Hormone Regulation of Human Prostate in Organ Culture

Marja T. Nevalainen, Pirkko L. Härmönen, Eeva M. Valve, Wu Ping, Martti Nurmi, and Paula M. Martikainen

ABSTRACT

We have established organ cultures of human prostate for in vitro analysis of the hormone responsiveness of prostatic carcinoma. Tissue samples were obtained from total prostatectomies for localized cancer. Normal prostate tissues with age-related hyperplastic changes were obtained from cystoprostatectomies of bladder cancer patients representing the same age group, and they were cultivated as controls. The explants of prostates were cultivated for 7 days in basal medium containing 5% dextran charcoal-treated fetal calf serum, insulin (0.08 IU/ml), and dexamethasone (10−7 M) with or without dihydrotestosterone (DHT) (10−9 M) or estradiol (10−9 M). Control prostates showed involutive changes of morphology when cultured in basal medium. These changes were prevented by DHT, which also maintained a strong epithelial immunostaining for PSA (prostate specific antigen), which was used as a marker for tissue-specific functions. The concentration of PSA in the medium was high. The rate of [3H]thymidine incorporation into DNA was stimulated by DHT in some cultures of control prostates, but no increase was seen in the others. Androgen stimulation of [3H]thymidine incorporation was consistently inhibited by the antihormone cyproterone acetate. The main morphological response of cultured control prostates to estradiol was induction of squamous metaplasia. This was associated with increased incorporation of [3H]thymidine, which was radioautographically localized to the basal layer of epithelium. Estradiol effects were counteracted by the antihormone tamoxifen. The expression of androgen receptor mRNA and protein in cultured control prostate was demonstrated by Northern blotting and immunohistochemistry, respectively. Also, the expression of estrogen receptor was demonstrated by the polymerase chain reaction analysis of total mRNA from cultured control and cancer prostate.

The cultured explants of prostate cancer maintained the overall morphology of the original carcinoma. However, the presence of DHT improved the morphology of cancerous acini in all better differentiated carcinomas (3 grade 1 and 5 grade II), and corresponding responses to DHT were observed in the rate of DNA labeling with [3H]thymidine. In 2 of 3 grade 1 carcinomas, DHT increased DNA synthesis, but in grade II cancers the patterns of hormone responses were more variable. The poorly differentiated grade III prostatic carcinomas did not respond to either hormone as measured by [3H]thymidine uptake, and no hormone effects could be observed in morphology. Immunostaining for PSA differed from that in control prostates: besides cancerous acini, the surrounding stroma was also intensively stained, which suggests unpolarized and impaired secretion of PSA by the cancer cells.

In conclusion, our study shows that organ cultures can be used to analyze the independent and combined actions of hormones and growth factors. Primary cultures of normal epithelial cells have been established, but they frequently differentiate and lose androgen sensitivity (23). Establishment of cell lines from prostate cancers has been difficult. Furthermore, cancer cell lines represent selected subpopulations of original tumor cells (24), which limits their use in predicting the behavior of an individual tumor (25). The hormone responsiveness and tissue specific functions have been, however, successfully maintained in organ cultures of the rat ventral (16, 19) and dorsolateral (21) prostate. This is probably caused by the presence of all tissue components and the interaction of epithelium and stroma, which is supposed to be important for maintenance of epithelial function (26–30).

In this work, we studied the possibility of using organ cultures in the analysis of hormone regulation and biology of normal and malignant human prostate. We demonstrate that the morphology and tissue-specific functions of the explants of both normal human prostate and prostatic carcinoma can be maintained in organ culture. In normal control prostates, androgen and estrogen exerted specific effects on morphology, proliferation, and secretory functions. These effects were largely counteracted by respective antihormones. The characteristic morphology of each cancer was maintained in culture, too, but hormone effects were seen in rather well-differentiated prostate cancers only.

INTRODUCTION

The clinical course of prostatic cancer is highly variable. By improved screening, many cases of prostate carcinoma are found before any clinical symptoms. In these cases of localized disease, it is not known how to distinguish the patients who would probably not need active treatment (1–3) from those who would benefit from rapid surgical intervention. However, the majority of patients have locally advanced or metastasized disease at the time of diagnosis. In these cases of advanced disease, there is disagreement concerning the timing and selection of appropriate therapy for individual patients (4, 5). There is a need for knowledge of the biology and regulation of cancer growth and responses that would help in looking for new prognostic markers.

All present forms of endocrine therapy are based on androgen deprivation (6). Androgens control development, growth, and function of the prostate (7), and they regulate prostatic cell turnover by means of proliferation (8, 9) and apoptosis (10, 11). Androgens are a prerequisite for the development of both prostatic hyperplasia and cancer, but several other factors are obviously also involved in their pathogenesis. Prostate also contains receptors for estrogen (12, 13), which seems to be involved in the development of benign prostatic hyperplasia and experimental prostatic cancer (14). The mechanisms by which androgens and estrogens regulate human prostate are poorly understood.

Other steroid hormones, such as glucocorticoids (15, 16), polypeptide hormones, such as insulin (17–19) and prolactin (20, 21), as well as polypeptide growth factors (22) are also supposed to have an important role in the function of normal prostate and pathogenesis of prostatic diseases.

Various in vitro models of the prostate have been developed to analyze the independent and combined actions of hormones and growth factors. Primary cultures of normal epithelial cells have been established, but they frequently dedifferentiate and lose androgen sensitivity (23). Establishment of cell lines from prostate cancers has been difficult. Furthermore, cancer cell lines represent selected subpopulations of original tumor cells (24), which limits their use in predicting the behavior of an individual tumor (25). The hormone responsiveness and tissue specific functions have been, however, successfully maintained in organ cultures of the rat ventral (16, 19) and dorsolateral (21) prostate. This is probably caused by the presence of all tissue components and the interaction of epithelium and stroma, which is supposed to be important for maintenance of epithelial function (26–30).

In this work, we studied the possibility of using organ cultures in the analysis of hormone regulation and biology of normal and malignant human prostate. We demonstrate that the morphology and tissue-specific functions of the explants of both normal human prostate and prostatic carcinoma can be maintained in organ culture. In normal control prostates, androgen and estrogen exerted specific effects on morphology, proliferation, and secretory functions. These effects were largely counteracted by respective antihormones. The characteristic morphology of each cancer was maintained in culture, too, but hormone effects were seen in rather well-differentiated prostate cancers only.

MATERIALS AND METHODS

Tissue Samples. The samples of prostatic carcinoma were obtained from 11 patients (age range, 56–73 years; mean age ± SD, 65.0 ± 5.7 years) undergoing radical prostatectomy. The carcinomas were grouped according to their histological grade. Three of the cancers were grade I (well differentiated), 5 grade II (moderately differentiated), and 3 grade III (poorly differentiated) carcinomas. Control prostate tissues were obtained from 17 patients undergo-
ing cystoprostatectomy because of bladder carcinoma. These patients were within the same age group (age, 45–71 years; mean age ± SD, 64.1 ± 7.2 years) as our cancer patients. All of these prostate glands showed at least microscopic hyperplasia, but were considered representative of normal control prostate, since the prevalence of benign prostatic hyperplasia in 61- to 70-year-old men has been shown to be 71 ± 7.2% (31). The experimentation was performed after approval by the Local Ethical Committee.

A 5-mm-thick horizontal section of the prostatic tissue was transported to the laboratory in ice cold medium 199 supplemented with G-penicillin (100 IU/ml), streptomycin sulfate (100 μg/ml), and glutamine (100 μg/ml). The basal medium also contained I (Insulin Lente; Novo Industries, Copenhagen, Denmark) and D (9α-fluoro-16a-methylprednisolone; Sigma Chemical Co., St. Louis, MO) at the concentrations of 0.08 IU/ml and 10^-7 M, respectively. The gas atmosphere was a mixture of O2, CO2, and N2 (40:5:55), and the temperature was 37°C. Water was kept in the incubator to ensure the maximal humidity. The explants were cultured for 7 days, and the medium was changed every other day. DHT (5α-androstan-17β-ol-3-one) 10^-7 M and E [1,3,5(10)-estratriene-3,17β-diol] 10^-4 M (both from Sigma Chemical Co., St. Louis, MO), as well as cyproterone 10^-5 M (Schering AG, Berlin, Germany) and toremifene 10^-7 M (Orion Corp., Farmos, Turku, Finland) were dissolved in propylene glycol (Fluka AG, Buchs, Switzerland). The final concentration of propylene glycol in the culture medium was 0.03%. From 2 to 5 parallel dishes were always cultured for each hormone combination in an experiment. For radioautography, slices of the labeled explants were coated with Kodak X-ray films (X-Omat; Kodak, Rochester, NY).

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The tissue was cut with a razor blade into small pieces of approximately 1 mm^3^ in a plain culture medium. The culture method of Trowell (32) was used with some modifications (19). Twenty pieces were transferred on lens papers lying on stainless steel grids in Petri dishes. The medium used was phenol-free medium 199 with Earle's salts (Flow Laboratories, Newcastle, United Kingdom) containing dialyzed 5% fetal calf serum, G-penicillin (100 IU/ml), streptomycin sulfate (100 μg/ml), and glutamine (100 μg/ml). The basal medium also contained I (Insulin Lente; Novo Industries, Copenhagen, Denmark) and D (9α-fluoro-16a-methylprednisolone; Sigma Chemical Co., St. Louis, MO) at the concentrations of 0.08 IU/ml and 10^-7 M, respectively. The gas atmosphere was a mixture of O2, CO2, and N2 (40:5:55), and the temperature was 37°C. Water was kept in the incubator to ensure the maximal humidity. The explants were cultured for 7 days, and the medium was changed every other day. DHT (5α-androstan-17β-ol-3-one) 10^-7 M and E [1,3,5(10)-estratriene-3,17β-diol] 10^-4 M (both from Sigma Chemical Co., St. Louis, MO), as well as cyproterone 10^-5 M (Schering AG, Berlin, Germany) and toremifene 10^-7 M (Orion Corp., Farmos, Turku, Finland) were dissolved in propylene glycol (Fluka AG, Buchs, Switzerland). The final concentration of propylene glycol in the culture medium was 0.03%. From 2 to 5 parallel dishes were always cultured for each hormone combination in an experiment.

**Morphology.** For morphological evaluation, from 3 to 5 explants from each dish were taken randomly into Baker's fixative at the end of the culture period. These tissue blocks were always cultured for each hormone combination in an experiment.

**Histology.** The sections were washed with PBS and preincubated with 5% normal rabbit serum for 30 min to minimize nonspecific binding of immunoglobulins. The normal rabbit serum was removed and the PSA polyclonal antibody (Dakopatts a/s, Denmark) was applied at a dilution of 1:1000 in 1% bovine serum albumin in PBS overnight. The slides were washed 3 times in PBS for 3 min each and a peroxidase-conjugated anti-rabbit IgG secondary antibody (Dakopatts a/s, Denmark) was applied for 30 min. After 3 washes in PBS, the horseradish peroxidase and rabbit-anti-horseradish peroxidase complex (Dakopatts a/s) were added for 30 min. The slides were washed with 3 changes in PBS, and peroxidase activity was visualized using a solution of 0.7% 3,3'-diaminobenzidine (Sigma), 0.02% H2O2 in 0.05 M Tris buffer (pH 7.6). The sections were counterstained with Mayer hematoxylin.

**Time Resolved Fluorimunnoassay of PSA.** The culture media (3 carcinomas and 9 control prostates) were collected from each hormone treatment group on day 7 of culture, 3 days after the previous medium change. The media were kept at −20°C. Determinations of PSA were done according to the instructions provided with the commercial PSA time-resolved fluorimunnoassay kit (Delfia PSA kit; Pharmacia, Wallace Oy, Turku, Finland).

**DNA Extraction and Poly(A)+ RNA Purification.** Total RNA was isolated from the cultured explants of control prostates according to the method of Chomczynski and Sacchi (36). Oligo(dt)-cellulose (0.015 g/each sample) was equilibrated in 300 μl of sterile 2X loading buffer [20 mM Tris HCl (pH 7.6), 50 mM NaCl, 1 mM EDTA, 1% SDS] and incubated with the sample. After enzymatic digestion, the column was washed with 900 μl of DEPC-H2O, 900 μl of 0.1 M NaOH, and 5 mM EDTA, and with 900 μl of DEPC-H2O, after which the pH of the column effluent should be less than 8.0. The total RNA remaining in the sterile DEPC-treated water was heated at 65°C for 5 min and added to the column with an equal amount of 2X loading buffer. The column was then spun overnight, washed with 5–10 column volumes of loading buffer, followed by 4 column volumes of loading buffer containing 0.1 M NaCl. The poly(A)+ RNA was eluted with 2–3 column volumes of elution buffer [10 mM Tris HCl (pH 7.5), 1 mM EDTA, 0.05% SDS], precipitated with 0.3 M sodium acetate (pH 5.2) and cold ethanol overnight at −20°C. The pellet was dissolved in sterile distilled DEPC-treated water.

**Northern Blot Analysis.** Poly A+ RNA (2.5 μg) was electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde and transferred to a Gene Screen membrane (New England Nuclear, Boston, MA) using 20X SSC (1X SSC is 0.15 M sodium chloride, 0.015 M sodium citrate). Androgen receptor DNA probe (75 basepair HindIII-EcoRI fragment of PS43, from Dr. Michael J. McPhaul) (37) was labeled by a random oligonucleotide priming method (38).

The labeled probe was added to the hybridization buffer (50% formamide, 1 M NaCl, 5 X Denhardt’s, 1% sodium dodecyl sulfate, 10% dextran sulfate, and 25 μg/ml single-stranded calf thymus DNA) and incubated with the blot overnight at 42°C. The filters were washed twice for 5 min at RT in 2X SSC; twice for 45 min at 65°C in 2X SSC, 1% sodium dodecyl sulfate; and twice for 30 min at room temperature in 0.1X SSC. The blots were then exposed to X-ray films (X-Omat; Kodak, Rochester, NY).

**Estrogen Receptor Reverse Transcription-Polymerase Chain Reaction.** Total RNA extracted from cultured carcinoma and control prostates was reverse transcribed to synthesize single stranded cDNA according to the instructions of commercial RNA-PCR kit (Gene Amp, Perkin Elmer Cetus). Poly A+ RNA from MCF-7 cells was used as a positive control. Briefly, 2 μg total RNA and 1 μg poly A+ RNA were incubated with 2.5 units reverse transcriptase in a 20-μl reaction volume containing 5 mM MgCl2, 1 mM for each dNTP, 1 unit RNase inhibitor, 1.25 μM random hexamers, and 1.25 μM oligo (dT) cellulose. One cycle of cDNA synthesis was performed in a thermocycler.

**References**

1. The abbreviations used are: I, insulin; D, dexamethasone; DHT, dihydrotestosterone; E, estradiol; TCA, trichloroacetic acid; PSA, prostate-specific antigen; DEPC, diethylpyrocarbonate; SSC, standard saline-citrate; RT, room temperature; ER, estrogen receptor; PCR, polymerase chain reaction; PBS, phosphate-buffered saline.

GLGRVYRPFRPSKTYRG was a gift of Dr. Michael J. McPhaul, University of Texas (35). The control sections were incubated with normal rabbit serum. A fluorescence isothiocyanate-conjugated swine anti-rabbit immunoglobulin (1:50 dilution in 0.01 M PBS, 0.015 M NaCl, pH 7.2) was added after the incubation with the primary antibody for 30 min at room temperature. The slides were washed in PBS 3 times (10 min each), and the sections were finally mounted in 100 mg/ml 0.1% NaNO2 in 50% glycerol in 2X PBS [1,4-diozobicyclo(2,2,2)octane; Sigma, St. Louis, MO].

Paraffin sections of cultured explants were used for PSA immunohistochemistry. The sections were deparaffinized by 3 washes in xylene for 10 min each, followed by rehydration in graded alcohol. The endogenous peroxidase activity was quenched by incubating the slides in a H2O2-methanol (6:1) solution for 8 min. The slides were washed in 3 changes in PBS and preincubated with 5% normal rabbit serum for 30 min to minimize nonspecific binding of immunoglobulins. The normal rabbit serum was removed and the PSA polyclonal antibody (Dakopatts a/s, Denmark) was applied at a dilution of 1:1000 in 1% bovine serum albumin in PBS overnight. The slides were washed 3 times in PBS for 3 min each and a peroxidase-conjugated anti-rabbit IgG secondary antibody (Dakopatts a/s, Denmark) was applied for 30 min. After 3 washes in PBS, the horseradish peroxidase and rabbit-anti-horseradish peroxidase complex (Dakopatts a/s) were added for 30 min. The slides were washed with 3 changes in PBS, and peroxidase activity was visualized using a solution of 0.7% 3,3'-diaminobenzidine (Sigma), 0.02% H2O2 in 0.05 M Tris buffer (pH 7.6). The sections were counterstained with Mayer hematoxylin.
Table 1 DNA and protein contents of cultured control prostates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DNA (µg/explant)</th>
<th>Protein (µg/explant)</th>
</tr>
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<tbody>
<tr>
<td>I-D</td>
<td>1.5 ± 0.19</td>
<td>244 ± 28</td>
</tr>
<tr>
<td>I-D-DHT</td>
<td>1.5 ± 0.19</td>
<td>252 ± 20</td>
</tr>
<tr>
<td>I-D-E</td>
<td>1.5 ± 0.18</td>
<td>247 ± 20</td>
</tr>
<tr>
<td>I-D-DHT-E</td>
<td>1.7 ± 0.26</td>
<td>242 ± 15</td>
</tr>
<tr>
<td>I-D-Cy</td>
<td>1.8 ± 0.19</td>
<td>260 ± 34</td>
</tr>
<tr>
<td>I-D-DHT-Cy</td>
<td>1.7 ± 0.21</td>
<td>283 ± 39</td>
</tr>
<tr>
<td>I-D-To</td>
<td>1.6 ± 0.19</td>
<td>264 ± 28</td>
</tr>
<tr>
<td>I-D-E-To</td>
<td>1.6 ± 0.19</td>
<td>279 ± 30</td>
</tr>
<tr>
<td>O-sample</td>
<td>3.7 ± 0.41</td>
<td>307 ± 31</td>
</tr>
</tbody>
</table>

The experiment was performed as follows: 15 min at 42°C, 5 min at 99°C, 5 min at 5°C, and 5 min at 25°C.

The oligonucleotide primer set flanked the human ER cDNA sequence from base 252 to 549 within the first exon of ER, as numbered by Green et al. (39), which corresponded to a part of the highly variable domain (A/B) of ER. A forward primer used was: 5'-CC AAA OCA TCT GGG ATG GCC CTA CTG CAT-3' and a reverse primer was 5'-CT CGG AG A CAC GCT GTT GAG-3'.

The cDNA fragments of ER were amplified by the addition of about 200 pmol of reverse and forward primers; 2.5 units Taq DNA polymerase; 1 min MgCl2, H2O; and 10X PCR buffer II (Perkin Elmer Cetus, Norwalk, CT) to the reverse transcriptase reaction mixture. The total reaction volume was 100 µl, and the reaction was performed for 35 cycles. Each cycle consisted of an incubation period of 2 min at 95°C, 2 min at 55°C, 2 min at 72°C, with the last phase of 72°C of the last cycle extended to 11 min.

Southern Blotting. The RT-PCR products were purified from primers and nucleotides by preparative electrophoresis in low melting agarose gel (NuSieve agarose; FMC BioProducts, Rockland, ME). After electrophoresis, the fragment bands were cut out, and the fragments were obtained by means of the freeze-squeeze technique (Ultrafree-MC Filter Unit, Millipore Corp., Bedford, USA). The purified RT-PCR products were fractionated by electrophoresis again, and the gel was denatured in 0.2 M NaOH and 0.6 M NaCl for 1 h and in 0.025 M NaHPO4/Na2HPO4, pH 6.5, for 1 h. The RT-PCR products were transferred to a Gene Screen membrane (New England Nuclear, Boston, MA) using 20X SSC as a transfer solution for 18 h. The membrane was prehybridized, hybridized, and washed as described for Northern blots. Human estrogen receptor DNA probe (1819-basepair EcoRI fragment of PKCR2, from Dr. S. Green) (39) was labeled by a random oligonucleotide priming method (38).

**Direct Nucleotide Sequencing.** The human prostate RT-PCR product of 297 basepairs, purified by electrophoresis, was subjected to direct nucleotide sequencing using Sequenase Version 2.0 T7 DNA polymerase according to the dideoxy method by Sanger et al. (40) with modifications. In brief, 500 ng of the double stranded RT-PCR product was denatured at 95°C for 5 min and simultaneously annealed with 5 pmol of the sense or antisense PCR primer. Then the template was subjected to labeling reaction with [α-32P]dATP (>1000 Ci/mmol, 10 mCi/ml; Amersham, United Kingdom) and Sequenase Version 2.0 T7 DNA polymerase (United States Biochemical Corporation) according to the manufacturer’s instructions. The G, A, T, and C terminated radioactive chains were synthesized at 37°C for 5 min by incorporating ddGTP, ddATP, ddTTP, and ddCTP, respectively. The chains were size fractionated by 8% polyacrylamide denaturing gel electrophoresis followed by autoradiography with X-ray film (NIF new RX-100; Fuji Film, Japan).

**Statistics.** DNA and protein contents (µg/explant) (Table I), the amount of PSA in the culture medium (µg/liter) (see Fig. 8), and the incorporation of [3H]thymidine into DNA (counts/min/100 µg of DNA) (Fig. see 2a) are presented as means ± SEM of the dishes of each treatment group from 3 to 17 separate cultures. The statistical analysis of the differences of DNA labeling...
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were included in the basal culture medium from the very beginning. The secretory appearance of the epithelium was preserved. The glandular
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metaplastic changes of the epithelium were pre
observed (Fig. 1b). When dihydrotestosterone was present in the
culture medium, the metaplastic changes of the epithelium were pre
lept or squamous. The epithelial cells were often arranged in
of the morphology of and androgen-stimulated secretion by cultured
endid by comparing control treatment against each hormone treatment
with a paired t test using the BMDP 4V program on the IBM microcomputer.
The statistical significance of the difference between hormone treatments, the analysis was
continuing to be clearly observed. Prostate explants were thus cultured for 7 days.
In addition to fetal calf serum (5%), dexamethasone and insulin
were included in the basal culture medium from the very beginning.
Addition of insulin has been shown to be optimal for the maintenance of the morphology of and androgen-stimulated secretion by cultured
human prostate (41). Insulin alone is a powerful mitogen for prostatic
epithelium both in organ cultures (17) and in primary cell cultures (18)
but together with corticoids, it maintains differentiated epithelium in
cultured human (42) and rat prostate (19, 21). Insulin alone is a powerful mitogen for prostatic
secretory appearance. The epithelium was thick and the cells in the upper layers
were flat and partly cuboidal. Keratinization of the epithelium was
often noted. There were many mitotic figures in the basal layers, and the
nuclei and nucleoli of the epithelial cells were prominent. Moreover,
the glandular luminae were very small (Fig. 1d). When dihydrotestosterone and estradiol were added together, the glandular luminae were large, and the epithelium was convoluted. The basal
epithelial cells were still arranged in layers as in the presence of only estradiol, but the epithelium of the uppermost layer was of a regular
columnar secretory type (Fig. 1e).

RESULTS

Hormone Effects on the Morphology of Control Prostates. The
that time of 7 days was found to be long enough for
the effects of hormone addition or withdrawal to be clearly observed.
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cultured human (42) and rat prostate (19, 21). Corticosteroids have
been shown to inhibit the proliferation of prostatic cells (15).
The tissues showed involutive changes after a 7-day culture in the
basal medium which contained insulin and dexamethasone. The
columnar, secretory epithelium, which was seen in the explants prior to
culture (Fig. 1a) was lost, and most of the epithelial cells looked
flattened or squamous. The epithelial cells were often arranged in
irregular layers and the glandular luminae were small. A fully developed squamous metaplasia with keratin formation was sometimes
observed (Fig. 1b). When dihydrotestosterone was present in the
culture medium, the metaplastic changes of the epithelium were prevented. The epithelial cells remained columnar and regular, and the
secretory appearance of the epithelium was preserved. The glandular luminae were large, and the acinar epithelium was convoluted (Fig.
1c).

In the presence of estradiol, the epithelium lost its normal columnar appearance. The epithelium was thick and the cells in the upper layers
were flat and partly cuboidal. Keratinization of the epithelium was
often noted. There were many mitotic figures in the basal layers, and the
nuclei and nucleoli of the epithelial cells were prominent. Moreover,
the glandular luminae were very small (Fig. 1d). When dihydrotestosterone and estradiol were added together, the glandular luminae were large, and the epithelium was convoluted. The basal
epithelial cells were still arranged in layers as in the presence of only estradiol, but the epithelium of the uppermost layer was of a regular
columnar secretory type (Fig. 1e).
The level of [3H]thymidine incorporation was significantly (P < 0.05) higher in the presence of estradiol than in the basal medium. Dihydrotestosterone also increased DNA labeling, although to a lesser extent. However, the addition of estradiol and dihydrotestosterone together induced no increase in [3H]thymidine incorporation. The effect of estradiol could be counteracted by an antiestrogen toremifene in 7 of 8 control prostates (P = 0.12), and the effect of dihydrotestosterone by an antiandrogen cyproterone (P < 0.01). The hormones did not significantly affect the specific activity of [3H]thymidine as judged on the basis of the analysis of the acid soluble fractions (data not shown).

The DNA contents of the explants mostly decreased during a 7-day culture, which indicated that the total cell number declined during the culture. The protein contents of the explants also decreased, although to a lesser extent. No significant differences were observed in DNA or protein contents of the explants of normal control prostates grown in the basal medium or in the presence of different hormones (Table 1).

Radioautographic studies showed that the labeling of DNA by cultured prostates occurred almost exclusively in epithelial cells. The labeled epithelial cells were located basally. Practically no tritiated thymidine was incorporated into DNA by stromal cells of cultured control prostate tissue (Fig. 2b).

PSA Immunohistochemistry in Control Prostates. The expression of PSA (prostate-specific antigen) in the explants of control prostates were studied by immunohistochemistry using a polyclonal antibody against human PSA. The level of PSA released into the medium of cultured prostates was determined with time-resolved fluoroimmunoassay.

In cultured control prostate, the immunohistochemical staining for PSA was almost exclusively located in the cytoplasm of acinar and ductal secretory epithelium. The basal cells were not stained with PSA. When cultured in the basal medium, very few acini of prostate tissue showed positive staining with PSA antibody (Fig. 3a). In the presence of dihydrotestosterone, the immunoreaction in most acini was intense, although there was some heterogeneity in the expression of PSA within the epithelium of the explants cultured. The glandular secretions also showed a positive immunoreaction (Fig. 3, b and c).

Demonstration of Androgen and Estrogen Receptor mRNA and Androgen Receptor Protein in Noncultured and Cultured Prostate. The presence of androgen receptors was studied by immunohistochemical staining of control prostate tissue prior to culture (Fig. 4a) and after a 7-day culture in the presence of a combination of insulin, dexamethasone, and dihydrotestosterone (Fig. 4b) using a specific polyclonal antibody to human androgen receptor (35). Androgen receptor staining was found mainly in the nuclei of secretory epithelial cells, although some nuclei of stromal cells also showed positive staining. The sections stained with normal rabbit serum occasionally showed faint background staining, but no nuclear staining was observed (Fig. 4c).

The expression of the mRNA for the androgen receptor in control prostate tissue cultured for 7 days in the presence of I, D, and DHT was also demonstrated by Northern blotting and hybridization with the specific cDNA probe (37). The sizes of mRNA (Fig. 4d) (7 and 10 kilobases) corresponded to those described previously (45).

Estrogen receptor mRNA was detected in cultured control prostates and carcinomas by a RT-PCR method. It was confirmed that the RT-PCR products (297 basepairs) corresponded to the part of the ER cDNA by direct nucleotide sequencing of the products. Moreover, the RT-PCR products were Southern blotted and hybridized with the specific estrogen receptor cDNA probe (39) (Fig. 5, a and b).

Morphology of Prostatic Cancer after a 7-Day Culture. The characteristic morphology of each prostatic cancer could be maintained in organ culture. After a 7-day culture, the structure and grade of differentiation corresponded to that of original tumor. The viability of stroma was preserved (Fig. 6). In some of the explants, epithelial cells migrated from glands and ducts, and epithelialized surfaces eventually covered the explants.

The glandular structures of the explants of the well differentiated prostatic carcinoma were preserved in culture. Dihydrotestosterone in the culture medium improved the differentiation of the cancer acini. The epithelium was mostly columnar, and the epithelial cells retained their polarity and height. Nuclear atypia and nucleoli could still be noted (Fig. 6, a, b, and c). Unlike control prostate, cultured cancer tissue did not respond to estrogen in morphology.

In poorly differentiated carcinomas, no specific hormone effects could be seen. However, the addition of both dihydrotestosterone and...
estradiol seemed to improve the viability of explants during the culture (Fig. 6, d and e).

**Hormone Response Patterns of DNA Synthesis in Cultured Prostate Cancers.** The level of DNA labeling in noncultured normal or carcinoma tissue was low, and well differentiated or poorly differentiated cancers did not differ from each other. All 11 prostate cancers (grade I, 5 grade II, and 3 grade III) were evaluated separately because the interindividual variation in hormone response patterns was wide.

In 2 of the well differentiated carcinomas (grade I), DHT increased incorporation of [3H]thymidine into DNA when compared to those explants that were grown in basal medium. Cyproterone counteracted the effect of DHT in one grade I cancer, which was the only one in which antihormone was tested, and also E suppressed DNA synthesis. In one of the grade I cancers, estradiol increased the level of [3H]-thymidine incorporation. In 3 of the 5 grade II carcinomas, dihydrotestosterone increased DNA synthesis, whereas 2 grade II cancers responded to DHT by a decrease in DNA synthesis. In 3 grade II cancers, also estradiol increased DNA synthesis. Poorly differentiated grade III prostatic carcinomas responded weakly to different hormones in a 7-day organ culture as measured by [3H]thymidine uptake. Neither dihydrotestosterone nor estradiol increased DNA labeling in these explants (Fig. 7a).

The contents of DNA in carcinoma explants decreased during the culture. The levels of protein also declined, mostly in parallel to those of DNA (data not shown).

In the cultured prostatic carcinoma, DNA labeling occurred predominantly in the tumor cells. Labeled cells were also noted in epithelial outgrowths surrounding the explants. Very few stromal cells were labeled (Fig. 7b).

**Immunohistochemistry and Time Resolved Fluorimmunoassay of PSA in Cultured Prostate Cancer.** The cultured cancer tissue showed considerably heterogeneous staining for PSA. In addition to epithelium, the stroma around the cancer acini also stained intensively. On the other hand, some acini did not stain at all (Fig. 8, a and b). No differences in PSA immunostaining were observed between the explants of prostate cancer cultured in the basal medium or in the presence of different hormones.

PSA was also determined with time-resolved fluorimmunoassay from the media of normal (n = 9) and malignant (n = 3) prostates cultured for 7 days. The media were changed 3 days before the determination. The amount of PSA (µg/liter) in the culture medium of carcinoma was less than 5% of that in the medium of control prostates (Fig. 8c).

**DISCUSSION**

In the present work, we have established organ cultures of human prostate for in vitro studies on the hormonal responses and biology of normal and malignant prostate. We also considered a possibility of developing an in vitro model for predicting biological behavior and therapeutic responses of individual tumors.

The morphological integrity of normal and malignant prostate could be readily maintained for up to 14 days in culture. In control prostates cultured in basal medium, prostatic involution was associated with epithelial reorganization, which resembled squamous metaplasia. Radioautographic studies showed that in normal cultured prostate DNA synthesis was mainly limited to basal layers of epithelium as also reported earlier (46). Basal cells have been regarded as functional stem cells which, upon appropriate stimulation such as androgens, are capable of differentiating into secretory cells (42, 47).

In the presence of DHT, the epithelium remained organized and the secretory appearance was preserved. The addition of DHT alone was associated with increased rate of DNA synthesis in some prostates but not in others. This might be explained by the ability of androgens to induce both cell division (20, 26, 43) and differentiation (9, 28, 41) of basal epithelial cells, and these two effects might follow one another. The DHT stimulation of [3H]thymidine incorporation was, however, counteracted by the antiandrogen cyproterone acetate, which suggests a specific androgen-receptor-mediated way of steroid effect. In addition to an agonistic effect on proliferation and differentiation, androgens have an antagonist effect on cell death in prostate (10, 48).
The ability of androgens to support the proliferation of prostatic epithelium without continuous influence of stroma has been questioned (18, 26, 29). In our studies, radioautography shows that DNA synthesis occurred in the epithelial cells of cultured prostatic explants. Moreover, androgen receptors were mainly located in the epithelial cells of cultured prostate, whereas prostatic stroma contained only few of them. However, because the stroma is present in our culture system, it is possible that androgens influence epithelial DNA synthesis also via stromal cells by blocking the action of a constitutive inhibitor (9, 49), or by inducing mitogenic substances (26, 50, 51), which act in an autocrine or paracrine manner. Human prostatic epithelium and stroma are shown to express a number of growth factors and receptors for them (22), which might also be involved in the transition from the androgen-dependent growth to autonomous proliferation in carcinoma.

Estrogen induced squamous metaplasia in the epithelium of control prostates. Corresponding effects have been described in the prostates of other species, too (14, 52). These changes in morphology were associated with a significant, estrogen-induced increase in [3H]thymidine incorporation which was localized, as shown by autoradiographic data, to basal epithelial cells. The epithelial cells did not show any tendency towards differentiation into secretory epithelium in response to estrogen. The addition of DHT prevented the E-induced increase in DNA labeling. In the presence of DHT and E, the morphology showed characteristic responses to both hormones: the uppermost layer retained the regular secretory morphology despite the continuously and actively proliferating basal cell layers. Taken together, our results clearly demonstrate the active proliferative response of normal elderly prostate to estrogens.

The expression of estrogen receptors could be clearly demonstrated by the PCR techniques both in cultured normal and malignant prostate, although the concentration was obviously low. Previous reports

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**Fig. 7. Different hormone response patterns in DNA synthesis of 11 prostate cancers.** Effects of various hormones on [3H]thymidine incorporation into DNA (10⁶ cpm/min/100 μg DNA) of the explants of 3 grade I, 5 grade II, and 3 grade III carcinomas (a). The radioautography shows the localization of DNA labeling in grade III carcinoma cultured in the presence of I 0.08 IU/ml, D 10⁻⁷ M, and DHT 10⁻⁷ M (b). Magnification, × 350; bar, 30 μm.

**Fig. 8. Immunohistochemistry of PSA in human prostatic carcinoma after a 7-day culture in the presence of I (0.08 IU/ml), D (10⁻⁷ M), and DHT (10⁻⁷ M) (a). Control section of cultured prostatic carcinoma stained with preimmune serum (b). Magnification, × 260; bar, 40 μm. c, quantitative determination of PSA in the medium (μg/liter) of cultured control prostates (n = 9) and cultured carcinomas (n = 3) in the seventh day of culture. The medium was changed 3 days before the determination. The method used was time-resolved fluoroimmunoassay.
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have shown that estrogen receptors are mainly localized in stroma of human prostate, but they have also been found in basal epithelial cells (12, 13). This is consistent with our observations on epithelial morphology and DNA labeling. The functional importance of low amounts of estrogen receptors in prostate has been questioned (53), and other mechanisms in estrogen action have been suggested (54, 55). The interactions of prostatic epithelium and stroma in organ culture also make possible the paracrine-like mechanisms suggested for estrogen action (56, 57).

Prostate cancer is thought to express considerable heterogeneity of androgen responsiveness. A single cancer may contain cell subpopulations with an absolute requirement for androgens, cells surviving in the absence of androgens, and cells totally independent of androgens (22). To predict the hormone response patterns and prognosis of individual cancers, the concentrations of androgen receptor have been widely studied (58, 59). However, the transition to steroid insensitivity in human cancers initially responsive to endocrine manipulation does not need to be accompanied by a loss of these receptors (60, 61).

We used DNA labeling by tritiated thymidine to assess the hormone response patterns of cultured explants of prostate cancer. It has been stated to be a suitable method for the analysis of reactivity of cancer tissues to hormones in vitro (62), and to reflect the proliferative fraction of a tumor (63, 64). Several of the better differentiated carcinomas (grades I and II) showed a specific response to androgen and/or estrogen. Parallel changes in morphologies suggest that the data on $[3H]$thymidine incorporation represented real hormone responses in these tumors. In other tumors, however, the pattern of net response varied from stimulation to suppression without marked changes in overall morphology, which suggests marked differences in the responses of various subpopulations of cancer cells within a tumor. The grade III prostatic cancers responded weakly to either hormone in 7-day organ culture as measured by $[3H]$thymidine uptake, which indicates the predominance of androgen-independent cell subpopulations in the tumor.

The tissue-specific functions of cultured normal and malignant prostate were demonstrated by the expression of PSA and prostate acid phosphatase (data not shown) proteins. In control prostates, PSA immunostaining was almost exclusively localized to the epithelial elements, which is consistent with previous findings on human prostate (65). In the presence of dihydrotestosterone, PSA immunostaining was more intense than in the explants grown in basal medium. This agrees with previous results that report increased cellular concentrations (30) as well as increased levels of PSA mRNA (66, 67) in response to androgen treatment. Androgens have been shown to increase the level of PSA mRNA by activating transcription (67) from the PSA gene, the promoter region that contains an androgen response element.

The concentration of PSA was more than 20 times lower in the culture medium of prostate cancer than in that of control prostate. Despite increased appearance of PSA in the circulation of prostatic cancer patients, no differences in PSA protein content (68) or PSA mRNA expression (69) have been demonstrated between carcinoma and hyperplasia specimens. In the cultured cancer epithelium, PSA staining was considerably heterogenous, and also the stroma was intensively stained, especially in those areas where most cancer acini existed. This suggests that the accumulation of PSA is directed towards the stromal compartment as a consequence of aberrations in ductal-acinar network and polarization of glandular epithelial cells, and this might account for the leakage of PSA into circulation in vivo (70). The absence of hormone effects in PSA immunostaining indicate impaired hormone responsiveness of malignant prostatic tissue.

In conclusion, our study shows that organ cultures can be used in the analysis of hormone regulation and/or biology of normal and malignant human prostate.

In this model, normal prostates with age-related hyperplastic changes showed a consistent morphological and functional response to androgens and estrogens, whereas prostatic carcinoma, which represented a clinically selected group of localized cancers, showed a considerable heterogeneity and variability of hormone responses. The clinical follow-up study will show how in vivo data on hormone responsiveness of prostate cancer correlate with the course of disease. The results of our study provide a basis for developing a method for in vitro testing of the individual hormone and drug responsiveness of a prostatic carcinoma.

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