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Lactate Dehydrogenase in Human Renal Carcinoma Tissues

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Summary. Lactate dehydrogenase (LDH) activity and isoenzyme were determined in 27 human renal carcinoma (RC) tissues. Although LDH-1 and LDH-2 were predominant in the normal kidney, a completely inverted isoenzyme pattern was observed in 16 carcinomas. The others, however, showed either a normal or an incompletely inverted pattern. The LDH activity in the tumours with a completely inverted pattern was significantly higher than the activity of incompletely inverted or normal kidney pattern. It was also found that the isoenzyme pattern was more closely correlated with cell type than the grade of malignancy of the tumour, 14 out of the 16 cases of the completely inverted pattern bing of the clear cell type, and 9 out of the 11 cases of incompletely inverted or normal kidney pattern being of either granular cell or mixed cell type. These results seemed to well substantiate an opinion that in clear cells anaerobic glycolysis provides a major energy source, whereas granular cells seek energy source primarily in TCA cycle and subsequent oxidative phosphorylation.

Key words: Renal carcinoma, Lactate dehydrogenase, Isoenzyme.

INTRODUCTION

It is well known that human renal carcinoma (RC) can be classified into two major categories of clear cell carcinoma (CCC) and granular cell carcinoma (GCC) depending on the cytoplasmic staining property, and in many of the RC cases both of these two cell types are represented (mixed cell type carcinoma, MCC). There are definite differences between these two cell types in histochemical or electron microscopic findings. For instance, while in CCCs, rich in glycogen, neutral lipid and phospholipid, intracellular organelles are underdeveloped, GCCs are high in mitochondria, well developed endoplasmic reticula and Golgi bodies, but have almost negligible levels of glycogen and lipid (6).

Based on the survival rate in several clinical follow-up surveys, it appears that GCC is more malignant than CCC (4, 15, 19), although opinions are still divided on this score (1). The biological significance of the differences in the structure noted between these types of cancer cells, probably both of which develop from the same origin – proximal tubular cells, is of interest. We describe the correlation between the histology and the isoenzyme pattern of lactate dehydrogenase (LDH) in renal carcinoma.

MATERIALS AND METHODS

In the present experiment 27 samples of RC tissue obtained by nephrectomy were used with normal renal cortical tissue obtained from 5 autopsy cases as controls. The clinical pictures and the postoperative course of these patients are represented in Table 1. Histological examinations were made on formalin fixed and paraffin embedded sections with ordinary hematoxylin-eosin staining. Grading of malignancy was based on the degree of nuclear atypia proposed by Skinner et al. (19); grades 1 and 2 were recorded as low grade of malignancy and grades 3 and 4 as high grade. The clear cell carcinoma means that RC is mainly composed of clear cells (more than 2/3 of carcinoma cells are clear ones) and the GCC of granular cells, depending on the cytoplasmic staining property. The MCC means that RC consists of approximately equal numbers of clear and granular cells. Clinical spread of the tumour was classified into two categories of

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Table 1. Patient profiles and clinical follow-up

Patient No.	Age	Sex	TNM[a]	Histology		Follow-up (months)
				Cell type	Grade	
1 N. M.	57	m	$T_2N_0M_0$	c[b]	2	7, alive-NED[e]
2 E. E.	54	f	$T_3N_0M_0$	c	2	41, alive with disease
3 Y. I.	51	m	$T_3N_0M_0$	c	2	21, alive-NED
4 M. Y.	58	m	$T_2N_0M_0$	c	2	16, alive-NED
5 Y. Y.	68	m	$T_3N_0M_0$	c	2	13, alive-NED
6 A. T.	62	m	$T_2N_0M_0$	c	2	11, dead
7 G. A.	65	m	$T_2N_0M_0$	c	2	27, alive-NED
8 K. I.	50	m	$T_2N_0M_0$	c	2	15, alive-NED
9 K. N.	60	m	$T_2N_0M_0$	c	2	23, alive-NED
10 M. W.	65	m	$T_3N_1M_0$	c	2	8, dead
11 Y. K.	74	m	$T_3N_0M_0$	c	2	39, alive-NED
12 H. S.	46	m	$T_4N_0M_{1b}$	c	3	3, alive with disease
13 Y. I.	56	m	$T_2N_0M_0$	c	3	21, dead
14 K. Y.	41	m	$T_4N_0M_0$	c	3	14, dead
15 H. M.	65	m	$T_3N_0M_{1b}$	c	3	7, dead
16 M. N.	68	m	$T_2N_0M_0$	c	3	19, alive-NED
17 S. I.	56	m	$T_3N_1M_{1a}$	m[c]	2	17, dead
18 T. K.	74	m	$T_2N_0M_{1b}$	m	2	4, alive with disease
19 H. K.	48	m	$T_4N_0M_0$	m	3	13, dead
20 N. Y.	47	m	$T_3N_1M_{1a}$	m	3	4, dead
21 K. O.	46	m	$T_3N_0M_0$	m	3	46, alive with disease
22 K. F.	64	m	$T_3N_0M_{1a}$	m	3	22, alive with disease
23 Y. Y.	32	f	$T_2N_4M_{1b}$	m	3	19, dead
24 F. C.	69	m	$T_3N_0M_0$	m	4	9, dead
25 S. A.	67	f	$T_2N_0M_{1c}$	g[d]	2	11, dead
26 S. M.	22	f	$T_2N_0M_{1c}$	g	3	14, dead
27 M. E.	52	f	$T_4N_1M_0$	g	4	4, dead

[a] Tumour-nodes-metastasis classification (Unio International Contre Cancrum, Geneva 1974)

[b] Clear cell carcinoma

[c] Mixed cell type carcinoma

[d] Granular cell carcinoma

[e] No evidence of disease

"localized" and "advanced", the former defined to be less than T_2 in TNM classification (23) without evidence of metastases to lymph nodes or distant organs. Venous thromboses were invariably resectable, hence were not taken into consideration in the classification of clinical stage. The postoperative survival rate was calculated by the life table method of Cutler and Ederer (5).

For LDH analysis tumour tissues was rinsed in saline to remove necrotic or fibrous elements and was homogenized at 0-4°C for about 10 sec using a tissue disperser (ILADO Laboratory, Type X-10/20) with 3 volumes of Tris-HCl buffer,

(100 mmol/l), pH 7.5, containing 1 mM EDTA and 1 mM thioglycerol. The homogenate was centrifuged at 4°C for 30 min at 1 x 10^4 rpm, and the resulting supernatant was used for determination of LDH activity, LDH isoenzyme pattern and protein.

For the measurement of total LDH activity, a mixture of 1 ml of phosphate buffer, (100 mmol/l) pH 7.4, containing 0.8 mg/ml lithium pyruvate and 0.1 ml of 10 mg/ml NADH was incubated at 37°C for 5 min, then 0.1 ml of the enzyme solution was added and incubated at 37°C for 30 min. To the reaction mixture, 1 ml of 0.2 mg/ml 2,4-dinitrophenylhydrazine dissolved in HCl (1 mol/l) was added, and placed in room temperature (20-30°C) for 20 min. Colour development was achieved by the addition of 10 ml of NaOH (0.4 mol/l), and determined colorimetrically at 500 nm within 30 min. LDH activity in an enzyme solution was calculated according to a standard line obtained with the use of serially diluted substrate solution, and was expressed in International Units (I.U.). Linearity of this assay response was evidenced between 0-600 I.U./L, and the enzyme solutions were appropriately diluted with the buffer used for homogenization when the LDH activity did not fall within the range of linearity.

LDH isoenzymes were separated by electrophoresis in 0.8% agar gel with barbital buffer pH 8.6 μ = 0.06, for 60 min at 4°C at a constant current of 5 mA/cm. Bands were made visible by incubating the gel at 37°C for 60 min with Tris-HCl buffer, (60 mmol/l) pH 7.4 containing sodium lactate (0.5 mmol/l), 1 mg/ml NAD, 0.5 mg/ml nitroblue tetrazolium and 20 μg/ml phenazine methosulfate. The enzymatic activity of each isoenzyme was determined by densitometry at 550 nM, using Shimazu CS-210 chromatoscanner. Peak areas were determined by cutting out scans and weighing and percentages of each isoenzyme were calculated.

Soluble proteins were measured by the method of Lowry et al. (11).

RESULTS

The clinical pictures of the cases studied showed that the degree of nuclear atypism was higher in MCC and GCC than CCC, and with the former it was often the case that the tumour was already advanced at the time of operation (Table 2). The postoperative survival rate reflects the difference in grade of malignancy and stage, the 3-year survival rate for MCC and GCC being 15%, while that for CCC was 55%.

The LDH activity levels in normal kidney and in renal carcinoma classified by the grade of malignancy and cell type are shown in Table 3 and Table 4. While in normal kidney this activity is almost constant, it is subject to extensive scatter in RC tissues, from 0.40 I.U./mg·protein to 5.99 I.U./mg·protein. The average is approximately the same as the normal kidney. The mean value of this activity was slightly higher with the low grade group and in the CCC in comparison with high grade group or MCC and GCC, but statistically insignificant.

In Table 3 the activities of LDH-1-LDH-5 are also shown in terms of percentage of total activity. In normal kidney, the contributions to total activity are higher with LDH-1 and LDH-2, but those of LDH-4 and LDH-5 are extremely low as has been reported (10, 18). In RC tissues the pattern in which LDH-4 and LDH-5 activity is high and LDH-1 and LDH-2 is low (conditions satisfying LDH-4 + LDH-5 $\geq$70%) is referred to as a completely inverted pattern of LDH isoenzyme and accounts for the majority of the cases, i.e. 16 out 27 cases. However, there is a wide range of variation in isoenzyme pattern, and there was for instance, a pattern closely similar to that of normal kidney (case 25) and a pattern in which activity is relatively high with LDH-2, LDH-3 and LDH-4 but rather low with LDH-5. The cases showing LDH-4 + LDH-5 <70% were referred to as incompletely inverted pattern of the isoenzyme.

As Table 5 indicates, there exists a definite correlation between the LDH isoenzyme pattern and the histological character. So, while with CCC LDH isoenzyme pattern was completely inverted in 9 out of 11 cases in the low grade group and all 5 cases in the high grade group, i.e. 14 out of 16 cases in total, the picture was entirely different with GCC and MCC, LDH isoenzyme pattern being incompletely inverted in 1 out of 3 cases in the low grade group, and all 8 cases in the high grade group, making 9 out of 11 cases in all. These differences were statistically significant ($P < 0.005$). The grade of

Table 2. Clinical spread and histopathological findings

Clinical spread	Clear cell carcinoma		Mixed and granular cell carcinoma	
	Low grade[a]	High grade[b]	Low grade[a]	High grade[b]
Localized	7	2	-	1
Advanced	4	3	3	7
Total	11	5	3	8

[a] grade 1, grade 2 [b] grade 3, grade 4

Table 3. Total LDH activity and percentage of the LDH isoenzymes in renal carcinoma tissue in comparison with normal renal tissue

Patient No.	Total LDH activity (IU[a]/mg·protein)	Percent of LDH isoenzymes				
		LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
1[b]	0.58	3.7	24.4	52.0	15.3	4.6
2	1.66	2.3	3.7	10.1	39.3	44.6
3	0.46	0.6	1.9	16.9	38.0	42.5
4	3.37	-	1.4	16.4	39.5	42.7
5	3.23	0.7	3.6	10.4	21.8	63.5
6	2.02	-	2.6	15.5	42.2	39.7
7	0.78	4.7	5.1	12.1	37.5	40.6
8	3.18	0.6	2.3	21.6	40.6	34.9
9	1.14	1.5	3.7	14.2	35.5	45.2
10	0.97	1.8	3.9	12.9	34.2	47.2
11[b]	0.40	15.2	29.5	36.7	10.4	8.3
12	0.88	1.1	3.3	13.4	36.2	46.0
13	3.99	0.8	3.6	20.7	42.4	32.4
14	2.17	0.8	5.6	23.4	39.1	31.1
15	5.51	0.4	3.0	21.3	37.7	37.6
16	1.64	0.5	1.1	11.2	53.1	34.1
17	1.79	0.4	3.6	18.5	38.7	38.8
18	5.99	0.2	2.1	18.3	38.3	41.1
19[b]	0.99	10,0	18.3	24.6	27.1	20.1
20[b]	0.74	1.9	8.8	28.7	38.2	22.4
21[b]	1.00	1.6	6.7	22.1	35.8	33.8
22[b]	0.48	14.0	21.3	33.0	22.4	9.3
23[b]	1.12	20.0	24.0	24.5	21.1	10.3
24[b]	0.34	7.2	15.6	27.7	31.9	19.9
25[b]	3.62	41.6	37.2	16.4	3.8	0.9
26[b]	0.97	12.4	41.0	45.7	0.9	-
27[b]	1.22	15.1	22.4	26.9	25.7	9.9
normal kidney[c]	2.02 ±0.27	40.70 ±5.04	33.58 ±0.96	17.40 ±3.90	6.58 ±1.77	1.72 ±0.63

[a] International unit

[b] Tumours of incompletely inverted type of LDH isoenzyme pattern

[c] Determination of five normal renal cortical tissues. The values are expressed in mean ± SD

Table 4. LDH activity in RC of different histological and enzymatic property

Classification of RC	LDH Activity (IU[a]/mg·protein) (mean ± SD)	
Total 27 cases	1.86 ± 1.54	
Low grade RC (14)	2.09 ± 1.61	N.S.[b]
High grade RC (13)	1.62 ± 1.50	(rs = 0.07)
Clear cell carcinoma (16)	2.00 ± 1.48	N.S.
Mixed and granular cell carcinoma (11)	1.66 ± 1.69	(rs = 0.07)
RC of completely inverted pattern (16)	2.42 ± 1.66	$0.001 < P < 0.01$
RC of incompletely inverted pattern (11)	1.04 ± 0.91	(rs = 0.57)

[a] International unit

[b] Not significant. Statistical analysis was performed by Spearman's non-parametric test

Table 5. LDH isoenzyme pattern and histological finding of RC

LDH Isoenzyme pattern	Clear cell carcinoma			Mixed or granular cell carcinoma		
	Low grade[a]	High grade[b]	(Total)	Low grade	High grade	(Total)
Completely inverted pattern (LDH-4 + LDH-5 >70%)	9	5	(14)	2	0	(2)
Incompletely inverted pattern (LDH-4 + LDH-5 <70%)	2	0	(2)	1	8	(9)
Total	11	5	(16)	3	8	(11)

[a] grade 1, grade 2

[b] grade 3, grade 4

Correlation between cell type and isoenzyme pattern was statistically significant ($X^2 = 10.26$, $P < 0.005$), while that between grade of malignancy and isoenzyme pattern was insignificant ($X^2 = 2.98$, $0.1 < P < 0.05$)

malignancy, however, did not show a significant correlation with the isoenzyme pattern of the tumour ($0.1 > P > 0.05$).

As shown in Table 4, total LDH activity is statistically higher in the tumours of completely inverted pattern than in those possessing the incompletely inverted one. In this study, novel LDH isoenzymes were not found in RCC tissues. On the basis of above data, it may be safely concluded that, while total LDH activity of RC ranged widely, it is almost independent of histological grade of malignancy or cell type. However the low grade group and CCC had rather higher mean values of activity, although not significantly so.

The isoenzyme pattern, however, showed close correlation with the cell type of the tumour, a completely inverted pattern predominating in CCC and an incompletely inverted pattern in MCC and GCC. Furthermore, when the total LDH activity is presented according to the type of its isoenzyme pattern, significantly higher activity is noted in tumours having a completely inverted pattern than in those with an incompeltely inverted one.

DISCUSSION

The variation of LDH activity and isoenzyme pattern in malignancy has been the subject of many investigations, and it is generally accepted that LDH activity is higher with an increasing proportion of LDH-4 and 5 in cases of malignant tumours (8, 9, 16, 18). A positive correlation between the proportion of LDH-4, LDH-5 and the histological grade of malignancy is noted in cases of human bladder cancer (2), whereas no such correlation is noted in most other kinds of tumour (8, 17).

In most cases in human RC tissues, it is already confirmed that LDH-5 level tends to increase (8, 10, 12, 14), but Nagahara and Ikoma (14) as well as Li et al. (10) became aware of the fact that in some cases of RC, and in GCC in particular, LDH isoenzymes were almost idential with that of normal kidney, and pointed this out as an observation worthy of further study. The result of our present experiments have supported the suggestion given by these authors, demonstrating that the LDH isoenzyme of RC is highly dependent upon the relative proportions of the clear and granular cells as integral components of the tumour.

Electron microscopic observations have shown a marked difference between these cell types as previously described. This ultrastructural difference suggests that anaerobic glycolysis provides the source of energy for cells of clear type, while TCA cycle and subsequent oxidative phosphorylation provides the main energy source for cells of granular type, and this difference appears to be in agreement with the observed difference in LDH isoenzyme pattern. On the other hand, the present experiments suggest that MCC and GCC are higher in their potential for malignancy than those of CCC, and apparently does not agree with the well-known fact that oxygen consumption of tumour decreases with increasing rate of proliferation (3, 20). The reasons for this discrepancy are uncertain.

GCC and CCC are both malignant, and it is not infrequent that cells of these two types coexist in the same tumour, (referred to as MCC). This suggests that malignant cells are dependent for energy production in some cases upon anaerobic glycolysis and upon TCA cycle in other cases. It might also suggest that each of these two types of cells can be convertted into the other, depending on the environmental conditions, although the relationship between the growth rate or size of tumour and the rate of oxygen supply is supposed to be the main determining factor (21). In the other words, it is not that cancer remains unaltered regardless of environment with regard to histological structure and biochemical properties, but that the biochemical properties become more or less adapted to the environment.

This duscussion is derived from the observed relationship between LDH isoenzyme and the ultrastructural characteristics of the individual cell types and is based on the "aerobic-anaerobic theory" proposed to explain the biological role of LDH isoenzymes (7, 13). This theory is, however, still open to question in the light of the available data on isoenzyme distribution and the in vivo findings about the mechanism of regulating enzymatic activity (22), and it is possible that the biochemical significance of the present experiment may be interpreted in some other way in the future.

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