Characterization and Localization of the *TCL-1* Oncogene Product

Tie-bo Fu, Laura Virgilio, Maria Grazia Narducci, Antonio Facchiano, Giandomenico Russo, and Carlo M. Croce


**Abstract**

The *TCL-1* gene maps at chromosome 14q32.1 and is activated in T cell leukemias and lymphomas by either chromosome translocations or inversions that juxtapose the *TCL-1* gene to the α/β or the β locus of the T cell receptor. The open reading frame of the *TCL-1* gene, coding for a protein of 114 amino acids, was expressed in bacteria and antisera were raised against it. The antibodies recognized the predicted *TCL-1* Mr 14,000 protein product in cells expressing *TCL-1* mRNA. Cell fractionation experiments indicated that the *TCL-1* protein is present in the microsomal fraction. These results were confirmed by confocal microscopy. The *TCL-1* protein has considerable sequence similarities to the product of the *MTCP-1* gene on chromosome Xq28, which is involved in T cell lymphoproliferative diseases. Thus, *TCL-1* may represent a member of a novel family of genes involved in lymphoid proliferation and/or survival and in T cell malignancies.

**Introduction**

Nonrandom chromosomal translocations and inversions are common in human hematopoietic malignancies (1). In B and T cell neoplasms such chromosomal rearrangements often involve the loci for human immunoglobulins (immunoglobulin) and TCR and occur during the process of recombination of the immunoglobulin or TCR genes (2). The rearrangements juxtapose cellular proto-oncogenes to enhancer elements present in the immunoglobulin and TCR loci leading to oncogene deregulation (2). In postthymic types of T cell leukemias, such as T-PLL, and acute or chronic T cell leukemias and in adult T cell leukemia and in T cell lymphomas, translocations or inversions involving bands 14q11 and 14q32.1 are quite common (3, 4). Such translocations or inversions juxtapose the α/β locus of the T cell receptor to a putative proto-oncogene we named *TCL-1* (5, 6). Less frequently a translocation between the TCR β locus and the *TCL-1* locus, (7;14) (q35;q32.1), may occur (7, 8). While we have postulated the existence of the *TCL-1* oncogene involved in the pathogenesis of T cell leukemias and lymphomas at 14q32.1 for some time, it has been difficult to identify and characterize the gene. We and others have cloned numerous breakpoints at 14q32.1 involved in T cell neoplasms (8–11). By mapping these breakpoints and by placing them on the map of the region we have been able to show that the breakpoints involve a chromosomal segment of approximately 400 kilobases and cluster in 2 regions (12). The centromeric region is predominantly involved in inversions, while the telomeric region is involved in simple translocations (12). These two regions bracket a segment of approximately 160 kilobases (6, 12). We postulated that if the oncogene activated by these different rearrangements is the same, it must reside between the two clusters of breakpoints (6). By using two different approaches, exon trapping and Northern blot hybridization with unique probes, we have identified the gene that is activated and deregulated by the chromosomal translocations and inversions and we have named it *TCL-1* (6). This gene is transcribed into a 1.3-kilobase mRNA and has an open reading frame of 342 nucleotides coding for a Mr 14,000 protein of 114 amino acids; we have expressed it as a recombinant product in bacteria (6).

In this study we have investigated the expression of the *TCL-1* protein in human cells, determined its cellular localization, and established its similarity to another protein product coded by a gene also involved in chromosomal translocations in T cell proliferative disorders.

**Materials and Methods**

**Immunological Methods.** The purified *TCL-1* p697 cDNA fragment (6) was ligated to the pQE30 expression vector (Qiagen) previously digested with *Bam*Hl and blunted with Klenow polymerase. Positive clones were grown in *Escherichia coli* strain M15 (REP4) and confirmed by DNA sequencing (6). Induction of expression of the *TCL-1* open reading frame and purification of the recombinant protein were carried out according to Qiagen recommendation (6). Rabbits were immunized against the recombinant *TCL-1* product. Antisera from such rabbits were able to detect the Mr 14,000 *TCL-1* band induced in bacteria by Western blotting (data not shown).

The detection of the expression of *TCL-1* in human cells was carried out by Western blotting using the anti-*TCL-1* rabbit antibodies essentially as described (13) and by immunoprecipitation. For immunoprecipitation human cells (5 × 10⁷ cells/ml) were suspended in DMEM (without methionine) and cultured for 1 h at 37°C. The medium was then replaced with fresh medium containing 10 µCi/ml of ³⁵S-methionine. After incubation at 37°C for 2–6 h, the medium was removed and the cells were washed with PBS. The cell pellets were lysed with lysis buffer (1% Triton X-100 and 1% SDS in PBS). After brief centrifugation, the supernatants were collected and centrifuged for an additional 20 min at 40,000 rpm. For antibody binding 15 µl protein A-agarose and 10 µl (1 µg) rabbit antibodies (IgG) were added to the supernatants and additional 20 µl at 40,000 rpm. For antibody binding 15 µl protein A-agarose and 10 µl (1 µg) rabbit antibodies (IgG) were added to the supernatants and incubated overnight at 4°C, followed by centrifugation at 2,500 rpm for 15 min. After the pellets were washed twice with PBS, they were suspended in 40 µl PBS. Twenty µl of each sample were mixed in 2× Laemmli loading buffer and denatured at 94°C for 5–10 min. Each sample was then loaded into a 14% SDS-PAGE.

In order to detect expression of *TCL-1* in human lymphoid cells, we have also used indirect immunofluorescence. Cells were plated on polylysine-coated coverslips in 60-mm Petri dishes. The coverslips were kept at room temperature for 10 min and then washed with prewarmed PBS, fixed in fresh 3.7% paraformaldehyde in PBS at 20°C, and thoroughly washed in PBS. The cells were permabilized with 0.1% Triton X-100 in PBS for 5 min at room temperature. After several washings with PBS containing 0.1% BSA and 0.05% Tween 20, the cells were treated with a 2% goat serum for 30 min at room temperature, followed by incubation with the first antibody (rabbit polyclonal anti-*TCL-1* antibody) overnight at 4°C, and washed three times with PBS. The cells were then incubated with a fluorescein-conjugated goat anti-rabbit antibody (IgG) (Cappel) for 30–120 min at room temperature and washed four times with PBS. Coverslips were sealed with 90% glycerol/PBS. Cells were observed by phase contrast microscopy and the localization of the *TCL-1* product was determined by confocal microscopy using a Zeiss Axiovert 100 microscope equipped with a MRC600 Bio-Rad laser.

**Subcellular Fractionation.** Subcellular fractionation was performed according to the method described by Storrie and Madden (14). Cells were homogenized in hypotonic medium (10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 18 U.S.C. Section 1734 solely to indicate this fact.

Received 11/2/94; accepted 11/10/94.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: TCR, T cell receptors; T-PLL, T-prolymphocytic leukemia; T-CLL, T cell chronic lymphocytic leukemia.
were suspended in the same buffer and homogenized by 6 strokes at 1700 rpm. The pellets (P4) from this centrifugation were kept as nuclear fraction and the supernatants (S3) were subsequently centrifuged at 17,000 × g for 20 min. The pellets from this spin were suspended in the same buffer and homogenized by 6 strokes at 1700 rpm using a motor-driven homogenizer. The homogenized pellets were then loaded on discontinuous Ficoll gradients and centrifuged at 59,000 × g for 60 min. The mitochondrial fractions were recovered as pellets at the bottom of the tubes and the sharp bands at the top were separated as the plasma membrane fraction. All the procedures were carried out at 4°C.

**Library Searches and Hydrophilicity Plot.** Library searches for homology to TCL-1 were performed using the FASTA algorithm on DNA and protein sequences, and the TFASTA algorithm was used to search for similarities between a query peptide sequence and a translated group of nucleotide sequences (15). The Swiss-prot database, containing 40,292 sequences, was searched, with KTup set on 1 and a PAM 250 matrix chosen for homology score calculations. The best alignment with the MTCP-1 protein was then performed by means of BESTFIT routine. A hydrophilicity plot was performed according to the Kyte and Doolittle index (16). Secondary structure analysis were carried out by means of the PEPTIDE STRUCTURE routine (GCG package) according to both the Chou-Fasman and Garnier methods. The antigenic index by Jameson and Wolf was used for the antigenic sites detection. Measures performed were then plotted by means of PLOT STRUCTURE routine (GCG package).

**Results**

Immunoprecipitation of 35S-metabolically labeled cell lines was carried out using rabbit anti-TCL-1 antibodies to determine whether we could detect expression of the TCL-1 protein in human cells. Previously we have shown expression of TCL-1 mRNA in pre-B cells and in B cells expressing surface IgM, but not in pro-B cells and in mature B cells. Immunoprecipitation experiments included 3 cell lines, U266 (multiple myeloma), RS4;11, and MV4;11 (two acute lymphoblastic leukemias with a t(4;11) chromosome translocation and a pro-B cell phenotype), that do not express TCL-1 mRNA (6) and 4 pre-B and endemic Burkitt cell lines (697, Daudi, P3HR1, and RS11846) that express different amounts of TCL-1 mRNA (6). The results are shown in Fig. 1 and parallel the findings observed at the mRNA level. The three cell lines that did not express TCL-1 mRNA (Fig. 1, Lanes 1, 2, and 3) did not express any TCL-1 product using the anti-TCL-1 antibodies for immunoprecipitation. In contrast, a Mr 14,000 specific band was clearly visible in all four cell lines expressing TCL-1 mRNA (Fig. 1, Lanes 4—7). The homology of TCL-1 is only with the longest ORF of the MTCP-1 gene. While organization of the two genes is very similar for exon length, organization, and amino acid composition, the homology described by Stern et al. (17) and the TCL-1 protein has 14% identity between the two amino acid sequences was 41% and the similarity was 61% (Fig. 4). The homology of TCL-1 is only with the longest ORF of the MTCP-1 gene. While organization of the two genes is very similar for exon length, organization, and amino acid composition, the homology
between the two DNA sequences is less striking and this is probably the reason why it has not been identified originally by the FASTA program.

The observed similarity suggests that the two proteins share a similar conformation in solution. In fact, according to the report of Sander and Schneider (18), the identity is well above the threshold accepted; this implies strong conformational homology. Similarity observed at the primary sequence level is even more evident at the hydropathy plot level, the plots being almost superimposable (Fig. 5). At this time any specific prediction concerning the tertiary conformation is too speculative. We have carried out, however, secondary structure predictions. Fig. 5 shows the secondary structure predictions according to Chou-Fasman and to Garnier algorithms, and other physicochemical features predicted on the basis of the TCL-1 sequence, namely the hydrophilicity, the surface exposure probability, flexibility plot, antigenic site prediction, and the glycosylation sites prediction. The analyses of the TCL-1 product agree in predicting α helix structure at the NH2-terminal half of the protein, and a few β-sheet and turn regions in the COOH-terminal half of the protein. No glycosylation sites have been predicted along the TCL-1 sequence, due to the lack of asparagine residues. As compared to the average percentage of amino acid composition of proteins TCL-1 shows the largest differences in asparagine content (0% in TCL-1 versus 4.4% average) and tryptophan content (4.4% in TCL-1 versus 1.2% average). MTCP-1 and TCL-1 proteins are both relatively rich in tryptophans and all 4 tryptophans of MTCP-1 match 4 of the 5 tryptophans of the TCL-1 sequence. Tryptophan is relatively rare in other protein sequences and in the entire PIR data bank; the high content in TCL-1 may indicate specific evolutionary constraints and may suggest a functional or structural role for the residue. The Genetics Computer Group Motifs program also revealed the existence of a casein Kinase II phosphorylation site (MAECP-TLGE-AVTDH) starting at amino acid 6.

Discussion

The results described in this study indicate that the TCL-1 oncogene product is expressed in lymphoid cells as a protein with a molecular weight of 14,000 that is predominantly localized in the microsomal fraction. Interestingly the TCL-1 product has sequence homology and similarity with another protein product coded by a gene, MTCP-1, on the human X chromosome that is also involved, although rarely, in chromosomal translocations in T cell proliferative diseases. One of the two peptides potentially encoded by the MTCP-1 gene, specifically the one containing 107 amino acids, has strong similarities with the TCL-1 protein. The similarities are quite striking when the hydropathy plots are considered, since they are almost superimposable. This suggests that the two proteins have a similar conformation in solution. These results indicate that TCL-1 and MTCP-1 are members of the...
CHARACTERIZATION AND MAPPING OF TCL-1 ONCOGENE PRODUCT

Fig. 5. Plot structure of TCL-1 (A) and MTCP-1 (B). The plotted measures of protein secondary structure of TCL-1 and MTCP-1 were obtained from the peptide sequences using the GCG Wisconsin software package.
same gene family; both cause T cell malignancies and most probably are involved in the control of lymphoid cell proliferation and/or survival.

Rearrangements at the TCL-1 locus are observed in approximately 75% of T-PLL (20) and in 10% of patients with the immunodeficiency syndrome ataxia-telangiectasia, as clonal expansions for years before the overt leukemia (17, 21). T-PLL has been recently classified as a postthymic type of leukemia characterized by splenomegaly (75%), lymphoadenopathy (53%), hepatomegaly (40%), skin lesions (27%), and high lymphocyte count (75%); morphologically it is characterized by nucleolated prolymphocytes and it has a very aggressive behavior (medium survival of 8 months) (20). According to the report of Matutes et al. (20) T-PLLs have probably been misdiagnosed in the past as T-CLL (especially the “knobby” type of T-CLL and the T-CLLS of helper/inducer phenotype) or, in the absence of membrane markers, as generic CLL or chronic lymphosarcoma cell leukemia. This observation is of great importance, since based only on morphology these cases are generally viewed as low grade lymphoproliferative disorders and therapeutic decisions are based on that assumption (22). T-cell leukemias in patients with ataxia-telangiectasia have a morphology and clinical course suggestive of T-PLL (3) and the presence of clonal expansions carrying rearrangements at the TCL-1 locus before the onset of the leukemia is of considerable importance for tumor progression and clinical evaluation.

The identification of these new oncogenes, TCL-1 and MTCP-1, and their proteins is obviously of great importance in the assessment of these diseases. Transgenic mouse experiments and gene knockout experiments will provide very important new information on the role of these genes in the development of the immune system and in B and T cell proliferation, differentiation, and survival.

References
Characterization and Localization of the TCL-1 Oncogene Product

Tie-bo Fu, Laura Virgilio, Maria Grazia Narducci, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/54/24/6297

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.