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Does The Lack of Regression-Associated mRNA Expression Render a Rat Ventral Prostate Epithelial Cell Line Androgen Independent?

Michael L. Montpetit and Martin P. Tenniswood

Department of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

A series of rapidly dividing epithelial (RDE) cell lines have been isolated from primary cultures of rat ventral prostate (RVP) epithelial cells. Unlike androgen-dependent secretory epithelial cells, the RDE cells in culture do not express the androgen-dependent secretory proteins, nor do they express the androgen-repressed cell death sequences (TRPM-2) found in the epithelial cells during prostatic regression. Screening of a cDNA clone library established from RDE cell mRNA has yielded a number of RDE cell-specific sequences. One of these, RDE-.25 is a 250-base mRNA. The sequence of RDE-.25 shows considerable homology with the rat growth hormone gene and two murine oncogene sequences. We believe that the absence of androgen-repressed cell death sequence expression confers androgen independence for survival and growth, while the expression of RDE-.25 may represent an autocrine growth stimulus which greatly increases the rate of cell division in these cells.

Key words: epithelial cells, putative growth factor, regression sequence, androgen-independent epithelial cells

The majority of hormonal therapies for the treatment of prostatic cancer are based on the assumption that by interfering with the androgen-receptor interaction in the prostate the androgenic stimulus for both replication and gene expression can be suppressed. However, despite a number of elaborate treatments that have been developed, the 5-year survival rate for patients undergoing ablative therapy is still relatively poor. This is thought to be due to the alteration in the sensitivity to androgens of the primary tumor and its metastases during hormonal therapy. The progression of the tumor to androgen independence is marked by the appearance of cells which are resistant to hormonal therapy [1]. These androgen-independent cells may arise as a result of mutations in the androgen-responsive cells which render them resistant to androgens or from a pool of androgen-independent cells that are present in the normal prostate which are selected for by the hormonal therapy.

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Attempts to develop new treatments for prostatic cancer based on the results obtained in animal models have been confounded by the biochemical diversity of the epithelial cells in different regions of the prostate [2], and the interactions between the epithelial and stromal cells of the gland [3,4]. During androgen ablation the androgen-dependent epithelial cells of the rat ventral prostate (RVP) undergo a programmed series of events. Among the first of these is the cessation of expression of androgen-dependent secretory proteins, including the prostatic steroid binding proteins (PSBP) and secretory acid phosphatase. Beginning approximately 48 h after castration a small number of androgen-repressed mRNA are transcribed de novo [5]. Among these is the TRPM-2 sequence found to be associated with the active and programmed death of the epithelial cell population [5,6] through the process of apoptosis.

We describe here the identification and characterization of androgen-independent epithelial cell lines isolated from the normal rat prostate, termed rapidly dividing epithelial-like (RDE) cells. These cells do not require androgens for survival or growth and do not express the normal androgen dependent prostatic markers, such as secretory acid phosphatase and prostatic steroid binding protein (PSBP) in the presence of physiological concentrations of testosterone. RDE cells also fail to express, either in the presence or absence of androgen, the androgen-repressed cell death sequence TRPM-2 [5,6]. A cDNA clone library of RDE sequences has yielded three RDE-specific cloned sequences (pRDEs). One of these, pRDE-.25, is complementary to a portion of a 250 base mRNA sequence. The DNA sequence of this clone bears a strong sequence homology to portions of the rat growth hormone gene (rat GH) and the murine proto-oncogenes c-abl II and pim-1. This raises the possibility that the rapid androgen-independent growth of the RDE cells is the result of the androgen independence confered by the absence of TRPM-2 expression and the inappropriate expression of the RDE-.25 mRNA which produces an autocrine growth factor, bypassing the usual control mechanisms for normal prostatic epithelial cell division and gene expression.

MATERIALS AND METHODS

RDE Cell Isolation and Culture

RDE cells were isolated as reported [7,8] from primary cultures of normal androgen-dependent RVP epithelial cells. The culture medium was supplemented with 34 nM testosterone unless otherwise indicated.

Northern Hybridizations

Total RNA was extracted with lithium chloride/urea from confluent cells [9]. Poly(A)$^+$ RNA was prepared using oligo(dT)-cellulose [10]. RNA preparations were electrophoresed on 1.5% agarose slab gels under denaturing conditions [11]. RNA was transferred to nylon filters (Hybond-N, Amersham, Oakville, Ontario, Canada) essentially as described by Thomas [12] for nitrocellulose filters. The filters were prehybridized for 16 hs at 42°C in a buffer containing 50% formamide, 5× Denhardt's solution (0.02% albumin, 0.02% Ficoll, 0.2% polyvinylpyrrolidone) and 250 μg/ml sonicated calf thymus DNA. The filters were then hybridized with ^{32}P-labeled pA34 (average specific activity 10^8 cpm/μg), a cDNA plasmid specific for the C3 component of PSBP [13] in a fresh quantity of the prehybridization buffer for 24 h at

42°C. The filters were then washed four times with 2× SSC, 0.1% SDS at room temperature for 5 min followed by two washes of 0.1× SSC, 0.1% SDS at 50°C for 20 min (20× SSC: 3M NaCl, 0.3 M sodium citrate, pH 7.0). Other cloned probes used were p21-04, coding for TRPM-2, a tissue regression and programmed cell death-associated protein [6] and the pRDE series of RDE specific messages. In situ cell blot hybridizations were performed on Hybond-N nylon membrane essentially as described by Yu and Gorovsky [14] for glass filters.

Cloning and Sequencing

Double stranded cDNA was prepared from poly(A)$^+$ RNA extracted from RDE cells using a modification [15] of the method of Gubler and Hoffmann [16]. Plasmids containing inserts of interest were initially identified by differential Grunstein-Hogness hybridization [17] using [^{32}P]-labeled cDNA reverse transcribed from poly(A)$^+$ RNA of RVP and RDE cells. Clones demonstrating greater signal strength with RDE cDNA were subjected to more rigorous screening by Northern hybridization. The inserts of clones determined to be specific for RDE cells were subcloned into the Ml3 mpl0 and Ml3 mpll vectors [18] to permit dideoxy-sequencing [19] of both strands of the inserted DNA. Computerized sequence analysis for restriction sites and comparison with the GenBank database was performed using Microgenie™ computer software (Beckman, Mississauga, Ontario, Canada) [20]).

RESULTS

Figure 1 is a Northern blot of poly(A)$^+$ RNA isolated from rat ventral prostate and the RDE cell lines and probed with ^{32}P-labeled pA34 (panel A) and p21-04 (panel B). The clone pA34 is a plasmid specific for the C3 component of the prostate steroid-binding protein (PSBP) [13], while p21-04 contains the TRPM-2 cell regression and programmed cell death sequence [6]. Lanes 1–4 show the hybridization of the probes to poly(A)$^+$ RNA from the ventral prostate of intact rats (lane 1), from rats castrated 4 (lane 2) and 11 (lane 3) days prior to RNA isolation, and poly(A)$^-$ RNA from intact rats (lane 4). By comparison to these controls, it is clear that the RDE cell lines 11.1, 11.4, 11.5, and 11.12 (lanes 5–8), grown to confluence in the presence of testosterone, do not express the C3 PSBP gene. The androgen-repressed TRPM-2 sequence is absent in the intact rat epithelial cells and only appears after castration as shown by the hybridization in panel B, lane 2. TRPM-2 sequences are not detectable in the RDE cells either in presence of androgens (lanes 5–8) or in the absence of androgen (not shown).

Northern hybridization of three clones isolated from the RDE library demonstrated RDE cell specificity (Fig. 2) relative to isolated RVP epithelial and fibroblast cells. These clones have been named to reflect their RDE cell specificity and mRNA size: pRDE-.25 codes for a 250-bp sequence, pRDE-1.5 hybridizes to a 1.5-kbp sequence, while pRDE-2.3 contains a portion of a 2.3-kbp message.

The presence of PSBP and pRDE-.25 expression in the different cell types within the prostate was analyzed by the highly sensitive cell blot methodology using gradient-separated RVP cells, RDE cells, and 2-week cultures of normal androgen-dependent primary cultures of RVP epithelial cells. As shown in Figure 3, the gradient effectively separates RVP fibroblast from epithelial cells as determined both morphologically (panel B) and by expression of the PSBP (panel A, series a).

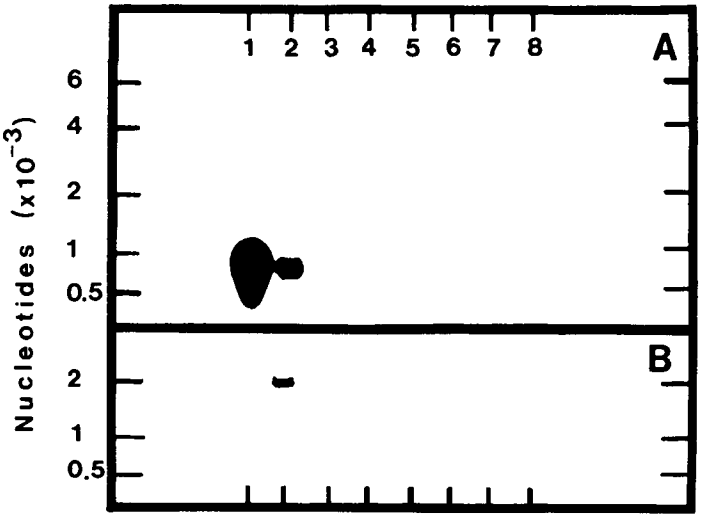


Fig. 1. Northern hybridization analysis of RNA from RDE cell. Poly(A)$^+$ RNA (3 μg/lane) was electrophoresed on denaturing DMSO/glyoxal agarose gels and transferred to nylon filters. The filters were hybridized to ^{32}P-pA34, the cDNA probe specific for prostate steroid-binding protein subunit C3 **(A)** or ^{32}P-p21-04 **(B)**, the probe specific for the TRPM-2 cell regression sequence. **Lanes 1–3:** Poly(A)$^+$ RNA from the prostate of rats castrated 0, 4, 11 days prior to RNA isolation. **Lane 4:** poly(A)$^-$ RNA from the prostate of intact rats. **Lanes 5–8:** poly(A)$^+$ RNA from RDE 11.1, 11.4, 11.5, 11.12, respectively.

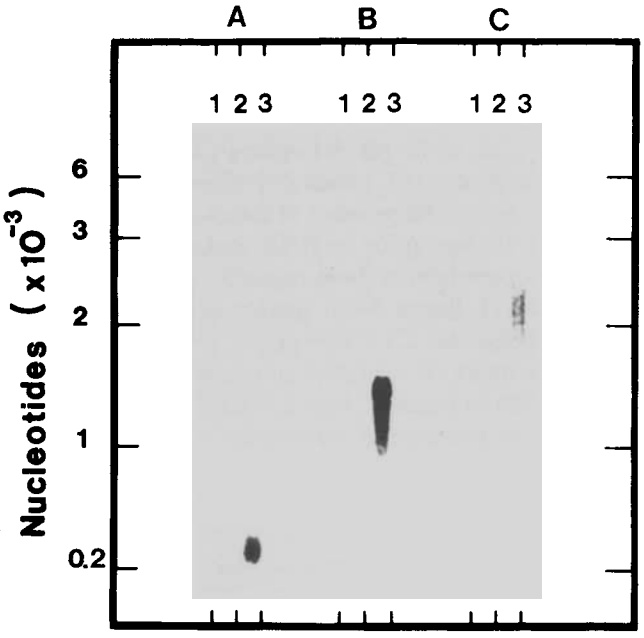


Fig. 2. Northern hybridization of RDE cell-specific cDNA clones. Poly(A)$^+$ RNA (5 μg/lane) was electrophoresed on denaturing DMSO/glyoxal agarose gel and transferred to nylon filters. The filters were hybridized with ^{32}P-labeled cDNA clones suggested by Grunstein-Hogness hybridization to be RDE cell-specific. **Lanes 1:** Poly(A)$^+$ RNA from intact RVP. **Lanes 2:** Poly(A)$^+$ RNA from rats castrated 4 days prior to RNA extraction. **Lanes 3:** poly(A)$^+$ RNA from RDE cells grown in the presence of 34 nM testosterone. **A:** Clone pRDE-.25. **B:** Clone pRDE-1.5. **C:** Clone pRDE-2.3.

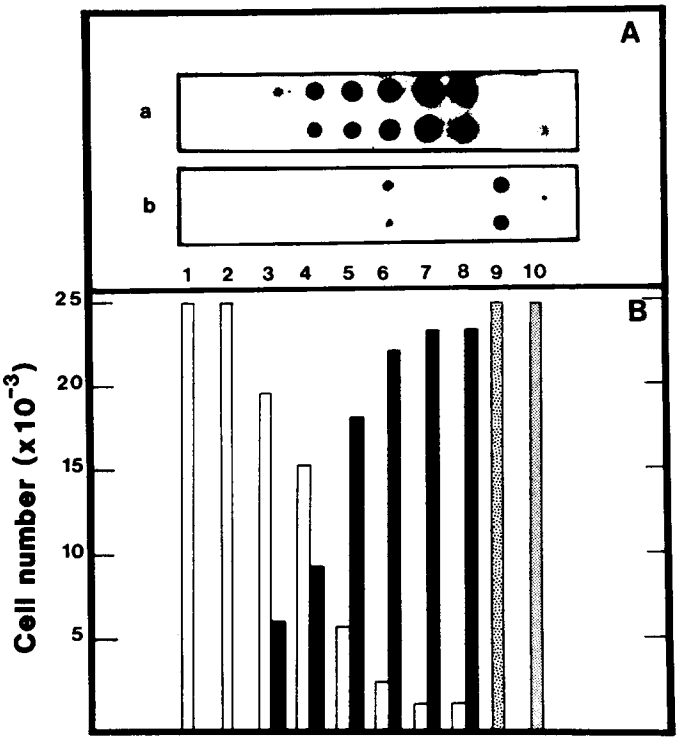
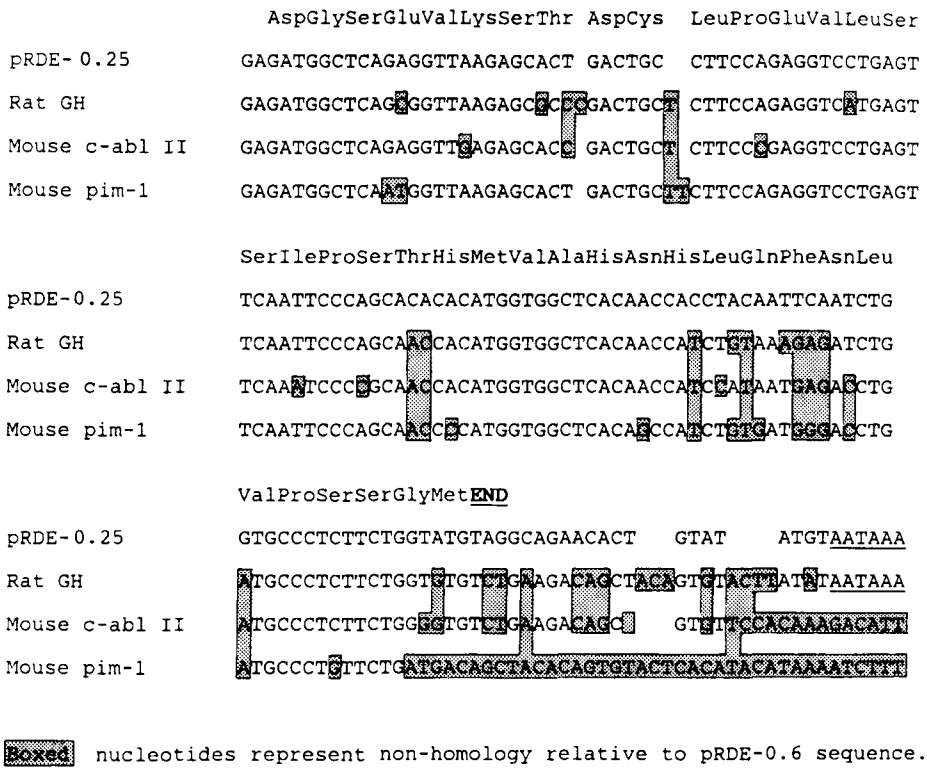


Fig. 3. In situ cell blot hybridization of rat ventral prostate cells and RDE cells. **A:** 25,000 cells from fractions of a Percoll step gradient fractionation of ventral prostate cells were dot blotted onto a poly-L-lysine coated nylon filter. After glutaraldehyde fixation the blots were hybridized to [^{32}P]-plasmid. **Series a:** Plasmid pA34 coding for the C3 component of prostate steroid binding protein. **Series b:** Plasmid pRDE-.25. **Columns 1–8:** Epithelial and fibroblast cells in fractions of the step gradient. **Column 9:** RDE cells. **Column 10:** 2-week primary cultures of androgen-dependent RVP epithelial cells. **B:** Relative proportions of epithelial and fibroblast cells in each step gradient fraction: dots 1–8. **Columns 1–8:** Epithelial (■) and fibroblast (□) cells in fractions of the step gradient. **Column 9:** RDE cells (□) grown to confluence in the presence of 34 nM testosterone. **Column 10:** 2-week primary cultures of androgen-dependent RVP epithelial cells (▩) grown in the presence of 34 nM testosterone. Cell numbers were determined morphologically in each step gradient.

Fibroblasts (columns 1, 2) and RDE cells (column 9) grown in testosterone supplemented medium (34 nM) do not express detectable levels of PSBP expression. Panel A, series b demonstrates that a subset of the epithelial cells from the normal prostate express the RDE-.25 sequence (column 6), at a level of approximately 1% that found for RDE cells (column 9). The remaining fractions of purified epithelial cells (columns 4, 5, 7, 8) do not express this sequence.

The sequence of the 3′ end of the pRDE-.25 cDNA (coding strand) contains a single extended open reading frame (ORF) (Fig. 4). pRDE-.25 has greater than 80% DNA sequence homology to rat growth hormone (GH) gene [21], the mouse pim-1 [22] and c-abl II [23] sequences. The sequence homology to the rat GH is with the message strand of DNA, the location of which is within each of two tandemly repeated sequences in the second intron of the gene [21]. The misalignments of pRDE-.25 relative to the rat GH intron sequences allow the maintenance of the pRDE-.25 ORF until the termination codon 17 base pairs upstream of the polyadenylation signal



Boxed nucleotides represent non-homology relative to pRDE-0.6 sequence.

Fig. 4. DNA sequence of pRDE-.25 and homologous rodent sequences. The pRDE-.25 insert was subcloned into Ml3 mp10 and Ml3 mp11 and sequenced by [^{32}P]-dATP-labeled dideoxynucleotide chain termination using a 17-base primer sequence. The samples were electrophoresed in 8% polyacrylamide gels at 2,500 V, 40 mA, and 50 W. The gels were fixed with 5% methanol, 5% acetic acid, then dried and autoradiographed for 18 h at room temperature.

A-A-T-A-A-A. Homology between pRDE-.25 and both pim-1 and c-abl II is with the complementary sequence of the proto-oncogenes and has no appropriate ORF within the portion of the pRDE sequence shown. The homology with pim-1 is to a portion of the genomic DNA beyond the 3′ end of the pim-1 mRNA [22]. The 3′ end of the pRDE-.25 sequence bears strong resemblance to a central portion of the 5′ exon of c-abl II [23].

DISCUSSION

The RDE cells described in this report differ from the androgen-independent RVP epithelial cells described by McKeehan et al. [24,25] in a number of characteristics including growth rates, growth in soft agar, the lack of requirement for cholera toxin or epidermal growth factor, and the formation of three-dimensional structures. RDE cells do not express the markers of androgen-dependent secretory activity, secretory acid phosphatase [5], and prostate steroid-binding protein, nor do they express the TRPM-2 tissue regression sequence in the presence or absence of androgens. TRPM-2 expression in the androgen-dependent population of RVP epithelial cells is thought to be responsible for the androgen-dependent survival of these cells.

Thus the absence of TRPM-2 expression in RDE cells, either in the presence or absence of testosterone, would seem to account for the androgen independence of the RDE cells. The RDE-specific sequences which have been isolated are also present in a subfraction of the freshly dissociated epithelial cells isolated from Percoll gradients, demonstrating that RDE cells have a normal counterpart in the rat ventral prostate. The low level of pRDE-.25 expression in a single fraction of the RVP cell gradient further suggests that the pRDE RNA sequence is expressed at normal levels in a small number of specific RDE cells rather that at low levels in a large number of normal epithelial or other cell type.

The portion of the pRDE-.25 cDNA clone sequenced to date shows no homology to prostatropin, a prostate epithelial growth factor [26]. It does, however, bear a high degree of DNA sequence homology to rat growth hormone (GH) and the murine proto-oncogenes pim-1 and c-abl II. This homology is of potential interest by virtue of the rapid division of RDE cells and the roles played by the homologous sequences in cell replication. The single open reading frame of pRDE-.25 is homologous to sequences within the second intron of the rat GH gene, and to noncoding regions of pim-1. In contrast to this, the homology to c-abl II is within the 5′ exon, but the homology of the coding strand of pRDE-.25 is with the opposite strand and orientation of the oncogene sequence. Sequence analysis suggests that the pRDE-.25 mRNA may represent another example of an abnormally spliced growth hormone mRNA [27], whose expression provides an autocrine stimulus to the epithelial cells in the absence of the modulating influence of the prostatic stroma. The nonexpression of TRPM-2 coupled to the expression of a growth hormone-like mRNA in these cells provides a useful model for the androgen-independent growth of the prostate that occurs after androgen ablation.

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