Abnormalities at 14q32.1 in T cell malignancies involve two oncogenes

Yuri Pekarsky*, Cora Hallas*, Masaharu Isobe†, Giandomenico Russo‡, and Carlo M. Croce*§

*Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107; †Toyama University, 3190 Gofuku, Toyama City 930-8555 Japan; and ‡Istituto Dermopatico dell’Immacolata-IRCCS, Via dei Monti di Creta 104, 00167 Rome, Italy

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ABSTRACT The TCL1 oncogene on human chromosome 14q32.1 is involved in the development of T cell leukemia in humans. Its expression in these leukemias is activated by chromosomal translocations and inversions at 14q32.1. Here we report the isolation and characterization of a new member of the TCL1 gene family, TCL1b, located at 16 kb centromeric of TCL1. The 1.2-kb TCL1b cDNA encodes a 14-kDa protein of 128 aa and shows 60% similarity to Tcl1. Expression profiles of TCL1 and TCL1b genes are very similar; both genes are expressed at very low levels in normal bone marrow and peripheral lymphocytes but are activated in T cell leukemia by rearrangements of the 14q32.1 region. Thus, translocations and inversions at 14q32.1 in T cell malignancies involve two oncogenes.

The TCL1 oncogene on chromosome 14q32.1 is involved in the development of chronic T cell leukemia (T-CLL) (1) and is activated in these leukemias by juxtaposition to the T cell receptor α/β locus, caused by chromosomal translocations t(14;11)(q11;q32) or t(7;14)(q35;q32) or inversion inv(14)(q11;q32) (1, 2). Normally, TCL1 expression is observed in early T cell progenitors (CD4+CD8−CD3−) (1) and lymphoid cells of the B cell lineage: pre B cells and immature IgM expressing B cells (1). Introduction of a TCL1 transgene under the control of an lck promoter caused mature T cell leukemia in mice after 15 months (3). However, some cases of T cell malignancies with abnormalities, such as gene amplification at 14q32.1, did not show activation of the TCL1 expression (4, 5), suggesting that perhaps an additional oncogene may be located in 14q32.1 (5).

The second member of the TCL1 gene family, MTCP1, is located at Xq28 and activated in rare cases of mature T cell leukemia with a t(X;14)(q28;q11) translocation (6).

Here we report the isolation and characterization of the third member of the TCL1 gene family, TCL1b, located at 14q32.1 and also activated by rearrangements at 14q32.1 in T cell leukemias.

METHODS

Cell Lines. Cell lines except Epstein–Barr virus (EBV)-transformed lymphoblastoid cell lines were obtained from American Type Culture Collection (Manassas, VA) and grown in RPMI 1640 medium with 10% fetal bovine serum. Lymphoblastoid cell lines were made from peripheral blood lymphocytes of patients with Alzheimer’s disease by transformation with EBV as reported previously (7).

Northern Blot, Rapid Amplification of cDNA Ends (RACE), and Reverse Transcription–PCR (RT-PCR) Analysis. These experiments were carried out as described previously (8) with the following exceptions. Human bone marrow and placenta mRNAs, and human immune system and human cancer cell line Northern blots were purchased from CLONTECH. Each line on Fig. 3 C and D contains 3 µg poly(A)⁺ RNA. PCR shown in Fig. 4 A was carried out for 25–35 cycles by using Multiple Tissue cDNA Panels (CLONTECH) and the manufacturer’s protocol. Primers were TC1 (GGCAGCTCTACCCGGGATGAA) and TC39 (ACAGACCTGAGTGGGACAGGA) (Fig. 4 A Top); TCLB (TCCTCTCTTGGCCAGGTAGTA) and TCLC (CAGTTACGGGTGCTCTTGCGT) (Fig. 4 A Middle); and control 3’ and 5’ RACE G3PDH primers (Fig. 4 A Bottom) (CLONTECH). The primers in Fig. 4 B (Middle and Bottom) were the same as above. PCR was carried out for 22 cycles with primers TC8 (ATGGCCTCGGAAGTTCCTGTCGCTTCCTGTCGCTTGC) and TC9 (AAATTGGCCCATGGTACTGCTTTTACTTTC) for 15 cycles. RACE primers were TC1 (for 3’ RACE) and TC5 (for 5’ RACE).

Pulse-Field Gel Electrophoresis (PFGE) Analysis and Chromosomal Localization. PFGE analysis was performed as described (9), except pulse time was 1–6 sec for 11 hr. Chromosomal localization of the TCL1b gene was carried out using GeneBridge 4 radiation hybrid-mapping panel (Research Genetics, Huntsville, AL) according to the manufacturer’s protocol. Primers were TC1 and TC4 (TGCTAGGACCGCTGCTCATAGA).

RESULTS

Identification of the TCL1b Gene. In some mature T cell leukemias with chromosomal abnormalities at 14q32.1, activation of the TCL1 gene at 14q32.1 was not observed (4, 5). To investigate the possibility that other, unknown TCL1 family member(s) may be involved, we searched the expressed sequence tag (EST) database for sequences homologous to the TCL1 and MTCP1 gene products. We found a single EST (accession no. A689513) that was homologous but not an exact match to both genes. Thus, we isolated a ~1.2-kb full-length cDNA by using 3’ and 5’ RACE procedure and human testis mRNA as a cDNA source. The 1.2-kb TCL1b cDNA encodes a 14-kDa protein of 128 aa (Fig. 1). It contains a starting ATG codon at position 28 within a perfect Kozak consensus sequence. The Tc1b protein has a 14-aa insertion compared with the Tc1 and Mtcp1 proteins (Fig. 1); it is 30% identical and 60% similar to Tc1 and 36% identical and 63% similar to Mtcp1 (Fig. 1).

Abbreviations: RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription–PCR; EBV, Epstein–Barr virus; Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. AF110465 (TCL1b gene), AF110466 (TCL1b cDNA), and AF110467 (TCL1b pseudogene)].

*To whom reprint requests should be addressed at: Kimmel Cancer Center, Thomas Jefferson University, BLSB Room 1050, 233 South 10th Street, Philadelphia, PA 19107. e-mail: croce@calvin.jci.tju.edu.
A radiation hybrid-mapping panel (GeneBridge 4) was used to determine the chromosomal localization of the human TCL1b gene. By analysis of PCR data at the Massachusetts Institute of Technology database (http://www-genome.wi.mit.edu), the TCL1b gene was localized to 3.05 cR from the marker D14S265, at 14q32. We also have discovered a TCL1b pseudogene and localized it to 5q12–5q13. The TCL1b pseudogene does not have the initiating ATG or introns and has a stop codon in the middle of the ORF (not shown).

Because both TCL1 and TCL1b are located at 14q32, we investigated whether TCL1 and TCL1b are linked physically. We screened the human BAC library and found several BAC clones containing TCL1 and TCL1b. The TCL1b gene is 6.5 kb in size and contains four exons of 189, 171, 69, and 697 bp, respectively (Fig. 2), but only the first three exons are coding. Pulse-field analysis of the positive BAC clone with both probes revealed that the TCL1 and TCL1b genes have opposite directions of transcription and are separated by only 16 kb (Fig. 2). Both genes are located in the ~160-kb region between two previously published sets of breakpoints observed in T cell acute lymphoblastic leukemia cases with translocations or inversions at 14q32.1 (1, 10).

**Expression of TCL1b Gene and Its Activation in T Cell Malignancies.** Because of the similarities in structure, sequence, and location between the TCL1 and TCL1b genes, it seemed possible that they would exhibit similar expression patterns. To verify this, we carried out a series of Northern blot and RT-PCR experiments (Figs. 3 and 4). Northern blot analysis in normal tissues was mostly negative for TCL1b (Fig. 3A), except that the 1.2-kb transcript was detected after several days of exposure in testis (not shown) and placenta (Fig. 3C). The TCL1 gene expression, however, was detected in most hematopoietic tissues after several days of exposure (Fig. 3A). Semiquantitative RT-PCR analysis (Fig. 4A) revealed that both TCL1 and TCL1b genes are expressed in spleen, tonsil, fetal liver, fetal kidney, and fetal thymus. However, the TCL1b gene is expressed in wider variety of tissues, including placenta, kidney, and fetal spleen (Fig. 4A). Northern blot analysis of commercial human cancer cell lines showed that TCL1 and TCL1b are expressed in only the Raji Burkitt’s lymphoma cell line (Fig. 3B), although TCL1 was expressed at a much higher level (Fig. 3B).

Because of the similarity of transcription patterns of the TCL1 and TCL1b genes and their physical linkage, we investigated whether the TCL1b gene also could be activated by rearrangements in 14q32.1. Fig. 3C and D (Middle) shows the activation of the TCL1b gene in a T leukemia cell line with a translocation at 14q32.1 (SupT11) compared with the normal bone marrow and with EBV-transformed lymphoblastoid B cell lines expressing TCL1. Because TCL1 and TCL1b normally are not expressed in postthymic T cells and postthymic T cell leukemias lacking 14q32.1 abnormalities (for example, in T cell acute lymphoblastic leukemia MOLT4 with no abnormalities at 14q32.1; Fig. 3B, lane 4), the expression of

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**Fig. 1.** Sequence comparison of Tcl1, Tcl1b, and Mtcp1. Identities are shown in black boxes, and similarities are shown in shaded boxes. For Tcl1 and Mtcp1, GenBank accession numbers are X82240 and Z24459, respectively.

**Fig. 2.** Genomic organization of the TCL1 and TCL1b genes. Vertical arrows refer to cloned 14q32.1 breakpoints (1, 10). Restriction sites are given for BssHII (B), ClaI (C), EagI (E), SfiI (F), KpI (K), MluI (M), NotI (N), NruI (R), and SauI (S). Solid boxes represent TCL1 and TCL1b exons.

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TCL1 and TCL1b in SupT11 cells carrying a t(14;14)(q11;q32.1) translocation indicates that juxtaposition of TCL1 and TCL1b to the α/δ locus of the T cell receptor regulates both genes.

To further investigate TCL1b expression, we analyzed four T cell leukemias and six EBV-transformed lymphoblastoid cell lines with elevated levels of TCL1. Fig. 4B shows the activation of the TCL1b expression in one leukemic sample from a patient with T cell prolymphocytic leukemia. Human T cell prolymphocytic leukemias carry the 14q32.1 translocation or inversion and overexpress TCL1 (1, 2). The TCL1b gene also was expressed in two of six EBV-transformed lymphoblastoid B cell lines (Fig. 3D Top, lanes 2–7).

**DISCUSSION**

In this report we have presented the cloning, mapping, and expression analysis of a novel member of the TCL1 gene family, TCL1b. The TCL1 and TCL1b genes are linked physically and show structural similarity, similar expression patterns, and involvement in T cell malignancies. Because the remaining two members of the TCL1 family are oncogenes (3, 11), it seems likely that TCL1b is also an oncogene. It is also likely that TCL1b activation would explain cases of T cell leukemia with amplification at 14q32 without activation of TCL1.

It is possible that two TCL1 genes are the result of duplication, although the TCL1b gene is slightly more homologous to the MTCP1 gene at Xq28 than to the TCL1 gene.

Neither the in vivo function of Tcl1 nor the mechanism(s) of its oncogenic potential is known, although its crystal structure (12) suggests that it may function as a transporter of small molecules, such as retinoids, nucleosides, or fatty acids. The same study (12) suggested that Tcl1 might function as a dimer, implying the possibility that Tcl1 and Tcl1b might form heterodimers.

Because TCL1 and MTCP1 transgenic mice develop mature T cell leukemia after only 15 months (3, 11), it will be of considerable interest to determine whether TCL1b transgenic mice also develop mature T cell leukemia late and whether TCL1 and TCL1b double-transgenic mice develop leukemia faster. Thus, it seems possible that translocations and inversions at 14q32.1 contribute to malignant transformation by activating two oncogenes at the same time.

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