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Comparative Study of Lactic Dehydrogenase Isozyme Patterns in Clonal Derivatives of Chinese and Armenian Hamsters

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Chinese hamster와 Armenian hamster에서 얻은 여러 細胞系에 대한 乳酸데히드로게나제 아이소자임 패턴에 관한 比較硏究

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摘 要

Chinese hamster, *Cricetulus griseus*와 Armenian hamster, *Cricetulus migratorius*의 正常細胞, 腫瘍瘤에서 由來된 細胞 및 바이러스로 再形質轉換된 細胞의 乳酸 데히드로게나제(LDH) 패턴을 電氣泳動法에 의해 測定하여 比較하였다. 이 두 종류의 電氣泳動移動度는 비슷했으며, 纖維芽細胞는 LDH-5만 나타내었고, adenovirus로 形質轉換된 上皮性인 細胞에서는 LDH-1-2-3-4-5, 2-3-4-5, 또는 3-4-5와 같은 패턴을 보여 주었다. 그러나 上皮性細胞가 自然的으로, 혹은 SV40 바이러스 處理로 因해서 纖維芽細胞로 변하였을 경우는 역시 LDH-5 패턴만 보여 주었다.

INTRODUCTION

The LDH (lactic dehydrogenase) isozyme is a phenotypic characteristic of somatic cells. This characteristic of cell gives the biologist another tool for studying changes in cellular metabolism. The relationships among closely related animals may be examined at various levels of organ, tissue, cellular and molecular organization. It has been generally accepted that tissue-specific patterns of LDH isozymes arise from differential activation of the genes in autosomes which control formation of the A and B subunits (Market and Ursprung, 1962; Dawson *et al.*, 1964). The early embryonic tissues of mammals contain LDH molecules with

predominantly, or solely, A subunits (LDH-5 and 4). During development the LDH isozyme patterns of most mammalian tissue change towards a pattern containing more B subunits (Engel, 1973).

Quantitative and qualitative variations in the distribution of isozymes have been reported during embryonic development (Philip and Vesell, 1962; Cahn, 1964; Engel, 1973), malignant tissue (Kadlecova et al., 1967) and among the various tissues of a given individual (Emery, 1967). The general tendency for embryonal fibroblast-like derivatives to exhibit only the LDH-5 isozyme pattern may serve to illustrate the need for extensive cloning and isolation of euploid cells with differing phenotypes. This must be done in order to provide greater depth to the approaches outlined in regarding somatic cell genetics. It is at this level of application that isolating and characterizing euploid cell types from inbred Chinese and Armenian hamsters will serve effectively in correlating chromosomal features to change in LDH isozymes.

MATERIALS AND METHODS

In this study, the following tissues were used as tissue extracts: diaphragm of both Chinese and Armenian hamsters; heart, kidney and skeletal muscle of Chinese hamster; adenovirus type 12-induced tumors in Chinese and Armenian hamsters; and parainfluenza type-3 (an RNA, non-oncogenic virus) transformed cheek pouch tumors of Chinese hamster. The cheek pouch tumors were obtained by implantation of viral transformed cells into cheek pouch of Chinese hamster. The materials for cell extracts were obtained from polyoma virus transformed cells of Chinese hamster, spontaneous and SV40 treated fibroblastic transformed cells from euploid derivatives of adenovirus type 12-induced tumors in both Chinese and Armenian hamsters, and also the derivatives of Chinese hamster cell lines transformed by parainfluenza type-3 virus, which were later treated by SV40 viruses.

Clonal isolation of sublines was made by seeding diluted cell suspensions in Petri dishes (500 to 1,000 cells in each Petri dish). One week after cell seeding, the clones were isolated using pasteur pipette and grown in separate culture vessels. Chromosomes were analyzed from each clones. Procedures for culturing cells and cytological preparations were described elsewhere (Yerganian and Lavappa, 1970).

For the final tissue extracts the organ and tumors were homogenated with 0.1 M tris-HCl pH 7.4 buffer (10 ml buffer solution to 1 ml of tissue) and centrifuged at 0°C and 17,000 rpm for 10 minutes. The final cell extracts were obtained by combining many farm bottles of monolayerly grown cells by trypsinization. Then the cells were washed twice by resuspensioning in 0.1M tris-HCl buffer and repelleting by centrifugation in the clinical centrifuge. Following these washing, the

cells were homogenized with tris-HCl buffer. From 12 to 20 farm bottles, the total volume of loosely packed cells were about 0.5 ml. Therefore, the volume of 0.1M tris-HCl buffer added to the homogenate for transfer was about 0.05 ml. The supernatants from homogenates were assayed for lactic dehydrogenase patterns by electrophoresis on cellulose acetate.

Cellulose acetate electrophoresis procedure was as follows; borate buffer pH 8.1 (0.3M boric acid, 0.06M NaOH) was used for soaking the cellulose acetate strips prior to the sample application and for filling the electrophoretic chamber. Between 0.005 and 0.01ml of tissue extracts and cell extracts were applied with a Beckman paper electrophoresis sample applicator. Electrophoresis was carried out at a constant voltage of 200V and the amperage allowed to vary between 6 and 9 ma. Following electrophoresis the strips were removed from the chamber and placed upside down in a shallow glass Pyrex dish containing the staining mixture for lactate dehydrogenase (200 ml of 1M lactic acid, 600 ml of nitro blue tetrazolium [1mg/ml, 60ml] of phenazine methosulfate [1 mg/ml,] 200 ml of phosphate buffer 0.1M pH 7.4, 2 grams of DPN, and 8 grams of NaOH). 50 ml aliquots of mixture was sufficient to stain 5 strips. The staining reaction was carried out in the dark at 25°C for 30 minutes and terminated by immersing the strips in distilled water for 1 to 2 minutes before blotting them dry. When completely dry, they were cleared by immersing them in mineral oil and then mounted in cellophane envelopes (Preston et al., 1965).

RESULTS AND DISCUSSION

The relative migration from the origin toward the anode of the different LDH bands was essentially the same for the two species. That is, the position of LDH-5 (AAAA) in the Chinese hamster was not different from that of LDH-5 in the Armenian hamster. This was also true for LDH-1, -2, -3, and -4 (BBBB-ABBB-AABB-AAAB). Hence, it appears that the overall change on the LDH molecules of the two species is very similar and therefore, possibly the amino acid composition is also very similar.

The great majority, if not all, of fibroblast-like cell strains and cell lines tested for LDH isozyme patterns have only the LDH-5 band which characterizes embryonal derivatives. In LDH determination (Table 1) 9 out of 17 lines studied had LDH isozyme patterns other than the LDH-5 alone. Six were epithelial line forms (11, 12, 13 in Table 1) from adenovirus type 12-induced tumors, while three others (16, 17 and 18) stem from parainfluenza type-3 transformed epithelial cells. Only one of the six Chinese hamster fibroblast-like derivatives (polyoma transformed), the 6PV (line 10 in Table 1) had a differing isozyme pattern, i.e., LDH-4-5,

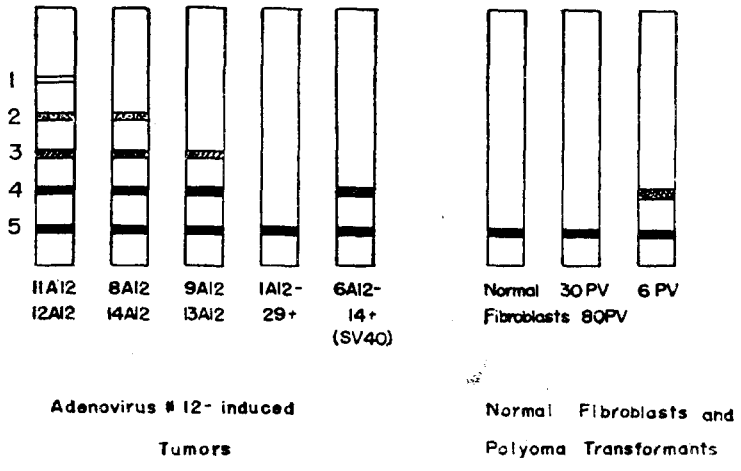

Fig. 1. Comparative LDH isozyme banding patterns.

while the remainders were LDH-5. Although fibroblastic in form, the 6PV cells had differing chromosome contraction ratios and telomeric associations. The cheek pouch tumors obtained by the implantation of tissue culture cells as well as viral transformed cells showed either 2-3-4-5 or all 5 bands which were different from the normal cheek pouch tissue. Analysis of epithelial-like adenovirus type 12-induced tumors showed either 3-4-5, 2-3-4-5, or 1-2-3-4-5 (BBBB-ABBB-AABB-AAAB-AAAA) (line 11, 12 and 13 in Table 1 and Fig. 1). Spontaneously transformed (epithelial-to-fibroblast) cell lines of adenovirus tumors, 1A12-29 and 6A12-14 (SV40), line listed in Table 1 (14 and 15) and Figure 1, had either an LDH-5 or an LDH-4-5 pattern; the latter having been exposed secondarily to SV40 virus, while still epithelial like, and transition to fibroblastic elements was virtually complete after two subcultivations (one week). The presence of the LDH-4 band in 6A12-14 (SV40) may be due to an incomplete dedifferentiation in response to the virus. These results are consistent with the idea that the viral genome influences cellular metabolism and morphology for in all cases cited, and transformation with a virus led to changes in isozyme pattern and morphology of the cells.

All male euploid fibroblast-like cell lines have exhibited the LDH-5 pattern (Fig. 1). Related euploid lines, 30PV and 84PV, were similar to mutant clones 80PV, 5PSP and 16PSP, which feature a medial deletion on the short arm of X_1, an inverted duplication of the long arm of the X_1, and deletion of the long arm of the X_1, respectively. Thus, evidence for the negative role of sex chromosomes in determining or influencing LDH isozyme patterns has been obtained, thereby confirming the autosomal pattern of inheritance first proposed by Market and Ursprung (1962).

Table 1. Lactic dehydrogenase isozyme patterns of Chinese and Armenian hamster cells (Gelman cellulose acetate strips).

	Isozyme Pattern***
A. Normal Adult Tissues	
*1. Diaphragm	1—2—3—4—5
**2. Heart	1—2—3—4—5
**3. Kidney	1—2
**4. Skeletal muscle	1—2—3—4—5
**B. Euploid Polyoma Transformed Fibroblast-like Cell Lines (male)	
5. Clone 30PV	5
6. Clone 84PV	5
**C. Pseudodiploid Fibroblast-like Cell Lines (male)	
7. Clone 80PV: Deletion in short arm of X_1	5
8. Clone 5PSP: Duplication in secondary constriction of long arm of X_1	5
9. Clone 16PSP: Deletion of 3/5ths of long arm of X_1	5
10. Clone 6PV: Medial deletion (partial monosomy) of short arm of chromosome #3	4—5
*D. Primary Euploid Adenovirus type 12 Tumors (both sexes) Epithelial-like Cell Lines	
11. Tumors 9A12, 13A12	3—4—5
12. Tumors 8A12, 14A12	2—3—4—5
13. Tumors 11A12, 12A12	1—2—3—4—5
E. "Spontaneous" Fibroblastic Transformation of Adenovirus type 12-induced Euploid Derivatives	
14. 1A12—29+	5
15. 6A12—14+ (SV40)	4—5
**F. Aneuploid Parainfluenza type 3-Transformed Epithelial-like Cell Lines (female)	
16. DP13 parent subline	1—2—3—4—5
17. Tumor of PD13 (SV40)	1—2—3—4—5
18. Tumor of clone 15DP13 (SV40): Derivative from earlier tumor	2—3—4—5
19. Cheek pouch tissue surrounding tumors (17 and 18)	4—5
20. Normal cheek pouch tissue mince	4—5

* Both species
** Chinese hamster only; others Armenian hamster
*** Uuderlined are the more prominent bands

Very little is yet known about the role of individual chromosome type and determining level or patterns of particular isozyme systems.

SUMMARY

Comparative LDH isozyme studies on normal, malignant and virus-transformed derivatives of Chinese and Armenian hamsters were illustrated.

With respect to LDH isozyme patterns, there were good indications that epithelial-like cells have varying levels of LDH-1-2-3 and -4, in addition to the "standard" LDH-5 band, which was exhibited by the majority of fibroblast-like cell lines.

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