A Variant Chromosome Translocation at 11q13 Identifying PRADI/Cyclin D1 as the BCL-1 Gene

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The 11q13 breakpoint region of t(11;14) (q13;q32), translocated to the Ig heavy chain locus at 14q32, has been designated as BCL-1 for B-cell leukemia/lymphoma-1, but the nature of the transcriptional unit has long remained unclear. Recently, the PRADI gene encoding cyclin D1, isolated from the 11q13 region, was proposed as a candidate BCL-1 gene on the basis of chromosome walking and concordant overexpression of PRADI mRNA in cell lines with t(11;14)(q13;q32). We report here molecular analysis of a variant translocation at the BCL-1 locus, t(11;22)(q13;q11), showing juxtaposition of the Ig light chain gene, Igκ, to the PRADI gene at its 3' end, resulting in overexpression of PRADI mRNA. Because only the PRADI gene is present between the Ig heavy chain and light chain gene breakpoints, an identity between BCL-1 and the PRADI/cyclin D1 gene is strongly indicated.

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Materials and Methods

Patient sample and cell lines. The patient sample was analyzed as a right buccal subcutaneous tumor diagnosed as a mantle zone lymphoma/intermediate lymphocytic lymphoma. Cytogenetic analysis indicated that 10 of 24 metaphases had 46, XX, del(1)(q11p12), t(11;22)(q13;ql1) and that the remaining 14 had an additional del(6)(q22q25). Cell surface markers were positive for CD5 (39.2%), CD19 (91.2%), CD20 (95.3%), CD21 (80.1%), CD38 (58.7%), HLA-DR antigen (96.3%), IgM (42.8%), and Igκ (42.1%). and were negative for CD3, CD4, CD8, and CD10. Detailed patient characteristics and clinical course were reported previously.17 Cell lines used were Raji, a Burkitt's lymphoma cell line without any malignancy, and KMS-12-BM, a multiple myeloma cell line with 11q13 abnormality, and KMS-12-BM, a multiple myeloma cell line with 11q13 abnormality, and KMS-12-BM, a multiple myeloma cell line with 11q13 abnormality.

Northern and Southern blot analyses. Northern and Southern blot analyses were performed as described previously.13,14 Ten micrograms of total RNA for Northern analysis and 10 μg of DNA digested either with BamHI or Pst I endonuclease for Southern analysis was applied in each lane. Probes used in this study are shown in Fig 1C. PRADI cDNA probes were kindly provided by Dr A. Arnold (Massachusetts General Hospital, Boston, MA) and Igκ probe was obtained through Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan).

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Fig 1. Northern and Southern blot analyses of the t(11;22)(q13;q11) lymphoma and schematic representation of the probes used. (A) Northern analysis of samples with 11q13 translocations. PBL, peripheral blood lymphocytes. The left panel illustrates PRAD1 cDNA probe a for detection of overexpressed signals in KMS-12-BM and the t(11;22) case. The dashed line indicates the 3.4-kb signal in the t(11;22) case comigrating with the signal for IgA probe c (right panel, the same blot was used as for the left panel). (Lanes are aligned in a mirror image for the IgA probe.) The sizes of the signals are shown on the left and the right of each panel, respectively. Ethidium staining of the gel is shown below. (B) Southern analysis with 3' PRAD1 and IgA probes. Probes b and c were used for the PRAD1 and IgA probes, respectively. Rearranged bands detected by probe b are indicated by arrows. Dashed lines show the comigration bands detected by probe c in either digestion. Size markers of AlHindlll are shown on the left of each panel. (C) Schematic representation of the PRAD1 cDNA and the genomic structure of the IgA gene and the probes. The coding region of the PRAD1 cDNA\(^\text{12}\) is depicted in the solid box. The shaded boxes at IgA2 and IgA3 mark direct repeats that share greater than 95% nucleotide sequence identity.\(^\text{19}\) Open boxes represent exons of the IgA genes. J, joining region; C, constant region. Probes are a, \text{Pst I/HindIII} 0.9 kb; b, \text{EcoRI/EcoRI} 1.6 kb; c, \text{EcoRI/HindIII} 3.4 kb.\(^\text{19,34}\) P, \text{Pst I}; H, \text{HindIII}; E, \text{EcoRI}.

Genomic library. A genomic library was made by partial digestion of high molecular weight DNA of the lymphoma by Sau3AI and was ligated in a Adash1l vector (Stratagene, La Jolla, CA) as described previously.\(^\text{13}\) Recombinant clones (3 \times 10\(^4\)) were screened with PRAD1 cDNA probe b (Fig 1C). Positive clones were subcloned into pBluescript vectors (Stratagene) and analyzed.

DNA sequencing. Nucleotide sequences were determined by the dideoxy chain termination method using a 7-deaza Sequenase version 2.0 kit (US Biochemicals, Cleveland, OH) described previously.\(^\text{13}\) Deletion mutants for sequencing were prepared with the Exo/Mung bean deletion system (Stratagene). Sequences were compared with those of both PRAD1 cDNA\(^\text{19}\) and the genomic IgA gene\(^\text{20}\) registered in the GenBank (LASL-GDB Rel.75).

Reverse transcriptase-polymerase chain reaction (RT-PCR). RT-PCR was performed as reported previously.\(^\text{23}\) Briefly, 5 \mu g of total RNA was reverse transcribed to cDNA in a total volume of 20 \mu L with random hexamers and 200 U of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Bethesda, MD). One-tenth of the cDNA was applied for PCR amplification in a total volume of 100 \mu L with 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.8 at 25°C), 1.5 mmol/L MgCl\(_2\), 0.1% Triton-X-100, 100 pmol of each primer, 20 mmol/L of each dNTP, and 2.5

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**THE BCL-1 GENE AT 11q13 IS PRAD1/CYCLIN D1**

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U of Taq polymerase (Promega, Madison, WI). Samples were overlaid with 100 μL mineral oil (Sigma, St Louis, MO). After 30 rounds of PCR (2 minutes at 95°C, 2 minutes at 58°C, and 1 minute at 72°C), 10 μL of PCR product was electrophoresed in a 1.4% agarose gel. Two RT-PCR products were isolated and sequenced.21 The sense primer, PRADI-S1, was 5'-ACTCTCAGGCAAGTTGGA-3' (nucleotides 3011-3030 of the PRADI cDNA) and the antisense primer, Ca-AS, was 5'-CAGTGGCCTGTGGGCTT-3' (nucleotides 15046-15065 of the Ig C3 region; see Fig 3B).20

RESULTS

Northern analysis with the PRADI cDNA probe a (Fig 1C) demonstrated smeary intense signals approximately 1.7 kb in size in the t(11;22) case, as shown in Fig 1A, with an intensity similar to its equivalent in KMS-12-BM with t(11;14). No such signals were detected in peripheral blood lymphocytes and a Burkitt's lymphoma cell line, Raji, under the present conditions, indicating overexpression of PRADI mRNA in the t(11;22) case. When compared with 4.5-kb and 1.7-kb PRADI signals of normal size in KMS-12-BM, no 4.5-kb signal was recognized in the t(11;22) case, but a truncated 3.4-kb signal was found, although at a lesser intensity (Fig 1A). Thus, it was speculated that the aberrant mRNA in the t(11;22) case was caused by either deletion within the gene or translocation with the Igλ gene at 22q11.

Southern blot analysis was conducted with PRADI cDNA probes. The PRADI cDNA probe b (Fig 1C) showed two rearranged bands (Fig 1B), indicating that the breakpoint is within this region. One of the rearranged bands was demonstrated to comigrate with a rearranged band detected by the Igλ probe c (Fig 1B and C). Because PRADI translocation with IgH takes place at its 5' side in a head-to-head orientation,22 it is speculated that the translocation with Igλ gene is in a head-to-tail orientation, as found in Burkitt's lymphoma.1 When the translocation breakpoint is located within the gene, PRADI-Igλ fusion transcripts may result as is the case for BCL-2-lg fusion transcripts.23 Such a possibility is suggested by comigration of the 3.4-kb signal in the t(11;22) case, with the signal detected with the Igλ probe c by Northern analysis (Fig 1A). These data prompted us to clone the breakpoint region.

A genomic library of the t(11;22) lymphoma was made and two clones were isolated using PRADI cDNA probe b. As shown in Fig 2A, comparison of the restriction maps of the two clones with that of the published germline allele22 showed the LMZ-2 to be of the germline allele and the LMZ-8 to be of the der 11 allele. The breakpoint was in PRADI exon 5 and the Igλ gene was introduced 3' to the breakpoint. The nucleotide sequence showed the breakpoint to be after the poly(A) signal for 1.7-kb mRNA in exon 5 and the PRADI was fused to the J-C intron of the Igλ3 gene in a head-to-tail orientation (Fig 2B). No N-segment–like sequence like that found in the BCL-2 translocation23 was present at the fusion site. The Ig recombination signal sequence, topoisomerase I-recognition sequence, and Alu element sequence, which had been suggested to be involved in chromosome translocations,24-26 were not observed.

Because the orientation of the fused PRADI and Igλ genes and the result of the Northern blot analysis (Fig 1A) suggested the presence of PRADI-Igλ3 fusion transcripts, an RT-PCR was conducted. RT-PCR analysis detected two chimeric fusion transcripts of 1.5 kb and 0.7 kb in the t(11;22) case, but no such band was observed in a negative control, Kasumi-1, an acute myeloid leukemia cell line (Fig 3A and B). The two kinds of PCR products were sequenced and the 1.5-kb band was read through transcripts, whereas in the 0.7-kb band, 784 bp were spliced out. The 3' end of this splicing was the J-C splicing site of the Igλ3 gene and both ends of the splice sites (nucleotides 14205 and 14988 of the Igλ gene) had the AGGT consensus sequence of the intron-exon junction.

DISCUSSION

The 11q13 region is not only the target for translocation with Ig genes but is also known as a region of amplification in various kinds of solid tumors.4,5,14,15 Candidate genes activated as a result of gene amplification at this region have long been pursued and PRADI, HSTI, INT2, as well as EMSI have all been identified in the same amplification unit.5 These are located in this order from centromere to telomere.27 In cells with 11q13 amplification, the PRADI and the EMSI genes have been shown to be overexpressed.16,27 Because the amplification unit includes several genes, it is difficult to identify which one is critical for growth advantage, ie, either PRADI or EMSI, both, or other genes yet to be elucidated might play a key role(s). Regarding the target gene for 11q13 translocation with IgH gene, the PRADI/cyclin D1 was suggested to be a candidate BCL-1 gene by gene walking28 and also by concordant PRADI mRNA overexpression with the presence of t(11;14)(q13;q32).13 However, it is possible that a gene(s) in the amplification unit other than the PRADI may be also activated by the IgH gene translocated to the BCL-1 locus because the IgH gene introduced to the BCL-2 locus was shown to affect the promoter of the BCL-2 gene more than 250 kb away.29 Indeed, the HSTI and INT2 are located 3' to the PRADI gene and within 300 kb from the BCL-1 major translocation cluster region (MTC).27 It has, therefore, been impossible to conclude unequivocally that the PRADI/cyclin D1 is the BCL-1 gene from the data so far accumulated. To examine the identity between the PRADI gene and the BCL-1 gene, we took advantage of chromosome translocation involving Ig genes. Breakpoints with the IgH gene and those with the Igλ genes have been shown to locate at the opposite side of the target genes, such as the MYC gene in Burkitt's lymphomas30 and the BCL-2 gene in follicular lymphomas.31-33 leading to the conclusion that target genes activated as results of Ig gene translocations are confined between the breakpoints with the IgH and Igλ genes. The present case showed that the breakpoint with the Igλ gene was located in the 3' portion of the PRADI gene (Fig 2A), indicating that the target gene for Ig gene translocation is confined in between. Because no transcripional unit has been identified between the BCL-1 MTC and the PRADI gene,9,12,28,30 the PRADI gene is the sole candidate known to be located between the breakpoints, indicating that the putative BCL-1 gene activated by chromosome translocation is in fact PRADI itself.
Fig 2. Molecular cloning of the breakpoint of the t(11;22) translocation. (A) Diagramatic representation of the 11q13 region and restriction maps of λMZ-2 and λMZ-8. An amplification unit from BCL-1 (MTC) to EMS1 is shown (11q13) and was reported to be within 1,000 kb. The BCL-1 (MTC) and PRAD1 were reported to be 120 kb apart and the distances between the PRAD1 and HST1 and between the HST1 and INT2 were reported to be 125 and 35 kb, respectively. The dotted region depicts the portion derived from the Iga gene. The solid boxes are the coding regions of the PRAD1 gene and the dotted box is the Iga constant region (CA3). The arrowhead shows the breakpoint. Arrows indicate the region sequenced. The dashed line in exon 5 denotes an alternative poly(A) site for the 1.7-kb signal.

(b) Comparison of nucleotide sequences around the breakpoint. The homologous region is indicated by asterisks. The arrowhead depicts the breakpoint. The 5' and 3' regions of the breakpoint correspond to exon 5 of the PRAD1 cDNA and the J-C intron of Iga, respectively. The nucleotide numbers are the same as those reported previously.

The mechanism of lymphomagenesis by BCL-1 translocation is not clear. Several lines of evidence suggested that the PRAD1 gene encoding cyclin D1 is an important cell cycle regulator, and, recently, overexpression of this cyclin D1 in NIH 3T3 cells was shown to give rise to tumors in nude mice. As shown in the present case, the coding region is not affected by the translocation because the fusion site is in the 3' untranslated region within PRAD1 exon 5. In our (11;22) case, Northern analysis showed two overexpressed PRAD1 mRNAs of 1.7 kb and 3.4 kb. Because the breakpoint is located after the poly A signal for 1.7-kb mRNA, the overexpressed 1.7-kb transcripts in the t(11;22) case are not altered in size. Although the detailed structures of chimeric mRNAs have not yet been fully analyzed, RT-PCR analysis demonstrated that PRAD1-IgA fusion transcripts exist, as found for BCL-2-Ig fusion transcripts in t(14;18)(q32;q21). It is not known whether all variant BCL-1 translocations will make chimeric mRNA, but it is unlikely that making such chimeric mRNAs itself is important for lymphomagenesis. Indeed, as for the BCL-2 translocation, t(14;18) cases do not always make chimeric mRNA. Thus, oncogenesis in this t(11;22) case is speculated to have resulted from the overexpression or deregulated expression of PRAD1 mRNA such as that of translocated BCL-2. The present study showed the target gene for BCL-1 translocation to be PRAD1. Attention should therefore now be focused on...
the PRAD1/cyclin D1 gene to facilitate understanding the molecular mechanisms underlying genesis of mantle cell lymphomas with a BCL-1 translocation.

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