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Genetics

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

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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 ≈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/δ locus, caused by chromosomal translocations t(14;14)(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 (GGCAGCTCTACCCCGGGATGAA) and TC39 (ACAGACCTGAGTGGGACAGGA) (Fig. 4*A Top*); TCLB (TCCTCCTTGGCAGGAGTGGTA) 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 (ATGGCCTCCGAAGCTTCTGTG) and TC39 (Fig. 4*B Top*). A portion (0.1 μl) of the reaction was used for the second PCR with nested primers TC10 (TGGTCGTGCGGTTCAATCCCT) and TC5 (AATCTGGCCATGGTCTGCTATTTC) 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 (TGCTAGGACCAGCTGCTCCATAGA).

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. AA689513) that was homologous but not an exact match to both genes. Thus, we isolated a ≈1.2-kb full-length cDNA by using 5′ and 3′ 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 Tcl1b protein has a 14-aa insertion compared with the Tcl1 and Mtcp1 proteins (Fig. 1); it is 30% identical and 60% similar to Tcl1 and 36% identical and 63% similar to Mtcp1 (Fig. 1).

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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)].

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TCL1      1  MAECPTLGEAVTDHPDRLWAWEKFVYLDEKQHAWLPLTIEIKDRLQ..............
MTCP1     1  ~~~~~MAGEDVGAPPDHLWVHQEGIYRDEYQRTWVAVVEEETSFLR..............
TCL1b     1  ~~MASEASVRLGVPPGRLWIQRPGIYEDEEGRTWVTVVVRFNPSRREWARASQGSRYEPS

TCL1     47  LRVLLRREDVVLGRPMTPTQIGPSLLPIMWQLYPDGRYRSSDSSFWRLVYHIKIDGVEDM
MTCP1    42  ARV..QQIQVPLGDAARPSHLLTSQLPLMWQLYPEERYMDNNSRLWQIQHHLMVRGVQEL
TCL1b    59  ITVHLWQMAVHTRELLSSGQMPFSQLPAVWQLYPGRKYRAADSSFWEIADHGQIDSMEQL

TCL1    107  LLELLPDD~~
MTCP1   100  LLKLLPDD~~
TCL1b   119  VLTYQPERKD
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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 Mctp1, GenBank accession numbers are X82240 and Z24459, respectively.

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. 3*A*), except that the 1.2-kb transcript was detected after several days of exposure in testis (not shown) and placenta (Fig. 3*C*). The *TCL1* gene expression, however, was detected in most hematopoietic tissues after several days of exposure (Fig. 3*A*). Semiquantitative RT-PCR analysis (Fig. 4*A*) 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. 4*A*). 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. 3*B*), although *TCL1* was expressed at a much higher level (Fig. 3*B*).

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. Fig. 3 *C* 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. 3*B*, lane 4), the expression of

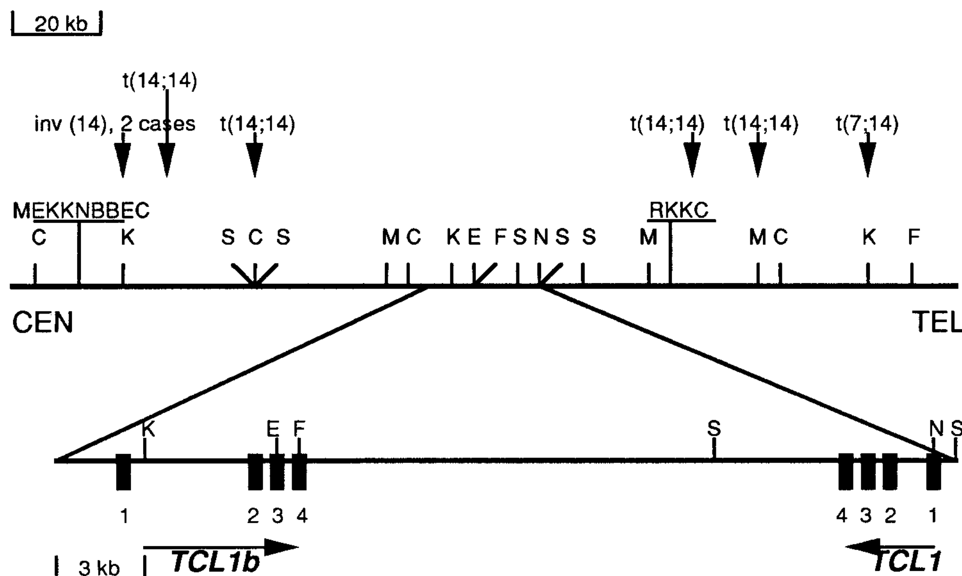

FIG. 2. Genomic organization of the *TCL1* and *TCL1b* genes. Vertical arrows refer to cloned 14q32.1 breakpoints (1, 10). Restriction sites are given for *Bss*HII (B), *Cla*I (C), *Eag*I (E), *Sfi*I (F), *Ksp*I (K), *Mlu*I (M), *Not*I (N), *Nru*I (R), and *Sal*I (S). Solid boxes represent *TCL1* and *TCL1b* exons.

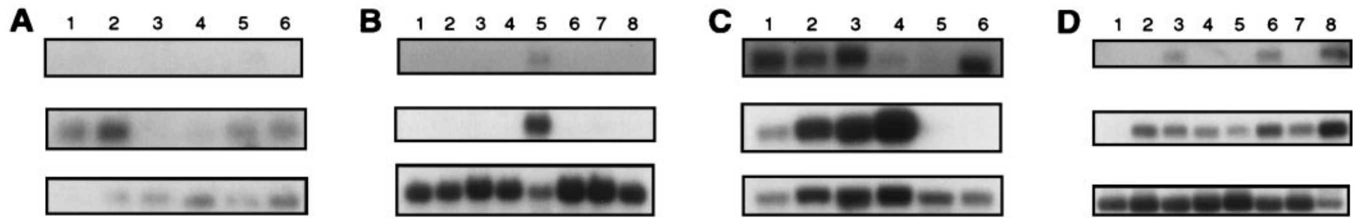

FIG. 3. Northern blot analysis of the *TCL1* and *TCL1b* genes. (*A*) Human immune system Northern blot. Lanes 1–6: spleen, lymph node, thymus, peripheral blood leukocyte, bone marrow, and fetal liver. (*B*) Human cancer cell line Northern blot. Lanes 1–8: promyelocytic leukemia, HL-60; Hela cells; chronic myelogenous leukemia, K-562; T lymphoblastic leukemia, MOLT-4; Burkitt's lymphoma Raji; colorectal adenocarcinoma, SW480; lung carcinoma, A549; and melanoma, G361. (*C*) Lanes 1–6: Burkitt's lymphoma Raji, Burkitt's lymphoma Daudi, Burkitt's lymphoma CA-46, SupT11, bone marrow, and placenta. (*D*) Lane 1, bone marrow; lanes 2–7, EBV transformed lymphoblastoid cell lines (Ado-1471, Ado-1476, Ado-1701, Ado-1727, Ado-2069, Ado-2199); lane 8, CA-46. (*A– D*) *TCL1b* probe (*Top*), *Tcl1* probe (*Middle*), and actin probe (*Bottom*).

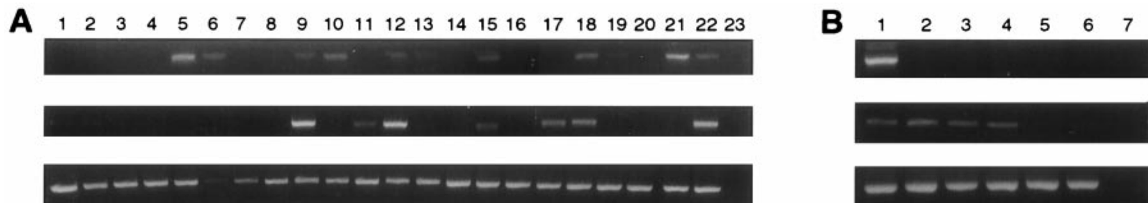

FIG. 4. RT-PCR analysis of *TCL1* and *TCL1b* genes. (*A*) Normal human tissues. Lanes 1–23: heart, liver, brain, muscle, placenta, kidney, lung, pancreas, spleen, lymph node, thymus, tonsil, peripheral blood lymphocytes (PBL), bone marrow, fetal liver, fetal brain, fetal lung, fetal kidney, fetal heart, fetal skeletal muscle, fetal spleen, fetal thymus, and negative control. (*B*) Lanes 1–4, T cell PLL samples (3047, 3046, 3050, 3048); lanes 5–6, bone marrow, PBL. (*A* and *B*) *TCL1b* primers (*Top*), *TCL1* primers (*Middle*), and control G3PDH primers (*Bottom*).

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 deregulates 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. 4*B* 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. 3*D 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, is 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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