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Gene Expression of Terminal Deoxynucleotidyl Transferase in Neoplastic Cells of Leukemia and Lymphoma

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Expression levels of terminal deoxynucleotidyl transferase (TdT) mRNA in fresh leukemia and lymphoma cells were measured by northern blotting analysis. Bands of 2.1 kb mRNA were detected in all of eight cases of TdT activity-positive leukemias: two cases of null-cell acute lymphoblastic leukemia (null-ALL), two of common ALL, one of pre-B ALL, one of T-ALL, and two of chronic myelogenous leukemia in blastic crisis. One of the null-ALL and one of the common ALL cases also showed large TdT mRNA (3.3 kb). Since all TdT activity-positive samples exhibited TdT mRNA, the TdT gene might be mainly regulated at the transcription level in leukemic cells. An elevated level of 2.1 kb TdT mRNA was also detected in one lymphoma case, where neither TdT activity nor immunoreactive TdT was detected. The extensive chromosomal abnormality demonstrated in this case might be associated with the translational anomaly of TdT.

Key words: Gene expression – Terminal deoxynucleotidyl transferase — Leukemia

Terminal deoxynucleotidyl transferase (TdT)[5] catalyzes the polymerization of deoxynucleotides to a pimer in the absence of DNA template.[1)] The characteristic localization of this enzyme among tissues of adult mammals is in most cortical thymocytes,[2)] and some bone marrow cells.[3)] TdT activity has been demonstrated in most cases of acute lympoblastic leukemia (ALL),[4)] in half of chronic myelogenous leukemias (CML) in blastic crisis,[5,6)] and in some acute myelogenous leukemia (AML),[7)] and has therefore been used as a biochemical marker for leukemic cells. The hypothesis that TdT functions in immunoglobulin heavy chain rearrangement[8)] has now been verified.[9)] Furthermore, T cell receptor β and γ gene rearrangement was reported to be associated with TdT expression.[10,11)]

Recent advances in TdT gene cloning[12,13)] enabled us to examine the expression level of TdT mRNA and its size: 2.1 kb mRNA was commonly detected in calf thymus and stable human T cell leukemia cell lines Molt 3 and Molt 4, whereas 3.3 kb mRNA was detected only in the leukemic cell lines.[14)] Our present study investigated the expression levels of TdT mRNA in various types of fresh human leukemic cells, and also looked for possible expression of TdT mRNA of other than the 2.1 kb size.

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[5] The abbreviations used are: TdT, terminal deoxynucleotidyl transferase; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML-bc, chronic myelogenous leukemia in blastic crisis.

MATERIALS AND METHODS

Patients and cells Ten hematology-oncology patients were examined: two cases of common ALL, two of null-ALL, one each of T-ALL, B-ALL and pre-B ALL, two of CML-bc, and one malignant lymphoma (Table I). Normal human thymus was obtained as a by-product at heart surgery from a patient with tetralogy of Fallot. ALL and CML-bc were differentially diagnosed by morphological analysis of leukemia cells. Phenotypic expression of leukemic cells was measured by immunofluorescence using monoclonal antibodies CALLA (CD10), B1 (CD20), B4 (CD19), 9.6 (CD2), Tp40 (CD7), Ia and surface IgM. Mononuclear cells obtained from peripheral blood or bone marrow aspirates before initiation of therapy were separated by Ficoll-Hypaque centrifugation.

Northern blotting Total RNA was extracted from cells by the guanidinium/cesium chloride method. Northern blotting was performed using polyA+ RNA according to the reported method.[15)] Nick-translated *Eco*RI fragment of human TdT cDNA was used as a probe.[14)]

Assays of TdT TdT activity was assayed in the Tris-Mn system reported previously.[16)] One unit is defined as the incorporation of 1 nmol of dGTP into an acid-precipitable form in one hour. Immunoreactive TdT was measured as reported previously.[17)]

RESULTS

We examined the experession levels of TdT mRNA in nine cases of leukemia, one malignant lymphoma and one

Table I. Expression of TdT Activity and mRNA in Leukemic Cells

Case	Age/Sex (yr)	Diagnosis	Peripheral blood		Bone marrow		Surface markers	Chromosome abnormality	Species	TdT activity ($U/10^8$ cells)	Expression of TdT mRNA
			WBC ($\times 10^9$/liter)	blast (%)	NCC ($\times 10^{10}$/liter)	blast (%)					
1	35/M	null-ALL	61.8	97	6.2	97	Ia+ CALLA−	47,XY,+19	PB	4.7	1.2 AU[f]
2	6/M	B-ALL	13.2	94	17.3	90	BI+ IgM+	46,XY	BM	ND	−
3	21/F	pre-B ALL (relapse)	100.8	88	104.0	95	Ia+ B4+ CALLA−	46,XX	PB	1.3	0.2
4	43/M	CML-bc	31.6	63	ND	49	*a*)	46,XY,t(9;22)	PB	23.1	5.0
5	56/M	CML-bc	92.7	100	75.0	89	ND[b]	45,X0,t(9;22) (chronic phase)	PB	13.3	1.4
6	6/M	T-ALL	166.0	81	54.0	99	9.6+ Tp40+	ND	BM	6.2	0.9
7	5/F	common ALL	7.4	60	100.0	90	CALLA+ Ia+	46,XX	BM	1.1	0.6
8	6/M	common ALL	549.0	96	85.9	99	CALLA+ Ia+	47,XY,+1, t(9;11)	BM	6.1	1.8
9	3/M	Tetralogy of Fallot hemato-logical : np							thymus	ND	1.0
10	65/F	malignant lymphoma (st.1V)	17.8	14	22.3	51	Ia+ B4+	*c*)	PB	−[e]	1134 cpm[g]
11	11/F	null ALL	33.4	79	60.2	88	Ia+ CALLA−	*d*)	BM	0.5	1721

a) Blast cells were positive for OKT10, Ia, MCS2, and My7. The differentiation stage was suspected to be near that of non-T, non-B cells.
b) Blast cells were peroxidase-negative.
c) 50,X,−X,+3,+19p+,1p−,6q+,11p−,17p+,+3mar.
d) 46,XX,del(4q−),−5,+der(5)t(5;?),+der(12)t(12;?).
e) Less than 0.01.
f) AU: An arbitrary unit of optical density of the bands on an autoradiogram per 4 μg polyA+RNA. The OD of 2.1 kb mRNA of thymus (case 9) was defined as 1 AU.
g) Radioactivity on the nitrocellulose membrane was measured by the use of a liquid scintillation counter.
ND: not determined. PB: peripheral blood. BM: bone marrow.

normal thymus. Partial clinical data, diagnosis, surface markers, chromosome analysis and TdT mRNA expression level are listed in Table I. TdT activity in neoplastic cells was measured in 9 of 10 cases; it varied from 0.5 to 23.1 units/10^8 cells. In experiment 1 (cases 1–9 of Table I), all the cases exhibited single 2.1 kb TdT mRNA, except case 8, in which 3.3 kb mRNA was also detected (Fig. 1A). Because of an unfortunate background around the 3.3 kb area of the autoradiogram, it was not clear whether large TdT mRNA was expressed in cases 1 and 4. No TdT mRNA signals were detected in one case of B-ALL (case 2). TdT assay was not available for this case, but most B-ALL is reportedly TdT-negative.[18] In experiment 2 (cases 10 and 11 of Table I), null-ALL showed a major 2.1 kb and a minor 3.3 kb TdT mRNA (case 2 in Fig. 1B). One malignant lymphoma showed a single and strong TdT mRNA signal of 2.1 kb, although no TdT enzyme activity was measured in the cells. Extensive chromosomal abnormality was observed in this case (Table I). Single 2.1 kb TdT mRNA was detected in normal human thymus, in accordance with the previous study of calf thymus.[14]

DISCUSSION

We previously reported that calf thymus experssed a single 2.1 kb TdT mRNA, and Molt 3 and Molt 4 predominantly expressed 2.1 kb mRNA but also 3.3 kb

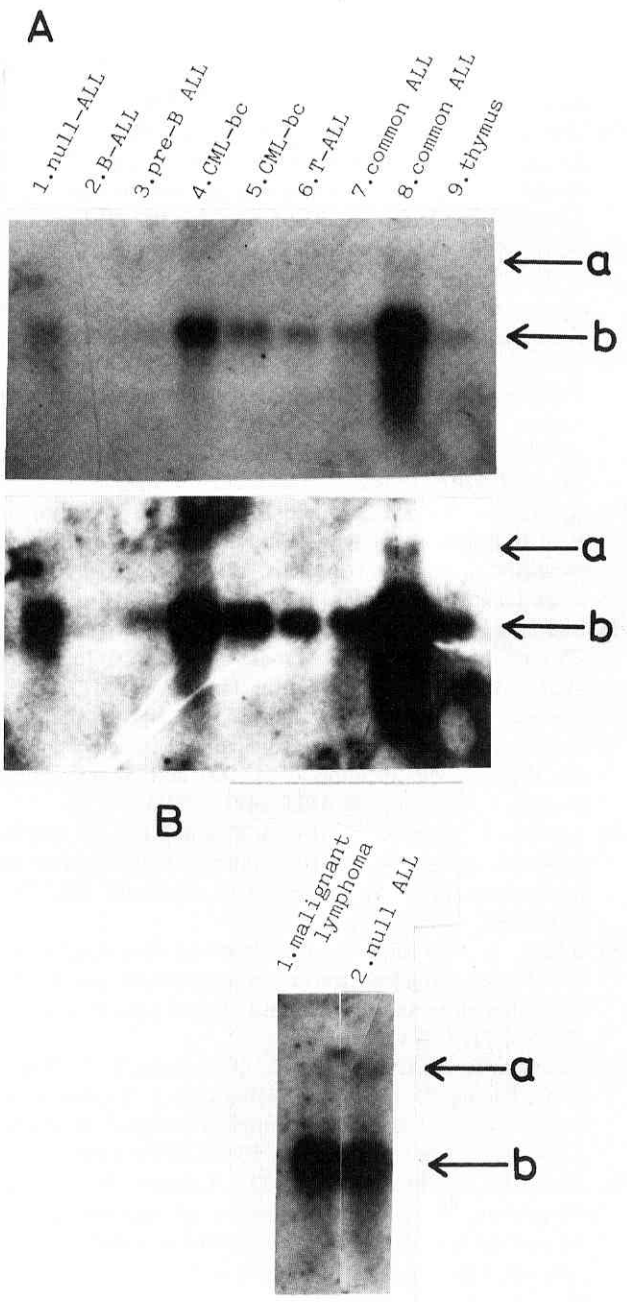

Fig. 1. Expression of TdT mRNA in fresh leukemic cells. Case numbers of lanes 1 to 9 in (A) are in the same order as in Table I and those of lanes 1 and 2 in (B) correspond to cases 10 and 11, respectively. Arrows, a and b, indicate the positions of 3.3 kb and 2.1 kb TdT mRNA, respectively. The upper part of (A) is an autoradiogram of short exposure time (overnight) and the lower part is one of long exposure time. The amounts of poly A+ RNA used in 1% agarose gel-running are 4 μg for lanes 1 to 6 and 9, and 10 μg for lanes 7, 8 and for experiment (B).

mRNA at low levels.[14] In the present study, we measured the expression and sizes of TdT mRNA in fresh leukemic cells by northern blotting. In 8 cases (2 cases of null-ALL, 2 of common ALL, 1 of pre-B ALL, 1 of T-ALL and 2 of CML-bc) with different levels of TdT activity, TdT mRNA was detected in all. Thus, TdT and its mRNA were the same in leukemic cells whose differentiation stages ranged from null cell through immature B or T cell. The major TdT mRNA were the same 2.1 kb size in all cases, in agreement with our previous study and others.[19, 20] In one case of common ALL (case 8 in Fig. 1A) and one case of null-ALL (case 2 in Fig. 1B), a larger TdT mRNA with 3.3 kb was also found. The relative amount of 3.3 kb to 2.1 kb mRNA is smaller than that previously found in Molt 3 and Molt 4. However, the large TdT mRNA was confirmed not to be an artifact in the cultured cell lines. Recently we also encountered one case of TdT activity-positive AML which showed 3.3 kb mRNA alone (data not shown). This larger mRNA might be a transient expression form during normal lymphocyte or hematopoietic cell differentiation. As no large TdT mRNA signal was detected in normal human or normal calf thymus,[14] we conclude that 2.1 kb mRNA is thymus-specific. Nevertheless, the possibility that the larger TdT mRNA is a different gene transcript from TdT's with a high level of homology can not be excluded. Since all TdT activity-positive samples exhibited TdT mRNA, TdT gene may be mainly regulated at the transcription level. This conclusion is supported by our recent demonstration of a positive linear correlation using several TdT-positive human leukemic cell lines (Oiwa *et al.*, unpublished result). One malignant lymphoma unexpectedly exhibited an elevated level of TdT mRNA of the thymus type, even though no TdT activity was detected. We tried to measure its TdT by the ELISA method, but found no immunoreactive TdT. This implies that the possible abnormality found in this case might be associated with the translational anomaly of TdT.

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