Detection of the Chromosomal Translocation t(11;14) by Polymerase Chain Reaction in Mantle Cell Lymphomas

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The t(11;14)(q13;q32) and its molecular counterpart, BCL1 rearrangement, are consistent features of mantle cell lymphoma (MCL). Rearrangement is thought to deregulate the nearby CCND1 (BCL1/PRAD1) proto-oncogene, a member of the cyclin G1 gene family, and thereby to contribute to tumorogenesis. We and others have previously shown that the BCL1 locus is rearranged in 55% to 60% of MCL patients and that, on chromosome 11, more than 80% of the breakpoints are localized within a 3-kbp DNA segment known as the major translocation cluster (MTC). We have determined the nucleotide sequence for a portion of the MTC region, and constructed chromosome 11-specific oligonucleotides that were in conjunction with a consensus immunoglobulin (lg) heavy chain joining region (JH) primer used to perform the polymerase chain reaction (PCR) to amplify t(11;14) chromosomal junctional sequences in DNA from 16 MCL patients with breakpoints in the MTC region. 15 of the 16 breakpoints that occurred at the MTC region were amenable to PCR detection. The sizes of the amplified bands, the existence or not of a Sac I site in the PCR products, and nucleotide sequencing of the amplified DNA from four patients showed that the breakpoints share a remarkable tendency to tightly cluster within 300 bp on chromosome 11, some of them occurring at the same nucleotide. On chromosome 14, the breakpoints were localized within the Ig JH. Our findings indicate that a BCL1 rearrangement can be detected using this approach in roughly one half of the MCL patients. This has implications for both the diagnosis and the clinical management of MCL.

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MALIGNANT NON-HODGKIN’S lymphomas and many other human tumors are often associated with specific chromosome translocations, and experimental evidence indicates that genes located at recurring chromosomal breakpoints are directly involved in tumor pathogenesis.1 Furthermore, several of these cytogenetic abnormalities are potentially valuable as diagnostic and prognostic aids. The most frequent translocation in human lymphoma is the t(14;18)(q32;q21),2 which can be detected by cytogenetic or molecular analyses in approximately 85% of follicular lymphomas. This translocation juxtaposes the proto-oncogene BCL2, from 18q21, with an immunoglobulin (lg) heavy chain joining region (JH) segment on the derivative chromosome 14. This results in a BCL2-lg fusion gene, chimeric transcripts, and transcriptional deregulation of the translocated BCL2.3,4 The chromosome breakpoints are remarkably well focused on chromosome segment 18q21 in two well-defined regions of 500 bp, known as the major and the minor breakpoint regions.5,6 Taking advantage of this situation, several authors have shown that the t(14;18) can be detected by polymerase chain reaction (PCR) amplification of DNA extracted from fresh tumor cells, frozen tissues, and even from formalin-fixed tissues.7,8 Moreover, because of its extremely high sensitivity, PCR amplification of the BCL2-lg junction offers a valuable means of detecting minimal residual disease in follicular non-Hodgkin’s lymphomas.

Mantle cell lymphoma (MCL) represents a clinicopathologically distinct subtype of malignant non-Hodgkin’s lymphomas that originates from immature virgin B cell in the primary follicle and mantle zone of peripheral lymphoid organs.9,10 It accounts for 5% to 10% of the overall number of malignant non-Hodgkin’s lymphomas in adults. The t(11;14)(q13;q32) and its molecular counterpart, BCL1 rearrangement, are consistent features of this lymphoma subtype.11-16 As a result of the t(11;14), the BCL1 locus is juxtaposed to an Ig enhancer sequence located on chromosome 14.17,18 The subsequent deregulation of CCND1 (PRAD1/BCL1), a member of the cyclin G1 gene family, is thought to perturb the G1-S transition of the cell cycle and thereby to contribute to tumor development.19-22 Although the chromosomal breakpoints are widely scattered on chromosome region 11q13, we and others have shown that more than 80% of them are clustered in a restricted DNA segment known as the major translocation cluster (MTC).13,15-18,24 In the present study we showed that 15 of the 16 t(11;14) breakpoints that occur at the MTC are amenable to PCR detection because they share a remarkable tendency to cluster tightly within 300 bp on chromosome 11. These findings should facilitate application of the PCR techniques for the diagnosis and the clinical management of MCL.

MATERIALS AND METHODS

Tumoral samples and cell lines. Characterization of the tumoral samples and cell lines used in this study have been previously reported (cases 1 to 39).25 All the MCL cases analyzed here showed a prominent diffuse pattern of growth. The human B-cell line Rec l (referred to as case 40) was established from a t(11;14)(q13;q32)-bearing lymphoma.26

DNA probes and hybridization procedures. The probe for the Ig JH was a gift from T.H. Rabbitts (MRC, Cambridge, UK). The MTC probe was kindly given to us by Y. Tsujimoto (Wistar Insitute, Philadelphia, PA) and consists of a 2.3-kbp Sac I-Sac I DNA genomic fragment. The EHO4 probe corresponds to an EcoRI-HindIII

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ACATCG-3'. Their relative location is denoted in Fig 2. For PCR amplification, one microgram of purified DNA was placed in 100 μL of PCR buffer (10 mmol/L TRIS HC1 pH 8.3/50 mmol/L KCl/1.5 mmol/L MgCl2/0.001% (wt/vol) gelatin/deoxyxynucleotide triphosphate at 200 μmol/L each) with primers P1 and P2 at 0.3 μmol/L. 2.5 U of Taq DNA polymerase (Perkin Elmer-Cetus, Norwalk, CT) were added and reaction mixtures were overlaid with paraffin oil. Capped tubes were subjected to 30 cycles of denaturation (1 minute at 95°C, first cycle 5 minutes), annealing (1 minute at 56°C), and extension (1 minute at 72°C, last cycle 10 minutes) in the Perkin Elmer DNA thermal cycler. Fifteen microliters of the reaction products was electrophoresed on a 2% agarose gel; after ethidium bromide staining the gel was blotted and the filter hybridized with the T4 polynucleotide kinase end-labeled oligonucleotide, P3.

**DNA sequencing of the PCR products.** To clone and sequence the amplified DNA, some PCR reactions were performed using oligonucleotides P1/P3 carrying the recognition sequence for BamHI or EcoRI at their 5' ends. The PCR products were eluted from agarose, digested with BamHI and EcoRI and cloned in the Bluescript vector. The inserts were sequenced using the double-stranded DNA sequencing technique (dideoxy chain termination procedure) with sequenase II (US Biochemical Corp., Cleveland, OH) as described by the manufacturer.

**RESULTS AND DISCUSSION**

The results of BCL1 rearrangement analysis for all the samples presented here have been reported in a previously published study. In this study, we showed that the BCL1 locus was rearranged in 19 of the 33 MCL (57%) patients tested, and that in 16 of them, the breakpoint was in the

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Fig 1. The COND1/BCL1 locus and restriction enzyme map surrounding the MTC region of chromosome 11 (band q13). The solid bars above the maps show the location of the major (MTC) and the minor (mTC1 and mTC2) translocation clusters (A), and the location of the 300-bp region that contains 15 of the 16 breakpoints analyzed (B). The double-headed arrows delimit the DNA segment whose sequence is shown in Fig 2. Slashed-horizontal lines indicate the position of the probes used in this study. Abbreviations: TEL, Telomere; CEN, Centromere; B, Bgl II; S, Sac I; H, HindIII; X, Xba I; E, EcoRI; Ba, BamHI.

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DNA fragment as mentioned in Fig 1. α-32P-labeling, prehybridization, hybridization and washing were performed as previously described.

**Preparation and analysis of λ phage DNA library.** DNA from the peripheral leukocytes of a normal donor was partially digested with Mbo I restriction endonuclease, DNA fragments averaging 15 to 20 kbp in length were selected by sucrose gradient centrifugation and ligated to BamHI arms of the EMBL 3 phage (Stratagene, La Jolla, CA). Library screening was performed with the MTC probe. The recombinant positive clones were characterized by restriction endonuclease mapping and subclones were prepared in Bluescript vectors (Stratagene).

PCR amplification of the BCL1-JH junction. A consensus Ig JH oligonucleotide primer (P1: 5'-ACCTGAGGAGACGGTGACA- GGGT-3') was used for chromosome 14. The chromosome 11q13-specific oligonucleotides had the following sequences: P2: 5'-GAAGGACTTGTGGG-CT-3'; P3: 5'-AGGCTGCTGTAC- CAGGGT-3'. Their relative location is denoted in Fig 2. For PCR amplification, one microgram of purified DNA was placed in 100 μL of 1× PCR buffer (10 mmol/L TRIS HC1 pH 8.3/50 mmol/L KCl/1.5 mmol/L MgCl2/0.001% (wt/vol) gelatin/deoxyxynucleotide triphosphate at 200 μmol/L each) with primers P1 and P2 at 0.3 μmol/L. 2.5 U of Taq DNA polymerase (Perkin Elmer-Cetus, Norwalk, CT) were added and reaction mixtures were overlaid with paraffin oil. Capped tubes were subjected to 30 cycles of denaturation (1 minute at 95°C, first cycle 5 minutes), annealing (1 minute at 56°C), and extension (1 minute at 72°C, last cycle 10 minutes) in the Perkin Elmer DNA thermal cycler. Fifteen microliters of the reaction products was electrophoresed on a 2% agarose gel; after ethidium bromide staining the gel was blotted and the filter hybridized with the T4 polynucleotide kinase end-labeled oligonucleotide, P3.

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Fig 2. Sequence of the MTC region showing locations of PCR oligonucleotides (P2 and P3) and translocation breakpoints. The nucleotide sequence is shown oriented in a telomere to centromere direction. At position 414, an A can be replaced by a C as a result of a DNA polymorphism. The underlined sequence corresponds to DNA binding site of the bp45 endogenous nuclease. Arrows denote location of the breakpoints. The sequence reported here has been deposited in the EMBL data base (accession number: X74150).
DETECTION OF t(11;14) BY PCR IN MCL

vicinity of the MTC region. In these 16 cases, no MTC/JH comigration was observed and we assumed that the rearranged fragment detected with the MTC probe was likely to originate from the derivative chromosome 11. To map more accurately the t(11;14) chromosomal breakpoints, we decided first to clone a probe capable of detecting the rearranged fragment originating from the derivative chromosome 14.

Screening of a genomic DNA library with the MTC probe allowed us to isolate several overlapping recombinant clones containing more than 5 kbp of the region telomeric of MTC (Fig 1). Following restriction endonuclease mapping analysis of this region we were able to isolate an EcoRI-HindIII DNA fragment (EH04) that is localized 1.5 kbp downstream of MTC (Fig 1). An EH04/JH comigration was observed in all the 16 cases studied here (data not shown). This implied that the EH04 probe detected the rearranged fragments that originate from the derivative chromosome 14 and that, on chromosome 11, the breakpoints were localized between the MTC and the EH04 probes. Moreover, as Southern blot analysis using EH04 probe detected a rearranged fragment in HindIII-cleaved DNA but not in EcoRI-digested DNA from 15 of the 16 patients, we were able to narrow the location of the breakpoints within a 2-kbp region delimited by the EcoRI site on the centromeric side. Patient 26 was the only one to show a rearranged band in EcoRI digest with EH04 probe, indicating that in this case, the break occurred in a region telomeric of the EcoRI site. Finally, we had previously noted that, when the breakpoints on chromosome 11 were localized within the SacI/SacI fragment which corresponds to the MTC probe, only one rearranged band could be shown in SacI digest. This indicated that the breaks, in these cases, might be very close to the MTC telomeric SacI site. Added to previous studies, these data clearly showed that the majority of the t(11;14) breakpoints are focused on a well-defined chromosome 11 region and that they could be detected by PCR amplification using Ig- and chromosome 11-specific primers.

To investigate further this region of chromosome 11 and the translocation breakpoints that were detectable with the MTC and EH04 probes, selected nucleotide sequencing was performed on germline chromosome 11 DNA containing and flanking the MTC and EH04 fragments. The resulting sequence (Fig 2) was used to construct oligonucleotides for use in PCR reaction in combination with a consensus Ig JH primer. These primers were used to study DNA specimens from a set of lymphomas that were selected solely because they had detectable chromosome 11 rearrangements on Southern blots using the MTC probe. When a chromosome 11-specific primer (P2) was used with the consensus JH primer (P1) on these DNA specimens, 15 of 16 were found to contain amplified DNA bands as detected after ethidium bromide staining of the control gel, or after hybridization of the filters with a radiolabeled oligonucleotide (P3) homologous to the amplified segment of chromosome 11. On ethidium bromide stained gel, several faint aspecific bands can be observed in all the samples, but are no longer present after hybridization with the radiolabeled oligonucleotide. Most of the specimens showed two major hybridization bands with different sizes. DNA from normal leucocytes and from patient 26 failed to result in detectable hybridization bands (Fig 3). This assay was capable of detecting 1 lymphoma cell in 10^5 total cells with 1 μg of genomic DNA (data not shown).

After cloning in the Bluescript vector, the PCR products from 4 patients (2 patients with a single amplified band and 2 patients with two amplified bands) were sequenced to determine the precise locations of each breakpoint on chromosome 11-DNA. In Fig 4, the nucleotide sequences of the four chromosomal junctions are compared with the ones of four other t(11;14) previously reported. The location of breakpoint sites within germline chromosome 11 DNA are shown as arrows in Fig 2. The data show that all breakpoints occurred within a 75-bp region of chromosome 11 DNA and that some of them occurred at the same nucleotide position. Sequencing of the PCR products also showed that in cases 20 and 25, which showed two amplified bands, the larger one is caused by the annealing of the consensus JH primer not only to the JH segment involved in the translocation, but also to a JH segment immediately centromeric of it (44 and J5 in case 20, J1 and J2 in case 25; data not shown).

Finally, the sizes of the amplified bands and the presence or not of a Sac I site in the amplified DNA enabled us to determine more accurately the location of 15 of the 16 breakpoints within a 300-bp region as mentioned in Fig 1. Interestingly, this region contains the following nucleotide sequence: GGGAGGACTGT, which represents a potential
target for an endogenous nuclease (bp45) recently identified. bp45-DNA interactions are thought to be involved in certain chromosome rearrangements including the (11;14) translocation.

All four breakpoints occurred within an Ig J segment. The chromosome 14 crossover sites for three of the breakpoints appeared to be in the joining segment J1 (case 25), J4 (case 20), and J6 (case 27). We were unable to determine which of the J segments was involved in case 4, probably as a consequence of somatic nucleotide mutations that occurred in the translocated I region. Between the clearly identifiable chromosome 11 and 14 portions of each sequence, insertions of variable length were observed, except in cases 4 and 20. The relative high GC content of these additional sequences makes them likely to represent N insertions similar to those found at the variable-diversity (V-D) and diversity-joining (D-J) joints of Ig gene. However, we cannot rule out the possibility that some of these sequences actually represent D segments and we are thus unable to confirm that the breakpoints shown here occurred as an error in the D-J or V-D joining reactions. In cases 4 and 20, the absence of an additional sequence between the chromosome 11 and 14 sequences suggests that the translocation may result from another mechanism involving, as a possibility, the bp45 protein mentioned above.

The analysis of a large series of MCL had allowed us to show that the BCL1 locus is rearranged in 57% of these lymphomas and that in 84% of the BCL1-rearranged MCL, the break on chromosome 11 involves the MTC region. In the present study, we show that most (15/16) of the (11;14) breakpoints that occur at the MTC are amenable to PCR detection using Ig- and chromosome 14 specific primers and thus, that a BCL1 rearrangement can be detected using this approach in roughly one half of the MCL patients.

MCL is a lymphoid malignancy whose histologic recognition and treatment remain problematic. According to the different histologic classifications, MCL is either considered as a low- or intermediate-grade B-cell lymphoma. However, it behaves like an aggressive tumor with a poor response to the chemotherapy regimens used so far, and its median survival is less than 5 years. Because of its simplicity and of its high sensitivity, PCR detection of BCL1 rearrangement in MCL patients provides new perspectives in the diagnosis and the treatment of this disease. In fact, this assay markedly refines the capacity to detect minimal residual disease in MCL patients and should improve the ability to determine the stage of the disease, to stratify treatment, and to evaluate therapy.

Beside MCL, BCL-1 rearrangement is also readily detectable in other B-cell malignancies, namely multiple myeloma, plasma cell leukemia and prolymphocytic leukemia where the incidence of the (11;14) ranges from 5 to 20%. PCR detection of BCL-1 rearrangement should also provide guidance for the clinical management of these tumors.

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