Identification and Characterization of an Androgen-Responsive Gene Encoding an Aci-Reductone Dioxygenase-Like Protein in the Rat Prostate

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The ALP1 [aci-reductone dioxygenase (ARD)-like protein 1] gene was identified in a comprehensive cDNA subtraction aimed at identifying genes regulated by androgens in the rat ventral prostate. ALP1 is homologous to the ARD/ARD' that were discovered in Klebsiella pneumoniae as enzymes that have the same polypeptide sequence and differ only in their metal content. This family of proteins is evolutionarily conserved from bacteria to humans and is involved in the methionine salvage pathway. Northern and Western blot confirmed the regulation of ALP1 by androgens in the rat ventral prostate. ALP1 mRNA is expressed in a variety of tissues; however, its regulation by androgens was specific to the prostate. ALP1 is expressed by the glandular epithelial cells of the rat prostate, with little or no expression in the stromal cells. ALP1 is down-regulated in the different rat Dunning tumor cell lines compared with the normal or castrated rat prostate. Expression studies showed that ALP1 overexpression is not tolerated by AT6.1 cells. Further studies demonstrated that ALP1 is also down-regulated in the human prostate cancer cell lines LNCaP, PC3, and DU145, and overexpression induces cell death in these cells. Taken together, our observations suggest that ALP1 may have an important role in androgen regulated prostate homeostasis as well as in prostate cancer progression by regulating cell death of prostate cancer cells. (Endocrinology 145: 1933–1942, 2004)

It has long been established that androgens play a critical role in regulating the growth, differentiation, and survival of epithelial cells in the prostate (1, 2). Androgens exert their effect on prostate cells through the androgen receptor (AR), a member of the superfamily of nuclear receptors (3–5). When complexed to testosterone or dihydrotestosterone, the AR induces the expression of various androgen response genes (6, 7) that are thought to control the growth, differentiation, and survival of prostate epithelial cells. In the rat, removal of androgens via castration results in a dramatic regression of the prostate due to massive apoptosis of the glandular epithelial cells (8–11). Administration of exogenous androgens then causes a rapid regrowth of the regressed prostate until it reaches near normal size, mainly due to the increased proliferation of the glandular epithelial cells. Whereas androgens stimulate DNA synthesis and cell proliferation in the castrated rat prostate, they do not have the same effect on a testis-intact rat prostate (12, 13). When the prostate has a normal number of cells, androgens stimulate secretions from the prostate but no longer activate DNA synthesis and/or cell proliferation (14). Thus, by regulating androgen response genes, androgens maintain the appropriate cell number in the rat prostate by balancing the rate of cell proliferation and cell death (15).

Androgens also have an important role in the two major diseases of the prostate, benign prostate hyperplasia (BPH), and prostate cancer. BPH is a major cause of decreased quality of life in older men, with symptoms occurring in 70–80% of the elderly population and surgical treatment needed in 25–30% of men who live to be 80 yr of age (16). Prostate cancer has become the most frequently diagnosed neoplasm and the second leading cause of cancer-related death in American men (17, 18). The current treatment for advanced stage, inoperable prostate cancer remains androgen-ablation therapy. However, this therapy eventually fails, leaving patients with an androgen-independent tumor for which there is no effective therapy (19, 20). The exact role of androgens in BPH and prostate cancer is still unclear. To better understand the mechanisms of androgen action in the prostate, we have identified 25 genes that were up-regulated by androgens and four that were down-regulated (21). One of the up-regulated genes, U-23 (up-regulated gene no. 23), encodes a protein of 179 amino acid residues with homology to aci-reductone dioxygenase (ARD). Thus, we rename U-23 ARD-like protein 1 (ALP1).

ARD and ARD' were discovered in Klebsiella pneumoniae as enzymes that have the same polypeptide sequence and differ only in their metal content, Ni2+ and Fe2+, respectively (22, 23). The two enzymes share the same substrate, 1,2-dihydroxy-3-keto-5-(methylthio)pentene, but yield different products. ARD' yields the α-keto precursor of methionine, thus forming part of the ubiquitous methionine salvage pathway that recycles methionine from 5'-methylthioadenosine.
(MTA) (24–26). This pathway is responsible for the tight control of the concentration of MTA, a powerful inhibitor of polyamine biosynthesis and transmethylation reactions. Polyamines are required for both cell growth and proliferation. ARD yields methylthiopropionate, carbon monoxide (CO), and formate preventing the recycling of MTA to methionine (22). The role of this pathway is unclear; methylthiopropionate is cytotoxic and has been implicated in the pathogenicity in plants (22). CO is a toxic waste product that has recently been shown to increase intracellular levels of cyclic GMP by activating guanylyl cyclase (27, 28).

This paper describes the characterization of ALP1 in the rat prostate and the prostate cancer cell lines LNCaP, DU145, PC3, and AT6.1. Northern blot and Western blot techniques demonstrate the regulation of ALP1, whereas overexpression studies have allowed for the determination of ALP1’s cellular localization and possible function. Our studies of the ALP1 gene product in the rat may represent the first analysis of a mammalian ARD enzyme and suggest the importance of the ALP1 gene in androgen action and prostate cancer.

Materials and Methods

Animals

Animal care and procedures are as described previously (21). Briefly, young adult male rats (Sprague Dawley, 250–300 g) purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN) were housed and maintained by Northwestern University Animal Facility. Castration and killing of the rats were performed in a room dedicated to animal surgery according to a protocol approved by the Northwestern University Animal Care and Use Committee (Chicago, IL). Castrations consisted of removal of the testis, fat pad, and epididymis. Androgen replacement was carried out by daily sc injections of testosterone propionate dissolved in propylene glycol (Fisher Scientific, Fair Lawn, NJ) at 2 mg/rat for up to 7 d. At various times after castration or androgen replacement, at least three rats were killed and their ventral prostate lobes removed, weighed, and frozen in liquid nitrogen. Other tissues isolated from the same animals include dorsal prostate, lateral prostate, seminal vesicles, liver, heart, kidney, brain, and muscle.

Cloning and (sequencing of full-length cDNA of rat) ALP1 (rALP1)

A full-length cDNA clone encoding for rALP1 was isolated from a λZAP phage cDNA library prepared using normal rat ventral prostate mRNA (21). In vivo excision of the λZAP phage yielded a plasmid pBluescript II SK with the rALP1 cDNA inserted at the EcoRI and XhoI sites, between the T3 and T7 promoters. Nested deletion mutants were generated using the erase-a-base kit (Promega, Madison, WI) and then sequenced using the Alpha Express automated sequencing machine (Amersham-Pharmacia, Piscataway, NJ). Both strands of the cDNA were sequenced completely. Sequence assembly and analysis were carried out using the DNASIS program (Hitachi Software, San Francisco, CA).

Cell culture

Dunning prostate cancer cell lines G, AT1, AT2, AT3.1, AT6.1, and MAT-LyLu (29) were generously provided by Dr. Allen Gao. These cells were cultured in RPMI 1640 with 10% fetal bovine serum, 1% glutamine, and 1% penicillin-streptomycin. 250 μg dexamethasone at 37 C in a humidified atmosphere containing 5% CO2.

PC3, DU145, and LNCaP human prostate cancer cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA). These cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% glutamine, and 1% penicillin-streptomycin at 37 C in a humidified atmosphere containing 5% CO2.

In situ hybridization

Sense and antisense digoxigenin (DIG)-labeled RNA probes were used for in situ hybridization. A full-length rALP1 cDNA inserted at the multiple cloning site of the pBluescript II SK plasmid was used in template preparation. Linearized, proteinase K-treated plasmid DNA templates were prepared and synthesis of the sense and antisense RNA probes was carried out by in vitro transcription using a DIG RNA labeling mix of nucleotides (Roche Molecular Biochemicals, Indianapolis, IN) with either T3 or T7 RNA polymerase (Promega, Madison, WI). In situ hybridization was carried out according to the method of Furlow et al. (31) with small modifications described in Cysric et al. (32).

Protein isolation and Western blot

Polyclonal antibodies were generated against two different peptides derived from the amino acid sequence of human ALP1, MDDAPGD-PRQPHRPDPGRPC, and KLDADKYENDELEKIRREC (Proteintech Group, Inc., Chicago, IL). These antibodies were then purified using a HIS-tagged human ALP1 fusion protein column. Proteins from mouse, rat, and humans were used to ensure specificity of the antibodies against the different species of ALP1 proteins.

Prostate extracts were prepared by homogenizing the prostate or cultured cells in a lysis buffer consisting of 1X PBS, 1% SDS, 10 mm EDTA, 100 μm phenylmethylsulfonyl fluoride, 10 μm leupeptin, 0.2 mm 4-(2-aminoethyl) benzaminesulfonyl fluoride, and 1 μm pepstatin. Insoluble materials were pelleted by centrifugation at 10,000 × g for 10 min at 4 C. Protein concentration was determined using a Bic-Rad (Hercules, CA) DC protein assay kit. Western blot analysis was conducted as previously described (33) using an anti-ALP1 polyclonal antibody. The secondary antibody used for detection was linked with horseradish peroxidase. The enhanced chemiluminescence (ECL) method was used to detect the conjugated horseradish peroxidase. A MAPK antibody (Zymed, San Francisco, CA) was used as a control for equal protein loading.

Transient transfection and cell death assay

The coding region for ALP1 was cloned into the pEGFP-C1 vector and pEGFP-C2 vector (CLONTECH, Palo Alto, CA) at the EcoRI and KpnI sites within the MCS (multiple cloning sites), creating green fluorescent protein (GFP) fusion proteins at the N terminus or C terminus. The construct was confirmed to be correct by restriction digestion and sequence verification, to ensure of no mutations and that ALP1 was in the correct reading frame. The plasmid DNAs for transient transfection were prepared using double-CaCl2 gradient banding.

Transient transfection for cell death genes has been described in Miura and Yuan (34). The pEGFP-C1, pEGFP-C1 + s100RV, or pEGFP-
C1+ ALP1 vectors were transfected into LNCaP, PC3, DU145, and AT6.1 cells via FuGene transfection reagent (Roche, Basel, Switzerland) as described by the manufacturer’s protocol. The transfections were carried out with a 1:1 ratio (FuGene: μg DNA), with 1 μg of DNA per well. Transfections were performed on cells grown on coverslips in a six-well plate (intracellular localization) or simply in a six-well plate (for quantitative analysis). The coverslips were then washed in PBS, fixed in 3.7% formaldehyde solution for 10 min, washed three times in PBS, transferred, and sealed to microscope slides using Vectashield (Vector Laboratories, Burlingame, CA). The slides were viewed and pictures taken on a Laser Scanning Confocal Microscope (LSM510, Zeiss, Jena, Germany) at the Cell Imaging Facility of Northwestern University between 24 and 72 h post transfection. Quantitative analysis was performed in six-well plates and viewed 48 and 72 h after transfection using a fluorescence microscope (Leica Corp., Wetzlar, Germany). At least 200 transfected cells in each transfection were counted. The cells that were detached, exhibited membrane blebbing, or fragmented nuclei were considered dead. The results were based on three independent experiments.

Cell death was also studied morphologically by staining the nuclei with Hoechst 33342 (Molecular Probes, Eugene, OR). Cultured cells were stained 48 or 72 h after transfection with 10 μm Hoechst 33342 for 10 min and then analyzed under a fluorescence microscope (Leica Corp.).

Results
Cloning and sequence analysis of the rALP1 cDNA
A small cDNA fragment corresponding to gene U-23 (ALP1), discovered previously (21), was used to isolate a 1.4-kb full-length cDNA clone from a rat ZAP-cDNA library. The cDNA sequence of the rALP1 gene was submitted to GenBank (accession no. AY346335). This cDNA contains the open reading frame for a 179-amino acid polypeptide (Fig. 1). The cDNA contains a short 5’-untranslated region and a 3’-untranslated region of 783 bp. A polyadenylation signal (AATAAA) and a poly (A)-tail are located at the end of the sequence. Sequence analysis of the ALP1 amino acid residues using PROSITE indicates that there are three potential casein kinase II (CK2) phosphorylation sites (Ser10, Ser62, and Thr136) and two potential tyrosine kinase (TK) phosphorylation sites (Tyr104 and Tyr142) (Fig. 1). The three histidines and one glutamic acid involved in the Ni2+ and Fe2+ binding sites are in boldface and underlined (Fig. 2) (35). These four amino acids are conserved between all members of the ARD family. The rALP1 protein shows high homology to a variety of hypothetical proteins from different organisms (Fig. 2), including human (FLJ10913), mouse (AL024210), Oryzta sativa (AC105729), Drosophila melanogaster (CG32068-P1), Saccharomyces cerevisiae (YMR009w), Schizosaccharomyces pombe (SPBC887.01), and Caenorhabditis elegans (F24F12.4) (Fig. 2). NCBI conserved domain search for ALP1 protein produced two significant alignments: ARD/ARD’ family (Fig. 2) and double-stranded β helix domain involved in carbohydrate binding and protein-protein interactions (data not shown).

ALP1 mRNA is regulated by androgens in the rat ventral prostate
The ALP1 gene was identified as one of the genes up-regulated by androgen replacement in a 7-d castrated rat ventral prostate (21). Figure 3 shows the time course of the androgen induction of ALP1 mRNA in the ventral prostate of a 7-d castrated rat. The mRNA induction occurs at 6.5 h after androgen replacement and reaches its maximum expression between 36 and 48 h.

![Fig. 1. cDNA and amino acid sequences of rALP1 (GenBank accession no. AY346335). Start codon (ATG) and stop codon (TAG) are both in boldface, as is the initial methionine and STOP. Ser10, Ser62, and Thr136 are all in boldface, indicating the potential CK2 phosphorylation sites. Tyr104 and Tyr142 are both in boldface and underlined indicating the potential TK phosphorylation sites.](https://academic.oup.com/endo/article-abstract/145/4/1933/2878722/fig1?size=large)
The rat prostate consists of ventral, lateral, and dorsal lobes, all of which are responsive to androgens. ALP1 mRNA is also regulated by androgens in the dorsal and lateral lobes as demonstrated by the down-regulation of ALP1 mRNA in the castrated rat compared with the normal (testis intact) rat (see Fig. 7). Up-regulation of ALP1 mRNA by androgen replacement in the dorsal and lateral lobes has also been demonstrated (data not shown). The ALP1 expression level in the ventral prostate is slightly higher than that in the dorsal and lateral prostate (data not shown).

**ALP1 protein is regulated by androgens in the rat ventral prostate**

Antibodies generated against human ALP, which also recognize rALP1, were used to determine whether ALP1 protein was also regulated by androgens in the rat ventral prostate. Figure 4A shows a short and long time course Western blot of the androgen induction of ALP1 protein in the rat ventral prostate using an anti-ALP1 polyclonal antibody. A, ALP1 protein induction in a 7-d castrated rat ventral prostate treated with androgens for the indicated times. B, The down-regulation of ALP1 protein after castration in the rat ventral prostate. The protein samples are from a normal rat (0) and rats castrated for the indicated number of days. ALP1 protein is indicated by the arrow at around 20 kDa, which correlates with the predicted value. MAPK expression level is seen in the bottom panels, MAPK is used as the control for equal protein loading.
the ventral prostate of a 7-d castrated rat. The short time course is from 2–24 h after addition of androgens. The long time course is from 14 h to 7 d post androgen replacement. Protein induction is detectable at 24 h after addition of androgens in the short time course. However, higher levels of the ALP1 protein are not detected until 48 h. The level of ALP1 protein continuously increases after 48 h until it reaches its maximum expression at around 3 d, at which point the prostate maintains this level of expression. ALP1 protein is also down-regulated by androgen ablation (castration) as seen in Fig. 4B. The level of protein begins decreasing 1 d after castration and is barely detectable at d 7. This result demonstrates that androgens are required and essential for ALP1 protein expression in the rat ventral prostate.

**ALP1 mRNA is expressed in a variety of rat tissues, but only regulated by androgens in the prostate**

The tissue specificity of ALP1 mRNA was studied using Northern blot analysis on different rat tissues during androgen manipulation (Fig. 5). ALP1 mRNA is expressed in all of the rat tissues surveyed, with the prostate having the most abundant expression. Also noted is that ALP1 regulation by androgens is only observed in the prostate tissue. The above observation indicates that although ALP1 is expressed in a

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**Fig. 5.** Northern blot analysis of the tissue specificity of ALP1 expression. N, indicates tissue from the testis-intact rat; C, indicates tissue from 7-d castrated rat; +, indicates tissue from the 7-d castrated rat plus 2-d androgen replacement. The ALP1 mRNA is indicated by an arrow. Total RNA quality and quantity were determined by staining the transferred nylon membrane with methylene blue (37).

**Fig. 6.** In situ hybridization analysis of ALP1 mRNA in the testis-intact (A) and 7-d castrated (B) rat ventral prostate. Both antisense (right panel) and sense (left panel) RNA probes were labeled with DIG and visualized with alkaline phosphatase conjugated anti-DIG antibody. Epithelial (E) and stromal (S) cells are indicated by arrows. The micrographs were taken with a ×40 objective.
variety of cell types, its regulation by androgens is prostate specific.

**ALP1 mRNA is localized to prostate epithelial cells**

The rat prostate consists mainly of epithelial cells (secretory cells that line the prostatic ducts) and stromal cells (which underlie the epithelial cells and provide structural support). To identify which cells of the prostate express ALP1 mRNA, we performed *in situ* hybridization experiments on normal rat ventral prostate sections (Fig. 6A). Results demonstrated that ALP1 mRNA is abundantly expressed in the epithelial cells with little or no expression in the stromal cells.

We also performed *in situ* hybridization experiments on a 7-d castrated rat ventral prostate (Fig. 6B). As expected, no expression of ALP1 was observed in the epithelial cells. This correlates with our Northern blot analysis, which displayed little or no signal for ALP1 in the 7-d castrated rat ventral prostate.

**ALP1 mRNA is down-regulated in the rat Dunning tumor cell lines**

Androgen action is intimately associated with prostate cancer pathogenesis. Because ALP1 appears to be an important androgen response gene, we investigated the expression of ALP1 mRNA in the Dunning rat prostate cancer cell lines: G, AT1, AT2, AT3.1, AT6.1, and MAT-LyLu (29). ALP1 mRNA was dramatically lowered in all cancer cell lines compared with the rat dorsal prostate from which these cancer cell lines were derived (Fig. 7A). ALP1 mRNA level in the rat Dunning prostate cancer cells was even lower than levels of ALP1 mRNA in the 7-d castrated rat dorsal and lateral prostates (Fig. 7A). Quantification of the mRNA signal for ALP1 is shown in Fig. 7B, values are shown in percentage of the dorsal prostate level.

**ALP1 is down-regulated in human prostate cancer cell lines compared with BPH**

To determine whether ALP1 was also down-regulated in the human prostate cancer cell lines LNCaP, PC3, and DU145, we performed Northern blot analysis on these cell lines along with BPH samples. Figure 8 demonstrates that ALP1 mRNA is down-regulated in all three of the cell lines in comparison to the BPH samples. LNCaP is an androgen-responsive cell line that has a functional, but mutated, AR. PC3 and DU145 are androgen insensitive, do not express the AR, and are much more aggressive than LNCaP cells. LNCaP cells have higher levels of ALP1 than do the androgen-insensitive cell lines PC3 and DU145.

**Overexpression of ALP1 induces cell death in prostate cancer cells**

To investigate ALP1 function, we transfected ALP1 expression vectors into the rat Dunning prostate cancer cell line AT6.1. At first we tried to select for stable expressants; however, stable transfection of ALP1 was never successful. Transient transfection experiments demonstrated that cells expressing GFP-ALP1 fusion proteins were unable to survive.

The transfected cells exhibited condensed nuclei and membrane blebbing, suggesting that rALP1 overexpression induced apoptosis. We performed Hoechst staining to provide further evidence that apoptosis was induced by rALP1 overexpression, and as expected, this process revealed the condensed and fragmented nuclei in the GFP-ALP1 transfected
cells (Fig. 9a). As a control, we used a GFP-S100RVP fusion protein to demonstrate that GFP fusion proteins do not cause cell death. S100RVP is a novel S100 calcium binding protein that is studied in our lab. Quantitative analysis (Fig. 9b) showed that cell death induced by GFP-ALP1 fusion proteins in AT6.1 cells was very efficient, with 88% ± 0.91% of the GFP-ALP1 transfected cells dead by 3 d after transfection. To determine whether GFP had any effect on ALP1 function we placed GFP at the C terminus of ALP1, where it proved equally efficient (88% ± 1.25%) at inducing cell death as GFP-ALP1 (Fig. 9b). In contrast, only 13% ± 0.89% of the GFP expression vector transfected AT6.1 cells showed apoptotic morphology and stained positive with Hoechst dye.

Transient transfections were used to determine whether ALP1 overexpression also induces cell death in the human prostate cancer cells PC3, DU145, and LNCaP. Overexpression of ALP1 in the human cell lines demonstrated a phenotype similar to that seen in the rat Dunning AT6.1 cells. Cells expressing GFP-ALP1 showed condensed nuclei and membrane blebbing and also demonstrated fragmented nuclei with Hoescht staining (Fig. 10a). Quantitative analysis demonstrated that 80% ± 0.79% of GFP-ALP1 transfected LNCaP cells were dead at 3 d post transfection (Fig. 10b), whereas 87% ± 1.19% of the GFP-ALP1 transfected PC3 and 90% ± 1.53% DU145 cells were dead by the same point (Fig. 10b). Again, ALP1-GFP showed efficiency at inducing cell death similar to GFP-ALP1 (LNCaP 79% ± 1.51%, PC3 94% ± 2.78%, and DU145 92% ± 1.21%), whereas GFP alone had little affect on the viability of the human prostate cancer cell lines (LNCaP 11% ± 1.75%, PC3 12% ± 1.89%, and DU145 8% ± 0.16%) (Fig. 10b). Interestingly, LNCaP cells exhibited a lower percentage of cell death relative to PC3 or DU145 cells in response to ALP1 overexpression, and the difference between LNCaP and DU145/PC3 is statistically significant (P < 0.01). These results confirm that human prostate cancer cell lines are also sensitive to ALP1 overexpression, and it appears that DU145 and PC3, which are more aggressive and express lower levels of endogenous ALP1, are more sensitive to ALP1 overexpression compared with LNCaP cells.

Discussion

This study identifies and characterizes an ARD-like protein in the rat prostate, which we term ALP1. The ARD/ARD' family of proteins is highly conserved with members in unicellular organisms (bacteria and yeast) and also in complex mammals (humans, rats, and mice) (Fig. 2). The ARD and ARD’ proteins were first identified in Klebsiella pneumoniae as enzymes that have the same polypeptide sequence and differ only in their metal content, Ni²⁺ and Fe²⁺, respectively (22, 23). The two enzymes share the same substrate, 1,2-dihydroxy-3-keto-5-(methylthio)pentene, but yield different products. rALP1 shows high homology to the ARD domain and contains the three histidines and one glu-
tamic acid implicated in Ni\(^{2+}\) and Fe\(^{2+}\) binding, as do all other members of the ARD family (Fig. 2) (35). Based on sequence homology, it is likely that ALP1 has ARD and/or ARD\(^{\prime}\) enzymatic activity in mammalian cells.

ARD and ARD\(^{\prime}\) are two proteins encoded by the same gene and differ only by their metal content. These proteins are highly conserved evolutionarily and are involved in two distinct pathways: a growth pathway promoting methionine salvage and polyamine synthesis (ARD\(^{\prime}\)), and a cytotoxic pathway whose function is unknown (ARD). Assuming that ALP1 functions as an ARD/ARD\(^{\prime}\) enzyme in the prostate could help explain how ALP1 is abundantly expressed in the normal rat prostate and also regulated by androgens. ALP1 could act like an ARD\(^{\prime}\) enzyme in this setting, promoting cell growth and polyamine synthesis (which occurs shortly after the induction of ALP1 mRNA and protein). However, in the cancer cell lines ALP1 (overexpression) could function like an ARD enzyme, producing a cytotoxic byproduct that induces cell death. Further studies will be needed to further characterize ALP1’s function in the normal and cancerous prostate cells and to determine whether ALP1 functions as an ARD/ARD\(^{\prime}\) enzyme in the prostate epithelial cells.

ALP1 may also play a role in different signaling pathways. Potential phosphorylation sites are found in the primary amino acid sequence for TK and also for CK2 (Fig. 1). ALP1 could potentially have posttranslational modifications that have a role in its activational state, either activating or inhibiting its enzymatic activity.

ALP1 was discovered in a comprehensive cDNA subtraction aimed at discovering androgen response genes in the rat ventral prostate. Here we demonstrate that ALP1 mRNA is androgen responsive by both castration and androgen replacement (Figs. 3 and 5). ALP1 mRNA expression is observed at 6.5 h after androgen replacement in the rat ventral prostate, classifying it as an early response gene. Early response gene induction occurred within 14 h after androgen replacement (21). Most genes discovered fell into the early response category and these are thought to be direct androgen response genes. ALP1 is responsive to androgens in all three lobes of the rat prostate: dorsal, lateral and ventral (Figs. 3 and 7). ALP1 is expressed in a variety of tissues in the rat; however, its expression is regulated by androgens only in the prostate (Fig. 5). ALP1 expression is also much higher in the prostate than the other tissues surveyed. Given these factors, we believe ALP1 plays a role in the androgen-regulated growth, differentiation, and/or survival of the rat prostate.

ALP1 protein is abundantly expressed in the normal and androgen-replaced rat ventral prostate, which correlates with the high mRNA expression observed in the same tissue.
As expected, ALP1 protein expression is also regulated by androgens in the rat ventral prostate (Fig. 4). ALP1 protein induction is detectable at 24 h after androgen replacement and the dramatic induction of ALP1 expression occurs between 2 and 3 d after androgen induction. The long lag between mRNA induction and protein induction may reflect the time required for prostatic epithelial cells to gain the ability to efficiently synthesize the ALP1 protein. This possibility is consistent with the observation that genes involved in protein synthesis are induced by androgens during the regrowth of the rat ventral prostate (36).

ALP1 mRNA is mainly localized to the prostate epithelial cells, with little or no expression in the stromal cells (Fig. 6A). The prostate epithelial cells are the most affected by androgen manipulation of the rat prostate, these are the cells that undergo massive apoptosis after castration and rapidly proliferate after androgen replacement. Androgen replacement also induces differentiation in these cells. The above information suggests that ALP1 plays a role in the androgen-regulated growth, differentiation, and/or survival of prostate epithelial cells.

To ensure that increases in ALP1 are not due to increases in epithelial cell numbers in the normal and androgen-replaced ventral prostate compared with the castrated prostate, in situ hybridization experiments were conducted on a 7-d castrated rat ventral prostate (Fig. 6B). As expected, no ALP1 is detected in the epithelial cells, which correlates with Northern blot analysis of the same tissue.

Prostate cancer is derived from the glandular epithelial cells, which express ALP1. To investigate ALP1’s role in prostate cancer cells, we determined the level of ALP1 mRNA expression in the rat Dunning tumor cell lines. Figure 7 shows that ALP1 expression is dramatically decreased in the Dunning tumor cells compared with the dorsal or lateral prostate, suggesting that ALP1 expression is not compatible with the cultured prostate cancer cells. Levels of ALP1 mRNA in the Dunning tumor cell lines were even lower than the levels in the castrated dorsal and lateral prostates.

ALP1 mRNA is also down-regulated in the human prostate cancer cell lines LNCaP, PC3 and DU145 compared with BPH tissue (Fig. 8). LNCaP cells express higher levels of ALP1 than do the PC3 and DU145 cells. Lower levels of ALP1 were expected in PC3 and DU145, which do not express the AR.

Transient transfection experiments were used to further identify ALP1’s role in the rat Dunning tumor cell line AT6.1. We first tried to establish stable cell lines that overexpressed a FLAG-tagged ALP1 protein, but after several failed attempts decided to use a GFP-ALP1 fusion protein construct that permits the visualization of the cells expressing the GFP-ALP1 fusion protein. Miura and Yuan (34) describe a variety of transient transfection assays for cell death genes, which we used to study ALP1 overexpression. This study demonstrated that cells expressing the ALP1 protein did not survive. Cells expressing the GFP-ALP1 protein exhibited membrane blebbing and fragmented nuclei at 3 d post-transfection (Fig. 9). Similar results were also seen in the human prostate cancer cell lines LNCaP, PC3, and DU145 (Fig. 10), indicating that ALP1 function is probably conserved between rats and humans. We used GFP-S100RVP (a novel S100 calcium binding protein that has been characterized in our lab; Oram, S., X. Cai, R. Haleem, J. Cyriac, and Z. Wang, manuscript in preparation) to demonstrate that GFP fusion proteins do not cause cell death in the prostate cancer cell lines. The finding that ALP1 overexpression induces cell death in prostate cancer cells was surprising given the high levels of ALP1 expression in the normal and androgen-replaced rat prostate. It is possible that ALP1 function in normal and cancer cells is different.

In summary, we showed that ALP1 is abundantly expressed and regulated by androgens in the prostatic epithelial cells. Overexpression of ALP1 induces dramatic cell death in prostate cancer cells, suggesting that ALP1 is suppressive to prostate tumor growth. Consistent with its tumor-suppressive role, ALP1 expression is down-regulated in all the surveyed prostate cancer cell lines. Our findings suggest that ALP1 plays an important role in androgen-regulated prostate homeostasis and its down-regulation represents an essential step in prostate cancer progression.

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