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SERUM DEOXYTHYMIDINE KINASE AS A PROGRESSIVE MARKER OF HEMATOLOGICAL MALIGNANCY

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ABSTRACT

The levels of serum deoxythymidine kinase (s-dTK) were investigated for 127 patients suffering hematological malignancy by a sensitive method using [^{125}I]-iododeoxy-uridine as substrate. It was found that the deoxythymidine kinase activity in the sera of these patients was higher than the normal levels in adults. It was also found that the levels of s-dTK in the progressive stage were much higher than those in the indolent stage. These results suggest that high levels of s-dTK in hematological malignancy may reflect the extent of disease and that this activity may be derived from proliferating leukemic cells. The deoxythymidine kinase activity in the extracts derived from cultured cell lines and clinical specimens indicated that leukemic cells contained much higher activity than normal cells.

Key Words: Serum deoxythymidine kinase, Hematological malignancy

INTRODUCTION

Different clinical staging systems of hematological malignancies help in evaluating prognosis for groups of patients, but are of less value when considering the individual case. Therefore, there is a great need for individual prognostic variables for routine clinical work. A useful approach to the search for tumor markers is the exploitation of alterations in cellular biochemical properties, such as isoenzyme type expression, with special reference to the transition of cells from the dormant to the dividing phase.[1] Deoxythymidine kinase (dTK), ATP: thymidine 5′ phosphotransferase (EC2.7.1.21), which catalyses the phosphorylation of deoxythymidine to deoxythymidine monophosphate, provides a "salvage" pathway for the synthesis of DNA. Three different dTK isoenzymes are known in human.[2] One of these, the cytosolar isoenzyme dTK-1, occurs in high amounts only in proliferating cells (G1 to S), and is more or less absent in resting cells.[3] The ability to measure elevated dTK levels in serum has earlier been demonstrated.[4,5] The development of a dTK assay optimized for dTK-1, utilizing [^{125}I]-iododeoxyuridine (IUdR) as substrate, facilitated the detection of normal serum dTK (s-dTK) levels. Using this assay, elevated s-dTK levels were found in patients with malignant disease.[6-13] Transiently enhanced s-dTK levels have also been found in acute stages of measles, rubella and different herpes virus infections.[14,15] In this paper, we describe an evaluation of s-dTK levels in hematological neoplasms as an aid in objectively estimating a tumor mass.

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MATERIALS AND METHODS

1. Deoxythymidine kinase assay

The levels of s-dTK were measured with Prolifigen® TK-REA Technique (AB Sangtec Medical, Bromma, Sweden) in our study. The outline of this assay system is shown in Fig. 1. This assay utilizes ^{125}I-IUdR (final concentration 10^{-7} M, 130–160 Ci/m mole), as substrate, and has been previously described in detail.[15] In order to minimize fluctuations in the assay, a biological control for the isotope was utilized, and all values were recalculated to units. Under the conditions used, 1 unit of enzyme corresponds to an activity of 1.2×10^{-18} katal, and gives approximately 1000 cpm per hour, with the amount of isotope used. In the preliminary examination, the optimal temperature and time for incubation were determined. At first, a count of control samples was made using incubation conditions at different temperatures for 4 hr. At 37 °C, the positive control sample (40 U/L) had the maximum count (Fig. 2). Next, different conditions of incubation time were analyzed at 37 °C. A linear turnover of substrate was given for 4 hr (Fig. 3). In all experiments, the control sample was simultaneously measured in addition to two control sera in order to estimate fluctuations in the assay, which was purified from herpes simplex virus (Type-1)-infected cell line BHK 21/13S.[15]

2. Serum sampling

Serum samples were collected from 57 normal subjects and 127 patients with hematological neoplasms. The patients with high s-dTK activity sera were categorized according to diagnosis of the disease and referred to the Branch Hospital, Nagoya University Medical School, between January 1985 and December 1988. Patients were categorized into seven types of disease as follows: 62 patients with acute myelogenous leukemia (AML), 6 patients with acute lymphocytic leukemia (ALL), 18 patients with chronic myelogenous leukemia (CML), 3 patients with chronic lymphocytic leukemia (CLL), 15 patients with malignant lymphoma (ML), 17 patients with myelodysplastic syndrome (MDS) and 9 patients with other diseases (i.e., aplastic anemia, idiopathic thrombocytopenic purpura and autoimmune hemolytic anemia). Moreover, all of these diseases were further divided into two groups, i.e., those in indolent stage and those in progressive stage. The indolent stage corresponds to remission in acute leukemia and lymphoma and to the non-transformation phase in CML, CLL and MDS; whereas, the progressive stage corresponds to relapse and transformation phase in these diseases.

3. Statistical methods

The resultant data were statistically analyzed by Willcoxon and Mann-Whitney's test with respect to s-dTK level between each type of hematological neoplasm and normal.

4. Preparation of the extracts from cultured cell lines and frozen blood cells

In order to check the intracellular dTK activity in various types of blood cells, the extracts were prepared by hypoosmolar shock method.

Briefly, 5×10^6 cells (cultured cell lines[16]) or 1×10^7 cells (frozen blood cells of patients) were harvested and washed two times with phosphate buffer serine (PBS). The resultant pellets were incubated with 1 ml distilled water for 1 min, after which, 2×PBS was added. After being centrifuged at 1000 xg for 10 min, the extracts were harvested for checking.

RESULTS

All samples derived from the 57 normal subjects had s-dTK levels below 10 U/L except one (Fig. 4, mean 4.75 U/L, S.D. 1.60). As shown in Fig. 5, there existed a statistically significant difference in s-dTK levels between four types of hematological malignancies and normal (all types, $p<0.001$). The s-dTK activities in acute leukemia were very high (mean 259.5, S.D. 282), although the level of s-dTK in all sera of the patients in remission were less than 20 U/L (mean 6.1, S.D. 7.0). The patients with chronic leukemia had a high activity (mean 271.8, S.D. 133), and the level of s-dTK in remission was relatively low (mean 56.4, S.D. 57.0). The s-dTK activity in the 12 patients in advanced stage of malignant lymphoma was also elevated (mean 307, S.D. 1022), whereas 3 patients in remission had low activity (mean 2.6, S.D. 0.38). In MDS, 9 patients in the progressive stage had very high activity (mean 173.6, S.D. 129), although 8 patients in the indolent stage had almost the same activity as that in normal (mean 8.0, S.D. 4.23).

In all cases, the mean s-dTK activity in the progressive stage was significantly higher than that in normal ($p<0.001$), although the activity in the indolent stage was almost the same as that in normal ($p>0.05$).

As shown in Table 1, the activity of dTK in the extracts derived from cultured cell lines was extremely high (mean 1290, range 233-6110), except for MOLT-4 and HSB-2 (both, T lymphoid). And all samples of frozen blood cells of patients, except 2 samples (CLL and AML), had significantly high activity (range 21.4-228).

DISCUSSION

The clinical course and the response to treatment of patients with hematological malignancy are highly variable. For these reasons, new markers giving both prognostic information for the individual patient and reflecting the effect of therapy are needed.

In the current study, we have analyzed dTK levels in serum with a recently developed method, utilizing [^{125}I]-iododeoxyuridine (IUdR) as substrate, which is very simple and sensitive enough to detect normal s-dTK levels. Our study in this paper clarified that s-dTK level in the progressive stage of hematological malignancy is extraordinarily high in contrast with that in normal, while that in the indolent stage is not significantly higher than that in normal.

The origin of the elevated s-dTK activity is not yet known. We investigated the s-dTK activities in the extracts derived from cultured and frozen cells. The dTK of supernatant derived from both cultured and frozen leukemic cells was significantly high. This result suggests that the release of the enzyme from proliferating tumor cells into serum may be responsible for the high activity of s-dTK in patients with progressive malignancy. These findings indicate that the s-dTK level in hematological neoplasms may be an excellent tool to estimate tumor mass and tumor cell turnover. And, because of the simplicity of this enzyme assay, it can easily be applied in routine clinical work.

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Table 1. The dTK activity in cell extracts

The extracts of cultured cell lines[16] (derived from 5×10^6 cells) and frozen blood cells of patients (derived from 1×10^7 cells) were prepared as described in Materials and Methods.

A. Cultured cell lines[16]

	Name of Cell Line	(U/L)
T lymphoid	CEM	1010
	MOLT-4	22.4
	HSB-2	1.58
B lymphoid	RAJI	1170
	SIMPSON	1200
nonT, nonB lymphoid	NALM-6	233
myeloma	ARA-10	1720
	SKO-007	540
myeloid	HL-60	1340
	KG-1	682
	NKM-1	262
monocyte	U937	1050
erythroid	K562	6110

B. Frozen blood cells

		Name of Patient	(U/L)
ALL		R.T. (9173*)	105
		I.H. (9247*)	81.4
		C.H. (B591*)	128
AML	M1	H.H. (9146*)	71.5
		Y.I. (B520*)	91.8
		E.K. (C1*)	84.1
	M4	M.Y. (7980*)	35.0
		T.Y. (C183*)	68.6
		K.U. (C593*)	126
	M5	Y.A. (9636*)	189
		K.M. (C568*)	4.26
CML (blast crisis)		R.T. (7140*)	150
		T.K. (9495*)	228
MDS (progressive condition)		K.M. (9759*)	21.4
CLL		I.S. (7233*)	0.26

* No. of frozen tube

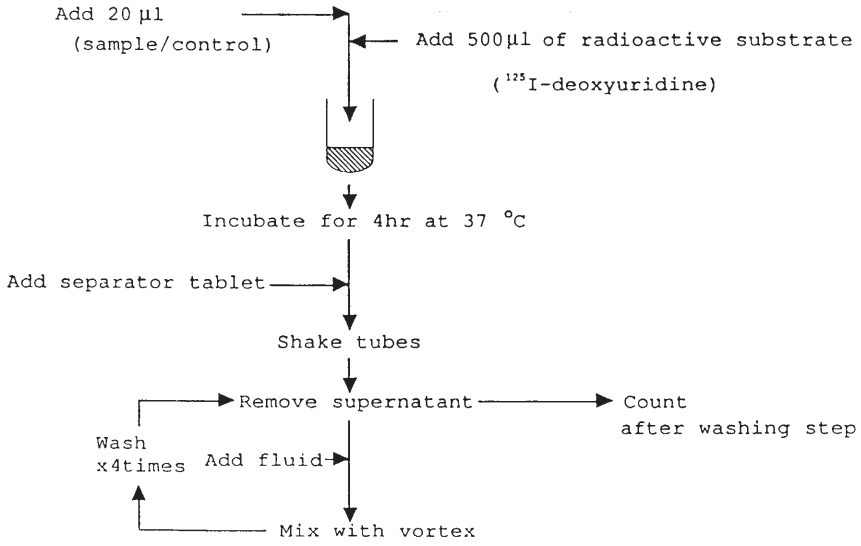

Fig. 1. Procedure for the measurement of s-dTK activity using the TK-REA technique

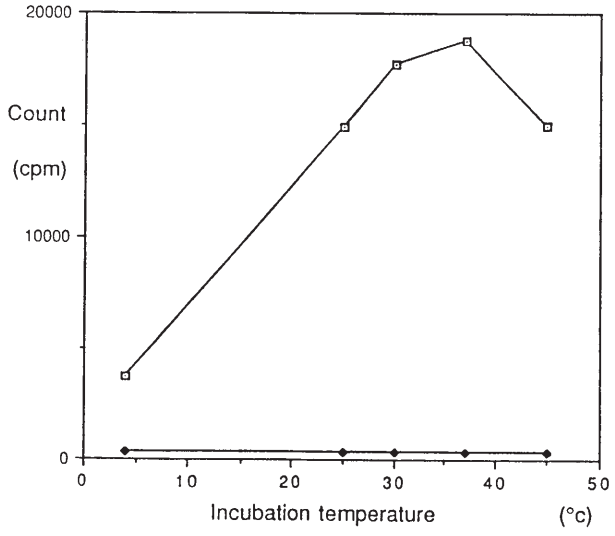

Fig. 2. The effect of incubation temperature in the measurement of s-dTK activity
The count of control samples was conducted using incubation at five temperatures (4 °C, 25 °C, 30 °C, 37 °C, and 45 °C) for four hours. At 37 °C, the positive control sample (40 U/L) had the maximum count, although the count of the negative control sample (0 U/L) was bit affected by temperature.
Symbol: □, positive; ■, negative.

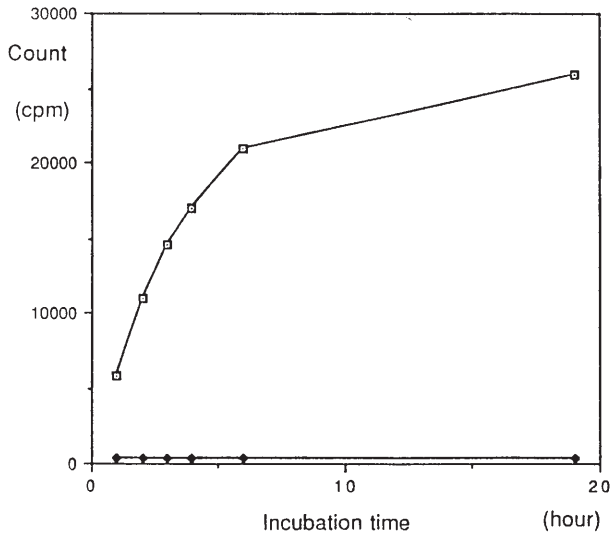

Fig. 3. The effect of incubation time on the measurement of s-dTK activity
Six different conditions of incubation time (1, 2, 3, 4, 6, and 19 hr) were analyzed at 37 °C. As the incubation time was prolonged, the count of the positive control sample (40 U/L) increased gradually. However, the rate of increase decreased after four fours.
Symbol: □, positive; ■, negative.

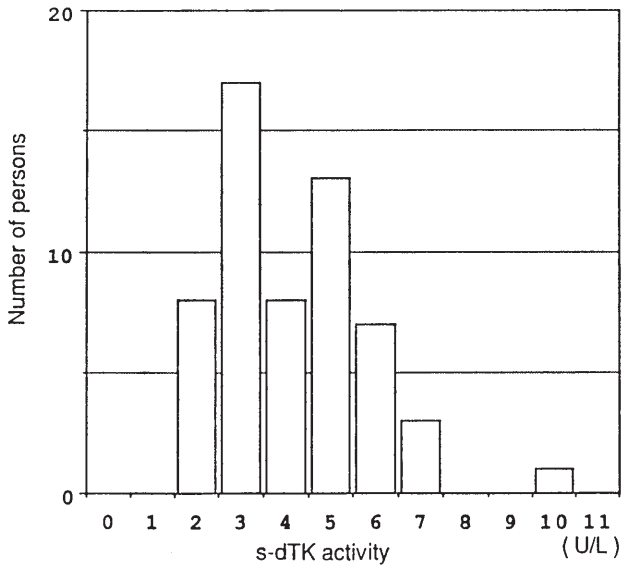

Fig. 4. Distribution of s-dTK values in normal sera
Serum samples were collected from 57 normal subjects, and the s-dTK activity was measured by the method as described.

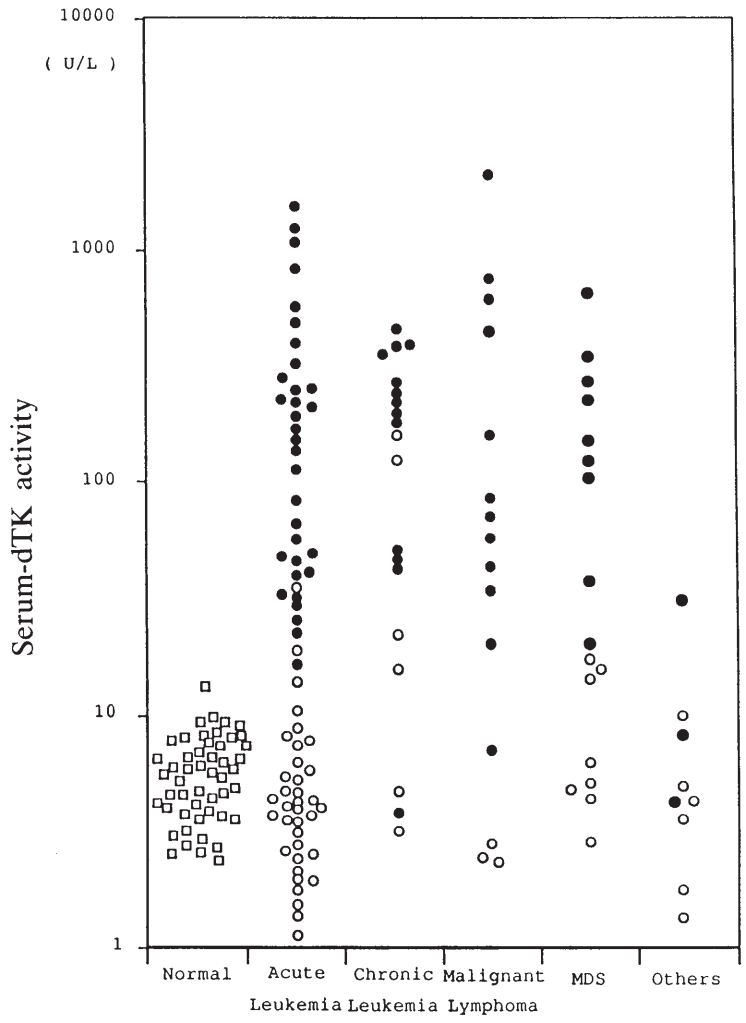

Fig. 5. Serum-dTK level in normal and hematological malignancy
Patients were categorized into five types of disease: 68 patients with acute leukemia, 21 patients with chronic leukemia, 15 patients with malignant lymphoma, 17 patients with myelodysplastic syndrome (MDS) and 9 patients with the other diseases (aplastic anemia, idiopathic thrombocytopenic purpura and autoimmune hemolytic anemia). The s-dTK activity was measured in sera of patients as described in Materials and Methods.
Symbols: □, normal; ●, progressive; ○, indolent.

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