Heterogeneous Expression and Functions of Androgen Receptor Co-Factors in Primary Prostate Cancer

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The androgen receptor (AR), a ligand-activated transcription factor of the steroid receptor superfamily, plays an important role in normal prostate growth and in prostate cancer. The recent identification of various AR co-factors prompted us to evaluate their possible roles in prostate tumorigenesis. To this end, we analyzed the expression of AR and eight of its co-factors by quantitative *in situ* RNA hybridization in 43 primary prostate cancers with different degrees of differentiation. Our results revealed nearly constant expression of AR and heterogeneous expression of AR co-factors, with increased expression of PIAS1 and Ran/ARA24, decreased expression of ELE1/ARA70, and no change in TMF1/ARA160, ARA54, SRC1, or TRAP220. Interestingly, whereas TMF1/ARA160, ELE1/ARA70, ARA54, RAN/ARA24, and PIAS1 were preferentially expressed in epithelial cells, another co-factor, ARA55, was preferentially expressed in stromal cells. Although the changes in levels of these co-activators did not correlate with Gleason score, their occurrence in high-grade prostatic intraepithelial neoplasia, suggests their involvement in initiation (or an early stage) of cancer. In addition, human prostate tumor cell proliferation and colony formation were markedly reduced by ELE1/ARA70. Together, these findings indicate that changes in levels of expression of AR co-factors may play important, yet different, roles in prostate tumorigenesis. (Am J Pathol 2002, 161:1467-1474)

Androgens mediate development and maintenance of normal prostate tissue and also seem to be involved in prostate tumor growth and progression.¹ Androgens act through the androgen receptor (AR), which belongs to the large family of nuclear receptors.² These receptors are hormone-activated transcription factors and structurally conserved. Activation of AR by androgens is a multistep process that involves androgen binding to the re-

ceptor, an accompanying structural change in the receptor, loss of associated heat shock/chaperone proteins, translocation of the liganded receptor to the nucleus, and binding of the liganded receptor to target genes. There is increasing evidence that the transcriptional activity of AR and other nuclear receptors depends on their interaction with various co-factors (co-activators and co-repressors).^{3,4} A variety of co-factors have been identified by their ability to bind various nuclear receptor domains and to alter the transcriptional activity of nuclear receptors after overexpression in cell lines.⁵ The best-studied group includes p300/CBP, the p160 family (SRC-1, TIF-2/GRIP-1, ACTR/P-CIP),⁵ and PCAF/GCN5 complexes (yeast SAGA, human STAGA).^{6,7} All have histone acetyltransferase activities and are thought to act mainly through histone acetylation and consequent chromatin structural perturbations, although they can also act through functional acetylation of activators⁸ and co-activators.⁹ A second group includes the TRAP/DRIP/ARC/SMCC/mediator complex,¹⁰ which shows subunit-specific interactions with both nuclear receptors (mainly through TRAP220) and other activators.¹⁰ This complex in turn facilitates the function of RNA polymerase II and the general initiation factors on DNA templates at postchromatin-remodeling steps.^{10,11} Of these various co-activators, p300/CBP^{12,13} and p160s^{12,14-16} have been shown to function with AR. Other co-factors implicated in the function of AR and, in most cases, other nuclear receptors, include the ARA group (ARA24, ARA54, ARA55, ARA70, and ARA160),^{17-20,35} ARIP3,²¹ SNURF,²² and AES.²³

Altered expression of nuclear hormone receptor co-factors has been implicated in the genesis and progression of breast cancers. Increased expression of TIF2, CBP, and steroid receptor RNA activator has been observed in breast tumor tissues.²⁴⁻²⁶ Peroxisome proliferator-activated receptor-binding protein (PBP/TRAP220) and SRC3/AIB1 genes are frequently amplified and overexpressed in breast tumors.^{27,28} In comparison, how-

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ever, little is known about the possibility of abnormal expression of co-factors in prostate cancer. Recently, Fujimoto and colleagues⁴⁰ found that the expression levels of ARA55 and SRC1 were higher in cancer specimens with a poor response to endocrine therapy than in those with a good response to endocrine therapy. To explore this question, we analyzed the levels of expression of both relatively AR-selective co-factors (TMF1/ARA160, ELE1/ARA70, ARA55, ARA54, Ran/ARA24, and PIAS1) and more general co-factors (SRC1, TRAP220) in human prostate cancer tissue by quantitative *in situ* hybridization. Among the tested co-factors, PIAS1 and RAN/ARA24 showed significantly higher expression levels in cancer tissue compared with benign tissue. In contrast, expression of ELE1/ARA70 was dramatically decreased in primary prostate tumor tissues. A subsequent analysis has demonstrated suppression of LNCaP cell growth by ELE1/ARA70. Collectively, these results imply that these co-factors likely play important but contrasting roles in prostate cancer differentiation and tumorigenesis.

Materials and Methods

Prostate Tissue Specimens and Pathological Evaluation

Prostate cancer and normal control tissues were derived from radical prostatectomy specimens of 43 prostate cancer patients treated at New York University Medical Center. The study protocol was approved by Institutional Review Board of New York University Medical Center. Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections of tissue (4 μ m) were cut and mounted on Superfrost Plus adhesion slides and used for histology, immunohistochemistry, and *in situ* hybridization. Prostate cancer foci were categorized as well differentiated (combined Gleason score 2 to 4; $n = 9$), moderately differentiated (combined Gleason score 5 to 6; $n = 17$), and poorly differentiated (combined Gleason score 7 and 8 to 10; $n = 17$). The histological features and the Gleason score of each individual specimen were confirmed by two pathologists (JM and PL).

Immunohistochemistry

The immunohistochemical staining was performed on an automated Ventana machine. Before staining, antigen retrieval was performed by heating the specimens in a microwave oven for 30 minutes in citrate buffer (pH 8.0) after dewaxing. A rabbit polyclonal anti-AR antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was applied to the sections at a 1:100 dilution, and sections were then incubated overnight at 4°C. A streptavidin-biotin peroxidase detection system was used according to the manufacturer's instruction (DAKO, Carpinteria, CA), with 3,3'-diaminobenzidine as substrate.

Table 1. List of Oligonucleotide Primers Used for Polymerase Chain Reaction

Protein	Primer	Sequence (5'-3')	Size (bp)
AR	T3/2263	accaatgtcaactccaggatgct	499
	T7/2762	cttcactgggtgtggaatagatg	
Ran/ARA24	T3/22	cagggtccagttcaaaactgtattggt	605
	T7/627	gagagcagttgtctgagcaacct	
ARA54	T3/863	ttgcccgttatgaccgc	474
	T7/1337	aaatgtttgaagggttgcctctagag	
ARA55	T3/-20	ctggagactaccacctcgacatg	608
	T7/588	actgcagcagccccagc	
ELE1/ARA70	T3/1344	tgagcctgagaagcataaagattc	491
	T7/1835	acatctgtagaggagttcgatataac	
TMF1/ARA160	T3/477	ttcaggggaaactctggcag	581
	T7/1058	tatccctgtcctgacaattcatcat	
PIAS1	T3/7	gacagtgccggaactaaagcaaatg	638
	T7/645	gaagtgatctcttctggacaactggt	
SRC1	T3/3235	tatcagtcaccagcatgaagg	499
	T7/3734	gggtattcagtcagtagctgctg	
TRAP220	T3/172	ttgttcagctgttggagacat	628
	T7/800	ttgtacacagcagatgttcctca	

T3 primers contain the T3 RNA polymerase promoter sequence gcaattaaccctcactaaaggg at the 3' ends and T7 primers contain the T7 RNA polymerase promoter sequence cgtaatacgactcactataggg at the 5' ends. Numbers indicate position of the primers' 5' ends on the cDNA sequences (the A of the ATG translation start codon was arbitrarily given the number 1).

In Situ Hybridization

The expression sequence tag (EST) cDNA clones of interest were obtained from Research Genetics, Inc (Carlsbad, CA). Oligonucleotides were designed to bear T7 promoter sequences on one end and T3 promoter sequences on the opposite end (Table 1) such that the sense and anti-sense probes were specified by the polymerase used. Fragments of corresponding genes (~500-bp DNA) were amplified using polymerase chain reaction. High-specific-activity ³³P-labeled RNA probes were synthesized by incubation of DNA with T7 or T3 RNA polymerase; 500 μ mol/L of GTP, ATP, and CTP; 3 μ mol/L of UTP; and 100 μ Ci α -³³P-UTP (6000 Ci/mmol) at 37°C for 45 minutes followed by DNase treatment for 15 minutes at 37°C. The probes were purified by chromatography on a Sephadex G-50 column. The yield and quality of the probes were assessed by trichloroacetic acid precipitation and scintillation counting, as well as by agarose gel electrophoresis and autoradiography.

After wax removal and rehydration, 4- μ m sections of formalin-fixed tissue were hybridized to the sense and anti-sense probes following the described procedures.³⁰ Then, slides were subjected to autoradiography by dipping in NTB-2 X-ray emulsion (Eastman-Kodak, Rochester, NY), exposed for 1 to 2 weeks, developed in D-19 developer (Eastman-Kodak), and fixed in G33C fixer. Lastly, the slides were counterstained with Gill's hematoxylin stain.

Image and Statistical Analyses

The benign and malignant tissue components were compared on the same section to eliminate tissue-to-tissue and slide-to-slide variations of grain signals. We first ex-

amined the consistency of quantification of a given case by analyzing randomly selected areas of nonneoplastic and cancer foci in the cancer specimens for five cases. The results from block to block were comparable in all cases (data not shown). These results validated the *in situ* hybridization approach with formalin-fixed and paraffin-embedded tissues for analyzing the expression of AR and its co-factors. Slides were evaluated under a microscope (Nikon Eclipse E400) equipped with a digital camera (Princeton Scientific Instruments, Inc., Monmouth Junction, NJ) interfaced to a computer with IPLab software. The specimens were categorized into four groups according to degree of differentiation by Gleason score; Gleason score 4, Gleason score 5 to 6, Gleason score 7, and Gleason score 8 to 10. The grain (*in situ* signal) numbers from the areas of interest (cancer, prostatic intraepithelial neoplasia, and normal) were captured and counted using IPLab software, and divided by the number of cells to quantify as grain number/cell. An average of 30 to 50 cells were analyzed for each case. Differences in expression levels of the genes of interest among these four groups were subjected to nonparametric Kruskal-Wallis analysis of variance analyses. Results were further grouped according to changes in RNA levels (malignant *versus* benign tissue) of less than twofold and greater than twofold and analyzed by the chi-square test.

Assays of Cell Growth Suppression

LNCaP cells were plated in six-well plates (35-mm wells) and grown in RPMI 1640 medium to ~70% confluency for transfection with plasmid DNA (pcDNA3.1 and pcDNA-ELE/ARA70) and 6 μ l of lipofectamine (Invitrogen Life Technologies, Carlsbad, CA). Two days later, cells were selected with G418 at 0.8 μ g/ml. After 4 weeks of selection with the medium change every 3 days, the cells were rinsed with phosphate-buffered saline (PBS), fixed with 2% formaldehyde in PBS for 15 minutes, stained with 0.5% crystal violet in PBS for 15 minutes, rinsed once or twice with distilled water, dried, and stored for subsequent quantification of colonies.

Results

Analysis of mRNA Expression by In Situ Hybridization

A large number of co-factors that regulate AR-driven transcription has been identified.⁴ To determine possible relationships to prostate tumorigenesis and prostate cancer progression, we investigated the expression levels of various AR co-factors in normal and tumor prostate tissues by *in situ* hybridization. To validate the *in situ* hybridization results, we performed both *in situ* hybridization and immunohistochemical analysis for AR with the same set of slides from the formalin-fixed, paraffin-embedded tissue blocks. Strong and uniform immunostaining of AR was observed in the nuclei of both epithelial and stromal cells in the benign areas of each of the 43 specimens (Figure 1G). Consistently, AR mRNA expression levels were

high in epithelial cells and lower in stromal cells revealed by *in situ* hybridization (Figure 1, A and B). As a negative control, only background signals were detected with the sense AR probe (Figure 1C), indicating that the signals obtained with the anti-sense AR probe were specific.

Expression of AR and Co-Factor mRNAs: Epithelial Expression Versus Stromal Expression

In the 43 cases studied, the expression levels and overall expression patterns of AR did not differ significantly (less than twofold) between normal prostate tissue (from the same section of and adjacent to the prostate cancer region) and prostate cancer tissue. There was no apparent relationship between the amount of AR and the degree of tumor differentiation. These results are in accordance with previous reports that AR is highly expressed in a variety of normal and malignant human prostate tissues.³¹ Expression levels of a panel of eight proteins described as modulators of AR function were then analyzed in the 43 prostate tumor samples. Two of these co-factors, SRC1 and TRAP220, interact with a broad spectrum of different nuclear receptors. The other six co-factors studied [the members of the ARA group (TMF1/ARA160, ELE1/ARA70, ARA55, ARA54 and Ran/ARA24) and PIAS1] are relatively specific for AR. The *in situ* hybridization results are summarized in Table 2. Expression of SRC1 and TRAP220 was detected in both stromal cells and epithelial cells, whereas expression of ARA54 was observed predominantly in epithelial cells. The expression levels of these mRNAs did not differ significantly between normal and tumor tissues. Although ARA55 mRNA was moderately expressed in stromal cells, it was undetectable in glandular epithelial cells (Figure 1, D and E), implying that ARA55 might regulate AR function in prostate stroma. Immunohistochemical staining with anti-ARA55 antibody on the same prostate tissues further confirmed that ARA55 was only expressed in stromal cells (Figure 1F). This is consistent with results of the previous study that ARA55 was detected only in cell lines derived from prostate stroma.³² In most of the 43 specimens studied, the expression level of ARA55 was lower in the stroma in regions of cancerous foci however quantification was not possible because of the scanty nature of stroma in cancerous foci.

Increased Expression of Ran/ARA24 and PIAS1 in Prostate Tumor Tissues

The 24-kd protein Ran/ARA24 belongs to the superfamily of GTP-binding proteins that use a structurally conserved G domain as a molecular switch for cycling between the GDP- and GTP-bound states.³³ Ran/ARA24 has been clearly implicated in the two-way traffic of macromolecules between the nucleus and the cytoplasm³⁴ and in microtubule assembly and spindle formation in cells in M phase. Recently, it has been shown that Ran/ARA24 physically interacts with the polyglutamine region of AR and enhances AR-dependent transcription.³⁵ Our *in situ*

Figure 1. Expression of AR and ARA55 in prostate tissues. **A** and **D**: Bright field (emulsion-coated) of AR and ARA55, respectively. **B** and **E**: Dark field of the same slide areas. **B** and **E** were hybridized with AR and ARA55 anti-sense probes, respectively, and **C** was hybridized with AR sense probes. **F** and **G**: Immunohistochemical analysis of ARA55 and AR in human prostate tissues. All slides were emulsion-coated, except **F** and **G**.

hybridization results for normal tissue showed only a low level of Ran/ARA24 mRNA expression that was present mainly in epithelial cells (Figure 2, A and B). Comparison of Ran/ARA24 expression in normal and tumor tissues

Table 2. Summary of *in Situ* Hybridization Data for AR and the Eight Examined Cofactors

Factors	Stroma	Epithelium	
		Benign	Tumor
AR	++	+++	+++
SRC1	+	+	+
TMF1/ARA160	+/-	+	+
TRAP220	+/-	+	+
ARA55	++	-	-
ARA54	+/-	+	+
Ran/ARA24	+/-	+	+++
PIAS1	+/-	+	+++
ELE1/ARA70	-	+++	+

-, Indicates undetectable levels of expression; +/-, +, ++, and +++ indicate slightly above background, low, moderate, and high levels of expression, respectively.

found overexpression (Figure 2, C and D *versus* A and B; Table 3) in 81% of the tumor specimens, with an average increase of $4.6(\pm 1.1)$ -fold and no change in 19% of the specimens. More dramatic changes (more than fivefold) were observed in 35% of the tumor specimens (Table 3). However, these changes did not correlate with prostate tumor grade (Gleason score) by nonparametric Kruskal-Wallis analysis of variance analysis and by the chi-square test when cases were further grouped according to changes of less than twofold and greater than twofold.

PIAS1 has been identified as a factor that binds to Stat1 (signal transducer and activator of transcription 1) and inhibits STAT-mediated signaling by interfering with the DNA binding of Stat1.³⁶ PIAS1 has also been identified as a co-activator for AR-, estrogen receptor (ER)-, and progesterone receptor (PR)-dependent transcription.^{37,38} In the current study, we observed higher PIAS1 expression levels in 33% of the tumor cases, with an average of 3.8-fold increase (Table 3). Although the percentage of cases showing an increase is significantly higher for Ran/ARA24 (81%) than for PIAS1 (33%), there

Figure 2. Increased expression of Ran/ARA24 in prostate cancer tissues. **Left** (A, C, E) and **right panels** (B, D, F) show bright and dark fields of the same areas of slides, respectively. A and B, C and D, and E and F show normal prostate, prostate tumor, and prostate intraepithelial neoplasia tissues, respectively. All slides are emulsion-coated.

Figure 3. Decreased expression of ELE1/ARA70 in prostate cancer tissues. **Left** and **right panels** show bright and dark fields of the same slide areas, respectively. A and B, C and D, and E and F show normal prostate, prostate tumor, and prostate intraepithelial neoplasia tissues, respectively. All slides are emulsion-coated slides.

is an 80% concordance between the increase for Ran/ARA24 and PIAS1. The PIAS1 expression patterns also did not correlate with prostate tumor grades by nonparametric Kruskal-Wallis analysis of variance and by the chi-square test.

Lower Expression of ELE1/ARA70 in Prostate Cancer

ELE1/ARA70 was identified first as a factor involved in the activation of the RET proto-oncogene in thyroid neoplasia³⁹ and later as a ligand-dependent transcriptional co-factor for AR.¹⁷ Our *in situ* RNA hybridization assays showed that ELE1/ARA70, like AR, is expressed at high

levels (and predominantly in epithelial cells) in normal tissue (Figure 3, A and B). However, ELE1/ARA70 expression was dramatically lower in prostate tumor tissues (Figure 3, C and D *versus* A and B). Expression was decreased twofold to fivefold in 42% of the cases and 5- to 30-fold in 38% of cases (Table 3), with an average decrease of 7.5(±1.4)-fold. We further observed that, in cases with increased expression of RAN/ARA24 and reduced expression of ELE1/ARA70, 70% of the cases showed reciprocal changes, indicating opposite effects of these co-activators in cancer. No obvious correlation between ELE1/ARA70 expression and prostate tumor grade was observed by nonparametric Kruskal-Wallis analysis of variance analysis or by the chi-square test

Table 3. Quantification of PIAS1, Ran/ARA24, and ELE1/ARA70 Expression

	PIAS1		Ran/ARA24			ELE1/ARA70		
fold	<2	2–7.5	<2	2–5	5–20	<2	2–5	5–30
cases	24	12	8	20	15	9	18	16
%	67	33	19	46	35	20	42	38

Figure 4. Growth suppression of the prostate tumor cells by ELE1/ARA70. LNCaP prostate cancer cells were transfected with 4 μ g of pcDNA3.1 (vector) or pcDNA-ELE1/ARA70 and selected for plasmid-containing cells with G418 for 4 weeks. Surviving cells were then fixed and stained with crystal violet. Colonies were counted and the data are presented as histograms.

when cases were grouped according to changes less than twofold and greater than twofold.

AR Co-Activator Expression in High-Grade Prostate Intraepithelial Neoplasia (HGPIN)

HGPIN is thought to be a prostate cancer precursor lesion as a result of abundant evidence based on morphological, topographical, immunohistochemical, and molecular studies. HGPIN was identified in the majority of our cases (40 of 43 cases), either within or away from cancerous foci. A comparative analysis of AR co-factor expression patterns in HGPIN located adjacent to the prostate cancer region showed changes similar to those observed in prostate cancer. These changes included enhanced Ran/ARA24 expression (Figure 2, E and F *versus* A and B) and decreased ELE1/ARA70 expression (Figure 3, E and F *versus* A and B). We did not observe a significant difference in co-activator expression according to location of HGPIN. These results support the concept that HGPIN is a precursor of prostate cancer and further indicate that abnormal expression of Ran/ARA24 and ELE1/ARA70 may be involved in prostate tumor initiation.

Suppression of Human Prostate Cancer Cell Proliferation and Colony Formation by ELE1/ARA70

The decreased expression of ELE1/ARA70 in prostate cancer suggest that this co-factor might negatively regulate prostate cell growth and proliferation. We therefore tested the ability of ELE1/ARA70 gene to suppress the growth of prostate tumor cells, using the metastatic prostate cancer cell line LNCaP, which expresses reduced levels of ELE1/ARA70 compared with normal primary prostate epithelial cells.⁴¹ Colony formation was suppressed by ELE1/ARA70 but not by the vector control (Figure 4). The colonies were small (containing a few

cells), even after 1 month of G418 selection (data not shown). These results indicate that ELE1/ARA70 suppresses tumor cell proliferation and colony formation and suggest that it may be a tissue differentiation factor or a potential tumor suppressor.

Discussion

Given the diverse functions of AR in different tissues, the large number of AR co-factors may provide means for cell- and promoter-specific regulation of AR activity.^{1,2} Most co-factors are not receptor-specific but also regulate the activity of many nuclear receptors as well as unrelated transcription factors.³ Furthermore, many co-factors are components of multiprotein complexes that have overlapping functions and nuclear receptor-binding sites.⁴² The challenge is to identify co-factors involved in AR function in the prostate, particularly in prostate growth and prostate cancer progression. Our results demonstrate the heterogeneous expression and functions of AR co-factors in the prostate. Significantly, we observed increased expression of PIAS1 and Ran/ARA24 and decreased expression of ELE1/RAR70 both in prostate cancer tissues and in HGPIN, relative to normal prostate tissue. Furthermore, our *in vivo* studies using a malignant prostate cell line raise the possibility that ELE1/RAR70 might be a tumor suppressor.

AR and Some Co-Factors Are Relatively Constant in Benign and Malignant Prostate Tissues

Enhanced AR activity has been correlated with prostate cancer formation and progression.⁴³ It also has been proposed that either AR gene mutation or AR gene amplification may enhance AR activity, thus promoting tumorigenesis or leading to androgen-independent prostate cancer.^{1,44} However, the relatively low incidence of AR mutation and amplification in primary prostate cancer suggests other causes. Consistent with this possibility, our *in situ* analyses have revealed that the levels and patterns of AR expression do not change significantly in primary prostate tumors of different grades. Down-regulation of SRC1, one of general nuclear receptor co-factors, is associated with tamoxifen resistance in breast neoplasms.⁴⁵ However, we did not detect a significant change in expression of SRC1 mRNA in prostate tumor tissue relative to normal tissue. TRAP220 was expressed in both epithelial and stromal cells, and the levels were not different in prostate cancer and benign prostate tissues. This might reflect rather broad functions of TRAP220 for various nuclear receptors and other activators.⁴²

Changed Expression of AR Co-Factors in Prostate Tumor

Recent studies have shown that various co-activators can bind to AR and augment the AR transcription activity in a ligand-dependent manner.^{1,2} Therefore, the activity of

co-factors might contribute to enhanced AR activity in primary prostate cancer. Our study shows that expression levels of Ran/ARA24 and PIAS1 are significantly higher in prostate tumor tissue compared with nonneoplastic prostate tissue. The higher levels of Ran/ARA24 and PIAS1 may contribute to overproliferation of prostate tumor cells. PIAS1 belongs to a family of PIAS proteins that, consistent with present results, are able to co-activate steroid receptor-dependent transcription.^{37,38} In contrast to increased expression of RAN/ARA24 in 81% of prostate tumor cases, enhanced expression of PIAS1 was observed only in 33% of the cases. These differences may reflect the involvement of different pathways for Ran/ARA24 and PIAS1 as well as the relative efficiencies in contribution to cancer formation.

Interestingly, PIAS1 was first cloned as a protein that inhibited Stat1,³⁸ which has been suggested to have an anti-oncogenic effect.⁴⁶ This correlates with our findings that the level of PIAS1 is increased, possibly to revert the proapoptotic activity of Stat1, in prostate tissue. Further investigations will be needed to determine whether high levels of PIAS1 promote prostate cell proliferation through the Stat1 pathway, the AR pathway, or both pathways. Similar to PIAS1, Ran/ARA24 is also involved in nuclear translocation and chromatin organization. It is however unclear which pathways are affected by the enhanced expression of RAN/ARA24.

Previous studies demonstrated that ELE1/ARA70 can serve as a co-activator of AR, ER, PR, and peroxisome proliferator activated receptor gamma (PPAR γ).^{17,29,47,48} Here we report a down-regulation of ELE1/ARA70 expression in prostate cancer compared with levels expressed in nonneoplastic prostate tissues. Consistent with our observations, ELE1/ARA70 expression is reduced in prostate cancer cell lines relative to primary cells from benign prostate epithelium primary cells.⁴¹ These observations suggest that ELE1/ARA70 may be involved in the development or progression of prostate cancer, particularly with respect to loss of androgen responsiveness. Overexpression of ELE1/ARA70 in a prostate cancer cell line suppresses cell proliferation and colony formation, suggesting that it might be a tumor suppressor or involved in the expression of genes required for prostate cell differentiation. AR in LNCaP cell harbors a mutation (codon 877, Thr to Ala) in the hormone-binding domain. This mutation confers an altered ligand-binding specificity.

The study of co-factor expression in prostate cancer should be of great importance for understanding AR function in prostate tumorigenesis and progression. A shift in the levels of various AR co-factors may influence the state of differentiation or proliferation of the prostate, possibly through the regulation of different AR responsive genes.

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