ART-27, an Androgen Receptor Coactivator Regulated in Prostate Development and Cancer*

Androgen receptor trapped clone-27 (ART-27) is a newly described transcriptional coactivator that binds to the N terminus of the androgen receptor (AR). Given the vital importance of AR signaling in prostate growth and differentiation, we investigated the role of ART-27 in these processes. Immunohistochemical studies indicate that ART-27 protein is expressed in differentiated epithelial cells of adult human prostate and breast tissue. In prostate, ART-27 is abundant in AR-positive prostate luminal epithelial cells, in contrast to the stroma, where cells express AR but not ART-27. The use of a rat model of androgen depletion/reconstitution indicates that ART-27 expression is associated with the elaboration of differentiated prostate epithelial cells. Interestingly, regulated expression of ART-27 in the androgen-sensitive LNCaP prostate cancer cell line inhibits androgen-mediated cellular proliferation while enhancing androgen-mediated transcription of the prostate-specific antigen (PSA) gene. Consistent with a growth suppressive function, we show that ART-27 expression levels are negligible in human prostate cancer. Importantly, examination of ART-27 protein expression in early fetal prostate development demonstrates that ART-27 is detected only when the developing prostate gland has proceeded from a solid mass of undifferentiated cells to a stage in which differentiated luminal epithelial cells are evident. Thus, ART-27 is an AR cofactor shown to be subject to both cell type and developmental regulation in humans. Overall, the results suggest that decreased levels of ART-27 protein in prostate cancer tissue may occur as a result of de-differentiation, and indicate that ART-27 is likely to regulate a subset of AR-responsive genes important to prostate growth suppression and differentiation.

Androgen steroid hormones direct the genetic program dictating prostate development and maturation in male development. They exert biological effects by binding to the androgen receptor (AR),1 a member of the steroid receptor family of transcription factors. Functional mapping of the androgen receptor shows that several regions are required for transcriptional activation (1, 2). These include a C-terminal domain, AF-2, as well as two regions in the N terminus, AF-1a and AF-1b. Recent evidence suggests that the AR cell- and promoter-specific transcriptional response is generated through interactions with regulatory proteins termed coactivators and corepressors with AF-1 and AF-2 (2–12).

Androgen stimulation results in cell proliferation in both the developing prostate and the malignant prostate. Current therapy for metastatic prostate cancer includes injections of luteinizing hormone-releasing hormone (LHRH) analogues to pharmacologically lower testosterone levels (androgen ablation), treatment with anti-androgens such as flutamide or bicalutamide, to block testosterone binding to the AR, or a maximal androgen blockade in which both treatments are combined. While such therapies are effective in the short term, cancers treated in this fashion will inevitably progress in an androgen-independent fashion.

An important determinant of AR transactivation function resides in the N terminus (1, 13, 14). The AR N-terminal residues 142–485 have been shown to activate a minimal promoter construct in a cell-free transcription system and to selectively interact with the transcription factors TFIIF and the TATA-binding protein (TBP), suggesting direct contact with general transcription factors (15, 16). A growing list of proteins has also been reported to interact with the AR N terminus (15, 17–22). The clinical importance of investigating the biological role of coactivators that bind the AR N terminus is underscored by a recent report describing a patient diagnosed with complete androgen insensitivity, but exhibiting no mutation in AR. The study suggested that a coactivator interacting with the N-terminal region of AR, apparently essential for genital development, was lacking in this patient (23).

The work described in this article investigates the biological role of the newly described androgen receptor coactivator, androgen receptor trapped clone-27 (ART-27). ART-27 binds to the AR N terminus and facilitates receptor dependent transcriptional activation. The ART-27 clone was isolated from an androgen-stimulated LNCaP cell cDNA library in a two-hybrid

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3 The abbreviations used are: AR, androgen receptor; AF-1, activation function 1; AF-2, activation function 2; ART-27, androgen receptor-trapped clone-27; ARE, androgen response element; IRES, internal ribosome entry site; PB, prostastic bud; PCNA, proliferating cell nuclear antigen; PIN, prostatic intraepithelial neoplasia; TBP, TATA box-binding protein; GST, glutathione S-transferase; PSA, prostate-specific antigen; RT, reverse transcription; HA, hemagglutinin.
screen designed to identify proteins that interacted with the AR-N terminus (24) and was previously cloned in an exon-trapping experiment (25). The ART-27 gene encodes a protein of 157 amino acids with a calculated molecular weight of 18 kDa. Functional characterization of ART-27 indicates that it is a nuclear protein that interacts with N-terminal rat AR amino acids 153–336, containing AF-1a and a part of AF-1b (24). Biochemical analysis of ART-27 indicates that it interacts with AR in nuclear extracts from LNCaP cells in a ligand-independent manner. In addition, ART-27 increases the transcriptional potencies of a variety of androgen-responsive promoters. Mechanistically, ART-27 is likely to function as part of a transcription complex since analysis of ART-27 behavior in density gradient sedimentation of HeLa nuclear cell extracts indicates that it co-sediments as part of a larger complex (24). Although the mechanism by which ART-27 functions as a transcriptional activator is unclear, it is likely that ART-27 acts as an adapter to assemble protein complexes at androgen-responsive promoters.

Steroid receptor coactivators and corepressors are likely important determinants of the response of a given hormone receptor in a given cellular context. As androgen stimulation results in differing cellular responses at various stages of prostate development and malignant transformation, we investigated the role of ART-27 in prostate epithelial cells.

EXPERIMENTAL PROCEDURES

Construction of Plasmids—The ART-27 GST fusion construct was made by isolating full-length ART-27 as a EcoR1Xho1 fragment from pEG202 and subcloning into EcoR1Xho1 sites in pEG-4T-1 (Amsersham Biosciences). FLAG-tagged ART-27 and hemagglutinin (HA)-tagged ART-27 were made by subcloning the ART-27 EcoR1/Xho1 fragment into a pcDNA3 vector with an N-terminal HA epitope (pcDNA-HA) or into a pcDNA3 vector with a C-terminal FLAG epitope (pcDNA3-FLAG).

Generation of Anti-ART-27 Polyclonal Antibodies—Generation of C-terminal anti-ART-27 antibodies has been previously described (24). C-terminal antibodies were affinity-purified by isolation of the IgG fraction on a protein A Sepharose column, followed by affinity purification using Affi-gel 15 resin (Bio-Rad) coupled to the ART-27 peptide immunogen. Antibody was eluted with 100 mM glycine, pH 2.7, and immediately neutralized. Antibodies against the ART-27-GST fusion protein were made by immunizing rabbits with the purified protein (Covance Research Products, Denver, PA).

Immunohistochemistry—All human specimens were used with approval of the New York University School of Medicine Institutional Review Board. Tissues utilized were obtained from paraffin-embedded human prostate and breast tissue. Consecutive sections from each tissue were dewaxed in xylene, rehydrated, and washed in phosphate-buffered saline (PBS), pH 7.4. For AR polyclonal N-20). Tissue sections were dewaxed in xylene, rehydrated, and washed in phosphate-buffered saline (PBS), pH 7.4. For antigen retrieval, paraffin sections were heated in a microwave oven (900 watts) in citrate buffer, followed by treatment with 3% H2O2 and blocking with 20% normal goat serum. Sections were then incubated with affinity-purified ART-27 (1:100) or AR antibody (1:200) followed by incubation with a biotinylated rabbit secondary antibody (Vector Labs). Heat-induced antigen retrieval was achieved by boiling the sections in citrate buffer. The sections were then incubated with the avidin-biotin complex (Vector Labs) and developed with diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide to visualize the antigen. The sections were mounted with Permount and viewed under a microscope.

RESULTS

ART-27 Is Expressed in Epithelial Cells of Human Prostate and Breast Tissues—To evaluate the biological role of ART-27, polyclonal antibodies were developed so that the cell and tissue-specific expression pattern of ART-27 could be determined in vivo. Characterization of anti-ART-27 polyclonal antibodies is shown in Fig. 1. Cells were transfected with tagged ART-27, and lysates were either immunoprecipitated with antibody against ART-27 or the tag or used directly for Western blot analysis. Fig. 1A (top panel) shows that a polyclonal antibody made against the C-terminal peptide of ART-27 recognizes overexpressed FLAG-tagged ART-27 (lanes 1 and 5), overexpressed HA-tagged ART-27 (lanes 2 and 6) as well as endogenous ART-27 protein (lanes 1, 2, 3, 4, and 5). A second polyclonal antibody made against full-length ART-27-glutathione S-transferase (GST) protein similarly recognizes these proteins (Fig. 1A, bottom panel) and confirms the identity of ART-27 protein. To verify detection of endogenous ART-27 protein, an untagged variant of ART-27 was expressed and compared with expression of endogenous ART-27 (Fig. 1B). The results establish that endogenous ART-27 is the same size as the untagged ART-27 and that the antibody is capable of specifically recognizing overexpressed and endogenous ART-27. As expected, the immune, but not the preimmune antisera recognizes ART-27 protein (Fig. 1C).

To determine the cell type specificity of ART-27 expression compared with AR expression, ART-27 and AR antibodies were separately incubated with freshly cut paraffin sections of human prostate and breast tissue. Consecutive sections from each
tissue were analyzed. Both ART-27 and AR are predominantly expressed in nuclei of differentiated epithelial cells of human prostate tissue. AR is almost exclusively nuclear, while ART-27 immunoreactivity appears to be present in both the cytoplasm and the nucleus (see higher magnification insets, Fig. 2, A and B). In breast tissue, once again, AR and ART-27 are both expressed in nuclei of epithelial cells. However, AR appears to be more abundant in the distal regions of the gland (observed as the smaller round gland indicated by the arrow in Fig. 2E) than in the more proximal part of the gland (the large central gland), while ART-27 protein appears to be abundant in both regions. Neither protein is ubiquitously expressed in epithelial cells of the colon (not shown).

As a control for antibody specificity, tissues were incubated in the presence of the primary antibody plus the immunizing peptide; these tissues show no immunoreactivity indicating that all of the observable staining is due to antibody recognition of the ART-27 C-terminal peptide. Additionally, incubation of the sections with secondary antibody only (Fig. 2, C and F) indicates that the secondary antibody does not contribute to the observed staining pattern. Therefore, the pattern of ART-27 protein expression is epithelial cell-specific.

Closer examination of immunoreactivity with the ART-27 antibody in human prostate tissue sections shows strong epithelial cell staining and little to none in the stroma of adult human prostate tissue (Fig. 3A). To further verify the cell type specificity of ART-27 protein expression, Western blot analysis was performed using lysates from explant cultures of primary human epithelial and stromal cells (28). Consistent with the immunohistochemistry results, ART-27 is highly expressed in epithelial cells and expressed at low levels, if at all, in stromal cells (Fig. 3B).

**ART-27 Is Expressed in Rat Prostate Epithelial Cells Undergoing Androgen-dependent Proliferation**—Since studies in human prostate tissue (above) indicated that ART-27 and AR are both expressed in prostate epithelial cells, a rat model of androgen depletion/reconstitution was used to examine the effects of androgen-mediated prostate growth and differentiation on ART-27 protein expression (29). Similar to the expression of ART-27 in human prostate tissue, immunohistochemistry performed on rat prostate tissue samples indicated that ART-27 protein is expressed in prostate luminal epithelial cells, but not in stromal cells (Fig. 4A). To androgen-deprive the animals, rats were castrated to cause withdrawal of testicular androgens and regression of the prostate gland. Androgens were then re-administered for 24 and 48 h, resulting in cellular proliferation and growth of the prostate. Prostate glands were dissected from the animals at the indicated time points, and lysates were prepared from each gland and used for Western blot analysis. The filters were incubated with antibodies against ART-27 as well as proliferating cell nuclear antigen (PCNA, a marker for cellular proliferation), clusterin (a protein that increases upon prostate involution and apoptosis, Refs. 30–32), and MAPK (used as an internal loading control). Following castration, PCNA expression is abolished and clusterin expression is greatly increased, supporting the induction of apoptosis in the prostate epithelium (Fig. 4B, cas). Upon administration of exogenous androgens, PCNA levels are increased with rapid reduction of clusterin expression, thereby confirming the mitotic role of androgens in this model (A24 and A48). ART-27 protein is dramatically reduced following androgen withdrawal (cas), but is abundant when androgens are available (con, A24 and A48). Although it is not evident in this figure, longer exposure of the film from the blot incubated with ART-27 antibody does detect ART-27 in the samples from the castrated animals (cas). Therefore, in this model, the expression of ART-27 is associated with an abundance of differentiated prostate epithelial cells and is reduced along with diminishing numbers of epithelial cells under conditions of androgen deprivation. Subsequent experiments were designed to determine if ART-27 may play a role in androgen-dependent prostate growth and/or differentiation.

**Regulated Expression of ART-27 in the Androgen-sensitive LNCaP Cell Line Causes Decreased Cell Proliferation**—To determine the effects of ART-27 on prostate cancer cell growth we constructed stable cell lines that express an epitope-tagged variant of ART-27 under the regulation of a tetracycline promoter in androgen-sensitive LNCaP prostate cancer cells. Parental LNCaP cells were transfected with the pTet-On vector and resulting clones were screened for Tet-dependent activation by measuring pRevTRE-luciferase reporter gene activity. A LNCaP clone displaying tight Tet-dependent activation was then transfected with pRevTRE-ART-27 (C-FLAG) prior to selection of resistant colonies. Fig. 5A shows expression of endogenous (ART-27) and ART-27 (C-FLAG) mRNA in one of the resulting clones in the presence and absence of R1881 and the tetracycline analog, doxycycline, as examined by Northern blot analysis. As expected, ART-27 (C-FLAG) rev/tet mRNA is up-

![Image](http://www.jbc.org/jbc/article-lookup/v182/p4/fig1.jpg)
regulated in the presence of doxycycline (Fig. 5A, lanes 2 and 4). However, neither endogenous ART-27 RNA levels (lower arrow) or ART-27 (C-FLAG) rev/tet RNA (upper arrow) levels are changed in response to the presence or absence of androgens (compare lanes 1 and 2 versus lanes 3 and 4) indicating that androgens do not regulate the transcription of ART-27.

Since subsequent experiments were designed to look at the effect of exogenous ART-27 on androgen mediated cell growth in LNCaP cells it was first determined if endogenous ART-27 protein levels are regulated by androgens, as might be suggested by the depletion of ART-27 protein levels in the castrated rat prostate cell lysates (Fig. 4B). For this purpose, ART-27 protein levels were determined in parental LNCaP cells in the presence and absence of R1881 (Fig. 5B). Western blot analysis of lysates made from androgen starved or androgen stimulated LNCaP prostate cancer cells is shown in Fig. 5B. The abundance of ART-27 protein in androgen-starved cells (Fig. 5B, lane 1) is identical to the levels of ART-27 in androgen-treated LNCaP cells (Fig. 5B, lane 2) suggesting that androgens do not affect the steady state levels of ART-27 protein.

Analysis of ART-27-FLAG protein expression in response to doxycycline is shown in Fig. 6A. As expected, treatment of an ART-27-FLAG clone 1 with increasing concentrations of doxycycline results in higher levels of ART-27 protein expression. As a preliminary step to the thymidine uptake experiments described below, it was also determined that endogenous levels of AR expression are not affected by treatment of the cell clones with doxycycline (Fig. 6B).

To determine the effect of ART-27 on androgen-mediated LNCaP cell proliferation, DNA synthesis was determined by incorporation of [3H]thymidine into cellular DNA. Fig. 6 (C and D) shows analysis of ART-27-FLAG clone 1 (C) and clone 2 (D) in the presence and absence of doxycycline and R1881. Both clones demonstrate regulation of ART-27-FLAG in response to doxycycline. Fig. 6C shows a representative experiment in which [3H]thymidine was added to the cells 1 h prior to sample analysis. As expected, there is very little incorporation of thymidine into DNA in the absence of androgens whether the cells are treated with doxycycline or not (Fig. 6C, 0 nM conditions). In the presence of R1881 (Fig. 6C, 0.05 nM) thymidine is incorporated into the cellular DNA as expected. However, cells expressing ART-27 FLAG (0.05 nM + Dox) show diminished thy-
midine incorporation compared with those that do not (0.05 nM
-Dox) at the 48-h and 72-h treatment with R1881. Analysis of
an additional clone (ART-27-FLAG clone 2) shows a similar
trend (Fig. 6D). Again the cells show thymidine incorporation
in response to androgens (compare 0 nM -Dox to 0.05 nM
-Dox). In this clone, expression of ART-27-FLAG in the pres-
ence of 0.05 nM R1881 (0.05 nM +Dox) also reduces thymidine
uptake compared with the cells grown in the absence of
ART-27-FLAG (0.05 nM -Dox). In fact, in clone 2, the presence
of ART-27-FLAG thymidine uptake is reduced to levels seen in
the 0 nM R1881 condition (compare 0.05 nM +Dox to 0 nM
+Dox). As a control it was shown that thymidine alone at the
concentration utilized does not inhibit parental LNCaP cell
thymidine uptake (not shown).

The decreased ability of ART-27-FLAG LNCaP cell clones to
synthesize DNA in the presence of doxycycline induced ART-27
gene expression is corroborated by the fact that we were not
able to make stable LNCaP cell lines that overexpressed
ART-27 constitutively. In these experiments ART-27 was sub-
cloned into the pIRESneo2 vector (Clontech) that utilizes an
internal ribosome entry site (IRES) to express the protein of
interest (ART-27) on the same transcript as a selectable
marker (neomycin). While hundreds of LNCaP colonies grew
upon selection when transfected with pIRESneo2 alone, no
colonies were evident on plates transfected with ART-27-
pIRESneo2, again suggesting that increased levels of ART-27
protein inhibit or prevent LNCaP cell growth.

To determine if ART-27-mediated LNCaP cell growth sup-
pression is linked to the up-regulation of androgen-responsive
genes involved in differentiated cell function, we examined the
effect of PSA gene transcription in the presence and absence
of doxycycline and R1881 by RT-PCR. PSA is an AR target gene
and product of differentiated epithelial cells. Fig. 6E shows the
DNA analysis of PCR products generated in RT-PCR reactions
using primers for either the PSA gene or the ribosomal protein
L19 (RPL19) gene included as a reaction and loading control.
The doxycycline-inducible ART-27 FLAG clone 1 was grown in
0.05 nM R1881 and treated with either doxycycline or R1881 for
72 or 120 h as indicated. Cells were treated with 0.05 nM R1881
to be consistent with conditions under which ART-27 suppres-
sion of cell growth was observed (Fig. 6, C and D). Since R1881
is typically used at concentrations between 1–100 nM to elicit

robust PSA transcription, it was anticipated that under condi-
tions of 0.05 nM R1881, PSA transcript levels would be low. Fig.
6E shows that PSA is marginally detectable in all the samples
and that the PSA gene product is increased in response to
ART-27 overexpression (compare -Dox +R1881 with +Dox
+R1881 at both the 72- and 120-h time points). We note that
this experiment does not indicate the mechanism by which
ART-27 enhances AR-mediated PSA gene transcription, and
experiments are underway to determine if ART-27 plays a
direct role. However, consistent with the hypothesis that
ART-27 plays a role in AR-mediated cellular differentiation
and growth suppression, transcription of PSA is enhanced un-
Fig. 7. Lack of ART-27 expression in prostate cancer. Normal prostate (A and B) or cancer containing prostate (C–F) were stained with AR antibody (right panels) or ART-27 antibody (left panels) as indicated. Cancer samples from three different individuals are shown in C–F and Fig. 8, A and B. As expected, both AR and ART-27 are predominantly expressed in the nuclei of normal prostate epithelium (A and B). The insets show the same samples at ×1000 magnification. Neither AR nor ART-27 exhibit staining in basal cells (vertical arrows A and B, insets). The horizontal arrow (B, inset) indicates an AR-positive staining stromal cell. A tissue region typical of prostate cancer containing small irregularly shaped glands is demarcated by the black box in C–F. While AR is present in the cancerous regions (D and F), ART-27 is not expressed in the cancerous glands (C and E). Both AR and ART-27 are expressed, however, in adjacent non-cancerous glands that are either normal or benign hyperplastic glands (indicated by arrows in C, E, and F). Images are shown at ×200 magnification.

Fig. 8. ART-27 expression in cancer versus in high grade PIN. Shows consecutive sections of prostate cancer tissue stained with either anti-ART-27 antibody (left panels) or with anti-AR (right panels). Samples are from different individuals than shown in Fig. 7. As above, adjacent photographs are from consecutive sections of the same tissue sample. A and B show expression of AR and ART-27 in prostate cancer. C and D show expression of AR and ART-27 in high grade PIN. Images are shown at ×400 magnification.

ART-27 Expression Is Decreased in Prostate Cancer—As the above results suggested that ART-27 inhibits androgen-mediated prostate cell growth, we evaluated the expression of ART-27 protein in benign (non-cancerous) and malignant (cancerous) human prostate tissue through immunohistochemical staining of paraffin-embedded radical prostatectomy (n = 22) specimens. Consecutive tissue sections stained with affinity purified ART-27 antibody or with an AR antibody show nuclear staining of normal prostate glandular epithelium (Fig. 7, A and B) as previously noted. Additionally, insets in A and B show that neither ART-27 nor AR exhibit immunoreactivity in prostate basal cells (see vertical arrows). Immunohistochemical staining of sections containing regions of adenocarcinoma (glandular epithelial cancer) indicate that ART-27 staining in prostate cancer is decreased (indicated by the black box in Fig. 7, C and E) relative to that observed in adjacent benign prostatic glands (indicated by arrows in Fig. 7, C and E). AR staining of a consecutive section shows uniform staining in benign and malignant glands, thereby providing a positive control for the integrity of the tissue specimen (Fig. 7, D and F). A higher magnification comparison of AR and ART-27 expression in an additional prostate cancer tissue specimen again confirms robust levels of AR and negligible levels of ART-27 (Fig. 8, A and B).

Decreased ART-27 staining was observed in all 22 cancer specimens examined. Decreased ART-27 signal could not be attributed to tissue damage (caused by overfixation, underfixation, cauterization, etc.) since only slides that showed positive immunoreactivity with anti-AR antibody were included in the study. The majority of samples tested were moderately differentiated (Gleason score 5–7), with a minority representing poorly differentiated disease (Gleason 8–10). In comparing the samples tested, however, staining patterns of the malignant glands appear similar regardless of the degree of differentiation.

In evaluating sections containing high grade prostatic intraepithelial neoplasia (HG-PIN), a condition thought to be a precursor to prostate cancer, strong ART-27 staining was noted within the dysplastic (abnormal looking, but non-cancerous) epithelium (Fig. 8, C and D). Thus, reduction in ART-27 expression appears to be a relatively late event in prostate carcinogenesis. Based collectively upon immunohistochemical staining patterns in prostate cancer and effect on prostate cancer cell growth, we speculate that ART-27 may play a role in promoting androgen-dependent epithelial differentiation thereby reducing tumor progression or proliferation rate. Its potential role in carcinogenesis remains to be defined.

ART-27 in Human Fetal Development—The disappearance of ART-27 protein in prostate cancer progression and consequent de-differentiation suggests that ART-27 may play a role in luminal epithelial cell differentiation. To examine ART-27 protein expression during developmental differentiation, we assessed the pattern of ART-27 protein in the region of the urogenital sinus from which the prostate develops in human fetal tissue. Sections through the urogenital sinus region of a 15-week-old fetus show a cell free region in the center which is the lumen of the urethra (indicated by the U in Fig. 9, A and B). Adjacent to the lumen are columns of epithelial cells that are surrounded by mesenchymal or stromal tissue (Str). Epithelial outgrowths from the urethra grow and branch into the surrounding mesenchyme to form the prostate buds (PB) or tu-
samples are incubated with either anti-ART-27 antibody (A, urogenital sinus. Prostatic buds (PB) are indicated by arrows). Tissue from the urogenital region of a 15-week-old fetus was also stained with ART-27 antibody. Since the urethra is much larger at this point in development (Fig. 9D, top), the presence of ART-27 is also detected in epithelial cells adjacent to the lumen of the urethra, and is not detected in the surrounding stroma (Str). However, ART-27 is now also detectable in some of the larger prostatic buds (PB) that have formed a lumen (positive ART-27 reactivity in the buds is indicated by arrows). B shows that AR is expressed in epithelial cells adjacent to the lumen of the urethra as well as in the stromal cells surrounding the glands and in the prostate buds. A higher magnification photograph of the epithelial cell layer between the lumen of the urethra (U) and stromal cells (Str) is shown in D. Staining of the two consecutive tissue sections with antibody against ART-27 (D, top) and AR (D, bottom) shows that ART-27 primarily stains the nuclei of the single layer of epithelial cells adjacent to the lumen while AR appears to stain multiple epithelial cell layers and stromal cells. Arrows are used to indicate three representative cells that stain positively for both ART-27 (D, top) and AR (D, bottom).

**Discussion**

Attempts to understand the role of AR in prostate cell growth or differentiation suggest that its effects are complex and dependent upon cellular context (33–36). Androgen receptor co-activators such as ART-27, are likely to modulate AR activity and determine differentiation and proliferation programs of prostate cell gene transcription.

Concise transcriptional regulation is a prerequisite for the development of highly specialized cell types that comprise multicellular organisms. One mechanism underlying such specificity is the restricted expression pattern of components of the transcriptional machinery to specialized cell types. For example, TAF51, a component of the transcription factor TFIID, is restricted to the granulosa cells of the ovary follicle and regulates a subset of genes necessary for normal ovarian follicular development (37). The striking cell type specific pattern of ART-27 protein localization during fetal urogenital development suggests that ART-27 regulates a subset of AR-respon-
sive genes in specialized cell types. For example, while AR is expressed in multiple cell layers of the urogenital sinus, ART-27 is expressed in a single cell layer adjacent to the lumen. In prostate, ART-27 is not present in prostatic buds at week 15, but becomes detectable at later stages that correlate with the differentiation of luminal epithelial cells and the appearance of a lumen. As the majority of proteins expressed by the differentiated prostate epithelium are secretory in nature, the presence of a lumen indicates cellular differentiation. Thus, ART-27 protein expression strongly correlates with prostate cell differentiation during organ development.

Within the mature prostate, ART-27 expression appears to remain unique to differentiated epithelial cells. Little to no expression is observed within the proliferative (basal cell) compartment of the glandular epithelium or the stroma. Within the luminal epithelium, variable intensity of ART-27 nuclear staining is often observed within cells uniformly staining for AR. This suggests a functional role for ART-27, perhaps dependent upon cell cycle, stage of cellular maturation, or secretory function.

As ART-27 is present in both benign and premalignant (HG-PIN) epithelium, but relatively absent in prostate cancer, the loss of ART-27 expression appears to be a relatively late event in carcinogenesis. Reintroduction of ART-27 into a prostate cancer cell line by stable transfection results in markedly decreased androgen-stimulated proliferation. Further, complete cessation of growth appears to occur upon attempts to constitutively express ART-27 within the cell line. These data are consistent with the hypothesis that ART-27 functions in initiation or maintenance of an AR mediated program of prostate cell differentiation and are supported by evidence that increased expression of ART-27 results in up-regulation of PSA gene transcription (Fig. 6), a gene expressed by differentiated prostate luminal epithelial cells.

Androgen receptor coactivators and corepressors are likely to play a role in androgen dependent and androgen-independent prostate cancer. The most effective means of treating prostate cancer is by androgen ablation therapy, which causes prostate tissue along with prostate tumors, to regress. While suppressing the supply of androgens is beneficial in the short term, some prostate cancer cells inevitably begin to grow in a hormone independent fashion, and at this point treatment options are poor. Multiple mechanisms might explain hormone refractory disease including androgen receptor mutation and/or up-regulation and altered recruitment of AR coactivators or corepressors to the AR (38, 39). The idea that steroid receptor coactivators might be instrumental in cancer progression has been realized with the discovery that Tamoxifen, the estrogen antagonist widely used to treat breast cancer, acts by recruiting the nuclear receptor coactivator, NcoR and preventing co-activator binding to ER (40–42). A recent report indicates that recurrent prostate cancers express higher than normal levels of the steroid hormone coactivators, transcriptional intermediary factor 2 (TIF2), and SRC-1 coactivator (steroid receptor coactivator-1) genes (43). In addition, in situ analysis of multiple androgen receptor coactivator expression levels showed that mRNA expression of the coactivators varied between normal and malignant prostate tissue samples (44). Effects on normal prostate cell growth have also been demonstrated in animal studies by targeted mutagenesis of the SRC-1 gene (45). However, these effects are relatively minor and therefore suggest that compensatory mechanisms exist and/or that additional co-regulators are required for androgen mediated cell growth.

Although the AR N terminus has been shown to be critical for AR transactivation, the expression of AF-1-binding proteins has not been evaluated during prostate cancer progression. Our data indicate that ART-27 expression is altered during prostate cancer progression, and therefore may play an important role in this disease. It is currently unknown how ART-27 is regulated in normal prostate cells or in prostate cancer. The cell type specificity of ART-27 expression suggests it is not directly regulated by androgens since ART-27 is expressed in a far more restricted pattern than AR. In addition, androgen treatments do not induce ART-27 mRNA or protein in LNCaP cells, and there are no decipherable androgen response elements in the promoter of ART-27. However, ART-27 protein expression may be indirectly regulated by localized secretion of cytokines or growth factors in response to androgen signaling.

A growing body of evidence suggests that co-regulators of steroid hormone receptors impart functional flexibility, thus allowing the receptors to respond differentially, contingent upon their biological context (46). Our results are consistent with the idea that the androgen receptor coactivator ART-27 is a cell type-restricted cofactor, and promotes androgen-dependent prostate epithelial cell differentiation, thereby inhibiting cellular proliferation.

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