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In Vitro Culturing and Characteristics of Transit Amplifying Epithelial Cells From Human Prostate Tissue

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Abstract The prostatic epithelium is functionally organized in stem cell units. This unit consists of a slow turn over stem cell within the basal epithelial layer which can replenish itself and provide progeny which differentiate down either a neuroendocrine or exocrine pathway. The maturation along the exocrine pathway initially involves transit amplifying cells within the basal layer proliferating and subsequently the progeny maturing into intermediate cells. These intermediate cells migrate into the luminal layer where they terminally differentiate into non-proliferative secretory luminal cells which express prostate specific differentiation markers, like PSA. A growing body of experimental evidence has identified the proliferating transit amplifying/intermediate cells as the cells of origin for the common prostatic adenocarcinomas. Using a series of growth characteristics, and mRNA and protein markers, we have validated that primary cultures can be established in serum free defined media from surgically resected human prostates which are composed of essentially pure population of transit amplifying cells. At each serial passage, the subsequent cultures undergo enhanced maturation into intermediate cells and by the 7–10th passage these cells eventually lose their proliferative ability. This study validates that these cells are a useful and relevant system for the determination of molecular events involved in prostatic carcinogenesis. J. Cell. Biochem. 91: 196–205, 2004.

Key words: basal cells; stem cells; transit amplifying cells; normal prostate; prostate cancer; PrEC

Normal adult prostatic epithelium is composed of a basal and luminal layer. This epithelial composite is continuously turning over with time, with the rate of proliferation balanced by an equal rate of death such that neither involution nor overgrowth of the gland occurs normally [Berges et al., 1995]. If an adult male is castrated, the serum testosterone level decreases rapidly and the prostate regresses because androgen chronically stimulates the rate of proliferation while simultaneously inhibiting the rate of prostatic epithelial death [Isaacs, 1984]. In steady-state, self-renewing tissue systems (e.g., skin, bone marrow, testes, gut, etc.), control over the total cell content of a tissue is determined by the total number of self-renewing stem cells [Lajtha, 1979]. A stem cell is defined as a clonogenic, pluripotent, self-renewing progenitor that can generate one or more specialized cell types [Lajtha, 1979]. Androgen-ablation/restoration cycling experiments [Isaacs, 1987] and flow cytometry [Bhatt et al., 2003], have demonstrated that the fraction of prostatic epithelial cells that are stem cells is low (i.e., $\approx 1\%$). This latter study documented that the prostatic stem cells are of basal origin and have a low growth fraction [Bhatt et al., 2003]. The majority of the cell production within the gland is due instead to the proliferation of a subclass of basal epithelial cells which, while progeny of stem cells, have differentiated to a state where they now have only a limited self-renewal capacity [Potten and Lajtha, 1982]. A characteristic of this subclass is that when physiological levels of androgen are present, it undergoes enhanced proliferative expansion with its progeny maturing into an intermediate phenotype which migrates (i.e., transits) from the basal to the luminal layer where it terminally differentiates into a non-proliferating secretory luminal cell. Although this transit subclass of cells can proliferate for

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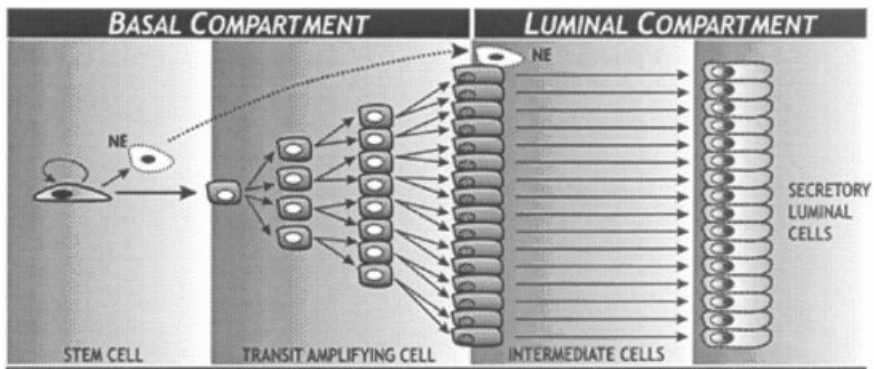
only limited numbers of cell divisions, due to the exponential nature of proliferations, this still results in a major amplification in the total number of epithelial cells, and thus these cells are termed, transit amplifying cells (Fig. 1). For example, if one stem cell gives rise to one transit amplifying cell which subsequently divides five times, this produces a 32-fold amplification in total cell number; if they can divide 10 times, this produces a 1,000-fold amplification. Such amplification results in the stem cells being a minority epithelial population in the prostate. The stem and transit amplifying cells are androgen-independent since they are able to survive even in long-term castrated animals. This point is emphasized by the fact that it is possible to castrate adult male rats, allow an extended period (i.e., >3 years) before replacing androgen, and yet still fully restore the gland [Isaacs and Coffey, 1989]. While being androgen independent, transit amplifying cells are sensitive to androgenic stimulation for enhancing the number of proliferations before dying [Isaacs, 1987]. Thus, restoration of the regressed prostate after castration by subsequent exogenous androgen replacement involves no increase in the rate of stem cell renewal, but instead is due to the increase in proliferation of the pool of



	CASTRATE MAJORITY OF BASAL CELLS REMAIN		CASTRATE MAJORITY OF SECRETORY CELLS DIE	
ANDROGEN RESPONSE	INDEPENDENT			DEPENDENT
ANDROMEDIN RESPONSE	INDEPENDENT	SENSITIVE		DEPENDENT
SELECTED PHENOTYPIC AND PROTEIN MARKER EXPRESSION				
Self Renewal Capacity	Extensive	Limited	Very Limited	None
Proliferative Index	Very Low	Much higher	Low	None
Keratin 5	+		+	−
Keratin 14	+		+	−
p63	+		−	−
Bcl-2	+		+	−
P cadherin	+		?	+
GST-pi	+		+/−	−
C-MET	+/−		+/−	−
$p27^{kip1}$	+	−	−/+	+
Keratins 8,18	−		+	+
NKX 3.1	−		−/+	+
PSA	−		−/+	+
PSAP	−		−/+	+
AR	−		−/+	+

Fig. 1. Stem cell model of prostatic epithelial cell compartmentalization. Prostate gland consists of a number of stem cell units which arise from one stem cell. Such a stem cell is located in the basal epithelial layer of the prostate and upon division gives rise to a population of transit amplifying cells. The latter divide in the basal layer and a fraction of them differentiate and move into the secretory luminal epithelial layer. As transit amplifying cells differentiate and move into a secretory luminal layer from the basal layer they acquire expression of a number of genetic markers, as indicated. NE denotes neuroendocrine cells; + denotes expression of marker; − denotes lack of detectable expression of marker.

preexisting transit amplifying cells [Isaacs, 1987].

There are epithelial cells within the prostate of an intact adult host that absolutely do require a critical level of androgen stimulation for their continuous presence since these cells die following castration and are restored following androgen replacement [Kyprianou and Isaacs, 1987, 1988]. These androgen-dependent cells are the terminally differentiated secretory luminal cells generated from the maturation of intermediate cells [Isaacs, 1987; Kyprianou and Isaacs, 1987, 1988; Isaacs and Coffey, 1989; Verhagen et al., 1992, Bonkhoff et al., 1994; Bonkhoff and Remberger, 1996; De Marzo et al., 1998a,b; Robinson et al., 1998; Hayward et al., 1999; Hudson et al., 2000; van Leenders et al., 2000; Collins et al., 2001; Bhatt et al., 2003; van Leenders and Schalken, 2003] (Fig. 1). These intermediate cells are defined by their simultaneous expression of cytokeratins 5/14, which are characteristic expressive markers of basal cells, and cytokeratins 8/18 which are characteristic of expressive markers for secretory luminal cells [van Leenders and Schalken, 2003] (Fig. 1). Transit amplifying cells express the p53 related p63 protein, c-Met, the plasma membrane receptor for hepatocyte growth factor (HGF), and pro-survival protein, bcl-2 but either do not express androgen receptor (AR) or express it at a very low level [Bonkhoff et al., 1994, 1998; Litvinov et al., 2003; van Leenders and Schalken, 2003]. A minority of stem cell progeny differentiate into neuroendocrine (NE) cells which secrete serotonin and NE peptides like bombesin, calcitonin, and parathyroid hormone-related peptide [Rumpold et al., 2002]. The majority of the stem cell progeny differentiate to become transit amplifying cells which eventually mature and migrate from the basal to the luminal layer where they progress through an intermediate phenotype before terminally differentiating into secretory luminal cells. Associated with this terminal differentiation, these secretory luminal cells now express AR and $p27^{Kip1}$cyclin-dependent kinase inhibitor and stop proliferating [Bonkhoff et al., 1994, 1998; van Leenders et al., 2000] (Fig. 1). Due to the hierarchical expansion during this maturation process, these non-proliferating AR/$p27^{Kip1}$ positive secretory luminal cells are quantitatively the major subtype of epithelial cells present in the normal prostate. These AR/$p27^{Kip1}$ positive secretory luminal cells also express the prostate specific differentiation markers [Litvinov et al., 2003], as outlined in Figure 1. The transcriptional expression of these prostate specific differentiation marker genes is induced by occupancy of the AR with physiologic androgen [dihydrotestosterone (DHT)] and the subsequent binding of the occupied AR at androgen response elements in the promoter and enhancer sequence of these genes within the nuclei of these secretory luminal cells [Litvinov et al., 2003].

In contrast to the regulation of transcription of these prostate differentiation marker proteins, AR in the nuclei of these secretory luminal cells does not directly regulate the survival of these luminal cells nor does it positively regulate the proliferation and survival of the prostatic epithelial stem, transit amplifying or intermediate cells. Instead, the survival of the secretory luminal cells and the proliferation of the transit amplifying and intermediate cells require the androgen dependent production of peptide growth factors by the prostatic stromal cells [Cunha et al., 1987; Hayward et al., 1992; Gao et al., 2001; Kurita et al., 2001]. Binding of DHT to the AR within the nuclei of these stromal smooth muscle cells inhibits their secretion of apoptotic molecules while enhancing their secretion of "andromedins" (i.e., androgen induced stromal peptide growth factors) [Lu et al., 1999; Planz et al., 1999]. These stromal andromedins diffuse into the epithelial compartment where they interact with their specific cognate receptors on the plasma membrane of secretory luminal cells generating intracellular signaling needed to repress the apoptotic death pathway in the secretory luminal cells [Kyprianou and Isaacs, 1989]. Binding of the andromedins to the plasma membrane receptors of the transit amplifying epithelial cells can also recruit them into the cell cycle. Without adequate "andromedins," prostatic transit amplifying epithelial cells remain proliferatively quiescent in G_o and do not enter the cell cycle, while prostatic secretory luminal cells undergo apoptosis [Kyprianou and Isaacs, 1989; Denmeade et al., 1996].

Although prostate cancer arises from the epithelial compartment, the identification of the specific epithelial cell subtype in which the carcinogenic process initiates has only recently been addressed. The precursor for prostatic carcinomas is believed to be high-grade

prostatic intraepithelial neoplasia [HGPIN] [McNeal and Bostwick, 1986]. HGPIN arises from low-grade PIN, which is derived from an "intermediate," prostatic epithelial cell. This "intermediate" cell is derived within the proliferative transit amplifying population which undergoes initial malignant molecular changes inducing gene expression and morphologic features of both basal and secretory luminal cells [Verhagen et al., 1992; De Marzo et al., 1998a,b; van Leenders et al., 2000, 2002; Meeker et al., 2002; van Leenders and Schalken, 2003]. The site of these phenotypically intermediate, initiated, cells appears not to be random within the prostate. Instead, they are enriched in sites of focal glandular atrophy where the atrophic appearing epithelial cells are quite proliferative and often surrounded by inflammation within the gland. Therefore, these sites have been termed "proliferative inflammatory atrophy [PIA]" [De Marzo et al., 1999]. Based on these findings, a new model of prostate carcinogenesis has been proposed whereby chronic and acute inflammation, in conjunction with dietary and other environmental factors, targets prostate epithelial cells for injury and destruction. Increased proliferation occurs as a regenerative response to lost epithelial cells. The increased proliferation occurs in cells with a transit amplifying/intermediate phenotype [Verhagen et al., 1992; Meeker et al., 2002; van Leenders et al., 2002; van Leenders and Schalken, 2003]. Thus, the appropriate "normal" prostatic epithelial cells to study in vitro as a relevant model for prostatic carcinogenesis are proliferating transit amplifying cells. Using a series of mRNA and protein markers as well as growth characteristics, we have validated that cultures can be established in serum free defined media from surgically resected human prostates which are composed of essentially pure transit amplifying cells and that for at least several serial passages, these cells retain a high proliferation ability and transit amplifying cell phenotype.

MATERIALS AND METHODS

Cell Culture and Reagents

Normal prostatic epithelial cells were obtained commercially (PrEC; Clonetics, Inc. Walkersville, MD) or derived ($n = 6$) from grossly normal areas of prostate tissue and characterized in a series of epithelial (E) cultures, as described earlier [Gao et al., 2001]. These cells were grown in prostate epithelial basal medium (PrEBM) supplemented with bovine pituitary extract, epidermal growth factor, insulin, transferrin, hydrocortisone, retinoic acid, epinephrine, tri-iodothyronine, and gentamycin–amphotericin solution (Clonetics Inc.). Early (p3–p5) and late passage (p7) cultures were used as mentioned. The synthetic androgen R1881 was obtained from Amersham Biosciences, Corp. (Piscataway, NJ).

Immunocytochemistry

Early passage PrEC (p3) were stained with antibodies to high molecular weight cytokeratins 5/14 or low molecular weight cytokeratins 8/18 followed by labeling with FITC or Rhodamine conjugated antibodies respectively, as per manufacturer's recommendation (Dako Corp., Carpinteria, CA). Cells were visualized by fluorescence microscopy at a magnification of 200× and positive cells were enumerated in a minimum of three fields/section. Total number of cells was visualized and enumerated using nuclear DAPI staining and percent of positively stained cells were determined.

Western Blotting

Western blots were performed using 50 μg of total protein per sample. Antibodies directed against PTEN (clone 6H2.1) were obtained from Cascade Bioscience (Winchester, MA), those directed against Rb (C-15), p53 (FL-393), AR (N-20), and p63 (A4A) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) while anti-p21cip and anti-p27kip monoclonal antibodies were obtained from BD Biosciences (San Diego, CA).

Secondary, HRP-conjugated anti-mouse or rabbit antibodies were used as appropriate and chemiluminescence was detected using ECL reagents (Amersham Biosciences, Corp.). Western blots for PSA, hK2, and PSMA were as described previously [Denmeade et al., 2003a].

RT-PCR

The RNeasy Kit from QIAGEN, Inc. (Valencia, CA) was utilized to isolate RNA from cultured cells. Fifty nanograms of RNA was subjected to RT-PCR analysis using TaqMan Reverse Transcription Reagents and SYBR Green PCR Master Mix from Applied Biosystems (Foster City, CA). Primer sequences are as follows:

- P27 (30 cycles; 60°C annealing): 5′primer-TGCAGAGACATGGAAGAGGC; 3′primer-CACCTTGCAGGCACCTTTG;
- PSCA (35 cycles; 66°C annealing): 5′ primer-TGACCATGAAGGCTGTGCTGCTT; 3′primer-TCGGTGTCACAGCACGTGATGTTC;
- GAPDH (glyceraldehyde-3-phosphate-dehydrogenase) (30 cycles; 60°C annealing): 5′primer-ACCACAGTCCATGCCATCAC; 3′primer-GTTCAGCTCAGGGATGACC;
- PSA (30 cycles; 60°C annealing): 5′primer-AACCCTGGCAGGTGCTTGT; 3′primer-TGTGTCTTCAGGATGAAACAG;
- P63 (30 cycles; 68°C annealing): 5′primerGTGAGCCACAGTACACGAACC; 3′primer-GAGCATCGAAGGTGGAGCTGG;
- AR (30 cycles; 66°C annealing): 5′primer-CCACAGGCTACCTGGTCCTG; 3′primer-TCCTCGTCCGGAGGTGCTG.

Cell Growth Assays

Live cells were quantitated using the CellTiter 96® non-radioactive cell proliferation assay (MTT) kit obtained from Promega Corp. (Madison, WI). Briefly, 3,200 normal cells were seeded in 96 well plates in growth factor containing media and allowed to attach overnight. The number of live cells was determined by the uptake of the MTT dye that was measured at $A_{570-630}$ absorbance using a microplate reader. Cell numbers for each sample were extrapolated from standard curves (absorbance vs. cell number) prepared for each cell line. In each experiment, measurements were obtained from 8-well repeats and averaged. One set of measurements was performed after allowing cells to attach (day 0) and represented the starting cell number. Cell numbers obtained on subsequent days were normalized to the starting cell number. The starting cell number was expressed as 100%. Data shown are mean ±SEM of three independent experiments.

Determination of Percentage of Proliferating Cells

Cells were seeded at a density of 8×10^5 cells/well in 6 well plates, incubated appropriately and harvested by trypsinization. Hundred microlitres of 2×10^5 cells/ml was cytospun onto poly-lysine coated slides, fixed in 3% paraformaldehyde (4°C for 30 min), washed in PBS containing 0.1% Triton X-100, and stored in cold methanol (−20°C) until needed. Cells were probed with an antibody to Ki67 (Immunotech Inc., Marseille, France), a nuclear antigen associated with cell proliferation at a dilution of 1:50. Biotinylated secondary antibody and streptavidin-HRP was obtained from DAKO (LSAB2 System-HRP kit) as described previously [Gao et al., 2001]. Endogenous peroxidases were inhibited in 0.6% hydrogen peroxidase and color development was performed using DAB substrate obtained from Vector Labs (Burlingame, CA). Cells were then coverslipped and enumerated using bright field light microscopy. Mean results were obtained from scoring at least three fields per sample. These experiments were performed three independent times.

RESULTS

Characteristics of Human Prostatic Cells Growing in Androgen and Serum Free Defined Media

Using androgen and serum free, growth factor supplemented media, it is possible routinely to develop proliferative cultures of cells from normal prostatic tissue [Chopra et al., 1996; Liu and Peehl, 2001]. These proliferating cultures can be serially passaged in serum free defined PREBM (Clonetics) supplemented with the following: insulin, hydrocortisone, epidermal growth factor, and bovine pituitary extract containing both acidic and basic fibroblast growth factors and nerve growth factor. By their second passage, these cultures contain no detectable stromal cells as assayed by α-smooth muscle actin or type I collagen using Western blotting. Whether derived in-house cells (e.g., 6E) or commercially obtained (i.e., PrEC cells) these early passage cultures are composed of a characteristic epithelial morphology (Fig. 2A). These cells also uniformly express high molecular weight cytokeratins 5 and 14 (Fig. 2B) and p63, mRNA (Fig. 3A), and p63 protein (Fig. 3B), which are characteristic markers of basally derived prostate transit amplifying cells [Verhagen et al., 1992; Tran et al., 2002; Garraway et al., 2003; van Leenders and Schalken, 2003]. In contrast, less than 5% of the early passage cells expressed the low molecular weight cytokeratins 8 and 18 alone characteristic of secretory luminal cells or in combination with cytokeratins 5 and 14 characteristic of "intermediate" basal cells [van Leenders and

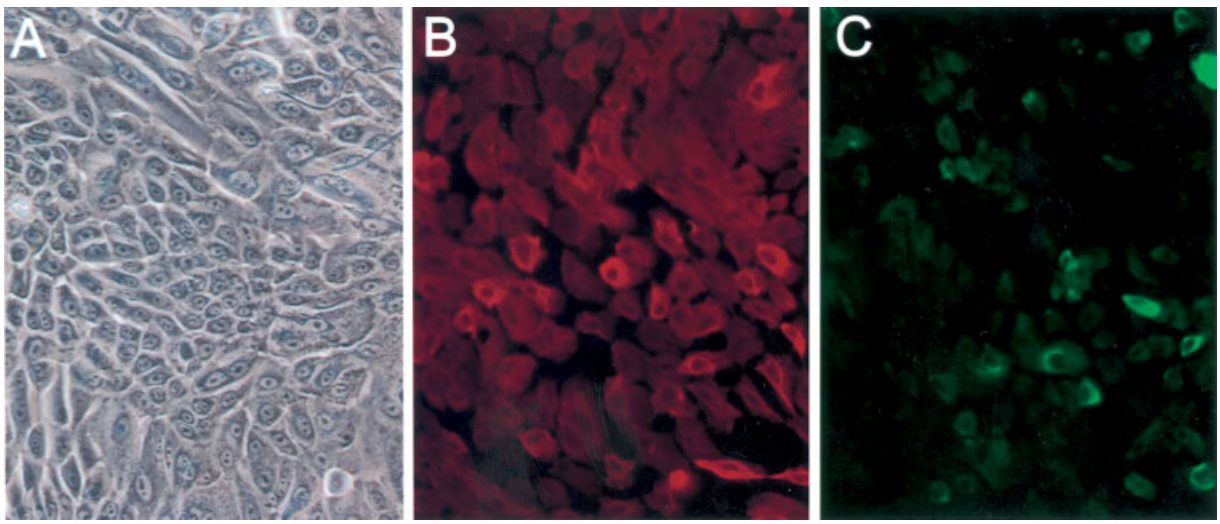


Fig. 2. Phase contrast microscopy (**A**) and immunocytochemical detection of cytokeratins 5 and 14 (**B**) and cytokeratins 8 and 18 (**C**) in early passage PrEC. [Color figure can be viewed in the online issue, which is available at: www.interscience.wiley.com.]

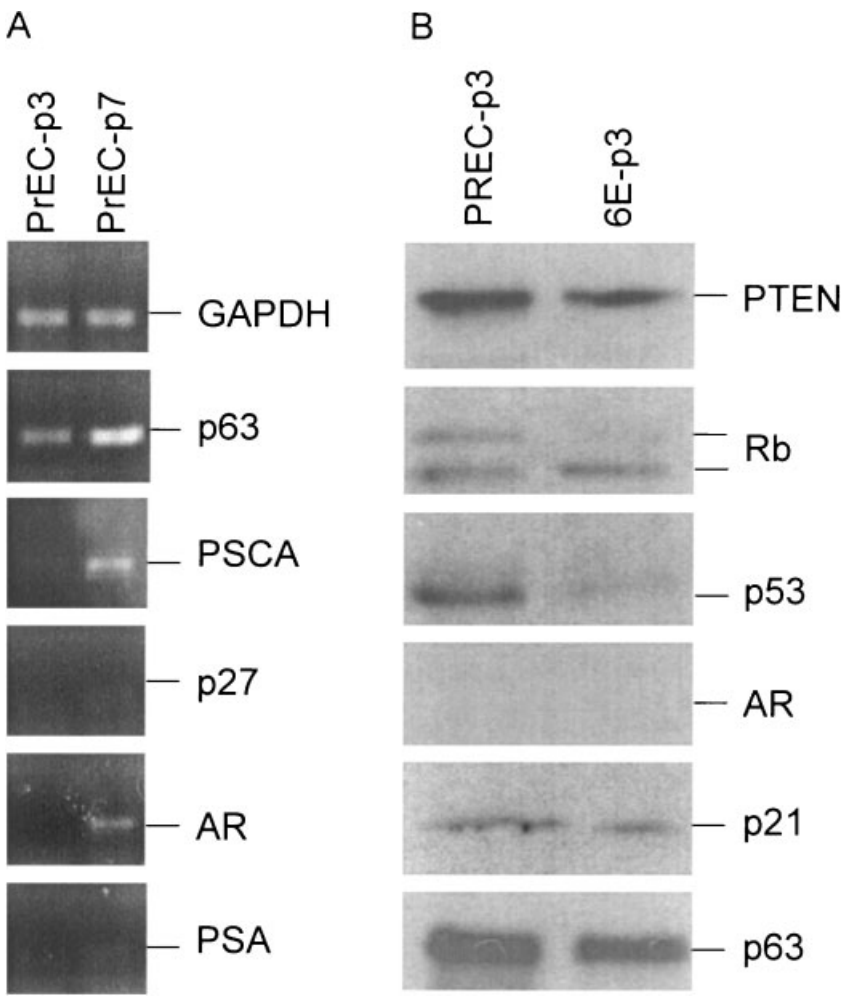


Fig. 3. RT-PCR (**A**) of early (p3) and late (p7) passage PrEC. RT-PCR was performed to detect mRNA of p63, PSCA, p27kip, AR, and PSA genes while monitoring GAPDH levels as a loading control. Western blots (**B**) of early (p3) passage of PrEC or in house produced 6E cells probed with antibodies against PTEN, Rb, p53, AR, p21, and p63, as shown.

Schalken, 2003] (Fig. 2C). Using Western blotting, these early passage basal like cells express detectable levels of PTEN, Rb, p53, p63, and p21cip proteins (Fig. 3B), but do not express detectable levels of p27kip mRNA (Fig. 3A), or protein (data not shown), which is again characteristic of basal epithelial cells [van Leenders et al., 2000; Tran et al., 2002].

In early passage, $65 \pm 6\%$ of these cells are proliferating as documented by immunocytochemical detection of expression of the proliferation specific Ki67 nuclear antigen (Fig. 4B). Such growing cultures have a doubling time of 42 ± 4 h (Fig. 4A). In early passage (i.e., <7 passages), these growing epithelial cells do not transcribe RT-PCR detectable levels of prostate stem cell antigen (PSCA) mRNA (Fig. 3A), whose expression is characteristic of late "intermediate" basal cells [Garraway et al., 2003], or mRNA for any of the prostate differentiation marker proteins characteristic of secretory luminal cells (i.e., prostate specific antigen (PSA), human kallikrein 2 (hk2) prostate specific membrane antigen (PSMA)), or the AR (Fig. 3A), and thus do not express detectable levels of AR protein (Fig. 3B) or PSA, hK2, or PSMA protein by Western blots (data not shown).

By their seventh passage, these normal prostatic epithelial cells continue to express p63 mRNA (Fig. 3A) and protein (Fig. 3B), but now also express AR and PSCA mRNA (Fig. 3A). While expressing the mRNA, these later passage cells do not express detectable levels of AR or PSCA protein (data not shown), nor mRNA expression of PSA (Fig. 3A). These results document that before passage 7, these cultures are composed of essential pure transit amplifying cells with less than 5% of the cells transiting into "intermediate" cells. After passage 7, the percentage of such transit-amplifying cells which mature into "intermediate" cells increases. Associated with this increased maturation, the cells undergo morphologic changes (i.e., the cells become flatter), and decrease their proliferative ability (i.e., doubling time increases to >72 h). By the 10th passage, the cells lose their proliferative ability (i.e., doubling time >10 days).

Further Characteristics of Transit Amplifying Cells in Culture

Besides these biochemical and growth characteristics, there are several other observations

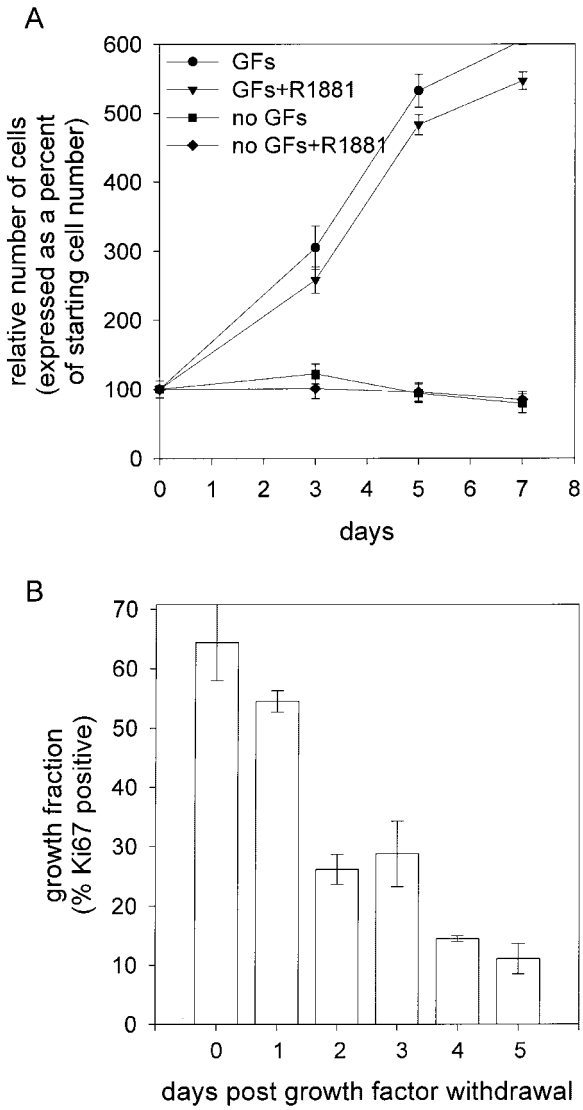


Fig. 4. **A**: Growth rate of early passage PrEC in the presence and absence of growth factors and the synthetic androgen, R1881. MTT assays were performed on PrEC grown in growth factor-free basal media (no GFs) or growth factor containing media (GFs) in the presence or absence of 1nM R1881 for a period of 7days. The percent cell number (compared to starting cell number at day 0, i.e., 100%) is represented as the mean ± SEM of three independent experiments. **B**: Growth fraction (i.e., percentage of cells expressing Ki67 in the nuclei) of PrEC maintained in media containing growth factors (day 0) or deprived of growth factors for a period of 5 days.

which are consistent with these early passage cultures being composed of the transit amplifying not stem or secretory luminal cells. The first is that these cells can be serially passaged for only approximately 10 times, before they express p27kip [Tran et al., 2002] and becoming proliferatively senescent and they do not express telomerase activity characteristic of stem

cells (data not shown). This is explainable since during serial passage, any slowly renewing stem cells which may have been present in early passage culture are rapidly outgrown by the highly proliferating transit amplifying cells and thus these stem cells are diluted out at each serial passage. Second, androgen does not directly enhance the expression of any of the prostatic luminal cell differentiation marker proteins nor does it stimulate the proliferation of these early passage cultures alone or in the presence of added growth factors (Fig. 4A). Third, while these early passage cells do not require androgen for their growth, such growth does require the addition of paracrine growth factors in the media (Fig. 4A). These added growth factors are required for maintenance of a high growth fraction, as assayed by Ki67 expression (Fig. 4B).

DISCUSSION

Normal prostatic epithelial cells display a precise balance of proliferation versus death [Berges et al., 1995]. In contrast, cells of HGPIN, the precursor of prostate cancer have an increased rate of proliferation that is not balanced by an equally increased rate of cell death [Berges et al., 1995]. Localized and metastatic prostate cancer cells have a similar imbalance such that malignant growth occurs continuously [Berges et al., 1995]. Thus, an understanding of the changes responsible for this imbalance has major implications for rational drug development for prevention and treatment of prostatic cancer. In order to evaluate the causal role of such malignant changes, appropriate in vitro test systems are needed which can be experimentally manipulated. Presently, there are a series of well-characterized in vitro prostate cancer cell lines required for such studies. Also required is the appropriate "normal" prostate epithelial cells from which prostate cancer develops which can also be experimentally manipulated for comparison. In order for such comparisons to be valid, however, the normal precursor and malignant cells must be evaluated under similar growth states. Since the growth fraction of human prostate cancer cell lines in vitro is characteristically greater than 70% [Denmeade et al., 2003b], similar high growth fraction cultures of normal prostate epithelial cells are required for appropriate comparative studies.

Production of such high growth fraction cultures in vitro is possible even though the overall growth fraction of the normal prostate epithelium in vivo is remarkably low (i.e., $<1\%$ of the total prostatic epithelial cells are in cycle [Berges et al., 1995]). This is because within the prostatic epithelial compartment, high rates of proliferation are restricted to the minor subset of transit amplifying cells [Bonkhoff et al., 1994, 1998]. These transit amplifying cells are located in the basal layer of the epithelial compartment and do not express AR protein nor prostatic differentiation marker proteins (e.g., PSA, hK2, and PSMA) [Bonkhoff et al., 1998; De Marzo et al., 1998a,b; Litvinov et al., 2003]. While this subset of AR negative basal cells does not respond directly to androgen, these cells do require adequate levels of the androgen stimulated, stromally derived, paracrine growth factors (i.e., andromedins) for their proliferation but not survival [Gao et al., 2001; Kurita et al., 2001]. These transit amplifying cells express the dominant-negative N terminal truncated (ΔN) form of the p53 related, p63 gene and are derived from prostatic epithelium stem cells [van Leenders et al., 2000; Tran et al., 2002; Garraway et al., 2003; van Leenders and Schalken, 2003]. Besides proliferating, these ΔNp63 expressing cells undergo a process of maturation into "intermediate" cells in the basal epithelial compartment [Tran et al., 2002; Garraway et al., 2003]. This maturation into "intermediate" cells involves the co-expression of cytokeratin protein isoforms characteristic of both basal and secretory luminal cells [van Leenders and Schalken, 2003] coupled with loss of ΔNp63 expression, decrease in their growth fraction, expression of AR mRNA (but not protein), and novel expression of PSCA mRNA and protein [Tran et al., 2002; Garraway et al., 2003]. The "intermediate" cells complete their maturation with their loss of PSCA protein expression during their migration (i.e., transit) into the luminal layer to become secretory cells [Tran et al., 2002]. These secretory luminal cells translate AR mRNA and thus express AR protein whose occupancy by androgen enhances their expression of prostate specific differentiation marker proteins like PSA and hK2, but it does not enhance their proliferation or survival [Gao et al., 2001; Litvinov et al., 2003]. While these secretory luminal cells are proliferatively quiescent due to their expression of high levels of the proliferation suppressive p27 kip protein,

they still require adequate levels of the androgen stimulated stromally derived andromedins for their survival [De Marzo et al., 1998a,b; Gao et al., 2001; Kurita et al., 2001].

Thus, when normal human prostates are used for culturing in serum free defined media containing known growth factors and hormones, only transit amplifying cells have a sufficiently high rate of proliferation to allow multiple sub-passaging before undergoing proliferative senescence. In contrast, the low proliferation rates of the prostatic stromal cells, epithelial stem cells, and the secretory luminal cells in androgen and serum free defined media result in the elimination of these cell types during the first several in vitro passages [Denmeade et al., 2003b]. Since these early passage culture cells are derived from the subset of proliferating (i.e., transit amplifying) normal prostatic epithelial cells in vivo and have a high growth fraction, they are a valid test system both for studies of prostatic carcinogenesis and for comparing the importance of various signaling pathways in their proliferation and survival versus prostatic cancer cells under essentially identical growth factor conditions.

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