Malignant Transformation in a Nontumorigenic Human Prostatic Epithelial Cell Line

Simon W. Hayward, 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 (CAF) 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\textsuperscript{TETD}-A and -B and the CAF-induced strains are designated BPH1\textsuperscript{CAF}-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\textsuperscript{CAF} strains formed colonies in soft agar, whereas the parental BPH-1 cells and the BPH1\textsuperscript{TETD} sublines did not. There was no immunocytochemically detectable expression of androgen (AR), α-estrogen (ER) 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 grown 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 adenocarcinomatous 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.

At present, there are a limited number of models of prostastic 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. Baue et al. and Jackson-Cook et al. (13, 14) used repeated cycling of the generally nontumorigenic P69SY40T cell line through nude mouse hosts to produce tumorigenic sublines. Webbet 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.

SV40\textsuperscript{T} 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). SV40\textsuperscript{T} interacts with and inactivates both p53 and pRb, thus eliminating these two important tumor suppressor pathways (17). However many SV40\textsuperscript{T}-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 SV40\textsuperscript{T}), 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\textsuperscript{3} 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\textsubscript{r} 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

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2 To whom requests for reprints should be addressed, at Department of Urologic 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@mcmail.vanderbilt.edu.

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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 non-tumorigenic 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.

CAFs were prepared from human prostate tumors as described previously (3). Briefly, tumors were identified using histopathological analysis of stained frozen sections. Five-mm³ 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 mesenchymal components by tryptic digestion, as described previously (4). Urogenital sinuses were dissected from fetuses and separated into epithelial and mesenchymal components by tryptic digestion, as described previously (36). The resultant tissue fragments were plated overnight in tissue culture flasks in a small volume (1.2 ml in a 25-cm² 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 µg/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 µl 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 CAF/TD 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% H₂O₂ 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 (PAI–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, Francisco laboratory animal resource center with food and drinking water ad libitum under controlled conditions (12 h light, 12 h dark, 20°C ± 2°C).

Mice carrying UGM + BPH-1 recombinants were treated hormonally by surgical implantation of Silastic capsules containing E₂ (17-β-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-cm² 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 µg/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.

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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 ERs (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 American International (Arlington Heights, IL). Biotinylated secondary antimouse immunoglobulin was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-p63 was purchased from Transduction Laboratories (San Diego, CA). Anti-p63 was purchased from Transduction Laboratories (San Diego, CA). Anti-p63 was purchased from Transduction Laboratories (San Diego, CA). Anti-p63 was purchased from Transduction Laboratories (San Diego, CA).

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 BPH1CAFTD-01, -03, -05 and -07), and an additional two strains were derived from T+E2-treated rUGM + BPH-1 tissue recombinants (designated BPH1TET-A and -B). The four BPH1CAFTD strains were grafted in collagen gel beneath the renal
capsule of athymic mouse hosts, in which they were formed tumors that were used to produce a second generation of BPH1 CAFTD cells (designated BPH1 CAFTD -02, -04, -06 and -08). The lineage derivation of these cell strains is outlined in Fig. 1. In addition to these cell lines a strain of cells derived from BPH-1 + rUGM grafts to untreated hosts was also derived and designated BPH1 UGM.

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, α-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 CAFTD and both of the BPH1 TETD strains (Table 1). Expression was slightly stronger in subconfluent than in confluent cultures. E-cadherin and β-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 UGM 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 CAFTD -04, which formed many fewer colonies than BPH1 CAFTD -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

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

<table>
<thead>
<tr>
<th>Cell Strain</th>
<th>E-cadherin</th>
<th>β-catenin</th>
<th>Pan keratin</th>
<th>HMW keratin</th>
<th>p63</th>
<th>Keratin 8</th>
<th>Vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental BPH-1</td>
<td>++**</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Parental BPH (subconf)</td>
<td>++</td>
<td>++ (membrane)</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -01</td>
<td>++</td>
<td>++ (membrane)</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -01 (subconf)</td>
<td>+</td>
<td>++ (membrane and cytoplasm)</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -02</td>
<td>++</td>
<td>(membrane)</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -02 (subconf)</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -03</td>
<td>+/−</td>
<td>+/−</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -03 (subconf)</td>
<td>+/−</td>
<td>+/−</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -04</td>
<td>+/−</td>
<td>+/− (cytoplasm)</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -04 (subconf)</td>
<td>+</td>
<td>+ (cytoplasm)</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -05</td>
<td>++</td>
<td>+/−</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -05 (subconf)</td>
<td>+/−</td>
<td>+/−</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -06</td>
<td>++</td>
<td>+/−</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -06 (subconf)</td>
<td>+</td>
<td>+/−</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -07</td>
<td>++</td>
<td>+/− (membrane)</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -07 (subconf)</td>
<td>+/−</td>
<td>+/− (membrane)</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -08</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 CAFTD -08 (subconf)</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 TETD -A</td>
<td>++</td>
<td>−/+ (cytoplasm)</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 TETD -A (subconf)</td>
<td>+/−</td>
<td>−/+ (cytoplasm)</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 TETD -B</td>
<td>++</td>
<td>+ (membrane)</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BPH1 TETD -B (subconf)</td>
<td>+/−</td>
<td>−/+ (cytoplasm)</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Staining: −, absent; −/+, weak (minority of cells); +/−, weak (majority of cells); +, uniform weak; +, moderate; +++, strong.

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**Table 2** Colony formation in soft agar

<table>
<thead>
<tr>
<th>Cell Strain</th>
<th>Colonies* per 10^5 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH-1</td>
<td>0.13</td>
</tr>
<tr>
<td>BPH1 CAFTD -01</td>
<td>8.83</td>
</tr>
<tr>
<td>BPH1 CAFTD -02</td>
<td>4.48</td>
</tr>
<tr>
<td>BPH1 CAFTD -03</td>
<td>19.05</td>
</tr>
<tr>
<td>BPH1 CAFTD -04</td>
<td>0.38</td>
</tr>
<tr>
<td>BPH1 CAFTD -05</td>
<td>2.05</td>
</tr>
<tr>
<td>BPH1 CAFTD -06</td>
<td>4.10</td>
</tr>
<tr>
<td>BPH1 CAFTD -07</td>
<td>6.66</td>
</tr>
<tr>
<td>BPH1 CAFTD -08</td>
<td>9.09</td>
</tr>
<tr>
<td>BPH1 TETD -A</td>
<td>0.38</td>
</tr>
<tr>
<td>BPH1 TETD -B</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* Mean number of colonies from an experiment performed in triplicate.
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 previously shown that human prostate CAFs can induce the BPH-1 but previously nontumorigenic human prostate epithelium. We have can elicit permanent malignant transformation in genetically initiated non resulted from congestion and areas of pneumonia, probably of the tumorigenic lines. In some cases, lungs appeared to be abnormal, and stromal cells is 0 in 125 attempts. Likewise BPH1 UGM 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+1 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 ERα) 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 CAPTD lines all share recurrent, and complex (harlequin) chromosomal 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-

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**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.

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

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Fig. 4. Gross appearance of parental BPH1-1 cells (a) and BPH1 CAFTD-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.
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+E1-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 tumorigenic 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 nontumorigenic cell to a fully tumorigenic 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-

Fig. 5. Histology of representative tumors formed by BPH1(EAPT) 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-α is detected as nuclear staining around blood vessels within the tumor but is not seen in the tumor epithelial cells themselves.
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 BPH1TETD strains shared the 11q and 20q amplifications seen in BPH1CAFTD 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 T/E/UGM (41). Unlike the situation in CAF-induced carcinogenesis, there are well-established pathways to tumorigenesis induced by TE/rUGM (41). Unlike the unbalanced translocations seen in the CAFTD cells, TETD cells 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 T/E/UGM (41). Unlike the situation in CAF-induced carcinogenesis, there are well-established pathways to tumorigenesis induced by TE/rUGM (41).

- 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 mechanisms by which sex steroid hormones, especially estrogens, can elicit reversion of highly malignant cells to benign cells.

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REFERENCES


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