Molecular Characterization of a t(11;14)(q23;q32) Chromosome Translocation in a B-Cell Lymphoma

Yukihiro Akao, Yoshhide Tsujimoto, Janet Finan, Peter C. Nowell, and Carlo M. Croce

INTRODUCTION

Chromosome abnormalities are a consistent feature of human malignancy (1). Hematopoietic neoplasms often exhibit specific chromosome translocations related to molecular events involving certain oncogenes, both known and previously unknown (2). For example, c-MYC at chromosome band 8q24 and BCL-2 at chromosome band 18q21 are juxtaposed to the IGH locus at chromosome band 14q32 in B-cell neoplasms (2). It is becoming clear that the activation of such oncogenes occurs as a consequence of their proximity to the IG genes and that translocations occur because of mistakes during physiological joining or switching (2).

The t(11;14)(q23;q32) chromosome translocation, which was observed in a B-cell lymphoma cell line, RC-K8 (3, 4), is uncommon, but other translocations involving 11q23 have been reported not only in acute lymphocytic leukemia but also in acute monocytic and myelomonocytic leukemias (5). It has been suggested that the q23 region of chromosome 11 may carry oncogenes, both known and previously unknown (6).

RESULTS

Production of Somatic Cell Hybrids. Somatic cell hybrids were made by fusing RC-K8 cells and hypoxanthine guanine phosphoribosyltransferase-deficient NP, mouse plasmacytoma cells in the presence of polyethylene glycol, and hybrids were selected in hypoxanthine-aminopterin-thymidine medium (6). The hybrids were then cloned by the limiting dilution method (6). Independent clones were expanded and examined for the presence or absence of the human 11q- and/or 14q+ chromosomes.

Cytogenetics. Chromosome analysis of RC-K8 and hybrid cells was performed by using the G-banding technique (7). Differential staining of mouse and human chromosomes in somatic cell hybrids was carried out using the G-11 technique (8).

Southern Blot Analysis. DNA extraction and Southern hybridization were carried out as described (9). Aliquots of 6–10 μg of DNA in human placenta and RC-K8 cells and of 15–20 μg of DNA in hybrid cells were digested with restriction endonucleases, BamHI, HindIII, and EcoRI. DNA probes were radiolabeled by nick translation.

Construction of Genomic DNA Libraries. DNA extracted from RC-K8 and hybrid cells was partially digested with Sau3A. DNA fragments of 15 to 23 kilobases were collected by using the sucrose gradient method. DNA inserts were cloned into the λEMBL3A phage vector digested with BamHI. The libraries were screened with the DNA probes radiolabeled by nick-translation using γ-32P-labeled deoxynucleotide triphosphates.

DNA Probes. The following probes were used: human IGH genes, Jα (10); constant region of the α-chain (Cα1) (11); constant region of the ε-chain (Cε1) (12); and constant region of the γ-chain (Cγ1) (13). The Cα1 and Cγ1 probes cross-hybridize to all other constant regions of α- and γ-chain genes, respectively. The probe for the switch region of the μ-chain (Sμ) also cross-hybridizes to all other switch regions (14).

Probes for the genes of BCL-1 (15), CD3 ε- and δ-chains (16, 17), ETS-1 (18), and T-cell receptor α-chain (TCRa) (19) were labeled by nick translation.

The 30-mer oligonucleotides specific for Cα1, Cα2, Cβ1, Cγ1, and Cγ4 were synthesized and used to differentiate the clones which contained the constant regions of the α- or γ-chains. The oligonucleotide probes were labeled by T4 polynucleotide kinase and γ-32P-ATP.

Karyotypic analysis of RC-K8 cells indicated that they were derived from a neoplastic clone carrying a t(11;14)(q23;q32) chromosome translocation, together with several other karyotypic changes that were essentially similar from cell to cell and remained stable over an extended period of time.

A hybrid cell line (669AA-1) and three sublines (BG4, BB7, and AG7) were used to localize the breakpoint of the t(11;14) translocation. We produced hybrid cells by fusing RC-K8 cells and NP3 hypoxanthine guanine phosphoribosyltransferase-deficient mouse myeloma cells. The segregating hybrids were analyzed with various DNA probes: TCRα (chr 14q11), BCL-1 (chr 11q13), and ETS-1 (chr 11q23).

The DNA of 669AA-1 hybridized with all of these probes and was subcloned to obtain three new hybrid cell lines, BG4, BB7, and AG7. DNA from BG4 cells was positive with the BCL-1 probe but negative with probes for ETS-1 and TCRα, suggesting that it carried the 11q- chromosome but not the normal 11 and 14 chromosomes or the 14q+ chromosome (Fig.
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Fig. 1. A Southern blot analysis of DNAs from human placenta (lane a), human parental cell line RC-K8 (lane b), mouse-human somatic hybrid cell lines 669AA-1 (lane c), carrying both 11q- and 14q+ chromosomes, BG4 (lane d), carrying 14q+ chromosome, and BB7 (lane e), carrying 11q- chromosome, and NP3 parental mouse cell line (lane f). A, hybridization with a C\textsubscript{ai} probe; B, hybridization with a C\textsubscript{7} probe. Size markers are in kilobases. Neither of the hybrid cell lines has normal chromosome 14.

Table 1 Characterization of somatic cell hybrids between RC-K8 B-cell lymphoma and NP3 mouse cell

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Status of human gene</th>
<th>Presence of human chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC-K8</td>
<td>+</td>
<td>11</td>
</tr>
<tr>
<td>669AA-1</td>
<td>+</td>
<td>11</td>
</tr>
<tr>
<td>BG4</td>
<td>+</td>
<td>11</td>
</tr>
<tr>
<td>BB7</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>NP3</td>
<td>-</td>
<td>11</td>
</tr>
</tbody>
</table>

* +, presence of the indicated probe or chromosome (>30% of the hybrid cells).

The Breakpoint on Chromosome 14 Is between the C\textsubscript{7} and C\textsubscript{72} Genes in the IGH Locus. To determine the region of the breakpoint on chromosome 14, Southern blots of DNA from RC-K8, the hybrid cell lines, and NP3 mouse cells were hybridized with several 32P-labeled IGH gene probes. We used the BB7 line because a larger fraction of the cells contained the 14q+ chromosome than did the AG7 cells. The C\textsubscript{ai} probe detected 23-, 20-, and 5.5-kilobase bands in BamHI-digested DNA from RC-K8. The 9.4-kilobase JH-hybridizing fragment represents a normal chromosome 14 which is not involved in the t(11;14) translocation (Fig. 2B). The 6.6-kilobase band is present in 669AA-1 and represents the 14q+ chromosome.

Fig. 2. A, gene order of IGH locus. The left side is the 5' region, which is telomeric on chromosome 14. B, restriction map of the C\textsubscript{7} region from germ line (20), normal chromosome 14 in RC-K8, and λ 669AA-1-\textsubscript{pγ} clone. Recombination has occurred in the switch region of C\textsubscript{7} gene on the nontranslocated chromosome 14. Restriction map of λ 669AA-1-\textsubscript{pγ} is consistent with the map which was previously reported as the region of C\textsubscript{7} gene (21). Total human DNA probe hybridized to the 5' flanking region of C\textsubscript{7} gene in λ 669AA-1-\textsubscript{pγ}. The specific 30-mer oligonucleotides of C\textsubscript{7i}, C\textsubscript{72}, and C\textsubscript{74} were synthesized with the phosphoramid method (27) and used to differentiate the clones which contain the constant region of \gamma or \gamma-chain genes. The recombinant clones were digested with HindIII (H), BamHI (B), EcoRI (E), XhoI (X), and PvuII (P). The probes, a and b, used for Southern blot analysis are also indicated. The solid boxes indicate the constant regions of \gamma-chain genes.

1). DNA from the BB7 and AG7 hybrid cells was positive with the probes for TCR\textsubscript{α}, ETS-1, and BCL-1 (Fig. 1). The results of cytogenetic analysis of the 669AA-1, BG4, and BB7 hybrid cell lines are summarized in Table 1.

The absence of a nontranslocated chromosome 14 in 669AA-1 cells was confirmed by Southern blot hybridization with the probe for J\textsubscript{H} (data not shown). This J\textsubscript{H} probe hybridized with the 9.4- and 6.6-kilobase bands in BamHI-digested DNA from RC-K8. The 9.4-kilobase J\textsubscript{H}-hybridizing fragment represents a normal chromosome 14 which is not involved in the t(11;14) translocation (Fig. 2B). The 6.6-kilobase band is present in 669AA-1 and represents the 14q+ chromosome.

The Breakpoint on Chromosome 14 Is between the C\textsubscript{7} and C\textsubscript{72} Genes in the IGH Locus. To determine the region of the breakpoint on chromosome 14, Southern blots of DNA from RC-K8, the hybrid cell lines, and NP3 mouse cells were hybridized with several 32P-labeled IGH gene probes. We used the BB7 line because a larger fraction of the cells contained the 14q+ chromosome than did the AG7 cells. The C\textsubscript{ai} probe detected 23-, 20-, and 5.5-kilobase bands in HindIII-digested DNA from RC-K8. The 23- and 5.5-kilobase fragments were also detected in HindIII-digested DNA from 669AA-1. The probes indicated that these two fragments were present on the chromosomes involved in the t(11;14) translocation and represented the C\textsubscript{ai} and C\textsubscript{ai} regions. The 5.5- and 23-kilobase fragments were also detected in HindIII-digested DNA from BG4 and BB7, respectively (Fig. 1A), implying that the breakpoint involved in the t(11;14) translocation of RC-K8 was between the C\textsubscript{ai} and C\textsubscript{ai} genes. Furthermore, the Southern blot analysis with the C\textsubscript{ai} probe indicated that hybridizing fragments of 18, 11, and 9-kilobases appeared separately in the lanes containing the DNA of BG4 (18 and 9 kilobases) and BB7 (11 and 9 kilobases), respectively (Fig. 1B). These data further indicated that the breakpoint involved in the chromosome translocation is located between the C\textsubscript{ai} and C\textsubscript{ai} genes, because the gene order from the 5' region of the IGH locus is C\textsubscript{ai}, C\textsubscript{ai}, C\textsubscript{ai}, C\textsubscript{ai}, and C\textsubscript{ai} (Fig. 2A) (20). Taken together, the results suggest that the breakpoint is between C\textsubscript{ai} and C\textsubscript{ai}. 

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Then we screened the genomic library from RC-K8 and 669AA-1 hybrid cells with probes for Cα, and/or Cγ3. Of the 11 λ phage clones, we obtained three that contained the Cα genes and eight that contained Cγ genes. The clones that contained the rearranged region 3’ of Cα did not have chromosome 11 sequences. All the clones that contained Cγ2, Cγ4, and Cγ7 were analyzed and the restriction map is shown in Fig. 2B. All the clones which contain the Cγ2 gene were from the normal chromosome 14.

A clone containing the Cγ7 gene was isolated from the library of 669AA-1 cells. The restriction map of this clone is identical to that reported previously (21). We subcloned the 3.4-kilobase PvuI-SalI fragment 3’ to the Cγ7 gene (Fig. 2B, probe A). Only the 8-kilobase fragment of the four fragments hybridized by probe A corresponds to the Cγ7 region. This 8-kilobase fragment was present in DNA from RC-K8, 669AA-1, and BG4 (Fig. 3A), indicating that the breakpoint of the t(11;14) translocation is 3’ to the Cγ7 gene. On the other hand, the probe for the BamHI-SalI 1.2-kilobase fragment 3’ to Cγ2 (Fig. 2B, probe B) hybridized to 3.5- and 4.0-kilobase fragments in BamHI-digested DNA from all hybrid clones except BG4 (Fig. 3B). The size of these two fragments is consistent with that of the restriction map of the fragment 3’ of the Cγ2 and Cγ4 genes from human germ line DNA (Fig. 2B). These data together indicated that the breakpoint on chromosome 14 is located between the Cγ7 and Cγ2 genes.

The Breakpoint on Chromosome 11 Is between the CD3 and ETS-1 Genes in Band q23. Southern blot analysis (of the parental cell line RC-K8) with probes for CD3λ and -ζ and for ETS-1 showed no rearranged band (data not shown). The ETS-1 probe hybridized to the 14- and 3-kilobase fragments in BamHI-digested DNA from all derived cell lines except BG4, which has the 11q- chromosome (Table 1). On the other hand, the probe for CD3ζ hybridized to 8-, 5-, and 3.2-kilobase fragments in HindIII-digested DNA from all cell lines studied except the NP3 mouse parental cells (Fig. 4). The probe for CD3λ also hybridized with digested DNA from BG4 (data not shown). These data indicated that the breakpoint at q23 on chromosome 11 is in the telomeric region between CD3ζ/ε and ETS-1.
DISCUSSION

We have localized the breakpoints on chromosomes 11 and 14 in the t(11;14)(q23;q32) chromosome translocation in a B-cell lymphoma cell line through DNA analysis of somatic cell hybrids retaining the 11q- and/or 14q+ human chromosome(s).

The breakpoint on chromosome 14 was mapped between the CPy and C72 genes. Although the distance between these two genes is not known precisely, due to lack of recombinant clones, this distance has been reported to be about 40 kilobases using pulse field gel electrophoresis analysis (22).

Two major types of mechanisms have been proposed in chromosome translocations in B-cell tumors. One involves the JH segment and the other the switch regions of the IGH locus (2). The JH segment includes the t(1;14) chromosome translocation in chronic lymphocytic leukemia (10), the t(14;18) in follicular lymphoma (23), and the t(8;14) in acute lymphocytic leukemia and endemic Burkitt’s lymphoma (14). Recently, it has been reported that a t(5;14) translocation in acute lymphocytic leukemia also involved the JH segment (24). These translocations are considered to occur at a pre-B-cell stage of differentiation when the IGH genes undergo physiological recombination (2).

Chromosome translocations in the switch regions are common in sporadic Burkitt’s lymphoma (2), and it has been proposed that these t(8;14) translocations are mediated by isotype-switching enzymes (2).

The t(11;14)(q23;q32) translocation in RC-K8 cells does not seem to belong to either of these two categories, because the breakpoint on the 14q+ chromosome was mapped between CPy and C72, and our Southern blots showed the switch region of C72 was not rearranged (data not shown). Therefore, it is possible that this translocation in RC-K8 cells is mediated by a different mechanism.

The breakpoint on chromosome 11 in the t(11;14) translocation was mapped between the CD3e/δ and ETS-1 genes. The map of this region appears to be: 11cen-CD3e/δ-breakpoint of t(11;14)-ETS-1-11q tel. This gene order is consistent with that reported recently (25). The distance between the CD3 genes and ETS-1 has been estimated by pulse field gel electrophoresis analysis to be approximately 280 kilobases (25). Various chromosome aberrations, including t(4;11) in acute lymphocytic leukemia, t(9;11) in acute monocytic leukemia, and t(X;11) in acute myelogenous leukemia, have been mapped between the CD3 and ETS-1 genes, a heritable fragile site at 11q23. This remains to be determined whether all these chromosomal rearrangements involve the same cellular gene. The cloned breakpoint of the t(11;14) translocation in RC-K8 cells should provide an important tool in the continuing efforts to answer these questions.

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REFERENCES

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