Characterization of Steroid Hormone Receptors in the Dunning R-3327 Rat Prostatic Adenocarcinoma

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ABSTRACT

We have characterized several histological variations of the transplantable R-3327 prostatic adenocarcinoma and correlated these histological types with steroid hormone receptor content. One type is clearly an adenocarcinoma. This tumor is hormonally responsive (grows better in male than in female rats) and contains substantial amounts of both androgen and estrogen receptors. In contrast another histological type, a fibrosarcoma developed in passages of the R-3327 tumor grows rapidly in female and in intact and castrated male rats. This histological type does not contain either the androgen or estrogen receptor as determined by sucrose density gradient centrifugation. There is also a third histological variant that is classified as a carcinosarcoma. These tumors contain histological elements of both adenocarcinoma and fibrosarcoma and are also hormonally responsive; they contain lower receptor levels than do the adenocarcinomas but more than the fibrosarcomas.

The androgen receptor found in the different histological forms of the tumors has a sedimentation coefficient of 7.8S, and the dissociation constant for methyltrienolone is about $4 \times 10^{-9}$ M. The estrogen receptor has a sedimentation coefficient of 8.3S, and the dissociation constant for estradiol is about $4 \times 10^{-10}$ M.

Plasma of the Copenhagen rat was shown to be devoid of androgen or estrogen receptors, and the normal prostate of Copenhagen rats was shown to contain low levels of an androgen receptor but no estrogen receptor.

INTRODUCTION

Although prostatic carcinoma is in general terms influenced by endocrine factors, the nature, mechanism, and degree of hormone dependence is not known. The identification and characterization of steroid hormone receptors in mammary carcinoma and the encouraging clinical applications of this work (2) have led us to believe that it would be most desirable to subject the histogenetically similar prostatic carcinoma to equivalent analysis. Recent preliminary work by Mobbs et al. (14) suggests that endocrine responsiveness of prostatic carcinoma can indeed be predicted from receptor studies.

Our laboratory is presently developing methodology for detection of steroid hormone receptors in prostatic tissue with a model system first described by Dunning (5) in 1963, the R-3327 transplantable prostatic adenocarcinoma grown in Copenhagen rats. The R-3327 tumor arose spontaneously in 1961 in a Copenhagen male rat, line 2331, and has been preserved since that time by s.c. transplantation into the same inbred line or into F1 hybrids from a Copenhagen × Fischer cross. The tumor grows very slowly and, during subsequent transplantation to Copenhagen or F1 hybrid hosts, it has retained its well-differentiated adenocarcinoma histology. Subsequent reports in 1974 and 1975 by Voigt and Dunning (18) and Voigt et al. (19) indicated that there were differences in growth rate of the tumor after transplantation to intact male rats or male rats that had previously been castrated. They also reported that the tumor contained the 5α-reductase enzyme that converted testosterone into 5α-dihydrotestosterone. These results showed that the tumor is androgen responsive (18). Preliminary studies demonstrated that both 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) and estradiol-binding proteins were present in the tumor (19).

The animal tumor appears to be a good model system for human prostatic cancer. A recent report by Smolov et al. (17) outlines certain characteristics of the R-3327 tumor that make it an appropriate model for human disease. In brief, these are spontaneous origin of the tumor in an aged animal, histological and histochemical similarity to human prostate cancer, hormonal dependence of the tumor (both androgen and estrogen), metastatic capacity, and ease of transplantation, thereby making the tumor accessible for study.

We report here on the characterization of steroid hormone receptors both in the hormonally responsive R-3327 tumor and in a rapidly growing, hormonally nonresponsive line that emerged during transplantation of the established R-3327 tumor. This rapidly growing line is histologically distinct from the established line as well as being hormonally nonresponsive as previously reported (19). Our efforts to characterize the androgen receptor in the tumor have been aided by the utilization of a synthetic androgen, R1881 (methyltrienolone), which has high affinity for the androgen receptor but low binding capacity for plasma proteins (3). Preliminary reports of our work have been presented (9, 10).

MATERIALS AND METHODS

Animal and Tumors. The frozen R-3327 tumor or tumor-
bearing Copenhagen rats were obtained from Dr. Arthur E. Bogden, Mason Research Institute, Worcester, Mass. After thawing, tumors were transplanted s.c. behind the front leg into adult male Copenhagen rats, strain 2331. Inoculations were made by trocar syringe with about 5 cu mm of tumor tissue. Tumors became palpable after about 2 months and were removed from the animals for receptor studies about 7 to 9 months after transplantation. To lower endogenous androgen levels, we castrated animals 16 to 24 hr prior to removal of tumor (or removal of blood or normal prostate). Sections of tumor were prepared for histological study, and the remaining tissue was stored in liquid nitrogen until receptor studies were performed.

Several tumor-bearing animals were also obtained from Dr. David M. Lubaroff, Department of Urology, University of Iowa, Iowa City, Iowa. These tumors were removed from the rats 4 to 5 months after transplantation and stored in liquid nitrogen until receptor studies were performed.

In other experiments, the R-3327 tumor (obtained from the Mason Research Institute) was used to prepare a cell suspension following the method of Lubaroff et al. (7). In this procedure the tumor was minced and digested with collagenase, DNase, and trypsin. Cell count was performed, and viability was determined by trypan blue exclusion. Cells were diluted to 10^7 viable cells/ml, and 0.1 ml was injected directly into the prostate or into the uterus in female Copenhagen rats. Three groupings of animals were used: females, intact males, and castrated males. Growth rate, pathological characteristics, and receptor status were analyzed in these tumors.

Tumor Pathology. With standard histological techniques, hematoxylin- and eosin-stained preparations were made of representative sections of all tumors.

Steroids. Unlabeled steroids were purchased from Steraloids, Wilton, N. H., or from Sigma Chemical Co., St. Louis, Mo. Tritium-labeled steroids were obtained from New England Nuclear, Boston, Mass. Stock solutions of all steroids were made up in absolute ethanol and stored at 0°. Steroids were checked for purity periodically by thin-layer chromatography and were used only if they were chromatographically pure.

Preparation of Cytosol. Frozen pieces of normal prostate or R-3327 prostate tumor were pulverized in a Mikro-dismembrator (VWR Scientific, Inc., Los Angeles, Calif.) at liquid nitrogen temperature. The frozen, pulverized tissue was diluted with 2 volumes of ice-cold 0.01 M Tris-1.5 mM EDTA-0.5 mM dithiothreitol, pH 7.4, and homogenized with a Polytron PT-10 ST tissue homogenizer (Brinkmann Instruments, Inc., Westbury, N. Y.). The homogenate was centrifuged in a Beckman L5-65 ultracentrifuge with an SW 56Ti rotor at 108,000 x g for 1 hr. The supernatant was carefully withdrawn from beneath the lipid layer with a syringe and stripped of endogenous steroids by stirring for 10 min at 0° with the pellet from an equal volume of dextran-coated charcoal (0.05% dextran T-70-0.5% radiolmunoassay grade charcoal in 0.01 M Tris-1.5 mM EDTA, pH 7.4). After centrifugation to remove charcoal, the cytosol was stored in an ice bath until use no more than 1 hr later. Cytosols yield an average protein concentration of 8 to 12 mg/ml as determined by the method of Lowry et al. (6).

Dextran-coated Charcoal Assay. Cytosol (0.1 ml) was added to 1.5-ml microfuge tubes in which varying quantities of 3H-steroid had been previously dried down under nitrogen. Incubations were performed in duplicate for 16 hr at 0°. For termination of the reaction, 1.0 ml of dextran-coated charcoal suspension was added to each of the microfuge tubes and incubated with periodic agitation for 10 min at 0°. The tubes were then centrifuged at 0° in a Beckman Microfuge B for 5 min. Charcoal efficiency was always better than 99.3%. Aliquots (0.5 ml) were counted in minivials with 5.0 ml of Biofluor (New England Nuclear, Boston, Mass.) in a Beckman LS 3150T scintillation counter at 36% efficiency. Scatchard plots (16) of total binding were constructed from these data. These plots were corrected for nonspecific binding as suggested by Chamness and McGuire (4) to obtain specifically bound 3H-steroid. Dissociation constants and total binding sites were determined from the plots of specifically bound 3H-steroid.

Sucrose Density Gradient Centrifugation. Cytosol (0.3 ml) was added to 1.5-ml microfuge tubes containing either 3H-steroid alone or 3H-steroid in the presence of a large molar excess of radioinert competitor. Steroids were taken to dryness under nitrogen just prior to incubation. The reaction mixture was incubated for 3 hr at 0°. Free and loosely bound steroids were removed by centrifuging the cytosol with the pellet from 1.5 ml of dextran-charcoal, vortexing, and incubating with periodic agitation for 10 min at 0°. Charcoal was removed by centrifugation at 0° in a Beckman Microfuge B. Aliquots (0.2 ml) of clear supernatant were removed and layered onto 5 to 40% linear sucrose gradients. Gradients were prepared with 0.01 M Tris-1.5 mM EDTA, pH 7.4, and formed in either 4.0- or 5.0-ml polyallomer tubes with a gradient former (Buchler Instruments Div., Fort Lee, N. J.). Gradient tubes were centrifuged in a Beckman L5-65 ultracentrifuge with an SW 56 Ti rotor at 400,000 x g for 16 hr at 0°. Fractions (0.1 ml) were collected from the bottom of each tube into minivials, and 5.0 ml of Sucrosolve (Beckman Instruments, Fullerton, Calif.) were added. Counting efficiency was 36% in the Beckman LS 3150T. Human serum albumin (4.6S) and human y-globulin (7.1S) were used as marker proteins to estimate sedimentation coefficients according to the method of Martin and Ames (11). When calculating total binding capacity by sucrose density gradient centrifugation, suppressible bindings in both the 8S and 4S regions are combined.

Plasma Binding of Steroids. In view of the possible occurrence of binding components in rat blood that could complicate determinations of steroid hormone receptors in cytosol, 3H-steroid binding by Copenhagen rat plasma was investigated. Free steroids were removed by diluting the plasma with 4 volumes of dextran-charcoal in 0.01 M Tris-1.5 mM EDTA-0.5 mM dithiothreitol, pH 7.4 and incubating with intermittent shaking for 10 min at 0°. The diluted plasma was then incubated with 3H-steroid for 3 hr at 0° either with or without 100-fold excess radioinert competitor. After incubation the reaction mixture was treated with dextran-charcoal to remove excess steroid and analyzed by sucrose density gradient centrifugation.
RESULTS

Pathological Characterization and Growth Characteristics of Histological Forms of R-3327 Prostatic Adenocarcinomas

Histopathological examination of the transplantable prostatic carcinoma from a number of rats shows that there are several distinct morphological variants and that there is some correlation of the histological type with the growth rate and biological activity.

Histologically, 1 tumor type was found to consist of a very cellular epithelial neoplasm with the pleomorphic tumor cells forming acinar structures (Fig. 1) that were closely packed and separated by a very thin fibrous stroma. The tumor cells were low cuboidal when arranged around the periphery of the acinar structures. There was piling up of the tumor cells, and they often filled the acini. The nuclei of these tumor cells were quite large, and there was a scant amount of cytoplasm. Nucleoli were quite prominent, and there were moderate numbers of mitotic cells present. These tumor cells were quite different from the normal rat prostate epithelium that is columnar and has a considerable amount of bright pink-staining cytoplasm. In this morphological tumor type, there was relatively little secretory material in the lumen of the acini. This variant of the transplantable prostatic tumor is considered to be a moderately differentiated adenocarcinoma.

The second histological group of tumors consisted of low cuboidal epithelial cells that are forming small to quite large acinar structures that contained a pink amorphous secretory material in the lumen (Fig. 2). These tumor cells were usually lined up on a basement membrane in a uniform manner 1 or 2 cell layers thick. The nuclei were smaller, and there was slightly more cytoplasmic content than in the first type. The epithelial tumor cells appeared to be more differentiated, and there were only occasional mitotic cells. In this tumor type there was an extensive proliferation of the stromal fibroblastic cells, and the connective tissue component often constituted a considerable area of the tumor. These fibroblastic cells were occasionally spindle shaped, but many of them were rounded up or only slightly elongated. The nuclei were large and hyperchromatic, and mitotic activity in the stromal component was quite marked in some tumors. Occasional binucleated fibroblastic cells were found. This histological variant was considered to be carcinosarcoma due to the mixed nature of the cell types. In metastatic foci both cell types also proliferated.

The third and most rapidly growing tumor type was a pure connective tissue neoplasm, and the tumor cells were densely packed spindle-shaped cells (Fig. 3). The nuclei were pleomorphic and varied from elongated, thin, dark-staining ones to others that were round to ovoid, contained nucleoli, and were quite pale staining. Mitotic activity was quite prominent in this tumor type, and there was a variable amount of pale-staining collagen. The metastatic foci of this tumor type were histologically the same as the primary site. This histological variant was classified as a fibrosarcoma, and the tumor cell type appeared comparable to the stromal cell of the carcinosarcoma. Grossly, these tumors were heavily encapsulated, the white capsular layer encasing a reddish brown tumor mass.

The growth characteristics of the different types of tumors varied as follows. The adenocarcinomas (which were obtained from Dr. Lubaroff) grew s.c. in the rats for about 4 to 6 months. The size of these tumors varied considerably at the time of excision; the diameter ranged from about 1 to up to 2.5 to 3 inches. The mixed tumors (obtained from Dr. Bodgen) grew slowly over a period of 6 to 9 months after s.c. injection into rats. These tumors were also quite variable in size at the time of removal. Their size range was similar to that of the adenocarcinomas. The fibrosarcomatous tumor that was obtained after preparation of a cell suspension (of a tumor from a rat supplied by Dr. Bodgen) and direct injection of the cells into the prostate or uterus of Copenhagen rats grew quite rapidly and reached a diameter of 1.5 to 2 inches within a period of 4 to 5 weeks. The sarcomatous tumors grew at the same rate whether injected into female rats, normal male rats, or castrated male rats and therefore appeared to be growing independently of their endocrine environment. On the other hand, the adenocarcinoma and the mixed tumor were hormonally sensitive since they grow better in male rats than in females.

Steroid Hormone Receptor Characterization of R-3327 Prostate Adenocarcinoma

Androgen Receptor. Titration of R-3327 tumor cytosol (obtained from the mixed tumor) with increasing amounts of [3H]R1881 revealed a specific binding component as analyzed by the dextran-charcoal procedure. Scatchard analysis (16) of these data after correction for nonspecific binding (4) produced a linear plot (Chart 1) suggestive of a single class of high-affinity binding sites. The average androgen receptor binding in 2 separate tumors was 46 fmol/mg cytosol protein (S.D., 1 fmol/mg). The average dissociation constant was 4.0 x 10^-9 M (S.D. for the 2 tumors analyzed was 3.0 x 10^-9 M) which agrees with previously reported values for R1881 binding to rat prostate cytosol receptors (3). In the adenocarcinoma total binding was 170 fmol/mg cytosol protein (S.D. for the 2 tumors analyzed was 14 fmol/mg). The dissociation constant was 3.2 x 10^-9 M (S.D. for the 2 tumors analyzed was 1.6 x 10^-9 M).

Molecular characteristics of the R1881 receptor from the R-3327 mixed tumor type were determined by sucrose density gradient centrifugation at low salt concentration. Under these conditions cytosol binding of [3H]R1881 was found at both 7.8S and 4.5 to 5S (Chart 2). This binding was completely suppressed by incubation with 100-fold molar excess radioiodinated R1881. Total suppressible binding was 40.7 fmol/mg cytosol protein in the tumor. However, we have seen binding levels ranging from barely detectable to about 65 to 70 fmol/mg cytosol protein in the mixed tumor. Although suppressible binding in the adenocarcinoma was considerably higher, close to 300 fmol/mg cytosol protein, the sedimentation coefficient was identical with that in the mixed tumor. Interestingly, in the adenocarcinoma between 80 and 90% of the suppressible binding sedimented at 7.8S; the remaining specific binding was in the 4.5 to 5S region. In the mixed tumor, binding in the 7.8 S region varied from 30 to 70%.

The androgen-insensitive tumor (fibrosarcoma) that grew
Steroid Hormone Receptors in Rat Prostate Cancer

the mixed tumor has a sedimentation coefficient of 8.3S; however, there was also suppressible binding at 4.5 to 55 (Chart 5). Total binding detected was about 13 fmol/mg cytosol protein. Again, as with the androgen receptor, there was a considerable range of estrogen receptor levels in the mixed tumor, varying from barely detectable to 35 to 40 fmol/mg cytosol protein. The adenocarcinoma contained considerably more estrogen receptor; total binding was about 290 fmol/mg cytosol protein. The sedimentation coefficient of the estrogen receptor was identical in both tumor types. Between 90 and 95% of the total suppressible estradiol binding in the adenocarcinoma sedimented at

![Chart 1](scatchard_plot_of_androgen_binding_in_cytosol_from_the_r_3327_prostatic_carcinosarcoma_constant_volumes_of_cytosol_were_incubated_for_16_hr_at_0_with_increasing_concentrations_of_[3h]r1881_scatchard_plot_of_total_binding_with_the_dextran_coated_charcoal_competitive_binding_assay_as_described_in_materials_and_methods_correction_for_nonspecific_binding_was_accomplished_with_the_method_suggested_by_chamness_and McGuire(4)._scatchard_analysis_of_specific_binding_indicated_a_k_d_of_1.2_x_10^-3_m_and_a_binding_capacity_of_45.3_fmol/mg_cytosol_protein.)

![Chart 2](sucrose_density_gradient_centrifugation_profile_of_androgen_binding_in_r_3327_prostatic_carcinosarcoma_gradients_are_from_5_to_40_sucrose_and_are_made_in_0.01_m_tris-1.5_m_edta_pH_7.4._vertical_arrows_at_7.15_and_4.65_indicate_the_positions_of_the_marker_proteins_human_y-globulin_and_human_serum_albumin_respectively_a_representative_profile_is_shown_for_binding_in_the_presence_of_20_n_m_[3h]r1881_alone(•)_or_with_a_3000-fold_molar_excess_of_radioinactive_r1881(A)._specific_binding_was_27.5_and_13.2_fmol/mg_cytosol_protein_in_the_7.8S_and_4.5_to_55_regions_respectively.)

![Chart 3](sucrose_density_gradient_centrifugation_profile_of_androgen_binding_in_hormonally_insensitive_tumor_conditions_are_as_described_in_the_legend_to_chart_2_twenty_n_m_[3h]r1881_binding_either_alone(•)_or_with_100-fold_molar_excess_radioinactive_r1881(A).)

equally well after injection of a cell suspension into female or intact or castrated male rats contained no detectable androgen receptors, as shown by sucrose density gradient centrifugation (Chart 3). Suppressible binding was not observed in either the 85 or 4.5 to 55 regions. There was, however, a nonsuppressible binding peak in the 4.5 to 55 region; this may have been due to a plasma-binding component. The central, reddish brown, noncapsular material was used for receptor analysis. Tumors grown in either male or female Copenhagen rats gave the same negative results. These results are in agreement with the preliminary characterization reported by Voigt et al. (19).

**Estrogen Receptor.** Titration of binding sites in cytosol from the hormonally responsive carcinosarcoma with increasing concentration of [3H]estradiol showed a specific binding component. After correction for nonspecific binding (4), Scatchard analysis of these data revealed a single class of high-affinity binding sites with a dissociation constant of \(4.1 \times 10^{-10} \text{ M}\) (mean value; S.D. for the 4 tumors was \(1.0 \times 10^{-10} \text{ M}\)) (Chart 4). In these 4 separate determinations, total binding capacity averaged 18.3 fmol/mg cytosol protein (S.D., 6.3 fmol/mg). In the adenocarcinoma total binding was 186 fmol/mg cytosol protein (S.D., in the 2 tumors analyzed was 84 fmol/mg). The dissociation constant was \(6.9 \times 10^{-10} \text{ M}\) (S.D., \(1.1 \times 10^{-10} \text{ M}\)).

Sucrose density gradient centrifugation at low ionic strength revealed that the estrogen-binding component in
The linearity of the corrected plot suggested a single class of high-affinity binding sites. Sucrose density gradient centrifugation was used to characterize the sedimentation properties of the cytosol form of the androgen receptor from the normal prostate of the Copenhagen rat. In low-ionic-strength buffer, the receptor has a sedimentation constant of about 8S. However, there was also suppressible binding in the 4.5 to 5S region (Chart 7). The amount of suppressible binding is quite low, 24 fmol/mg cytosol protein, but agrees quite well with the value determined from Scatchard analysis on the same tissue (shown in Chart 7). Four separate experiments with

8.3S, whereas in the mixed tumor binding in the 8.3 S region varied from 35 to 75% of total suppressible binding. The slow-growing, androgen-insensitive tumor was completely devoid of suppressible estradiol binding in the 8S and 4.5 to 5S regions (Chart 6). There was, however, a nonsuppressible binding peak in the 4.5 to 5S region that may have been due to a plasma binding component.

**Normal Prostate Receptor Characterization in the Copenhagen Rat**

Characteristics of the steroid hormone receptors in normal prostatic tissue from Copenhagen rats were also studied for comparison with those in the R-3327 prostatic adenocarcinoma. Prostates were removed from several normal, adult male Copenhagen rats 16 to 24 hr after castration, and the entire prostate glands were pooled for receptor studies. Prior to analysis cytosol was stripped of free steroids by dextran-charcoal treatment (see "Materials and Methods"). Androgen binding in normal prostatic tissue was analyzed as a function of R1881 concentration, and the Scatchard plot (16) of total binding is shown in Chart 7. A high-affinity binding component was detected after correction for nonspecific binding as suggested by Chamness and McGuire (4). A value of $3.8 \times 10^{-8}$ M (S.D. for 2 separate pooled prostate samples was $2.5 \times 10^{-8}$ M) was obtained for the dissociation constant, and the number of binding sites was 20 fmol/mg cytosol protein (S.D., 1 fmol/mg). The linearity of the corrected plot suggested a single class of high-affinity binding sites.

Sucrose density gradient centrifugation was used to characterize the sedimentation properties of the cytosol form of the androgen receptor from the normal prostate of the Copenhagen rat. In low-ionic-strength buffer, the receptor has a sedimentation constant of about 8S. However, there was also suppressible binding in the 4.5 to 5S region (Chart 8). The amount of suppressible binding is quite low, 24 fmol/mg cytosol protein, but agrees quite well with the value determined from Scatchard analysis on the same tissue (shown in Chart 7). Four separate experiments with
different pooled prostates gave a range of 5 to 24 fmol/mg cytosol protein (mean value, 16 fmol/mg; S.D., 7 fmol/mg). In 3 of these prostate samples the proportion of 8S and 4.5 to 5S binding was about 33 and 67%, respectively, whereas in the fourth 100% of the suppressible binding sedimented in the 4.5 to 5S region.

Normal prostate was devoid of an estrogen receptor as analyzed by either sucrose density gradient centrifugation (no suppressible binding in the 8S or 4.5 to 5S regions) or by titration of prostate cytosol with increasing concentrations of [3H]estradiol (which indicated essentially no specific binding).

**Plasma Binding of Steroid Hormones**

Since prostate tissue (normal and malignant) obtained for the receptor studies is vascularized, there is significant contamination of cytosol with plasma proteins. For elimination of the possibility that saturable, 8S binding observed in cytosol from the R-3327 prostatic adenocarcinoma was caused by steroid-binding components in blood, steroid binding in plasma from Copenhagen rats was investigated. Sucrose density centrifugation indicated that there was no suppressible binding of either [3H]estradiol or [3H]R1881 in the 8S or 4.5 to 5S regions.

**Other Steroid Hormone Binding in the R-3327 Tumors**

Cytosol from R-3327 prostatic adenocarcinoma was also analyzed for progestin and glucocorticoid binding. With 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (a synthetic progestin), sucrose density gradient centrifugation on 3 separate specimens from either adenocarcinoma or mixed tumor indicated the complete absence of suppressible binding in the 8S region and little or no suppressible binding in the 4.5 to 5S region. Identical aliquots of the same cytosols incubated with [3H]R1881 showed significant suppressible binding in the 8S region (from 50 to 125 fmol/mg cytosol protein). Analysis of R-3327 prostatic adenocarcinoma cytosol for glucocorticoid receptor by sucrose density gradient centrifugation revealed either no binding or very low levels (<5 fmol/mg cytosol protein) of dexamethasone and triamcinolone acetonide binding. When present, suppressible binding of glucocorticoids was found only in the 8S region.

**DISCUSSION**

The availability of an appropriate animal tumor model for human prostate carcinoma is a valuable asset for research aimed at understanding the clinical disease state. In this report we have characterized the steroid hormone receptors found in the R-3327 transplanted prostatic adenocarcinoma carried in male Copenhagen rats and first described by Dunning (5). Recent reports (17-19) have shown that this rat tumor is hormonally sensitive and responds to endocrine ablation. Further studies by Voigt et al. (19) indicated that 3.5S binding components for both androgen and estrogen were present in the hormonally sensitive R-3327 tumor but that the androgen-binding protein was absent in R-3327A, an androgen-insensitive line derived from transplantation of R-3327.

Our work has confirmed the histological variations and extended the steroid-binding studies; preliminary reports of this work have been published (9, 10). We have described several histological tumor variations derived from the R-3327 prostatic adenocarcinoma. One type is clearly an adenocarcinoma, another group of tumors contains a mixture of epithelial and stromal elements and is called a carcinosarcoma, and yet another type is a connective tissue neoplasm and is classified as a fibrosarcoma. In general, both the adenocarcinoma and carcinosarcoma contain androgen- and estrogen-binding proteins that satisfy the physical-chemical and kinetic criteria for classification as steroid hormone receptors. First, the steroid hormones were bound with high affinity. By Scatchard analysis (Ref. 16; Charts 1 and 4), the specific receptors have dissociation constants...
(K_d) in close agreement with those reported for comparable steroid hormone receptors in other tissue and cells. The androgen receptor bound [3H]R1881 with a K_d of about 4 x 10^{-8} M, whereas the estrogen receptor exhibited a K_d of about 4 x 10^{-10} M for binding of [3H]estradiol. Second, steroid binding was saturable and of low capacity. Corroboration between sucrose gradient and dextran-charcoal analysis indicated that there were between 100 to 300 fmol/mg cytosol protein androgen receptor in the adenocarcinomas, whereas the mixed tumor showed a wide range of receptor levels from barely detectable to about 65 to 70 fmol/mg cytosol protein. Estrogen receptor levels in the adenocarcinoma varied between 100 and 300 fmol/mg cytosol protein, and in the mixed tumor levels they ranged from barely detectable to 35 to 40 fmol/mg cytosol protein. Finally, the specific receptors sediment consistently at approximately 8S as determined by sucrose density gradient centrifugation in low salt concentration. In the carcinomas we see suppressible binding in the 4S region at times equal to or greater than the quantity of 8S binding. However, we are not sure of the significance of this form of the suppressible binding component. Interestingly, in the adenocarcinomas the major portion of suppressible binding sediments in the 8S region. These findings distinguish the specific receptors from albumin, sex hormone-binding globulin, and other low-affinity, steroid-binding components in plasma that sediment only in the 4S region. Furthermore, sucrose density gradient centrifugation of tumor-bearing Copenhagen rat plasma revealed the complete absence of suppressible binding of R1881 or estradiol. Additionally, steroid competition studies (data not shown) indicate that the androgen and estrogen receptors are distinct and unique proteins.

Two problems normally encountered in androgen receptor analysis in clinical prostate material have been virtually eliminated in our studies with the rat model systems. One of these, the interference of plasma-binding proteins with receptor determination, has been eliminated by use of the synthetic steroid R1881, which Asselin et al. (1) and Bonne and Raynaud (3) have shown does not bind to sex hormone-binding globulin. In our studies with the R-3327 tumor in Copenhagen rats in which there is no sex hormone-binding globulin (1, 15), this does not present a problem. However, sex hormone-binding globulin contamination in human prostate tissue is a significant complication, and the potential for use of R1881 to eliminate this problem is of considerable importance. One problem encountered with R1881 in studies with human benign prostatic hypertrophy, however, is the affinity of the synthetic hormone for the progestin receptor (1). This is not a complication in our studies with the R-3327 tumor since there is no progestin receptor present.

The second problem in androgen receptor determination in prostate tissue is the presence of endogenous steroids that block available receptor sites. This problem has been mentioned by others working with human prostate tissue (8, 13, 15). In our studies, however, endogenous androgens do not appear to be a significant problem. First, animals are castrated 16 to 24 hr prior to removal of tumors to lower the endogenous steroid levels. Second, the tumors are located at a remote site from the source of androgen synthesis, thus androgen concentrations might be diluted as compared to the concentrations, for example, in human benign hyperplastic prostate tissue where there is a high content of endogenous dihydrotestosterone (13). The complete absence of suppressible binding in the anaplastic (fibrosarcoma) R-3327 tumor is, therefore, attributed to the absence of receptors (both estrogen and androgen) rather than to excessively high androgen levels in this tumor. In contrast, the low levels of androgen receptor in the normal rat prostate may in part be due to endogenous androgens blocking receptor sites. Attempts to measure androgen receptors by an exchange assay with R1881 have thus far not been successful.

As indicated previously, receptor levels varied in the different histological forms of the R-3327 tumor. It appeared that the higher receptor levels were found in the adenocarcinoma and that the levels were considerably diminished in tumors with considerable stromal character. The hormonally responsive tumors grew quite slowly. By contrast the hormonally insensitive tumor, which grows quite rapidly and equally well whether injected into female or intact or castrated male rats and is completely devoid of receptors (Charts 3 and 6), is characterized histopathologically as a fibrosarcoma. It is possible that there are variable amounts of at least 2 cell types in the R-3327 tumor line: the epithelial cell type containing hormone receptors, and, therefore, being hormonally sensitive; and the stromal fibroblast being devoid of receptors and hormonally insensitive. This may account for the rather wide range of receptor levels that we have observed in different histological forms of the tumor. In this regard, the tumor with the highest level of receptors consisted mostly of epithelial cells in well-differentiated acini, negligible stroma, and with variable but active secretory processes; tumors with significantly lower receptor levels contained more stromal elements, and most dramatically in the fibrosarcomatous form of the tumor which is hormonally unresponsive and completely stromal, there were no demonstrable receptors. The characteristics of the 3 histological types are summarized in Table 1.

Since the presence of the steroid hormone receptors in the R-3327 prostatic adenocarcinoma correlates with the hormonal responsiveness (presence of receptors, hormonally sensitive tumors; absence of receptors, hormonally insensitive tumors), it is possible that there is a variable amount of hormonally responsive epithelial cells in the other tumors. This is supported by the observation that tumors which contained hormonally responsive epithelial cells grew more slowly than tumors with a high stromal content.
insensitive tumors), we feel that this animal tumor is a good model system for the development of tests based on steroid hormone receptor determination to predict hormonal responsiveness in patients with primary, metastatic, or recurrent prostate cancer, similar to those used in breast cancer patients (12).

ACKNOWLEDGMENTS

We would like to acknowledge the skillful technical assistance of Laura Lee, Wesley Naritoku, and Davoud Molayem. We are grateful to Dr. Paul C. Hsieh for helping with the histopathological characterization of the tumors during the early stages of this work. We are also indebted to Dr. T. A. Khwaja and Jan Varven of the University of Southern California Cancer Center for assisting with tumor transplantation.

REFERENCES


Fig. 1. Typical section of adenocarcinoma, with closely packed differentiated glandular structures. H & E, × 200.

Fig. 2. Typical section of the mixed tumor, showing islands and nests of functional glandular tissue separated by actively proliferating fibrous stroma. This tumor is designated a carcinosarcoma. H & E, × 200.

Fig. 3. Typical section of the anaplastic tumor. As can be easily seen, this tissue appears to be indistinguishable from fibrosarcoma. The field is covered with spindle-shaped cells arranged in a storiform manner. H & E, × 200.

SEPTEMBER 1978
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