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ORIGINAL RESEARCH

Analysis of lactate dehydrogenase activities and isoenzyme patterns in colorectal cancer tissues

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Abstract

AIM: To investigate the relationship between lactate dehydrogenase (LDH) activity or LDH isoenzyme patterns and the pathogenesis of colorectal cancer.

METHODS: Activities of tissue LDH and LDH isoenzyme patterns in 16 patients with colorectal cancer were assayed using spectrophotometric procedures and agarose gel electrophoresis, respectively.

RESULTS: The total and specific activities of LDH were significantly higher in colorectal cancer tissues than those in adjacent noncancerous tissues ($P < 0.001$). The LDH isoenzyme pattern was also different from that in the control. The percentage of LDH_5 doubled and the ratio of $LDH_4 + LDH_5/LDH_1 + LDH_2$ was 3.6 ± 1.4 in cancer tissue, significantly greater than in the control.

CONCLUSIONS: The increased LDH activity in colorectal cancer tissues resulted mainly from the increased LDH_5, suggesting that the alteration of LDH activity and isoenzyme patterns were related to the pathogenesis of colorectal cancer.

Key words: Colonic neoplasms; Rectal neoplasms; Lactate dehydrogenase; Lactate dehydrogenase isoenzymes

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INTRODUCTION

Studies on lactate dehydrogenase (LDH) isoenzyme patterns in colorectal cancer tissues have rarely been reported although its total and specific activities have been measured by many authors[1,2]. To study the pathogenesis of colorectal cancer and provide a certain theoretical basis for diagnosis, in the present study we determined the total and specific activities and isoenzyme patterns of LDH in colorectal cancer tissues and in adjacent noncancerous tissues.

MATERIALS AND METHODS

Materials

All samples were obtained surgically and histological examinations were made routinely. The samples were washed with ice cold normal saline to remove contaminated blood and stored at 30 ℃.

In our experiment specimens were obtained from rectal cancer (13 cases), colonic cancer (3 cases) and noncancerous tissues taken at 5-8 cm proximal or distal to the edges of the tumor of the same patient. Nine men and seven women were included in the group. All reagents used were "Anala R" grade.

Methods

Preparation of tissue homogenate supernatants 0.3 g tissues were homogenised in 3 mL of 0.01 mol/L Tris HCl buffer (containing 0.001 mol/L DTT, 0.001 mol/L EDTA, pH7.5) and centrifuged at 20000 × *g* for 20 min at 4 ℃ (DUPONT RC5C). The supernatants were collected for assay.

Determination of LDH activity Enzymatic activities in tissue extracts were measured by spectrophometric procedures with 2,4-dinitrophenylhydrazine[3]. 1 μmol pyruvate produced at 37 ℃ for 15 min represented one unit.

Isoenzyme patterns Isoenzyme patterns were assayed by agarose electrophoresis modified according to Lou *et al*[4]. Gels were scanned at 500 nm using a Dual Wavelength Chromato Scanner (Shimadzu CS-930).

Determination of protein content Protein content was measured by the method of Bradford[5], with bovine serum albumin as standard.

Statistical analysis

All values were expressed as $\bar{x} \pm s$ and Student's *t* test was used

Table 1 Lactate dehydrogenase activities in colorectal cancer tissues and adjacent noncancerous tissues ($\bar{x} \pm s$)

Tissues	*n*	Total activities (u/g tissue)	Specific activities (u/mg protein)
Cancer tissue	16	62.70 ± 13.50	63.41 ± 12.41
Adjacent Control			
Proximal tissue	16	43.15 ± 22.95[d]	38.22 ± 19.77[b]
Distal tissue	16	44.81 ± 17.24[b]	39.92 ± 15.15[b]

[b]$P < 0.01$, [d]$P < 0.01$ *vs* cancer tissue

Table 2 Lactate dehydrogenase isoenzyme patterns in colorectal cancer tissues and adjacent noncancerous tissues ($\bar{x} \pm s$)

Tissues	*n*	LDH isoenzyme (%)					$LDH_4 + LDH_5/LDH_1 + LDH_2$
		1	2	3	4	5	
Cancer tissue	16	1.65 ± 1.42	15.54 ± 3.80	26.59 ± 6.25	36.63 ± 6.80	19.13 ± 8.05	3.6 ± 1.4
Adjacent control							
Proximal tissue	16	4.93 ± 6.19	19.18 ± 5.29[a]	30.05 ± 4.04	37.03 ± 8.24	8.76 ± 6.04[e]	2.3 ± 1.2[a]
Distal tissue	16	4.28 ± 2.55[b]	22.17 ± 4.57[b]	34.38 ± 5.75[e]	30.76 ± 5.83[a]	8.11 ± 6.32[e]	1.7 ± 0.9[e]

[a]$P < 0.05$, [b]$P < 0.01$, [e]$P < 0.001$ *vs* cancer tissue. LDH: lactate dehydrogenase.

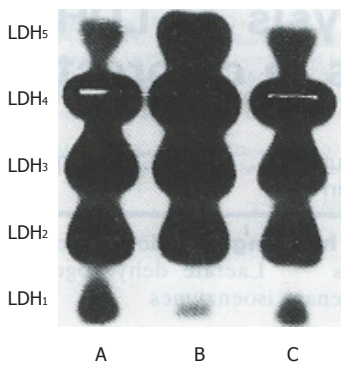

Figure 1 Lactate dehydrogenase isoenzyme patterns. A: proximal tissue; B: cancer tissue; C: distal tissue. LDH: lactate dehydrogenase.

for intergroup comparison.

RESULTS

LDH activities

Table 1 shows that both the total and specific LDH activities in tumors were significantly higher than those in the adjacent noncancerous tissues ($P < 0.001$).

LDH isoenzyme patterns

The electrophoretograms of LDH isoenzymes in the diseased foci showed a shift towards the M type (Figure 1). The percentages of LDH_1 and LDH_2 in tumors decreased significantly in comparison with proximal and distal noncancerous tissues; the percentage of LDH_3 decreased while that of LDH_4 increased in comparison with distal tissues; the percentage of LDH_5 was 2.2 and 2.4-fold higher than that in proximal and distal tissues, respectively. The ratio of $LDH_4 + LDH_5/LDH_1 + LDH_2$ was 3.6 ± 1.4, above the control (Table 2).

DISCUSSION

It is well known that glycolysis in cancer tissue increases significantly as a consequence of an important enzyme of the glycolytic pathway LDH that may manifest with a higher activity in a cancer patient's serum and tissues. Our data showed a significant increase of total and specific LDH activities in cancer tissues, about 140% of the control. These results were consistent with the reports by Carda-Abella *et al*[6] and Hong *et al*[7].

Because of the tissue distribution specificity, LDH isoenzymes may be expressed in different levels. It was necessary to assay LDH isoenzyme patterns while total and specific activities were determined. Our results indicated that the increased LDH_5 contributes to the increase of total LDH activity in tumors; the ratio of $LDH_4 + LDH_5/LDH_1 + LDH_2$ also increased greatly, *i.e.* 3.6 ± 1.4, suggesting that LDH isoenzyme pattern shifts towards the M type. It is the M type LDH that promotes the conversion of pyruvate to lactate, while the H type LDH mainly catabolizes the utilization of lactate. Therefore M type LDH can be found predominantly in colorectal cancer tissues in which anaerobic glycolysis is increased abnormally. Market *et al* thought that the patterns of isoenzymes were biochemical phenotypes of genes. H and M subunits were controlled by A and B genes, respectively. The findings that LDH isoenzyme patterns shift towards the M type may be related to its abnormal expression of genes, suggesting that studying the expression of LDH genes in colorectal tumors will help to elucidate its pathogenesis. In the comparison of malignant tissues with the control at the distance of 1, 2, 4, 6 and 8 cm from the edge of cancer, Onos[8] found that LDH activity in cancer tissues was very high and it gradually decreased in control tissues surrounding the tumor with a distance from cancer. By studying LDH isoenzyme patterns in precancerous polyps, Onos also found that it shifts towards the M type, indicating that the deviation of LDH isoenzyme patterns in normal tissue could be regarded as early signs of malignancy before the morphological changes.

Our results suggest that the alteration of LDH activity and its isoenzyme patterns are related to the pathogenesis of colorectal cancer and more details will be studied in our laboratory.

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