Oncodevelopmental Enzymes of the Dunning Rat Prostatic Adenocarcinoma

Margot Hall, Lawrence Silverman, Allen S. Wenger, and Don D. Mickey

Department of Pathology [M. H., L. S.] and the Division of Urology, Department of Surgery [A. S. W., D. D. M.], School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

ABSTRACT

In this paper, data are presented which demonstrate that adenylate kinase and creatine kinase are oncodevelopmental enzymes in the rat prostate.

The Dunning tumor (dorsal rat prostate) was used as a model system; four sublines of the tumor (R3327-H, R3327-AT, MAT Lu, and MAT LyLu) were studied. The tumor lines were maintained as solid tumors in syngeneic rats (Copenhagen) and as monolayers in tissue culture. The appearance of adenylate kinase with malignant transformation of the dorsal prostate was demonstrated. The disappearance of the CK-M subunit of creatine kinase and decreasing levels of creatine kinase were demonstrated with increasing anaplasia. The lactate dehydrogenase (LDH) concentration increased with increasing anaplasia, and the LDH isoenzyme pattern shifted to a more glycolytic pattern (LDH-4, LDH-5). The malignant isoenzyme pattern was reversible with the use of a differentiating agent (dimethyl sulfoxide).

Prostates from neonatal rats and castrated adult male rats exhibited patterns of creatine kinase and adenylate kinase similar to those of the undifferentiated tumor. The oncofetal isoenzyme pattern of the castrated rat prostate was reversible with physiological levels of exogenous testosterone.

INTRODUCTION

It has been said that cancer is a disease of cell differentiation (30), and indeed, there is considerable morphological and biochemical evidence to support this. The list of oncofetal antigens includes, among others, α-fetoprotein (1), carcinoembryonic antigen (17), and carcinoembryonic antigen (12). Fetal type isoenzyme patterns have also been reported in various types of cancers (15, 26, 40).

The possibility of an oncofetal isoenzyme pattern accompanying prostatic adenocarcinoma has been suggested by Silverman et al. (42, 43), who reported finding elevated serum levels of CK-BB3 in patients with advanced prostatic cancer (42). The link between prostatic cancer and differentiation has also been made by Brawn (5), who reported that prostatic adenocarcinomas dedifferentiate over time.

The dependence of the prostate on normal testicular function is well recognized and has been used as the basis for clinical treatment. Furthermore, prostatic diseases are not seen in patients with impaired testicular or pituitary function (32). While prostatic cancer is rare before age 40, it increases in incidence with increasing age (44). It is known that testosterone/dihydrotestosterone serum levels of the human male prostate decrease with age (9). Since it is also known that some isoenzymes exhibit a developmental pattern, the hypothesis was formulated that malignant prostate cells exhibit an enzyme pattern reflective of an earlier stage of differentiation.

In the present study a rat prostate adenocarcinoma tumor in vivo and in vitro was analyzed for the following enzymes: creatine kinase, adenylate kinase, and lactate dehydrogenase. We report (a) the normal developmental enzyme pattern of the rat prostate; (b) the effect of hormone (testosterone) depletion and replacement on the enzyme patterns of normal rat prostates; (c) a comparison of enzymes from normal and malignant rat prostates; and (d) the effect of a differentiating agent (DMSO) on the enzyme patterns of malignant rat prostates. Two oncodevelopmental enzymes of the malignant rat prostate are proposed.

MATERIALS AND METHODS

Materials

All reagents were reagent grade unless otherwise noted.

Protein and Enzyme Analyses

Cells from tissue culture were washed twice in PBS (Grand Island Biological Co.) and suspended in 2 ml of PBS. Tissues and solid tumors were surgically removed from rats, placed in 2 ml of PBS, stored in liquid nitrogen, and allowed to thaw at room temperature. The cells were disrupted by sonication. Creatine kinase activity was determined by the method of Oliver (34) using a kinetic enzyme analyzer (AminoTech Rotochem, Travenol, Inc.). Optimized substrate reagent (Worthington, Inc.) based on the formulation described originally by Rosalki (39) was used. The reaction uses a coupled enzyme system and measures the production of NADPH spectrophotometrically at 340 nm. The substrate formulation contains an adenylate kinase inhibitor (AMP) and an enzyme stabilizer (N-acetyl cysteine).

Creatine kinase isoenzymes were separated electrophoretically using the Corning ACI agarose electrophoresis system, pH 7.8, at 90 V. The isoenzyme bands were visualized using the Corning CPK reagent substrate based on the work of Roe et al. (38) and then quantitated densitometrically using the Corning ACI fluorescent densitometer. The substrate formulation includes an inhibitor (AMP) to human RBC adenylate kinase and an enzyme stabilizer (N-acetylcysteine). The reaction uses a coupled enzyme system and measures the production of NADPH fluorometrically (excitation wavelength, 365 nm; emission wavelength, 460 nm).

Adenylate kinase activity was determined by the method of Caynys (6) using a kinetic enzyme analyzer (AminoTech Rotochem, Travenol, Inc.). Optimized substrate reagent (Helena, Inc.) based on the formulation originally described by Caynys et al. (7) was used. The reaction uses a coupled enzyme system and measures the production of NADPH spectrophotometrically at 340 nm.

Adenylate kinase was separated electrophoretically from the creatine kinase isoenzymes using a Corning ACI agarose system (90 V; MOPSO...
buffer, pH 7.8), visualized using the Helena CP- (AK) substrate reagent, and measured densitometrically using the Corning ACl densitometer (340 nm). The substrate formulation was originally described by Cayans et al. (7). The reaction uses a coupled enzyme system. CPK isotrol was used as a negative control and human RBC adenylate kinase as a positive control. In high concentration, adenylate kinase could also be visualized on the creatine kinase isoenzyme plates as a band which migrated cathodally.

Lactate dehydrogenase activity was determined by the method of Wroblewski and La Due (46) using a kinetic enzyme analyzer (Aminco Rotochem, Travenol, Inc.). Optimized substrate reagent (Worthington, Inc.) based on the formulation described by Wroblewski and La Due was used (46). The reaction measures the production of NADH spectrophotometrically at 340 nm. Lactate dehydrogenase isoenzymes were separated electrophoretically using a Beckman Paragon Agarose Electrophoresis system (pH 8.2, 100 V). The isoenzyme bands were visualized using the Beckman Paragon LDH substrate based on the formulation originally described by Elevitch et al. (13). The reaction uses a coupled enzyme system and is measured at 600 nm using the Beckman Paragon Densitometer. Ortho LDH isoenzyme control serum was used as a control.

Total protein was determined by the method of Bradford (4) using the Quant T-test kit from Quantimetrix, Inc. Control curves were determined using an albumin-globulin control mixture from Quantimetrix. All enzyme activities were expressed as international units per gram of protein (IU/g). Mean values, standard deviations, and P-values were reported where appropriate. Isotrol (Sigma Chemical Co., St. Louis, MO) and lactate dehydrogenase control (Ortho Diagnostics, Raritan, NJ) were used for control isoenzyme distributions. Ortho normal human serum control (Ortho Diagnostics) was used to validate the enzyme assays. It was possible to detect creatine kinase in the presence of adenylate kinase because the substrate for the creatine kinase assay includes an adenylate kinase inhibitor; and it was possible to detect adenylate kinase in the presence of creatine kinase because there is no creatine phosphate present in the substrate for the adenylate kinase assay.

Tissue Culture

RPMI 1640 medium (Grand Island Biological Co.) enriched with 20% fetal calf serum (heat inactivated), porcine insulin (1 unit/ml), amphotericin B (2.5 μg/ml), and gentamycin (50 μg/ml) was used for all cell culture (45). Cells were incubated at 37°C and at pH 7.4, pO2 was 5%, pCO2 was 18%, and pO2 was 77%.

Surgery

Normal adult male Copenhagen rats (250 g) were used unless otherwise noted. The rats were given sufficient ether to make them drowsy and then anesthetized with a 1:1 mixture of acepromazine maleate (10 mg/ml) (Anecof Co., Inc.) and ketamine hydrochloride (100 mg/ml) (Bristol Laboratories) injected intraperitoneally (dose, 0.1 ml/100 g). Subcutaneous implant or castration was performed, and the animal was given 3.0 ml of PBS (pH 7) intraperitoneally and then warmed under a sun lamp for 1 h. During this time a blood sample was drawn from the tail. Subcutaneous tumor implants consisted of a 4-mm cube of a Dunning tumor (R3327-H, R3327-AT, MAT Lu, MAT LyLu). Subcutaneous capsule implants used testosterone propionate capsules (50 mg/capsule or 5 mg/capsule) (Innovative Research of America, Inc.).

Solid Tissue

Test and control rats were anesthetized as described above. The appropriate tissues (prostate lobes, tumor tissue) were removed and frozen in liquid nitrogen.

Testosterone Analysis

Serum testosterone concentration was determined by the method of Chen et al. (8).

Model System

The Dunning tumor (R3327 H) is a spontaneous malignant tumor of the Copenhagen rat prostate which closely resembles human prostatic adenocarcinoma. Four sublines (R3327-H, R3327-AT, MAT Lu, MAT LyLu) of the Dunning tumor (14) were used in vivo. R3327-H is a well-differentiated, androgen sensitive, non-metastatic, and slowly growing tumor that is believed to have arisen spontaneously from the dorsal lobe of the prostate. The R3327-AT arose from R3327-H. It is an anaplastic, androgen-insensitive, non-metastatic, and quickly growing tumor. Both MAT Lu and MAT LyLu arose from R3327-AT. Each is anaplastic, androgen insensitive, metastatic, and quickly growing. MAT Lu metastasizes to the lung, whereas MAT LyLu metastasizes to both lymph nodes and lung. The MAT LyLu subline (45) was also examined in tissue culture.

Experimental Design

All surgically removed tissue was stored in liquid nitrogen prior to enzyme analysis.

Normal Tissue

Developmental Study. Male Copenhagen rats were sacrificed at 14, 17, 19, 21, 25, 29, 41, and 65 days postnatally. The dorsal prostates were removed surgically and pooled in groups of four. Adult rats were 65 days or older, whereas rats that were 21 days or younger were defined as neonates.

Castrated Rats. Normal adult male Copenhagen rats were castrated and sacrificed at 1, 2, 3, 6, and 10 weeks post castration. The dorsal prostates were removed surgically.

Castrated Rats with Testosterone Implants. Ten normal adult male Copenhagen rats were castrated. Five rats were sacrificed 6 weeks post castration, and their dorsal prostates were removed. Five rats received testosterone implants (physiological levels, i.e., 5 mg capsules) 6 weeks post castration. One week after the testosterone implant they were sacrificed, and their dorsal prostates were removed.

Excess Hormone. Normal rat prostate was grown in medium containing either DHT (0.1 μg/ml) or DHT (2.0 μg/ml) (Sigma Chemical Co.). Cells were harvested after passages 1–12. Normal rat prostate controls were grown in medium without DHT. Testosterone propionate pellets (50 mg/animal) were inserted subcutaneously into five normal adult male Copenhagen rats. After 3 weeks the animals were sacrificed, and their dorsal prostates were removed.

Tumor

Dunning Sublines. Tumors (R3327-H, R3327-AT, MAT Lu, MAT LyLu) were implanted into the flanks of normal adult male Copenhagen rats. Three weeks later the tumors were removed. The dorsal prostate was removed from normal animals and used as a control. The following cell lines were maintained in tissue culture for at least 60 passages: normal rat prostate (NRP); R3327-H; and MAT LyLu (ML). The normal rat prostate cell line was derived from a retired male Copenhagen breeder rat. The tumor cell lines were derived from Dunning tumor taken from the flank of a Copenhagen rat. Tissue was minced into small fragments and incubated with RPMI 1640 medium (Grand Island Biological Co.), supplemented with 20% fetal calf serum, amphotericin B (2.5 μg/ml), and gentamycin (50 μg/ml). The fragments were immobilized under a coverslip, and cells were observed to attach and divide after approximately 24–36 h. The cell monolayer has been serially subcultured (see above) weekly for at least 60 passages.

Differentiating Agent (DMSO). MAT LyLu and R3327-H were grown in culture medium with DMSO (1.5, 2.0, and 2.25% v/v) added. Cells were taken from passage 3 through passage 60. These two sublines were also grown in medium that had the DMSO removed and sampled at 1 and 2 passages after the DMSO was removed. Normal rat prostate...
was used as a control. DMSO (5% v/v) was added to the drinking water of five normal and five tumor-implanted (Mat LyLu) adult male Copenhagen rats. The animals were maintained on this regimen for 3 weeks and then sacrificed. Five naïve rats and five tumor (Mat LyLu)-implanted rats not exposed to DMSO were used as controls. Both normal rat dorsal prostate and primary tumor implants were used for enzyme studies.

Differentiating Agent (Testosterone/DHT). Testosterone propionate pellets (50 mg/animal) were inserted subcutaneously into five normal and ten MAT LyLu-implanted adult male Copenhagen rats. DMSO (5% v/v) was added to the drinking water of five of the tumor-bearing rats. The animals were sacrificed after 3 weeks. Their dorsal prostates and primary tumors were removed. MAT LyLu and MAT LyLu with 2.25% DMSO were grown in cell culture medium containing either DHT (0.1 ng/ml) or DHT (2.0 μg/ml) (Sigma Chemical Co.). Cells were harvested after passages 1–12. Control cells were grown in medium without DHT.

RESULTS

Normal Tissue

Developmental Study. Enzymes were measured from the dorsal prostates of Copenhagen rats ranging in age from 14 days to adulthood (Chart 1). AK activity was higher in the neonatal rat prostate and decreased to zero in the adult prostate. Creatine kinase activity was also elevated in the neonatal rat prostate relative to the adult. The lactate dehydrogenase activity did not change appreciably. Isoenzyme analysis was also performed on these samples. The major finding was an absence of AK in the adult prostate and the presence of AK in the neonate. Prior to 19 days the dorsal prostate exhibited only trace amounts of CK-MM and CK-MB. All five isoenzymes of LDH were present in all of the samples (data not shown). The neonatal rats had serum testosterone levels which ranged from 86 ng/dl for the 14-day rat to 151 ng/dl for the 29-day rat.

Castrated Rat Experiments. Enzymes were measured on dorsal prostates taken from rats at times ranging from 0–10 weeks post castration (Chart 2). Both creatine kinase and adenylate kinase activities were increased post castration. There was no demonstrable change in lactate dehydrogenase activity. Isoenzyme analysis was also performed on these samples. The adenylate kinase band increased, while the CK-MM and CK-MB bands decreased with castration. The lactate dehydrogenase exhibited all five isoenzyme bands similarly to the normal adult male (data not shown). All castrated rats had serum testosterone levels of <25 ng/dl (normal adult levels, 85–500 ng/dl) and exhibited progressive involution of the prostate.

Castrated Rats with Exogenous Testosterone. Enzymes were measured on dorsal prostates of adult male Copenhagen rats 6 weeks post castration and on rats comparably treated but with 1 week of subsequent exogenous testosterone at physiological level (5 mg/rat) (Table 1). Creatine kinase and adenylate

Chart 1. Developmental enzymes of the dorsal rat prostate: AK, AK, and LDH reported in IU/g (4–10 prostates were pooled for each age). Electrophoretic enzyme distribution estimated as ++, +, ±, or −.

Chart 2. Enzymes of dorsal prostates taken from castrated normal adult rats: CK, AK, and LDH reported in IU/g, one sample per point. Electrophoretic enzyme distribution estimated as ++, +, ±, or −.

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>Testosterone</th>
<th>CK (IU/g)</th>
<th>AK (IU/g)</th>
<th>LDH (IU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal prostate, castrated rat</td>
<td>−</td>
<td>4759 ± 606a (5)</td>
<td>171 ± 88 (5)</td>
<td>780 ± 422 (5)</td>
</tr>
<tr>
<td>Dorsal prostate, castrated rat</td>
<td>+</td>
<td>1910 ± 35 (2)a</td>
<td>46 ± 2 (2)a</td>
<td>385 ± 10 (2)a</td>
</tr>
<tr>
<td>Normal adult, dorsal prostate</td>
<td>−</td>
<td>2218 ± 593 (4)a</td>
<td>0 (6)a</td>
<td>946 ± 328 (7)a</td>
</tr>
</tbody>
</table>

* Mean ± SD.

 Numbers in parentheses, number of determinations.

a p < 0.05.

b p < 0.001.
kinase activities were increased in the castrated rat and returned to nearly normal levels after 1 week of physiological concentration of exogenous testosterone. Lactate dehydrogenase activity did not change significantly with hormone manipulation. Isoenzyme analysis was performed on these samples (Table 2). Prostates from castrated rats exhibited trace amounts of CK-MB but did not change significantly with hormone manipulation. Isoenzyme analysis was performed on these samples (Table 2). Prostates from castrated rats exhibited trace amounts of CK-MB but did not change significantly with hormone manipulation.

**Excess Hormone.** There was no significant change in the enzyme concentrations or isoenzyme patterns (data not shown) of either normal rat prostate or MAT LyLu (MLL) grown in tissue culture in the presence of two concentrations of DHT.

Enzymes were measured in solid tissues from whole animals that were maintained on a regimen of 10 times physiological dosage of testosterone (50 ng/rat implant) (data not shown). There was a very slight increase in the adenylate kinase and lactate dehydrogenase levels of the normal dorsal prostate in rats treated with testosterone. There was no change in the creatine kinase levels. There was no difference in the isoenzyme patterns of treated and control animals. Serum testosterone analysis was performed, and each animal served as his own control. The testosterone level of the control animals was 189 ± 76 ng/dl, and the testosterone level of the implanted animals was greater than 1526 ng/dl.

**Tumor Sublines.** Enzyme activities were measured in prostates from normal adult male Copenhagen rats and the Dunning tumor sublines R3327-H, R3327-AT, MAT Lu, and MAT LyLu (Table 3). We observed a decrease in total CK, an increase in total LDH, and the appearance of AK accompanying increasing anaplasia. The creatine kinase decreased, while the adenylate kinase increased with increasing anaplasia. The LDH increased slightly in R3327-H and did not change in Mat LyLu. The isoenzyme patterns were also examined (Table 6). The creatine kinase/adenylate kinase pattern shifted from a pattern of mostly CK-BB (CK-BB >50% quantitated by densitometry) to a predominantly AK pattern (little or no CK-BB) with increasing anaplasia. There was an increase in growth rate, loss of contact inhibition, and increased rounding up of the cells with increasing anaplasia (data not shown).

**Use of a Differentiating Agent (DMSO).** Enzyme activities 

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>Testosterone</th>
<th>AK</th>
<th>MM</th>
<th>MB</th>
<th>BB</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal prostate (castrated rat)</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dorsal prostate (castrated rat)</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Normal adult, dorsal prostate</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2

In vivo effect of exogenous testosterone on enzymes of prostates from castrated rats

Physiological levels of exogenous testosterone were administered for 1 week to rats castrated 6 weeks previously. Control animals were sacrificed 6 weeks post castration.

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>CK (IU/g)</th>
<th>AK (IU/g)</th>
<th>LDH (IU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal dorsal prostate</td>
<td>75 ± 15 (5)</td>
<td>6 (1)</td>
<td>5454 ± 814 (7)</td>
</tr>
<tr>
<td>R3327-H</td>
<td>39 ± 10 (5)</td>
<td>37 (1)</td>
<td>7348 ± 1144 (7)</td>
</tr>
<tr>
<td>MAT LyLu</td>
<td>57 ± 11 (6)</td>
<td>52 ± 26 (9)</td>
<td>5639 ± 26 (7)</td>
</tr>
</tbody>
</table>

Table 3

In vivo enzymatic activity of rat prostates

Solid tissue from adult male Copenhagen rats was used. Unpaired t-test was applied to the data with the value for normal dorsal prostate used as control.

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>CK (IU/g)</th>
<th>LDH (IU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal dorsal prostate</td>
<td>2218 ± 593 (4)</td>
<td>946 ± 328 (7)</td>
</tr>
<tr>
<td>R3327-H</td>
<td>628 ± 182 (8)</td>
<td>1927 ± 350 (8)</td>
</tr>
<tr>
<td>R3327-AT</td>
<td>445 ± 108 (9)</td>
<td>4334 ± 694 (8)</td>
</tr>
<tr>
<td>MAT Lu</td>
<td>155 ± 43 (12)</td>
<td>4454 ± 898 (9)</td>
</tr>
<tr>
<td>MAT LyLu</td>
<td>78 ± 28 (10)</td>
<td>4801 ± 974 (6)</td>
</tr>
</tbody>
</table>

Table 4

In vivo electrophoretic enzyme distribution of rat prostates

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>CK (IU/g)</th>
<th>LDH (IU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat prostate</td>
<td>7348 ± 1144 (7f)</td>
<td>5639 ± 26 (7)</td>
</tr>
<tr>
<td>R3327-H</td>
<td>4334 ± 694 (8)</td>
<td>5454 ± 814 (7)</td>
</tr>
<tr>
<td>MAT LyLu</td>
<td>39 ± 10 (5)</td>
<td>7348 ± 1144 (7)</td>
</tr>
</tbody>
</table>

Table 5

In vitro enzymatic activity of rat prostates

Cell culture was done on a normal rat prostate line and on two cell lines established from the Dunning rat tumor. The unpaired t-test was applied to the data using normal rat prostate as the control.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CK (IU/g)</th>
<th>LDH (IU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat prostate</td>
<td>57 ± 11 (6)</td>
<td>52 ± 26 (9)</td>
</tr>
<tr>
<td>R3327-H</td>
<td>7348 ± 1144 (7)</td>
<td>5639 ± 26 (7)</td>
</tr>
</tbody>
</table>

Table 6

In vitro electrophoretic enzyme distribution of rat prostates

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CK (IU/g)</th>
<th>LDH (IU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat prostate</td>
<td>7348 ± 1144 (7f)</td>
<td>5639 ± 26 (7)</td>
</tr>
<tr>
<td>R3327-H</td>
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</tr>
<tr>
<td>MAT LyLu</td>
<td>39 ± 10 (5)</td>
<td>7348 ± 1144 (7)</td>
</tr>
</tbody>
</table>

Table 7

In vitro enzymatic activity of rat prostates

Cell culture was done on a normal rat prostate line and on two cell lines established from the Dunning rat tumor. The unpaired t-test was applied to the data using normal rat prostate as the control.
were measured in cell lines established from normal adult rat prostate and the Dunning tumor subline MAT LyLu (MLL) treated with a differentiating agent, DMSO (Table 7). There was no appreciable change in either AK or LDH with DMSO treatment. By contrast, the total CK, primarily CK-BB, increased with increasing concentrations of DMSO. With removal of the DMSO, creatine kinase returned to its pretreatment level. Isoenzymes were measured in these samples (Table 8). Adenylate kinase decreased, and the CK isoenzyme pattern shifted dramatically toward increasing concentrations of CK-BB with increasing DMSO. This shift was reversible with the removal of DMSO from the medium. There was a quantitative increase in the amount of LDH-4 with increasing concentration of DMSO. Again the effect was seen to be reversible with the removal of DMSO. In addition, the growth rate of MLL was decreased in the presence of DMSO, and the cellular morphology became less spindle-shaped and more round (data not shown).

Enzyme activities were measured on tissues removed surgically from animals that had 5% DMSO added to their drinking water (data not shown). The DMSO treatment did not appear to have any appreciable effect on the creatine kinase levels of either the dorsal prostates of Mat LyLu implanted rats or of the surgically unmanipulated rats. However, the total creatine kinase increased in the primary tumors of rats treated with 5% DMSO relative to the untreated rats. There was no change in either adenylate kinase or lactate dehydrogenase levels above that seen in the control samples. Isoenzyme analyses on these samples revealed no differences between the primary tumors in the DMSO-treated animals and the untreated animals (data not shown). DMSO causes a quantitatively significant increase in creatine kinase (more normal pattern) in the primary tumor.

**DISCUSSION**

The main findings of this study were the appearance of adenylate kinase and the loss of the CK M-subunit of creatine kinase with loss of differentiation. The data presented in this paper support the hypothesis of an oncopetal enzyme pattern (CK and AK) in the malignant rat prostate.

In order to establish the normal developmental enzyme pattern, prostate tissues were obtained from normal rats varying in age from 14 days to adulthood and assayed for CK, AK, and LDH. Fetal prostates were not tested because of the difficulty in sexing these immature animals. In the developmental study the neonatal rat prostate exhibited increased AK and CK and decreased CK-M subunit when compared with the normal adult prostate.

Differentiation has been shown to be dependent on hormonal status in other systems (18, 20), and the androgen-dependent nature of prostatic epithelium is well known (25). Therefore, hormonal manipulation was used to simulate the different stages of maturation (differentiation). The neonatal enzyme pattern was duplicated in prostates from castrated (decreased testosterone) normal adult male rats. There was progressive involution of the normal adult male prostate with increasing time post castration. These prostate tissues concomitantly exhibited increased adenylate kinase and creatine kinase (decreasing CK-M) when compared with the normal adult prostate. The enzyme pattern in the prostates of castrated rats could be reversed by administering physiological dosages of exogenous testosterone to the animals prior to sacrificing them. In further experiments, pharmacological levels of exogenous testosterone (in vivo) had a negligible effect on the enzyme and isoenzyme patterns of normal rat prostates, as did DHT in a normal rat prostate cell line. From these data we concluded that adenylate kinase and creatine kinase (CK-B subunit) are fetal enzymes found in the rat prostate and that the adult enzyme pattern is induced under the stimulus of testosterone/dihydrotestosterone.

The Dunning tumor is a spontaneous malignant neoplasm derived from the dorsal lobe of the Copenhagen rat prostate. Four sublines (R3327-H, R3327-AT, MAT Lu, and MAT LyLu) of the Dunning tumor were compared with normal dorsal rat prostate. The sublines varied in their degree of differentiation, growth characteristics, and metastatic capabilities. We observed the appearance of adenylate kinase, the disappearance of CK-M subunit (decreasing creatine kinase), and increasing amounts of lactate dehydrogenase with a glycolytic shift towards LDH-5 accompanying increasing anaplasia in the solid tumors. The normal and malignant enzyme pattern was recapitulated in vitro (normal rat prostate, R3327-H, and MAT LyLu). Thus the malignant cells expressed enzyme patterns (increased AK, decreased CK-M) similar to those of the prostates obtained from neonates and castrates.

In an attempt to change the enzyme pattern of the anaplastic tumor (Mat LyLu), a differentiating agent, DMSO, was added to the cells in vitro. Dimethyl sulfoxide partially reversed the enzyme pattern of MAT LyLu and decreased the growth rate (data not shown). The removal of DMSO from the culture medium caused a...
ONCODEVELOPMENTAL ENZYMES OF PROSTATE CANCER

...the cells to revert to their faster growth rate and enzyme pattern. We concluded that DMSO allowed the anaplastic MAT LyLu cells to become more differentiated and to express a more normal adult enzyme pattern. The reversibility of DMSO-induced enzyme expression argues against cytotoxicity of the drug for a subpopulation of the cell line and supports the concept of an altered state of differentiation. In vivo, DMSO had little effect on the anaplastic MAT LyLu tumor enzyme expression. Perhaps this was due to low serum/tissue concentrations of the drug achieved, since DMSO was presented to the animals as a 5% solution in their drinking water. In control experiments pharmacological levels of dihydrotestosterone and testosterone had a negligible effect on the enzyme patterns of MAT LyLu both in vitro and in vivo. These data support the concept that MAT LyLu has no functional androgen receptors (27).

The results of this study support the hypothesis of an oncofetal enzyme pattern (AK and CK) in the malignant rat prostate. While others have looked at enzymes in nonprostatic tissues and in serum from patients with prostatic cancer, this is the first report measuring these enzymes in the prostate. Our data are consistent with those of Turley,4 who reported an increase in adenylate kinase in a variety of malignant human tumors. Adenylate kinase has also been reported as elevated in serum of human leukemia patients (31). Criss et al. (11) reported finding adenylate kinase decreased in rat hepatoma, and Greengard et al. (19) reported decreases in adenylate kinase seen with human lung cancer tissue.

Our work is also consistent with the work of Silverman et al. (42, 43), who reported finding elevated levels of CK-BB in serum from patients with extracapsular prostatic cancer. Hoag et al. (24) and Lemar et al. (29) also reported increased human serum CK-BB in patients with prostatic carcinoma. Hoag et al. (24) have also reported elevated levels of CK-BB in the serum of a patient with testicular cancer, and Coolen and Pregay (10) have reported similar findings in patients with oat cell carcinoma of the lung. Hall et al. (22) have reported finding significantly elevated levels of CK-BB in human amniotic fluid in a case of teratocarcinoma. Our data are also supported by the work of Oliver et al. (35) and Hein et al. (23), who reported finding an increase in the LDH 5:1 ratio of human malignant prostate. However, in our study there was no correlation between the glycolytic LDH shift seen with cancer and the neonatal pattern.

We demonstrated the reappearance of fetal enzymes (AK and CK-BB) and the disappearance of adult isoenzymes (CK-MM and CK-MB) in the malignant rat prostate. Since our sublines are composed of heterogeneous cell types, there is no proof that the enzymes are all being synthesized by the same cell type. However, the positive regulation of fetal gene expression and the concomitant down regulation of adult gene expression suggest increased expression of a cellular oncogene (3) and not expansion of a single clone.

It would be most useful to have a definitive marker of differentiation comparable to the synthesis of hemoglobin by erythrocytes in the Friend leukemia cell system (16). In the absence of a good differentiation marker, one can look for changes that mimic the maturational changes. In our study, the changes in enzyme patterns (in vitro) following the administration of DMSO were comparable to the changes seen in the developmental study. This strongly suggests that DMSO was inducing differentiation and not acting as a selective cytotoxic agent. Reversion of the cells to their original growth and enzyme pattern upon removal of DMSO from the medium substantiated true differentiation of the cell line.

There have been reports (21, 37) of changes in serum prostatic acid phosphatase accompanying malignant transformation. However, in our system the prostatic acid phosphatase levels were too low to be reliable. This may have been due to the insensitivity of the methodology used (enzymatic inhibition).

There are a number of genetic and epigenetic models that are consistent with our data. One possible explanation for the reappearance of fetal enzyme expression (AK and CK-BB) would include the turning of a c-oncogene (2, 3). This could result from mutation or rearrangement of a regulatory gene leading to increased transcription of oncogenes and oncodevelopmental proteins. One might expect to see positive regulation of promoter sequences controlling fetal gene expression and down regulation of other promoters controlling adult gene expression. Such a model would explain the concomitant appearance of AK and disappearance of CK-M. The model would also explain: the loss of hormonal control of the undifferentiated tumor (MAT LyLu); the rapid growth; the morphological/histological changes of the tumor which are at least partially reversible with the use of a differentiating agent (DMSO); the fetal/adult enzyme pattern; the metastasis (similar to fetal cell motility); and the escape from the host's immune system. Another possible explanation would consist of a chromosomal rearrangement involving the translocation of a promoter sequence (28) from the region of the CK-M gene locus to the region of the AK locus. This model would require that the promoter(s) also affect the genes coding for growth, cell motility, hormonal dependence, morphology, and antigenicity. The MAT LyLu karyotype (45), which includes several marker chromosomes with translocations, would support either this model or the oncogene model, depending on which gene promoter(s) is being translocated. Unfortunately, we do not know the location of the creatine kinase or adenylate kinase genes in rats. The insertion of extra genetic material (36) leading to increased production of specific mRNAs is another very attractive explanation, as it would reflect the increase in chromosomal number (modal number = 58) seen in MAT LyLu (45). If the extra genetic material contained an oncogene, one could easily rationalize the observed data. Other possible explanations include: gene amplification (41); the clonal expansion of primitive cells which naturally produce low titers of the oncodevelopmental enzymes and not the adult form of the enzymes (33); and the unmasking of an operator leading to constitutive transcription of enzyme (28). It is difficult to see how any of these would lead to all the observed changes in the malignant prostate. Gene amplification would not explain the decreased synthesis of CK-M. Clonal expansion of primitive cells would not explain a chromosomal modal number of 58. The constitutive transcription model would require that both adenylate kinase and LDH-5 be controlled by the same operator, which is unlikely unless one hypothesizes the derepression of an C-oncogene operator. In contrast, the data is explicable if a mutation, genetic rearrangement (translocations), or insertion of extra genetic material leads to the promotion of oncofetal transcription.

In this study we quantitated enzymes in normal and malignant prostatic tissue. This allowed us to correlate our findings directly

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4 P. Turley, private communication, Arkansas Children's Hospital, Little Rock, AR.
with the tissue source. Other authors have looked at enzymes in serum, a technique which has the obvious disadvantage of reflecting the physiology of the entire body and not just the prostate. While it would be indeed useful to develop a screening test for human prostatic cancer using serum enzyme levels, we felt that it was essential to first demonstrate that there is a correlation between enzyme levels and prostate-specific disease. The development of an immunoassay should improve sensitivity to the lower enzyme concentrations found in serum and in ejaculate and lead to potentially useful clinical screening tests.

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Oncodevelopmental Enzymes of the Dunning Rat Prostatic Adenocarcinoma


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