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MOLECULAR ANALYSIS AND CHARACTERIZATION OF PrEC, COMMERCIALLY AVAILABLE PROSTATE EPITHELIAL CELLS

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SUMMARY

Adenocarcinoma of the prostate comprises 95% of all prostate cancer. Commercially available primary cultures of "normal" prostate epithelial cells, PrECs, have been used as a convenient model to investigate neoplastic transformation. Here PrECs were characterized for the expression of lineage- and developmental-specific markers cytokeratin (CK) 8 and 18, p63, chromogranin A, TMEPAI, S100P, NKX 3.1, ANKH, and FN 1 as well as androgen receptor and prostate-specific antigen by Western blot and Northern blot analyses, immunohistochemistry, reverse transcriptase–polymerase chain reaction (RT-PCR), and quantitative real-time PCR. Immunohistochemical staining detected PrECs positive in varying degrees for p63, CK 8, and CK 18, with only the rare cell being positive for chromogranin A. The PrECs also tested positive for p63 protein by Western blot analysis. RT-PCR with PrEC cDNA showed products for FN 1 and S100P but not for ANKH and androgen receptor or prostate-specific antigen. This profile of markers in PrEC cells is consistent with that expected for pubertal prostate epithelial cells.

Key words: prostate cancer; endocrinology; prostate epithelial cells; prostate-specific antigen; androgen receptor

INTRODUCTION

The prostate is a gland that is part of the male reproductive system and provides the prostatic fluid in the ejaculate. Prior to puberty, the prostate is approximately 1 to 2 g in weight and grows rapidly during puberty to approximately 20 g at maturity (Isaacs, 1987). This rapid growth rate is due to the increased levels of serum testosterone (androgen) at puberty. The prostate requires androgens for growth as well as function.

The functional unit of the prostate is the glandular acinus, which is composed of both stromal and epithelial cells. The epithelial cells that make up the prostate are lumenal (secretory), basal, and neuroendocrine. The lumenal cells secrete prostate-specific antigen (PSA) and prostatic acid phosphatase, two important clinical biomarkers for prostate cancer. These cells express androgen receptor (AR) and are dependent on androgens for function and viability. Reduction of androgen by medical or surgical castration after maturity results in involution of the prostate largely due to apoptosis of lumenal cells (Denmeade et al., 1996). There are discrepancies in the literature as to whether basal and neuroendocrine cells express AR (Bonkhoff and Remberger, 1993; Harper, 1998). Reports that these cells do not express AR are consistent with the fact that they do not undergo apoptosis in response to androgen ablation (Bonkhoff and Remberger, 1993; Harper, 1998; Krijnen et al., 1993; Nakada et al., 1993). Markers used to distinguish prostate epithelial cells include cytokeratin (CK) 8 and 18 for lumenal cells, CK 5 and 14 and p63 for basal cells, and chromogranin A for neuroendocrine cells. The majority of adenocarcinomas of the prostate exhibit a lumenal-like phenotype based on their expression profiles of CKs, AR, PSA, and their response to androgen ablation. The AR and PSA are still expressed in most hormone refractory tissues from patients with advanced prostate cancer (Visakorpi et al., 1995).

Adenocarcinoma of the prostate comprises 95% of all prostate cancer and is one of the leading causes of cancer death in aging males. Morbidity results from the transformation of the normal prostatic epithelium to a metaplastic adenocarcinoma. Research examining the molecular mechanisms underlying carcinogenesis has taken advantage of several different in vitro and in vivo model systems (Sobel and Sadar, 2005a, 2005b). However, there is a lack of "normal" prostatic lumenal cells available for experimentation in vitro. Viral transformation of benign cells for sustained growth in tissue culture results in cells that are no longer normal. One commercial source of "normal" prostate epithelial cells that are maintained as a monolayer in culture and can be passaged are PrECs from Clonetics. These cells have been used to represent "normal" cells for studies comparing changes in gene expression, testing potential chemotherapeutics, and molecular mechanisms associated with adenocarcinoma (Porkka and Visakorpi, 2001; Reagan-Shaw and Ahmad, 2005; Voelkel-Johnson, 2003). Here PrECs were further characterized for expression of lumenal, basal, and neuroendocrine epithelial markers as well as for expression of genes regulated during the pubertal stage of prostate development.

MATERIALS AND METHODS

Reagents. Antibodies were obtained from various suppliers: AR 441 (Santa Cruz sc-7305, Santa Cruz, CA), PSA (clone ER-PR8 from DAKOCytomation,

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TABLE 1

SEQUENCES OF PRIMERS USED FOR PCR AND EXPECTED SIZED OF cDNA AND GENOMIC DNA AMPLICONS

Gene	Primers	5′-sequence-3′	Size cDNA	Size genomic	Reference
ANKH	Forward	CATCACCAACATAGCCATCG	160 bp	NA[a]	This paper
	Reverse	CAGGCCCACATTTTTGAAGT			
AR[b]	Forward	TACTTCGCCCCTGATCTGGTTTTC	509 bp	NA	This paper
	Reverse	CATGAGCTGGGGTGGGGAAATAGG			
β-actin	Forward	GGACCTGACTGACTACCTCATGAA	523 bp	523 bp	This paper
	Reverse	TGATCCACATCTGCTGGAAGGTGG			
FN 1	Forward	AGGCTCAGCAAATGGTTCAG	155 bp	985 bp	This paper
	Reverse	TCGGTTCCTCCATAACAAG			
GAPDH[c]	Forward	CCGAGCCACATCGCTCAGA	~350	NA	This paper
	Reverse	CCCAGCCTTCTCCTGGTG			
NKX 3.1	Forward	GGCCTGGGAGTCTCTTGAGCTCCACGAC	234 bp	1198 bp	Skotheim et al., 2003
	Reverse	ATGTGGAGCCCAAACCACAGAAAATG			
PSA[d]	Forward	GGCAGGTGCTTGTAGCCTCTC	521 bp	850 bp	This paper
	Reverse	CACCCGACGAGGTGCTTTTGC			
S100P	Forward	CGAGGGCAGCACGCAGACC	110 bp	2898 bp	Dhanasekaran et al., 2001
	Reverse	GAGCAATTTATCCACGGCATCCTT			
TMEPA1	Forward	CCTTCTCTTCCCCTTTCCATCTCC	147 bp	147 bp	Dhanasekaran et al., 2001
	Reverse	GTCCCGCCAACCCCAAATCTATCT			

[a] NA = not available.
[b] AR = androgen receptor.
[c] GAPDH = glyceraldehyde-3-phosphate dehydrogenase.
[d] PSA = prostate-specific antigen.

Mississauga, Ontario, Canada), CK 8 (clone 35βH11 from DAKOCytomation), β-actin (Abcam ab8227, Abcam, Cambridge, MA), p63 (D9) (Santa Cruz sc-25268) and chromogranin A (DAKOCytomation). The androgens employed in theses studies were mibolerone (NEN, Boston, MA), also referred to as R1881 and dihydrotestosterone (DHT) (Sigma, Oakville, Ontario, Canada).

Cell culture. The PrECs (Clonetics, East Rutherford, NJ) used for these studies were derived from a 14-yr-old Caucasian male (lot no. 1F1808) and were grown according to manufacturer's directions in serum-free PrEGM (Clonetics) supplemented with bovine pituitary extract (BPE) (Clonetics). Where indicated, PrECs were "starved" for 24 h in PrEGM media lacking BPE. No obvious cell death was observed. The LNCaP cells (ATCC, Manassas, VA) were grown in RPMI 1640 supplemented with 2 μ*M* L-glutamine (StemCell Technologies, Inc., Vancouver, British Columbia, Canada) with 5% (v/v) fetal bovine serum (HyClone, Logan, UT) and 100 units/ml penicillin and 100 μg/μl streptomycin (Invitrogen, Burlington, Ontario, Canada). LNCaP cells were starved in phenol red-free, serum-free media for 24–48 h and then treated for the indicated times with 10 n*M* androgen.

Immunohistochemistry. Cells ($1.3–1.5 \times 10^4$) were plated onto eight-chambered glass slides or on sterile, poly-L-lysine–coated coverslips. After 0–24 h of treatment, the cells were fixed with methanol for 10 min at RT, air-dried for 2 h, and stored at 4° C until stained as previously described (Wang et al., 2001).

Western analysis. Nuclear and cytoplasmic protein fractions were prepared from cells as previously described (Hewish and Burgoyne, 1973a, 1973b). Briefly, following cell lysis, nuclei were pelleted (5 min at $1000 \times g$ at 4° C.), and the supernatant was retained as the cytoplasmic fraction. Whole cell lysates were obtained by boiling cells for 5 min in 1× SDS-PAGE sample loading buffer with DTT (1 m*M*). Protein concentrations were determined using the BCA assay reagents as per manufacturer protocols (Pierce, Nepean, Ontario, Canada). Total protein (15 μg) was electrophoresed in 12% Tris-glycine SDS-PAGE gels and transferred to Immobilon-P membrane (Millipore, Nepean, Ontario, Canada) using 1× Towbin's transfer buffer with 20% methanol (Towbin et al., 1979). The membrane was blocked for 1 h in TBS containing 0.1% Tween (TBST) and 5% dry milk (w/v). The blots were incubated for either 2 h at RT or overnight at 4° C with anti-AR (1:1000), anti-PSA (1:100), anti-β-actin (1:3–5000), and anti-p63 (1:500), washed, and incubated with a horseradish peroxidase (HRP)–conjugated secondary antibody for 1–2 h at RT. Membranes were washed 3 × 10 min in TBST, developed with reagents for ECL (Amersham Biosciences, Baie d'Urfe, Quebec, Canada), and exposed to film. After probing for PSA, AR, or p63, the immunoblots were stripped with Restore (Pierce), washed, and reprobed with anti-β-actin.

Northern analysis. RNA was isolated from cells using TRIzol reagent (Invitrogen). Total RNA (15–20 μg) was fractionated by agarose (1.2%) gel electrophoresis with formaldehyde. Membranes were hybridized at 42° C overnight with ^{32}P-α dCTP labeled complementary DNA (cDNA) probes for PSA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Ueda et al., 2002), washed twice (2× SSC, 0.1% SDS for 20 min at 65° C), and exposed overnight to a phosphorimage screen (Molecular Dynamics, Baie d'Urfe, Quebec, Canada). The bands were visualized on the Molecular Dynamics phosphorimager and the blot then reexposed to X-ray film (Kodak, Rochester, NY) for various time periods over several days.

Reverse transcriptase–polymerase chain reaction. Total RNA (~1–2 μg) were reverse transcribed into cDNA with Superscript II polymerase (Invitrogen) according to manufacturer protocol using either a random hexamer or oligo dT primer. For reverse transcriptase–polymerase chain reaction (RT-PCR), 1-μl template cDNA was used per 20-μl reaction and subjected to 35 cycles with an annealing temperature of 55° C for 1.5 min and 72° C extension for 45 s. Details regarding the primers used and size of expected amplicons are in Table 1.

Quantitative real-time PCR. Total RNA was treated with DNAse I, purified with E.Z.N.A. Total RNA kit I columns (Omega Bio-Tek, Lilburn, GA), and quantified before preparing cDNA using oligo dT primer. The Platinum SYBR Green qPCR SuperMix (Invitrogen) or the Bio-Rad supermix kit was used as recommended by the respective manufacturer. Reaction conditions were as follows: 95° C for 15 s, 60° C for 45 s, and 72° C for 45 s for a total of 45 cycles. ROX reference dye was included to account for pipetting differences and reaction volumes. Reactions were run in triplicate on an ABI prism 7900HT (Applied Biosystems, Streetsville, Ontario, Canada) or an iCycler (Bio-Rad, Inc., Mississauga, Ontario, Canada), and GAPDH was used as the reference gene for calculating normalized mean expression values using Q-Gene software (Muller et al., 2002). The β-actin primers, which give the same-size band for both genomic and cDNA, were employed to generate a standard curve from known amounts of genomic DNA to estimate the quantities of cDNA present in the gene-specific reactions (data not shown).

Results

PrECs express a mixture of epithelial and neuroendocrine markers. In prostate tissue, expression of CKs 8 and 18 are restricted to prostate lumenal epithelium, p63 distinguishes the basal epithelium, and chromogranin A is a marker for neuroendocrine cells (Bonkhoff et al., 1994). Examples of these staining patterns and cell

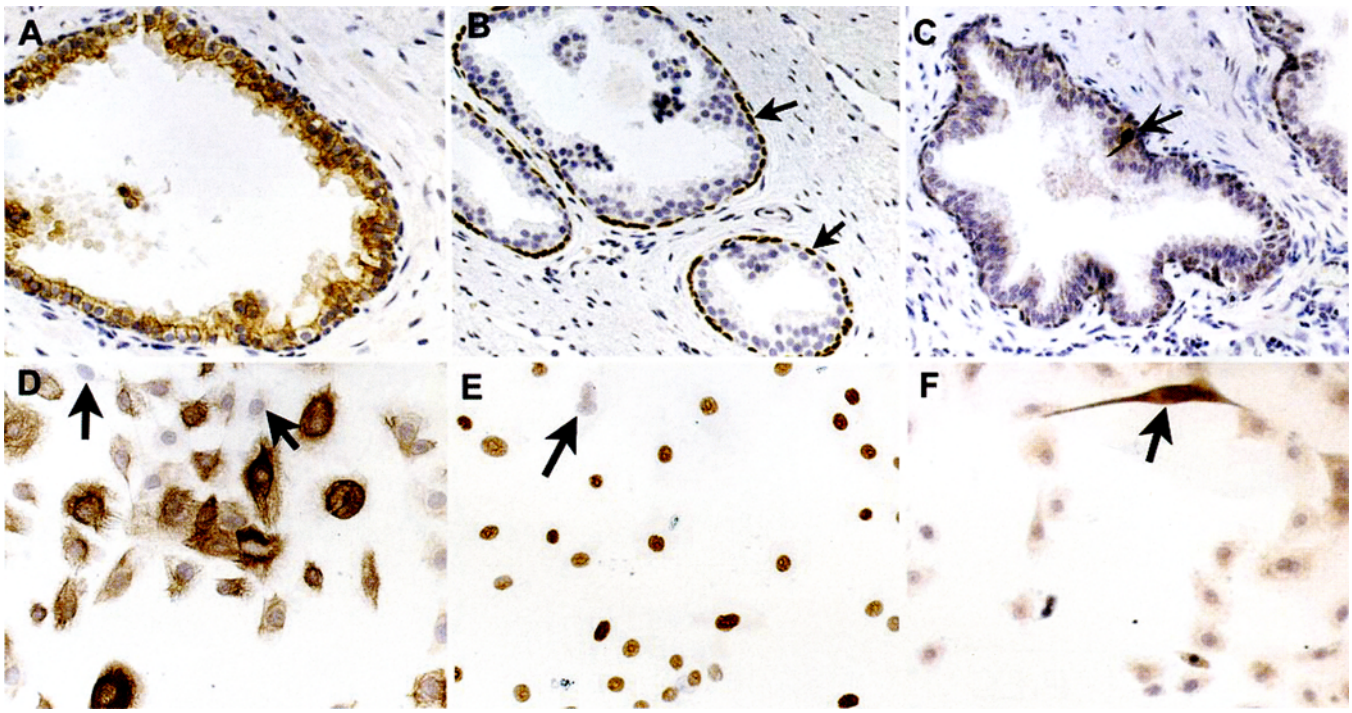

FIG. 1. Expression of markers of lumenal, basal, and neuroendocrine cells in prostate tissue and prostate epithelial cells (PrECs). Sections of human adult benign prostate tissue (*A–C*) and PrECs (*D–F*). Cells were stained for cytokeratin 8 (*A* and *D*), p63 (*B* and *E*), and chromogranin A (*C* and *F*). *Arrows* point to unstained cells (*D* and *E*), rare cells that stained for chromogranin A (*C* and *F*), or cells positively stained for p63 (*B*). All images were captured at ×400 magnification, except F, which was captured at ×100 and graphically enlarged.

types in benign prostatic tissue are shown in Figure 1. The cytoplasm of lumenal cells stained positive for CK 8, while the basal cells were negative (Fig. 1*A*). Basal epithelium showed strong nuclear staining for p63 (Fig. 1*B*). These cells formed the perimeter of the duct in a noncontinuous band, consistent with our previous observations (Wang et al., 2001). Neuroendocrine cells, as detected by chromogranin A, were rare in the benign prostate epithelial compartment and exhibited the expected unique morphology (Fig. 1*C*). Note that multiple sections had to be tested before detection of a single cell that stained for chromogranin A.

Immunohistochemical staining was conducted to determine which type of epithelial cells made up the majority of the population of cells in cultures of PrECs. To determine if PrECs were lumenal cells, expression of CK 8 and 18 was investigated. The majority of cells stained positive for both CK 8 (Fig. 1*D*) and CK 18 (data not shown), although expression of CK 8 was not uniform across the field. Some cells did not stain for CK 8 (depicted by arrows). The majority of PrECs expressed CKs 8 and 18, markers that are associated with lumenal cells.

Next, PrEC cells were examined for expression of p63, a marker for basal epithelial cells. The majority of PrECs showed strong positive nuclear staining for p63 with only the occasional unstained cell(s) (Fig. 1*E*; note unstained cells designated by the arrow). These results are consistent with a previous report showing positive p63 staining for PrEC cells (Garraway et al., 2003). Thus, the majority of cells present in cultures of PrECs express markers for both lumenal and basal cells.

The PrEC cells were immunostained to examine the expression of the neuroendocrine marker chromogranin A. Most PrEC cells failed to express this marker. However, an occasional cell that resembled the expected morphology of a neuroendocrine cell did stain positive for chromogranin A (Fig. 1*F*). Thus, cultures of PrECs contain a small number of neuroendocrine cells. Taken together, these data suggest that cultures of PrECs contain a heterogeneous population of cells comprised mainly of cells that express markers for both lumenal and basal cells.

PrECs fail to express PSA or AR protein but do express the basal marker p63. Normal adult prostate lumenal cells express PSA and AR, while basal cells do not (Bonkhoff et al., 1994). Therefore, levels of PSA, AR, and p63 proteins were measured by Western blot analysis using nuclear, cytoplasmic, or total cell lysates prepared from PrEC cells, using LNCaP cells as a positive control for lumenal cells. LNCaP cells were isolated from a mature male with prostate cancer (Horoszewicz et al., 1980), exhibit a lumenal-like phenotype, and express AR and PSA (Nagle et al., 1987; Brinkmann et al., 1989; Hasenson et al., 1989).

As expected, AR protein was detected in both nuclear and cytoplasmic fractions from untreated LNCaP cells but not in PrEC cells (Fig. 2*A*). PSA protein was detected in the cytoplasmic fraction of LNCaP cells (Fig. 2*B*). The PrECs failed to show expression of PSA in cytoplasmic, nuclear, or total cell extracts. In agreement with the immunohistochemistry results for p63, a band at the expected size was observed in both nuclear and total cell lysates but not in the cytosolic extract of lysates from PrECs (Fig. 2*C*). No bands could be detected for p63 using protein extracts from LNCaP cells. Equal loading of protein in all lanes was assessed by detection of β-actin (Fig. 2*D*).

Expression of PSA is induced by androgen in LNCaP cells by a

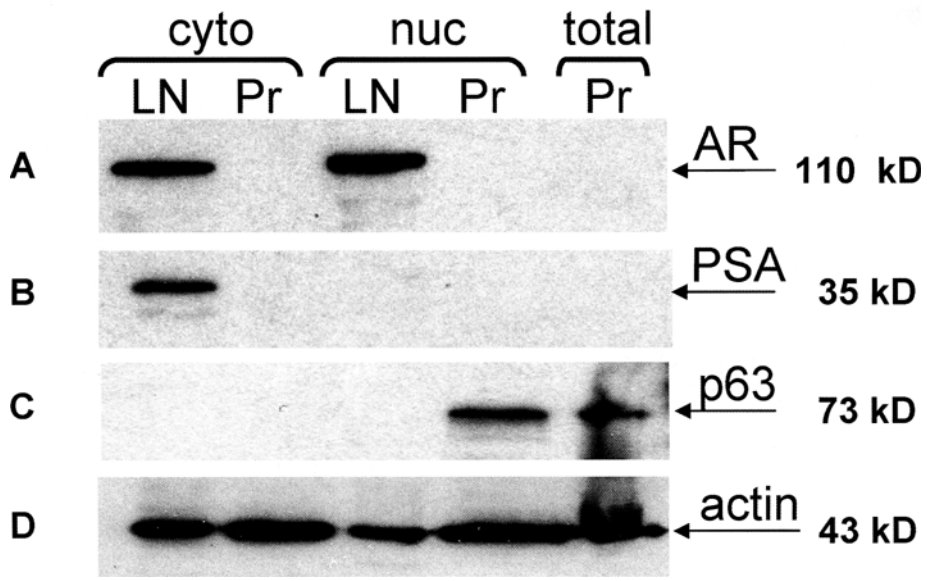

FIG. 2. Western blot analysis failed to detect expression of androgen receptor (AR) and prostate-specific antigen (PSA) in prostate epithelial cells (PrECs). Cytosolic (cyto), nuclear (nuc), and total protein lysates were prepared from LNCaP (LN) and PrEC (Pr) cells and probed for AR (*A*), PSA (*B*), p63 (*C*), and β-actin (*D*) as a loading control.

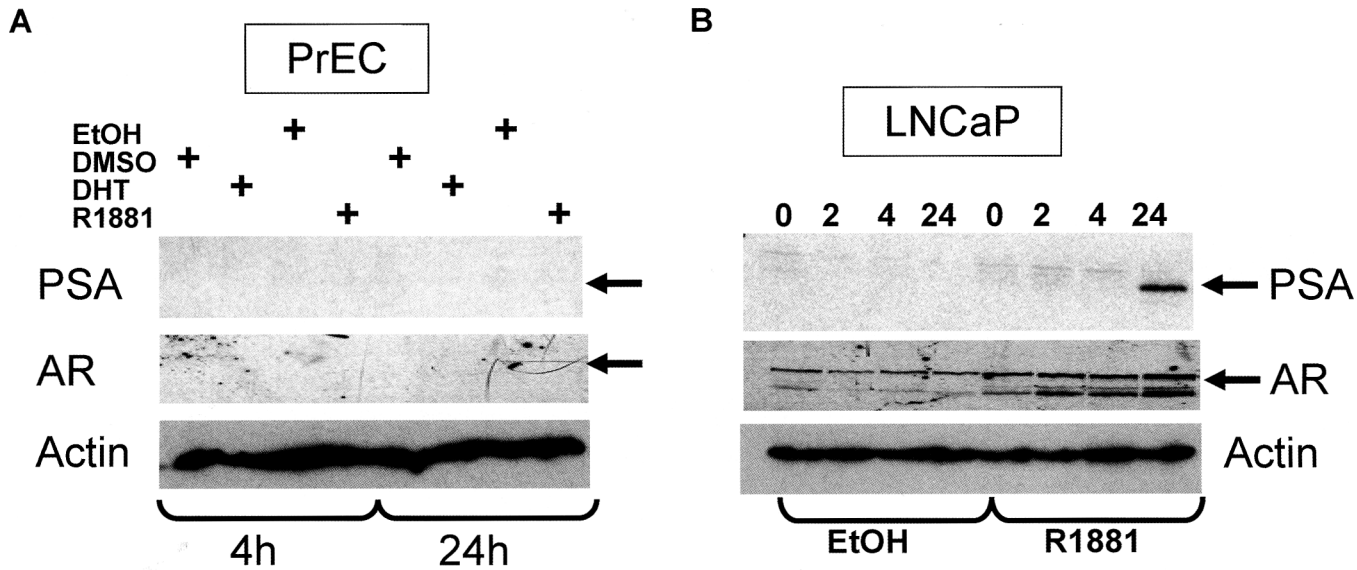

FIG. 3. Western blot analysis failed to detect expression of androgen receptor (AR) and prostate-specific antigen (PSA) in prostate epithelial cells (PrECs) in response to androgen. (*A*) PrECs were treated for 4 to 24 h with the androgens R1881 or DHT or ethanol (EtOH) and DMSO the vehicle controls. (*B*) LNCaP cells were treated for 0, 2, 4, or 24 h with R1881 or EtOH vehicle control. Whole cell protein lysates were prepared and probed for PSA or AR. β-actin was probed as a loading control.

mechanism dependent on the presence of a functional AR (Brinkmann et al., 1989). Therefore, PrEC and LNCaP cells (positive control) were treated with the androgens DHT or R1881 and then subjected to Western blot analysis to examine the levels of PSA and AR protein. Expression of PSA could not be detected in PrECs treated for 4 or 24 h with either R1881 or DHT (Fig. 3*A*, uppermost panel). This result is consistent with the failure to detect AR protein in PrECs (middle panel). As expected, LNCaP cells showed increased levels of PSA protein between 4 and 24 h after administration of R1881 (Fig. 3*B*, uppermost panel) and expression of the AR (Fig. 3*B*, middle panel). Treatment of LNCaP cells with R1881 for 2 h showed an increase in levels of AR protein expression that remained elevated for at least 24 h (Fig. 3*B*, middle panel). This is consistent with previous reports that the AR protein is stabilized in the presence of androgen (Zhou et al., 1995). Thus, PSA and AR protein were not detectable in lysates from PrECs by Western blot analysis.

PrEC cells fail to express PSA or AR mRNAs. PSA and AR proteins could not be detected by Western blot analysis using lysates from PrEC cells. It is possible that these genes are transcribed but not translated or are translated at levels undetectable by this approach. To examine this possibility, total RNA from PrECs and LNCaP cells was subjected to Northern blot analysis using a cDNA probe specific for PSA mRNA. Consistent with Western blot analysis, no PSA transcript was detected by Northern blot analysis using RNA from PrECs (Fig. 4*A*, top panel). A band for PSA transcript

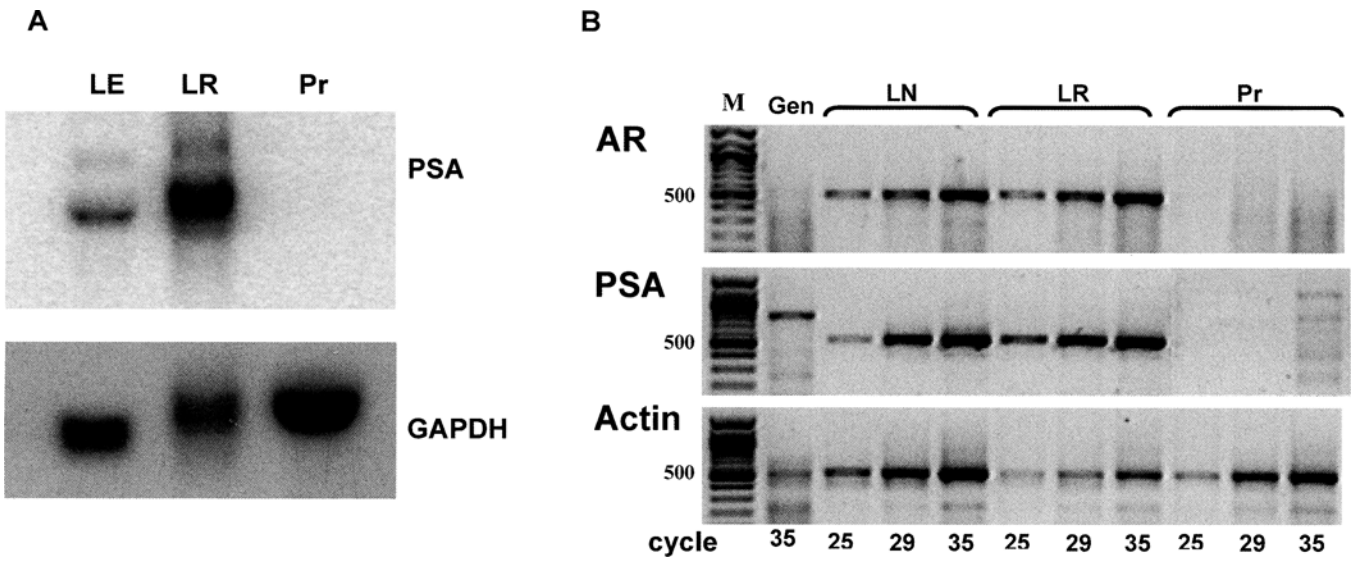

FIG. 4. Prostate epithelial cells (PrECs) failed to express prostate-specific antigen (PSA) and androgen receptor (AR) transcript. (*A*) Northern blot analysis for PSA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (loading control) mRNAs using total RNA isolated from PrECs (Pr) or LNCaP treated with ethanol vehicle control (LE) or androgen (LR). (*B*) Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis using cDNA prepared from total RNA isolated from LNCaP cells that were untreated (LN) or treated with androgen (LR) or PrECs (Pr). Primer pairs and expected RT-PCR and genomic DNA amplicon sizes for PSA, AR, and actin are listed in Table 1. Samples were removed from the PCR reactions at the cycle number indicated below each lane. The RT-PCR with actin is included as a normalizing control. No products were observed in reactions without the addition of template (data not shown). M = 100-bp DNA ladder; Gen = genomic DNA template.

was detected using RNA from LNCaP cells, as expected (Fig. 4*A*, top panel). For relative quantitation and comparison, loading of RNA to the gel was assessed by stripping the membranes and re-probing for GAPDH (Fig. 4*A*, bottom panel).

To further confirm the lack of expression of either PSA or AR, the highly sensitive technique of RT-PCR was employed to determine if low levels of PSA or AR mRNA could be detected in PrEC cells. Total RNA was reverse transcribed into cDNA and subjected to PCR with primer sets that span introns for both the AR and the PSA, respectively (see Table 1). Both androgen-treated and -untreated LNCaP cells produced the expected RT-PCR products for AR and PSA (Fig. 4*B*). RT-PCR of PrEC cDNA failed to yield cDNA bands for either PSA or AR but did yield a product for β-actin. Thus, PrECs do not express either PSA or AR mRNA at levels that are detectable by RT-PCR analysis. These data are consistent with the results from Western blot analyses and demonstrate the lack of expression of AR and PSA in PrECs.

PrEC cells express pubertal genes and fail to express other androgen-regulated genes. A recent study comparing gene expression profiles of pubertal and adult prostate specimens found 131 genes that were up-regulated and 244 that were down-regulated in pubertal prostates compared to adult tissue (Dhanasekaran et al., 2001). Expression of genes in pubertal prostates that are up-regulated include S100P and FN 1, while TMEPAI, ANKH, and NKX 3.1 are down-regulated compared to adult tissues (Dhanasekaran et al., 2001). Quantitative real-time PCR (qPCR) was performed to examine the levels of mRNA for these five genes relative to GAPDH using cDNAs prepared from total RNA isolated from PrECs and androgen-treated and -untreated LNCaP cells (Fig. 5). Levels of S100P and FN 1 PCR products were markedly enhanced, while levels of ANKH, NKX3.1, and TMEPAI PCR products were decreased in PrECs compared to untreated LNCaP cells. Levels of PCR product for ANKH, TMEPAI, and NKX3.1 increased in LNCaP cells that were exposed to androgen, consistent with previous studies suggesting that these are androgen-regulated genes (Dhanasekaran et al., 2001). Taken together, these results suggest a gene expression profile of PrECs that is consistent with pubertal cells.

DISCUSSION

Commercially available "normal" prostate epithelial cells, PrECs, can be maintained as a monolayer and passaged numerous times. These cultures have been applied as the "normal" reference for gene expression changes, testing chemotherapeutics, and delineating the molecular mechanisms for adenocarcinoma of the prostate (Porkka and Visakorpi, 2001; Voelkel-Johnson, 2003; Reagan-Shaw and Ahmad, 2005). Here, we characterized these PrECs and show the following: (1) expression of p63, CK 8, and CK 18 in a majority of the cells; (2) expression of chromogranin A in a small percentage of cells; (3) no expression of AR; (4) no expression of the androgen-regulated genes, PSA, or NKX3.1; and (5) an expression profile that resembles pubertal prostate.

Primary cultures of normal epithelial cells may contain basal, lumenal, neuroendocrine, and possibly stem cells (Peehl, 2005). Expression of the transcription factor p63 and CKs 5 and 14 is restricted to basal cells, while CKs 8 and 18 are limited to the lumenal cells. There are approximately equal numbers of basal and lumenal epithelial cells in "normal" prostatic acini. Thus, if all epithelial cells were successfully cultured with similar doubling times, this ratio would be preserved in cultures of PrECs. Immunohistochemistry revealed that this is not the case; instead, the majority of the cells expressed both p63 and CK 8. These results

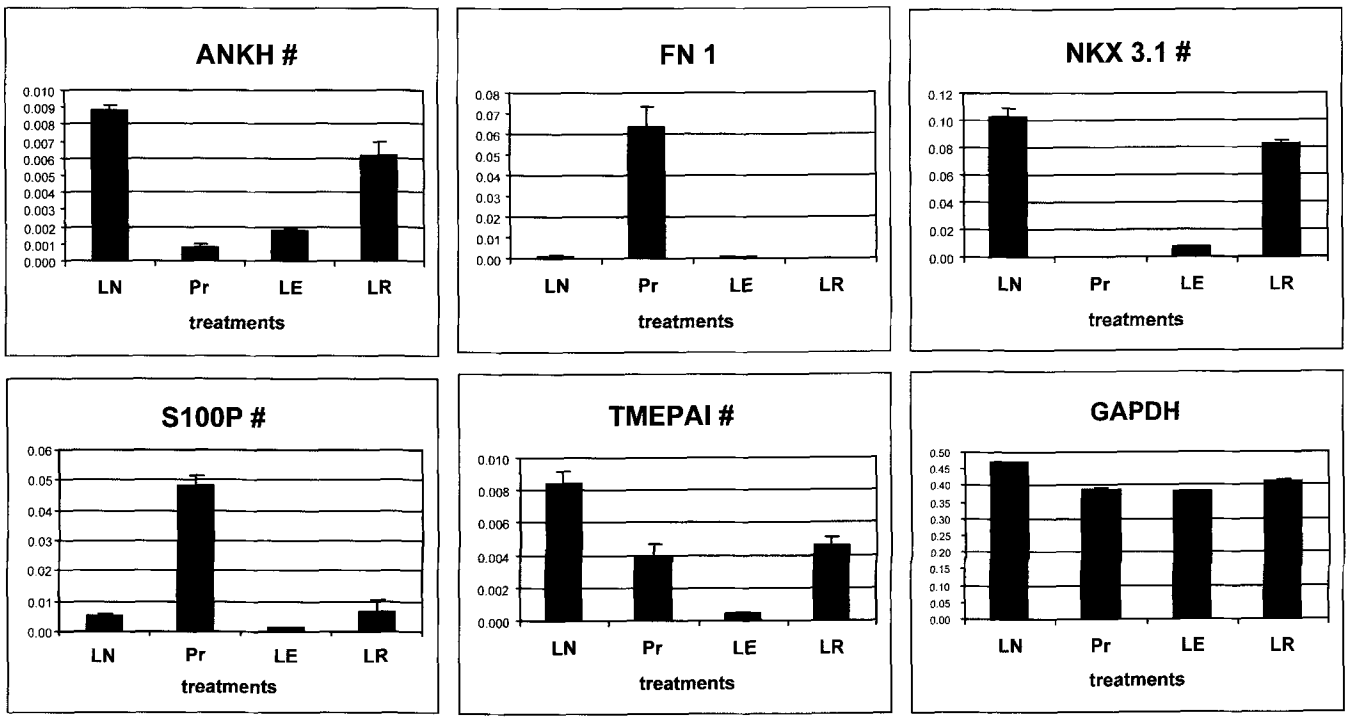

FIG. 5. Expression of genes differentially expressed between pubertal and adult prostate. Quantitative real-time polymerase chain reaction (PCR) analyses for ANKH, FN 1, NKX3.1, S100P, TMEPAI, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from cDNAs prepared from treated and untreated LNCaP and prostate epithelial cells (PrECs). Cell treatment abbreviations are as described in previous figure legends. # indicates genes that have been reported to be regulated by androgen. Data are calculated as mean expression normalized to GAPDH using Q-Gene software.

are consistent with a previous report that PrECs express CKs 5 and 14 in addition to CK 18 (Garraway et al., 2003). A possible explanation for PrECs expressing markers for both basal and lumenal cells is that these cells may be derived from a basal epithelial stem cell and are in the process of becoming lumenal cells. As the cells transit from basal to lumenal cells, they may acquire lumenal markers and gradually lose basal cell markers. Thus, there would be a period during which these cells exhibit a molecular phenotype intermediate between basal and lumenal (Garraway et al., 2003). Such a cell type is referred to as transit-amplifying or intermediate cells (Garraway, 2003; Peehl, 2005). Thus, cultures of PrECs may be comprised predominantly of transit-amplifying cells. However, it cannot be discounted that CK expression may be altered as an artifact of being grown as a monolayer in vitro (for a review, see Peehl, 2005).

Neuroendocrine cells in the prostate have also been suggested to be derived from local stem cells and may simultaneously express chromogranin A, basal cell–specific CKs, or PSA, a marker for lumenal cells (Bonkhoff et al., 1994). These studies did not test for the simultaneous expression of markers but did show that only rare cells with a unique morphology exhibited positive staining for chromogranin A. Thus, cultures of PrECs probably contain a heterogeneous population that includes a low number of neuroendocrine cells. This interpretation is consistent with the origin of these cells and their low passage number from primary cultures.

A hallmark characteristic of prostatic lumenal epithelial cells is their dependence on androgen for viability and function (Coffey et al., 1968; Bruchovsky et al., 1975; Denmeade and Isaacs, 1996). The effects of androgens are mediated through the AR, and the expression of more than 500 genes have been reported to be altered in the LNCaP cells in response to androgens (DePrimo et al., 2002). The PrECs failed to express AR at the protein or RNA levels in either the presence or the absence of androgen. Consistent with the lack of expressed AR, genes regulated by this transcription factor such as PSA (Young et al., 1991) and NKX3.1 (Bieberich et al., 1996; He et al., 1997; Prescott et al., 1998; Xu et al., 2000) were also not detected in PrECs. NKX3.1 is a homeobox gene that is essentially silent in pubertal prostate (Dhanasekaran et al., 2001). Other genes that showed similar trends in expression in PrECs compared to LNCaP that were consistent with results obtained for puberty versus adult tissues were S100P, FN 1, ANKH, and TMEPAI. S100P is a calcium-binding protein (Becker et al., 1992) and has been reported by several groups to be increased by androgen in prostate cells (Averboukh et al., 1996; Amler et al., 2000; Hammacher et al., 2005). However, expression of this gene was ninefold greater in PrEC as compared to LNCaP cells. The expression trend of S100P was similar to that obtained for fibronectin (FN 1), a gene that is highly expressed in pubertal but not adult tissue (Dhanasekaran et al., 2001). Based on the expression of genes investigated here, the PrECs derived from a 14-yr-old exhibited a pubertal phenotype as compared to mature lumenal cells, but it is unknown if this is a general phenotype of all PrECs regardless of the age of the donor. Thus, PrECs may provide a useful model for investigating development of the prostate but do not unequivocally provide a reliable reference of "normal" lumenal cells for comparison to most adenocarcinoma of lumenal-like phenotype.

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