Changes in lactic dehydrogenase isoenzyme pattern in muscle of tumor-bearing mice[1]

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Summary. The lactic dehydrogenase isoenzyme distribution shifts gradually toward the isoenzyme pattern of the tumor in mouse muscles located distally from a transplanted mammary carcinoma.

Shifts in the pattern of isoenzymes of lactic dehydrogenase, LD (EC 1.1.1.27), have been observed in cancers grown in laboratory animals and in cancers found in humans as compared to normal control tissues[2-5]. The shift is usually toward the M-type (muscle type) with an increase in the LD-4 and LD-5 isoenzymes, and frequently represents a net increase in the synthesis and total LD activity[6]. An increase in the total activity and a shift toward the M-type have been found, also, in the serum of the tumor-bearing host[7]. In the present study utilizing mice, we have observed that the LD isoenzyme pattern of a tumor-free muscle located distally from a growing transplanted mammary carcinoma shifted toward the pattern of the tumor. 2 weeks after resection of the tumor, the LD isoenzyme pattern partly reverted toward the pattern of the control muscle.

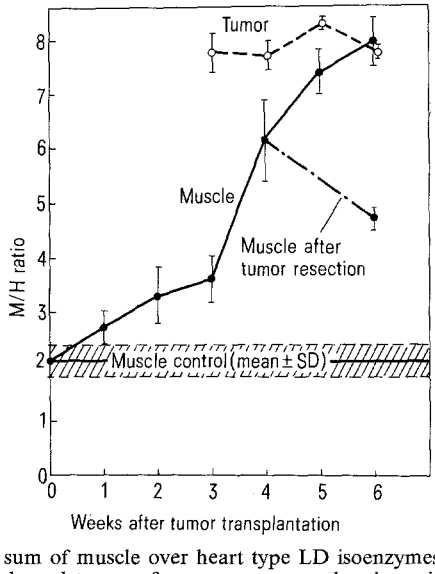
C3H/He female mice (Jackson Laboratories, Bar Harbor, Maine), 6–10 weeks old, were used. Within a given experiment, mice of a similar age were used. They were fed Purina laboratory chow and water ad libitum and were kept under standardized environmental conditions (lights on at 06.00 h, off at 18.00 h; room temperature of 25.5±0.5 °C. A spontaneous mammary carcinoma from a C3H/Bi mouse was used. This tumor grows locally and does not metastasize; it reaches a size of 2–2.5 cm in diameter by the 5th post-transplantation week and kills the animal by inducing cachexia. Most animals are dead by the 8th week.

The effects of tumor growth on the LD were evaluated at weekly intervals after tumor transplantation. For every study point a group of experimental mice received a piece of tumor approximately 2×2×2 mm s.c., and the control animals underwent a sham operation under ether anesthesia. The number of tumor-bearing mice varied from a maximum of 18 to a minimum of 9 at the 6 study points. At the time of the experiment the animals were killed by breaking their neck. Tumor from the tumor-bearing group and the anterior thigh muscles from both groups were removed and frozen in dry ice. Samples of muscle were homogenized in a Potter homogenizer in 200 volumes of ice-cold normal saline and centrifuged to remove cellular debris. All homogenates were analyzed within a few hours after preparation. Total LD activity was assayed using the method of Hanson and Freier and expressed as IU/g of tissue[8]. LD isoenzymes were separated on plates of agarose gel in barbital buffer, pH 8.6, in a Beckman Microzone electrophoretic cell[9]. The patterns were then stained, scanned, and the area under each isoenzyme peak estimated as a percentage of the total LD activity. The proportions of H (heart type) and M (muscle type) LD subunits were calculated from the percentage of the 5 isoenzymes, taking into account the subunit content of each isoenzyme, according to the following formula:

subunit, H = LD1 + 3/4 LD2 + 1/2 LD3 + 1/4 LD4

subunit, M = LD5 + 3/4 LD4 + 1/2 LD3 + 1/4 LD2

In a group of 8 animals, after 4 weeks of growth, the tumor was resected under ether anesthesia, and 2 weeks later the total LD activity and the isoenzyme pattern of the muscle were determined.



Ratio of sum of muscle over heart type LD isoenzymes in tumor-free muscle and tumor of mammary cancer-bearing mice. Control ratio represents the mean of the pooled values of controls from all 6 study points.

The mean LD activity in normal muscle was 433±47 IU/g. After tumor transplantation, the total muscle LD activity gradually increased to 558±48 IU/g at 3 weeks. The LD isoenzyme distribution in the muscle changed markedly. In normal muscle the LD-5 was 40±2.1% and increased gradually to 62±3.9% in tumor-bearing mice after 6 weeks (table 1). The most significant change, however, was in the M/H ratio which in the muscle of tumor-bearing mice kept increasing progressively with tumor growth, and after 6 weeks its value reached that of the tumor tissue (figure 1). It is interesting that 2 weeks after resection of a 4-week-old tumor, the M/H ratio declined and tended to return toward the control values (figure). The total LD activity, isoenzyme distribution and the M/H ratio in the tumor remained constant during the 6 weeks of growth (table 2 and figure).

Shifts in the LD isoenzyme distribution pattern toward that of malignant cells have been observed in preinvasive cancer and in normal tissues adjacent to a malignant growth[10-15]. Following infection of chicks and hens with Rous sarcoma virus, Parina et al. found that LD5 increased in the pectoral muscle during the tumor growth while LD1 and LD2 decreased[16]. Lundholm et al. studied total LD activity in muscle distally located from the growing tumor; they found an increased activity in mice with a methylcholanthrene-induced sarcoma but a decreased activity in patients with a variety of cancers[17]. The effect of isoenzyme distribution in distally located tissues of mammals bearing cancer has not been reported before.

It appears that these observed changes represent a humoral effect of cancer on the gene expression of normal cells. Such changes in tumor-free tissues of the host may explain the recent observations by Holroyde et al. that the lactate production increased in patients with colorectal cancer, although the magnitude of the increase correlates poorly with the size of the tumor[18].

Table 1. Lactic dehydrogenase activity and isoenzyme distribution in muscle of mice bearing a mammary carcinoma and of controls

Weeks after transplantation	Total LD (IU/g)	Isoenzyme (%) LD 1	LD 2	LD 3	LD 4	LD 5
0	443±47	11±1.2	12±1.6	15±1.8	22±2.1	40±2.1
1	440±30	6±1.6	12±1.6	14±0.7	19±1.9	40±2.8
2	459±28	5±0.75	9±1.04	14±0.5	19±1.1	53±2.4
3	588±48	5±0.55	8±0.85	11±0.9	20±1.2	56±2.7
4	441±42	0	5±0.5	6±0.7	29±0.9	60±1.5
5	402±43	0	6±0.2	8±0.2	26±1.2	60±2.3
6	340±49	0	0	5±0.5	33±0.67	62±3.9
2 weeks after resection of a 4-week tumor	394±44	1±0.6	6±0.7	10±1.5	28±1.9	55±2.5

Table 2. Lactic dehydrogenase activity and isoenzyme distribution in the mammary carcinoma

Weeks of growth of tumor	Total LD (IU/g)	Isoenzyme (%) LD 1	LD 2	LD 3	LD 4	LD 5
3	245±28.6	0	0	5±0.5	36±2.1	59±2.7
4	213±27.1	0	0	9±0.3	25±1.1	66±1.8
5	139±18	0	0	6±0.2	31±0.2	63±1.5
6	180±27.5	0	0	6±0.3	34±1.5	60±1.9

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Estimation of nonspecific lectin-mediated staining of glutaraldehyde-fixed cells

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Summary. Lectin-mediated stainings are widely used for the visualization of carbohydrate-carrying cellular components using the electron microscope. The use of glutarladehyde-fixed cells for these stainings introduces the possibility of low nonspecific lectin-trapping by the glutaraldehyde which coats the cells. This trapping was estimated by means of peroxidase-binding to human leukocytes, *Tetrahymena pyriformis* and *Escherichia coli* cells and was shown to be prevented by rinsing the glutaraldehyde-fixed cells in an amino acid solution before exposure to the lectin.

The use of concanavalin A-mediated peroxidase-binding to cells for ultrastructural cytochemistry was first described by Bernhard and Avrameas[1]. The method is based on the specificity of Con A for α-D-glucopyranosyl, α-D-mannopyranosyl or β-D-fructofuranosyl residues[1,2] and on its ability to react at one of its active sites with a cell-bound sugar and at the other active site with the sugar of horseradish peroxidase. The bound peroxidase is revealed by the diaminobenzidine (DAB) method of Graham and Karnovsky[3]. In the controls, 0.2 M α-methyl mannoside is added to the Con A and peroxidase solutions[1]. The sugar addition inhibits the specific binding of Con A to the cells and the peroxidase-binding to nonspecifically bound Con A. It does not prevent nonspecific Con A-binding to the cells. While nonspecific Con A trapping is negligible when untreated cells are used, glutaraldehydefixed cells[1,4,5] may trap some Con A due to remaining free aldehyde groups[6,7]. This trapped lectin will then be stained similarly to the specifically bound lectin and will not be detected in those controls which contain the free sugar in the peroxidase solution[1].