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Expression of *TCL1* in Hematologic Disorders

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Key Words
Apoptosis · Hodgkin's disease · Leukemia · Non-Hodgkin's lymphoma · Oncogene · *TCL1*

Abstract
Objective: *TCL1, MTCP1* and *TCL1b* are three members of a new family of oncogenes that are expressed in T cell leukemias of ataxia telangiectasia patients (T-PLL, T-CLL). *TCL1* is located at 14q32.1 and activated by juxtaposition to the α/δ-locus at 14q11 or β-locus at 7q35 of the T cell receptor during the reciprocal translocations t(14;14)(q11;q32), t(7;14)(q35;q32), or inversion inv(14)(q11;q32). *TCL1* encodes a predominantly cytoplasmic protein of 114 aa (14 kD) of unknown function. Recent studies suggest that *TCL1* promotes cell survival rather than stimulating cell proliferation, as previously proposed. ***Methods:*** In an attempt to clarify the contexts in which *TCL1* is expressed, we investigated *TCL1* expression in 114 lymphoma and leukemia patients by Northern blot, RT-PCR and immunohistochemistry. ***Results:*** *TCL1* expression is restricted to lymphoid cells, and is found in neoplastic (T and B cell neoplasms, and Hodgkin's disease) and nonneoplastic proliferations (reactive lesions). Out of 114 cases, 18 neoplasms of myeloid and 4 cases of epithelial origin were *TCL1*-negative. In lesions of the lymphoid system, both low- and high-grade lymphomas were found to express *TCL1*. ***Conclusions:*** We propose that *TCL1* expression especially in high-grade B cell non-Hodgkin's lymphomas might interfere with B cell differentiation and promote the transition from low- to high-grade lymphoma.

Introduction

Recurrent chromosomal aberrations, notably nonrandom chromosomal translocations, are associated with specific types of leukemias or lymphomas with distinct clinicopathologic features [1–6]. Molecular cloning of chromosomal translocation breakpoints has shown that the molecular anatomy of oncogenes located at these sites may be altered [6]. In lymphoid neoplasia, chromosomal translocations often result in the juxtaposition of an oncogene or part of an oncogene and immunoreceptor genes. A classical example is Burkitt's lymphoma with the t(8;14) translocation involving the c-*myc* oncogene on chromosome 8q24, and the Ig heavy-chain gene on chromosome 14 [7]. The most common type of low grade B cell lymphoma, follicular lymphoma, is associated with t(14;18) rearrangement involving the *bcl-2* oncogene [8]. In mantle cell lymphoma, which often contains t(11;14), the breakpoints on chromosome 11q13 include the *bcl-1* and *PRAD-1/cyclin D1* loci [9–11].

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In peripheral T cell neoplasias, the long arm of chromosome 14 is frequently the site of chromosomal alterations [1, 12, 13]. These neoplasias are classified as T prolymphocytic leukemias (T-PLL), and constitute very aggressive mature T cell proliferations occurring late in life (median age of 69 years) and with high frequency in patients with ataxia telangiectasia (AT). Furthermore, adult T cell leukemias (ATL) may be associated with human T lymphotropic virus type I (HTLV-I) endemic infection and show many similarities with T-PLL or T chronic lymphocytic leukemias (T-CLL) that also occur in AT patients at a younger age [14–16]. The most common rearrangements in these neoplasias are reciprocal translocations t(14;14)(q11;q32), t(7;14)(q35;q32), or inversions inv(14)(q11;q32). The breakpoint region of these chromosomal alterations was cloned, and a gene adjacent to these altered regions was isolated, sequenced and named 'T cell lymphoma/leukemia-1' *(TCL1)* at 14q32.1 [17]. *TCL1* is activated and overexpressed in these leukemias by juxtaposition to the α/δ-locus at 14q11 or β-locus at 7q35 of the T cell receptor. In T-CLL of AT, *TCL1* activation is already noticeable in T cell clonal expansions at the preleukemic stage. Therefore, *TCL1* activation may be the first step in the neoplastic process, followed by secondary genetic events that could then result in overt leukemia [18]. Interestingly, another possible way of enhancing *TCL1* expression in the absence of micro- or macrorearrangements has to be considered in the rare cases of ATL related to endemic infection with the HTLV-I virus. Among these, it seems that the HTLV-I products (transcriptional transactivator *tax,* a weak oncogene in vitro), may be responsible for *TCL1* overexpression and induction of leukemia [19].

TCL1 expression is normally observed in early T cell progenitors (CD4–, CD8–, CD3–), in the lymphoid cells of the B cell lineage (pre-B cells and immature B cells) and weakly in CD19+ peripheral blood lymphocytes [17, 20–22].

TCL1 encodes a predominantly cytoplasmic protein of 114aa (14kD) of unknown function and is also found in small quantities in the nucleus of lymphoid cells [23]. Its restriction to the cytoplasmic microsomal fraction suggests an intracellular function [24]. Moreover, another oncogene named 'mature T cell proliferation 1' *(MTCP1)* [20, 25–27] located at Xq28, with partial amino acid or nucleotide sequence similarity with *TCL1* (41% identical and 61% similar), seems to be activated in rare cases of mature T cell leukemia, e.g. T-PLL, and in benign clonal proliferations by translocations of t(x;14)(q28;q11) [24, 28]. As *TCL1* and *MTCP1* show no similarity to any other known oncogenes, they may represent two members of a new lymphoid-specific gene family [25]. A third member of this *TCL1* gene family, named *TCL1b* and located at 14q32.1 was recently cloned [21]. It also shows physical linkage and similarity of structure and expression with *TCL1* and is also activated by rearrangements at 14q32.1 in T cell leukemias.

To provide a better understanding of *TCL1* function, a transgenic mouse carrying *TCL1* was developed. The expression of the murine *TCL1* homologue was controlled by the T cell specific Lck promoter. Young mice expanded a preleukemic T cell population expressing *TCL1;* over the age of 15 months, the development of T cell leukemia was observed. The phenotype of murine leukemias is CD4–CD8+ in contrast to human leukemias, which are predominantly CD4+CD8–.

The function and the expression of *TCL1,* the first member of the oncogene family located at 14q32.1, remain unknown. Contradictory results have been published regarding the selective expression of *TCL1* in either T cell or B cell malignancies [22, 26]. The present study focuses on the expression of *TCL1* in a broad range of hematopoietic neoplasma of myeloid and lymphoid origin, including both T and B cell lymphomas and Hodgkin's disease (HD).

Materials and Methods

Patients

Peripheral blood and bone marrow cells from untreated patients with various types of leukemia and lymphoma were obtained at time of diagnosis, and an aliquot was immediately subjected to diagnostic procedures. The remaining cells were isolated over a Ficoll density gradient and cryopreserved. In the leukemias, diagnosis was based on the French-American-British [27] morphologic and cytochemical criteria, when they were applicable. Thirty-one patients with leukemia were studied: 16 acute myeloid leukemias (AML), including 7 cases of M2-AML with maturation, 4 cases of M1-AML without differentiation, 4 cases of M3 acute promyelocytic leukemia and 1 case of M4 acute myelomonocytic leukemia, 2 chronic myeloid leukemias (CML; Ph1-positive), 1 acute undifferentiated leukemia (AUL), 1 B prolymphocytic leukemia (B-PLL), 9 acute lymphocytic leukemias (ALL) and 2 B cell chronic lymphocytic leukemias (B-CLL). Three patients with normal bone marrow were included in this study as controls.

Tissue samples of lymphoma patients were snap-frozen and kept at –70°C until further processing. Part of the tissue was used for diagnosis, fixed in formalin and embedded in paraffin. The diagnosis was established on the basis of combined morphologic, immunophenotypic and sometimes genotypic analyses. These genotypic analyses, including gene rearrangement studies of B and T lineage cells, TCR and IgH by Southern blot, and demonstration of *bcl2* rearrangement by PCR, where this was necessary for diagnosis. Conventional HE morphology, Giemsa, PAS and Silver stains were done in each

Table 1. *TCL1* in different types of lymphoma

n	R.E.A.L. classification	RT PCR *TCL1*-p9A			Northern blot *TCL1*			Immuno-histochemistry *TCL1*		
		+	–	ND	+	–	ND	+	–	ND
7	B-CLL	6	1		5	1	1	3		4
5	Follicular grade I	4	1			1	4	5		
8	Follicular grade II	7	1		5	1	2	4		4
2	Lymphoplasmacytoid	2			2					2
13	DLBCL	10	3		6	3	4	9		4
4	DLBCL, secondary	4			4			2		2
1	Lymphoblastic lymphoma	1			1			1		
1	T cell-rich B cell lymphoma		1			1				1
1	Low-grade NHL, NOS	1			1					1
1	T-CLL			1	1			1		
6	Sézary syndrome	3	3				6			6
4	High-grade T-NHL	1	3			3	1			4
13	Hodgkin's lymphoma, ns	2	6	5	4	9			3	10
4	Hodgkin's lymphoma, mixed		2	2	2	2				4
13	LAD	11	1	1	11	1	1	13		
4	Adenocarcinoma		4			4			4	
16	AML			16		16			2	14
2	CML			2		2				2
2	B-CLL			2	2			1	1	
1	B-PLL			1	1			1		1
1	AUL			1	1					1
9	ALL			9	7	2		5		4
3	Normal bone marrow				2					3

NOS = Not otherwise specified; ns = nodular sclerosing type; ND = not done; R.E.A.L. = Revised European American lymphoma classification.

case. For immunohistochemistry, cytospin preparations of tumor cells were carried out. The diagnosis of lymphoma was based on the Revised European American Lymphoma classification [28]. Seventy patients with lymphoma and 13 patients with nonneoplastic lymph node disorders were included in this study. 53 of the lymphomas were non-Hodgkin's lymphoma (NHL) and 17 HD cases. NHLs were of B cell (41 cases) and T cell origin (12 cases). In the group of B-NHL, 22 cases were low-grade and 19 cases were considered high-grade lymphomas. In the group of T-NHL, 8 cases were low-grade and 4 cases were considered high-grade lymphomas. 13 HD cases were of nodular sclerosing type and 4 of mixed cellularity type. As controls, 4 patients with carcinoma were included (table 1). No cytogenetic data concerning the 14q were available in either leukemia or lymphoma cases.

Cell Lines

The cell lines were obtained from the American Type Culture Collection. The Burkitt's lymphoma lines Daudi and Raji, the promyelocytic leukemic cell line HL-60, the CML cell line K 562 and the T lymphoblastic cell lines Jurkat and MOLT-4 were cultured in McCoy's medium (Gibco, Grand Island, N.Y., USA) supplemented with 10% fetal bovine serum in a humified atmosphere containing 5% CO_2 at 37°C. Stimulation with phytohemagglutinin was carried out at a final concentration of 0.1% for 3 days.

RNA Analysis

Total RNA was extracted from cell lines, leukemic cells and lymph nodes by the guanidinom thiocyanate-phenol-chloroform method [29]. The RNA was analyzed by Northern blot hybridization with a *TCL1* c-DNA probe and by RT-PCR using the primers indicated below. Positive controls for all RNA tests included RNA from the Daudi cell line and RNA from a patient with T-CLL, known to express *TCL1* [17]. The *TCL1*-negative cell line HL-60 was used as control. Because the intensity of the positive signal for *TCL1* was variable, the cases were scored strongly positive for *TCL1* when there was a strong band in Northern blot and/or a distinct band indicating amplification of the *TCL1* transcript. Cases with a narrow but still visible band were considered weakly positive.

Northern Blot

The RNA samples were size-separated by an agarose-formaldehyde gel (1% wt/vol) and transferred to a nylon membrane (Hybond, Amersham, Amersham, UK). Hybridization with random primer

^{32}P-labeled probes (1–2 × 106 cpm/ml hybridization solution) was performed for 36 h at 42°C. Filters were washed to a final stringency of 0.1–0.25% SSC at 55°C and exposed for 16–96 h at –70°C to XM films (3M, Trimax, Ferrania, Italy). Purified inserts of *TCL1* (1.25 kb, *Eco*RI) from vector pBSII were used as human cDNA probe [17].

RT-PCR

c-DNA was made from total RNA using the MuLV reverse transcriptase (Stehelin, Basel, Switzerland), 1 μg RNA, respective buffers and oligo(dT) according to the specifications by the manufacturer. PCR amplification was performed using the *TCL1* primers p9A (5′-TGCTGCCAGATGACTGATGT-3′) and RevIII (5′-CAAAT-GGAATCCTCCTTGGC-3′) [17]. PCR was performed under the following conditions: 40 cycles with 1 min at 94°C, 1 min at 66°C and 1 min at 72°C. A 5-min step at 94°C was included before the first cycle to allow a hot start using AmpliWax (Perkin Elmer) and a final step at 72 or 60°C was carried out for 10 min in the last cycle. PCR amplicons were electrophoresed on 1.0% agarose gels, blotted on nitrocellulose membranes and hybridized with an appropriate DIG-labeled probe (5′-TGAGCCTGGCCAGCCTACAAT-3′). Hybridization was performed at 54°C and prehybridization at 68°C using 10 pmol probe/ml. The internal PCR standard used was amplification with primers specific for the β-actin gene. Primers specific for amplification of a 154-bp product of the housekeeping β-actin gene were A1 (5′-TCATCACCATTGGCAATGAG-3′) and A2 (5′-CAGTGTGTTGGCGTACAGGT-3′). The *TCL1* amplification product is of 280 bp.

Immunohistochemistry

Immunohistochemistry staining was performed on cytospin preparations using alkaline phosphatase-anti-alkaline phosphatase (APAAP) and avidin-biotin complexes (ABC). As a primary antibody, a polyclonal anti-*TCL1* rabbit antiserum was used [24]. Chromogens were diaminobenzidine for ABC and Fast-Red TR plus AS-MX sodium salt and levamisole for APAAP. Negative controls without primary antibody were included. Immunostaining was performed using streptavidin-AP and substrate (ELF-Kit, Molecular Probes, NSA).

Immunohistochemistry on sections of formalin-fixed, paraffin-embedded tissues was performed using the same polyclonal antibody in combination with a conventional immunoperoxidase technique (diaminobenzidine as chromogen, no special antigen retrieval method).

Results

TCL1 *Transcription in Cell Lines*

The RNAs of a number of tumor cell lines were tested by Northern blot for the transcription of *TCL1. TCL1* is transcribed in cell lines of lymphoid origin but not in cell lines of nonlymphoid origin (HL-60, K 562). It is transcribed in B cells of endemic Burkitt's lymphomas (Raji and Daudi), but not in cell lines derived from T-ALL (Jurkat, MOLT-4). The detected transcript is of 1.3 kb. In all cell lines transcribing *TCL1, TCL1* was demonstrated by immunohistochemistry, localized in small granules in the cytoplasm.

Transcription of TCL1 *in Human Leukemias*

The RNA of 31 leukemia patients was investigated by Northern blot and in 7 of these cases *TCL1* was also detected by immunohistochemistry. None of the nonlymphoid leukemias (16 AMLs and 2 CMLs) showed any *TCL1* transcription. In lymphoid leukemias 1/1 B-PLL, 2/2 B-CLLs, 1/1 AUL and 7/9 ALLs did transcribe *TCL1* (table 1). A representative blot is shown in figure 1. In positive cases, immunohistochemistry showed a fine granular reaction in the cytoplasm of the cells (fig. 2a). RNA extracted from normal human bone marrow mononuclear cells showed *TCL1* mRNA (by Northern blot) in 2/3 cases.

Transcription of TCL1 *in Lymphomas and Reactive Lymph Node Lesions*

Seventy patients with malignant lymphomas (53 NHL and 17 HD cases), 13 patients with reactive lymphoid lesions (LADs) and 4 patients with carcinoma were screened for the expression of *TCL1* by RT-PCR and Northern blot. Where Northern blot and RT-PCR were carried out on parallel, the results were identical except for 1 case of high-grade B-NHL (diffuse large B) which was negative in Northern blot but positive in RT-PCR.

Among B-NHLs, 19 out of 22 low-grade lymphomas strongly transcribed *TCL1,* including 6/7 B-CLL, 4/5 follicular lymphomas grade I, 7/8 follicular lymphomas grade II and 2/2 lymphoplasmocytic lymphomas. In the group of high-grade B-NHLs 15/19 transcribed *TCL1* (table 1). Interestingly, 4 of these positive cases were secondary high-grade lymphomas which had evolved from low-grade follicular lymphomas, or in 1 case from B-CLL (Richter's syndrome).

In the group of T-NHLs, 3/6 Sézary syndromes transcribed *TCL1,* as well as a case of T-CLL, a peripheral T-NHL of low grade and 1/2 T-ALL (table 1). Analysis of the clinical history of these Sézary cases revealed that 3 *TCL1*-expressing cases had an unfavorable clinical course while the other 3 had a good clinical outcome.

Six out of 17 cases of classical HD (2 mixed cellularity, 4 nodular sclerosis) efficiently transcribed *TCL1.* All these HD were cases with a normal phenotype of HD/RS cells, that is CD30+, CD15+ and CD20– (table 1).

Of the 13 patients with LADs, 11 were shown to transcribe *TCL1,* either by Northern blot or RT-PCR (table 1). The LAD cases consisted of granulomatous, involutive (Castleman-like) and nonspecific LADs.

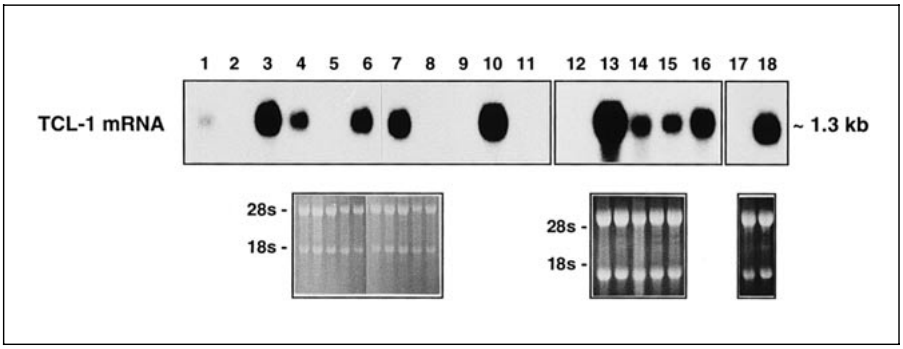

Fig. 1. Northern blots showing representative cases of the study: 1–5, 7, 10: ALL; 6: PLL; 8, 9, 11: AML; 12: T-ALC; 13: diffuse large B-NHL; 14, 15: LAD; 16: HD, 17: HL-60, negative control; 18: Daudi, positive control.

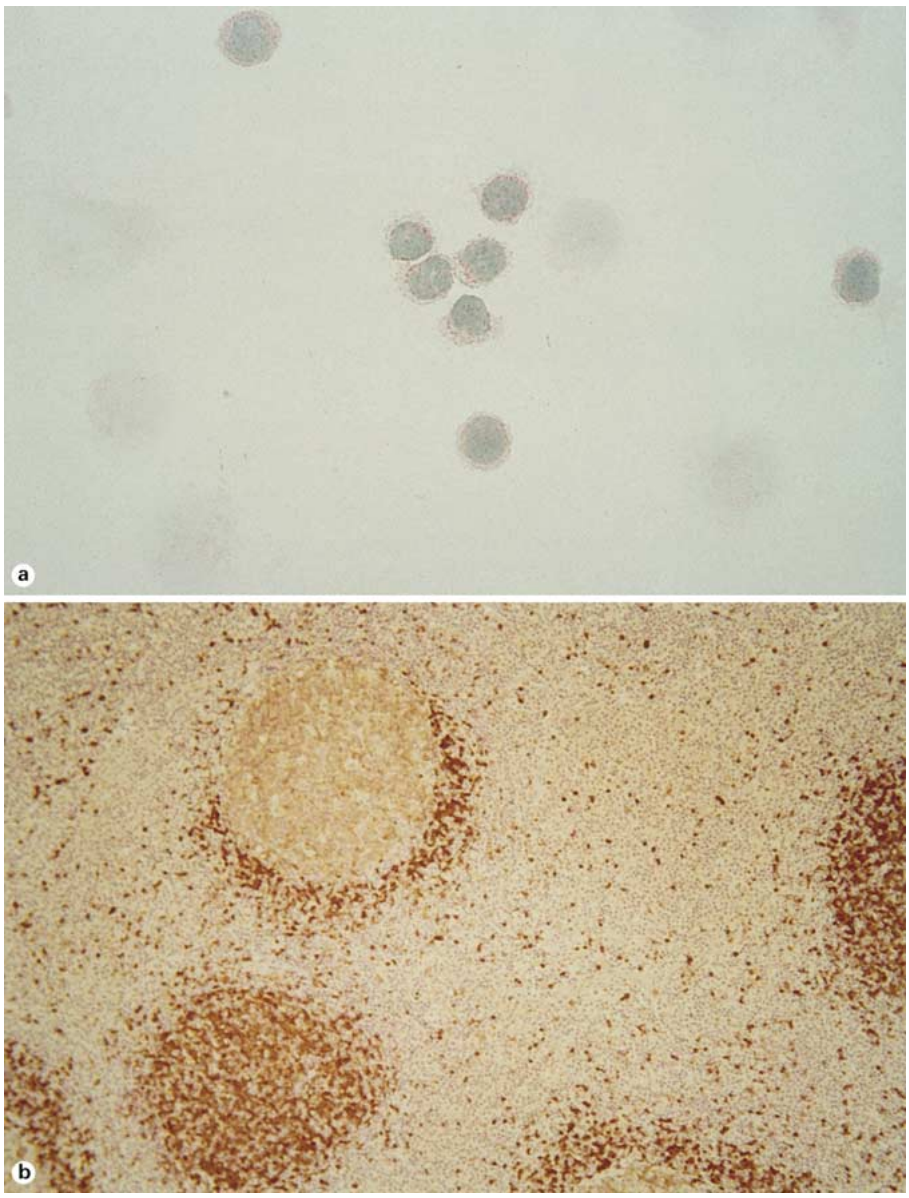

Fig. 2. a Peripheral blood cells, cytospin preparation, immunohistochemistry for *TCL1:* T-CLL, *TCL1*-positive in Northern blot; note the fine granular staining of the neoplastic cells, indicating a cytoplasmic localization of *TCL1.* **b** Reactive lymph node, immunohistochemistry for *TCL1:* mantle zone lymphocytes as well as scattered lymphocytes in the parafollicular region are strongly and germinal center cells are weakly *TCL1*-positive.

NHLs staining for *TCL1* were positive in lymphoma cells. A case was considered positive, when more than 10% of the lymphoma cells were positive. Due to tissue/cell shortage, only 52 cases of lymphomas/leukemias or reactive lesions were investigated by immunohistochemistry (table 1). Percentage and pattern of immunoreactivity were variable in different lymphoma types. In HD, the neoplastic HD/RS cells were negative, whereas surrounding bystander cells expressed *TCL1.* In reactive lesions, mantle zone cells and to a lesser extent germinal center cells and some cells in the interfollicular areas were positive (fig. 2b).

Discussion

Following its initial description, the *TCL1* oncogene was found to be involved in the development of human T cell leukemias (75% T-PLL, 10% AT leukemia showing a rearrangement at the *TCL1* locus at 14q32.1) [17, 18]. The *TCL1* oncogene product was also detected in other types of lymphoid tissue, e.g. B cell lymphomas and leukemias, AIDS immunoblastic lymphoma, plasmocytoid cell lines and LAD [20–22, 26, 30].

Although the oncogenic potential of *TCL1* is established, its function is still unknown. Some recent studies suggest an antiapoptotic effect of *TCL1,* and that *TCL1* may become activated in a multistep sequence of events resulting in neoplasia after a long latency [21, 23, 30]. A newly proposed mechanism is the promotion of cellular survival and proliferation by *TCL1* coactivating the Akt kinase [31, 32]. We therefore focused our RT-PCR and Northern blot examinations on *TCL1* transcription in a broad array of hematologic disorders. Leukemias of myeloid and lymphoid origin, NHLs, including both T and B cell lines, HD and nonneoplastic reactive lymphoid disorders were investigated. In selected cases, *TCL1* was detected by immunohistochemistry in specific subsets of normal B lymphocytes and a majority of B cell neoplasias, but neither in T cell neoplasias nor in HD [22].

We found *TCL1* transcription in the majority of B cell lymphomas and leukemias, in a few T cell lymphomas, in HD as well as in most of the LADs. In nonneoplastic cells, *TCL1* is known to be transcribed early in B cells and in pre-B cells expressing high levels of CD19, and continues to be expressed in immature IgM+ B cells in fetal bone marrow, in immature thymocytes as well as in mantle zone cells of lymph nodes [17, 30]. In addition, Said et al. [29] have shown recently that *TCL1* expression is high in naïve B cells, reduced in germinal center B cells, and absent in memory B cells and plasma cells.

This suggests that *TCL1* expression, first detected in T cell leukemia, actually occurs more often than initially thought. Although in our series there were no cytogenetic data available for translocation at the *TCL1* locus 14q32.1, the transcription of *TCL1* often seems to occur without rearrangement. This has been shown in numerous cell lines. Virgilio et al. [17] show that except for the cell line SupT11 which carries the t(14;14)(q11;q32.1) and expresses high levels of *TCL1,* expression is detectable in several other cell lines in absence of *TCL1* translocation. According to previous studies, a rearrangement at the *TCL1* locus was restricted to rare T cell lymphoproliferative disorders, such as T-PLL and AT. The same phenomenon was described for other types of oncogenes, such as *bcl-2,* which is detectable in neoplastic cells in cases of human lymphoma carrying the t(14;18) chromosomal translocation, but also without rearrangement as in normal T and B lymphoid cells, and in a variety of lymphoproliferative disorders and solid tumors [33]. A similar behavior can be observed in *bcl-1* and *PRAD-1/cyclin D1,* both frequently found in mantle cell lymphoma with a t(11;14) translocation. Activation of the latter gene has also been described in several diseases lacking a rearrangement, such as benign breast disease and breast carcinoma [34]. A possible mechanism for the activation of *TCL1* expression in the absence of chromosomal rearrangements is the loss of methylation of one promoter allele [35].

In our group of high-grade B cell NHLs, 79% (15 out of 19) transcribed *TCL1* as did all secondary high-grade B cell NHLs (4/4). They all showed a clinically and histologically proven transformation from low- to high-grade lymphoma. A high frequency of *TCL1* gene expression in low-grade B NHLs has been found in other series of NHL, whereas a lower frequency of *TCL1* expression has been reported in high-grade lymphomas, ranging from 18 to 25% of diffuse large B cell lymphoma (DLBCL) [29, 36]. The group of Teitell et al. [30] reported a high percentage of AIDS-DLBCL expressing *TCL1* and propose that downregulation of *TCL1* is dependent on normal immune functions, to explain the difference between AIDS and immunocompetent lymphoma patients. In our series, we found a high percentage of *TCL1* expression especially in secondary DLBCLs in immunocompetent patients. Along these lines, transformation to high-grade lymphoma could be linked to alterations of the immune system manifesting themselves by *TCL1* expression. On the other hand, it could be speculated that *TCL1* activation

interferes with B cell differentiation and promotes the transition from low- to high-grade lymphoma. However, the mechanism of progression from low- to high-grade lymphoma remains largely unexplained.

Given that *TCL1* transgenic mice develop mature T cell leukemia after a long latency period (15 months) and that human lymphomas progress from low to high grade in several years, an antiapoptotic effect of *TCL1* could be suggested. Recent studies attribute the promotion of cell survival to *TCL1,* rather than stimulation of cell proliferation, as previously proposed. This suggestion is supported by the fact that *TCL1* is found in regions of cell survival, e.g. as mantle B zone of reactive lymph nodes and in decreased levels in follicle centers, which are areas of extensive apoptotic activity [22]. Interestingly, *TCL1* and *bcl-2* distributions are similar in this context. It remains to be investigated whether an antiapoptotic role of *TCL1* could explain the long period of latency in these lymphoproliferative disorders.

As the *TCL1* gene family is growing, it can be postulated that more than just one oncogene needs to be activated early in the neoplastic process, before the occurrence of secondary genetic events [21]. This theory is partially supported by the findings that early *TCL1* translocation is present in T cell clonal expansions at the preleukemic stage, before the appearance of an overt AT leukemia [18].

We have shown that all the lymphoid neoplasms studied may express *TCL1.* However, this expression is not the only reason for neoplastic lymphoid progression, since we have demonstrated frequent *TCL1* activation in lymph nodes (11/13) with nonmalignant reactive changes, as well as in normal mononuclear bone marrow cells (2/3).

In our series of T cell NHLs, we observed *TCL1* transcription in 50% (6/12) of the cases, including 2 low-grade cases (i.e. T-CLL, fig. 2) and 50% of our Sézary syndromes (3/6). Sézary syndromes are neoplastic disorders of T lymphocytes initially involving the skin. Only in later stages of the disease do the tumor cells spread from the skin to other sites, especially the blood. *TCL1* is transcribed in all cases, indicating an unfavorable clinical course, with tumor cells becoming morphologically more blastic and appearing in the peripheral blood.

Thus, in our series of neoplastic T cell lesions, *TCL1* transcription seems to coincide frequently with clinical and histopathologic progression. This has to be further investigated and confirmed with larger groups of patients. It is of special interest that we have shown *TCL1* transcription in 24% of HD cases (4/17). These results stand in contrast to the recently described absence of *TCL1* expression in HD [22]. HD is composed of the neoplastic HD and Reed-Sternberg cells and numerous 'bystander' cells. *TCL1* might be derived from reactive small bystander lymphocytes. The expression of *TCL1* in our cases of HD could result from the admixture of small lymphoid cells, similarly to the expression of *TCL1* in reactive lymphoid tissues. As far as the HD cases in our study are concerned, there are four cases showing *TCL1* transcription, two of the nodular sclerosis and two of the mixed cellularity type. No specific clinical or histological features were observed in these cases.

In the myeloid leukemia group, no *TCL1* transcription was observed neither in our series nor in those of others [22, 26]. In contrast, *TCL1* transcription was found in T-CLL and other leukemias of lymphoid derivation including the B cell line. As already shown for tumor cell lines, it appears that the expression of *TCL1* is restricted to neoplasias of the lymphoid system. One case of AUL expressing *TCL1* in our series, turned out to be of lymphoid origin after revision of the diagnosis. The cell lines (HL-60, K 562, Raji and Daudi, Jurkat, MOLT-4) tested initially also showed this restriction to lymphoid cells. Cytogenetic data were not available for any patient of our study, because these cases were retrieved from tissue and cell archives. Our results with human leukemias are in agreement with those obtained with cell lines.

In conclusion, *TCL1* is widely expressed and restricted to the lymphoid system including normal, reactive and neoplastic disease, NHL and HD. In malignant tumors, *TCL1* expression seems to coincide with disease progression. The presence of *TCL1* in areas of cell survival and the slow development and progression of B cell lymphoma positive for *TCL1* (in humans and mice), suggest that *TCL1* is linked with protection against apoptosis [30]. From recent observations adding other members to the family of *TCL1* oncogenes, such as *MTCP1* and *TCL1b,* it could be speculated that a possible rearrangement at 14q32.1 may contribute to malignant transformation by activating two oncogenes at the same time [21]. This is further evidence that *TCL1* is only one part of the puzzle of multistep lymphomagenesis, but not at all restricted to the T cell lineage.

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