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A Novel Coculture Model for Benign Prostatic Hyperplasia Expressing Both Isoforms of 5α-Reductase*

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ABSTRACT

We have developed a coculture system for primary fibroblast and epithelial cells derived from benign prostatic hyperplasia (BPH) that retained many of the characteristics of the intact human prostate. In contrast to separately cultured prostate fibroblast and epithelial cells, cocultures of fibroblasts and epithelial cells maintained messenger ribonucleic acid expression and functional activity for both isoenzymes of 5α-reductase (type I and type II) as well as maintained expression of androgen receptors and prostate-specific antigen. Furthermore, levels of prostate-specific antigen secreted by cocultured epithelial cells were increased by treatment with androgens, mimicking the situation in the human gland. This contrasted with conventionally cultured fibroblasts or epithelial cells, which failed to express 5α-reductase type II and rapidly lost expression of androgen receptors and androgen sensitivity upon being placed into culture. Electron microscopy demonstrated intracellular structures indicative of the differentiated state of the cocultured cell types, including round nuclei, tonofibrils, and microvilli in epithelial cells and elongated nuclei; large amounts of Golgi and cilia; along with immature collagen fibers in fibroblasts. The present study demonstrates that the coculture model reflects more closely the *in vivo* system for human BPH and is thus a far more suitable model for investigating the molecular and cellular events that underlie BPH than current *in vitro* systems. (*J Clin Endocrinol Metab* **83:** 206–213, 1998)

BENIGN prostatic hyperplasia (BPH) is an almost inevitable feature of male aging (1) and is considered to be responsible for urinary symptoms in the majority of men over the age of 50 yr (2). There are few clues to the origin of the disease, but there is likely to be an endocrine element involved, as castration of males before puberty prevents BPH (3), and prostate development is absent in males with inherited 5α-reductase syndrome (4). Even so the precise role for androgens in the pathogenesis of BPH is unclear, and there may be other putative factors involved in the regulation of prostate growth.

The investigation of the events that lead to BPH has been hampered by the lack of suitable *in vitro* model systems. Although primary cells derived from prostate can be maintained in culture *in vitro*, and a number of prostate derived cell lines have been established (5–10), all of these systems have many limitations compared to human BPH in particular with regard to their androgen sensitivity/hormone responsiveness. Androgen receptors are rapidly lost upon primary culture of prostate cells and are either absent or present at low levels in established cell lines (11), making it difficult to investigate the role of androgens in prostate growth and differentiation. Furthermore, prostate epithelium and stroma interact during the normal development and function of the gland (12), but in a conventional culture system, epithelial cells are grown in isolation and, as a result, behave abnormally. This is particularly evident with immortalized cell lines that suffer from clonogenicity and phenotypic/genotypic alterations (13, 14). These alterations are highlighted by the inability of cultured epithelial cells to secrete prostate-specific antigen (PSA) (11, 15) and to express 5α-reductase type II (5α-RII) (15).

To improve our understanding of the processes involved in the development of BPH, there is a need for a model that maintains the *in vivo* characteristics of fibroblast and epithelial cells. Here we describe an *in vitro* model for BPH that maintains many of the characteristics of the *in vivo* state and does not suffer from the same shortcomings as earlier models.

Materials and Methods

Culture of human BPH cells

Establishment of primary cultures. BPH tissue was obtained from men undergoing transurethral resection of the prostate. The histological status of the tissue was checked by an independent pathologist. Primary cultures of separated fibroblast and epithelial cells were established as previously detailed (5, 16). In brief, the prostate tissue was washed with phosphate-buffered saline to remove all traces of blood before being diced into approximately 1-mm^3 pieces using forceps and scissors. The diced tissue was then incubated for 20 h at 37 C in a collagenase solution. After digestion with collagenase, the epithelial acini and fibroblast cells were separated by centrifugation. The epithelial acini were resuspended in WAJC 404 (Kyoto Pharmaceutical Co., Tokyo, Japan) supplemented with 0.5% FCS, 2.5 μg/L fungizone, 100,000 IU/mL penicillin, 100,000 μg/mL streptomycin, and 0.5 μg/L. The fibroblast cells were resuspended in RPMI 1640 (Life Technologies, Paisley, Scotland) supplemented with 200 mmol/L L-glutamine, 100,000 IU/L penicillin, 100,000 μg/mL streptomycin, 2.5 μg/L fungizone, and 10% FCS. The separated cells were then incubated at 37 C in 5% CO_2. The identity and purity of the separated cultures were confirmed by immunohistochemistry and phase contrast microscopy as previously described (5, 16).

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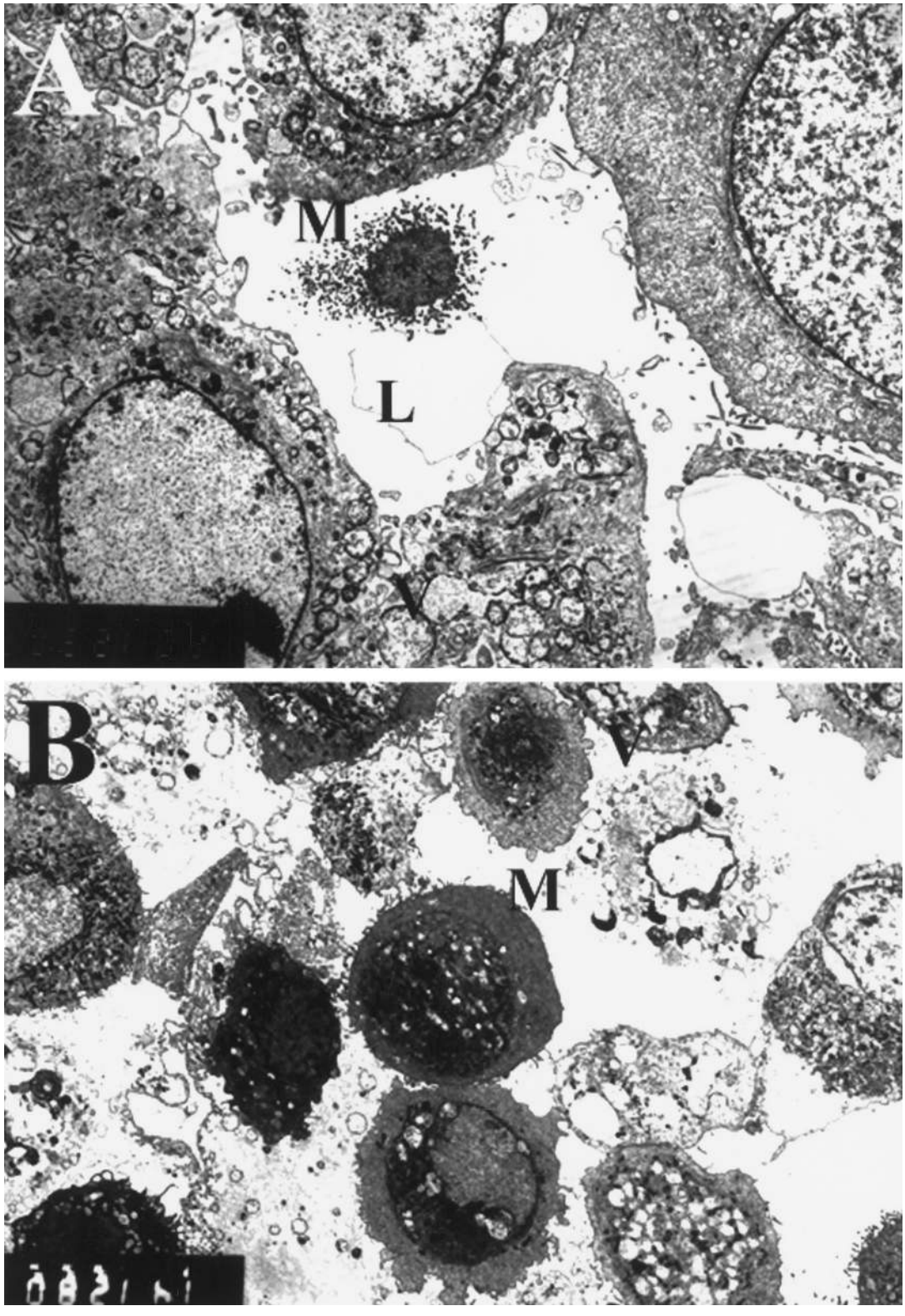

FIG. 1. Electron micrographs of primary and cocultured epithelial cells. A, Epithelial cells cocultured with fibroblasts for 10 days, showing the development of lumen (L) and exhibiting abundant microvilli (M) and numerous secretory vesicles (V; magnification, 3888). B, Primary epithelial cells cultured on plastic for 10 days and showing few microvilli (M) and secretory vesicles (V; magnification, ×2817).

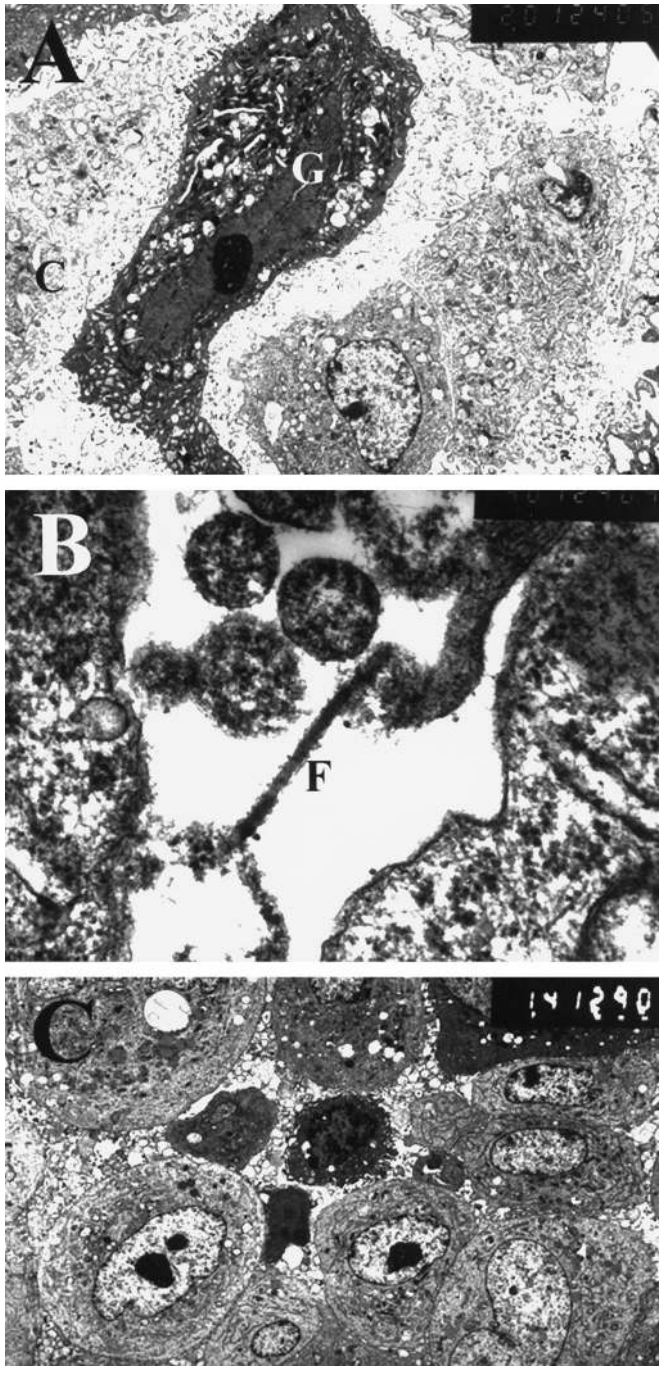

FIG. 2. Electron micrographs of primary and cocultured fibroblast cells. A (magnification, ×3888), Fibroblasts cocultured with epithelial cells for 10 days, demonstrating the presence of cilia (C). B (magnification, ×86400), Collagen fibrils (F) and an abundance of Golgi apparatus (G) were also found (magnification, ×3888). C, Primary fibroblasts grown on plastic for 10 days.

Establishment of cocultures. Confluent cultures of epithelial cells were harvested by trypsinization and seeded onto six-well plates at a density of 10,000 cells/well in 2 mL of a 50:50 (vol/vol) mix of epithelial cell growth medium [WAJC 404 (Kyoto Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 0.5% FCS, 2.5 μg/L fungizone, 100,000 IU/mL penicillin, 100,000 μg/mL streptomycin, and 0.5 μg/L insulin] and fibroblast cell growth medium [RPMI 1640 (Life Technologies, Paisley, Scotland, UK) supplemented with 200 mmol/L L-glutamine, 100,000 IU/L penicillin, 100,000 μg/mL streptomycin, 2.5 μg/L fungizone, and 10% FCS]. The epithelial cells were then incubated at 37 C in 5% CO_2 for 2 h to allow attachment. Fibroblasts harvested after initially being placed in culture were subsequently seeded at a density of 2,000 cells/well in 2 mL of the same medium onto microporous membrane inserts (Millicell, Sigma Chemical Co., Poole, UK) that were placed into separate 6-well plates and left for 2 h to allow cell to attach. The inserts containing fibroblast were then removed using sterile forceps and placed into the wells containing the epithelial cells, thus allowing for interpopulation communication by means of diffusible elements but without any direct contact between epithelial cells and fibroblasts. Cocultured cells were maintained for up to 8 days. Cells were examined by electron microscopy for typical intracellular structures characteristic of epithelial cells and fibroblasts. This also served to determine the purity of the cultures.

Measurement of functional 5α-reductase activity

5α-Reductase activity was assayed as described previously (17). Immediately before the assay, 120,000 cells were harvested by trypsinization, pelleted by centrifugation, resuspended in RPMI 1640, and counted. For assays, cells were then recentrifuged and resuspended in 200 μL sodium phosphate buffer (4 mmol/L) at either pH 7.5 (5α-RI) or pH 5.5 (5α-RII) containing 0.32 mol/L sucrose and 1 mmol/L dithiothreitol. Cell suspensions were added to glass tubes containing 1 μCi [^{3}H]testosterone ([1,2,6,7-^{3}H]testosterone; SA, 105 Ci/mmol; Amersham International, Aylesbury, UK) and an NADPH-generating system consisting of 0.5 mmol/L NADP, 0.1 U/mL glucose-6-phosphate, and 5 mmol/L glucose-6-phosphate dehydrogenase, and the final assay volume was adjusted to 1 mL by the addition of the appropriate pH buffer. Reactions were agitated at 37 C for 30 min in a water bath. Reactions were stopped by the addition of an equal volume of diethylether containing 500 cpm [^{14}C]dihydrotestosterone ([^{14}C]DHT; SA, 50 mCi/mmol; Amersham International) and 25 μg each of unlabeled 3α-androstanediol, 3β-androstanediol, testosterone, DHT, and androstenedione (all from Sigma). After vortexing, steroids were extracted by evaporating to dryness in a vacuum oven at 40 C. The residue was resuspended in 50 μL ethanol, and steroids were separated on TLC plates (Gelman Sciences, Ann Arbor, MI) in dichloromethane-diethylether (9:1, vol/vol). Steroids were visualized by spraying with phosphomolybdic acid reagent spray, and the radioactive metabolites were quantified using a Tri-Carb liquid scintillation counter (Canberra Packard, Panbourne, Berks, UK) as previously described (5, 18). Conversion of [^{3}H]testosterone was linear for at least 60 min for both 5α-RI and 5α-RII under the conditions used in these experiments. At both pH 7.5 (5α-RI assay) and pH 5.5 (5α-RII assay), DHT was the major metabolite and accounted for approximately 70% and 60% of the total metabolites formed by the cocultured fibroblast and epithelial cells after a 30-min incubation.

Ribonucleic acid (RNA) preparation and reverse transcription-PCR (RT-PCR) amplification of 5α-reductase isoenzyme expression

Total cellular RNA was extracted by the method of Chomczynski and Sacchi (19). The presence of messenger RNA (mRNA) encoding 5α-RI and 5α-RII was then determined by RT-PCR. One microgram of total RNA was reverse transcribed using a commercial RT kit (Promega, Southampton, UK) following the manufacturer's protocol. Twenty microliters of the RT reaction were then used for PCR analysis in a reaction volume of 100 μL containing 8 μL 10 × assay buffer (pH 9.0; Promega), 0.2 μL *Taq* polymerase (5000 U/mL), 2 μL deoxy-NTP mix (1.25 mmol/L), and 10 μL intron-spanning primers (50 μg/mL) as follows.

The 5α-RI primers used were 5′-TGCTGATGACTGGGTAACAG and 5′-GTTGGCTGCAGTTACGTATTC amplifying a 171-bp DNA fragment between nucleotides 453–624; for 5α-RII, the primers used were 5′-CCTTGTACGTCGCGAAGC and 5′-CCACCCATCAGGGTATTCAG amplifying a 350-bp fragment between nucleotides 98–447. PCR reactions were carried out in a Hybaid Thermal reactor (Hybaid, Teddington, UK). Conditions for 5α-RI were 30 cycles of 96 C for 1.5 min, 52 C for 1 min, and 72 C for 1.5 min. Conditions for 5α-RII amplification were 35 cycles of 96 C for 1.5 min, 56 C for 1 min, and 72 C for 1.5 min. PCR products were separated on 1% agarose and visualized by ethidium bromide staining under UV transillumination. Control reactions from which complementary DNA (cDNA) or RT had been omitted were

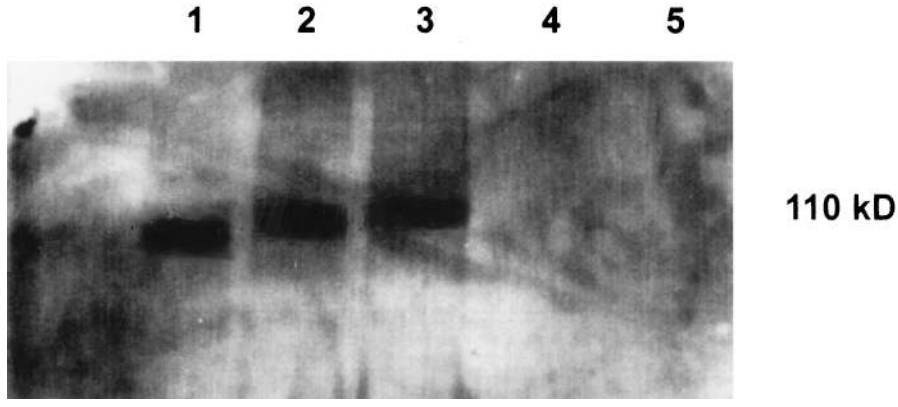

FIG. 3. Expression of androgen receptors (AR) in primary and cocultured prostate fibroblasts and epithelial cells analyzed by Western blotting. One hundred-microliter aliquots of cell lysates (1×10^7 cells) were run on 4% PAGE at 20 mamp and then immunoblotted with AR antibody. Lane 1, LNCaP; lane 2, cocultured epithelial cells; lane 3, cocultured fibroblasts; lane 4, primary cultured epithelial cell; lane 5, primary cultured fibroblast.

included in each set of PCR reactions. The authenticity of all PCR products was verified by restriction analysis as previously described (20).

PAGE and Western blot analysis for androgen receptor

Cells to be analyzed by PAGE were lysed directly in 1 mL loading buffer [100 mmol/L Tris-HCl (pH 6.8), 200 mmol/L dithiothreitol, 4% SDS, 0.4% bromophenol blue, and 20% glycerol]. One hundred-microliter samples (25 μg protein/mL) were electrophoresed on 6% SDS-polyacrylamide gels following the method of Laemmli (21). Gels were electroblotted onto nitrocellulose and incubated with a rabbit polyclonal antibody to human androgen receptor (Novocastra Laboratories, Newcastle, UK). Protein (54 μg/mL) from LNCaP cells (a prostate cancer cell line known to express androgen receptors) was included as a positive control. Primary antibodies were detected using an ECL kit (Amersham).

Cell proliferation and response to androgens

Cellular responsiveness to androgens was determined by replacing normal growth medium with medium supplemented with 10% FCS stripped of endogenous steroids by treatment with dextran-coated charcoal. After a 24-h incubation period, the cells were supplied with fresh stripped medium containing 100 nmol/L testosterone (Sigma) and maintained for a further 4 days. Cells were subsequently removed by trypsinization, their densities were estimated with a hemocytometer, and counts are given as the number of cells per well. Controls that received no testosterone were similarly analyzed. All results are reported as the mean ± SEM of three replicates.

PSA measurement and expression

Immunoenzymetric assay. Cells were maintained in the same medium for 4 days, after which conditioned medium from six wells was collected then dialyzed against H_2O for 24 h in membranes with a 6000–8000 mol wt cut-off. The dialysate was lyophilized and reconstituted in 100 μL sterile H_2O, and PSA was measured using a Tandem-MP assay kit (Hybritech, Liege, Belgium) according to the manufacturer's protocol. Results were expressed as the mean (nanograms per mL) ± SD of three replicates.

Immunohistochemistry of PSA. Epithelial cells grown under normal or coculture conditions were fixed directly in six-well plates in 1% formaldehyde in phosphate-buffered saline. After washing twice in Tris-buffered saline [TBS; 60 mmol/L Tris-HCl (pH 7.4) and 137 mmol/L NaCl], endogenous peroxidase activity was blocked with 3% H_2O_2. Nonspecific binding of antibodies was blocked with 20% sheep serum for 20 min at room temperature. Incubation was carried out overnight at 4 C with anti-PSA monoclonal antibody (Bionostics, Wyboston, UK) diluted in TBS (1:50,000). Cells were given two 5-min washes with TBS, then incubated in biotinylated sheep antimouse antibody (Dako, High Wycombe, UK) for 30 min at room temperature, followed by two five-min washes in TBS. After a 30-min incubation with horseradish peroxidase-conjugated streptavidin, cells were washed twice with TBS, and the peroxidase enzyme was visualized by immersion in 0.05% (wt/vol) diaminobenzidine for 10 min. Cells were counterstained with hematoxylin for 1 min. For each staining experiment, a negative control with the primary antibody omitted from the staining procedure and a positive control (LNCaP cell line) were included.

Electron microscopy

Separated primary and cocultured epithelial and fibroblast cells were harvested by trypsinization, pelleted by centrifugation, and fixed with 3% glutaraldehyde in 0.1 mol/L cacodylate-HCl buffer at 4 C overnight. Secondary fixation of samples was carried out with osmium tetroxide in deionized water for 45 min at room temperature. Samples were impregnated with EMIX resin and cut into 90-nm sections, which were mounted on 300-μm mesh copper grids before staining using the uranyl acetate-lead citrate method (22). After processing, the samples were visualized using a JEOL 100CXII transmission electron microscope (JEOL, Peabody, MA) operating at 60 kV.

Statistical analysis

Statistical significance was determined using two-tailed Student's *t* test.

Results

Morphology of prostate epithelial and stromal cells grown in coculture

Cells grown in coculture were examined by electron microscopy for specific intracellular structures characteristic of prostate fibroblast and epithelial cells. The morphology of these cells was also compared to that of separated primary culture cells maintained on plastic. Cocultured epithelial cells (Fig. 1A) exhibited an abundance of tonofibrils, maintained a complex microvilli structure, and possessed a large number of secretory vesicles. They also showed greater differentiation than separated primary epithelial cells, which grew in monolayer and exhibited very few microvilli (Fig. 1B). The primary and cocultured epithelial cells were also stained for specific markers of basal epithelial cells using the mouse antihuman high mol wt cytokeratin antibody from Dako, which reacts with cytokeratins 1, 5, 10, and 14. This demonstrated good staining for the primary cultured epithelial cells, highlighting the basal nature of the cells. The cocultured cells also expressed these basal epithelial cell markers, indicating that the epithelial cells grown in coculture are also basal in nature (results not shown).

Cocultured fibroblasts (Fig. 2), on the other hand, were characterized by the presence of cilia, which are known to be

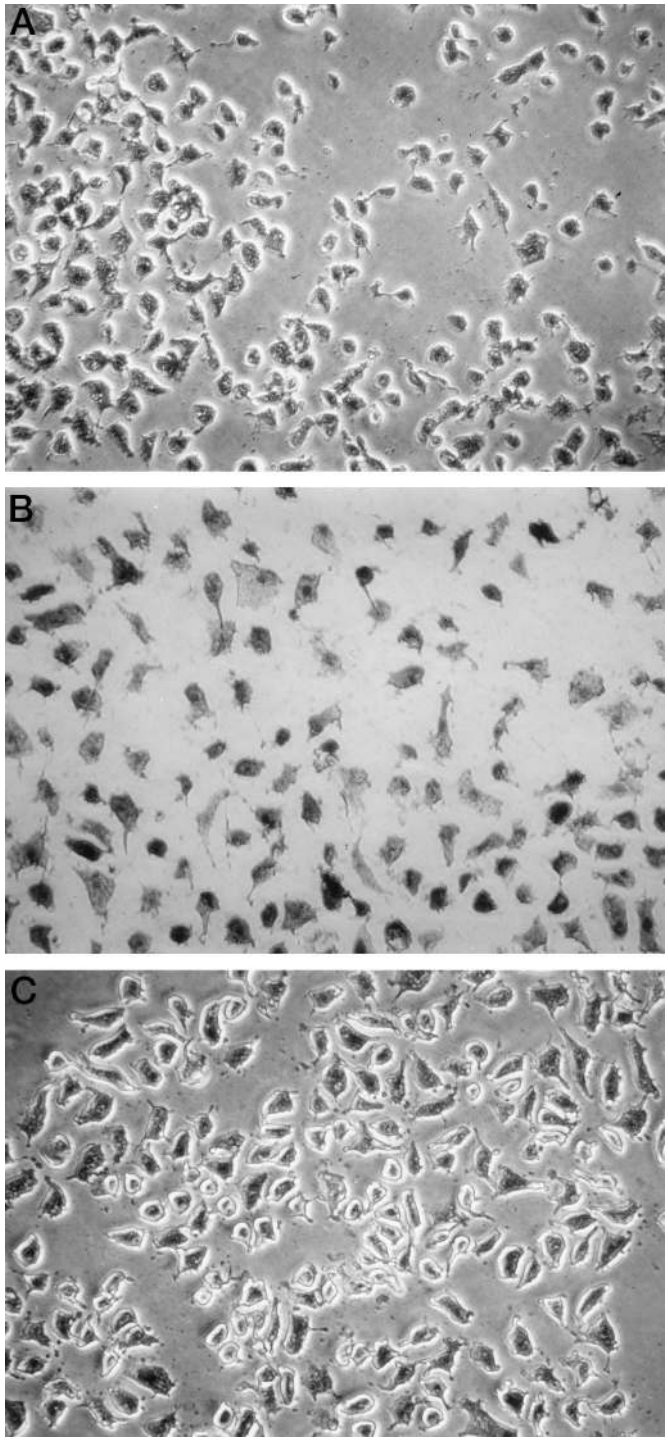

FIG. 4. Immunoperoxidase staining of separated and cocultured primary cultures of epithelial cells derived from BPH. Primary monoclonal anti-PSA antibody was used at dilution of 1:50,000. Sections were counterstained with hemotoxylin. a, Cocultured epithelium processed in the absence of the primary antibody; b, cocultured epithelium incubated with PSA monoclonal antibody; c, primary epithelial cells processed in the presence of the primary antibody.

specific to this type of cell and the formation of collagen. The difference between primary and cocultured fibroblast cells is demonstrated in Fig. 2, A–C. The cocultured fibroblast cells shown in Fig. 2A demonstrate the characteristic shape of a fibroblast cell, abundant cilia, Golgi, and collagen production (Fig. 2B). The primary fibroblast cells shown in Fig. 2C are rounder in appearance and demonstrate little cilia and no collagen production, but have large amounts of Golgi. There is no evidence of smooth muscle cells in either the cocultured epithelial or fibroblast.

Characterization of stromal and epithelial cells grown in coculture

Expression of androgen receptors. Androgen receptor expression in fibroblast and epithelial cells at passage 1 (P1) was examined by Western blot analysis and compared to expression in the same cell types in coculture (Fig. 3). Cocultured epithelial and fibroblast cells contained a single immunoreactive band at 110 kDa corresponding to the protein for human androgen receptor and coincident with the 110-kD immunoreactive protein in LNCaP cells, an androgen receptor-expressing prostate cell line (11). No similar bands were seen in separated primary cultured fibroblast or epithelial cells (Fig. 3).

PSA expression in cocultured epithelial cells. Medium conditioned by cocultured epithelial cells for 4 days contained 0.2 ± 0.0015 ng/mL PSA, whereas PSA was undetectable in medium conditioned by separately cultured epithelial cells over the same time period. Furthermore, exposure of cocultured epithelial cells to exogenous testosterone (100 nmol/L) elicited a 2-fold increase in PSA to 0.4115 ± 0.0057 ng/mL. We were unable to detect any PSA in the separated primary cultured epithelial cells even after these cells were exposed to testosterone. In all experiments, PSA measurements were normalized by cell count. As in intact prostate, PSA expression was restricted to the epithelial cells in the cocultures. PSA expression in the cocultured cells has been detected up to 21 days after initialization of the coculture procedure.

Immunostaining employing a specific antibody to the PSA demonstrated positive PSA staining in cocultured epithelial cells (Fig. 4b), in contrast to separately cultured epithelial cells, which showed no PSA staining (Fig. 4c). No PSA immunoreactivity was seen in fibroblasts either separately cultured or cocultured.

Androgens stimulate proliferation of cocultured cells

Two-day treatment of fibroblast and epithelial cells grown in coculture with DHT at 100 nmol/L produced no significant increase ($P < 0.05$) in cell numbers compared to those of control cells (Fig. 5) in the absence of androgens. However, longer exposure to the androgens (4 days) yielded a significant increase ($P < 0.05$) in number of cocultured epithelial and fibroblast cells compared to that in control cocultures (Fig. 5). Noteworthy are our earlier studies on separated primary cultures of fibroblast and epithelial cells that demonstrated no significant increase in cell numbers after exposure to exogenous DHT compared with controls (11).

Cocultured cells express both 5α-RI and 5α-RII

Examination of total RNA isolated from co-cultured and separated primary epithelial and fibroblast cells demonstrated striking differences in the extent of 5α-RI and 5α-

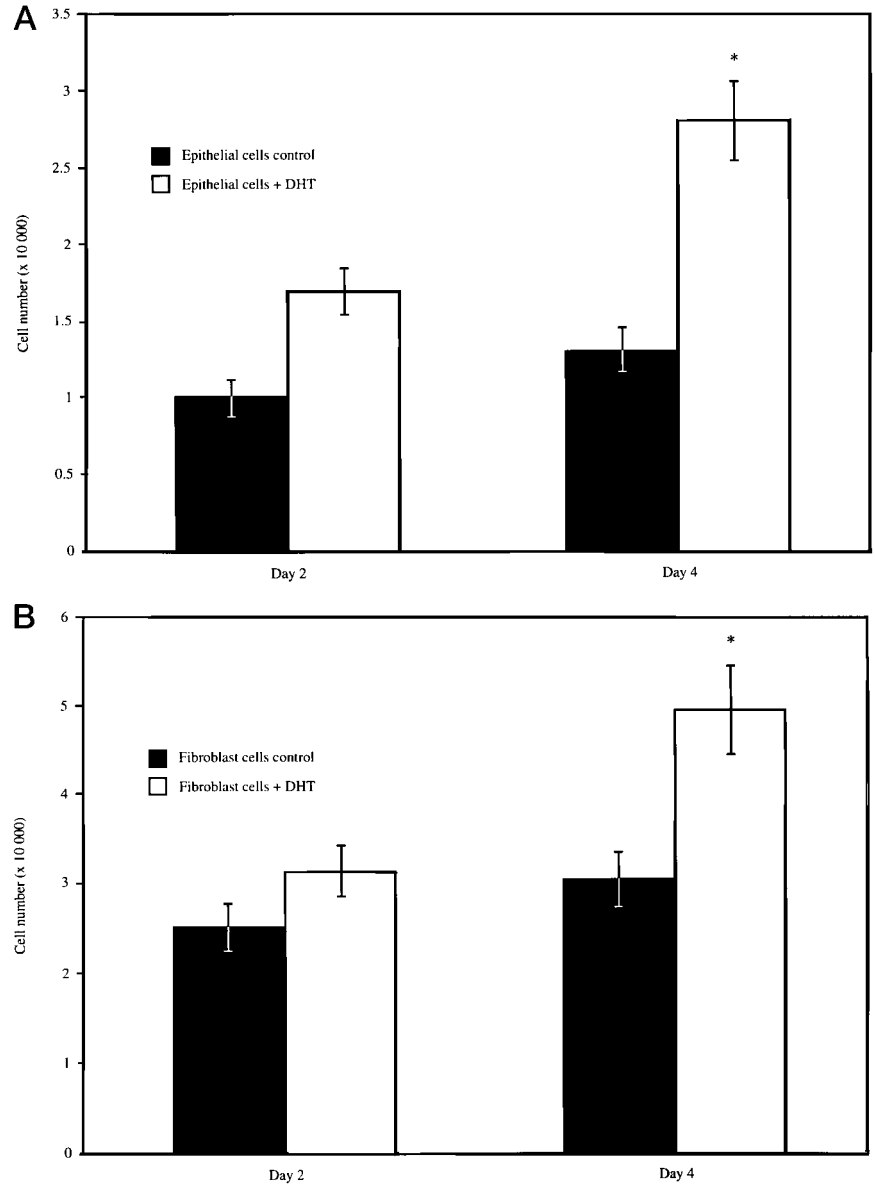

FIG. 5. The effect of DHT (100 nmol/L) on the growth of cocultured epithelial cells (a) and fibroblasts (b). Cell counts were assessed on days 2 and 4 and compared to those in the control culture with no androgens. Each *data point* represents the mean from three wells ± SEM. *, $P < 0.05$.

RII gene expression as assessed by RT-PCR (Figs. 6 and 7). Whereas separately cultured fibroblast and epithelial cells derived from BPH specimens clearly contained a 5α-RI mRNA easily detectable by RT-PCR (Fig. 6), no amplification of 5α-RII cDNA was seen. In contrast, RT-PCR carried out on total RNA isolated from cocultured fibroblast or epithelial cells (from the same tissue sample) generated a very strong 5α-RI and 5α-RII signal in the RT-PCR reaction (Fig. 7). This demonstrates that cocultures of fibroblast and epithelial prostate cells maintained the expression of 5α-RII and 5α-RI seen in intact prostate (20) in contrast to separately cultured cells that expressed the type I isoenzyme only.

To verify that the 5α-RI and 5α-RII mRNA expressed in cocultured cells were translated into functional enzyme, we compared the activities of the two 5α-reductase isoenzymes in cocultured and separately cultured epithelial cells and fibroblasts. The results confirmed the data generated from the mRNA analyses. Cocultured fibroblasts and epithelial cell suspensions (~60,000 cells/mL) exhibited high levels of 5α-RI and 5α-RII activities, as measured by the conversion of [^{3}H]testosterone to [^{3}H]DHT at pH 7.5 (5α-R1) and pH 5.5 (5α-RII; Table 1). In contrast, the separately cultured fibroblast and epithelial cells showed much lower levels (~30-fold) of 5α-RI activity, and 5α-RII activity was undetectable (Table 1). We also noted that whereas cocultured epithelial cells expressed similar levels of 5α-RI and 5α-RII activities, cocultured fibroblasts contained 6 times more 5α-RII activity than 5α-RI enzyme activity (Table 1).

Discussion

Until recently, much of the testing was carried out on immortalized cell lines (LNCaP, PC-3, DU-145, and TSU-PRI) derived essentially from metastatic prostate cancer (13, 14, 24, 25) that maintained little of the differentiated character-

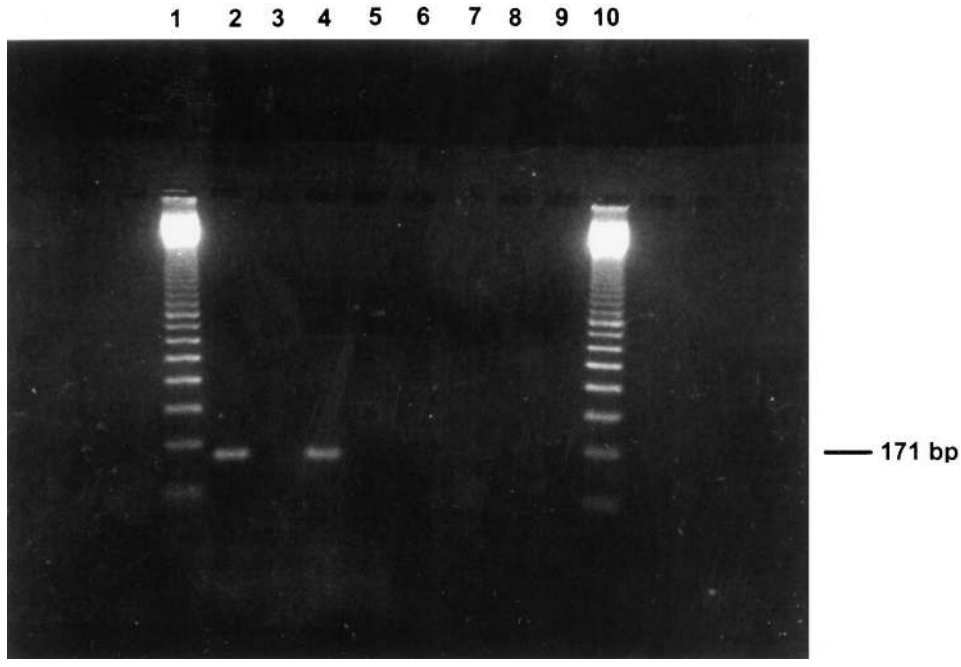

FIG. 6. Amplification of 5α-RI and 5α-RII cDNA fragments from primary epithelial and fibroblast cells. Total RNA (1 μg) isolated from the primary cultures was used to prepare cDNA in an oligo(deoxythymidine)-primed RT. cDNAs were subjected to either a 30-cycle (5α-RI) or a 35-cycle (5α-RII) PCR reaction with the 5α-RI or 5α-RII primers, and the resulting fragments were separated on 1% agarose gel containing ethidium bromide. The PCR products were visualized under UV luminator. Lane 1, One hundred-base pair ladder; lane 2, epithelial cells/5α-RI; lane 3, epithelial cells/5α-RII; lane 4, fibroblasts/5α-RI; lane 5, fibroblasts/5α-RII; lane 6, no DNA control/5α-RI epithelium; lane 7, no DNA control/5α-RII epithelium; lane 8, no DNA control/5α-RI fibroblast; lane 9, no DNA control/5α-RII fibroblast; lane 10, 100-bp ladder.

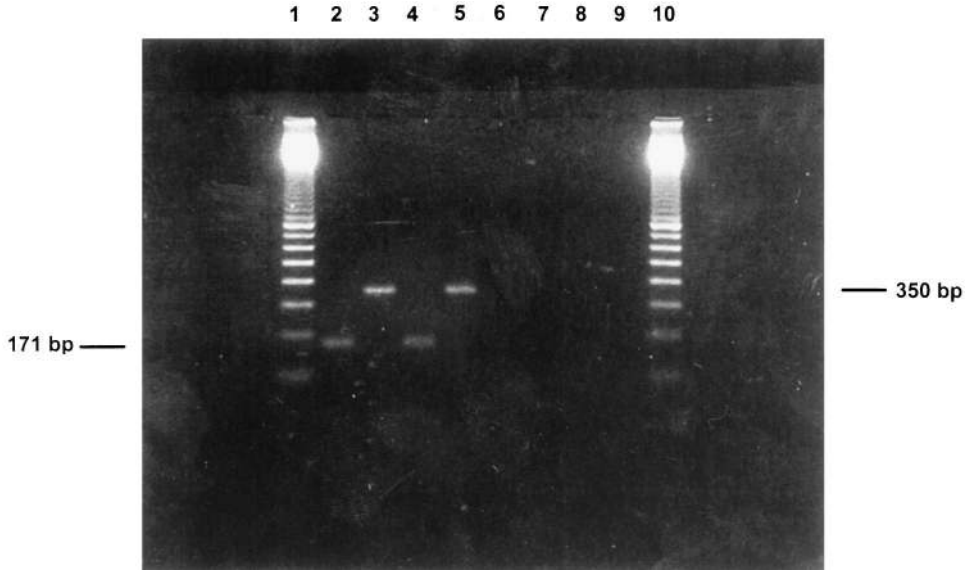

FIG. 7. Amplification of 5α-RI and 5α-RII cDNA fragments from cocultured epithelial and fibroblast cells. Total RNA (1 μg) isolated from the cocultures was used to prepare cDNA in an oligo(deoxythymidine)-primed RT. cDNAs were subjected to either a 30-cycle (5α-RI) or a 35-cycle (5α-RII) PCR reaction with the 5α-RI or 5α-RII primers, and the resulting fragments were separated on a 1% agarose gel containing ethidium bromide. The PCR products were visualized under UV illumination. Lane 1, One hundred-base pair ladder; lane 2, cocultured epithelium/5α-RI; lane 3, cocultured epithelium/5α-RII; lane 4, cocultured fibroblast/5α-RI; lane 5, cocultured fibroblast/5α-RII; lane 6, no DNA control/5α-RI epithelium; lane 7, no DNA control/5α-RII epithelium; lane 8, no DNA control/5α-RI fibroblast; lane 9, no DNA control/5α-RII fibroblast; lane 10, 100-bp ladder.

istics of their benign counterpart. Primary cultured human prostate epithelial and fibroblast cells have also been used, but these, again, exhibited few of the properties of the cells of origin (5–8, 11, 26–29). Furthermore, none of the *in vitro* models eluded to, with the notable exception of LNCaP, express androgen receptors and secrete PSA (31). Clearly, for prostate epithelial cultures to be truly representative of epithelial cells *in vivo*, they must exhibit androgen dependence in both growth and gene expression (11), and this demands the presence of a fully functional androgen receptor protein.

To overcome the manifested limitations of earlier models, we have herein described an *in vitro* cell model for investigating prostate cell function and differentiation. Cocultured fibroblast and epithelial cells maintained high levels of 5α-RI and 5α-RII expression, expressed PSA, and were responsive to androgens, thus mimicking the *in vivo* situation. Furthermore, the coculture system allowed separation of the two cell types at the end of the culture period, permitting separate analysis to be performed on the two cell types. This model has very significant advantages over other *in vitro* models described, all of which have limitations in their use and validity (5–8, 11–14, 24–29). In particular, the growth of the prostate gland is dependent on androgens; none of the *in vitro* models currently in use, with the notable exception of the LNCaP cell line, contains functional androgen receptors, and therefore, they do not exhibit androgen-sensitive cell growth and gene expression. In contrast, our cocultured prostate cells both expressed androgen receptors and showed androgen-induced alterations in cell properties, and the growth of both the fibroblast and epithelial components of the coculture was stimulated by the presence of exogenous androgens. To the best of our knowledge, this is the first

example where androgen has been noted to stimulate the proliferation of fibroblasts in prostate tissue.

Our studies also showed that the cultivation of prostate epithelial cells on standard tissue culture flasks reduced their capacity to secrete PSA and promoted a dedifferentiated morphology. However, once the epithelial cells were cocultured with fibroblasts, they regained many of the *in vivo* characteristics of the differentiated human prostate gland, including round nuclei as well as the presence of tonofibrils and microvilli, whereas the fibroblasts showed elongated nuclei and the presence of Golgi and cilia. In addition, we observed enhanced PSA secretory activity, which was increased significantly after androgen stimulation. The importance of the fibroblast to the clonal growth of prostate epithelial cells had been demonstrated previously (31), and a subsequent report Fong *et al.* (9) demonstrated that human prostate epithelial cells grown on basement membrane promoted PSA secretion, which was potentiated by the presence of androgens. In that study the prostate epithelium was in contact with the basement membrane, whereas in our own coculture system the two cell populations were separated by a microporous membrane, thus allowing for epithelial/fibroblast cross-talk by means of diffusible elements. It is evident from these studies that cell to cell contact is not required for PSA expression, but the results suggest that the promotion of PSA secretion is influenced by secondary products from the fibroblast; these factors remain to be identified.

The present report also showed that the coculture microenvironment was crucial to the maintenance of 5α-reductase activity in both fibroblasts and epithelial cells. Expression of the isoenzyme types I and II was observed in both cell types, and this was further confirmed by assays of functional activities at pH 5.5 (5α-RII) and 7.5 (5α-RI), thus reflecting the patterns seen in human BPH (20). The loss of 5α-RII expression in separated primary cultures highlights the importance of epithelial/fibroblast cross-talk to maintain the activity of this isoenzyme. It is likely that a diffusible factor(s) produced by one or both cell types is responsible for the expression of this isoenzyme. In the absence of the factor(s), as in primary cultures of fibroblasts or epithelial cells grown in isolation, the responsible mechanism(s) is switched off, and no 5α-RII is detected. We are at present attempting to elucidate the nature of this mechanism(s).

Collectively, the findings reported in this study demonstrated that many of the properties of differentiated epithelium and fibroblast are preserved in the coculture system described here. This model allows for a better analysis of any possible *in vivo* effects of a test compound on the prostate than was previously possible using the earlier model systems. It also opens up opportunities for the investigation of fibroblast/epithelial cell interactions that may be important in understanding the processes involved in the development of not only BPH but also prostate cancer.

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