MALIGNANT TRANSFORMATION-LINKED IMBALANCE: DECREASED XANTHINE OXIDASE ACTIVITY IN HEPATOMAS

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1. Introduction

Previous work demonstrated that the activity of glutamine phosphoribosylpyrophosphate (PRPP) amidotransferase (EC 2.4.2.14) was increased in all examined rat hepatomas [1,2]. The activity of this key purine biosynthetic enzyme is opposed by that of the rate-limiting enzyme of purine catabolism, xanthine oxidase (EC 1.2.3.2). This paper reports that xanthine oxidase activity was decreased in all hepatomas irrespective of growth rate or differentiation. Thus, in the liver tumors the ratio of the activity of amidotransferase/xanthine oxidase was markedly increased. This enzymatic imbalance, that yields a predominance of the synthetic over the opposing catabolic enzyme of purine metabolism, should confer selective advantages to the cancer cells.

2. Materials and methods

2.1. *Animals and tissues*

The transplantable hepatomas of different growth rates were carried subcutaneously in inbred strains of male Buffalo or ACI/N rats of 150–250 g of weight. Normal rats of the same strain, sex, age and weight were sacrificed along with the tumor-bearing animals. The studies on regenerating liver and developing rats, the killing of animals and excision of livers and tumors were conducted as previously [3]. The biological aspects [4] and biochemical properties [3] of the tumor lines in the spectrum of hepatomas of different growth rates were reported.

2.2. *Enzyme preparation*

5% homogenates (w/v) were prepared from normal liver and tumor tissues in 0.25 M sucrose. The homogenate was centrifuged at 100 000 *g* for 30 min at 3°C in Beckman L5-50 preparative centrifuge. Uricase activity (EC 1.7.3.3) was not present in the supernates; thus, it did not interfere with the xanthine oxidase assay.

2.3. *Assay system of xanthine oxidase*

A standard assay was developed which was an adaptation of that of DeLamirande et al. [5] to the kinetic conditions of our rat liver and hepatoma systems. The supernate was preincubated for 40 min at 37°C and then added to the reaction mixture which contained in final concentrations: xanthine (0.17 mM); phosphate buffer (33 mM, pH 7.5); and a suitable amount of enzyme (supernate). The reaction was carried out at 37°C and was stopped at 0 and 20 min by addition of 0.1 ml of 100% (w/v) trichloroacetic acid. The mixture was centrifuged at 10 000 *g* for 15 min. In the clear supernates the uric acid produced from the substrate, xanthine, was measured by the increase in absorbancy at 293 nm, in a Gilford 2400S recording spectrophotometer. Blanks contained the identical reaction mixture without xanthine. The enzyme activity was provided by the difference between the rate in the complete reaction and that in the blank. Xanthine oxidase activ-

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Table 1
Decreased xanthine oxidase activity in hepatomas

Tissues	Growth rate (months)	Xanthine oxidase activity μmoles/h/mg protein × 10^{-2}	Amidotransferase activity μmoles/h/mg protein × 10^{-2}	Amidotransferase/ xanthine oxidase ratios
Normal liver (Buffalo)				
Control for 9618A		11.0 ± 0.4	5.5 ± 0.7	0.5 ± 0.02
Control for 20		7.8 ± 0.5	6.0 ± 0.4	0.8 ± 0.02
Control for 9618B		7.0 ± 0.3	5.5 ± 0.4	0.8 ± 0.01
Control for 16		7.0 ± 0.2	6.9 ± 0.5	1.0 ± 0.02
Control for 47C		10.8 ± 0.7	6.8 ± 0.3	0.6 ± 0.01
Control for 28A		8.2 ± 0.3	5.5 ± 0.4	0.7 ± 0.02
Control for 8999		10.8 ± 0.4	6.9 ± 0.1	0.6 ± 0.03
Control for 9633		10.1 ± 0.6	7.3 ± 0.3	0.7 ± 0.02
Control for 7800		9.0 ± 0.4	5.5 ± 0.4	0.6 ± 0.02
Normal liver (ACI/N)				
Control for 3924A		10.0 ± 0.3	7.5 ± 0.3	0.8 ± 0.02
Control for 3683		10.9 ± 0.4	5.4 ± 0.4	0.5 ± 0.02
Hepatomas				
9618A	12.4	3.5 ± 0.2 (32)[a]	12.0 ± 0.7 (218)[a]	3.4 ± 0.1 (689)[a]
20	11.6	3.9 ± 0.1 (50)[a]	13.0 ± 0.6 (217)[a]	3.3 ± 0.1 (412)[a]
9618B	10.8	1.1 ± 0.1 (16)[a]	9.3 ± 0.4 (169)[a]	8.4 ± 0.3 (1050)[a]
16	8.3	0.7 ± 0.1 (10)[a]	10.1 ± 0.6 (146)[a]	14.4 ±0.9 (1469)[a]
47C	7.1	2.3 ± 0.1 (22)[a]	17.1 ± 0.8 (251)[a]	7.4 ± 0.7 (1333)[a]
28A	6.8	3.9 ± 0.1 (48)[a]	9.6 ± 0.5 (174)[a]	2.5 ± 0.1 (357)[a]
8999	6.3	5.4 ± 0.2 (50)[a]	22.0 ± 0.6 (319)[a]	4.0 ± 0.5 (666)[a]
9633	5.2	5.9 ± 0.2 (58)[a]	11.2 ± 0.5 (153)[a]	1.0 ± 0.2 (271)[a]
7800	1.2	5.5 ± 0.2 (61)[a]	13.9 ± 0.7 (253)[a]	2.7 ± 0.2 (450)[a]
3924A	0.9	1.0 ± 0.1 (10)[a]	15.5 ± 0.5 (207)[a]	15.5 ± 0.8 (1937)[a]
3683	0.5	1.1 ± 0.1 (10)[a]	15.1 ± 0.9 (280)[a]	13.7 ± 0.8 (2740)[a]

The data are given as means ± S.E. of 4 to 10 rats in the various groups with percentages of corresponding control liver values in parentheses. The specific activities are to be multiplied by the exponential given to arrive at the actual values. Growth rate is expressed as the mean transplantation time in months between inoculation and growth to 1.5 cm diameter.

[a]Values statistically significantly different from the respective controls ($p < 0.05$).

ity was calculated in μmoles uric acid produced per h/g wet weight of tissue and was expressed, as specific activity, in μmoles per h per mg protein. Through such investigations the optimum assay conditions and the various kinetic constants were established for the crude enzyme in liver and hepatomas.

3. Results and discussion

3.1. *Comparison of properties of xanthine oxidase in normal liver and hepatomas*

Kinetic studies showed that the affinity of xanthine oxidase to the substrate, xanthine, was the same in normal liver and in slow- and rapidly-growing hepatomas (K_M = 6 to 8 μM). For both liver and hepatoma the optimum pH was at 8.0; at pH = 7.4 the activity was approximately 80% of that at the pH optimum. Under our conditions xanthine oxidase activity was linear during 60-min incubation, and it was proportionate with amounts of protein of supernate added over a range of 0.5 to 3.0 mg.

3.2 *Comparison of xanthine oxidase activity in normal rat liver and in hepatomas of different growth rates*

Table 1 shows that in normal liver the xanthine oxidase specific activity ranged from 7.0 to 11.0 μmol substrate metabolized per h per mg protein × 10^{-2}. The specific activities in the slow, medium and rapidly growing hepatomas were 2- to 10-fold decreased. Since in the assays the enzyme activity was proportionate with enzyme amount, it is assumed that the decreased enzyme activity present in all the hepatomas represents decreased enzyme concentration in the tumors.

Since xanthine oxidase is the rate-limiting enzyme of purine catabolism [5], and glutamine PRPP amidotransferase a key enzyme of purine biosynthesis [6], it was of interest to examine the relationship of these two opposing key enzymes in the same hepatomas. As table 1 shows, for liver the specific activities were lower for amidotransferase than for xanthine oxidase. That the liver amidotransferase/xanthine oxidase ratios were low and unfavorable for de novo purine synthesis is underlined by the high K_M of amidotransferase for the substrates, glutamine (K_M = 1.7 mM) and PRPP (K_M = 0.9 mM) [2], in contrast to that of xanthine oxidase for its substrate, xanthine (K_M = 0.7 μM). Thus, in the reprogramming of gene expression that occurred in all hepatomas examined, the amidotransferase activity increased, whereas xanthine oxidase activity decreased. In consequence, amidotransferase/xanthine ratios were increased to 2.7- to 27-fold of those in the corresponding normal liver. This reprogramming of gene expression should confer selective advantages to the neoplastic cells.

Previously we reported antagonistic behavior for opposing key enzymes in the hepatoma spectrum. The activities of key enzymes of glycolysis increased and those of gluconeogenesis decreased in parallel with the increase of tumor growth rate and malignancy [3]. Similar antagonistic behavior was recognized for activities of DNA synthetic enzymes (DNA polymerase, ribonucleotide reductase, thymidine kinase) that increased and the rate-limiting degradative enzyme (dihydrouracil dehydrogenase) that increased with the rise in hepatoma growth rate [3]. This study demonstrates reciprocal behavior in gene expression manifested in the activities of two opposing key enzymes that are increased in all hepatomas (amidotransferase) and decreased in all hepatomas (xanthine oxidase).

3.3. *Xanthine oxidase activity in regenerating and developing liver*

Xanthine oxidase activity in regenerating liver was not altered from that of the liver of sham-operated controls at 12, 24, 48, 72 and 96 h after operation.

Investigation in rats of different age groups showed that at 6 days post-natal age xanthine oxidase activity in the average liver cell was less than 5% of the activity of adult liver. The activity rose to 18, 46, 76 and 94%, respectively, at post-natal age of 18, 25, 30 and 40 days (fig.1).

3.4. *Specificity to neoplasia of decreased xanthine oxidase activity in hepatomas*

The decreased xanthine oxidase activity seems specific to neoplastic transformation in the liver and it does not merely indicate proliferation, because this activity did not change in regenerating liver. Since the decrease in enzyme activity occurred in all hepatomas irrespective of growth rate and degree of histological differentiation, this all-or-none behavior can be sharply contrasted with the differentiation-linked events that are expressed during post-natal development in normal liver (fig.1). These results also underline the

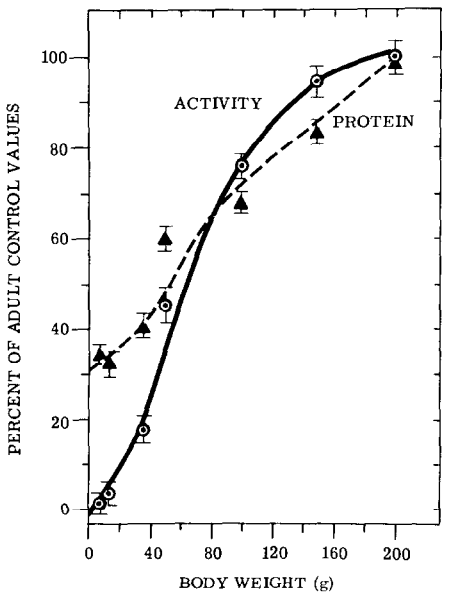

Fig.1. Behavior of xanthine oxidase activity in developing liver. Activities were calculated per average cell and expressed as percentages of the normal adult liver values.

malignancy-linked nature of the imbalance in the ratios of amidotransferase/xanthine oxidase.

The presently reported decrease in xanthine oxidase in 11 lines of hepatomas is in agreement with that observed in several types of primary and transplantable rodent neoplasms [5,6]. We also observed that in the MK-3 renal carcinoma xanthine oxidase specific activity was decreased to 50% of that of the kidney of control normal rats. Since the decreased xanthine oxidase activity is consistently associated with neoplasia, it has a relatively high probability of being an essential biochemical determinant of transformation.

3.5. *Malignancy-linked alterations: biological significance*

That xanthine oxidase activity was decreased in all the hepatomas, irrespective of the degree of malignancy, differentiation and growth rate, is important, since it suggests that the reprogramming of gene expression that occurs in malignant transformation is linked with a decrease in the expression of this key purine catabolizing enzyme activity. In our laboratory recently we discovered in the hepatomas six such malignant transformation-linked alterations in enzyme activities and five of these relate to an *increased* potential in directing of precursors to strategic biosynthetic processes. Thus, the increase in the activities of glucose 6-phosphate dehydrogenase and transaldolase provides an increased potential for channeling glycolytic intermediates into pentose phosphate biosynthesis [7]. The elevated UDP kinase activity should yield a higher capacity for RNA and de novo DNA biosynthesis [8]. The increased adenylosuccinate synthetase activity should lead to increased IMP utilization for nucleic acid biosynthesis [9]. The high activity of glutamine PRPP amidotransferase should provide a heightened ability for de novo purine biosynthesis [1,2]. The sixth alteration observed in all hepatomas is the *decrease* in xanthine oxidase activity which depresses the ability to degrade purines. Thus, purines can be recycled for nucleic acid biosynthesis. These six enzymes may be used in the biochemical diagnosis of hepatomas as markers of neoplastic transformation and indicators of liver malignancy.

4. Conclusions

Xanthine oxidase was decreased 2- to 10-fold in all examined rat hepatomas irrespective of the malignancy, growth rate and degrees of histological differentiation of the neoplasms. The affinity to substrate (K_M = 6–8 μM) and the pH optimum (8.0) of the liver and hepatoma enzymes were the same. The reprogramming of gene expression, as manifested in the decreased activity of this key purine metabolizing enzyme, appears to be specific to neoplastic transformation. Since glutamine PRPP amidotransferase activity was increased but the opposing enzyme, xanthine oxidase, was decreased in all the hepatomas, the reprogramming of gene expression results in an imbalance that favors synthesis against catabolism. This enzymatic imbalance should confer selective advantages to the cancer cells.

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