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Lactate dehydrogenase isozymes in teratoma 402AX and other tumor cell lines

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Summary

Lactate dehydrogenase (LDH) isozymes of mouse teratoma 402AX, teratocarcinoma F9, and human epidermoid carcinoma A431 were studied. The isozyme patterns were analyzed by activity staining of the isozymes on the polyacrylamide gel after electrophoresis of the crude extract of each cell type and by densitometric tracing of the activity bands. The LDH isozyme patterns of 402AX and F9 cells thus obtained were almost the same as those obtained by DEAE-Sephadex A-50 column chromatography of the extracts. In 402AX cells, LDH-5 and LDH-4 represented about 87 % and 13 %, respectively, of the total lactate dehydrogenase activity. Only trace activity was detected for LDH-3, LDH-2, and LDH-1. The isozyme pattern of 402AX cells was similar to that of skeletal muscle or spleen rather than that of cartilage. Although the treatment of 402AX cells with both retinoic acid and dibutyryl cyclic AMP, which are known to induce cell differentiation of various teratocarcinoma cell lines, affected the total lactate dehydrogenase activity, the isozyme pattern was not changed.

Introduction

Teratocarcinoma stem cells are malignant cells which are similar in many respects to

the inner cell mass cells of the early embryo. Teratocarcinoma cells injected subcutaneously into host animals can differentiate into multiple types of cells¹⁾. And when injected into a blastocyst, they can even participate in normal development²⁻⁴⁾. Differentiation of teratocarcinoma cells can also be induced in culture by the addition of various factors^{5,6)} and compounds that inhibit DNA synthesis and cell proliferation⁷⁾. Teratoma 402AX was originally prepared by Stevens and Jackson⁸⁾ from testicular teratoma of strain 129 mouse, and was later adapted to culture⁹⁾. Since 402AX cells injected into host 129 mice differentiated more readily into cartilage than into other tissue types, it was thought to have cartilaginous nature⁸⁾. Therefore, 402AX cells in culture could be expected to be an useful model for the studies on the factors that affect the differentiation and development of cartilage cells, and for the studies on the action mechanisms of such factors.

Lactate dehydrogenase (LDH) is known to have a tetrameric structure with two types of subunits, H and M. According to the possible combinations of these two subunits there are five isozymes: LDH-1 [H_4], LDH-2 [H_3M_1], LDH-3 [H_2M_2], LDH-4 [H_1M_3], and LDH-5 [M_4]^{10,11)}. The facts that tissues of adult mammals have their own specific LDH isozyme patterns, and that the pattern

in each tissue changes during the development of the animal¹²⁾, suggest the possibility that the LDH isozyme pattern could be used as a marker of cell differentiation induced under culture conditions.

In the present paper, we examine the LDH isozyme pattern in teratoma 402AX cells in culture, and the pattern is compared with those of various mouse tissues and of other tumor cell lines. The effect of treatment of 402AX cells with retinoic acid (RA) and dibutyryl cyclic AMP (dBcAMP) on LDH isozymes in the cells is also described.

Experimental Procedures

Culture conditions—Mouse teratoma 402AX cells obtained from Dr. K. Hosoi, Department of Physiology, Josai Dental Univer-

sity, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 3 % fetal bovine serum (FBS). They were kept at 37 °C in an atmosphere containing 5 % CO₂. The cells were split once a week in a 1 : 20 or 1 : 40, and the medium was changed once a week. Mouse teratocarcinoma F9 cells were kindly given by Dr. Y. Nishimune, Research Institute of Microbial Diseases, Osaka University. The cells were cultured in minimal essential medium of Eagle (MEM) supplemented with 3 % FBS under the same conditions as for the 402AX cells. Human epidermoid carcinoma A431 cells were cultured in DMEM supplemented with 7 % FBS and 7 % bovine serum (BS), 100 units/ml of penicillin and 100 µg/ml of streptomycin.

Fig. 1 Analysis of lactate dehydrogenase isozymes of various cell lines. Crude extracts from mouse teratocarcinoma F9 and 402AX cells and from human epidermoid carcinoma A431 cells were subjected to polyacrylamide gel electrophoresis, and the lactate dehydrogenase isozymes on the gel were localized by staining for their activity according to Dietz and Lubran¹⁴⁾. Lactate dehydrogenase of mouse kidney was also examined and shown for identification of the isozymes.

Table 1 Isozyme patterns of lactate dehydrogenase in cultured cells and various mouse tissues

Source	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
402AX	ND	ND	ND	13	87
402AX*	ND	ND	1	10	89
F9	2	10	26	32	30
F9*	8	14	26	26	26
A431	ND	ND	6	34	60
Brain	43	12	15	20	9
Heart	32	21	20	12	15
Kidney	21	16	21	21	21
Adrenal	3	4	18	35	40
Costal cartilage	4	7	13	22	53
Submandibular gland	1	<1	5	34	59
Lung	2	2	3	23	70
Intestine	1	1	4	7	87
Muscle	<1	<1	2	8	88
Spleen	ND	ND	ND	9	91
Liver	ND	ND	ND	<1	99

All values were calculated from densitometric tracings of activity bands obtained by polyacrylamide gel electrophoresis except in the case of the cells indicated by asterisks, where the values were based on the elution profile obtained by DEAE-Sephadex A-50 column chromatography. Numbers refer to the percentages of the total LDH activity.

Extraction and analysis of the Enzyme

—Crude extracts from 402AX and F9 cells were prepared as follows: cells on the bottom of a culture dish (35-mm diameter) were washed three times with 0.5 ml of phosphate-buffered saline (PBS (—)) and then collected with rubber policeman in 0.5 ml of 50 mM Tris-HCl, pH 7.4, and put into a small tube. The cells were sonicated at 0°C for two 10-sec intervals with a 5-sec pause in between. The sonicate was centrifuged at 105,000×g for 60 min and the crude extract was obtained as the supernatant. The enzyme from various murine tissues was extracted by homogenizing the materials with 3 volumes (V/W) of 50 mM Tris-HCl, pH 7.4, in a glass homogenizer with a Teflon pestle. Polyacrylamide gel electrophoresis of the crude extracts was carried out according to Davis¹³⁾, and the enzyme activity on the gel after electrophoresis was stained by the method of Dietz and Lubran¹⁴⁾. Lactate dehydrogenase isozymes of 402AX and F9 cells were also analyzed by DEAE-Sephadex A-50 column chromatography according to Nadal-Ginard

and Market¹⁵⁾. The activity of lactate dehydrogenase was assayed by a photometrical method according to Stolzenbach¹⁶⁾. Protein was determined by the method of Lowry *et al.*¹⁷⁾.

Materials—Various tissues were dissected from an IRC mouse (male) immediately after the animal had been killed, and washed three times with a large amount of PBS (—), and stored at –80°C until used. NAD⁺ and nitroblue-tetrazolium were obtained from the Oriental Yeast Co. and Dohjin Laboratories, respectively.

Results

Isozyme Patterns of Lactate Dehydrogenase in Various Cell Lines

Lactate dehydrogenase (LDH) isozymes in various cell lines were separated by polyacrylamide gel electrophoresis of the crude extracts from cells and visualized by staining of the enzyme activities on the gel (Fig. 1). The activity of each LDH isozyme was quantitatively analyzed by densitometric tracing of the activity bands on the gel (Fig. 2, Table

1.). LDH isozymes of 402AX and F 9 cells were also separated by DEAE-Sephadex A-50 column chromatography of the crude extracts from the cells (Fig. 2, Table 1). The ratio of each LDH isozyme activity to total enzyme activity obtained from the densitometric tracing of the activity bands on the gel was almost the same as that obtained from the elution profile from the DEAE-Sephadex column. The LDH isozyme patterns of various mouse tissues were examined in the same manner and are shown in Table 1 for comparison. 402AX cells were rich in the M-type subunit (M:L=30:1), thus having a high content of LDH-5 and LDH-4 isozymes. The isozyme pattern of 402AX cells was similar to that of skeletal muscle or spleen rather than that of costal cartilage. In the cartilage, LDH-5 was also predominant, but the ratio of M-to L-type subunits was about 3.7:1. The specific activity of LDH in the extract from the 402AX cells was also measured in terms of wet weight or g protein and compared with that in extracts from mouse tissues (Table 2).

Effect of Retinoic Acid and Dibutyryl Cyclic AMP on Lactate Dehydrogenase of

Teratoma 402AX Cells

Since retinoic acid (RA) and dibutyryl cyclic AMP (dBcAMP) are known to induce differentiation of teratocarcinoma F 9 cells^{5,6)}, the effect of these compounds on 402AX LDH was examined. By the 7 day treatment of the cells with these compounds, the amount of protein extracted from one dish was decreased to about 80 % of that of control (untreated) cultures. The LDH isozyme pattern analyzed by electrophoresis and activity staining of the enzyme was not significantly affected by these compounds. In the control cultures total LDH specific activity (per mg of protein) increased as the cells grew. Such an increase in the enzyme activity, however, was not observed in the treated cells: the specific activity in cells treated with RA and dBcAMP for 5 days or 7 days was 50 % or 20 %, respectively, of that of the cells in each control culture (Table 3), indicating that the treatment inhibited the increase in total LDH activity in the cells.

Discussion

LDH isozyme patterns of 402AX cells and

Fig. 2 Comparison of lactate dehydrogenase isozyme analysis by densitometric tracing and by DEAE-Sephadex column chromatography. A: Densitometric tracing of the activity bands of LDH isozymes on a polyacrylamide gel after electrophoresis of the crude extract from 402AX cells. The crude extract was prepared as described in the text, and 60 μ l of the extract was applied onto a column of polyacrylamide gel. B: Separation of LDH isozymes of 402AX cells by DEAE-Sephadex A-50 column chromatography. The cells from a 35-mm dish were sonicated in 0.5 ml of 50 mM Tris-HCl buffer, pH 7.4, for 20 sec. The sonicate was centrifuged for 60 min at 100,000 $\times$ g. A 6-ml aliquot of the supernatant was applied onto a DEAE-Sephadex A-50 column (1.0 $\times$ 90 cm) equilibrated with 33 mM phosphate buffer, pH 7.2, containing 1 mM 2-mercaptoethanol, and the isozymes were eluted with a linear gradient of NaCl from 0 M to 0.5 M in the buffer.

Table 2 Specific activities of lactate dehydrogenase in teratoma 402AX cells and in various mouse tissues

Source	Lactate dehydrogenase activity	
	Activity per g of wet tissue (U/g)	Activity per g of protein extracted (U/g)
402AX.	11.6	20.2
Brain	6.20	205
Heart	43.6	124
Kidney	32.5	359
Adrenal	39.8	60.8
Costal cartilage	0.10	1.30
Submandibular gland	46.5	91.0
Lung	23.2	137
Intestine	53.6	371
Muscle	126	759
Spleen	21.3	94.4
Liver	8.70	230

other tumor cell lines were examined by activity staining of the isozymes on the polyacrylamide gel after electrophoresis and by densitometric tracing of the activity bands. The isozymes were quantitatively analyzed by this method as well as chromatographic method on DEAE-Sphadex A-50 column (Fig. 1, Table 1). Although 402AX cells were thought to have cartilagenous nature⁸⁾, the LDH isozyme pattern of the cells was found to be similar to that of skeletal muscle or spleen rather than that of costal cartilage (Table 2). As Dawson *et al.*¹¹⁾ described that malignant tumor cells with high glycolytic activity were rich in the M-type subunit of LDH, LDH-5 was the predominant isozyme in both 402AX and F9 cell lines (Table 1), while the isozyme pattern of these cell lines were quite different from each other (Fig. 1). In human epidermoid carcinoma A431, the M-type subunit was also predominant over the H-type subunit (Fig. 1, Table 1).

On the other hand, Nadal-Ginard and Market¹⁵⁾ reported that the M-type was rather the predominant subunit of LDH in tissues which produce lactic acid by reduction of pyruvic acid, and that, *vice versa*, the H-type predominates in tissues in which oxidation

Table 3 Effect of treatment of 402AX cells with retinoic acid and dibutyryl cyclic AMP on lactate dehydrogenase activity

Duration of treatment (days)		Culture conditions	
		Control	RA + dBcAMP
3	LDH activity (units/mg protein)	30.4	31.9
	Relative activity (%)	100	105
5	LDH activity (units/mg protein)	79.4	35.2
	Relative activity (%)	100	44
7	LDH activity (units/mg protein)	202	38.9
	Relative activity (%)	100	19

of lactic acid to pyruvic acid predominates over reduction of pyruvic acid to lactic acid. Therefore, we examined the effect of the addition of 5 mM pyruvate or 5 mM lactate into the culture medium on the LDH isozyme pattern of 402AX cells. Neither of these compounds affected, either the total activity or the isozyme pattern of LDH (data not shown).

RA and dBcAMP have been widely used to induce cell differentiation of teratocarcinoma cells in culture^{5,6)}. Strickland *et al.*⁶⁾ examined the effect of these compounds on LDH of teratocarcinoma F9 cells, and demonstrated that the LDH activity in F9 cells treated with the compounds for 4 days decreased to about 10 % of the level in the control cells. Although the isozyme pattern was not examined, they discussed that the decreased level of LDH activity was thought to reflect cell differentiation toward that of parietal endodermal cells because rather low LDH activity is one of the characteristics of such endodermal cells⁶⁾.

In the case of 402AX, treatment of the cells with RA and dBcAMP inhibited the cell growth-correlated increase in LDH activity (total LDH activity per mg protein extracted): LDH activity in the cells treated

with these compounds for 7 days was approximately 20 % of that in the control 402AX cells (Table 3). Nevertheless, the LDH isozyme pattern itself was not altered by the treatment with these compounds. These results suggested: 1) that some properties of 402AX cells were altered by RA and dBcAMP; and 2) that these compounds, however, might not be sufficient to fully induce cell differentiation of 402AX in culture. Recently, Liesi *et al.*¹⁸⁾ reported that by the treatment of teratocarcinoma F9 cells with nerve growth factor together with RA and dBcAMP the cells exhibited immunoreactivity to tyrosine hydroxylase, a rate-limiting enzyme of

catecholamine synthesis specific for adrenergic neurons, and that the treatment with nerve growth factor alone, however, showed no effect. Their observations suggested that the combination of some hormones or growth factors with RA and dBcAMP could induce further cell differentiation of teratocarcinomas in culture.

It is under elucidation on such hormones and other factors which when used together with RA and dBcAMP induce further cell differentiation of 402AX. It will also be interesting to know whether the LDH isozyme pattern is altered when the cells are treated with such factors.

抄録: マウス・テラトカルシノーマ F9 および 402AX 細胞の乳酸脱水素酵素 (LDH) アイソザイムについて、ポリアクリルアミドゲル電気泳動および活性染色法を用い、成体マウス各組織のものと比較検討した。402AX 細胞では、LDH-5 と LDH-4 が総 LDH 活性の 87 % および 13 % を占めており、これ以外のアイソザイムはほとんど検出されなかった。402AX 細胞の LDH アイソザイムパターンはマウス骨格筋のパターンに類似していた。また電気泳動・活性染色法によって得られた 402AX 細胞あるいは F9 細胞のアイソザイムパターンは抽出液を DEAE-Sephadex A-50 カラムクロマトグラフィによって分析した結果とよく一致していた。402AX 細胞をレチノイン酸およびジブチルサイクリック AMP 存在下で 7 日間培養することにより、総 LDH 活性は対照の細胞に比べて約 20 % に減少した。しかし、LDH アイソザイムパターンには有意な変化は見られなかった。

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