Short Communication

The Isoenzymes of Hexokinase in Normal and Neoplastic Tissues of the Rat[1]

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Key Words

Isoenzyme

Hexokinase

Neoplasm

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Abstract. The isoenzymes of hexokinase in fetal, adult and neoplastic tissues of the rat were analyzed by starch gel electrophoresis. All tumors contained significant amounts of hexokinase III, whether or not their respective tissues of origin expressed this variant.

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DISCLAIMER

to suggest
There is ample evidence/that the sum total of known metabolic potentialities expressed in different neoplasms varies between narrower limits than it does among their tissues of origin [3, 6]. This diminished metabolic versatility of neoplasms is due, in part, to the loss of organ-specific functions and is generally more pronounced among tumors growing at faster rates. We have recently shown that what appears to be a restriction of genomic expression occurs also among those functions which persist in neoplasms: enzymes which exist in multimolecular forms and exhibit in normal organs of adult rats tissue-specific profiles, exhibit in neoplasms a single pattern common to all tumors examined, irrespective of their origin. The three enzymes then studied [2] exhibited in ten different tumors solely or predominantly the enzyme variant characteristic of fetal tissues.

The present note reports on the isoenzymic distribution of hexokinase (EC 2.7.1.1) in eight of the same tumors and their control tissues. The nature and origin of these transplanted tumors were previously described [2]. Normal adult controls were of the same strains as those in which the tumors were carried; the fetal tissues were from rats of the inbred NEDH strain. No strain differences in isoenzymic patterns were observed. High speed supernatant solutions of 10-25% tissue homogenates were assayed for hexokinase activity as described by Jamdar and Greengard [4]. Thirty µl were subjected to electrophoresis in starch gel, prepared in a 1:20 dilution of the following stock solution: 0.02 M EDTA, 0.5 M boric acid and 0.9 M Tris, pH 8.6. The electrode compartments contained a 1:7 dilution of the same stock solution; 280 v (25 mamps) were applied for 14 h in the cold. The gel was then immersed in a solution containing in 100 ml: 0.5 mM glucose,

1 mM ATP, 0.13 mM NADP, 30 units glucose-6-phosphate dehydrogenase, 0.5 ml of a 1.3% solution of nitro blue tetrazolium, 0.3 ml of a 1.2% solution of phenazine methosulfate and 2 mM KCN, for 1.5 h at 37°C in the dark.

Figure 1 illustrates the electrophoretic resolution of the hexokinase isoenzymes in some hepatic tissues. The numbering system is that of Katzen and Schimke [5], with "a" variants added. The other rat tissues contained, in varying amounts, one or more of the four isoenzymes seen in figure 1. The relative amounts of the isoenzymes, based on visual estimation, are given in table I for all tissues examined. Total activity is assigned a score of 6, so an isoenzyme designated as 2 represents, for example, one third of the total; zones of activity estimated to contain less than 15% of the total were not scored (to examine the scoring system, compare figure 1 with entries for corresponding tissues in table I).

The total hexokinase activities, determined in tissue extracts (last column of table I) are in approximate agreement with a previous study in which this activity in tumors was found to be proportional to their growth rate [7]. The several fold increase in mammary gland during lactation has also been observed previously [1]. Extracts of a whole fetus at 12 days' and of fetal kidney and muscle at 17-18 days' gestation contain only hexokinase I which identifies this form as the fetal isoenzyme. It is retained in all tissues examined. Hexokinase Ia distinguishes fetal liver from other hepatic tissues, but small amounts of this form are also seen in adult kidney, bone marrow and one mammary carcinoma. Muscle, and mammary gland during pregnancy and lactation, acquire hexokinase II in significant proportions, while small amounts are present in most adult tissues. Shatton et al. [9] found high levels of isoenzyme III in several types of tumors; the isoenzyme distributions reported by Sato et al. [8] are in disagreement with both the

present results and with those of Shatton et al. [9]. In the present study a pronounced preponderance of hexokinase III marks all tumors as well as the host livers of tumor-bearing animals, as if the increase in total activity over normal liver in these tissues were due to the activation of the gene coding for this isoenzyme. This holds true whether or not the tissue of origin expresses this variant of hexokinase: kidney carcinomas, mammary tumors and rhabdomyosarcoma express hexokinase III while their respective tissues of origin do not. Hepatomas and the livers of tumor-bearing rats express hexokinase III to a greater extent than normal liver. Bone marrow and spleen, two rapidly growing tissues in the adult animal, also contain significant amounts of this variant, whereas intestine does not.

Our previous studies showed that the fetal forms of lactate dehydrogenase, pyruvate kinase and aldolase are the ones which predominate in tumors. In contrast, the present work shows that hexokinase III, which predominates in tumors, is absent from fetal tissues and thus serves to distinguish neoplastic tissues from normal, undifferentiated tissues. However, the isoenzymes of hexokinase have not been sufficiently purified and their structural relationships studied to rule out the possibility that hexokinase III is coded for by the fetal gene and undergoes post-translational modification. At all events, hexokinase shares two features with the three enzymes on which we have previously reported: a) the same enzyme variant predominates in all tumors, irrespective of their origin; b) the same isoenzyme that predominates in the tumors is also the most abundant enzyme variant in some normal adult proliferating tissues. These observations argue for the view that the profiles of biochemical components converge upon a common pattern in tumors and that this pattern may be uniquely suited for rapidly growing tissues.

FOOTNOTE

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Table I. Summary of the isoenzymic distribution of hexokinase in different tissues

Tissues	Hexokinase Isoenzymes I	Ia	II	III	Activity in tissue extract (units/g)
Hepatic Tissues					
Fetal liver (15 days)	3+	3+			0.37 ± 0.08 (3)
Neonatal liver (2 days)	2+	2+	+	+	0.37
Adult liver	3+			3+	0.18 ± 0.04 (3)
Regenerating liver (41 h)	3+			3+	0.16
Hepatoma 7800	2+			4+	0.36
Hepatoma 7777	+		+	4+	0.42
Host liver	+		+	4+	0.36 ± 0.06 (7)
Renal Tissues					
Fetal kidney (18 days)	6+				0.12 ± 0.04 (4)
Adult kidney	4+	+		+	0.26 ± 0.04 (3)
Carcinoma MK-2	2+			4+	0.7
Carcinoma MK-3	+		+	4+	0.90 ± 0.11 (3)
Mammary Tissues					
Virgin gland	6+				0.12 ± 0.04 (4)
Pregnant gland (19 days)	4+		2+		0.28 ± 0.06 (4)
Lactating gland (1-10 days)	4+		2+		0.79 ± 0.11 (9)
Carcinoma DMBA 1C	3+	+		2+	1.6
Carcinoma DMBA 5A	2+		+	3+	0.87 ± 0.10 (3)
Carcinoma Walker	2+		+	3+	1.05

Muscle Tissues					
Fetal muscle (17 days)	6+				0.12
Adult muscle	2+		4+		0.26
Rhabdomyosarcoma NS1[4]	2+		+	3+	0.18, 0.29 (2)
Miscellaneous Tissues					
Bone marrow	2+	+		3+	0.78 ± 0.14 (3) [6]
Spleen	3+		+	2+	0.83 ± 0.15 (3)
Intestinal epithelium	4+		+		0.42 ± 0.10 (3)
Whole fetus (12 day)	6+				0.22 ± 0.06 (3)

Each tissue was electrophoresed three times or more and gave reproducible results. The scoring system is explained in the text. The unit of activity is defined as the amount of enzyme which will produce 1 μmol of glucose-6-phosphate per min. Activities are means (± SD) with the number of preparations assayed in parentheses or results with single preparations.

LEGEND

Figure 1. Photograph of a starch gel depicting the electrophoretic separation of hexokinase isoenzymes in, from left to right, hepatoma 7800 (7800), adult male liver (liver) and 18 day fetal liver (fetal liver), each in duplicate.