Animal Models of the Hormone-sensitive and -insensitive Prostatic Adenocarcinomas, Dunning R-3327-H, R-3327-HI, and R-3327-AT

John T. Isaacs, Warren D. W. Heston, Robert M. Weissman, and Donald S. Coffey

Abstract

The Dunning R-3327-H is a well-differentiated transplantable rat prostatic adenocarcinoma that contains both hormone-sensitive and -insensitive cells. The component composed of hormone-insensitive cells has been permitted to grow in a castrated male, and a new slow-growing, well-differentiated hormone-insensitive subline of the tumor has been established and designated R-3327-HI. In addition, a rapidly growing hormone-insensitive anaplastic tumor has been developed, R-3327-AT. These three tumor lines have been characterized, and their histological and biochemical profiles are compared.

Introduction

The treatment for disseminated human prostatic cancer has remained essentially unchanged for the past 35 years. Androgen ablation through castration or estrogen therapy is still the major treatment, even though this therapy provides only a questionable or limited extension of life span and very few permanent remissions. The almost universally observed relapse from hormonal therapy is due to the growth of hormone-insensitive cells. At present, adequate hormonal therapy is available to regulate the growth of androgen-sensitive cells; however, only limited insight has been obtained into the control of hormone-insensitive cells.

The investigation of this complex problem has been assisted by the availability of several animal models that appear to be appropriate for the study of prostatic cancer (4, 18, 19, 23, 24). Selecting a single animal model for prostatic cancer may not be realistic because the human cancer counterpart is itself a variable and multifaceted disease. Prostatic adenocarcinoma in humans is often characterized by diversity in relation to pathology, state and variability of cellular differentiation, uniformity of growth rate, and differences in therapeutic responsiveness to hormonal, nonhormonal, and radiation treatment. Indeed, many of these variables can sometimes be observed within one patient. Because of this variability in the types of human prostatic cancer, it is possible that more than one animal model may be required to correspond to these different states of human cancer. This study provides the comparative characterization of 3 forms of transplantable rat prostatic adenocarcinomas: R-3327-H, a well-differentiated hormone-sensitive tumor; R-3327-HI, a similar tumor that is slow growing, well differentiated, and hormone insensitive; and R-3327-AT, a rapidly growing anaplastic tumor, which is also hormone insensitive. All of these transplantable prostatic tumors originated as sublines from the original Dunning R-3327, which was first described by W. F. Dunning in 1961 as a spontaneous tumor originating in the prostate of an aged, syngeneic Copenhagen rat (4). The tumors can also be carried s.c. in F1 male hybrids of Fischer female cross. The original tumor is hormone sensitive and metabolizes testosterone to dihydrotestosterone (23). The R-3327-H hormone-sensitive tumor has been characterized in detail in relation to growth properties, morphology, histochemistry, and therapeutic responsiveness (18, 19).

Materials and Methods

The Dunning R-3327-H hormone-sensitive prostatic adenocarcinoma was originally obtained from the Mason Research Institute and is carried in the F1 hybrids of male Copenhagen x female Fischer rats. This R-3327-H tumor gave rise in our laboratory to the R-3327-H1 (hormone-insensitive) and R-3327-AT (anaplastic tumor) lines.

The animals were housed in cages containing 3 to 5 animals. One Copenhagen breeding male serviced 3 to 4 female rats. With these methods healthy litters with a survivorship exceeding 90% were obtained once the good breeding mothers were selected. The F1 hybrid adult males 60 to 80 days old carried the tumor lines and were given s.c. transplants of cell suspensions of the tumors. Once the tumor had reached 2 cm in diameter, it was excised, minced with scissors, passed through 2 wire mesh gauze strainers (1.5 and 0.5 mm), and resuspended in cold Roswell Park Memorial Institute Tissue Culture Medium 1640. Cell suspensions were then made up to a dose of 10^6 cells/ml. The resultant cell suspension (1 ml) was then injected s.c. into the flank of the recipient animal.

After the s.c. injection of tumor cell suspension, tumors were measured in all animals at various times with microcalipers. Three diameters of the mass were determined, and the tumor volume was calculated according to the formula \( V = \frac{4}{3} \pi r^3 \) described by Janek et al. (10). When the tumor was excised, the actual volume and tumor weight were measured. Total tissue DNA, RNA, and protein were measured according to the method described by Coffey et al. (1). Knowledge of the tumor volume and weight and the total DNA/mg tissue, as well as the DNA content/isolated nucleus, made it possible to determine the total number of cells per tumor.

The measured tumor volumes along the growth curve of each tumor were analyzed semilogarithmically. The slope...
of the semilog plot, determined by statistical linear regression, determined the actual growth rate of each tumor, as described previously (19).

Tumor biopsies, all normal sex accessory tissue, and the control tissues of liver and kidney were stained routinely with hematoxylin and eosin, periodic acid-Schiff reagent, and Masson trichrome stain.

All enzyme assays were performed on aliquots of whole homogenates of tissue. Homogenization was by means of an all-glass conical homogenizer. Tissue was diluted with 50 mM Tris buffer, pH 7.4, to a final concentration of 1:10 (w/v). 5α-Reductase was assayed by the method of Moore and Wilson (14). 3α- and 3β-hydroxysteroid dehydrogenase was assayed according to the method of Jacobi and Wilson (9). 7α-Hydroxylase was assayed by the method of Isaacs and Coffey. Acid phosphatase was assayed by the method of Roy et al. (17). Alkaline phosphatase determinations were according to the method of Roy (16). The method of Talalay et al. (21) was used for β-glucuronidase determinations. γ-Glutamyltransferase was assayed according to the method of Tate and Meister (22). Leucine aminopeptidase was assayed by the methods of Goldbarg and Rutenberg (5). Glucose-6-phosphate dehydrogenase was assayed according to the method of Taketa and Watanabe (20). Glutathione reductase was assayed by the method of Pinto and Bartley (15). Catalase was assayed by the method of Luek (12). LDH was assayed according to the method of Kornberg (13). LDH isozymes were determined by the procedure of Dietz and Lubrano (3).

Results and Discussion

**Hormone-sensitive R-3327-H.** The histology of this well-differentiated adenocarcinoma indicates abundant acini with secretions, and the pathology is almost identical at the light and electron microscopic level with that of a well-differentiated human prostatic adenocarcinoma (19). The tumor epithelial cell plasma membranes contain microvilli and secretory granules. The cell and nuclei shapes appear malignant.

Cell kinetic and growth rate studies indicate that 93% of the inoculated tumor cells grow s.c. in an adult intact male; the doubling time of the tumor is approximately 20 days. The absence of androgen (obtained by castration or estrogen or antiandrogen therapy) reduced the size of the tumor growth by 84 to 92% (Table 1).

Although the R-3327-H tumor attains a larger size when grown for 6 months in the presence of androgens, some limited growth was observed even in the castrate animals. When the growth of the tumor in the castrate was monitored and the log of the tumor volume was plotted against time, a straight line was obtained, which did not extrapolate to the origin (for details see Ref. 19). These cell kinetic studies indicated clearly that a specific fraction or clone (8 to 29%) of the total original tumor cells inoculated were hormone-insensitive cells that were capable of growing in the absence of androgens.

**Hormone-insensitive R-3327-HI.** When the R-3327-H tumor was placed in a castrate animal and grown for 180 days, the small fraction or clone of hormone-insensitive cells within the original inoculum continued to grow at a similar tumor-doubling rate of approximately 25 days (Table 1). The fraction of tumor cells growing in the castrate for 180 days in the absence of hormone yielded a different histology (compare Fig. 1). This hormone-insensitive tumor is composed of small acini possessing secretion, and the epithelial cells appear almost cuboidal. This hormone-insensitive tumor tissue has been removed, transplanted, and carried in castrate animals as a new slow-growing hormone-insensitive prostatic adenocarcinoma line termed R-3327-HI. The histology and growth rate of this tumor has remained essentially constant through multiple passages. The importance of this new line (R-3327-HI) is that it represents the type of cells that grow following relapse to hormonal therapy, the type of cells requiring nonhormonal or special therapeutic considerations. Like human prostatic adenocarcinomas, castration and estrogen therapy will not cure animals carrying the R-3327-H tumor because of the small clone of these hormone-insensitive cells (R-3327-HI) that continues to grow.

Markland and Lee (13) reported that the Dunning tumor contained androgen and estrogen cytoplasmic receptors. These studies have been extended by Heston et al. (8), who have compared the receptors in the hormone-sensitive (R-3327-H) and -insensitive (R-3327-HI) cells. The insensitive R-3327-HI line has a 68% reduction in the cytoplasmic androgen-binding protein, but the level is still higher than in the normal dorsal-lateral prostate tissue (8). In contrast, the cytoplasmic estrogen receptor level does not change and remains in both tumors almost 10-fold higher than in the normal prostate. Small levels of the progesterone receptor appear in the hormone-insensitive lines (8).

**Anaplastic Tumor (R-3327-AT).** A third tumor line, a hormone-insensitive anaplastic tumor, has arisen from the original R-3327-H on a transfer to intact males. The reason for the transformation is unknown. This rapidly growing anaplastic tumor grows equally well in female, intact male, and castrate male rats and has remained stable through over 50 passages.

The R-3327-AT tumor does not contain acini structures but contains sheets of anaplastic cells, and there is no evidence of secretory activity. The histology of all 3 tumor lines is compared in Fig. 1.

Comparison of the 3 Types of Prostatic Tumors. The general properties of the 3 tumor lines are compared in Table 2. The R-3327-H tumor was thought to arise originally from the dorsolateral prostate of the Copenhagen rat. The enzymatic profile of 13 different enzymes is compared for each of the 3 tumors and for the normal sex accessory tissues. The activity is expressed on a cellular basis by normalizing to a unit amount of DNA (see Table 3). A comparison of the relative rates, normalized to 1.0 for the R-3327-H tumor, indicates the similarity of the enzymatic profile to that of the dorsolateral prostate (Chart 1).

As the hormone-sensitive tumor (R-3327-H) becomes hormone insensitive (R-3327-HI) or anaplastic (R-3327-AT), there is a marked shift in the enzymatic profile, with a progressive decrease in 5α-reductase, 7α-hydroxylase, and

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9 J. Isaacs and D. S. Coffey. Characterization of the 3β Hydroxy Steroid 7α-Hydroxylase Activity of Rat Ventral Prostate, submitted for publication.

4 The abbreviation used is: LDH, lactic dehydrogenase.
Rat Prostatic Adenocarcinomas

Table 1

<table>
<thead>
<tr>
<th>Hormonal status</th>
<th>Tumor inoculations containing $1.5 \times 10^5$ viable R-3327-H tumor cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>6-mo. treatment&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intact</td>
<td>None</td>
</tr>
<tr>
<td>Intact</td>
<td>Androgen (TP)</td>
</tr>
<tr>
<td>Intact</td>
<td>Estrogen (DES)</td>
</tr>
<tr>
<td>Intact</td>
<td>Antiandrogen (flutamide)</td>
</tr>
<tr>
<td>Castrate</td>
<td>None</td>
</tr>
<tr>
<td>Castrate</td>
<td>Androgen (TP)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Animals were treated daily as indicated with testosterone propionate (TP), 20 mg/day; diethylstilbestrol (DES), 100 μg/kg/day; or flutamide, 50 mg/kg/day.

Table 2

Properties of the transplantable Dunning prostatic tumors in rats

<table>
<thead>
<tr>
<th>Type</th>
<th>Origin</th>
<th>Histology</th>
<th>Androgen sensitivity</th>
<th>Growth rate</th>
<th>Tumor doubling time</th>
<th>Metastatic rate</th>
<th>Biochemical profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-3327-H</td>
<td>Well-differentiated prostatic adenocarcinoma</td>
<td>Large, well-developed acini filled with secretions</td>
<td>Hormone sensitive; maximal growth in intact adult males; heterogeneous, 80% of cells are hormone sensitive and 20% are hormone insensitive</td>
<td>Slow</td>
<td>15-20 days</td>
<td>&lt;1%</td>
<td>Enzyme profile of tumor similar to dorsal lateral prostate tissue; moderate activity of 5α-reductase; androgen and estrogen cytoplasmic receptors present; large tumor mass reduces size of sex accessory tissue and elevates serum acid phosphatase levels</td>
</tr>
<tr>
<td>R-3327-HI</td>
<td>Well-differentiated prostatic adenocarcinoma</td>
<td>Smaller acini with less secretions</td>
<td>Hormone insensitive; growth rate equal in both intact and castrate males</td>
<td>Slow</td>
<td>15-20 days</td>
<td>Probably &lt;1%</td>
<td>Moderate levels of 5α-reductase; decrease in level of androgen cytoplasmic receptor, but no decrease in estrogen receptor; appearance of progesterone receptor; elevated serum acid phosphatase levels</td>
</tr>
<tr>
<td>R-3327-AT</td>
<td>Anaplastic prostatic tumor; not a squamous cell carcinoma</td>
<td>No acini; sheets of anaplastic cells</td>
<td>Hormone insensitive; growth rate equal in both intact and castrate males</td>
<td>Fast</td>
<td>2 days</td>
<td>Slow local invasion to lymph nodes, but no distant metastasis detected</td>
<td>Low biochemical correlation to normal lobes of rat prostate; low in 5α-reductase activity; androgen receptor absent; no effect of tumor mass on sex accessory tissue; no increase in serum acid phosphatase levels</td>
</tr>
</tbody>
</table>

alkaline phosphatase activity. In contrast, other enzymes increase, such as 3α-hydroxysteroid dehydrogenase and LDH (Chart 2).

The increase in LDH was resolved into the 5 isozyme patterns (I to V). This was of interest because Denis et al. (2) reported a relative increase in the LDH V isozyme of prostatic carcinoma but not for benign prostatic hyperplasia. Goldman et al. (6) have also shown a preferential increase
Table 3
Comparison of enzyme profiles in normal sex accessory tissue and Dunning tumors

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal tissue</th>
<th>Tumor tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seminal vesicles</td>
<td>Ventral prostate</td>
</tr>
<tr>
<td>5α-Reductase</td>
<td>12.8 ± 4.84</td>
<td>32.9 ± 5.62</td>
</tr>
<tr>
<td>3α-Hydroxysteroid reductase</td>
<td>841 ± 235</td>
<td>2080 ± 574</td>
</tr>
<tr>
<td>3β-Hydroxysteroid reductase</td>
<td>365 ± 17</td>
<td>138 ± 46</td>
</tr>
<tr>
<td>7α-Steroid hydroxylase</td>
<td>200 ± 53.5</td>
<td>5000 ± 500</td>
</tr>
</tbody>
</table>

Hydrolytic enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal tissue</th>
<th>Tumor tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>8.5 ± 0.47</td>
<td>10.9 ± 0.52</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>291 ± 9.4</td>
<td>615 ± 97.7</td>
</tr>
<tr>
<td>α-Glucuronidase</td>
<td>6.35 ± 1.18</td>
<td>7.8 ± 1.15</td>
</tr>
<tr>
<td>γ-Glutamyl transferase</td>
<td>318 ± 55</td>
<td>4.6 ± 0.58</td>
</tr>
<tr>
<td>Leucine aminopeptidase</td>
<td>5 ± 0.59</td>
<td>13.9 ± 0.63</td>
</tr>
</tbody>
</table>

Metabolic enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal tissue</th>
<th>Tumor tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>112 ± 11.8</td>
<td>75 ± 5.8</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>71 ± 11.8</td>
<td>81 ± 5.8</td>
</tr>
<tr>
<td>Catalase</td>
<td>22.4 ± 1.5</td>
<td>25.3 ± 2.9</td>
</tr>
<tr>
<td>LDH</td>
<td>1053 ± 47</td>
<td>948 ± 144</td>
</tr>
</tbody>
</table>

a Enzyme units: a, pmol/hr/100 μg DNA; b, nmol/min/100 μg DNA; c, μmol/min/100 μg DNA.
Chart 1. Comparisons of the enzymatic profile of the dorsal-lateral and ventral prostate to the R-3327-H tumor. All activities are expressed as relative values compared to the activities of the R-3327-H tumor, the levels of which are set at 1.0. dehyd., dehydrogenase.

Chart 2. Comparisons of the enzymatic activities in the various Dunning tumor lines. Values are relative to the activities of the R-3327-H tumor, the levels of which are assigned a value of 1.0. dehyd., dehydrogenase.

Chart 3. LDH isozyme patterns. The relative migration rate is in decreasing order from LDH I to LDH V.

in LDH V isozymes for many different malignant tissues in comparison to benign tumors. Grayhack et al. (7) have presented evidence that the prostatic cancer causes an increase in the LDH V:LDH I ratio above 2 in expressed prostatic fluid but not from benign prostatic hyperplasia. It was of interest to compare the LDH isozyme pattern. There was a marked increase in the LDH V isozyme patterns in all of the tumors (Chart 3), and isozyme V increased with increasing levels of total LDH. The meaning of this shift in isozyme pattern has not been resolved.

In summary, a spectrum of animal models for prostatic cancer are now available and have been characterized. The Dunning R-3327 series mimics many of the properties of human prostatic cancer including initial response to hormonal therapy followed by relapse to a hormone-insensitive state. It is anticipated that these animal models will provide new insight into the understanding of prostatic cancer. The ideal animal models for prostatic cancer do not exist; they would need to fulfill the 16 criteria listed in Table 4. The
Dunning R-3327 models approach most of the points in Table 4 (Points 1 through 13), with the exception of Point 8. The limitation of the Dunning tumors is the low incidence of metastasis, and this may be due to the s.c. sites of the tumor. At present, it is being determined whether these models predict past clinical experiences to existing therapeutic regimens.

The ultimate test will concern the predictability and accurate testing of new therapeutic approaches. This study contributes to the final point of providing a wide spectrum of characterized animal models for the study of prostatic cancer.

In conclusion, animal models are not sufficient within themselves to assess the full clinical importance of a future therapeutic approach; however, well-characterized animal models do provide a unique opportunity to develop new concepts that are most difficult to obtain through available clinical studies and within reasonable cost, time, and ethical restraints. Appropriate animal studies and careful clinical trials must be done in concert to assist the search for new and more effective management of prostatic cancer.

Acknowledgments

The authors wish to express their gratitude for the excellent professional help given by Bobby J. Trotter, William Isaacs, and Diane Gomolka, without whose help this project could not have been accomplished.

References

Fig. 1. Comparative histology. A, normal dorsal-lateral prostate; B, R-3327-H; C, R-3327-Hi; D, R-3327-AT. × 410.
Animal Models of the Hormone-sensitive and -insensitive Prostatic Adenocarcinomas, Dunning R-3327-H, R-3327-HI, and R-3327-AT


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