

# Malignant Transformation in a Nontumorigenic Human Prostatic Epithelial Cell Line<sup>1</sup>

Simon W. Hayward,<sup>2</sup> Yuzhuo Wang, Mei Cao, Yun Kit Hom, Baohui Zhang, Gary D. Grossfeld, Daniel Sudilovsky, and Gerald R. Cunha

Departments of Urology [S. W. H., Y. K. H., G. D. G., G. R. C.], Anatomy [Y. W., M. C., B. Z., G. R. C.], and Pathology [D. S.], University of California-San Francisco, San Francisco, California 94143

## ABSTRACT

The human prostatic epithelial cell line BPH-1 is normally nontumorigenic in nude mice. The present report demonstrates that this cell line can be permanently transformed by its microenvironment to become tumorigenic. The establishment of a series of tumorigenic sublines based on this parental cell line is described. BPH-1 cells were induced to form tumors either by recombination with human prostatic carcinoma-associated fibroblasts (CAFs) or by exposure to carcinogenic doses of testosterone and estradiol (T+E2) after recombination with rat urogenital sinus mesenchyme. Epithelial cells isolated from these tumors were established as cell strains in culture. When regrafted to nude mouse hosts epithelial cells isolated from CAF- or T+E2-induced tumors were found to be consistently tumorigenic even in the absence of CAF or T+E2. The T+E2-induced cell strains have been designated BPH1<sup>TETD</sup>-A and -B and the CAF-induced strains are designated BPH1<sup>CAFTD</sup>-01 through -08. *In vitro*, the cells had an epithelial morphology with a less well-defined cobblestone pattern than the parental line. They express SV40 large T antigen, confirming their derivation from the parental BPH-1 line. The BPH1<sup>CAFTD</sup> strains formed colonies in soft agar, whereas the parental BPH-1 cells and the BPH1<sup>TETD</sup> sublines did not. There was no immunocytochemically detectable expression of androgen (AR),  $\alpha$ -estrogen (ER $\alpha$ ), or progesterone (PR) receptors by the parental BPH-1 cell line or by any of the tumor-derived cell strains. The cells uniformly coexpressed both basal and luminal cell-type cytokeratins and the basal cell marker p63. When grafted beneath the renal capsule of athymic mouse hosts, all of the tumor-derived cell strains consistently formed tumors. These were predominantly poorly or moderately differentiated squamous or adenosquamous tumors, similar in organization to the primary tumors from which the cell strains were derived. The cell strains continued to express both basal- and luminal-type cytokeratins *in vivo*. Some of the cell strains also coexpressed vimentin. E-cadherin expression was absent from many of the cells, although patches of cells expressing this marker were seen. The cells continued to express SV40T antigen. These cell strains, which are all derived from a common nontumorigenic progenitor, represent a useful resource for examining genetic and phenotypic changes during carcinogenesis.

## INTRODUCTION

The nontumorigenic human prostatic epithelial cell line, BPH-1, can be induced to form tumors either by association with a tumorigenic stromal microenvironment or by treatment with hormonal carcinogens (1–4). This report demonstrates that epithelial cells derived from such tumors have undergone permanent malignant transformation and are tumorigenic in athymic mice. The establishment and characterization of a series of cell strains derived from the nontumorigenic BPH-1 human prostatic epithelial cell line is described.

Received 6/4/01; accepted 9/18/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> Supported by NIH Grants DK52721, DK52708, CA64872, DK47517, CA59831, and CA89520.

<sup>2</sup> To whom requests for reprints should be addressed, at Department of Urology Surgery, A 1302 Medical Center North, Vanderbilt University Medical Center, Nashville, TN 37232-2765. Phone: (615) 322-5823; Fax: (615) 322-8990; E-mail: simon.hayward@mcm.vanderbilt.edu.

At present, there are a limited number of models of prostatic carcinogenesis (5). Prostate cancer is a slowly developing disease of aging men and dogs and has an extremely low incidence of spontaneous occurrence in laboratory animals. A variety of methods, including hormonal induction and targeted expression of oncogenes have been used to induce prostate cancer in rats and mice (6–9). In a few cases, it has been possible to examine the range of tumor types derived from a common rodent precursor cell or tumor strain (10, 11).

There is a dearth of model systems to examine the induction of tumors in human prostatic epithelium. Although models that manipulate already malignant human prostatic cells and tissues are available (12), there are few models in which cancer is induced in a previously benign human prostatic epithelium. Bae *et al.* and Jackson-Cook *et al.* (13, 14) used repeated cycling of the generally nontumorigenic P69SV40T cell line through nude mouse hosts to produce tumorigenic sublines. Webber *et al.* and Bello *et al.* (5, 15) have used both Ki-ras and the chemical carcinogen methylnitrosourea (MNU) to derive a series of tumorigenic sublines from the nontumorigenic RWPE-1 line.

SV40T<sup>3</sup> was introduced into primary cultures of prostatic epithelial cells using the Zipneo viral construct containing a selectable *neo* cassette to generate the immortalized BPH-1 cell line (16). SV40T interacts with and inactivates both p53 and pRb, thus eliminating these two important tumor suppressor pathways (17). However many SV40T-expressing cells, including the BPH-1 cell line, are not tumorigenic (16, 18). The production of tumors in immuno-incompetent hosts is a clear marker of tumorigenicity and is easily demonstrated. The opposite of tumorigenicity (nontumorigenicity) implies an inability to form tumors. However the mere absence of tumors after transplantation into an immuno-incompetent host does not of itself prove that a cell line or graft is “nontumorigenic.” This terminology requires that the cells or tissues, which were grafted or injected, have the ability to survive at the graft site and not to grow to form a tumor. We have previously demonstrated that parental BPH-1 cells grafted to athymic hosts can be recovered from the graft site for up to 1 year after grafting and that these cells do not form tumors at the site (0/125 attempts; Ref. 4). BPH-1 cells can however be considered to be genetically initiated, in that they have suffered a major genetic insult (attributable to the expression of SV40T), which renders them susceptible to further genetic damage and to progression along a pathway to malignancy.

Prostatic carcinogenesis involves genetic alterations to epithelial cells including activation of oncogenes (19–22) and inactivation of tumor suppressor genes (23, 24). Alterations in tumor suppressor genes such as the *RB*<sup>3</sup> and *p53* genes have been suggested to play a role in the development of human prostate cancer (23, 25–27). The *RB* gene encodes a *M<sub>r</sub>* 110,000 nuclear protein involved in cell cycle control (28). *RB* gene mutations have been reported in 16.4% of primary human prostatic cancers, which suggests that inactivation of

<sup>3</sup> The abbreviations used are: SV40T, SV40 large T (antigen); RB, retinoblastoma; CAF, carcinoma-associated fibroblast; rUGM, rat urogenital sinus mesenchyme; FBS, fetal bovine serum; TD, tumor-derived; AR, androgen receptor; ER, estrogen receptor; E<sub>2</sub>, estradiol; T+E2, testosterone and E<sub>2</sub>; PR, progesterone receptor; IGF, insulin-like growth factor.

*RB* may play a role in carcinogenesis in at least a subset of prostatic carcinomas (29–31). Estimates of the levels of p53 mutations in prostate cancer vary widely. Commonly reported figures suggest 20–50% of advanced stage tumors contain these mutations (32). However estimates of p53 mutations as high as 56% in high-grade prostatic intraepithelial neoplasia and 72% in prostatic carcinoma have been made, based on immunohistochemical data (33).

Androgens have long been known to elicit prostatic epithelial differentiation as a result of paracrine actions dependent on receptors located in the urogenital sinus mesenchyme (34). In contrast, differentiated function of prostatic epithelium (expression of secretory markers) is apparently dependent on the presence of ARs located in the epithelial cells (35). More recent data show that ERs in both stromal and epithelial cells play a role in mediating estrogenic effects in the prostate (36). We have previously demonstrated that the nontumorigenic BPH-1 cell line can be induced to form tumors either by the influence of stromal cells that are derived from human prostate tumors (3) or by recombination with rUGM and stimulation with a combination of T+E2, presumably as a result of paracrine interactions (4). This communication describes the isolation and characterization of a series of 10 tumorigenic sublines with various degrees of invasive potential derived from the nontumorigenic parental BPH-1 line. The data presented here demonstrate for the first time that genetically initiated but nontumorigenic human prostatic epithelial cells can undergo permanent malignant transformation as a result of their previous exposure to CAFs. We further demonstrate that cells derived from hormonally induced tumors are likewise tumorigenic. These cell lines will be a useful resource with which to investigate the genetic and phenotypic lesions induced by the *in vivo* process of carcinogenesis.

## MATERIALS AND METHODS

**Preparation and Processing of Grafts.** Two types of cellular recombinants were prepared using previously described methods. Briefly BPH-1 cells were from our own stocks (16). Cells were routinely maintained and passaged in RPMI 1640 with 5% FBS. BPH-1 cells were released from tissue culture plastic with trypsin, washed in growth medium containing 20% FBS, and viable cells were counted using trypan blue exclusion and a hemacytometer.

CAF was prepared from human prostate tumors as described previously (3). Briefly, tumors were identified using histopathological analysis of stained frozen sections. Five-mm<sup>3</sup> tissue fragments immediately adjacent to identified carcinoma were used. Specimens were digested with collagenase and hyaluronidase (37) and placed into culture in RPMI 1640 containing penicillin, streptomycin, and Fungizone supplemented with 10% FBS. After 10 days of growth, the fibroblastic cells were separated from contaminating epithelial and endothelial cells by differential trypsinization. Subsequent immunocytochemical characterization confirmed their fibroblastic nature, as described previously (3).

Pregnant rats were obtained from Simenson (Gilroy, CA). rUGM was prepared from 18-day embryonic fetuses (copulatory plug date denoted as day 0). Urogenital sinuses were dissected from fetuses and separated into epithelial and mesenchymal components by tryptic digestion, as described previously (38). rUGM was then further reduced to single cells by a 90-min digestion at 37°C with 187 units/ml collagenase (Life Technologies, Inc., Grand Island, NY). After digestion, the cells were washed extensively with RPMI 1640 tissue culture medium. Viable cells were then counted using a hemacytometer, and viability was determined by trypan blue exclusion.

Cell recombinants were prepared by mixing 100,000 epithelial (BPH-1) cells with 250,000 rUGM cells or CAFs in suspension. For control grafts, 350,000 cells of each cell type (BPH-1, rUGM, or CAF) were used. Cells were pelleted and resuspended in 50  $\mu$ l of neutralized rat tail collagen prepared as described previously (39). The recombinants were allowed to set at 37°C for 15 min and were then covered with growth medium (RPMI 1640 + 5% FBS) containing testosterone ( $10^{-8}$  M) and were cultured overnight. Recombinants were then grafted beneath the renal capsule of adult male outbred athymic mice (Simenson). All of the animals were housed in the University of California-San

Francisco laboratory animal resource center with food and drinking water *ad libitum* under controlled conditions (12 h light, 12 h dark, 20°C  $\pm$  2°C).

Mice carrying rUGM + BPH-1 recombinants were treated hormonally by surgical implantation of Silastic capsules containing E<sub>2</sub> (17- $\beta$ -estradiol benzoate; Sigma Chemical Co.) and testosterone propionate (Sigma Chemical Co.) as described previously (4). Control animals received empty Silastic tubing. Three months postgrafting, hosts were killed by anesthetic overdose followed by cervical dislocation. Kidneys were excised, and grafts were dissected free of the host kidney, weighed, and then processed for immunohistochemistry and for cell isolation.

**Isolation of Cell Strains.** Grafts, which had formed tumors, were cut into small fragments using forceps and scalpel. Control grafts composed of BPH-1 + rUGM from untreated hosts were treated in the same manner. The resultant tissue fragments were plated overnight in tissue culture flasks in a small volume (1.2 ml in a 25-cm<sup>2</sup> flask) of tissue culture medium (RPMI 1640 containing 5% FBS). The following day, the medium and any unattached fragments were aspirated and replaced with 5 ml of fresh medium. After 1 week of growth, the medium was additionally supplemented with 250  $\mu$ g/ml G418 (Clontech, Palo Alto, CA). G418 selection was applied for 2 weeks, during which time all of the nonresistant cells died. The resulting cell populations were expanded in culture and characterized as described below.

**Regrafting Experiments.** Epithelial cells (350,000 cells) of each of the derived cell strains were suspended in 50  $\mu$ l of rat tail collagen gel, as described above, and grafted to the kidney capsules of male athymic mouse hosts. After 1 month of growth, the hosts were killed, and the grafts were removed from the kidney, weighed, fixed in formalin, and processed to paraffin. Before fixation, small pieces of the CAFTD grafts were removed and placed in culture as described above to isolate a “second generation” of TD cells. These were again grown in athymic mouse hosts for 1 month, excised and weighed, and fixed for immunohistochemical analysis. The lineage of these cell strains are shown in Fig. 1.

**Immunohistochemistry and Immunocytochemistry.** Formalin-fixed tumor sections were deparaffinized, hydrated, and blocked for 30 min with 0.5% H<sub>2</sub>O<sub>2</sub> in methanol, washed in PBS (pH 7.4), and treated with 5% goat or donkey serum for 30 min. The sections were then incubated with the primary antibodies overnight at 4°C or with nonimmune mouse IgG at the same concentration. In these experiments, rabbit polyclonal anti-AR antibody (PA1-111A; 1:100) was purchased from Affinity BioReagents (Golden, CO). The anti-SV40 T antibody PaB 101 was a generous gift from Dr. John Lehman (Albany Medical College, Albany, NY). Anticytokeratin antibodies, all mouse monoclonals (LE41, LE61, and LL001 against keratins, 8, 18, and 14, respectively) were generously provided by Dr. E. B. Lane, University of Dundee,

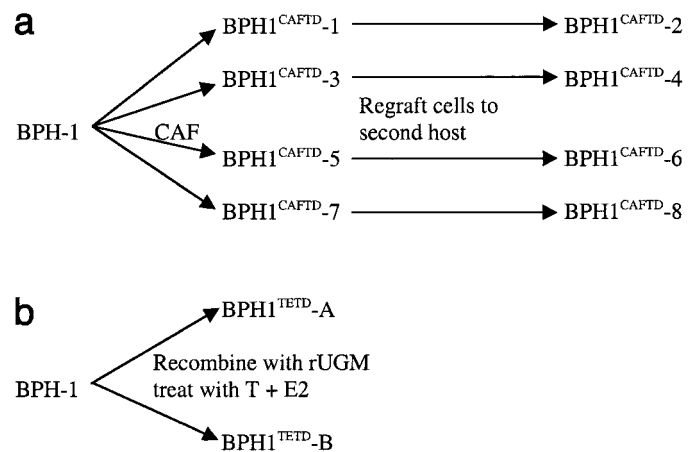


Fig. 1. *a*, BPH1<sup>CAFTD</sup> lineage. BPH-1 cells were recombined with CAFs and grafted beneath the renal capsule of male athymic mouse hosts to produce tumors. Tissue from these tumors was then put into cell culture, and G418-resistant cells were selected. Cell strains were designated BPH1<sup>CAFTD</sup>-01, -03, -05, and -07. Each of these cell strains was then regrafted beneath the renal capsule of athymic mice to produce tumors. Further cell selection was performed from which BPH1<sup>CAFTD</sup>-02, -04, -06, and -08 were derived. *b*, BPH1<sup>TETD</sup> lineage. BPH-1 cells were recombined with rUGM and grafted beneath the renal capsule of male athymic mouse hosts. The hosts were treated with T+E2 for three months. The resulting tumors were excised; epithelial cells were grown out and selected by resistance to G418.

Dundee, United Kingdom. Mouse anti-E-cadherin monoclonal antibody was purchased from Transduction Laboratories (San Diego, CA). Anti-p63 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). p63 is a p53 homologue that is essential for regenerative proliferation in epithelial development. We have demonstrated that, in prostate, p63 is coexpressed with cytokeratin 14 and is, thus, a good nuclear marker of basal epithelial cell type (40). Anti-human ER $\alpha$  (clone 1D5) was purchased from Dako (Carpenteria, CA). Anti-Ki67 was purchased from Immunotech (Westbrook, ME). Purified rabbit and mouse IgGs were obtained from Zymed Corp. (South San Francisco, CA). Biotinylated antirabbit and antimouse IgG were obtained from Amersham International (Arlington Heights, IL). Biotinylated anti-goat antibody was purchased from Sigma Chemical Co. Peroxidase-linked avidin/biotin complex reagents were obtained from Vector Laboratories (Burlingame, CA). After incubation with primary antibody, sections were washed with PBS and incubated with appropriate biotinylated secondary antimouse immunoglobulin diluted with PBS at 1:200 for 30 min at room temperature. After incubation with the secondary antibody, sections were washed in PBS (three 10-min washes) and then incubated with avidin-biotin complex (Vector Laboratories, Foster City, CA) for 30 min at room temperature. After a further 30-min of washing in PBS, immunoreactivity was visualized using 3', 3'-diaminobenzidine (DAB) in PBS and 0.03% H<sub>2</sub>O<sub>2</sub>. Sections were counterstained with hematoxylin and were dehydrated in graded alcohols. Control sections were processed in parallel with mouse or rabbit non-immune IgG (Dako) at the same concentration as the primary antibodies.

For immunocytochemical analysis cells were grown on sialized microscope slides, fixed briefly in 100% ethanol, and washed in PBS. Cells were then blocked and processed as described above, except that epitope retrieval was not performed.

**Proliferation Assays.** Proliferation assays were initiated by plating 25,000 BPH-1 or experimental cells in 0.5 ml of 5% FBS-containing medium into one well of a 24-well plate at 37°C and grown overnight to allow cells to attach. After attachment, serum-containing medium along with nonattached cells were aspirated. Experimental culture medium [RPMI 1640 (0.5 ml per well) with 2.5% dextran coated charcoal-stripped heat inactivated serum] was added, and cultures were continued another 5 days with one medium change.

Cultures were terminated for proliferation assays by aspirating culture medium and fixing cells with 0.2 ml of 11% glutaraldehyde per well on a rotary shaker for at least 20 min at room temperature. Glutaraldehyde was then removed, and plates were washed thoroughly with tap water and allowed to air-dry (~1 h). Cells were subsequently stained with 0.5 ml of 0.1% crystal violet per well in 200 nM boric acid (at pH 6.3) for 20 min on a rotary shaker. Crystal violet staining solution was aspirated from each well, and plates were again washed extensively with tap water and allowed to air-dry.

Proliferation measurement was started by adding 0.2 ml of 10% acetic acid per well for 20 min on a rotary shaker to solubilize the bound dye. Absorbance of the dye was measured at 590 nm with an Elx800UV automated microplate reader (BIO-TEK Instruments, Winooski, VT). Cell proliferation values were determined via extrapolation from a standard curve established with BPH-1 cells. Results are presented as the mean of at least three replicates with SE at 95% probability.

**Preparation of Soft Agar Plates.** A bottom layer of 1 ml was prepared with a 1:1 mixture of 1% agar (~50°C) and warm 2 $\times$  DMEM and was poured into a well of a 6-well plate. This layer was allowed to solidify at 4°C for at least 15 min. A top layer was then constructed by mixing 500  $\mu$ l of warm 2 $\times$  DMEM with 200  $\mu$ l of sterile dH<sub>2</sub>O and 300  $\mu$ l of 1% agar (~50°C) along with the cells at the appropriate density. Typically, 2 wells of a 6-well plate were each seeded with 10<sup>3</sup>, 10<sup>4</sup>, or 10<sup>5</sup> cells per well. The top layer with cells was poured on top of the solidified bottom layer and allowed to gel. After both layers had set, cultures were fed with 1 ml of RPMI 1640 supplemented with 5% FBS. Medium was gently removed and feedings delivered at least three times a week. After 4 weeks of culture, as much medium as possible was removed and cultures were stained with 10  $\mu$ g/ml of tetrazolium violet (Sigma Chemical Co.) in medium overnight. Anchorage-independent growth was assessed by counting cell colonies of more than 200  $\mu$ m in diameter in a single well of a 6-well plate. Data are presented as colonies present per 10,000 cells originally plated.

## RESULTS

Grafts of BPH-1 epithelial cells recombined with CAF or recombined with rUGM followed by treatment with T+E2 gave rise to tumors, as described previously (3, 4). ARs and ERs were not detected in the epithelial component of these tumors. However, immunoreactivity to steroid receptors was visualized in both the rUGM and the CAF-derived stromal cells. Grafts of BPH-1 + rUGM in untreated hosts did not form malignant tumors, but gave rise to glandular architecture, as previously described (3, 4). After culture and selection with G418, four cell strains were derived from BPH-1 + CAF tumors (designated BPH1<sup>CAFTD</sup>-01, -03, -05 and -07), and an additional two strains were derived from T+E2-treated rUGM + BPH-1 tissue recombinants (designated BPH1<sup>TETD</sup>-A and -B). The four BPH1<sup>CAFTD</sup> strains were grafted in collagen gel beneath the renal

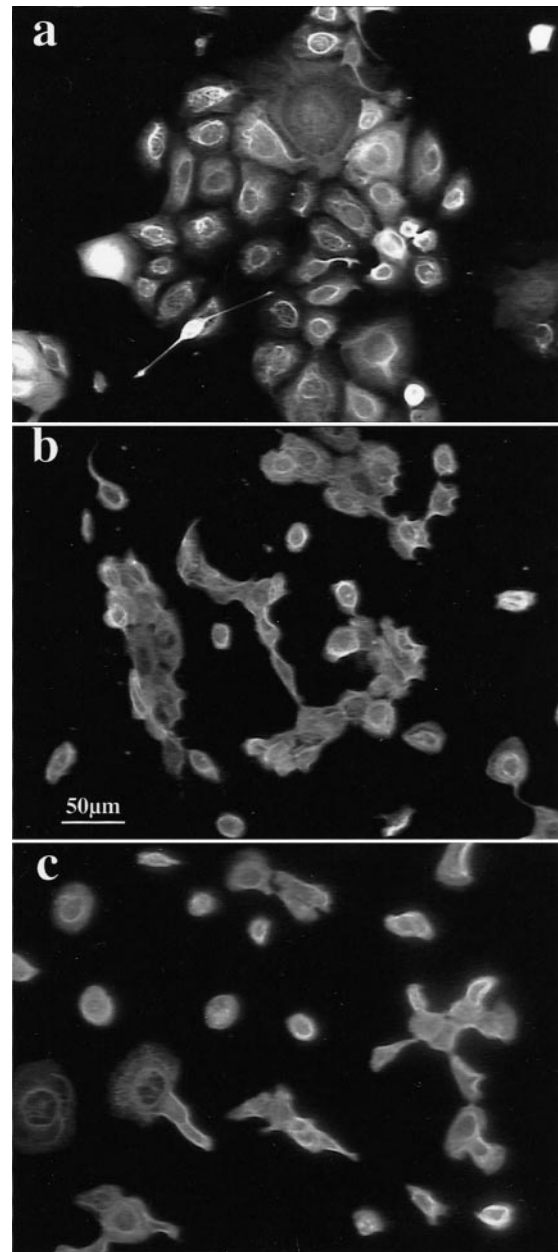


Fig. 2. Immunofluorescence images (at the same magnification) showing cytokeratin expression in the BPH-1 cell line (a) and in BPH1<sup>CAFTD</sup>-01 (b) and BPH1<sup>CAFTD</sup>-02 (c) *in vitro*. Note the cobblestone morphology of the parental line with evenly shaped colonies as compared with the smaller more elongated TD cells forming more angular groups.

Table 1 Summary of the *in vitro* phenotypic characteristics of the BPH-1-derived lineages

Confluent and subconfluent (subconf) cells were stained immunocytochemically to determine expression and localization of epithelial markers.

	E-cadherin	$\beta$ -catenin	Pan keratin	HMW keratin	p63	Keratin 8	Vimentin
Parental BPH-1	+++ <sup>a</sup>	++	+++	+++	+++	+	-
Parental BPH1 (subconf)	+++	++ (membrane)	+++	+++	+++	+	-
BPH1 <sup>CAFTD</sup> -01	++	++ (membrane)	+++	+++	+	+	-/+
BPH1 <sup>CAFTD</sup> -01 (subconf)	+	++ (membrane and cytoplasm)	+++	+++	+	+	+
BPH1 <sup>CAFTD</sup> -02	++	+	+++	+++	++	+	-/+
BPH1 <sup>CAFTD</sup> -02 (subconf)	++	+/-	+++	+++	++	+	-/+
BPH1 <sup>CAFTD</sup> -03	+/-	+/-	+++	+++	+++	+	-
BPH1 <sup>CAFTD</sup> -03 (subconf)	+/-	+/-	+++	+++	+++	+	-
BPH1 <sup>CAFTD</sup> -04	+/-	+	+++	+++	+++	+	-
BPH1 <sup>CAFTD</sup> -04 (subconf)	-	+	+++	+++	+++	+	-
BPH1 <sup>CAFTD</sup> -05	++	+/-	+++	+++	+++	+	-
BPH1 <sup>CAFTD</sup> -05 (subconf)	+/-	+/-	+++	+++	+++	+	-
BPH1 <sup>CAFTD</sup> -06	+/-	+/-	+++	+++	+++	+	-
BPH1 <sup>CAFTD</sup> -06 (subconf)	++	+/-	+++	+++	+++	+	-
BPH1 <sup>CAFTD</sup> -07	+/-	++ (membrane)	+++	+++	+++	+	-/+
BPH1 <sup>CAFTD</sup> -07 (subconf)	+/-	++ (membrane)	+++	+++	+++	+	-/+
BPH1 <sup>CAFTD</sup> -08	+	+	+++	+++	+	+	-
BPH1 <sup>CAFTD</sup> -08 (subconf)	+	+	+++	+++	+	+	-
BPH1 <sup>TETD</sup> -A	+/-	-/+ (cytoplasm)	+++	+++	++	+	-/+
BPH1 <sup>TETD</sup> -A (subconf)	+/-	-/+ (cytoplasm)	+++	+++	++	+	+
BPH1 <sup>TETD</sup> -B	+/-	++ (membrane)	+++	+++	+	+	-
BPH1 <sup>TETD</sup> -B (subconf)	+/-	-/+ (cytoplasm)	+++	+++	+	+	-/+

<sup>a</sup> Staining: -, absent; -/+, weak (minority of cells); +/-, weak (majority of cells); +, uniform weak; ++, moderate; +++, strong.

capsule of athymic mouse hosts, in which they were formed tumors that were used to produce a second generation of BPH1<sup>CAFTD</sup> cells (designated BPH1<sup>CAFTD</sup>-02, -04, -06 and -08). The lineage derivation of these cell strains is outlined in Fig. 1. In addition to these cell lineages a strain of cells derived from BPH-1 + rUGM grafts to untreated hosts was also derived and designated BPH1<sup>UGM</sup>.

When grown on tissue culture plastic, the TD cell strains had a cobblestone or slightly elongated morphology. Although there were strain-to-strain differences, the TD cells were in general less tightly adherent to each other than the parental BPH-1 cells (Fig. 2). *In vitro* the cell strains uniformly expressed SV40T antigen, confirming their origin. The basal cell-specific markers, high-molecular weight cytokeratins and p63, were uniformly expressed as was the luminal cell-specific marker, cytokeratin 8 (Table 1). ARs,  $\alpha$ -ERs, and PRs were not detected by immunocytochemistry in the parental cell line or in any of the TD cell strains. Vimentin was not detected in the parental BPH-1 cell line but was weakly expressed in three of the BPH1<sup>CAFTD</sup> and both of the BPH1<sup>TETD</sup> strains (Table 1). Expression was slightly

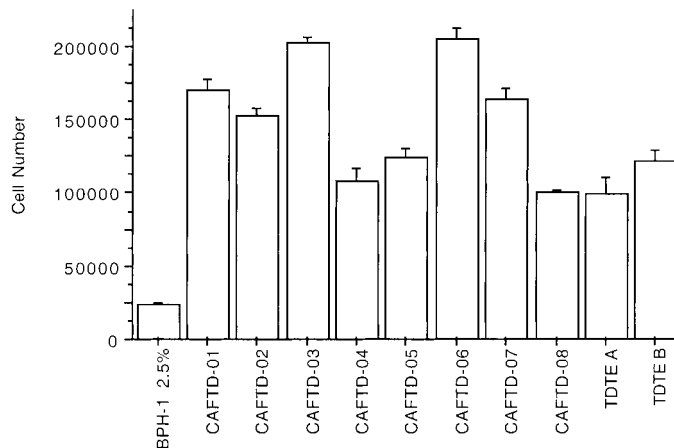


Fig. 3. Cell number (mean and SE) after 5 days of culture. For each well, 25,000 cells were plated and allowed to attach in RPMI 1640 supplemented with 5% FBS. The medium was then switched to RPMI 1640 supplemented with 2.5% dextran-coated charcoal-stripped heat-inactivated FBS for the rest of the experiment. Under these conditions, the parental cell line does not proliferate, whereas the TD substrains increase in number between 4- and 8-fold.

Table 2 Colony formation in soft agar

Cell strain	Colonies <sup>a</sup> per 10 <sup>4</sup> cells
BPH-1	0.13
BPH1 <sup>CAFTD</sup> -01	8.83
BPH1 <sup>CAFTD</sup> -02	4.48
BPH1 <sup>CAFTD</sup> -03	19.05
BPH1 <sup>CAFTD</sup> -04	0.38
BPH1 <sup>CAFTD</sup> -05	2.05
BPH1 <sup>CAFTD</sup> -06	4.10
BPH1 <sup>CAFTD</sup> -07	6.66
BPH1 <sup>CAFTD</sup> -08	9.09
BPH1 <sup>TETD</sup> -A	0.38
BPH1 <sup>TETD</sup> -B	0.13

<sup>a</sup> Mean number of colonies from an experiment performed in triplicate.

stronger in subconfluent than in confluent cultures. E-cadherin and  $\beta$ -catenin expression was patchy in the tumorigenic sublines, with some cells showing no expression, others expressing poorly, and some demonstrating normal levels of protein. The expression of a range of markers by these cell strains is summarized in Table 1. The BPH1<sup>UGM</sup> cells expressed the same markers, had the same morphology, and behaved in the same way, in all of the *in vitro* and *in vivo* assays, as the parental BPH-1 line.

A comparison of the relative growth rates of the epithelial sublines demonstrated that, in the presence of 5% FBS, all of the cells grew rapidly. In contrast, when the medium was changed to RPMI 1640 supplemented with 2.5% dextran-coated charcoal-stripped, heat-inactivated FBS, the parental BPH-1 cell line became essentially growth-quiescent, whereas the TD strains increased in number between 4- and 8-fold over a 5-day assay (Fig. 3).

The parental BPH-1 and the TETD cell lines grow extremely poorly in soft agar (Table 2). In contrast the CAFTD sublines all exhibited some ability to form colonies in soft agar. The lone exception was BPH1<sup>CAFTD</sup>-04, which formed many fewer colonies than BPH1<sup>CAFTD</sup>-03, from which it was derived. This may reflect chance selection of a population of cells with low colony-forming ability during the selection of this strain. Results of the soft agar assay are summarized in Table 2.

BPH-1 cells grafted beneath the renal capsule of athymic mouse hosts in the absence of other cell types have never produced tumors, even in the face of hormonal carcinogens (4). The incidence of tumors in parental BPH-1 cells grafted to athymic mouse hosts in the absence

Table 3 Characteristics of tumors formed by grafting 350,000 cells of each of the BPH-1 sublines beneath the renal capsule of male athymic mouse hosts for 28 days

The mean wet weight of the parental BPH-1 cells, when grafted under these conditions, is always less than 10 mg (mean 3 mg). This low weight and benign behavior of the parental cell line is maintained for at least 1 year.

Cell strain	Differentiation	Invasion	Wet weight (mg) mean $\pm$ SD	Ki67 labeling index (%)	Necrosis
BPH1 <sup>CAFTD</sup> -01	Poor, focall, squamous	Limited to stroma	15.5 $\pm$ 2.6	11.7	No
BPH1 <sup>CAFTD</sup> -02	Poor, adenosquamous	Limited to stroma	158 $\pm$ 123	20.8	No
BPH1 <sup>CAFTD</sup> -03	Moderate, squamous	Limited to stroma	94 $\pm$ 22	25.6	No
BPH1 <sup>CAFTD</sup> -04	Moderate, squamous	Into renal parenchyma	348 $\pm$ 58	17.2	Yes
BPH1 <sup>CAFTD</sup> -05	Moderate, squamous	Into renal parenchyma	244 $\pm$ 32	21.8	Yes
BPH1 <sup>CAFTD</sup> -06	Poor, squamoid	Into renal parenchyma	238 $\pm$ 51	35.6	Yes
BPH1 <sup>CAFTD</sup> -07	Moderate, squamous	Limited to stroma	18 $\pm$ 10	6.7	No
BPH1 <sup>CAFTD</sup> -08	Moderate, squamous	Limited to stroma	20 $\pm$ 9	17.1	No
BPH1 <sup>TETD</sup> -A	Poor, squamoid	Into renal parenchyma	461 $\pm$ 161	20.9	Yes
BPH1 <sup>TETD</sup> -B	Poor, adenosquamous	Into renal parenchyma	305 $\pm$ 40	20.8	Yes

of stromal cells is 0 in 125 attempts. Likewise BPH1<sup>UGM</sup> cells formed small grafts with a mean wet-weight of 4 mg and a benign histology. In contrast, after tumorigenesis induced either by interaction with CAF or by treatment with T+E2 and subsequent G418 selection, all of the cell strains formed tumors at 100% efficiency when grafted beneath the renal capsule of athymic mice. Grossly, the tumors grew at a variety of rates (Table 3) but engulfed the host kidney given sufficient time (Fig. 4). Histologically, the tumors were squamous or adenosquamous (Fig. 5), similar to the tumors from which they were derived. Some tumors grew as noninvasive proliferative lesions on the surface of the host kidney. Others formed invasive lesions that engulfed and destroyed kidney tissue (Fig. 5). The tumors continue to express SV40T antigen. They coexpress markers characteristic of both basal and luminal epithelial cells (high-molecular weight cytokeratins, cytokeratin 14, p63, and cytokeratins 8 and 18). Ki67 labeling index (percentage of epithelial nuclei exhibiting positive staining with an anti-Ki67 antibody, based on counts of at least 2000 tumor epithelial cells) is comparable with, and in some cases elevated over, the 15% level previously described in BPH-1 epithelial cells recombined with rUGM in untreated nude mouse hosts (Ref. 4; Table 3). E-cadherin expression was patchy in the tumors, and in many places, the protein was not detectable. In some areas, apparently normal levels of membrane-localized protein were seen. In other areas, the protein was diffusely localized throughout the cell. Steroid hormone receptors (AR, PR, and ER $\alpha$ ) were not detected in the epithelial cells. ER was seen in cells of the kidney capsule and in some blood vessels within the tumor (Fig. 5).

Necropsy revealed no evidence of metastasis in hosts carrying any of the tumorigenic lines. In some cases, lungs appeared to be abnormally firm, but histological examination revealed that this phenomenon resulted from congestion and areas of pneumonia, probably attributable to the deteriorating health of the hosts before being killed.

## DISCUSSION

The present communication demonstrates that stromal environment can elicit permanent malignant transformation in genetically initiated but previously nontumorigenic human prostate epithelium. We have previously shown that human prostate CAFs can induce the BPH-1 epithelial cell line to form tumors (3). The present study shows that the tumorigenic behavior, as a result of recombination with CAF, resulted in permanent malignant transformation of the epithelial cells.

Malignant transformation in this model is most likely to be caused by genetic changes in the cells, a phenomenon described more fully in the article by Phillips *et al.* (41). It is significant that there are characteristic patterns of genetic change that occur in four separate lineages of BPH-1 cells exposed to CAFs, although these were derived from separate experiments using different CAF populations. The BPH1<sup>CAFTD</sup> lines all share recurrent, and complex (harlequin) chro-

somal rearrangements resulting in loss of 8p, 11p, and 20p, and high-level amplification of 1p, 11q, and 20q (41). This type of specific genetic change is an important observation that underlines the concept that stromal microenvironment may play a crucial role in both promoting carcinogenesis and in eliciting genetic changes during malignant progression. The mechanism by which such common genetic changes could be induced by stromal environment is at present unclear, although the changes in cellular adhesion seen in these tumors may provide a clue to the cause of the genetic changes. We previously demonstrated that malignant changes are associated with a reduction or loss of membranous E-cadherin localization in the BPH-1 epithelial cells that form the tumors (3, 4). The cell lines described here likewise have a general tendency toward reduced expression and a loss of membrane localization of adherens junction proteins. Interactions between E-cadherin and p53 have been postulated to result in genomic instability (42). In a broader context, cellular adhesion has been proposed to modulate neoplastic processes by altering the p53 pathways that control genomic stability (43). However, in the present model, the p53 pathway is disrupted in both the benign and malignant phenotypes (because of the presence of SV40T), which suggests that this route may not be involved in progression from the nontumorigenic to the tumorigenic state here.

One model, which is in some ways similar, is a study showing that the human prostate cancer cell line LNCaP C4-2, when injected into nude mice, is capable of inducing genetic changes in host stromal cells around the injection site (44). In this instance, genetically damaged and tumorigenic epithelial cells apparently induce "neoplastic transformation in stromal cells of the host organ by some, as yet unknown, epigenetic mechanism(s)" (44). In the present model, the situation is somewhat different in that apparently genetically normal but pheno-

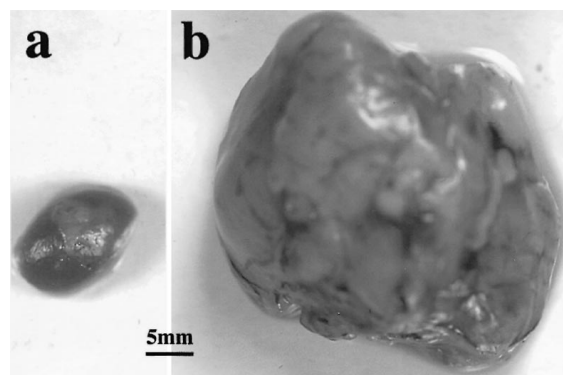


Fig. 4. Gross appearance of parental BPH-1 cells (a) and BPH1<sup>CAFTD</sup>-04 (b) 4 months after subrenal capsule grafting to an athymic mouse host. Notice the four small discreet grafts formed by the parental cells, as compared with the large well-vascularized tumor mass formed by the two grafts of TD cells, which, in this period, totally engulf and destroy the kidney.

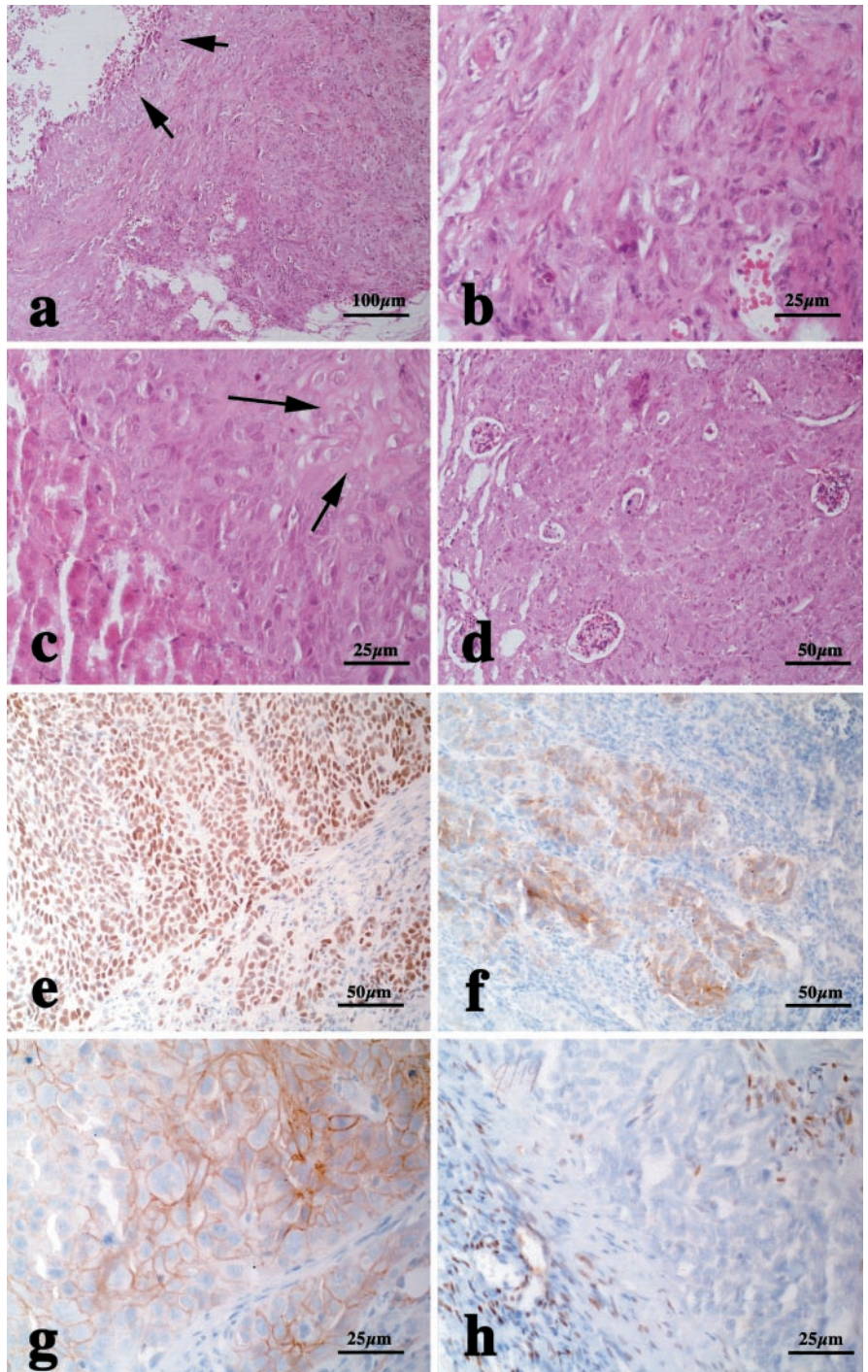


Fig. 5. Histology of representative tumors formed by BPH1<sup>CAF<sup>TD</sup></sup> substrains following subrenal capsule grafting to an athymic mouse host. *a*, tumors demonstrate cystic minimally invasive, predominantly squamous areas (*arrows*) as well as infiltrative growth (*lower right*). *b*, some infiltrative areas recapitulate small acinar prostatic carcinoma and form small abortive acinar structures and small groups composed of cells with bubbly amphophilic cytoplasm and indistinct cell borders. *c*, other areas demonstrate squamous features as sheets of cells with dense cytoplasm and distinct cytoplasmic borders with a broad pushing margin and occasional angulated nests. Focal areas of keratinization are also noted (*arrows*). *d*, tumors growth was noted permeating residual renal structure. *e*, SV40T expression confirms the origin of the epithelial cells forming the tumor mass. *f*, cytokeratin 14 delineates small invasive squamous nests permeating residual renal structures. *g*, E-cadherin expression is patchy, with some areas of strong membrane localized expression and other areas in which staining is weak or absent. *h*, ER- $\alpha$  is detected as nuclear staining around blood vessels within the tumor but is not seen in the tumor epithelial cells themselves.

typically modified stromal cells are inducing permanent malignant transformation in genetically initiated but nonmalignant epithelial cells. It is, however, entirely possible that similar epigenetic mechanisms are involved in this process.

Our model of tumorigenesis in the BPH-1 cell line raises the issue of the relative importance and role of genetics *versus* epigenetic factors in tumorigenesis. CAF-induced tumorigenesis of BPH-1 cell is clearly an epigenetic effect that leads to further genetic alterations in the course of the tumorigenetic process. T+E2-induced carcinogenesis in BPH-1 cells requires interaction with rUGM as well as treatment with T+E2. Hormonal carcinogenesis induced by T+E2 in rUGM+BPH-1 recombinants, therefore, is also likely to involve

epigenetic as well as genetic mechanisms. Thus, the pathway to hormonal carcinogenesis surely can involve both epigenetic and genetic mechanisms. The dominance of genetic *versus* epigenetic mechanisms in determining progression to tumorigenesis or the tumorigenetic state *per se* appears to vary on an individual case-by-case basis.

There are many instances in which the single addition of a dominant acting oncogene is sufficient to convert a nontumorigenetic cell to a fully tumorigenetic cell. Such observations emphasize the important genetic change as a key determinant of malignancy. On the other hand, studies with highly malignant teratocarcinoma cells emphasize the dominance of epigenetic factors in the expression of benign *versus* malignant growth. Small numbers of teratocarcinoma cells trans-

planted into a host will consistently form tumors that will lead to the demise of the host (45). Such highly malignant cells, when microinjected into mouse blastocysts, will participate in normal development producing a range of benign tissues representing all germ layers. Indeed, because of contribution of teratocarcinoma cells to the germ line, it is possible to derive mouse strains from teratocarcinoma cells (46). Thus, despite the genetic alterations within the parental teratocarcinoma cells, epigenetic factors can elicit reversion of highly malignant cells to benign cells.

The ability of T+E2 to induce malignancy in BPH-1 cells has been previously demonstrated (4). The original description of these tumors emphasizes that stromal cells are required for sex hormone-induced carcinogenesis. We also demonstrated that transplantable tumors can be established from these T+E2-induced tumors. The present study demonstrates that epithelial cells that are isolated from these hormone-induced tumors retain their malignant potential. The BPH1<sup>TETD</sup> strains shared the 11q and 20q amplifications seen in BPH1<sup>CAFTD</sup> but also had high-level amplifications of 7p encompassing the *v-erb* (epidermal growth factor receptor) region. In addition to the unbalanced translocations seen in the CAFTD cells, TETD cells also had examples of reciprocal translocations, the exact genetic implication of which is currently unclear but may reflect the unique pathways to tumorigenesis induced by TE/rUGM (41). Unlike the situation in CAF-induced carcinogenesis, there are well-established mechanisms by which sex steroid hormones, especially estrogens, can induce genetic change. Metabolism of natural and synthetic estrogens generates free radicals that are capable of DNA damage (47). Both diethylstilbestrol and estradiol can induce sister chromatid exchange (48–50). T+E2 treatment also increases DNA strand breakage as a result of free radical action (51).

There are a number of signaling mechanisms that could act to promote carcinogenesis; these include the Wnt/frizzled pathway, which may act to regulate cellular adhesion through adherens junctions (for a recent summary see Ref. 52). Growth factors are also obvious potential mediators of paracrine interactions between epithelial and stromal cells. A number of growth factors, notably members of the IGF and the fibroblastic growth factor (FGF) families have been implicated as potentially playing a role in models of prostate cancer. As an example; attention has focused on IGFs because of data suggesting that they may be linked to human prostate cancer progression (53–55). Mutations of IGF-1 or its receptor are known to delay prostatic development (56), whereas overexpression of the ligand is suggested to induce prostatic carcinogenesis (57). In murine models of prostate cancer, IGF-1 levels were also seen to rise in conjunction with progression of the disease (58). Clearly this is a complex field that is not, at present, well understood.

A comparison of the *in vivo* and *in vitro* characteristics of the various cell lines reveals that behavior in the two environments is only loosely linked. Thus, for example, in the present study, only tumorigenic epithelial cells formed colonies in soft agar. However, the inability to form colonies under these conditions does not preclude a cell strain from being highly tumorigenic and invasive *in vivo*. These observations reinforce the importance of determining the true *in vivo* potential of cells to form tumors before extrapolating from data obtained *in vitro*.

The present communication describes a series of cell substrains with a variety of malignant phenotypes generated from a clonally derived nontumorigenic human prostatic epithelial cell line. These cells have the advantage that the sublines have a common genetic heritage, having all been originally derived from a single cell. This will allow a characterization of the effects of specific genetic insults. BPH-1 was immortalized with SV40 large T only, rather than the whole SV40 early region, resulting in a clonally derived cell line that,

although immortalized, is not tumorigenic in nude mice. We have previously demonstrated that this cell line can undergo some aspects of prostatic differentiation and can be induced to form tumors both by sex steroid hormones and by its stromal microenvironment (3, 4). The lines described retain epithelial characteristics and are tumorigenic but nonmetastatic. Both invasive and noninvasive phenotypes were seen, although these did not correlate with other characteristics examined.

In summary, this report demonstrates that stromal microenvironment and hormonal carcinogens can elicit permanent malignant transformation in human prostatic epithelial cells. We further characterize a series of cell strains that will be useful tools to further examine the processes involved in carcinogenesis elicited by various mechanisms.

## REFERENCES

- Hayward, S. W., Grossfeld, G. D., Tlsty, T. D., and Cunha, G. R. Genetic and epigenetic influences in prostatic carcinogenesis. *Int. J. Oncol.*, *13*: 35–47, 1998.
- Grossfeld, G., Hayward, S., Tlsty, T., and Cunha, G. R. The role of stroma in prostatic carcinogenesis. *Endocrine-related Cancer*, *5*: 253–270, 1998.
- Olumi, A. F., Grossfeld, G. D., Hayward, S. W., Carroll, P. R., Tlsty, T. D., and Cunha, G. R. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res.*, *59*: 5002–5011, 1999.
- Wang, Y. Z., Sudilovsky, D., Zhang, B., Haughey, P. C., Rosen, M. A., Wu, D. S., Cunha, T. J., Dahiya, R., Cunha, G. R., and Hayward, S. W. A human prostatic epithelial model of hormonal carcinogenesis. *Cancer Res.*, *61*: 6064–6072, 2001.
- Webber, M. M., Quader, S. T., Kleinman, H. K., Bello-DeOcampo, D., Storto, P. D., Bice, G., DeMendonca-Calaca, W., and Williams, D. E. Human cell lines as an *in vitro/in vivo* model for prostate carcinogenesis and progression. *Prostate*, *47*: 1–13, 2001.
- Noble, R. L. Production of Nb rat carcinoma of the dorsal prostate and response of estrogen-dependent transplants to sex hormones and tamoxifen. *Cancer Res.*, *40*: 3547–3550, 1980.
- Pollard, M. P., Luckert, P. H., and Schmidt, M. A. Induction of prostate adenocarcinomas in Lobund Wistar rats by testosterone. *Prostate*, *3*: 563–568, 1982.
- Kasper, S., Sheppard, P. C., Yan, Y., Pettigrew, N., Borowsky, A. D., Prins, G. S., Dodd, J. G., Duckworth, M. L., and Matusik, R. J. Development, progression, and androgen-dependence of prostate tumors in probasin-large T antigen transgenic mice: a model for prostate cancer. *Lab. Invest.*, *78*: 319–333, 1998.
- Greenberg, N. M., DeMayo, F., Finegold, M. J., Medina, D., Tilley, W. D., Aspinall, J. O., Cunha, G. R., Donjacour, A. A., Matusik, R. J., and Rosen, J. M. Prostate cancer in a transgenic mouse. *Proc. Natl. Acad. Sci. USA*, *92*: 3439–3443, 1995.
- Isaacs, J. T., Isaacs, W. B., Feitz, W. F., and Scheres, J. Establishment and characterization of seven Dunning rat prostatic cancer cell lines and their use in developing methods for predicting metastatic abilities of prostatic cancers. *Prostate*, *9*: 261–281, 1986.
- Isaacs, J. T. Development and characteristics of the available animal model systems for the study of prostatic cancer. *In*: D. S. Coffey, N. Bruchovsky, W. W. Gardner, Jr., M. I. Resnick, and J. P. Karr (eds.), *Current Concepts and Approaches to the Study of Prostate Cancer*, pp. 513–576. New York: A. R. Liss, 1987.
- van Bokhoven, A., Varella-Garcia, M., Korch, C., Hessels, D., and Miller, G. J. Widely used prostate carcinoma cell lines share common origins. *Prostate*, *47*: 36–51, 2001.
- Bae, V. L., Jackson-Cook, C. K., Brothman, A. R., Maygarden, S. J., and Ware, J. L. Tumorigenicity of SV40 T antigen immortalized human prostate epithelial cells: association with decreased epidermal growth factor receptor (EGFR) expression. *Int. J. Cancer*, *58*: 721–729, 1994.
- Jackson-Cook, C., Bae, V., Edelman, W., Brothman, A., and Ware, J. Cytogenetic characterization of the human prostate cancer cell line P69SV40T and its novel tumorigenic sublines M2182 and M15. *Cancer Genet. Cytogenet.*, *87*: 14–23, 1996.
- Bello, D., Webber, M. M., Kleinman, H. K., Wartinger, D. D., and Rhim, J. S. Androgen responsive adult human prostatic epithelial cell lines immortalized by human papillomavirus 18. *Carcinogenesis (Lond.)*, *18*: 1215–1223, 1997.
- Hayward, S. W., Dahiya, R., Cunha, G. R., Bartek, J., Deshpande, N., and Narayan, P. Establishment and characterization of an immortalized but non-tumorigenic human prostate epithelial cell line: BPH-1. *In Vitro*, *31A*: 14–24, 1995.
- Ludlow, J. W. Interactions between SV40 large-tumor antigen and the growth suppressor proteins pRB and p53. *FASEB J.*, *7*: 866–871, 1993.
- Webber, M. M., Bello, D., Kleinman, H. K., Wartinger, D. D., Williams, D. E., and Rhim, J. S. Prostate specific antigen and androgen receptor induction and characterization of an immortalized adult human prostatic epithelial cell line. *Carcinogenesis (Lond.)*, *17*: 1641–1646, 1996.
- Cooke, D. B., Quarmby, V. E., Mickey, D. D., Isaacs, J. T., and French, F. S. Oncogene expression in prostate cancer: Dunning R3327 rat dorsal prostatic adenocarcinoma system. *Prostate*, *13*: 263–272, 1988.
- Thompson, T. C., Kadmon, D., Timme, T. L., Merz, V. W., Egawa, S., Krebs, T., Scardino, P. T., and Park, S. H. Experimental oncogene induced prostate cancer. *In*: J. T. Isaacs (ed.) *Prostate Cancer: Cell and Molecular Mechanisms in Diagnosis and Treatment*, pp. 55–72. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1991.
- Peehl, D. M. Oncogenes in prostate cancer. *Cancer (Phila.)*, *71*: 1159–1164, 1993.

22. Wang, Y. Z., and Wong, Y. C. Oncogenes and tumor suppressor genes in prostate cancer: a review. *Urol. Oncol.*, *3*: 41–46, 1997.
23. Sarkar, F. H., Sakr, W., Li, Y. W., Macoska, J., Ball, D. E., and Crissman, J. D. Analysis of retinoblastoma (*RB*) gene deletion in human prostatic carcinomas. *Prostate*, *21*: 145–152, 1992.
24. Isaacs, W. B., Bova, G. S., Morton, R. A., Bussemakers, M. J., Brooks, J. D., and Ewing, C. M. Molecular biology of prostate cancer. *Semin. Oncol.*, *21*: 514–521, 1994.
25. Bookstein, R., Rio, P., Madreperla, S. A., Hong, F., Allred, C., Grizzle, W. E., and Lee, W. H. Promoter deletion and loss of retinoblastoma gene expression in human prostate carcinoma. *Proc. Natl. Acad. Sci. USA*, *87*: 7762–7766, 1990.
26. MacGrogan, D., and Bookstein, R. Tumour suppressor genes in prostate cancer. *Semin. Cancer Biol.*, *8*: 11–19, 1997.
27. Yeh, S., Miyamoto, H., Nishimura, K., Kang, H., Ludlow, J., Hsiao, P., Wang, C., Su, C., and Chang, C. Retinoblastoma, a tumor suppressor, is a coactivator for the androgen receptor in human prostate cancer DU145 cells. *Biochem. Biophys. Res. Commun.*, *248*: 361–367, 1998.
28. Weinberg, R. A. The retinoblastoma protein and cell cycle control. *Cell*, *81*: 323–330, 1995.
29. Bookstein, R., Shew, J. Y., Chen, P. L., Scully, P., and Lee, W. H. Suppression of tumorigenicity of human prostate carcinoma cells by replacing a mutated *RB* gene. *Science (Wash. DC)*, *247*: 712–715, 1990.
30. Phillips, S. M., Barton, C. M., Lee, S. J., Morton, D. G., Wallace, D. M., Lemoine, N. R., and Neoptolemos, J. P. Loss of the retinoblastoma susceptibility gene (*RBI*) is a frequent and early event in prostatic tumorigenesis. *Br. J. Cancer.*, *70*: 1252–1257, 1994.
31. Kubota, Y., Fujinami, K., Uemura, H., Dobashi, Y., Miyamoto, H., Iwasaki, Y., Kitamura, H., and Shuin, T. *Retinoblastoma* gene mutations in primary human prostate cancer. *Prostate*, *27*: 314–320, 1995.
32. Bookstein, R. Tumor suppressor genes in prostatic oncogenesis. *J. Cell Biochem. Suppl.*, *19*: 217–223, 1994.
33. Tamboli, P., Amin, M. B., Xu, H. J., and Linden, M. D. Immunohistochemical expression of *retinoblastoma* and *p53* tumor suppressor genes in prostatic intraepithelial neoplasia: comparison with prostatic adenocarcinoma and benign prostate. *Mod. Pathol.*, *11*: 247–252, 1998.
34. Cunha, G. R., and Chung, L. W. K. Stromal-epithelial interactions. I. Induction of prostatic phenotype in urothelium of testicular feminized (*Tfm/y*) mice. *J. Steroid Biochem.*, *14*: 1317–1321, 1981.
35. Donjacour, A. A., and Cunha, G. R. Assessment of prostatic protein secretion in tissue recombinants made of urogenital sinus mesenchyme and urothelium from normal or androgen-insensitive mice. *Endocrinology*, *131*: 2342–2350, 1993.
36. Risbridger, G., Wang, H., Young, P., Kurita, T., Wang, Y. Z., Lubahn, D., Gustafsson, J. A., Cunha, G., and Wong, Y. Z. Evidence that epithelial and mesenchymal estrogen receptor- $\alpha$  mediates effects of estrogen on prostatic epithelium. *Dev. Biol.*, *229*: 432–442, 2001.
37. Hayward, S., Cox, S., Mitchell, I., Hallows, R., Deshpande, N., and Towler, J. The effects of interferons on the activity of  $\alpha$ -glycerolphosphate dehydrogenase in benign prostatic hyperplasia cells in primary culture. *J. Urol.*, *138*: 648–653, 1987.
38. Hayward, S. W., Haughney, P. C., Rosen, M. A., Greulich, K. M., Weier, H. U., Dahiya, R., and Cunha, G. R. Interactions between adult human prostatic epithelium and rat urogenital sinus mesenchyme in a tissue recombination model. *Differentiation*, *63*: 131–140, 1998.
39. Hallows, R. C., Bone, E. J., and Jones, W. A new dimension in the culture of human breast. *In*: R. J. Richards and K. T. Rajan (eds.), *Tissue culture in medical research*, Vol. 2, pp. 213–220. Oxford: Pergamon Press, 1980.
40. Wang, Y. Z., Hayward, S. W., Cao, M., Thayer, K. A., and Cunha, G. R. Cell differentiation lineage in the prostate. *Differentiation*, in press, 2001.
41. Phillips, J. L., Hayward, S. W., Wang, Y. Z., Vasselli, J., Pavlovich, C., Padilla-Nash, H. M., Wangsa, D., Ghadimi, B. M., Grossfeld, G. D., Linehan, W. M., Cunha, G. R., and Ried, T. Correlation of chromosomal aneuploidies with gene expression profiles in cell line models for prostate carcinogenesis. *Cancer Res.*, *61*: 8143–8149, 2001.
42. Bukholm, I. K., Nesland, J. M., Karesen, R., Jacobsen, U., and Borresen-Dale, A. L. Expression of E-cadherin and its relation to the p53 protein status in human breast carcinomas. *Virchows Arch.*, *431*: 317–321, 1997.
43. Tlsty, T. D. Cell-adhesion-dependent influences on genomic instability and carcinogenesis. *Curr. Opin. Cell Biol.*, *10*: 647–653, 1998.
44. Pathak, S., Nemeth, M. A., Multani, A. S., Thalmann, G. N., von Eschenbach, A. C., and Chung, L. W. Can cancer cells transform normal host cells into malignant cells? *Br. J. Cancer.*, *76*: 1134–1138, 1997.
45. Pierce, G. The Benign Cells of Malignant Tumors. *In*: T. King (ed.), *Developmental Aspects of Carcinogenesis and Immunity*, pp. 3–22. New York: Academic Press, Inc., 1974.
46. Mintz, B. Genetic mosaicism and *in vivo* analyses of neoplasia and differentiation. *In*: G. Saunders (ed.), *Cell Differentiation and Neoplasia*, pp. 27–56. New York: Raven Press, 1978.
47. Newbold, R. R., Jellinck, P. H., Metzler, M., and McLachlan, J. A. Ontogeny of peroxidase activity in epithelium and eosinophils of the mouse uterus. *Teratog. Carcinog. Mutagen.*, *11*: 267–278, 1991.
48. Forsberg, J. G. Estrogen effects on chromosome number and sister chromatid exchanges in uterine epithelial cells and kidney cells from neonatal mice. *Teratog. Carcinog. Mutagen.*, *11*: 135–146, 1991.
49. Hillbertz-Nilsson, K., and Forsberg, J. G. Estrogen effects on sister chromatid exchanges in mouse uterine cervical and kidney cells. *J. Natl. Cancer Inst. (Bethesda)*, *75*: 575–580, 1985.
50. Hillbertz-Nilsson, K., and Forsberg, J. G. Genotoxic effects of estrogens in epithelial cells from the neonatal mouse uterine cervix: modifications by metabolic modifiers. *Teratog. Carcinog. Mutagen.*, *9*: 97–110, 1989.
51. Ho, S. M., and Roy, D. Sex hormone-induced nuclear DNA damage and lipid peroxidation in the dorsolateral prostates of Noble rats. *Cancer Lett.*, *84*: 155–162, 1994.
52. Arias, A. M. Epithelial mesenchymal interactions in cancer and development. *Cell*, *105*: 425–431, 2001.
53. Chan, J. M., Stampfer, M. J., Giovannucci, E., Gann, P. H., Ma, J., Wilkinson, P., Hennekens, C. H., and Pollak, M. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study [see comments]. *Science (Wash. DC)*, *279*: 563–566, 1998.
54. Wolk, A., Mantzoros, C. S., Andersson, S. O., Bergstrom, R., Signorello, L. B., Lagiou, P., Adami, H. O., and Trichopoulos, D. Insulin-like growth factor 1 and prostate cancer risk: a population-based, case-control study [see comments]. *J. Natl. Cancer Inst. (Bethesda)*, *90*: 911–915, 1998.
55. Peehl, D. M., Cohen, P., and Rosenfeld, R. G. The role of insulin-like growth factors in prostate biology. *J. Androl.*, *17*: 2–4, 1996.
56. Hayward, S. W., and Cunha, G. R. Development and physiology. *In*: H. Hricak and P. R. Carroll (eds.), *Prostate Gland: Clinically Relevant Approach to Imaging*, pp. 1–14. Philadelphia: Saunders, 2000.
57. DiGiovanni, J., Kiguchi, K., Frijhoff, A., Wilker, E., Bol, D. K., Beltran, L., Moats, S., Ramirez, A., Jorcano, J., and Conti, C. Deregulated expression of insulin-like growth factor 1 in prostate epithelium leads to neoplasia in transgenic mice. *Proc. Natl. Acad. Sci. USA*, *97*: 3455–3460, 2000.
58. Kaplan, P. J., Mohan, S., Cohen, P., Foster, B. A., and Greenberg, N. M. The insulin-like growth factor axis and prostate cancer: lessons from the transgenic adenocarcinoma of mouse prostate (TRAMP) model. *Cancer Res.*, *59*: 2203–2209, 1999.