Establishment and characterization of a prostate cancer cell line from a prostatectomy specimen for the study of cellular interaction

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Though human prostate cancer (PCa) heterogeneity can best be studied using multiple cell types isolated from clinical specimens, the difficulty of establishing cell lines from clinical tumors has hampered this approach. In this proof-of-concept study, we established a human PCa cell line from a prostatectomy surgical specimen without the need for retroviral transduction. In a previous report, we characterized the stromal cells derived from PCa specimens. Here, we characterized the epithelial cells isolated from the same tumors. Compared to the ease of establishing prostate stromal cell lines, prostatic epithelial cell lines are challenging. From three matched pairs of normal and tumor tissues, we established one new PCa cell line, HPE-15. We confirmed the origin of HPE-15 cells by short tandem repeat microsatellite polymorphism analysis. HPE-15 cells are androgen-insensitive and express marginal androgen receptor, prostate-specific antigen and prostate-specific membrane antigen proteins. HPE-15 expresses luminal epithelial markers of E-cadherin and cytokeratin 18, basal cell markers of cytokeratin 5 and p63 and neuroendocrine marker of chromogranin A. Interestingly, HPE-15 Cells exhibited no tumorigenicity in different strains of immune-deficient mice but can become tumorigenic through interaction with aggressive cancer cell types. HPE-15 cells can thus serve as an experimental model for the study of PCa progression, metastasis and tumor cell dormancy.

Introduction

One of the major hurdles in prostate cancer (PCa) research is the lack of representative cell lines from primary lesions to simulate clinical progression under experimental conditions. Currently, all PCa cell lines derived from primary tumor tissues require genetic manipulation. All the available cell lines are either derived from metastatic tumors or from normal or malignant prostate tissues through gene transduction using retroviral vector encoding human telomerase reverse transcriptase (hTERT), simian virus SV40 or human papillomavirus 18 (HPV-18). With exogenous viral oncogenes supporting proliferation and survival, these cell lines are probably not suitable for investigating the natural history of PCa development and progression.

We conducted ex vivo tumor culture to establish new PCa cell lines of both mesenchymal origin and epithelial cell

Key words: prostate cancer, cell line, transit amplifying, and cell–cell interaction

Abbreviations: AR: androgen receptor; CgA: chromogranin A; CK: cytokeratin; CO2: carbon dioxide; ELISA: enzyme-linked immunosorbent assay; FBS: fetal bovine serum; H&E: hematoxylin and eosin stain; IHC: immunohistochemical staining; luc: luciferase; PBS: phosphate buffered saline; PCa: prostate cancer; PSA: prostate-specific antigen; PSMA: prostate-specific membrane antigen; RFP: red fluorescence protein; STR: short tandem repeat

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lineage. In a previous report on the mesenchymal compart-
ment, we established and characterized three pairs of mesen-
chymal stromal cell lines from tumor and the matched “normal” zone.6 Using a direct coculture system to simulate in vivo cancer–stromal interaction, we determined that the main effect of cancer-associated stromal cells was to preserve epithelial cancer cell vitality. We propose that in the absence of stromal cells, the survival of cancer cells is severely com-
promised by the loss of outside-in signaling.6 We further used cancer-stromal coculture to demonstrate that cancer-associated stromal cells could promote PCa cell heterogeneity.7 Characterizing newly established cell lines from clinical specimens provides a unique opportunity to uncover new behavioral traits of prostate tumors, as well as their interactions with cells in the tumor microenvironment.

This report characterizes a newly established epithelial cell line from the same clinical PCa specimens used to establish the HPS-15 prostate stromal cell line.6 This new cell line, HPE-15, is unique because it can be propagated indefinitely but is nontumorigenic. Though these cells do not form xenograft tumor in athymic mice, HPE-15 cells can be used as a tool to study the mechanism of cellular interaction and reprograming that promotes phenotypic and behavioral transition of PCa cells from indolent to aggressive states.

Materials and Methods

Human PCa cell lines

The LNCaP (RRID:CVCL_0395) cell line established from lymph node metastasis8,9 was a kind gift from the late Dr. Gary Miller (University of Colorado, Denver, CO).10 The PC-3 (RRID:CVCL_0035) cell line, derived from a bone aspirate sample, was obtained from American Type Cell Culture (ATCC, Manassas, VA). Passage 4 of the PC-3 cells from the time of purchase was used in our study. These cells were cultured in RPMI 1640 medium (Life Technologies, Carlsbad, CA) containing 10% fetal bovine serum (FBS, Atlanta Biologicals, Lawrenceville, GA), penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C, in humidified atmospheric air supplemented with 5% CO₂. Primary culture of human prostate epithelial PrEC cells was purchased from Lonza (Rockland, ME) and cultured in the recommended PrEGM medium (Lonza) for four passages before being used for whole cell lysate preparation. IH10 and IIG5 cells represent selected tumorigenic ARCaP (RRID:CVCL_4830) subclones established originally from the ascites of a PCa patient with widely disseminated PCa to bone and soft tissues.11,12 IH10 and IIG5 cells were cultured and maintained in T-medium10 (Formula LS0020056DJ, Life Technologies) supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml). We have reported the isolation and characterization of HPS-14 and HPS-15, matched pair of patient-derived prostate stromal cell lines.6 All human cell lines used in the study have been authenticated using STR profiling.

Culturing prostate epithelial cells from clinical PCa specimens

Three matched pairs of clinical prostate specimens from three patients were used in our study, as reported previously.6 To establish epithelial cell lines, a similar protocol to the one for establishing stromal cell lines was used.6 Briefly, diced prostate tissue specimens were digested with Dispase II (Roche Diagnostics, Indianapolis, IN) and cultured in a thin layer of T-medium10 for outgrowth. Cells with epithelial morphology were isolated based on weaker attachment to the plastic surface than the stromal cells and were stored as passage 1 (p1) in vapor phase liquid nitrogen. Continued passaging was initiated by replating p1 cells at a 1:3 ratio. Cell detachment was achieved by treatment with trypsin (Life Technologies), accutase (Sigma-Aldrich, St. Louis, MO) or citric saline (135 mM potassium chloride and 15 mM sodium citrate treatment). The method of limiting dilution was used for cloning cells of interest.

Analyses for androgen responsiveness

Changes in cell proliferation, androgen receptor (AR) level and prostate-specific antigen (PSA) production were assayed as parameters of androgen response, following our previously reported protocols.13,14 Briefly, cells under androgen-starvation conditions for 48 hr were treated for 24 hr with the synthetic androgen analog methyltrienolone (R1881, Sigma-Aldrich). The cells were then subjected to crystal violet staining for cell proliferation.6 PSA production was determined from the culture medium by enzyme-linked immunosorbent assay (ELISA) as we reported.6

Western blotting

The protocol for western blotting was previously reported.14 Antibodies to human AR (sc-7305), p63 (sc-8431), chromogranin A (CgA, sc-13090), cytokeratin 5 (CK5, sc-32721), cytokeratin 18 (CK18, sc-6259) and β-actin (sc-69879) were obtained from
Santa Cruz Biotechnology (Dallas, TX). Antibodies to E-cadherin (E-cad, #5296) and FOXA2 (#8186) were from Cell Signaling Technology (Danvers, MA). The I591 monoclonal antibody to prostate-specific membrane antigen (PSMA) was generously provided by Dr. Neil Bander of Cornell University.

**Xenograft tumor formation assay**

Tumorigenicity of the human PCa cells was assessed by xenograft tumor formation in immune compromised mice. Male mice between the age of 4–8 weeks underwent bilateral subcutaneous (s.c.) or intratibial (i.t.) tumor cell inoculation following our reported protocols. After recovery from anesthesia, the mice were kept for a 12-month observation period for in vivo tumor formation and metastasis assay. Mouse strains used in our study included nude (Ncrprimemice, NCI, Frederick, MD), SCID (NOD.Scid/NCr, NCI, Frederick, MD) and NSG mice (NOD scid gamma, Jackson Laboratory, Bar Harbor, ME). Matrigel has been shown to promote xenograft tumor formation. In some experiments, to facilitate tumor formation cells were premixed with an equal volume of Matrigel (BD Biosciences, Bedford, MA) before inoculation. Protocols for the tumorigenicity and metastasis assay and bioluminescence imaging (BLI) have been reported.

**Genotyping analysis**

Authentication of isolated cell lines was based on microsatellite polymorphism through short tandem repeat (STR) analysis. For each analysis, 1 × 10⁶ cells in 100 μl phosphate buffered saline (PBS) were spotted onto a sample collection card and submitted for STR profiling analysis by Cell Line Authentication Service in DDC Medical (Fairfield, OH) or ATCC.

**Karyotype analysis**

The protocol used for chromosome preparations and staining was reported previously. Cells at 70% confluence in fresh media were exposed to Colcemid (20 ng/ml, Sigma-Aldrich) at 37°C for 2 hr, and then to hypotonic solution (75 mM KCl) for 20 min at room temperature and fixed with cold fixative solution of methanol and glacial acetic acid (3:1). Optically aged slides were G-banded using trypsin solution and stained in Giemsa. Images were captured using a Nikon 80i microscope equipped with karyotyping software from Applied Spectral Imaging Inc. (ASI, Vista, CA).

**Fluorescence protein and luciferase tagging**

Red fluorescence protein (RFP) tagging was performed by transfecting plasmid DNA (1 μg) of the expression vector pCDH-CMV-MSC-EF1-RFP-puro (CD516B-2, System Biosciences, Palo Alto, CA) following our reported protocol. HPE-15REF, a representative clone of RFP-tagged HPE-15 cells after 2 weeks of puromycin (2 μg/ml, Life Technologies) selection and limiting dilution, was used in the study. To track xenograft tumor formation in NSG mice, HPE-15REF cells were further tagged with luciferase protein (luc) by transfection with the MSCV Luciferase PGK-hygro plasmid (Addgene, Cambridge, MA). HPE-15REF cells expressing luciferase, HPE-15REF/luc were selected with 200 μg/ml hygromycin (Gemini Bio-products, West Sacramento, CA).

**Coculture with inductive PCa cells**

Our previously reported cell coculture protocol was used to test if HPE-15 could be induced to gain tumorigenic and metastatic potential. To determine if the cell phenotype can be switched after coculture, we determined the optimal in vitro conditions to be three-dimensional (3D) spheroid coculture. The indolent HPE-15REF/luc cells (1 × 10⁶) were mixed with 5 × 10⁵ aggressive IH10 or IIG5 cells in a 10-cm Ultra-low attachment culture dish (Corning Inc., Corning, NY) in RPMI 1640 medium containing 10% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml) for 4 days. HPE-15REF/luc cells were then isolated from the coculture by fluorescence-activated cell sorting (FACS) to collect top 10% counts with the highest red fluorescence. To amplify recovered HPE-15REF/luc cells, sorted cells (1 × 10⁵) were plated onto a 15-cm culture dish in 25 ml culture medium containing 4 μg/ml puromycin for 14 days to purge any IH10 or IIG5 contamination at the same time. Purified HPE-15REF/luc without visible IH10 or IIG5 contamination was used for further analyses.

**Phase contrast and fluorescence microscopy**

Phase contrast images of cultured cells were documented with an Eclipse Ti inverted microscope (Nikon Instruments Inc., Melville, NY). Fluorescence imaging was obtained with an EVOSFl inverted microscope and the Eclipse Ti microscope with 488 nm laser excitation. To facilitate comparison, an identical exposure time was set for all fluorescence imaging with red fluorescence.

**Immunohistochemical staining**

Xenograft tumors were harvested and fixed in 10% neutral-buffered formalin for tissue processing and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (H&E) for histology. Our previously reported protocol was used for immunohistochemical (IHC) staining. Primary antibodies to RFP (ABIN129578, Antibodies-online), pan-cytokeratin (pan-CK, Santa Cruz), and EpCAM (Novus Biologicals, Littleton, CO) were used. Images were acquired by Digital Sight DS-SM camera (Nikon, Melville, NY).

**Results**

We used a specially formulated T-medium to culture clinical prostate specimens, with the expectation of obtaining matched pairs of prostate stromal and epithelial cell lines for the study of cancer–stromal interaction. As previously reported, we established matched pairs of prostate stromal cell lines from three patients with confirmed localized PCa. In the current study, we focused on the establishment and characterization of prostate epithelial cells from the same surgical specimens.
General features of ex vivo prostate tissue culture

With our ex vivo culture protocol, all three matched pairs of prostate specimens produced similar types of morphologically distinctive outgrowth from diced tissues. An epithelial cell population crept out 4 days into the culture (Fig. 1a), forming a monolayer of fast-growing, mostly cobblestone-like cells reminiscent of cell lines with known epithelial cell properties.12,19 As the outgrowth proceeded, the monolayer became interspersed with long fiber-like cells (Fig. 1b) with a neuroendocrine cell morphology20 as defined in the culture of PrEC cells, which were considered as normal primary human prostate epithelial cells. Cells with stromal cell morphology started to appear later, around 2 weeks of culture.6 Taking advantage of the differences in outgrowth time and attachment strength, we obtained large numbers (≈4 × 10^7) of epithelial cells from each of the six prostate tissue specimens, three each from normal and tumor zones, for the generation of prostate stromal and prostate epithelial cells. We concluded that primary prostate epithelial cells could be expanded readily from clinical specimens.

Though it was quite easy to obtain epithelial cells from primary prostate tissue culture, continued culture of these cells was difficult, since these cells appeared unable to survive replating. Most cells that survived replating showed markedly retarded growth and ceased proliferation completely within 15 passages (Supporting Information Table S1). Notably, after testing many commercially formulated cell culture media including RPMI 1640, αMEM, DMEM and K-SFM, even a combination of popular growth factors for primary cell culture in the case of PrEGM medium,21 we found no culture medium or culture conditions we have tested could reverse the tendency of growth arrest and cell death in cultured PCa epithelial cells. Our observation is in agreement with previous ex vivo culture results which concluded that prostate epithelial cells could undergo only limited numbers of cell division.22,23 The inability to survive ex vivo seems an inherent trait of primary prostate epithelial cells.

Despite the low survival rate, certain epithelial cells from one tumor specimen did survive repeated plating in continuous culture. Derived from the same tumor specimen as the HPS-15 cancer-associated prostate stromal cells,6 this surviving epithelial cell-like population was named HPE-15. Within the primary (p1) HPE-15 cell population, while most cells would die upon replating, a few colonies always arose in secondary culture, in the middle of many surviving but senescent cells. Based on the number of colonies in secondary culture, we estimated that there could be between 6 and 15 colony-forming units among 1 × 10^6 cells of the primary culture. The colony-forming cells probably reflected the presence of a small number of cancer stem cells, which are known to be present in the primary setting.6
fraction of cells with extraordinary growth and survival potential in the original tumor specimen.

**HPE-15 as a newly established prostate epithelial cell line**

The colonies of HPE-15 cells in secondary culture could be propagated through replating in T-medium. We conducted three separate experiments to confirm that HPE-15 cells could be cultured continuously for more than 60 passages without showing any signs of senescence (Figs. 1c and 1d). These cells grew slowly in the first 10 passages, and acquired a moderately accelerated rate of growth afterward, with a doubling time of about 31 hr. In contrast, the long neuroendocrine-like cells kept their slow growth rate and disappeared after the first 20 passages. The remaining HPE-15 cells were quite tolerant to different culture media, and after 20 passages the cells grew equally well in either RPMI 1640 or T-medium with 5% FBS. In all three experiments, HPE-15 cells grew continuously beyond 60 passages, demonstrating immortality. Though out of three pairs of prostate tissues specimens, we were successful in establishing only a single epithelial cell line, HPE-15 is the first PCA cell line established spontaneously from the outgrowth of a primary PCA tissue.

**Androgen insensitivity of the HPE-15 cell line**

Androgen-responsive PSA production is considered as a marker of prostate epithelial cells. When examining culture media for PSA production, however, we found that tumor specimens would rapidly lose PSA production in continued culture (Fig. 2a). In all six ex vivo cultures, PSA concentration was dropped beyond passage 5 and became undetectable at the 10th passaging. These observations were in accord with previous findings in which prostate tissue in ex vivo culture rapidly lost PSA expression, even under 3D microgravity culture conditions that more closely simulated in situ tumor growth.

Similarly, growth and survival of HPE-15 cells were not affected by androgen. In a comparative study with LNCaP cells, to which the addition of androgen R1881 would generate a biphasic growth by androgen. In a comparative study with LNCaP cells, to which the addition of androgen R1881 would generate a biphasic growth response, that is, stimulatory at lower R1881 concentration but suppressive at 5 nM, as documented previously, the HPE-15 cells were not sensitive to androgen. After treatment under androgen-deprivation conditions, the addition of R1881 did lead to increased PSA production by LNCaP cells, but this was not observed in HPE-15 cells (Fig. 2b).

In two limiting dilution studies with HPE-15 cells at the 25th and 34th passages, no colonies were formed from 384 cells in 1,152 wells or from 576 cells in 1,152 wells, respectively. In parallel experiments, the addition of androgen (R1881, 1 nM) did not help HPE-15 cells survive limiting dilution. In the third limiting dilution study, in which 576 HPE-15 cells mixed with equal numbers of red fluorescent LNCaP RL1 cells were plated to 2,304 wells, 37 red fluorescent colonies were counted (≈6.4%) while still no HPE-15 colonies were observed. These results indicated that HPE-15 had only a little intrinsic colony formation capability, a phenotypic feature not seen in other prototypical human PCA cell lines.

**Unique features of the HPE-15 cell line**

**Cell line authentication.** To exclude any possibility that HPE-15 is an artifact of cross-contamination, we submitted HPE-15 cells for genotyping. STR analysis revealed that HPE-15 cells have a unique STR profile differing from any known cells of human origin (Fig. 2c). Furthermore, STR analysis showed that HPE-15 shared an identical STR genotype with its matched stromal HPS-15 pair, confirming that these cell lines were isolated properly from the same tumor specimen.

**Karyotypic features.** The potential of indefinite cell growth is often accompanied by gross chromosomal abnormalities. We conducted karyotype analysis to investigate whether HPE-15 cells harbored visible abnormalities. At passage 64, all 20 HPE-15 cells examined were hyperdiploid aneuploid, with chromosomal numbers ranging from 45 to 54 and a modal number of 48 (Fig. 2d). Polysomy 20 was frequent (≈75%), X chromosome was present in all the cells, whereas Y chromosome was present in 17 of 20 cells. A marker chromosome (M1) was found in 19 of the 20 cultures. Relative to the complex chromosomal rearrangements seen in commonly used PCA cell lines of LNCaP, DU145, PC-3, ARCaP and CW22Rv1, HPE-15 cells seem to contain the simplest chromosomal abnormalities (Table 1), much like virally transformed RWPE-1 prostate epithelial cells.

**Expression of differentiation markers.** Prostate epithelial cells are known to express different marker proteins during the process of differentiation. We used western blotting to determine marker protein expression. This series of analyses revealed that HPE-15 cells had complicated patterns of marker protein expression. Among the four marker proteins of prostate luminal epithelial cells, HPE-15 cells did not express detectable AR or PSMA proteins but expressed CK18 and E-cad (Fig. 2e). Meanwhile, the same cells expressed basal cell marker proteins of p63 and CK5, together with expression of the neuroendocrine marker protein CgA. Importantly, none of the marker proteins expressed in HPE-15 cells was affected by androgen, because similar levels of marker protein expression were observed under regular culture conditions, during androgen starvation and upon androgen stimulation (Fig. 2e). The results from these analyses suggest that the HPE-15 cell line is a unique cell type which, under normal conditions, is probably present in a transitional stage from basal- to luminal-epithelial cell differentiation.

**Lack of xenograft tumorigenicity in the HPE-15 cell line**

As an epithelial cell line isolated from a clinical prostate tumor and with genomic abnormality, HPE-15 was expected to harbor tumorigenic potential. Surprisingly, no tumorigenicity was detected through repeated xenograft tumor formation assays (Table 2). Three strains of immunocompromised mice were used to investigate the incidence of tumor formation. Though immune compromised, NCr nu/nu and SCID mice might have remnant immune capacity. The NCr nu/nu mouse, for instance, reserves a certain capacity for antibody production
Characterization of a new prostate cancer cell line

Figure 2. Legend on next page.
and even T-cell function. The fact that HPE-15 cells could not form tumors in any of the tested mouse strains suggests that null tumor formation was an intrinsic feature of this cell line, independent of the host immunity.

**HPE-15 cell xenograft tumor formation after interaction with PCa cells**

Though HPE-15 cells were nontumorigenic, we found that these cells were highly susceptible to interaction with other cancer cells under 3D spheroid coculture conditions. For instance, a 4-day coculture with aggressive IH10 and IIG5 cells, sublines of the ARCaP PCa cells, rendered HPE-15 cells tumorigenic in mice. Using our reported xenograft tumor formation assay protocol, we determined that both sublines were highly tumorigenic by themselves, each forming palpable s.c tumors within 2 weeks of inoculation, yielding 100% tumor formation in 8 weeks (n = 9). To track the growth of xenograft tumors, dual RFP- and luc-tagged HPE-15 cells of the HPE-15RFP/luc clone were cocultured with either IH10 or IIG5 PCa cells (Table 3 and Fig. 3). After 4 days in 3D coculture with IH10 or IIG5 cells, HPE-15RFP/luc cells were purified and amplified by 2 weeks of high-dose puromycin selection (Fig. 3a). HPE-15RFP/luc cells after the coculture became highly clonogenic in colony formation assays with rate of colony formation increased from the pre-coculture < 5% to 43.2 and 30.7%, respectively. Importantly, HPE-15RFP/luc cells from the coculture became capable of forming s.c tumors in NSG mice (Fig. 3b). The tumors became palpable at 2 weeks after inoculation, and reached 1 cm in the largest dimension in 2 months when the assay was terminated. In these experiments, purity of HPE-15RFP/luc cells from IH10 or IIG5 contamination was validated by STR analyses (Fig. 2c and Table 4). Though an extra D8S1179 allele was detected in the HPE-15RFP/luc cells after 3D coculture with IH10 cells, it could be a result of the cell-cell interaction rather than a contamination, because the same alleles were absent from IH10 cells. The xenograft tumors displayed glandular structure (Fig. 3c) with expression of pan-cytokeratin (CK) and EpCAM, together with RFP indicative of the HPE-15 origin of the tumors (Fig. 3d). Intriguingly, HPE-15RFP/luc cells recovered from xenograft tumors through ex vivo culture were found to have a karyotype similar to the parental HPE-15 cells as shown in Figure 2d, probably indicating that the malignant transition was mainly an epigenetic event. Though the underlying molecular and cellular mechanism has yet to be elucidated, these findings support our previous finding that a cell can acquire tumorigenicity from interactions with other cells in the tumor microenvironment.

**Discussion**

After isolation and characterization of a panel of human prostate mesenchymal stromal cell lines, we established matched pairs of PCa epithelial cell lines from the same tumor specimens. These specimens were from patients whose PCa tumors were confirmed with indications for radical prostatectomy. This study demonstrates that, compared to mesenchymal stromal cells, PCa epithelial cell lines are much more difficult to establish (Supporting Information Table S1), since most of these cells become senescent in continuous passaging and die after prolonged growth arrest. These observations agree with previous failures in establishing PCa cell lines, as virtually no cell line has been established directly from freshly harvested PCa tumor tissues without the use of viral gene transduction. Though the biological function of prostate epithelial cells is maintained by androgen stimulation, the addition of androgen in ex vivo tumor culture has not facilitated isolation and establishment of PCa cell lines. The cause of this failure remains unresolved.

The difficulty with prostate epithelial cells ex vivo could be attributed to several reasons. In our prostate tissue culture, we

**Table 1. Chromosomal numbers in HPE-15 and other common PCa cell lines**

| Cell line | Number of modal chromosomes | Number of marker chromosomes | Reference |
|-----------|-----------------------------|-----------------------------|-----------|
| LNCaP     | 76–91                       | 7                          | 7         |
| DU145     | 61–64                       | 6                          | 23        |
| PC-3      | 55–62                       | 10–20                      | 24        |
| ARCaP     | 68–95                       | 11                         | 9         |
| 22Rv1     | 49–52                       |                             | 25        |
| HPE-15    | 45–54                       | 1                          | 26        |
| RWPE-1    | 45–51                       |                             |           |

Figure 2. Characteristics of the HPE-15 cell line. General features of HPE-15 are (a) loss of PSA production in ex vivo culture. ELISA was used to detect PSA levels in cell culture medium. Quadruplet assays were conducted for each sample. Fresh medium was used to culture each prostate tissue for 48 hr, followed by ELISA for PSA detection. Inset: passages from each surviving epithelial culture are shown. (b) Nonresponsiveness to androgen stimulation. Cells were first treated in androgen starvation medium for 48 hr, then replenished with regular medium (Control), kept in androgen-deprivation medium (Starvation), or stimulated with 5 nM R1881 in androgen-deprivation medium (R1881). After another 24 hr, culture medium from each treatment was subjected to PSA detection. (c) Unique STR genotype profile. An electropherogram of HPE-15 STR analysis is shown. The STR genotype was determined to be identical to pair-matched HPS-15 stromal cells but different from any known established cell lines of human origin. Some genotype data are not shown to protect the identity of the donor. (d) Aneuploidy of HPE-15 cells. HPE-15 cells at passage 64 were used for analysis. Representative results from 20 metaphase spreads are shown. (e) Unique marker protein expression. HPE-15 cells at passage 34 under regular culture (Control), androgen starvation (Starvation) and androgen stimulation (R1881, 5 nM) for 24 hr were subjected to western blotting analyses. PC-3 cells were used as a positive control for FOXA2 expression. PrEC cells at passage 5 were used as a “normal” prostate epithelial cell control.
observed drastic death of primary epithelial cells in the first passage. A majority (≥85%) of primary epithelial cells, from a full monolayer with high vitality, would undergo apoptotic cell death immediately after the first passaging (data not shown).

We tested several mild detachment agents, such as diluted trypsin (0.0025%), dispase II and accutase (1:10) and citric saline, and found that none could help the cells avoid widespread postpassaging death. These observations indicate that death is not caused by harsh treatment of cells during detachment, but rather that the primary epithelial cells per se are sensitive to any standard forms of monolayer disintegration.

Detachment-induced apoptosis, or anoikis, is a biological mechanism for maintaining structural integrity.37,38 Both cell–cell adhesion and cell–extracellular matrix attachment are supported by surface protein-mediated intercellular signaling. The dimeric E-cad protein, for example, binds to another E-cad dimer on an apposed cell in a Ca2+-dependent and homophilic manner, while heterodimeric integrins bind to specific extracellular matrix epitopes. Importantly, the subcellular region of the surface adhesion proteins is linked to mechanisms of cell growth, survival and differentiation.39 It is probable that abrupt detachment during replating nullifies outside-in survival signaling. Elucidation of the adhesion-dependent survival mechanism may help establish PCA cell lines from prostate specimens, and may also help better understand PCA development and progression.

It is not clear why only one of the tumor specimens yielded the established HPE-15 cell line, although the same ex vivo culture protocol was applied to all the prostate specimens. HPE-15 was derived from the tumor of a late-life Caucasian with a Gleason score over 7 at diagnosis, without detectable extraprostatic metastasis. We speculate that the success in culturing HPE-15 cells was particular to the intrinsic properties of the tumor specimen in which HPS-15 was shown to be one of the most effective stromal fibroblasts tested for sustaining the survival of PCa cells in an experimental coculture model.6 Unlike the other five specimens, this specimen could also have contained certain cells with stronger survival potential and robust proliferation capability suitable for ex vivo culture conditions. This postulation is supported by the fact that only a small fraction of cells could survive initial replating to form colonies in secondary culture.

Though the established HPE-15 cells share morphologic features with PrEC prostate epithelial cells (Fig. 1), HPE-15 has unlimited growth potential while PrEC cells have only limited proliferation. HPE-15 shares with PrEC cells a minimal PSA level and androgen insensitivity when cultured in vitro. In fact, we identified a rapid loss of PSA expression in all prostate epithelial specimens during ex vivo culture (Fig. 2). It seems that with the exception of LNCaP cells, prostate epithelial cells tend to lose PSA expression and androgen responsiveness in ex vivo culture. With the availability of a homotypic HPS-15 stromal cell line, it would prove to be of interest to investigate if prostate stromal cells can activate AR and PSA in vitro and in vivo if recombined or cocultured with HPE-15 cells.

In the initial characterization of this newly established cell line, HPE-15 was determined to share a unique STR profile with its matched stromal cell line (Fig. 2c and Table 4), confirming

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### Table 2. Null tumorigenicity in HPE-15 cells as determined by xenograft tumor formation

| Subject mouse strains | Group size | Route of inoculation | Cell numbers per site | Total number of sites | Matrigel coinjection | Number of tumors formed |
|-----------------------|------------|----------------------|----------------------|----------------------|---------------------|------------------------|
| NCr<sup>−/−</sup>nu   | 5          | s.c.                 | 2 × 10<sup>6</sup>   | 10                   | Yes                 | 0                      |
|                       | 5          | s.c.                 | 5 × 10<sup>6</sup>   | 10                   | Yes                 | 0                      |
|                       | 6          | i.f.                 | 2 × 10<sup>6</sup>   | 12                   | Yes                 | 0                      |
| SCID                  | 5          | s.c.                 | 2 × 10<sup>6</sup>   | 10                   | Yes                 | 0                      |
|                       | 5          | s.c.                 | 5 × 10<sup>6</sup>   | 10                   | Yes                 | 0                      |
|                       | 5          | s.c.                 | 2 × 10<sup>7</sup>   | 10                   | Yes                 | 0                      |
| NSG                   | 5          | s.c.                 | 2 × 10<sup>6</sup>   | 10                   | Yes                 | 0                      |
|                       | 5          | s.c.                 | 5 × 10<sup>6</sup>   | 10                   | Yes                 | 0                      |

Cells between passages 35 and 45 were used in the assay.

1Cells premixed with 50% Matrigel were used in the inoculation.

2Xenograft tumor formation was determined 12 months after the inoculation.

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### Table 3. Acquired tumorigenicity of HPE-15 derivative cells from 3D coculture

| Cells                  | Source of the HPE-15 cells                      | Rate of xenograft tumor formation<sup>1</sup> |
|------------------------|------------------------------------------------|----------------------------------------------|
| HPE-15<sup>RFP/luc/10</sup> | 3D monoculture of HPE-15<sup>RFP/luc</sup> cells | 0/10 (0%)                                    |
| HPE-15<sup>RFP/luc/I10</sup>  | 3D coculture of HPE-15<sup>RFP/luc</sup> with I10 cells | 10/10 (100%)                                |
| HPE-15<sup>RFP/luc/I10</sup>  | 3D coculture of HPE-15<sup>RFP/luc</sup> with I10 cells | 8/8 (100%)                                  |

<sup>1</sup>Results were obtained 6 months after s.c inoculation in NSG mice.
that HPE-15 represents a new prostate epithelial cell line. Though these cells are aneuploid with visible chromosomal rearrangement (Fig. 2d) and indefinite proliferation potential, HPE-15 is nontumorigenic as evaluated in experimental mice with different degrees of immune compromise (Table 2). In future studies, orthotopically inoculating HPE-15 cells directly to the prostate gland of athymic mice could determine whether tumorigenicity in this cell line depends on the prostatic stromal microenvironment of the host. It is, however, not uncommon that a tumor-derived cell line is nontumorigenic in experimental mice. LNCaP cells, for example, are nontumorigenic, though this cell line was derived from PCa lymph node metastasis.8,9

To assess the pathological relevance of the HPE-15 cell line, we compared its marker protein expression to PrEC, LNCaP and PC-3 cells (Fig. 2e). HPE-15 cells may represent a unique cell type in the transit amplifying stage from basal cell to luminal epithelial cell differentiation.40,41 Though controversial, epithelial cells at this stage could contribute to PCa oncogenesis and development.42 With this possibility in mind, we investigated especially whether HPE-15 cells could be induced to become tumorigenic cells through cellular interaction instead of using genomic manipulation with exogenous genes. We demonstrated that HPE-15 cells acquired aggressive phenotype and tumorigenicity simply through in vitro 3D coculture with

| STR locus | HPS-14 | HPS-15 | HPE-15<sup>5</sup> inoculated<sup>3</sup> | HPE-15<sup>5</sup> recovered<sup>4</sup> | ARCaP derivative clones |
|-----------|--------|--------|---------------------------------|-----------------|----------------------|
| DSS818    | 10, 13 | 10, 13 | 10, 13                          | 10, 13          | 10, 11               |
| D13S317   | 11     | 11     | 11                              | 11              | 11                   |
| D75820    | 10, 13 | 10, 13 | 10, 13                          | 10, 13          | 10, 11               |
| D165539   | 11, 12 | 11, 12 | 11, 12                          | 11, 12          | 11                   |
| vWA       | 14, 17 | 14, 17 | 14, 17                          | 14, 17          | 18, 19               |
| TH01      | 9.3    | 9.3    | 9.3                             | 9.3             | 8, 9.3               |
| TPOX      | 8      | 8      | 8                               | 8               | 8, 10                |
| CSF1PO    | 10, 11 | 10, 11 | 10, 11                          | 10, 11          | 10, 13               |
| Amelogenin| X, Y   | X, Y   | X                               | X               | X, Y                 |

1Not all the results from 18 loci were shown to protect patient information. Omission of results does not affect the study conclusion.
2A representative STR microsatellite electrophoretic profile of HPE-15 cells is shown in Figure 2c.
3HPE-15<sup>RFP/luc</sup> cells isolated from 3D coculture with cells of the ARCaP IH10 clone and amplified to be inoculated for xenograft tumor formation (Fig. 3a).
4HPE-15<sup>RFP/luc</sup> cells recovered from ex vivo culture of the s.c mouse xenograft tumor.

Figure 3. Acquisition of tumorigenicity through interaction with highly tumorigenic PCa cells. HPE-15<sup>RFP/luc</sup> cells, recovered from three-dimensional (3D) coculture with two aggressive PCa cells, IH10 or IIG5, were found to have acquired tumorigenic potential by s.c xenograft tumor formation. (a) Photograph of HPE-15<sup>RFP/luc</sup> cells isolated from 3D coculture with IH10 cells and amplified under puromycin selection conditions (HPE-15<sup>RFP/luc/IH10</sup>). No contaminating IH10 cells were seen after the cells were red fluorescent. (b) Representative BLI results of NSG mice bearing s.c HPE-15<sup>RFP/luc/IH10</sup> tumors. (c) Representative H&E staining of s.c xenograft tumors of HPE-15<sup>RFP/luc/IH10</sup> and HPE-15<sup>RFP/luc/IIG5</sup> cells (200x). (d) Representative IHC images of HPE-15<sup>RFP/luc/IH10</sup> tumors, which expressed pan-CK and EpCAM epithelial markers. Strong RFP staining confirmed the origin of the tumor formation to be from HPE-15<sup>RFP/luc/IH10</sup> cells (100x).
aggressive tumorigenic PCa cells (Table 3 and Fig. 3). In this regard, HPE-15 cells were found to be particularly susceptible to induction by other cancer cells through direct cell–cell contact. Because initial HPE-15 lacks any tumorigenicity, this cell line should be a promising model for studying the role of cellular interaction and intercellular programming in the coevolution observed in PCa tumors.53,44 Further studies are ongoing in our laboratory focusing on the mechanisms of induction and phenotypic transition of PCa cells from indolent to aggressive behaviors.

In conclusion, we isolated and established a new PCa cell line, HPE-15, directly from clinical prostate tumor specimens. Both genomic and gene expression results show that this cell line uniquely represents a new cell type of prostate epithelium. As these cells are highly susceptible to modulation by exogenous inductive cues, HPE-15 will be an excellent experimental model for PCa oncogenesis and antitumor treatment. This cell line may also prove valuable in defining the underlying molecular mechanisms accounting for the transition of prostate basal cells to luminal epithelial cells during prostate carcinogenesis.

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