sequence revealed that the first 39-base pair sequence of the 5' end of the cDNA was in the conserved region c (Figs. 1A and 2A). The orientation of the sequence is directed to the centromere, indicating that the promoter region and the possible first exon of 39 base pairs of the RCK gene are decapitated from its coding region and that the coding region on der (11) is likely to be under the influence of the immunoglobulin heavy chain gene. Cloning analysis of der (11) confirmed that the region juxtaposed to RCK was a part of immunoglobulin genes (data not shown).

Localization of the RCK gene at 11q23 was demonstrated by in situ hybridization using a cDNA clone, SA-30. As shown in Fig. 2B, it hybridized to chromosome 11 band q23 of 20-metaphase peripheral blood lymphocytes, confirming that the transcriptional unit is from this region. Northern blot analysis with cDNA probe revealed a 7.5-kilobase mRNA (Fig. 3A) and it was found that the expression was ubiquitous in various tissues (data not shown). Although RCK is at the t(11;14) breakpoint region, the pattern of RCK expression in RC-K8 and other cell lines as well as in normal peripheral blood lymphocytes are not detectably altered (Fig. 3A). The mechanism of the RCK gene leading to malignant transformation by this translocation needs to be studied.

**Probes Isolated from yB22B2 YAC Clone.** We showed previously that the breakpoints of t(11;19) are detectable by PFGE with the CD3D probe (5). Thus, we attempted to isolate phage clones, which can detect rearrangements and transcriptional units, from a YAC clone with a 300-kilobase insert containing CD3D/G, but not CD3E (Fig. 1B). A genomic library was constructed with yB22B2 DNA after BamHI complete digestion and screened with radiolabeled total human DNA. Seven independent clones, 1a, 7y, 3h, 8r, 3i, 6n, and 7z, were mapped on yB22B2 with the reiterated sequence-free probe of 6n (Fig. 4, A and C). On the other hand, the unique probe from 7z also detected rearranged bands (a part of the results with 6n is shown in Fig. 4, A and C). On the other hand, the unique probe from 7z also detected rearranged bands with different sizes from those detected by 8r, 3i, and 6n unique probes (Fig. 4, B and D). These data indicated that some of the breakpoints of t(4;11) and t(11;19) lines are between 6n and 7z clones.

An attempt was then made to detect transcriptional units. A mixture of reiterated sequence-free probes from 6n detected 12- and 11-kilobase mRNA signals, the size and the pattern of which are similar to those of the MLL/ALL-1 gene which has been reported by two groups of investigators (1, 2). The expression in cell lines with t(4;11) and t(11;19) is not significantly different from RC-K8 or peripheral blood lymphocytes, which is in agreement with the previous reports by van der Poel et al. (1) and Cimino et al. (2).

**RCK and MLL/ALL-1 Genes.** The unique probes from 8r, 3i, and 6n detected rearranged bands in all t(4;11) cell lines except Karpass 422 and in all three t(11;19) cell lines by NotI and NruI digestion, respectively (a part of the results with 6n is shown in Fig. 4, A and C). On the other hand, the unique probe from 7z also detected rearranged bands with different sizes from those detected by 8r, 3i, and 6n unique probes (Fig. 4, B and D). These data indicated that some of the breakpoints of t(4;11) and t(11;19) lines are between 6n and 7z clones.
t(11;19) cell lines by NotI digestion (Fig. 4E). The observation that 1a, 7y, and 3h probes derived from yB22B2 hybridized to the same NotI fragment detected by RCK probe indicated that the 690-kilobase NotI fragment adjoins to the 96-kilobase NotI fragment where t(4;11) and t(11;19) breakpoints are located (Fig. 1B). Although the NotI fragments adjoin each other, the size of transcriptional units near the breakpoints of RCK and MLL/ALL-1 is different, suggesting that the gene involved for t(11;14) is different from the one for t(4;11) and t(11;19) translocations.

The present study showed that a novel gene, RCK, is found at the breakpoint region of t(11;14) and is a candidate gene to be...
involved in this translocation. Although MLL/ALL-i cDNA has not been cloned yet, the transcriptional units detected by van der Poel et al. (1) and Cimino et al. (2) are most likely to be derived from the candidate gene associated with t(4;11) and t(11;19). Thus, at least two different genes, RCK and MLL/ALL-i, are involved in the translocations at 11q23 region. Further study on these genes as well as the genes on the donor chromosomes will bring new aspects in understanding hematopoietic malignancies with 11q23 translocations.

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