Identification of the TCL1/MTCP1-like 1 (TMLI) Gene from the Region Next to the TCL1 Locus

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Abstract

The region on chromosome 14q32.1 is frequently involved in chromosomal translocations and inversions with one of the T-cell receptor loci in human T-cell leukemias and lymphomas. The breakpoints of the different rearrangements segregate into two clusters: inversion on the centromeric side and simple balanced translocations on the telomeric side. If the target gene activated by these different types of chromosomal rearrangements is the same, the gene must reside between the two breakpoints in a region of ∼160 kb. By screening of a placenta cDNA library using genomic probes from the vicinity of TCL1 locus, we have identified a gene coding for a 1.7-kb transcript that is expressed in leukemic cells carrying a t(14;14)(q11;q32) chromosome translocation. The cognate cDNA sequence reveals an open reading frame of 384 nucleotides encoding an M, 15,000 protein with ~30% of homology with both p14^{TCL1} and p13^{MTCP1} oncoproteins. The genomic organization of the TCL1 locus was characterized, with three exons located 15 kb from and tail-to-tail in relation to TCL1 locus. Because of its location and sequence similarity with TCL1 and MTCP1 oncoproteins, this gene, named TMLI (TCL1/MTCP1-like 1) is a candidate gene that is potentially involved in leukemogenesis.

Introduction

Nonrandom chromosomal translocations are characteristic of most human hematopoietic malignancies (1). In B and T cells, chromosomal translocations and inversions often occur as a consequence of mistakes during the normal process of recombination of the genes for immunoglobulins or TCRs. These rearrangements juxtapose enhancer elements of the immunoglobulin or TCR genes to oncogenes, the expression of which is then deregulated (2). In T-cell tumors, the TCR genes located on chromosomes 14q11 (TCRA/D; Refs. 3 and 4), 7q35 (TCRB; Ref. 5), and 7p15 (TCRG; Ref. 6) occasionally cause translocations or inversions as a consequence of the faulty joining of genes during the physiological process, leading to VDJ recombination. Chromosome region 14q32.1 is commonly involved in chromosomal rearrangements with TCR loci in several T-cell neoplasms. Chromosome abnormalities, such as the inversion inv(14)(q11q32) and translocations t(14;14)(q11;q32) and t(7;14)(q35;q32), are frequently observed in: T-cell prolymphocytic leukemia, a rare form of acute and chronic T-cell leukemias arising in patients with the immunodeficiency syndrome AT; and non-

malignant clonal expansion of T cells of patients with AT. We and others have cloned numerous breakpoints at 14q32.1 involved in T-cell neoplasms (7–11). By placing these breakpoints on the map of the region, we found that the breakpoints involve a chromosomal segment of ∼400 kb and cluster in two regions (12). The centromeric region is mainly involved in inversions, whereas the telomeric region is involved in simple translocations. These two regions enclose a segment of ∼160 kb. We postulated that, if the oncogene activated by these different rearrangements is the same, it must reside between these two clusters of breakpoints. Within this region, we have previously identified a gene named TCL1 (13) that is activated and deregulated by the chromosomal translocations and inversions. The sequence of the TCL1 gene revealed that it is highly homologous to that of the MTCP1 (B1) gene, which has been isolated from the breakpoint of t(X;14)(q28;q11) translocation, found in rare cases of AT (14). The MTCP1 (B1) gene at Xq28 was also activated by juxtaposing with TCRα/δ region at 14q11. The TCL1 and MTCP1 genes encode two homologous proteins, p14^{TCL1} and p13^{MTCP1}, which share no similarities with any other known proteins. Both TCL1 and MTCP1 transgenic mice developed T-cell leukemia at an old age (15, 16). In contrast to AT patients, who develop evident clonal expansion of T cells by the age of 20 years, the occasional clonal expansion of T cells in TCL1 transgenic mice was not observed until at least 12 months after birth. Furthermore, the size of region affected by translocations with TCR loci (160 kb) is far larger than that of TCL1 locus (<10 kb). These results suggested that there might be an additional gene that contributes to the development of clonal expansion of T cells at a younger age. Thus, we screened human cDNA libraries to look for genes in this affected region. By using a cosm id clone derived from this region as a probe, we were able to identify a novel gene that has significant homology with the TCL1 and MTCP1 genes and is expressed in T-cell leukemias carrying the translocation t(14;14)(q11; q32.1).

Materials and Methods

Cell Lines and Lymphocytes. The majority of the cell lines used in this study were obtained from American Type Culture Collection (Manassas, VA). The SupT1 cell line was derived from patient NL (17). PBLs were isolated from whole blood by centrifugation on a Ficoll-Hypaque gradient, followed by a 1-h adherence to Petri dishes to remove the monocytes. Stimulation with PHA at a final concentration of 0.1% was carried out for 3 days.

Isolation of Cosmid Clones Surrounding the TCL1 Locus. A YAC clone, 96D10, was identified from the CEPH mega YAC library (Centre d’Étude du Polymorphisme Humain, France) by screening with PCR using primers corresponding to TCL1 locus. DNA from YAC 96D10 was partially digested with the Sau3AI restriction enzyme and fractionated into 40–50-kb fragments by sucrose gradient ultracentrifugation. The resulting DNA fragments were ligated into pMFG2 cosm id vector, which was constructed by introduction of bacteriophage T3 and T7 promoters on both sides of a BamHI site and two NorI sites on both sides of a BamHI site in a pHSG274 vector. The ligated DNA was transformed into Escherichia coli HB101 after in vitro
packaging. The colony hybridization was performed by using \([^{32}\text{P}]\text{dCTP}\)-labeled human Cot DNA as a probe to obtain human cosmid clones.

**Direct cDNA Library Screening.** The human placenta cDNA library constructed in Agt10 vector was purchased from Clontech (Palo Alto, CA). Cosmid DNA that was used as a probe was prepared by a standard alkaline lysis method, purified by ethidium bromide-CsCl centrifugation to eliminate any *E. coli* DNA contamination, and then labeled by nick translation using \([^{32}\text{P}]\text{dCTP}\). The labeled cosmid DNA was precipitated with 20 μg of human Cot DNA (Roche Biochemicals, Basel, Switzerland) and 10 μg of pMFG2 vector in ethanol and dissolved in 10 μl of 5× SSC, followed by denaturation in boiling water for 5 min and incubation at 65°C for 1 h. Hybridization of preannealed cosmid probe onto the cDNA library was carried out in a solution of 50% formamide, 4× SSC, 3× Denhardt’s solution, and 125 μg/ml denatured salmon sperm DNA at 37°C overnight. Filters were washed twice at 3× SSC, followed by denaturation for 2 min at 70°C for 30 cycles. The labeled cosmid DNA was precipitated with 20 μl of 3 M sodium acetate, 0.1 M EDTA, pH 7.5, and ethanol precipitation. Filters were incubated with 2× SSC, subjected to a final rinse with 2× SSC at room temperature, and then exposed to Kodak X-ray film for 48 h.

**5’ RACE.** The 5’ RACE was performed by using the Marathon cDNA amplification kit (Clontech), per the manufacturer’s recommendations. First-strand cDNA synthesis was performed using 1 μg of human Cot DNA (Roche Biochemicals, Basel, Switzerland) and 10 μg of pMFG2 vector in ethanol and dissolved in 10 μl of 5× SSC, followed by denaturation in boiling water for 5 min and incubation at 65°C for 1 h. Hybridization of preannealed cosmid probe onto the cDNA library was carried out in a solution of 50% formamide, 4× SSC, 5× Denhardt’s solution, and 125 μg/ml denatured salmon sperm DNA at 37°C overnight. Filters were washed twice at 55°C for 10 min in 2× SSC and once at 55°C for 10 min in 1× SSC, subjected to a final rinse with 2× SSC at room temperature, and then exposed to Kodak X-ray film for 48 h.

**RT-PCR.** First-strand cDNA synthesis was performed using 1 μg of total RNA with Superscript II reverse transcriptase (Life Technologies, Inc, Gaithersburg, MD) and oligo(dT) as a primer; 10% of the reaction mixture was subsequently used for each single PCR amplification. Amplification of each cDNA from human cell lines and human normal tissues was carried out under the following conditions: denaturing for 1 min at 95°C, annealing for 1 min at 55°C, and elongation for 2 min at 72°C for 30 cycles. The TML1-specific primers TML1 14S (5’-CGAGGGATTGAGACCGACGAC-3’) and TML1 137AS (5’-CTGGCCGGAGGAGAGTAGC-3’) were used for the amplification. Amplification with the TCL1-specific primers TML1 14S (5’-CCCGGTTGCGAAGTTCGTAGTCC-3’) and TML1 127AS (5’-CTGGCCGGAGGAGAGTAGC-3’) and the TML1-specific primers TML1 12S (5’-CTGGCTCTTGCCCTTTTCC-3’) and TCL1 386AS (5’-GTCTGGCAACGACCCATGTCGT-3’) were used for the amplification. Amplification with the G3PDH-specific primers G3PDH-5’ (5’-ACCACAGTCCATGGCCAC-3’) and G3PDH-3’ (5’-TCCACCACCGTGTCAAC-3’) was performed as a control. The resulting products were detected by staining with ethidium bromide after fractionation of a 6% polyacrylamide gel or a 1.5% agarose gel. The specificity of each amplified product was confirmed by hybridization with digoxigenin-labeled gene-specific oligonucleotide.

**In Vitro Translation.** A plasmid, pTML1 ORF, containing full-length *TML1* cDNA was linearized by digestion with *Xho*I and transcribed and translated in vitro. The resulting products were detected by staining with ethidium bromide after fractionation of a 6% polyacrylamide gel or a 1.5% agarose gel. The specificity of each amplified product was confirmed by hybridization with digoxigenin-labeled gene-specific oligonucleotide.

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Fig. 1. Genomic and cDNA organization of the TML1 and TCL1 genes. A, restriction map of the TML1 and TCL1 loci on chromosome 14q32.1 (11). Vertical arrows, cloned breakpoints in the literature (7, 9, 17, 23, 24). Horizontal arrows with open arrowheads, orientation of TCR region with respect to breakpoints. Horizontal bars, positions of P1 clones 7-4 and 20-7 (11) and cosmid clone cos231. b, close-up of restriction map corresponding to both loci. c, organization of three exons of the TML1 gene and four exons of the TCL1 gene are indicated. Arrows, direction of the transcripts. [ ] 5’ and 3’ untranslated regions; ■, coding sequences.

Northern Blot Analysis. Poly(A) RNA was isolated by using PolyATtract system 1000 kit (Promega, Madison, WI). Ten μg of poly(A) RNA were electrophoresed on a 1% agarose gel. RNA was then transferred to nitrocellulose and hybridized with a [32P]dCTP-labeled pTML1 cDNA probe overnight at 37°C in 50% formamide, 5×3 SSC, 5×3 Denhardt's solution, 0.1% SDS, and 20 μg/ml denatured salmon sperm DNA. This was followed by one wash in 2×3 SSC-0.1% SDS at room temperature for 10 min, two washes in 0.1×3 SSC-0.1% SDS at room temperature for 10 min, and one wash in 1×3 SSC-0.1% SDS at 50°C for 10 min. The filter was wrapped and subjected to autoradiography using Kodak X-ray films (Eastman Kodak, Rochester, NY).

Results

Cloning and Sequence Analysis of the TML1 Gene. To identify transcribed sequences between the two clusters of breakpoints as illustrated in Fig. 1, we first isolated cosmid clones from YAC clone (964B10) encompassing TCL1 locus. We obtained total of 250 human cosmid clones from YAC 964D10. We then used them for the direct screening of several human cDNA libraries. When we screened a placenta cDNA library with one of the cosmid clones, cos231 containing TCL1 locus as a probe, one positive clone was obtained and designated pPL1. Sequence analysis of cDNA clone pPL1 revealed the presence of an ORF lacking translation initiation codon. Thus, we further proceeded to 5′ RACE experiments using mRNAs from placenta to obtain cDNA corresponding to the 5′ region and yielded many overlapping cDNA clones of 0.3 kb. One of the longest cDNA clones was named pMPLB4.

All cDNA clones were entirely sequenced, and the complete nucleotide sequences of pPL1, and pMPLB4 produced a combined sequence of 1753 bp, followed by 30-bp poly(A) tail, as shown in Fig. 2. Sequence analysis showed the presence of two long ORFs. Frame 1 contains an ORF of 384 nucleotides with a starting ATG codon at position 32 in a stretch of sequence GCCATGG that matches Kozack consensus sequence [(A/G)NNATG(A/G)] and a stop codon at position 416 (Fig. 2). This ORF potentially encodes for a protein of 128 amino acids, with a predicted molecular mass of 15 kDa. Frame 2 contains an ORF with a starting ATG codon at position 1089 in a stretch of sequence TTCATGT and a stop codon at position 1520, to give a putative protein with a mass of 15.8 kDa. However, the sequence surrounding ATG in frame 2 does not match Kozack consensus and the ORF in frame 2 only presents in the last exon. Thus, it is less likely that the ORF in frame 2 is translated. To confirm the presence of the ORF and its ability to encode a protein, the cDNA containing either a frame 1 or frame 2 sequence was subcloned, and an in vitro translation was performed. The specific product of a Mr 15,000 protein was only detected in transcript coding for frame 1 (data not shown). Interestingly, the homology search of the deduced amino acid sequence from frame 1 revealed a significant homology with p14TCL1 and p13 MTCP1, as shown in Fig. 3. The sequence has identities of 29 and 32% and similarities of 36 and 36% with human p14 TCL1 and p13 MTCP1, respectively. Thus, we designated this gene and its deduced product as TML1 gene and p15 TML1 protein.

Genomic Structure of the TML1 Gene. To study the genomic structure of TML1 gene, we isolated and sequenced subclones corresponding to the entire TML1 gene from P1 clones (P1 7-4 and P1 20-7). The map and structure of the TML1 gene are shown together with those of the TCL1 gene in Fig. 1. The TML1 locus maps 15 kb centromeric to the TCL1 locus with a tail-to-tail orientation. Thus, the TML1 and TCL1 genes use independent promoter regions. The TML1 gene is composed of three small exons with a 3′ untranslated region of 1383 nucleotides. Donor and acceptor signal sequences are in good agreement with the consensus signal sequences, except for the exon-intron boundary of exon 2 (AG/gc instead of AG/gt) (18).
Expression of the TML1 Gene in Tumors and Normal Human Tissues. To determine whether the isolated gene is deregulated in cells with the t(14;14)(q11;q32) translocation, we carried out a Northern blot analysis comparing the amount of TML1 transcript present in resting PBLs, PHA-activated PBLs, and SupT1 cells [a cell line established from a patient with acute T-lymphocytic leukemia with a resting PBLs, PHA-activated PBLs, and SupT11 cells [a cell line established from a patient with acute T-lymphocytic leukemia with a

Discussion

The TML1 gene is located in a chromosomal region banded by two clusters of breakpoints. In its strategic position, between the two clusters of breakpoints, the TML1 gene becomes juxtaposed to TCR-Cx regulatory elements in both types of rearrangements involving 14q32.1. The TML1 gene can be activated by the control elements of the TCR gene, where they are positioned 5’ to the TML1 gene, as in inversions, or 3’ to TML1, as in translocations. A similar situation has been observed in the TCLI gene because of its proximity to the TML1 gene (19). The expression of the TML1 gene in leukemic T cells with the t(14;14) translocation but not in leukemic T-cell lines with other types of chromosomal rearrangements suggested that this gene becomes deregulated as a consequence of its juxtaposition to the TCRα/D locus.

The sequence analysis of the deduced protein of the TML1 gene revealed that p15TML belongs to the third member of TCL1/MTCP1 family. The results of crystal structure analysis indicated that p14TCL1 and p13MTCP1 consist of an eight-stranded antiparallel beta barrels with novel topologies (20, 21). Because the sequences in the regions that formed beta strands in p14TCL1 and p13MTCP1 are also well conserved in p15TML1, this protein probably forms structures that are similar to those of the other two proteins. Moreover, it has been reported that purified recombinant p14TCL1 forms dimers in solution (22). Although further studies are required, it is possible that p15TCL1 also forms homodimers with itself or heterodimers with p14TCL1 in tumor cells coexpressing TML1 and TCLI gene such as endemic Burkitt’s or T-cell tumors with 14q32.1 involvement. To date, no information is available to imply the molecular function of TCLI/MTCP1 family. The amino acid sequence similarities among p15TML1, p14TCL1, and p13MTCP1 suggest that their function may be analogous, and they are most probably involved in the control of lymphoid cell proliferation and/or survival.

Except for the expression of TML1 in placenta and testes, the expression pattern of the TML1 gene is well correlated with that of TCLI. This suggests that concomitant expression of both genes may play an important role in the clonal expansion of T cells and leukemogenesis. Thus, it would be interesting to know whether the TML1 gene is capable of forming tumors by itself in transgenic mice or accelerating the clonal expansion and/or tumor formation in combination of double transgenic mice harboring both the TML1 and TCLI genes, as in clonal T cells and leukemic T-cells with t(14;14) translocations in AT patients.

In conclusion, the TML1 gene is a strong candidate for an oncogene because it is deregulated by translocation with TCR locus, and the deduced protein of TML1 gene has a striking homology with the p14TCL1 and p13MTCP1 oncoproteins.
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Note Added in Proof

During submission of this paper, a new gene called TCL1b was reported from the region centromeric to the TCL1 locus (26). The sequence of TCL1b was almost identical to that of TML1. Thus, we conclude that TCL1b is the same gene as TML1.

References

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