Characterization of a Human, Sex Steroid-responsive Transitional Cell Carcinoma Maintained as a Tumor Line (R198) in Athymic Nude Mice

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ABSTRACT

We have established a transplantable tumor line R198, derived from a papillary (transitional cell) carcinoma of the human urinary bladder. In nude mice, the tumor line exhibits properties attributable to both prostatic and transitional epithelia. In tumor-bearing animals given androgens, the neoplasm has a rapid growth rate, possesses low levels of measurable androgen receptors, produces tartrate-inhibitable acid phosphatase, and forms well-encapsulated, cystic tumors composed of transitional, glandular, and squamous cells. The administration of estrogens or transplantation of the tumor into female mice causes regression of the tumor. In a small percentage of the transplants placed into females or estrogenized animals, selection occurs for tumor cells which can grow under these conditions. The resulting tumors are infiltrating scirrhous carcinomas that closely resemble squamous cell carcinomas of the urinary bladder. These neoplasms grow slowly and do not possess androgen receptors or secretory squamous cells. They are composed of a homogeneous population of squamous cells which are locally invasive.

The paradox of a bladder tumor with some prostatic characteristics may be explained by the fact that the tumor was derived from the trigone region of the bladder, which embryologically is formed by an admixture of tissue from the Wolffian duct and the urogenital sinus. Some trigone-derived neoplasms have characteristics of both bladder and prostate. We hypothesize that sex steroid-sensitive R198, with characteristics of both bladder transitional cells and prostatic epithelium, is a tumor which phenotypically expresses the embryological origins of these tissues. As such, the tumor line will serve as a useful model for studying sex steroid-responsive cells of the urogenital epithelium.

INTRODUCTION

Nude mice are unable to reject most allo- and xenografted tissue because their congenital athymia renders the animals immunologically deficient (11, 23, 27–29). Many types of human neoplasms have been routinely grafted into these mice which serve as hosts for serially transplantable tumor lines (11, 20, 21, 23, 25, 27, 29, 32). Sufrit et al. (37) have reported a success rate of approximately 40% when surgical specimens of human bladder tumors were transplanted into nude mice. These transplants maintained the same morphology as did the inoculated primary tumors. In the present study, we report the establishment of a transplantable tumor line, R198, derived from human bladder carcinoma cells. This tumor line has characteristics resembling those of neoplasms of both prostatic and urinary bladder epithelia. Extensive characterization of the tumor line indicates that it is derived from multipotential epithelia of the bladder.

MATERIALS AND METHODS

Origin of the Tumor

The neoplasm was obtained from an 81-year-old white male who had been admitted with symptoms of urinary tract obstruction. The patient had had diabetes mellitus for several years which was controlled by diet and daily injections (24 units/day) of insulin. The patient had previously undergone a transurethral prostatectomy in 1960. In 1975, he was admitted to the urological service with symptoms of urinary retention. Cystoscopy revealed a mass that was partially obstructing the prostatic urethra. A suprapubic prostatectomy was subsequently performed. During the operation, a papillary mass adjacent to the right ureter was found and removed along with the obstructing mass. A portion of the prostatic mass and the entire papillary tumor were submitted for histopathological studies.

Histologically, the prostatic mass was characteristic of BPH, while the bladder lesion was diagnosed as a papillary (transitional cell) carcinoma, Stage A, Grade 1, according to the classification of Jevett (14). No evidence of a primary prostatic carcinoma was found in multiple sections of the hyperplastic tissue. Neoplastic epithelial cells, however, were found clinging to the surfaces of the resected BPH fragments. At this time, the origin of the cells was not apparent, but their cytological features and subsequent transplantation studies indicated that they were neoplastic urothelium.

The patient made an uneventful recovery. One year later, he again presented for obstruction of the bladder neck. Cystoscopy revealed numerous raised foci scattered throughout the mucosa of the bladder. A diagnosis of disseminated transitional cell carcinoma was made. The patient elected not to receive treatment and was lost to follow-up.

Mice

BALB/c nude mice were part of a breeding colony established initially in Dr. G. Sato's laboratory (University of California, La Jolla) and thereafter in Dr. Reid's laboratory (Albert Einstein College of Medicine). Homozygous nude males were bred to heterozygous females to yield offspring, approximately 50% of which were nude mice. All animals were maintained in isolation in rooms with HEPA-filtered air and in laminar-flow, HEPA-filtered units. The food, water, and bedding were sterilized. Four times a year, the colony was screened for viral and bacterial pathogens and for the general state of health of the animals.

Development of the Transplantable Tumor Line in Athymic Nude Mice

The surgical specimen of hyperplastic tissue containing adherent neoplastic transitional cells was transported to the laboratory in chilled...
serum-free medium [Dulbecco's modified Eagle's medium: Ham's F-12 medium (1:1)]. In the laboratory, the sample was rinsed for 1 to 2 min in 70% ethanol to ensure sterility. It was then minced, a portion was fixed in buffered formalin, and the remainder was injected s.c. into 2 6-week-old male BALB/c nude mice. Each mouse received a 20-mg pellet of 5α-DHT (Calbiochem). The pellets were inserted s.c. on the contralateral side of the tumor implant. After 2 months, a neoplasm began to develop in one of the 2 mice. After another 2 months, the tumor was sufficiently large (more than 1 cm in diameter) to be transplanted. It was transplanted into 4 male nude mice, each pelleted with 20 mg of 5α-DHT. Tumors developed in all the mice. Thereafter, stock lines of the tumor were maintained by serial passage into male nude mice pelleted with 5α-DHT. To passage the tumor, a mouse carrying the tumor was killed, and the tumor was dissected out aseptically. The tumor was mechanically dissociated by pressing it through a collector (Belloco Glass Co., Vineland, NJ) with a No. 2 coarse grid. The dissociated tumor tissue was centrifuged at 900 rpm, rinsed twice with PBS, and brought up in Dulbecco's modified Eagle's medium: Ham's F-12 medium (1:1) supplemented with 10% fetal bovine serum at a concentration of 0.5 ml of packed tumor mince per ml of medium. Tumor mince, 0.2 to 0.3 ml of mince plus medium, was injected s.c. into each male mouse. A tumor, 1 cm or more in diameter, generally developed in 6 to 8 weeks.

Karyotype Studies

Portions of the tumor from several mice were dissected under sterile conditions and prepared for primary cultures by procedures described below. After several days in culture, the cells were collected and treated with anti-mouse antiseraum by the procedures described by Reid and Rojkind (31). Using this technique, the majority (greater than 99%) of the mouse cells were eliminated leaving only the human tumor cells. Karyotyping of the cultures was done by procedures described by Worton and Duff (39). The cultures were treated with Colcemid (0.04 μg/ml) overnight. The cells were harvested with 0.5% trypsin:0.2% EDTA and then exposed to a 0.075 M KCl hypotonic solution at 37° for 35 min. They were fixed in several changes of acetic acid:methanol (1:3). Slides were made by air drying and then stained in a 4% Giemsa solution in 0.05 M Sorensen's buffer (pH 6.8) for 10 min. More than 30 cells were examined (Fig. 1).

Growth Curves

The tumor was evaluated for its ability to grow in the following groups of animals under various conditions (Charts 1 and 2): Group 1, untreated males or females or castrated males; Group 2, males treated with 20-mg pellets of either 5α-DHT or 17β-estradiol (made from powder from Calbiochem); Group 3, females treated with 20-mg pellets of 5α-DHT or 17β-estradiol.

The experiments were initiated by inoculating s.c. 0.2 ml of minced tumor into 4- to 5-week-old adult mice. Pellets containing the steroid were implanted s.c. in the side contralateral to the transplanted tumor. Once or twice each week, caliper measurements were made on any palpable neoplasm that formed at the inoculation site.

Histological Studies

At each passage, a portion of the tumor, along with samples of the liver, lungs, spleen, and lymph nodes, was fixed in buffered formalin; dehydrated with an ethanol series; and embedded into paraffin. Sections 6 to 8 μm thick were stained with hematoxylin: eosin.

Ultrastructural Studies

Tumor specimens were fixed for 2 hr in 2% paraformaldehyde:2.5% glutaraldehyde in 0.1 M phosphate buffer and were subsequently post-fixed in 1% osmic acid:0.1 M phosphate buffer at 4°, dehydrated with a graded series of ethanol solutions, and embedded in Epon 812. Thin

chart 1. Growth curves of R198 in BALB/c nude mice in passage 5. BALB/c nude mice, 4 to 8 weeks old, were given 0.2 ml s.c. injections of minced tumor. After inoculation, the mice were examined twice a week for the development of neoplasms. Any neoplasms which developed were measured with calipers in several directions, and an average diameter was calculated. The development and growth of the neoplasms was followed for up to 6 months. The tumor diameters in each growth curve are the averages of tumors from different animals grown under the same conditions. In Table 1 are given standard deviations on those variables for which statistics could be calculated and the ranges for those with insufficient data for statistical analysis. •, O: growth curves of tumor in castrated animals and growth curves of tumors in intact, unpelleted animals; D, growth curves of tumors in animals (males or females) pelleted with 5α-DHT; A, growth curves of tumors in animals (males or females) pelleted with 17β-estradiol. A, growth of R198 observed in adult male mice (N = 25). The lag time to first appearance of the tumor in untreated animals was 7 to 10 days, and the tumors grew slowly and steadily thereafter. With pellets of 5α-DHT, the tumors grew more rapidly and to a larger size. If the mice were pelleted with 20 mg of estradiol, all tumors regressed within the first month and did not survive. B, growth of R198 in adult female mice (N = 5) pelleted with 20 mg of 5α-DHT. The rate of growth and the lag time to first appearance of the tumor was equivalent to that in untreated male mice. If the females were pelleted with 17β-estradiol, the tumors regressed and did not survive. C, if adult male mice (N = 10) were castrated and then 1 week later inoculated with R198, the tumor underwent complete regression in 100% of the mice and reappeared after 2 months in 50% of the animals. Once tumor growth started, the growth rate was equivalent to that observed in normal male mice. O, growth curve of tumors that underwent selection and grew; D, growth curves of tumors that did not survive. D, if R198 was inoculated into adult female mice (N = 10), the tumor showed poor survival (20%) (O). However, in those mice in which it did survive, the tumors underwent an initial, complete regression. The tumors which did regrow (O) regrew after a 6- to 12-week lag and then grew at rates comparable to those growing in untreated animals.

sections were double stained with uranyl acetate followed by lead citrate and examined in a Phillips 300 electron microscope.

AK Analysis

AK isoenzyme analysis was used to "fingerprint" tissues, whether normal or neoplastic, since the isoenzyme patterns are considered to be organ specific (33). Tissue was homogenized and the AK isoenzymes assessed at different pHs by isoelectrofocusing techniques. For these studies, the profiles were made on: (a) normal human bladder and normal prostate, both derived at autopsy (samples of the 2 tissue types were obtained from 35- to 50-year-old men; (b) surgical specimen of an oat cell carcinoma; (c) a surgical specimen of an ovarian cystadenocarcinoma; (d) surgical specimen of a colon carcinoma; (e) a human prostatic carcinoma cell line, PC3, derived from a bone metastasis (16); (f) a surgical specimen from a primary tumor of prostatic carcinoma and; (g) a surgical specimen of a prostatic carcinoma metastatic to the liver (same patient as the one from which the primary tumor was derived). These profiles were then compared to those of the transplantable tumor line.
Primary Cultures. Primary cultures were prepared by mincing freshly dissected tumor and plating it onto 60-mm Falcon tissue culture dishes. In later studies, it was realized that the cells plated better on Falcon Primaria culture dishes (60 mm). The cells were cultured in RPMI 1640 supplemented with 10% calf serum and 20% conditioned medium from GH3 cells. Epithelial cells grew out of the mince and became confluent on the plates within several days.

RESULTS

Development of the Tumor Line

In the initial passage, the tumor grew in only one of the 2 androgen-treated mice inoculated with minced tissue. This growth occurred 6 months after the initial transplant. The tumor grew slowly and was passed at 3 to 4 months. This neoplasm was designated R198. Subsequently, its passage time (the time required for 0.2 ml of mince to form a neoplasm greater than 2 cm in diameter) was reduced to approximately 2 months. The tumor retained its morphological and functional characteristics and was maintained as a stock line in male BALB/c nude mice pelleted with 20-mg pellets of 5α-DHT for more than 6 years. A summary of its development along with morphological descriptions are presented in Table 1.

Karyotype Studies (Proof of Species of Tumor Cells)

In the more than 30 cells examined, the n number was highly variable, ranging from 64 to 90. Three cells were examined in detail following G-banding with trypsin. Almost all chromosomes appeared to be unaltered and could be assigned to specific human homologues. A number of marker chromosomes were also present. They consisted of 3 to 6 large acrocentrics. One marker chromosome consisted of 2 large intensely staining bands adjacent to the centromere with another dark band in a terminal position. These dark bands are separated by a lightly stained band, a dark band, and a terminal pale band. Additionally, a medium-sized ring chromosome was observed in some metaphases. Fig. 1 is a representative karyotype from the tumor cells.

Influence of Sex Hormones on the Growth of and on the Morphology of R198 Tumors

The ability of R198 to grow and the resultant morphological and functional features of the tumor differed greatly, depending upon whether the tumor was carried in untreated, castrated, or sex steroid-treated nude mice. In the first 2 passages, the neoplasm failed to grow in intact males not treated with androgens. During subsequent passages, tumor size was dependent on endogenous testosterone or on 5α-DHT treatment of male mice. In later passages, some tumor transplants were able to grow in untreated males, in castrates, and in females. The results of these hormonal influences on tumor growth rate are presented in Charts 1 and 2 and in Table 1.

Untreated Male Mice. In passage 5, the tumor survived transplantation into untreated male mice but grew slowly (Chart 1A). Two months after inoculation, the tumors were 0.5 to 1.0 cm in diameter and firm. When cut, small cysts containing a milky fluid were found. Microscopically, the epithelial cells, which

PC3, a human prostatic carcinoma cell line, was obtained from Dr. Edward Kaighn (NIH, Bethesda, MD). The cell line was established from a bone-metastatic lesion of prostatic carcinoma and has been characterized elsewhere (16, 38).

Cell Lines. GH3 cells, from a rat pituitary carcinoma cell line were obtained from Dr. Norman Fleischer (Albert Einstein College of Medicine) and cultured in 75-cm² tissue culture flasks (Corning) in RPMI 1640 (Grand Island Biological Co., Grand Island, NY) supplemented with 10% calf serum (Grand Island Biological Co.). Conditioned medium was collected weekly from confluent cultures, sterilized by filtration through 0.22-μm Nalgene filtration units, and stored frozen at −20°C until used. The cell line is known to secrete growth hormone and prolactin into the medium. Its secretory properties and other characteristics have been described elsewhere (41).
formed the walls of the cysts, were surrounded by thin bands of stroma. The cysts were sharply delineated, and no evidence of stromal invasion was present (Fig. 2). Frequently, numerous papillary projections extended into the central cavity. Some of the projections contained fluid-filled spaces giving these structures an acinar appearance (Figs. 2 and 3).

At passage 14, R198 grew rapidly in untreated male mice (Chart 2A). The morphology of the tumor was similar to that observed in passage 5 in untreated male mice.

Androgen-treated Male Mice. Tumors in passage 5 (Chart 1A) and in passage 14 (data not shown) grew faster and attained a greater size in 2 months following 5α-DHT inoculation than was the case for those carried in untreated mice (Charts 1A and 2A). Tumors measuring 2 to 3 cm in diameter were usually found at this time. The tumors were soft and well encapsulated. Numerous cysts were present which contained a milky to bluish fluid. Histologically and cytologically, these neoplasms were identical to those from untreated mice except that the cysts were more numerous and had larger lumens. Neoplastic cells were compactly arranged, piled one upon another, progressing from a basal layer to a dilated lumen. Nuclear pleomorphism was frequently observed in the tumors, slower growth rates, and tumors with a scirrhous, invasive growth pattern. R198 maintained its bipotentiality of response to androgen and estrogen environments after 14 passages in nude mice.

Castrated Untreated Male Mice. In all the animals, the tumors regressed and were not palpable for varying periods of time (Table 1). In approximately 50% of the mice, neoplasms began to appear from 7 to 8 weeks and generally grew at rates faster than those in the intact males or males treated with of 5α-DHT (Chart 1C). In those mice in which the tumors had survived the selection process, the masses at 3 to 4 months were usually less than 1 cm in diameter and were firm. Grossly, the tumors were soft and rarely contained cysts. In these neoplasms, cells were piled one upon another, similar to those in androgen-treated and untreated male mice. However, degenerative changes such as cytoplasmic vacuolation and nuclear pyknosis were observed in some cells (Fig. 5).

Untreated Intact Females and Females Treated with Estrogen. As was observed for castrated males, R198 in females underwent a selection process (Table 1; Chart 1D). Fewer transplants, however, survived the selection process in females and even fewer survived in females treated with estrogen. At the fifth passage, in 6 of 8 transplants into untreated females, the tumors regressed and were not palpable for periods of up to 6 months. At autopsy, no residue of the tumor was found. In 2 of 8 transplants in untreated females, neoplasms began to reappear after 5 to 7 weeks in the mice and grew at a rate comparable to that of the tumors in males (Chart 1D). By the 14th passage, R198 continued to show very poor survival when transplanted into untreated female nude mice (0 of 5 mice with tumors). However, at the 14th passage, the tumor grew in 2 of 6 female

### Table 1

<table>
<thead>
<tr>
<th>Nuggets</th>
<th>No. of mice with growing tumor</th>
<th>Latency until tumor appearance (days)</th>
<th>Passage time (wk)</th>
<th>Morphology of tumor</th>
</tr>
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<tbody>
<tr>
<td>Sex</td>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>None</td>
<td>22/22</td>
<td>11.8 ± 2.6d</td>
<td>8.5 ± 3.6</td>
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<td>Males</td>
<td>5α-DHT</td>
<td>32/30</td>
<td>10.5 ± 1.9</td>
<td>7.5 ± 2.7</td>
</tr>
<tr>
<td>Males</td>
<td>Castrated</td>
<td>5/10</td>
<td>26 ± 12.6</td>
<td>12.8 ± 2.8</td>
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<tr>
<td>Males</td>
<td>17β-Estradiol</td>
<td>2/30</td>
<td>30, 42</td>
<td>8, 10</td>
</tr>
<tr>
<td>Males</td>
<td>17β-Estradiol</td>
<td>1/4d</td>
<td>20</td>
<td>Little growth</td>
</tr>
<tr>
<td>Females</td>
<td>None</td>
<td>2/15/50</td>
<td>5, 12</td>
<td>14, 24</td>
</tr>
<tr>
<td>Females</td>
<td>5α-DHT</td>
<td>6/9</td>
<td>13.5 ± 1.8</td>
<td>8 ± 2.2</td>
</tr>
<tr>
<td>Females</td>
<td>17β-Estradiol</td>
<td>2/10</td>
<td>32, 41</td>
<td>12, 14</td>
</tr>
<tr>
<td>Females</td>
<td>17β-Estradiol</td>
<td>2/6</td>
<td>12, 32</td>
<td>26, 30</td>
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</tbody>
</table>

* The number of weeks for 0.2 ml of minced tumor injected s.c. into mice to yield a tumor greater than 1 cm in diameter.

** BALB/c nude mice, 4 to 6 weeks old, were inoculated with 0.2 ml of minced tumor. On the contrastral side, the mice were pelleted with 20-mg pellets of the appropriate steroid. The controls were pelleted with 20-mg pellets of cholesterol. To prepare castrated mice, 4- to 6-week-old males were orchiectomized 1 week prior to inoculation with the tumor.

<sup>a</sup> Mean ± S.D.
<sup>b</sup> From an experiment testing transplants in the 14th passage. In this experiment, the 2 females with growing tumors died at approximately 2 months after inoculation of unknown causes. Because the animals were closely watched, the deaths of these mice were realized in time to preserve the tumors for histological studies.

### References

- *Cancer Research*.
- *The American Journal of Pathology*.
- *Journal of Pathology*.
- *American Journal of Anatomy*.

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mice treated with 17β-estradiol (Chart 2B). The lag time was approximately 40 days, and the growth rate was equivalent to that observed for the tumor line in male mice. In these cases, the neoplasms were scirrhous, were poorly encapsulated, and were dissected with difficulty. Histologically, the neoplasms were arranged in cords or aggregated into nests, separated by dense bands of fibrous connective tissue (Fig. 6). No central cavity or fluid was observed in these tumors. The pattern closely resembled that observed in infiltrating squamous cell carcinomas which provoke a pronounced desmoplastic response. Nuclear pleomorphism was commonly observed. The cytoplasts of neoplastic cells were usually deeply eosinophilic and often appeared hyalinized. In some instances, cells within nests appeared to have undergone squamous metaplasia.

Estrogen-treated Male Mice. In the experiments detailed in Table 1, only 6% of the transplants into estrogenized mice grew. Similar to those tumors transplanted into females with and without estrogen treatment, they grew after a long (several months) selection process. They had a morphology similar to that of tumors grown in intact females (compare Fig. 6 with Fig. 7).

Androgen-treated Intact Females. The tumors grew at a rate and pattern similar to those in untreated males (Chart 1B). Histologically, the tumors were identical to those seen in untreated males.

Ultrastructural Studies of R198 in an Estrogen Environment

The cells from tumors grown in male mice treated with 20-mg pellets of 5α-DHT had a variable appearance. Cells closest to the lumens occasionally had a glandular morphology (Fig. 8). In many regions of the tumor, transitional cells predominated (Fig. 9). In addition to the glandular and transitional cells, a less numerous population of cells were found that exhibited characteristic squamous differentiation (Fig. 11). All 3 cell types contributed to the walls of the acini. Glandular and transitional cells had short microvilli which projected into the acinar lumens. These cells were joined together at their apical margins by junctional complexes (9). The glandular cells were characterized by considerable amounts of rough endoplasmic reticulum, Golgi membranes, lipid droplets, and various types of lysosomes including multivesicular bodies (Fig. 8). The transitional cells were piled atop one another. Their cytoplasmic organelles were usually sparse and appeared to be uniformly distributed around the nucleus (Fig. 9). In some instances, the perinuclear band of organelles, which included multivesicular bodies, appeared well developed (Fig. 10). Contours of nuclei, found in cells featuring squamous differentiation, were more angular than those of the other 2 cell types, and the heterochromatin appeared more condensed adjacent to the nuclear envelope.

Ultrastructural Studies of R198 in an Estrogen Environment

Tumors grown in female mice treated with estrogen were primarily composed of a morphologically homogeneous population of cells. The nuclei were extraordinarily furrowed, and many contained unusual aggregates of nuclear material (Fig. 12). Large multiple nucleoli were common. The cytoplasts of these cells had a squamous appearance characterized by numerous bundles of tonofilaments. Desmosomes were frequently found joining adjacent neoplastic cells (Fig. 12). Although intracellular lumens (1) were observed in tumors from androgen-treated mice, they were much more prevalent in the tumors carried in estrogenized females (Fig. 13). Fibroblasts near the tumor-stromal interface appeared to be very active (desmoplastic response) and were usually separated from the tumor by broad bands of collagen fibers (Fig. 14).

AK Isoenzyme Profiles

Isolelectrofocusing techniques have shown (33) that the AK isoenzymes have activity profiles which are organ specific. By assaying the AK activity along a continuous pH gradient, one can obtain a profile which can be useful in identifying tissue type. In preliminary studies, the AK isoenzyme profile was found to be similar for all urogenital tissues studied. Thus, the AK isoenzyme profile was used to ascertain whether or not R198 was of urogenital origin. As shown in Chart 3, the AK isoenzyme profiles of normal human bladder and prostate were found to be indistinguishable. Different tumor types have different AK activity profiles which correlate with those found for the normal tissues. In Chart 4 are shown 6 AK isoenzyme profiles of various neoplastic human tissues and an established, poorly differentiated prostatic carcinoma cell line, PC3 (16, 38). For all the normal and neoplastic prosthetic tissue profiles studied, AK activity peaks were observed near pH 4.9, 7.6, 8.0, and 9.5. As shown, the AK activity peaks near pH 4.9 and 9.5 are characteristic for normal human urogenital tissue and at an activity ratio of 1:3. However, an unusual aspect of R198 was the finding that its AK activity varied depending upon whether the tumor was grown in males or females (Chart 5). The AK isoenzyme activities from R198 grown in female mice, when compared with activity profiles of R198 in a male, showed a small but reproducible peak at pH 2.3, a shift of the peak with a marked increase in magnitude to pH 4.3, and loss of some of the activity peaks between pH 7.0 and pH 9.0 (Chart 5). The AK activity profiles of R198 grown in male mice showed a pattern which was more similar to that shown in Chart 4 for the prostatic tumors except for the diminution of the peak near pH 9.5. Thus, the tumor when passaged in males has a profile similar to those for urogenital tissues. In females, the tumor shows a marked redistribution of isoenzyme activity and apparent change of isoelectric point values indicating a responsiveness to hormonal environment.

PAP Levels

The PAP levels were found to be significant in R198 tumors and, more importantly, were found to be influenced by sex steroids (Table 2). The influence of sex steroids on PAP was most dramatically seen in the levels in the fluid present in the tumors. The amount of fluid and its PAP content varied and correlated with the amount of androgens, the highest amounts of fluid and PAP levels being found in males treated with androgens and no fluid being found in castrated males or females. The levels were approximately equal in homogenates of the tumors grown in males, castrated males, and males treated with 5α-DHT. However, there was no detectable PAP in homogenates of tumor grown in females.

Androgen Receptor Assays of R198 Tumors (Table 2)

Very low levels of cytosolic androgen receptors were found in R198 tumors in their 11th passage in male mice with and without treatment with 5α-DHT. No detectable levels of androgen recep-
Charts 3 to 5. AK isoenzyme profiles. It has been shown previously (33) that AK isoenzymes have profiles by isoelectrofocusing techniques which are organ specific. By assaying the AK isoenzyme activity along a continuous pH gradient, one can obtain a profile which is useful in the identification of tissue type. The normal or tumor tissue was extracted and electrofocused for about 60 hr at 4°C in 1% Ampholine (pH 3 to 10). Each profile represents a minimum of 3 separate samples in duplicate. Total enzyme activity recoveries were greater than 80%.

Chart 3 (top). AK isoenzyme activity profiles were similar for normal human prostate and bladder, showing activity peaks at pH 6 and 9 for prostate and pH 5 and 9 for bladder.

Chart 4 (bottom left). AK isoenzyme profiles of a lung tumor (an oat cell carcinoma), an ovarian cystadenocarcinoma, a colon carcinoma, a primary prostatic carcinoma, a metastatic lesion of a prostatic carcinoma (to the liver), and a prostatic carcinoma cell line, PC3. The profiles for tumors are often similar to those for the normal tissue but may have additional peaks. For the prostatic tumor tissue profiles studied, AK activity peaks were observed near pH 4.9, 7.6, 8.0, and 9.5. As shown, the AK activity peaks near pH 4.9 and 9.5 are characteristic for normal human bladder and prostate.

Chart 5 (bottom right). AK activity in R198 tumors varied depending upon whether the tumor was grown in males or females. In female mice, the profiles showed a small but reproducible peak at pH 2.3, a large peak at 4.3, and small peaks between pH 7.0 and 9.0. In male mice, R198 tumors showed a profile similar to that observed for prostatic carcinomas.
Table 2
PAP and androgen receptor levels in R198 tumors grown in BALB/c nude mice

<table>
<thead>
<tr>
<th>Sex of mouse</th>
<th>Treatment</th>
<th>PAP (IU/g protein)</th>
<th>Androgen receptor levels in the tumors (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>None</td>
<td>1.80 ± 0.61</td>
<td>0.46 ± 0.20</td>
</tr>
<tr>
<td>Males</td>
<td>Castrated</td>
<td>2.05 ± 0.20</td>
<td>No fluid</td>
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<tr>
<td>Males</td>
<td>5α-DHT</td>
<td>2.75 ± 0.37</td>
<td>1.51 ± 0.97</td>
</tr>
<tr>
<td>Females</td>
<td>None</td>
<td>ND</td>
<td>No fluid</td>
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<tr>
<td>Females</td>
<td>5α-DHT</td>
<td>ND</td>
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</tr>
</tbody>
</table>

* Mice were either untreated, castrated as described in "Materials and Methods," or treated with sex steroids by placing a 20-mg pellet of the designated steroid s.c. in the mouse at the time of inoculation with tumor mince.

ND, not detectable.

DISCUSSION

Tumors with histological and cytological features of urothelium grew in nude mice following the transplantation of a human BPH specimen that included adherent bladder carcinoma cells. The human derivation of the tumor line is confirmed by our karyotyping which includes G-banding studies. The karyotype of the tumor indicates aneuploidy, a 2n chromosome number of 64 to 90, and a number of distinctive marker chromosomes. Although the precise anatomical origin of the neoplastic urothelial cells is uncertain, it is likely that they were shed from the papillary carcinoma of the bladder that was removed along with fragments of hyperplastic prostate. Alternatively, the cells could have originated from an undetected neoplasm in the excretory ducts of the prostate gland or in the urothelium of the prostatic urethra. While uncommon, transitional cell carcinomas from these regions are histologically similar to those arising from other urothelial sites (15). Sherwood (36) and Shade et al. (34) have reported the presence of preneoplastic and neoplastic lesions in extravesicular portions of the urinary tract in a large percentage of patients undergoing cystectomy for primary carcinoma of the bladder.

The conclusion that R198 arose from bladder urothelium is derived from several lines of evidence. The histopathology of R198 was found to be similar to that of the papillary carcinoma, removed from the patient. As discussed in more detail below, the histopathology and ultrastructural studies of R198 indicate characteristics typical of bladder epithelia. An indirect line of evidence suggesting a vesicular origin for the tumor was that the cells grew in nude mice. Although human bladder tumors have been successfully xenografted into nude mice (20, 21, 37), differentiated human prostatic carcinomas have almost never been successfully xenografted unless the mice are immunosuppressed (29, 30). It has become clear in recent years that nude mice have various immunological and host defense mechanisms which can effect the rejection of certain allogeneic and xenogeneic transplants (13, 28). With respect to prostatic tumors, the host defense mechanisms include one(s) regulated by interferon which can be optimally blocked with antiserum to mouse interferon (29). R198 grew in athymic nude mice without immunosuppression, providing circumstantial evidence that R198 is derived from bladder rather than prostate.

To the best of our knowledge, this is the first report of a transplantable, sex hormone-responsive bladder carcinoma tumor line. Perhaps the most intriguing aspect of R198 is the apparent ability of sex steroids to modulate histological, cytological, and biochemical features of the tumor. For example, in the presence of androgens, R198 exhibited sustained growth which was characterized by the formation of cysts and acini in well-encapsulated tumors. The fluid-filled cysts were not present in androgen-deprived tumors. Therefore, these structures must represent a specific phenotypic expression influenced by the male sex hormone. On the other hand, when the R198 tumor line was implanted into female hosts, equally unique morphological changes, attributable to estrogen, occurred. The female hormone appeared to be responsible for the disappearance of transitional and glandular cells and for the emergence of a homogeneous population of neoplastic squamous cells. As the tumor evolved into a squamous cell carcinoma, it was characterized by local invasion of the surrounding stroma which was accompanied by a severe desmoplastic reaction. Thus, it appears that estrogen is antagonistic to the glandular and transitional cell components of the tumor and selectively promotes the proliferation of a specific cell type that exhibits a propensity to invade stroma.

The unique sensitivity of this tumor to sex steroids is reflected not only by the modifications in its growth rate but also by alterations in its morphology and certain biochemical characteristics. The fastest growth rates were observed in male mice pelleted with androgens or in tumors grown in females. Intermediate growth rates were observed in untreated males and castrated males. In most females and in about 50% of the castrated males, the tumor completely regressed and was not detectable at autopsy after 6 months. The presence of cytosolic androgen receptors [also reported by Shane et al. (35) in some human prostatic neoplasms] and the nature of the AK isoenzyme activity profile were found to be dependent upon whether the tumor was grown in an androgen or an estrogen environment. The mechanisms by which sex steroids influence these parameters are presently unclear.

The transitional, glandular, and squamous cells, which we identified in R198, have been found by other investigators to coexist in spontaneous carcinomas of the human urinary bladder (2, 3). Although some characteristics of R198 were similar to those of bladder carcinomas, we found that this tumor also had features found in prostatic carcinomas. While the majority of tumor cells resembled transitional epithelia, some of the glandular as well as the transitional cells had cytological features reminiscent of those described ultrastructurally in human prostatic carcinomas (17). Intracytoplasmic lumens, present in R198, have been reported to occur in prostatic carcinomas (17). Lipid droplets and secretory vacuoles are commonly observed in neoplastic prostatic cells, especially in Grade III and Grade IV tumors (17). Histochemically, acid phosphatase has been demonstrated in secondary lysosomes and secretory vacuoles of normal and neoplastic human prostatic epithelium (5, 12). The multivesicular bodies in R198, which resemble secretory vacuoles in prostatic tissue, may have been the site of acid phosphatase storage in the androgen-stimulated tumors. The synthesis of this enzyme...
by prostatic epithelia has long been considered to be an androgen-mediated function. When tumor-bearing male mice were treated with androgen, R198 produced large amounts of tartrate-inhibitable acid phosphatase. This isoenzyme is considered to be of prostatic origin and has been used clinically as a means for distinguishing the source of elevated enzyme levels in the circulation of patients suspected of harboring prostatic carcinoma (10).

The paradox of a bladder tumor with some prostatic characteristics can be better understood when examined in the light of studies by Cunha et al. (6, 7). Bladder-prostate epithelial cell differentiation is interchangeable depending on stromal influences (6, 7). Embryological studies have shown that the trigone and the prostate are derived from the pelvis, which is in turn receives cellular constituents from both the Wolffian and Müllerian ducts (24). Thus, it is significant that the trigone of the human bladder is frequently the site of Brunn’s nests and adenocarcinomas (19). Pund et al. (24) have noted that some adenocarcinomas arising from the trigone have histological features reminiscent of both transitional and prostate neoplasms. Male hormones may influence the development of bladder tumors since these transitional cell carcinomas occur more frequently in human males than in females (26). Furthermore, experimentally induced bladder tumors occur more frequently in male (8) or androgen-treated (18, 22) rats than in females.

In conclusion, we have established a tumor line that possesses qualities of transitional cell carcinoma of the bladder and prostatic carcinoma. Because the morphology, growth rate, and biochemical features of this tumor line respond significantly to hormone stimulation in Nb rats. Invest. Urol., 18: 285–288, 1980.


Ware, J. L., Paulson, D. F., Mickey, G. H., and Webb, K. S. Spontaneous

Fig. 1. Karyotype studies in tumor line R198. Primary cultures of tumor cells were established and then evaluated in karyotype studies by methods detailed in "Materials and Methods." Shown is a karyotype of chromosomes treated with trypsin to reveal G-banding. The 2n number was 64 and included the 6 (unnamed) marker chromosomes shown at the bottom.

Figs. 2 to 7. Histopathology of R198 in passage 5.
Fig. 2. Cyst in R198 grown in an intact untreated male nude mouse. The neoplastic cells form a compact multilayered wall which surrounds a central cavity (cyst). A papillary projection of the tumor wall extends into the central cavity. The neoplasia is sharply demarcated from the surrounding stroma. × 63.
Fig. 3. Cross-sectioned papillary projections in the central cavity of a cyst. A small lumen is found in the interior of each projection giving the structures an acinar appearance. From an intact male. × 100.
Fig. 4. Detail of a cyst wall in a tumor grown in a 5α-DHT-treated male. It consists of a stratified epithelium which surrounds a central lumen and is distinctly demarcated from the surrounding tissue by a thick stromal capsule. Invasiveness of tumor cells into the stroma is not evident. Note nuclear pleomorphism. × 250.
Fig. 5. Tumor grown in a castrated male and sampled during regression. Epithelial cells are stratified in a manner similar to the organization found in intact males. However, many cells appear to be undergoing degeneration. Note nuclear pyknosis. × 250.
Fig. 6. R198 tumor grown in an untreated female nude mouse. These neoplasms are solid, scirrhous, and devoid of cysts or lumens. The neoplasms are characterized by an extensive desmoplastic response in the stroma. Squamous differentiation is evident. Intracytoplasmic lumens, typical of bladder tumor cells, are observed in many of the tumor cells (see Fig. 13). × 250.
Fig. 7. R198 tumor grown in an estrogen-treated male. These tumors are histologically similar to those grown in untreated female mice (compare with Fig. 6). × 250.

Figs. 8 to 14. Ultrastructure of R198 in passage 5.
Fig. 8. Luminal region within R198 tumor taken from a 5α-DHT-treated male mouse. Numerous microvilli are present at the apical surfaces of the neoplastic cells which are joined together by means of junctional complexes (J). Although some cells at the surface have features of superficial transitional cells of the bladder, they lack fusiform vesicles and have progressed toward a glandular cell phenotype characterized by abundant rough endoplasmic reticulum (RER). Lipid droplets and autophagic vacuoles (AV) are commonly present in the luminal cells. × 4,600.
Fig. 9. Transitional cells in R198 from a 5α-DHT-treated male mouse. The transitional cells are usually characterized by sparse rough endoplasmic reticulum and other cytoplasmic organelles. × 5,100.
Fig. 10. Detail of transitional cell from a 5α-DHT-treated male. A perinuclear band of organelles including multivesicular bodies (arrows) is present. × 14,000.
Fig. 11. Squamous cells in R198 from a 5α-DHT-treated male mouse. The angular contours of the squamous cell nuclei (S) may be contrasted with the rounded contours of transitional cell nuclei (T). Bundles of tonofilaments (TF) are prominent in the squamous cells. × 9,900.
Fig. 12. R198 tumor cells in female mouse treated with estrogen. The tumor cells, with markedly furrowed nuclei, have undergone squamous differentiation indicated by tonofilaments (TF) and desmosomes (D). The tumor-stromal interface includes wide bands of collagen fibers (C). × 7,200.
Fig. 13. Tumor cells in estrogen-treated female mouse. Intracytoplasmic lumens (L), a characteristic feature of bladder tumor cells (T), are frequently encountered in the estrogen environment. × 7,000.
Fig. 14. Tumor-stromal interface of R198 in an estrogen-treated female mouse. The fibroblasts (F) appear very active and are associated with wide bands of collagen fibers (C). × 6,800.
Characterization of a Human, Sex Steroid-responsive Transitional Cell Carcinoma Maintained as a Tumor Line (R198) in Athymic Nude Mice

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