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CREATINE KINASE ISOZYMES IN HUMAN TUMORS

Marlene DeLuca, Norman Hall, Rene Rice and Nathan O. Kaplan

Departments of Chemistry, Medicine and Cancer Center
University of California, San Diego
La Jolla, California 92093

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SUMMARY

The creatine kinase content and isozyme distribtution in 33 human tumors carried in athymic mice has been determined. All of the tumors except two melanomas contained significant amounts of the BB isozyme, the mammalian fetal type enzyme, but very little of the MM isozyme. Many of the tumors of diverse origin contained a significant amount of the mitochondrial isozyme. Some of the serum from mice carrying these tumors showed elevated levels of either BB or mitochondrial creatine kinase, however, this does not seem to be specifically associated with any single type of tumor.

INTRODUCTION

In 1967 Eppenberger et al. (1) demonstrated the existence of three isozymes of creatine kinase (CK); namely, MM found in muscle, BB from brain tissue and the hybrid MB. Jacobs et al. (2) demonstrated a fourth isozyme that was uniquely associated with mitochondria. The enzyme is immunologically distinct from MM & BB forms and will not hybridize with the cytoplasmic enzymes (3, 4).

A number of workers have studied the appearance of CK isozymes during development (5,6). At birth there is no detectable mitochondrial creatine kinase in mouse hearts. At six days after birth it represents about 1% of the total creatine kinase and does not reach adult level (9%) until 25 days after birth (6). In general the early fetus contains predominantly the BB isozyme which is replaced by MB & MM during the later stages of development.

Recently while studying the CK isozyme composition of tumor tissue, we found an unusual increase in the mitochondrial creatine kinase in certain

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melanomas. This led us to investigate the creatine kinase distribution in a number of different tumor tissues.

MATERIALS AND METHODS

Freshly excised tumors were weighed and homogenized in cold 0.1 M sodium phosphate pH 7.4, 10 mM β-mercaptoethanol 1 mM EDTA. Three to 5 mls of buffer per gram of tissue was used. Cellular debris was removed by centrifugation at 12,000 x g for 10 minutes. The supernatant was assayed for total creatine kinase activity by the method of Rosalki (7). Electrophoresis and staining of the isozymes was performed as described previously (8). Hybridization experiments were done according to the procedure reported previously (3).

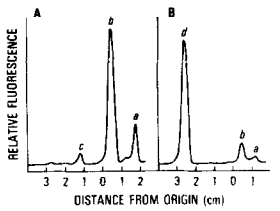
Tumor mitochondria were obtained by extracting with 0.25 M sucrose, 0.05 M Tris pH 7.4. Cellular debris was removed by centrifuging at 70 x g for 15 minutes. This was followed by centrifugation at 12,000 x g for 15 minutes and the mitochondrial pellet was washed two times with sucrose. They were then extracted with 0.1 M sodium phosphate pH 7.4, 10 mM β-mercaptoethanol, 1 mM EDTA.

The tumor lines used were obtained from: Sharp Hospital, University Hospital, Doctors Hospital, Mercy Hospital, Scripps Memorial Hospital, and Scripps Clinic and Research Foundation. All tumor lines were established from patient primary neoplasm then xenographed into the athymic mice, with the exception of line M-21 melanoma tumor which was derived from cells established in tissue culture. Passages in the athymic mice range from the primary xenograph of T-422 colon carcinoma to the 62nd passage of T-24 astrocytoma. All of the tumors have been serially transplanted. The percent of mouse elements in the tumor tissues involved was determined by the amount of mouse lactic dehydrogenase as measured by gel electrophoresis for each passage. The tumor tissues involved contained no mouse LDH with the exception of the T-245 colon which has 15% mouse elements. Properties of some of the tumor lines have been described in other publications (9,10).

RESULTS

Figure 1A shows the pattern obtained when a normal athymic mouse heart extract is subjected to electrophoresis and stained for enzymatic activity. The majority of the activity remains near the origin, peak b, and is the MM isozyme. A small amount moves toward the anode, peak c, the MB form. Another fraction moves toward the cathode peak a, the mitochondrial isozyme. Figure 1B shows the pattern obtained from an athymic mouse brain extract. The predominant form of the enzyme is a rapidly anodal migrating enzyme, peak d, the BB isozyme. There are also detectable amounts of MM and mitochondrial isozyme.

Figure 2A shows the electrophoretic pattern obtained after mixing extracts from mouse muscle and brain tissue. When this same mixture is



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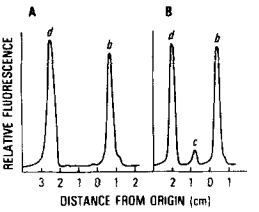

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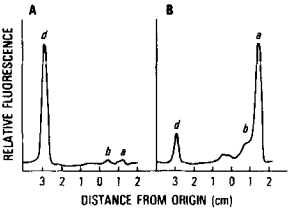

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Figure 1 Isozyme patterns obtained after electrophoresis of (A) mouse heart extract and (B) mouse brain extract. Peaks are a, mitochondrial; b, MM; c, MB; and d, BB isozymes, respectively. Migration to the right of origin is cathodal; to the left is anodal.

Figure 2 Isozyme patterns obtained after electrophoresis of (A) a mixture of muscle and brain extract; (B) the same mixture after freezing and thawing. Peak c is the hybrid MB isozyme.

Figure 3 Isozyme pattern obtained after electrophoresis of (A) extract of colon tumor T-347, and (B) extract of colon tumor T-398. Peaks are labelled as indicated in Figure 1.

subjected to three cycles of freezing and thawing, and then electrophoresed (Figure 2B), the hybrid enzyme MB, peak c, is now present. This independently confirms the identification of these enzymes as the mouse BB and MM isozymes.

Figure 3A shows the isozyme distribution obtained from an extract of a colon tumor (T-347). This tumor contains 96% of the total creatine kinase as the BB isozyme, with only traces of the MM and mitochondrial forms. Another colon tumor (T-398) is found to contain a quite different isozyme distribution, Figure 3B. This tumor contains 70% of the mitochondrial isozyme, 10% of the MM form, and 14% of the BB enzyme. The identification of the peak a as mitochondrial in origin was substantiated in two ways: Attempts were made to hybridize this enzyme by freeze-thawing with either MM or BB enzymes, and in no case was there any evidence of hybrid formation. Secondly, when the tumor was extracted in isotonic sucrose and the mitochondria isolated by differential centrifugation, it was shown that peak a creatine kinase remained with the mitochondrial pellet, while peak d (BB) remained in the supernatant fraction.

We have examined 33 human tumors for creatine kinase activity and isozyme composition. These data are presented in Table I. Within each type of tumor,

Table I. Creatine Kinase Composition of Various Tumors [1/]

TUMOR		Passage of Tumor in Athymic Mouse	Total U/gm	Per Cent of Total Units BB	MM	Mito	
COLON - Adenocarcinoma							
1. T-245		16	87.0	100	0	0	
2. T-348		13	76.0	98	0	2	
3. T-347		8	37.7	96	2	2	
4. T-362		10	34.1	90	1	7	
5. T-183		30	12.2	95	1	4	
6. T-401		2	11.9	70	10	15	
7. T-219		35	10.9	20	5	70	
8. 84		46	7.0	40	5	50	
9. T-398		6	6.0	14	10	70	
10. T-379		5	4.6	70	5	15	
11. T-380		5	3.3	75	17	3	
12. T-422		Primary	2.1	88	1	8	
RECTAL							
13. T-157		25	422.0	100	0	0	
14. T-348		13	158.0	100	0	0	
LUNG							
15. T-293	Oat Cell	22	59.4	98	0	2	
16. T-404A	Large Cell	3	40.7	94	2	4	
17. T-222	Epidermoid	30	20.4	30	30	40	
18. T-404	Metastasis from 404A	5	10.5	85	5	10	
19. T-402	Adenocarcinoma	3	6.4	45	45	10	
20. T-392	Epidermoid	2	2.7	40	25	35	
21. T-417	(see footnote 2/)	6	1.9	90	5	5	
22. T-291	Adenocarcinoma	20	0.5	60	20	10	
MELANOMA							
23. T-355		15	2.0	0	30	60	(10% MB)
24. T-354	Amelanotic	11	1.6	5	25	50	(2% MB)
25. T-242		35	1.0	50	15	35	
26. TM-21		11	0.6	20	20	40	
BREAST							
27. T-112		19	17.0	50	35	12	
28. T-386		10	9.2	65	5	30	
29. T-378	Chondrosarcoma	4	5.5	93	3	4	
LIVER							
30. Li-16		34	67.5	97	2	1	
31. Li-7		57	38.4	94	2	4	
32. T-23	Sarcoma	21	1.6	85	3	12	
33. T-24	Astrocytoma	59	43.2	95	3	2	

1/ In tumors where the percentage of BB, MM and mitochondrial does not add up to 100, this is due to a small amount of adenylate kinase present which shows up as a small peak near the margin.

2/ This tumor has as yet not been definitely diagnosed. It may be either a primary lung or a metastasized tumor from breast. The patient had existing breast cancer. It is a poorly differentiated adenocarcinoma.

they have been listed in order of decreasing total creatine kinase activity.

While there does not appear to be any unique isozyme pattern associated with

any tumor some general observations can be made: (a) Most of the tumors contain a significant amount of BB isozyme which has usually been considered the fetal type. The rectal tumors have only this form present and appear to contain the highest levels of creatine kinase. The melanomas have a lower content of BB than other tumors. (b) The total creatine kinase activity in the tumors extends over an enormous range with the melanomas having an unusually low content. The melanomas also have significant levels of the MM isozyme. (c) The mitochondrial isozyme is found in some colon tumors, some lung, all of the melanomas and one breast tumor. It is of interest that T-354 and T-355 are melanomas excised from the same patient. One contained large amounts of melanin; the second is amelanotic.

Sera from 21 athymic mice carrying human tumors, as well as sera from three athymic mice which carried no tumors were analyzed for creatine kinase. The three controls showed isozyme profiles containing MM CK, and 2-5% BB. Of the 21 sera from mice carrying tumors, only 5 differed significantly from the controls. Three had significant levels of mitochondrial CK in the serum, which was not found in the controls. These sera were from mice carrying one breast tumor (T-112) and several adenocarcinomas (T-84 and T-219). Two mice carrying rectal cancers (T-348 and T-151) had sera with significantly higher levels of BB than those found in controls.

The athymic mouse may not be a good model for detecting release of CK from tumors since there is a membrane surrounding these tumors which could prevent release of the enzymes. However, it is of interest that the mice with high serum BB were animals with the rectal cancer which have the highest BB levels.

DISCUSSION

Creatine kinase is an important enzyme in regulating cellular energy reserves. It catalyzes the phosphorylation of ADP by phosphocreatine and is thus in a position to maintain the ATP supply. Jacobus & Lehninger (11) suggested that the mitochondrial CK catalyzes the phosphorylation of creatine

from the cytoplasm so that creatine phosphate and not ATP is exported from mitochondria. Thus the four enzymes of CK could be expected to play a key role during growth and development and might be expected to be greatly altered during tumor development. Recently Thomson et al. (12) reported that in some breast cancers the serum levels of the BB isozyme was elevated. Also, Liu et al. (13) reported a cathodally migrating CK in the serum of patients with breast cancer. While the enzyme was not characterized the data would indicate that it is mitochondrial CK. Likewise, Meyer et al. (14) have reported the presence of a variant CK in sera obtained from patients with breast, stomach and large intestine adenocarcinomas that is different from MM and BB. It is of interest to note that except for two of the human tumors, no MB hybrid was detected, and in these two cases the percentage of the hybrid was quite small. Since the MB hybrid can readily be formed in vitro from the parent MM and BB hybrids, there must be specific situations for the formation of MB hybrid in vivo.

While the present observations regarding the distribution of CK in tumor cells allow some broad generalizations, it is clear that much more work needs to be done to clarify the distribution of this enzyme in various tumors as well as normal human tissue. It is important to note that most tumors contain significant levels of the BB isozyme, therefore any diagnostic procedures based on the detection of B-creatine kinase in serum must be cautiously evaluated.

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REFERENCES

1. Eppenberger, H.M., Dawson, D., and Kaplan, N.O. (1967) J. Biol. Chem. 242, 204-209.

2. Jacobs, H., Heldt, H.W., and Klingenberg, M. (1964) Biochem. Biophys. Res. Comm. 16, 516-521.

3. Hall, N., Addis, P., and DeLuca, M. (1979) Biochem. 18, 1745-1751.

4. Roberts, R., and Grace, A.M. (1980) J. Biol. Chem. 255, 2870-2877.

5. Ingwall, J.S., Kramer, M.F., and Friedman, W. (1980) in Heart Creatine Kinase (W.E. Jacobson and J.S. Ingwall, Eds.) p. 9-16, Williams and Wilkins, Baltimore, Maryland.

6. Hall, N., and DeLuca, M. (1975) Biochem. Biophys. Res. Comm. 66, 988-994.

7. Rosalki, S.B. (1967) J. Lab. Clin. Med. 69, 696-705.

8. Hall, N., and DeLuca, M. (1976) Anal. Biochem. 76, 561-565.

9. Giuliani, F.C., and Kaplan, N.O. (1980) Can. Res. 40, 4682-4687.

10. Giuliani, F.C., Zirvi, K.A., and Kaplan, N.O. (1981) Can. Res. 41, 325.-335.

11. Jacobus, W.E., and Lehninger, A.L. (1973) J. Biol. Chem. 248, 4803-4810.

12. Thomson, R.J., Rubery, E.D., and Jones, H.M. (1980) Lancet, 673-675.

13. Liu, T.Z., Shen, J.T., Lee, Y.N., and Shohet, S.B. (1980) Clin. Chem. 26, 1765

14. Meyer, I.J., Thompson, J.A., Ernest, M.S., Kiser, J., and Haven, G.T. (1980) Amer. Soc. Clin. Path. 74, 332-336.