Expression of the TCL1 Gene at 14q32 in B-Cell Malignancies but Not in Adult T-Cell Leukemia

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The TCL1 gene was recently cloned as a candidate target within the 14q32.1 breakpoint cluster region observed in T-cell malignancies. We examined the TCL1 gene expression in 21 patients with adult T-cell leukemia (ATL) and 5 cell lines, because ATL is reported to have frequent chromosome 14 band q32 aberrations. However, 20 of the ATL patients and all 5 cell lines lacked any TCL1 expression on northern blot analysis, and TCL1 transcripts were only very faintly detected in the remaining one patient. Expansion of our analysis to include other types of hematopoietic malignancies revealed strong expression of the TCL1 gene in almost all tumor cells of B-cell lineage except myelomas. However, no TCL1 signals were encountered in cells of T-cell or myeloid lineages. In normal human tissues TCL1 was found to be expressed in the spleen, lymph nodes and B-lymphocytes of peripheral blood. These results indicate that TCL1 is not a major target gene for ATL, but that it may play a role in B-cell differentiation and proliferation.

Key words: TCL1 gene — Adult T-cell leukemia — B-cell malignancies

In hematopoietic malignancies particular types of translocations are associated with histologically distinct neoplasms.1) Several breakpoints at 14q32.1, designated as the TCL1 (T-cell lymphoma/leukemia-1) locus, have been analyzed and found to be scattered over about 400 kb of DNA.2, 3) A large proportion of patients with T-cell prolymphocytic leukemia (T-PLL) and T-cell leukemia developing from ataxia telangiectasia carry t(14;14)(q11;q32), inv(14)(q11;q32) or t(7;14)(q35;q32), which juxtapose the TCL1 locus to the α/δ or β loci of the T cell receptor (TCR).4–6) This points to the presence of a gene which is activated by the juxtaposition of TCR, as found for MYC or BCL2 translocations with activation by the immunoglobulin gene (Ig) in B-cell malignancies. Virgilio et al.7) recently identified a transcript from the TCL1 locus and designated it as the TCL1 gene, although direct evidence for activation by juxtaposition to TCR genes remains elusive. The open reading frame of this gene encodes a 14-kDa protein that is predominantly localized in the microsomal fraction.8) The product has a sequence similarity to the protein encoded by the MTCP-1 (mature T cell proliferation-1) gene on chromosome Xq28, which is known to be involved in T-cell lymphoproliferative diseases.9, 10)

Although adult T-cell leukemia (ATL) is associated with human T-cell leukemia virus type-I (HTLV-I), the virus alone cannot explain the development of ATL because of the presence of a long latent period between HTLV-I infection and manifestation of the disease, as well as the very low occurrence rate of ATL among carriers of the virus. Therefore it has been considered that ATL leukemogenesis might be the result of accumulation of a number of genetic events.11) A recent cytogenetic study of 107 ATL cases revealed that chromosomal translocations occurred most frequently in band 14q32,12) suggesting that a gene involved in leukemogenesis of ATL exists at this locus. To determine whether TCL1 might play a role, its expression in ATL as well as various hematopoietic malignancies was investigated in the present study.

MATERIALS AND METHODS

Patients Clinical features of the 21 ATL patients are listed in Table I. Diagnosis was made using the criteria described previously by Shimoyama et al.13) All patients were positive for serum antibody to HTLV-I and CD4 surface markers. HTLV-I proviral genome integration was examined by Southern blot analysis for 18 cases. Karyotypic analysis has been performed in 8 patients. None of the patients showed chromosomal abnormalities involving 14q32. In UPN001, who showed very faint TCL1 expression, the karyotype was normal; 46, XY. Patients with other types of T-cell malignancies, including four with acute lymphocytic leukemia (T-ALL), two with lymphoblastic lymphoma (T-LBL), three with T-PLL and one
with a diffuse lymphoma of T-cell type (T-DL), were also studied. The karyotype of the UPN201 patient who showed faint TCL1 expression is 44, XY, der(2) t(2;3)(p21;p13), del(3p13), del(7q22), −7, −8, der(7)t(7;14)(q32;q24), t(8;11)(p21;q13), t(7;12;14)(q22;q32;q24).

**Cell lines** Sixty-five hematopoietic cell lines (13 from T-cell tumors, 31 from B-cell tumors, 13 from myeloid tumors and 8 others) were examined (Table II). Some of these cell lines were kindly provided by Drs. A. Karpas, A. Epstein, N. Kamada, T. Nakagawa, K. Yanagisawa, M. Ogura, M. Abe, I. Kubonishi, S. Nakazawa, M. Nitta, T. Ohtsuki and S. Shimizu.

**Phytohemagglutinin or IL-2 stimulation of peripheral blood mononuclear cells** Peripheral blood mononuclear cells (PBMNCs) from normal individuals were separated by Ficoll-Hypaque gradient centrifugation and cultured in RPMI 1640 with 5% fetal calf serum (FCS)(RPMI-FCS) in the presence of 0.1% phytohemagglutinin-P (PHA-P; Difco Laboratories, Detroit, MI) in a 5% CO2 incubator at 37°C for 72 h. Anti-CD19 beads (MBL, Nagoya) were used for cell fractionation analysis, conducted according to the manufacturer’s protocol.

**Northern blot analysis** Total RNA was isolated by ultracentrifugation on a guanidine isothiocyanate/CsCl2 gradient and northern blot analysis was performed as described previously. The TCL1 probe was synthesized by means of reverse transcription-polymerase chain reaction using Daudi cell line mRNA with 5′-CGGGGATCCGACGC-3′ and 5′-GCTGAA TTCT-3′ and cloned into the pBluescript II vector at the BamHI/EcoRI site. The TCL1 sequence was confirmed by dideoxy sequencing (Sequencing kit, Amersham-Japan, Tokyo). mRNA signals were normalized to the corresponding 28S rRNA levels visualized by ethidium bromide staining. Hybridization signals of mRNA were quantitated by scanning densitometry (Image Analyzer V10, TOYOBO, Osaka). mRNA of the SP-49 cell line was applied to every gel for northern analysis as a standard, and the SP-49 signal was arbitrarily defined as 10. The human TCRβ chain gene probe, CTβ, and the human IgM gene probe, Cμ, were also used to evaluate cell types in blots of PBMNCs.

**RESULTS**

**TCL1 expression in normal human tissues** Northern blot analyses of TCL1 expression in various human tissues (Fig. 1) revealed signals in the spleen and lymph

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**Table I. Clinical Features and TCL1 Expression in ATL Patients**

| Case | Age/Sex | Subtype | Sample | HTLV-I integration | Expression of TCL1 |
|------|---------|---------|--------|-------------------|-------------------|
| UPN001 | 58/M | A | PB | + | 0.1 |
| UPN002 | 70/M | A | PB | + | − |
| UPN003 | 43/F | A | Acites | + | − |
| UPN004 | 34/M | A | PB | + | − |
| UPN005 | 66/M | A | PB | ND | − |
| UPN006 | 64/F | A | PB | + | − |
| UPN007 | 78/F | A | PB | + | − |
| UPN008 | 71/F | A | PB | + | − |
| UPN009 | 73/F | A | PB | + | − |
| UPN10 | 57/F | A | PB | + | − |
| UPN11 | 47/F | A | PB | + | − |
| UPN12 | 51/M | A | PB | + | − |
| UPN13 | 47/M | A | LN | + | − |
| UPN14 | 53/F | A | PB | + | − |
| UPN15 | 52/M | A | PB | + | − |
| UPN16 | 49/F | C | PB | + | − |
| UPN17 | 40/F | C | PB | + | − |
| UPN18 | 54/M | C | PB | + | − |
| UPN19 | 72/M | UD | PB | ND | − |
| UPN20 | 52/F | UD | PB | ND | − |
| UPN21 | 71/M | UD | PB | + | − |

Abbreviations: UPN, unique patient number; ND, not done.

a) Subtype: A, acute type; C, chronic type; UD, undefined.
b) The level of expression was normalized by arbitrarily defining the SP-49 signal as 10.
nodes, with intensities of one-third and two-thirds, respectively, of those of the SP-49 signal. A very weak signal was also found in PBMNCs at one-fiftieth of the SP-49 level. No signals were detected in the thymus or other tissues. These results indicate that the TCL1 expression is restricted to lymphoid organs.

### TCL1 expression in tumor samples of T-cell malignancies

We examined the TCL1 expression in 12 hematopoietic T-cell lines including five ATL cell lines, four T-ALL cell lines, one T-LBL cell line (HT-1), one CTCL cell line and one Ki-1 lymphoma cell line (Karpas-299), but no signals were detected in any case (Fig. 2, Table II). HT-1 cells established from a patient with lymphoblastic lymphoma carrying inv(14)(q11;q32) did not exhibit any TCL1 signals. We next examined 21 cases of ATL patients and 20 were found to be negative (Fig. 3, Table I), the remaining patient UPN001 showing a very faint TCL1 signal at one-hundredth of SP-49. Ten patients with other types of T-cell malignancies (4 with T-ALL, 3 with T-PLL, 2 with T-LBL, and one with T-DL) were also studied (Fig. 3). Only one T-PLL case demonstrated a signal (two twenty-fifths of the SP-49 signal).

#### Table II. Expression of TCL1 mRNA in Hematopoietic Cell Lines

| Cell line | Type/origin | Northerna | Cell line | Type/origin | Northerna |
|-----------|-------------|------------|-----------|-------------|------------|
| AML cell lines |             |            | AML cell lines |             |            |
| HL-60     | AML M2      | —          | NALM-6    | Pre-B(ALL)  | 23.1       |
| KASUMI-1  | AML M2      | —          | NALM-18   | Pre-B(ALL)  | 7.5        |
| SKNO-1    | AML M2      | —          | NALL-1    | Pre-pre-B(ALL) | 4.0   |
| NKM-1     | AML M4      | —          | RS4;11    | Pre-pre-B(ALL) | 0.7   |
| KOCL-48   | PreB(ALL) →M4 | —       | KOPN-1    | Pre-B(ALL)  | 63.2       |
| HE-1R     | AML M4E     | —          | KOCL-45   | Pre-B(ALL)  | 5.8        |
| NOMO-1    | AML M5      | —          | KOCL-58   | Pre-B(ALL)  | 36.9       |
| THP-1     | AML M5      | —          | KOCL-33   | Pre-B(ALL)  | 62.3       |
| HEL       | AML M6      | —          | KOCL-44   | Pre-B(ALL)  | 49.3       |
| CMK       | AML M7      | —          | KOCL-50   | Pre-B(ALL)  | 3.4        |

CML cell lines

| Cell line | Type/origin | Northerna |
|-----------|-------------|------------|
| K562      | CML-BC (E)  | —          |
| NCO-2     | CML-BC (My) | —          |
| MEG-01    | CML-BC (Meg)| —          |

T-Lymphoid cell lines

| Cell line | Type/origin | Northerna |
|-----------|-------------|------------|
| CCRF-CEM  | T(ALL)      | —          |
| HPB-ALL   | T(ALL)      | —          |
| Jurkat    | T(ALL)      | —          |
| MOLT-3    | T(ALL)      | —          |
| MOLT-4F   | T(ALL)      | —          |

Karpas-299

| Cell line | Type/origin | Northerna |
|-----------|-------------|------------|
| Hut-78    | T(CTCL)     | —          |
| HT-1      | T(LBL)      | —          |
| Hut-102   | T(ATL)      | —          |
| ATN-1     | T(ATL)      | —          |
| IPAT-1    | T(ATL HTLV-I(−)) | — |

WHN-2

| Cell line | Type/origin | Northerna |
|-----------|-------------|------------|
| ATN-5T    | T(ATL)      | —          |

AST-1

| Cell line | Type/origin | Northerna |
|-----------|-------------|------------|
| KPNT-1    | T(HTLV-I(+))| —          |

Other cell lines

| Cell line | Type/origin | Northerna |
|-----------|-------------|------------|
| U937      | Histioytic  | —          |
| RC-K8     | Histioytic(DL)| —     |
| SU-DHL-1  | Histioytic  | —          |

a) The level of expression was normalized by arbitrarily defining the SP-49 signal as 10.0.
level), although no 14q32 abnormality was evident on karyotypic analysis.

**TCL1 expression in samples of B-cell and myeloid-cell malignancies** We expanded our analysis to include various types of B-cell malignancies (Fig. 4, Table II) and found the *TCL1* gene to be strongly expressed in all of 12 cell lines derived from pre-B ALL and 12 cell lines derived from B-cell lymphomas. No signal was found in the SU-DHL-10 cell line derived from a B-NHL or in seven myeloma cell lines. Eleven cell lines derived from either AML or CML were also studied (Fig. 2, Table II) but no TCL1 signals were observed in any case.

**TCL1 expression in normal peripheral blood mononuclear cells stimulated by phytohemagglutinin (PHA)** Since the cell line study suggested TCL1 expression to be associated with the B-cell lineage, we investigated fractionated normal PBMNCs with anti-CD19 beads. The TCL1 signal was found in CD19-positive cells, but not in CD19-negative cells (Fig. 5). This result indicated that the TCL1 expression is restricted to B-lymphocytes in peripheral blood. In an attempt to study TCL1 expression in response to a growth signal, we investigated the mRNA expression of normal PBMNCs stimulated with PHA-P. TCL1 mRNA levels decreased to one-tenth of the unstimulated case after 6 h, but the signal was then increased to 31% at 24 h, 81% at 48 h and 102% at 72 h relative to unstimulated PBMNCs. Since the kinetics of both Cµ and CTβ mRNAs were similar to that of TCL1 when the membrane was reprobed with CTβ and Cµ (Fig. 6), it is not possible to distinguish which lineage is more involved in the TCL1 expression on PHA-stimulation. However, this experiment demonstrated that the TCL1 expression is regulated upon growth stimulation.

![Fig. 1. Northern analysis of TCL1 expression in normal human tissues.](image)

![Fig. 2. Representative northern analysis of TCL1 expression in T-cell and myeloid cell lines.](image)
DISCUSSION

Chromosomal translocations involving 14q32 have been reported to be the most frequent in ATL patients. The report showed that 25 out of 78 cases (32%) with acute and lymphoma type ATL had 14q32 abnormality. Recently, a candidate gene in the TCL1 locus activated by a juxtaposed TCR gene was isolated. This prompted us to examine the expression of this gene in ATL cell lines and patients to assess its involvement. Northern blot analysis was carried out as shown in Fig. 3 and 4. The results demonstrated that TCL1 expression was significantly increased in ATL cell lines as compared to normal B-cell lines. These findings suggest that TCL1 may play a crucial role in the pathogenesis of ATL.
ysis, however, produced no signals in any of the five ATL cell lines or in 20 of the 21 ATL patients, and only a very weak signal in the remaining case, UPN001. This suggests that the TCL1 gene is not activated in the majority of ATL patients’ cells, and is in direct contradiction to the data recently presented by Narducci et al.16) who found overexpression of TCL1 products in all ATL cases examined by immunostaining. Our analysis, on the other hand, revealed that the majority of B-cell malignancies other than myelomas show very intense TCL1 signals. Furthermore, the fact that TCL1 expression was seen in normal spleen, lymph nodes and PBMCs, but not in other tissues examined, including the thymus, suggests that TCL1 is transcribed predominantly in lymphoid cells of B-cell lineage. Indeed, cell fractionation analysis using anti-CD19 beads demonstrated that CD19-positive cells are responsible for the TCL1 in PBMCs. Thus, it should be emphasized that the expression of TCL1 in ATL leukemic cells needs to be more carefully examined, because contamination by B cells might generate strong positive signals. It is therefore significant that Narducci et al.’s data showing positive reaction on ATL cells do not correlate to the percentage of leukemic cells in the samples.16) Furthermore, the expression of various cell lineages examined in their report5,8,16) has not been examined by means of northern blot analysis, but only reverse transcriptase-polymerase chain reaction. Therefore, the positivity on ATL cells needs to be re-examined in conjunction with mRNA levels, which should clarify the relevance of TCL1 gene involvement in ATL.

In an attempt to study their possible role in TCL1 expression, PBMCs were stimulated with PHA. In response, the TCL1 signal was reduced to about one-tenth after 6 h, and gradually increased thereafter. Although the precise function of the TCL1 gene remains unknown, this decrease caused by PHA might indicate some role in cell proliferation. The down-regulation of the signal during the first 6 h after the stimulation also suggests that TCL1 may be expressed during a limited period of the cell cycle.

The present study revealed one PLL patient with significant TCL1 expression. Because the number of blastic cells in this patient was 76,300/µl and 95% of the cells were CD3-positive, it is unlikely that contaminating B-cells were responsible for this level of mRNA expression. This suggests that either a distinct type of T-cell malignancy might aberrantly express TCL1, or a T-cell subset, presenting itself as such a T-cell malignancy, might express TCL1 as a normally regulated gene expression in the course of development. Although the TCL1 expression in one PLL patient raises the possibility that TCL1 can indeed be aberrantly expressed in ATL, the fact that the 21 cases we examined did not show any strong mRNA expression suggests that TCL1 does not represent a major gene alteration in ATL. Thus, it is important to study TCL1 in many T-cell and B-cell malignancies of various types, as was done in the present study to clarify the role of the TCL1 gene in cell differentiation and proliferation.
In conclusion, our data presented here demonstrate that TCL1 gene involvement is not a major cause of ATL development, but rather indicate that this gene expression may play some role in B-cell development.

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