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PROSTATE PROSPECTS

Molecular Regulation of Androgen Action in Prostate Cancer

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Abstract Androgens are critical regulators of prostate differentiation and function, as well as prostate cancer growth and survival. Therefore, androgen ablation is the preferred systemic treatment for disseminated prostate cancer. Androgen action is exerted in target tissues via binding the androgen receptor (AR), a nuclear receptor transcription factor. Historically, the gene expression program mediated by the AR has been poorly understood. However, recent gene expression profiling and more traditional single-gene characterization studies have revealed many androgen-regulated genes that are important mediators of androgen action in both normal and malignant prostate tissue. This review will focus on the androgen-regulated gene expression program, and examine how recently identified androgen-regulated genes are likely to contribute to the development and progression of prostate cancer. We will also summarize several recent studies that have attempted to unravel how these genes are deregulated in androgen depletion independent prostate cancer. J. Cell. Biochem. 99: 333–344, 2006. © 2006 Wiley-Liss, Inc.

Key words: androgen receptor; prostate cancer; gene expression; transcription

Prostate cancer is the most frequently diagnosed male cancer and second leading cause of cancer deaths in North America [Jemal et al., 2004]. The transcriptional program activated by the androgen receptor (AR) plays a critical role in overall function of the prostate as well as growth and survival of normal and malignant prostate tissue. Therefore, the treatment regimen for locally advanced, relapsed, or metastatic prostate cancer is based on inhibiting AR transcriptional activity. Although this approach is initially effective it is not curative, and with time prostate cancer will recur in a form resistant to further hormonal manipulations. Mounting evidence suggests that the AR is able to retain activity at this stage of the disease through aberrant mechanisms of activation. Therefore, many of the AR-regulated target genes that promote the growth and survival of prostate cancer cells are likely still expressed. An important aim of prostate cancer research is to identify what these key AR-regulated genes are, with the goal of exploiting them as therapeutic targets in advanced prostate cancer. In this review, we will focus on the gene transcription program regulated by the AR in the prostate. Specifically, we summarize the enormous amounts of data that have recently been generated by gene expression profiling and determine how these studies have extended our knowledge of androgen action in normal and malignant prostate cells.

THE ANDROGEN SIGNALING AXIS

The AR is a 110 kDa phosphoprotein and member of the nuclear receptor transcription factor superfamily [Lamb et al., 2001]. The AR is the key mediator of androgen action in target tissues such as the prostate. Testosterone is produced by Leydig cells in the testes and is the most abundant (~90%) androgen in the circulation. Androgens produced by the adrenal cortex,

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such as dehydroepiandrosterone (DHEA) and androstenedione (4-dione), make up the remaining 10%. Both DHEA and 4-dione are converted to testosterone in peripheral tissues [Labrie et al., 2001].

The AR shares a common modular structure with other nuclear receptors, containing an N-terminal transactivation domain, a central DNA binding domain, and a C-terminal ligand-binding/transactivation domain [Heinlein and Chang, 2004]. Testosterone is able to diffuse into target cells and directly bind to and activate the AR. However, in target tissues such as the prostate, testosterone is first converted to dihydrotestosterone (DHT) by the enzymes 5α-reductase type I and II [Steers, 2001; Navarro et al., 2002]. DHT is a more potent AR ligand than testosterone because it dissociates more slowly from the AR and induces a receptor conformation more resistant to degradation [Heinlein and Chang, 2004]. In the basal, unliganded state, the AR exists in the cytoplasm in a complex that includes molecular chaperones and cochaperones from the heat shock protein (Hsp) family such as Hsp90, Hsp70, and Hsp56 [Heinlein and Chang, 2004]. This complex is essential for the generation of a high-affinity, ligand-binding conformation of the AR. After binding to androgen, there is a change in the conformation and composition of this complex, which leads to AR nuclear translocation. In the nucleus, the AR binds androgen response elements (AREs) as a dimer in the promoter and enhancer regions of various target genes [Heinlein and Chang, 2004].

To transcriptionally activate target genes, ARE-bound AR relies on the activity of coactivator proteins. Many of these coactivators are important for transcriptional activation by other steroid hormone receptors, and include the p160 family (SRC-1, GRIP1/TIF2, RAC3/pCIP/ACTR/AIB1/TRAM1), P/CAF, CBP, and p300 [Grossmann et al., 2001]. These coactivators possess intrinsic histone acetyltransferase (HAT) activity, which can be directed towards histone as well as other proteins [Roth et al., 2001]. In addition to coactivators with HAT activity, the AR has been shown to specifically recruit the AR-associated (ARA) coactivators ARA70, ARA55, and ARA54 [Grossmann et al., 2001]. The AR-induced assembly of these multi-protein complexes results in a finely regulated level of target gene transcription.

In addition to the long-recognized role of the AR as a transcription factor, recent studies have revealed an important role for AR as a cytoplasmic signaling molecule. The first hints at this phenomenon were provided when it was discovered that ER antagonists could block androgen-induced proliferation of LNCaP prostate cancer cells [Migliaccio et al., 2000]. Subsequent studies demonstrated that this was due to an androgen- or estrogen-induced complex formed between AR, estrogen receptor (ER)α or ERβ, and c-Src. Indeed, androgen or estrogen stimulation results in rapid (2–15 min) activation of the Src/Raf-1/ERK pathway, which can be blocked by either antiandrogens or antiestrogens. These effects are completely independent of AR DNA binding, but rather result from direct association between a proline-rich region in the AR N-terminus and the c-Src SH3 domain [Migliaccio et al., 2000]. Importantly, this non-genotropic mode of AR signaling appears to be intact in osteoblasts, thus mediating the antiapoptotic activities of estrogens and androgens in these cells [Kousteni et al., 2001]. Furthermore, in NIH3T3 murine fibroblasts, which express barely detectable levels of AR protein, very low concentrations of androgens can stimulate DNA synthesis while higher concentrations can induce cytoskeletal changes. Both of these effects appear to be mediated via non-genotropic AR signaling pathways [Castoria et al., 2003]. As a result of these important discoveries, a full understanding of androgen action must also consider the cellular activities regulated by cytoplasmic AR.

ANDROGEN ACTION IN ADULT PROSTATE TISSUE

The prostate is a small, walnut-sized sex accessory gland surrounding the urethra at the base of the bladder. Secretions from the prostate comprise a major fraction of the total seminal plasma volume of human ejaculate. The prostate epithelium forms glandular acini, which are separated from a supporting stroma by a basement membrane interface. Androgens mediate key physiological processes in these prostate tissue compartments such as differentiation, secretory function, metabolism, morphology, proliferation, and survival. The prostate epithelial cell compartment consists of three discrete cell types: basal cells situated along the basement membrane, luminal columnar secretory

cells, and a small fraction of neuroendocrine cells. The lineage and differentiation of these epithelial cells is regulated by the surrounding stroma [Sung and Chung, 2002]. In the adult prostate, the AR is expressed in both the epithelial and stromal cells [Litvinov et al., 2003]. Androgen ablation results in prostate involution, and the loss of epithelial cells via apoptosis. Subsequent androgen re-administration induces the prostate to regain normal size and function through rapid proliferation and differentiation of stem cells in the basal epithelial cell compartment [Litvinov et al., 2003]. Tissue grafting experiments in rodents have suggested that the AR functions primarily to maintain the differentiated secretory function of prostate epithelial cells [Kurita et al., 2001]. The androgen-dependent survival of the epithelial compartment is regulated in large part by paracrine factors expressed and provided by the supporting AR positive stromal cells [Kurita et al., 2001]. This complex signaling relationship between the epithelial and stromal compartments results in homeostasis whereby the proliferative and apoptotic indices in the prostate are balanced at approximately 1%–2% [Litvinov et al., 2003].

ANDROGEN ACTION IN PROSTATE CANCER

The normal human prostate is divided into three discrete regions, defined by their location in relationship to the urethra. Importantly, the cells within these zones vary significantly in their contribution to the prevalence of prostate cancer [Che and Grignon, 2002]. For example, the transition zone comprises 5%–10% of glandular tissue, and accounts for 20% of prostate adenocarcinomas. The central zone comprises approximately 25% of the glandular tissue; however cancer arising from these tissues is rare at 1%–5%. The peripheral zone makes up the bulk of prostate tissue (~70%) and accounts for the majority (~70%) of cancers. Regardless of the origin of the neoplasm, these cancer cells initially remain dependent on androgens for growth and survival. Therefore, if surgery is not curative, castration and/or administration of AR antagonists are the mainstays of systemic therapy. Initially, this androgen ablation results in marked AR inhibition, as indicated by reduced expression its target gene, prostate-specific antigen (PSA), and concomitant tumor regression [Feldman and Feldman, 2001]. Invariably, however, prostate cancer relapses in a form that is resistant to these hormonal manipulations, and further treatment is essentially palliative [Grossmann et al., 2001]. This stage of the disease is referred to as androgen-independent, androgen-refractory, or androgen depletion-independent (ADI) [Roy-Burman et al., 2005]. Although ADI prostate cancer is resistant to further attempts at blocking androgen action, the AR remains a critical factor for the growth and survival of the majority of these tumors [Feldman and Feldman, 2001; Grossmann et al., 2001; Litvinov et al., 2003; Heinlein and Chang, 2004]. For example, most ADI prostate cancer retains high levels of AR expression [Buchanan et al., 2001]. Moreover, the *PSA* gene continues to be expressed at this stage of the disease [Heinlein and Chang, 2004]. Indeed, ribozyme, antisense, and small-interfering RNA (siRNA) approaches have shown that targeted inhibition of the AR decreases PSA expression, cell proliferation, and survival in various cell-based models of ADI prostate cancer [Eder et al., 2000; Zegarra-Moro et al., 2002; Haag et al., 2005; Liao et al., 2005]. These findings suggest that ADI prostate cancer cells continue to proliferate and survive through aberrant mechanisms of AR activation, and thus the AR signaling axis remains a valid target for therapy. A major goal has been to elucidate the critical downstream targets of AR signaling which play a critical role in the growth and survival of ADI prostate cancer cells.

GLOBAL PROFILING OF ANDROGEN ACTION IN THE PROSTATE

There have been many studies in the past several years aimed at comprehensively unraveling the complete androgen-regulated gene expression program in normal and cancerous prostate cells. The huge mass of data associated with these reports can be attributed to new technologies allowing researchers to study the expression of thousands of individual genes in response to androgenic stimuli. A summary of these studies is depicted in Table I.

Model Systems Used to Discover Androgen-Regulated Genes

The majority of large-scale gene expression studies have been performed in LNCaP cells, the most widely used model cell line for prostate cancer research [Waghray et al., 2001; Xu et al.,

TABLE I. Expression Profiling of Androgen-Regulated Genes

Study	Tissue source	Conditions	Profiling platform
Amler et al. [2000]	1. CWR22	1. AD[a] tumors established in male mice, followed by castration for 0–480 h.	Incyte UniGEM 1.0 cDNA array
	2. AI[a] subline	2. AI[a] tumors established in female mice	
Asirvatham et al. [2005]	rVPECs[a]	48 h serum starve, followed by 2–12 h 10 nM R1881	Affymetrix RAE 230A oligonucleotide array
Clegg et al. [2002]	LNCaP	24 h with 10% CSS[a], followed by 24 h ±1 nM R1881	EST[a] sequencing
DePrimo et al. [2002]	1. LNCaP	1. 48 h with 10% CSS[a], followed by 8–72 h 1 nM R1881	Custom cDNA arrays
	2. MDAPCa2a	2. 48 h with 10% CSS[a], followed by 24 h with 10-1,000 nM DHT	1. 24,000 cDNA elements
	3. MDAPCa2b 4. LAPC4	3. Culture in 10% CSS[a] with 1 nM R1881, followed by 48–72 h in 10% CSS[a]	2. 9,600 cDNA elements
Desai et al. [2004]	1. Rat VP[a]	1. Castrate rats for 72, 120, 168 h	AP Biosciences CodeLink UniSet Rat Bio array
	2. Rat DLP[a]	2. Castrate rats for 72 h, followed by daily administration of testosterone propionate (500 μg/dl) for 48 h	
Eder et al. [2003]	LNCaP	1. 48 h with AR antisense 2. 48 h with 10% CSS[a] 3. 48 h with bicalutamide	NIH 10 K cDNA array
Febbo et al. [2005]	LNCaP	48 h with 10% CSS[a], followed by 0–18 h with 0.1 nM R1881	Affymetrix U95A2 array
Jiang and Wang [2003]	1. Rat VP[a] 2. Rat DLP[a]	Castrate rats for 168 h, followed by administration of testosterone propionate (2 mg/rat) for 14 h	Incyte rat toxicity array
Meehan and Sadar [2004]	LNCaP	24 h serum starve, followed by 48 h ±10 nM R1881	ICAT 2-D LC MS/MS[a]
Nelson et al. [2002]	LNCaP	24 h with 10% CSS[a], followed by 0–48 h 1 nM R1881	Custom cDNA array with 3000 prostate specific cDNAs
Nantermet et al. [2004]	Rat VP[a]	Castrate rats for 216 h, followed by administration of 5α-DHT[a] (3 mg/kg) for 6–24 h.	Affymetrix RGU34A oligonucleotide array
Oosterhoff et al. [2005]	1. LNCaP 2. AI[a] subline	48 h with 5% CSS[a], followed by 1–72 h 0.1 nM R1881	Agilent Human 1A cDNA array
Pfundt et al. [2005]	1. Normal rat prostates	1. Prostates from intact mice versus 336 h castrate mice	Sigma Genosys 5K oligonucleotide set
	2. Tumors from Dunning model	2. H tumors (AD[a]) versus H1 (AI[a]) tumors from Dunning rat model	
Segawa et al. [2002]	LNCaP	120 h with 10% CSS[a], followed by 0–24 h with 0.1 or 10 nM R1881	Affymetrix HuGeneFL array
Shi et al. [2004]	1. LNCaP 2. 3 AI[a] sublines	120 h with 5% CSS[a] or 120 h complete serum	Affymetrix Human Genome U95A oligonucleotide array
Velasco et al. [2004]	LNCaP	24 h with 5% CSS[a], followed by 2–72 h 10 nM DHT[a]	Affymetrix Hu6800FL oligonucleotide array
Waghray et al. [2001]	LNCaP	72 h with 5% CSS[a], followed by 24 h ±1 nM DHT[a]	1. SAGE[a] 2. 2-DGE/ MALDI-TOF MS[a]
Xu et al. [2001]	LNCaP	120 h with 5% CSS[a], followed by 24 h ±10 nM R1881	SAGE[a]

[a]Abbreviations: AD, androgen-dependent; AI, androgen-independent; CSS, charcoal-stripped (steroid-depleted) serum; DHT, dihydrotestosterone; DLP, dorsolateral prostate; EST, expressed sequence tag; ICAT 2-D LC MS/MS, isotope coded affinity tag 2-dimensional liquid chromatography tandem mass spectrometry; SAGE, serial analysis of gene expression; VP, ventral prostate; 2-DGE/ MALDI-TOF MS, 2-dimensional gel electrophoresis/matrix-assisted laser desorption ionization-time-of-flight mass spectrometry.

2001; Clegg et al., 2002; DePrimo et al., 2002; Nelson et al., 2002; Segawa et al., 2002; Eder et al., 2003; Meehan and Sadar, 2004; Shi et al., 2004; Velasco et al., 2004; Febbo et al., 2005; Oosterhoff et al., 2005]. The strength of this system is that it is one of the few cell-based models that retains several of the salient features of human prostate cancer. For example, LNCaP cells are epithelial in origin, express the AR as well as AR-regulated genes, and are androgen sensitive for growth and survival in culture and as xenografts. Furthermore, as is the case for the majority of prostate cancer, androgen ablation only temporarily affects the growth and survival of these cells, resulting in eventual progression to an ADI phenotype. However, the major caveat of this cell line is a T877A mutation in the AR that renders it responsive to other steroids, such as glucocorticoids and progestins, as well as antiandrogens such as flutamide. Therefore any studies with respect to AR function in these cells must be interpreted in the context of a mutant receptor. The second most frequently used model system

has been the rat prostate [Jiang and Wang, 2003; Desai et al., 2004; Nantermet et al., 2004; Pfundt et al., 2005]. The main caveat of this system is the fundamental anatomical differences between human and rat prostates. Whereas the human prostate is an encapsulated organ, the rat prostate is composed of distinct dorsal, lateral, ventral, and anterior lobes. The ventral prostate is most commonly studied because this lobe behaves in a manner similar to normal human prostate tissue, wherein the epithelial compartment undergoes massive apoptosis in response to androgen ablation. Re-administration of androgens results in restored size and function. While data can be interpreted in the context of a wild-type AR when the rat ventral prostate is studied, care must be taken because results arise from the heterogeneous mixture of cell types within this organ. Other model systems that have been employed include rat ventral prostate epithelial cells (rVPECs) [Asirvatham et al., 2005] and a variety of human prostate cancer cell lines such as CWR22 [Amler et al., 2000], MDACaP2a, MDACaP2b, and LAPC4 [DePrimo et al., 2002].

Androgen Responsive Genes in Prostate Cancer Cells

Profiling the expression of thousands of genes in cells of prostate origin has allowed estimates to be made regarding the total number of genes expressed in prostate cells, referred to as the prostate transcriptome. Serial analysis of gene expression (SAGE) and massively parallel signature sequencing (MPSS) are two similar technologies that allow the transcriptome to be determined for an RNA sample isolated from a collection of cells. SAGE and MPSS are not selective for mRNAs that encode proteins, which is an important consideration in light of recent estimates that the human genome contains roughly 25,000 distinct protein-coding genes and over 100,000 distinct non-coding polyadenylated mRNAs [Claverie, 2005]. Three independent studies using either SAGE [Waghray et al., 2001] or MPSS [Lin et al., 2005; Oudes et al., 2005] have identified between 10,570 and 15,541 individual cDNAs and expressed sequence tages (ESTs) in LNCaP and LNCaP-derived cells. A separate study of the LNCaP transcriptome using SAGE identified 23,448 individual known genes or ESTs [Xu et al., 2001]. Therefore, the LNCaP transcriptome is likely to be between 10,570 and 23,448 polyadenylated RNAs. Remarkably, regardless of the androgen concentration, time-course, platform, or method of data analysis used in various expression profiling studies, the percentage of the LNCaP transcriptome that is either directly or indirectly regulated by androgens is consistently found to be between 1.5% to 4.3%. SAGE-based approaches have provided low-end estimates, suggesting that 1.5% (351/23,448) to 2.1% (351/16,750) of detected genes are androgen-regulated in LNCaP cells [Waghray et al., 2001; Xu et al., 2001]. Mid-range estimates have arisen from oligonucleotide array experiments, which have suggested that 2.8% (98/3500) to 3.7% (251/6502) genes are androgen-regulated [Segawa et al., 2002; Velasco et al., 2004]. A high-end estimate comes from a cDNA array hybridization experiment, which suggested that 4.3% (195/4,491) of detected genes are androgen-regulated [Oosterhoff et al., 2005]. In a protein expression profiling experiment, 1,031 individual proteins were detected, 44 of which displayed a change in intensity in response to androgens [Waghray et al., 2001]. This finding suggests that a similar fraction of cellular proteins are androgen-regulated (4.3%). However, this study concurrently analyzed transcript expression by SAGE, and very little correlation between the androgen regulation of transcripts and proteins was observed [Waghray et al., 2001]. This could be due to the lack of sensitivity of current protein profiling technologies compared to the sensitivity of gene expression profiling technologies. This is demonstrated by the recent use of isotope-coded affinity tag (ICAT) labeling coupled with 2-dimensional liquid chromatography and tandem mass spectrometry (LC-MS/MS) for the identification of androgen-regulated proteins in LNCaP cells. This approach identified 139 proteins, 77 of which quantitatively displayed androgen-regulation [Meehan and Sadar, 2004]. However, ICAT-LC-MS/MS does have a greater potential to provide deeper coverage, as a more recent study obtained quantitative profiles of 940 proteins in LNCaP and LNCaP-derived ADI cells [Lin et al., 2005].

MOLECULAR REGULATION OF ANDROGEN ACTION IN THE PROSTATE

Collectively, the numerous gene expression profiling studies have provided important

global overviews of androgen action in prostate cells. However, the ultimate goal of such studies is the identification of androgen-regulated genes that are key to fundamental processes in normal and cancerous prostate tissue. Sophisticated bioinformatics-based analysis tools have played the most significant role in advancing prostate cancer research towards this goal. Assigning identified androgen-regulated genes to common pathways or regulatory processes, or groups with common kinetics of induction/repression are examples of these approaches. As a result, these methods of analyses have identified sets of genes that are candidate regulators of prostate function, growth, and survival. In addition, the bioinformatics-based identification of putative regulatory AREs for these androgen-regulated genes has led to a candidate list of direct transcriptional targets of the AR [Nelson et al., 2002; Nantermet et al., 2004; Asirvatham et al., 2005]. In the next section, we will discuss how gene expression profiling coupled with validation and thorough bioinformatic analysis has furthered our molecular understanding of androgen action in normal and cancerous prostate tissue. In addition, we will discuss several recent reports that have identified and characterized androgen-regulated genes that are likely key mediators of growth and survival of prostate cancer. These molecular determinants of androgen action are summarized in Figure 1.

Androgen Regulation of Differentiation and Prostate Function

The primary function of the prostate is to produce secretions rich in proteins, organic solutes, lipids, and cholesterol. These secretions, which are a major component of seminal fluid, support male fertility and insemination and also protect the lower urinary tract from infection. Androgens are key regulators of this function. Indeed, some of the most abundant proteins in human semen, such as PSA, kallikrein 2 (hK2), and prostatic acid phosphatase, are also the best-characterized androgen-regulated genes [Nelson et al., 1998]. PSA is a serine protease responsible for liquefaction of semen and is initially secreted as an inactive zymogen, which must first be catalytically activated by

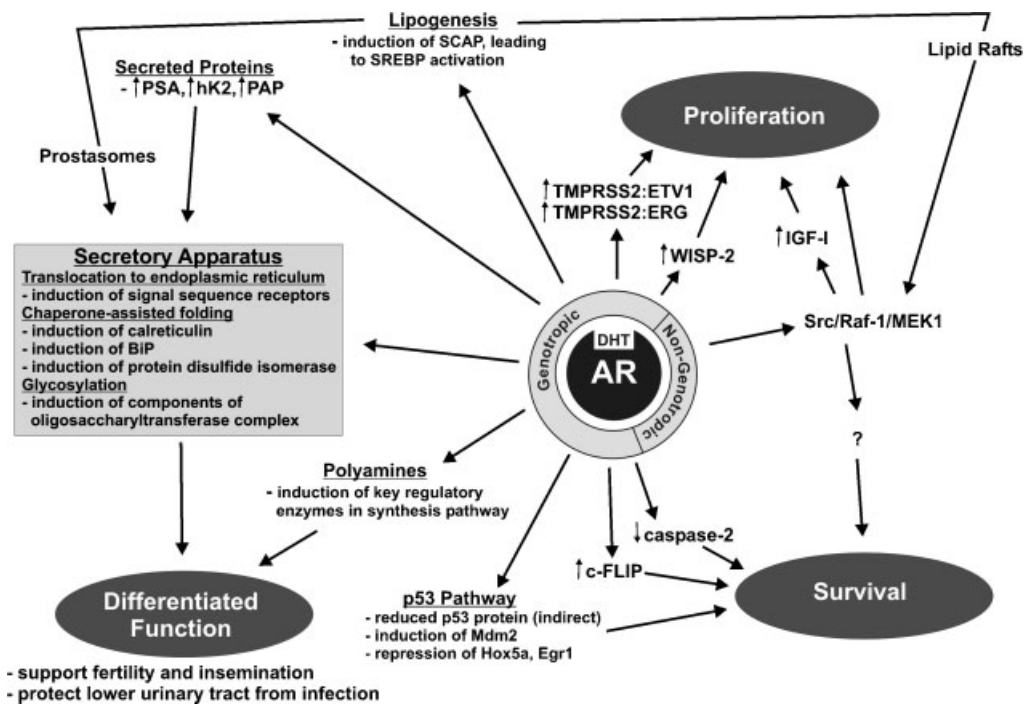

Fig. 1. A schematic representation of androgen action in prostate cancer cells. The androgen receptor (AR) modulates key effector molecules through transcriptional activation/repression and non-genotropic signaling. These key effector molecules mediate the effects of the active AR on differentiated cell function, cell proliferation, and cell survival. Genes and pathways modulated by the AR are described in detail in the text.

the serine protease hK2 [Lilja, 2003]. Prostatic acid phosphatase is a non-specific phosphatase and its precise role in seminal plasma has not been clearly elucidated.

In addition to these well-characterized androgen-regulated genes, many of the additional candidates identified in LNCaP and rat ventral prostate gene expression profiling experiments are predicted to be secreted proteins, or play a role in prostate secretory function. For example, a recent study investigated the genes that are activated within 6 or 24 h by DHT in the rat ventral prostate following 9 days of castration [Nantermet et al., 2004]. This time period was chosen because it precedes the massive revascularization and proliferation of the ventral prostate, thus providing a greater opportunity to identify bona fide androgen-regulated genes. Of the 234 androgen-regulated genes identified in this study, 62 are known to play a role in protein synthesis, degradation or maturation, and 24 are known to play a role in regulating secretory activity. Remarkably, most of the 86 genes involved in these processes displayed induction in response to DHT. In contrast, for the remaining androgen-regulated genes identified in this study, the number of genes induced was similar to the number of genes repressed. In line with these findings, a study that analyzed the response of LNCaP cells to R1881 over a 72 h time course found that over 200 of a total 517 androgen-regulated genes contribute to the synthesis and modification of secretory proteins [DePrimo et al., 2002]. Genes induced by androgens in these studies have been shown to participate in all steps of maturation of secreted proteins, including transport to the endoplasmic reticulum, folding, and glycosylation. For example, in the rat ventral prostate, signal sequence receptor γ (SSR3), which regulates nascent polypeptide entry into the endoplasmic reticulum, was induced fivefold within 6 h, and 10.4-fold within 24 h of DHT administration [Nantermet et al., 2004]. Similarly, signal sequence receptor Δ (SSR4) was induced 2.6-fold within 24 h [Nantermet et al., 2004]. Sequence search algorithms identified three putative AREs upstream of the SSR4 transcription start sites conserved across human, mouse, and rat genomes [Nantermet et al., 2004]. Genes that regulate chaperone-assisted folding, such as calreticulin, protein disulfide isomerase, and BiP were also characterized as androgen-induced [Nantermet et al., 2004]. Genes involved in glycosylation such as UDP-galactose 4-epimerase (2.8-fold at 6 h, 18.2-fold at 24 h), UDP-galactose: *N*-acetylglucosamine β1,4-galactosyltransferase (3.2-fold at 6 h, 15.3-fold at 24 h), and α1,3-fucosyltransferase (7.2-fold at 6 h, 15.1-fold at 24 h) were some of the genes most strongly induced by androgens in the rat ventral prostate [Nantermet et al., 2004]. Components of the endoplasmic reticulum oligosaccharyltransferase complex such as ribophorins I and II, defender against cell death-1 (DAD1), and glucosidase-1, were also identified as androgen-induced genes [Nantermet et al., 2004]. Importantly, a separate gene expression profiling study also reported *DAD1* as a gene induced by androgens in the ventral, dorsal, and lateral lobes of the rat prostate [Jiang and Wang, 2003]. A rapid and dose-dependent induction of DAD1 by androgens was further confirmed via Northern blot of RNA from rat prostates as well as LNCaP cells [Jiang and Wang, 2003]. Additionally, three putative AREs were identified in regulatory regions upstream of the DAD1 transcription start sites in human, mouse, and rat genomes [Nantermet et al., 2004].

Prostatic secretions are also rich in polyamines such as choline, spermine, and spermidine. Ornithine decarboxylase (ODC) is an enzyme that catalyzes one of the first committed steps of polyamine synthesis and is a well-described direct transcriptional target of the AR. S-adenosylmethionine (SAM) decarboxylase is another enzyme that catalyzes one of the first steps of polyamine synthesis. Studies in LNCaP cells [DePrimo et al., 2002] as well as the rat ventral prostate [Nantermet et al., 2004] have shown that SAM decarboxylase is rapidly induced by androgens. In addition, spermine synthase and spermidine/spermine N-acetyltransferase have both been demonstrated to be androgen-regulated in LNCaP cells and the rat ventral prostate [DePrimo et al., 2002; Nantermet et al., 2004]. Proteomic analysis of LNCaP cells also revealed that spermine synthase is androgen-induced at the protein level [Meehan and Sadar, 2004]. These findings suggest the high polyamine content of prostate secretions is due in large part to the coordinate androgen regulation of key enzymes involved in polyamine biosynthesis.

Prostatic secretions provide the bulk lipid content of seminal plasma. Indeed, androgen stimulation of LNCaP cells results in marked

lipogenesis, leading to accumulation of fatty acids and cholesterol [Swinnen et al., 2004]. The physiological significance of lipid accumulation in response to androgens is not clear. This accumulation may simply provide the building blocks to make the cellular membranes needed for cell division. Alternatively, increased lipid content may alter the composition, concentration, and/or function of lipid rafts, which are ordered lipid domains that spatially and temporally control cell signaling. Yet another hypothesis is that increased lipogenesis is necessary for the synthesis of prostasomes, which are prostate secretory vesicles in human semen [Ronquist and Nilsson, 2004]. Regardless of the role of increased lipid content in the prostate, most gene expression profiling studies have demonstrated that a network of genes involved in lipogenesis is under androgenic control [Swinnen et al., 2004]. Most of the genes within this network are likely not direct transcriptional targets of the AR, but rather are transcriptional targets of sterol regulatory element binding proteins (SREBPs). SREBPs are synthesized in an inactive form and retained in endoplasmic reticulum membranes in association with SREBP cleavage activating protein (SCAP) [Swinnen et al., 2004]. SCAP regulates the activation of SREBPs in response to decreased cellular lipid concentrations [Swinnen et al., 2004]. Recently, SCAP has been shown to be a direct transcriptional target of the AR, via an ARE in intron 8 of the gene [Heemers et al., 2004]. This finding likely explains the mechanisms underlying the wealth of gene expression profiling data demonstrating androgenic regulation of lipogenic enzymes. This serves as an excellent example of how androgenic regulation of a key regulatory enzyme is likely to render an entire metabolic pathway responsive to androgens in prostate cells.

Androgen Regulation of Proliferation

Some of the most sought-after androgen-regulated genes are those involved in cell-cycle regulation. The success of androgen ablation therapy in prostate cancer is attributed in large part to the induction of cell-cycle arrest [Heinlein and Chang, 2004]. Key questions, therefore, are what are the direct AR targets that regulate normal and malignant prostate cell proliferation, and are these targets aberrantly activated in ADI prostate cancer? Based on androgen concentrations used in LNCaP cells, many gene expression profiling studies have not been adequately designed to answer these questions. For example, LNCaP and LAPC4 prostate cancer cells display a biphasic proliferative response to androgen stimulation: androgen concentrations of 0.1 nM stimulate proliferation, while 10 nM inhibits proliferation and induces differentiation. At an androgen concentration of 1 nM, which is most commonly used for gene expression profiling studies, LNCaP and LAPC4 cells do not display a proliferative response [de Launoit et al., 1991]. Conversely, The MDA PCa 2a and 2b cell lines proliferate in response to 1 nM androgens [Ye et al., 1999]. Indeed, in comparing the gene expression profiles of these four prostate cancer cell lines in response to 1 nM R1881, DePrimo et al. [2002] observed that a distinct proliferation-associated cluster of genes was induced in MDA PCa 2a and 2b, but remained unaffected in LNCaP and LAPC-4 cell lines. This finding could explain why most gene expression profiling studies have been unable to reveal the critical androgen-regulated genes that drive cell proliferation. Nevertheless, several proliferation- and transformation-associated genes have recently been reported that could play important roles in androgen-dependent prostate cancer growth.

Wnt-induced secreted protein-2 (WISP-2), also known as connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed 5 (CCN5) is a secreted growth factor that binds and activates integrins and stimulates mitosis as well as other cellular processes [Brigstock, 2003]. Previous gene expression profiling of estrogen-regulated genes in breast cancer cells revealed WISP-2 induction. Indeed, subsequent studies demonstrated that WISP-2 is a direct ER transcriptional target [Brigstock, 2003]. In cultured rat ventral prostate epithelial cells (rVPECs), WISP-2 is induced by androgens within 12 h as assessed by both array-based profiling and quantitative RT-PCR confirmation. Further analysis revealed two putative AREs upstream of the transcription start site [Asirvatham et al., 2005]. Taken together, these findings suggest that WISP-2 is a common target of both the AR and ER signaling pathways, and may play an important role in the development and progression of these hormone-related cancers.

Increased insulin-like growth factor-I (IGF-I) levels in the serum have been associated with an

increased risk of prostate cancer [Kaaks et al., 2000]. In model systems, IGF-I has been linked to prostate cancer cell proliferation in culture and the progression of prostate cancer xenografts to an androgen refractory phenotype. Previous studies of a potential link between IGF-I signaling and androgens suggested that IGF-I modulates AR activity by activating the PI3K/Akt or Ras/MAPK pathways, leading to AR phosphorylation and sensitization to low concentrations of androgens [Heinlein and Chang, 2004]. Interestingly, the IGF-I receptor (IGF-IR) is induced by androgens at the mRNA and protein level in LNCaP cells [Pandini et al., 2005]. Quantitative RT-PCR demonstrated that IGF-IR induction is rapid, but is independent of AR DNA-binding activity. Rather, IGF-IR androgen responsiveness appears to be dependent on the Src/MAPK pathway, since various modes of inhibition of this pathway can block IGF-IR induction. Functionally, this non-genotropic induction of IGF-IR was shown to sensitize prostate cancer cells to IGF-I-induced proliferation as well as chemoinvasion [Pandini et al., 2005].

One of the most significant recent findings with respect to prostate cancer development and progression is the existence of recurrent chromosomal translocations, which result in novel fusions between the *TMPRSS2* gene and the Ets transcription factor family members, ERG or ETV1 [Tomlins et al., 2005]. *TMPRSS2* was first discovered as an androgen-regulated gene by microarray-based profiling of LNCaP cells [Lin et al., 1999]. As determined via Northern blot, TMPRSS2 mRNA is induced within 2 h of androgen stimulation and reaches a maximum level within 24 h [Lin et al., 1999]. Subsequent studies have shown that TMPRSS2 expression is largely prostate restricted in its expression, localized to prostate luminal epithelial cells [Afar et al., 2001; Vaarala et al., 2001], and likely androgen-regulated by virtue of a putative ARE located 148bp upstream from the transcription start site [Lin et al., 1999]. The development and application of a novel bioinformatic tool termed cancer outlier profile analysis (COPA) led to the identification of fusions between TMPRSS2 and ERG or ETV1 [Tomlins et al., 2005]. COPA was applied to existing gene expression data sets to search for marked overexpression of oncogenes in subsets of cancerous versus normal tissues. Strong outlier profiles were identified for ERG and ETV1, and overexpression of these genes was confirmed for a subset of cancerous prostate tissue. ERG or ETV1 overexpression was not observed in prostate intraepithelial neoplasia (PIN), the precursor to prostate cancer, or benign prostate tissue. Using a combination of genomic analysis techniques, ERG or ETV1 overexpression was attributed to chromosomal translocations, which result in various fusions between the 5′ end of the *TMPRSS2* gene and the 3′ end of either ERG or ETV1. Indeed, as would be predicted, these translocations render expression of ERG and ETV1 androgen-responsive. It is tempting to speculate that these fusion products are the long sought-after key regulators of prostate cancer development. However further studies are necessary to address this hypothesis. At the present time, the exact prevalence of these mutually exclusive translocations in prostate cancer is unknown. However, initial estimates suggest that it could occur in up to 80% of prostate cancers [Tomlins et al., 2005].

Androgen Regulation of Apoptosis/Survival

Another set of sought-after androgen-regulated genes are those involved in cell survival. Perhaps the best-described apoptotic regulator is the *p53* tumor suppressor gene. While *p53* has not been described as an androgen-regulated gene, gene expression profiling studies have suggested that *p53* is under androgenic control in the prostate. For example, in LNCaP cells, androgens significantly decrease cellular p53 levels in a dose- and time-dependent manner [Rokhlin et al., 2005]. Similarly, in the rat ventral prostate, androgens decrease the overall level of cellular p53, which correlates with decreased expression of p53 in the nucleus. The decreased expression of p53 observed in the rat ventral prostate is blocked by cyclohexamide, thus suggesting an indirect mechanism of repression [Nantermet et al., 2004]. Indeed, expression profiling demonstrated that androgens induce the expression of the negative p53 regulator Mdm2, and decrease the expression of the positive p53 regulators, Hox5a and Egr1 [Nantermet et al., 2004]. Furthermore, gene expression changes consistent with decreased p53 function have been observed in the rat ventral prostate in response to androgens: the p53-activated genes *Rb*, *Scyd1*/fractalkine, *IGFBP3*, and *BTG-2*/*TIS21*/*PC3* are all repressed, while the p53-repressed gene, hexokinase

II, is induced [Nantermet et al., 2004]. Therefore, androgens likely influence prostate cell survival in part by negatively regulating the p53 pathway.

An additional apoptotic regulator, caspase-2, has recently been shown to be a direct target of the AR in LNCaP and other prostate cancer cells [Rokhlin et al., 2005]. Caspase-2 is highly conserved across species and is unique in its class in that it possesses both initiator and effector caspase activities [Zhivotovsky and Orrenius, 2005]. In investigating the mechanisms by which androgens protect LNCaP cells from death induced by tumor necrosis factor α (TNFα), Rokhlin et al. [2005] found that caspase-2 expression is decreased by androgens in a dose-dependent manner. They further identified a consensus ARE within intron 8 of the caspase-2 gene and confirmed its occupancy by the AR using chromatin immunoprecipitation (ChIP). Their findings demonstrate that caspase-2, a critical regulator of apoptosis in response to TNFα, is repressed by androgens in prostate cancer cells.

Androgens also protect cells from apoptosis by inducing the expression of c-FLIP, an inhibitor of Fas/FasL-mediated apoptosis [Gao et al., 2005]. c-FLIP inhibits pro-apoptotic Fas signaling by directly blocking caspase-8 activation at the death-induced signaling complex (DiSC) [Thome and Tschopp, 2001]. c-FLIP is directly induced by androgens in LNCaP cells via a cluster of four AREs within a 156 bp region downstream from the transcription start site [Gao et al., 2005]. The importance of c-FLIP for prostate cancer survival has been further demonstrated by overexpression in LNCaP cells. c-FLIP overexpression accelerated the transition to androgen refractory growth for LNCaP xenografts in castrated mice by inhibiting apoptosis [Gao et al., 2005].

CONCLUDING REMARKS

High-throughput gene expression profiling approaches coupled with traditional characterization of candidate genes have provided important glimpses into the molecular basis of androgen action in normal and malignant prostate tissue. These studies have revealed several key androgen-regulated genes that are likely responsible for the maintenance of prostate function, growth, and survival. Additional mechanistic studies are necessary to fully confirm the importance of these candidate genes for their predicted cellular processes. A critical remaining question is whether these targets are aberrantly activated in ADI prostate cancer. Recent comparisons of the gene expression profiles of androgen-dependent and ADI prostate cancer cells have provided initial clues to this question. For example, in comparing androgen-dependent LNCaP with ADI LNCaP-LN0 cells, Oosterhoff et al. [2005] demonstrated that 21% of the genes with constitutively higher expression in LN0 versus LNCaP are androgen-induced genes in LNCaP cells, and 14% of the genes with constitutively lower expression in LN0 versus LNCaP are androgen-repressed genes in LNCaP cells. Similarly, Amler and colleagues monitored gene expression profiles as androgen dependent CWR22 xenografts were subjected to androgen ablation and progressed to an ADI stage (CWR22-R). Their study demonstrated that the vast majority of the genes in CWR22 xenografts that were altered in response to castration were eventually restored in CWR22-R to the levels initially observed in intact mice [Amler et al., 2000]. Taken together, these findings suggest that many androgen-regulated genes display aberrant constitutive activation or repression in ADI prostate cancer. This supports the hypothesis that the AR is able to achieve critical levels of activity through various mechanisms of aberrant activation at this stage of the disease. Therefore, the AR as well as deregulated AR-responsive genes should be considered as valid targets for therapeutic intervention in ADI prostate cancer.

REFERENCES

Afar DE, Vivanco I, Hubert RS, Kuo J, Chen E, Saffran DC, Raitano AB, Jakobovits A. 2001. Catalytic cleavage of the androgen-regulated TMPRSS2 protease results in its secretion by prostate and prostate cancer epithelia. Cancer Res 61:1686–1692.

Amler LC, Agus DB, LeDuc C, Sapinoso ML, Fox WD, Kern S, Lee D, Wang V, Leysens M, Higgins B, Martin J, Gerald W, Dracopoli N, Cordon-Cardo C, Scher HI, Hampton GM. 2000. Dysregulated expression of androgen-responsive and nonresponsive genes in the androgen-independent prostate cancer xenograft model CWR22-R1. Cancer Res 60:6134–6141.

Asirvatham AJ, Schmidt M, Gao B, Chaudhary J. 2005. Androgens regulate the immune/inflammatory response and cell survival pathways in rat ventral prostate epithelial cells. Endocrinology.

Brigstock DR. 2003. The CCN family: A new stimulus package. J Endocrinol 178:169–175.

Buchanan G, Irvine RA, Coetzee GA, Tilley WD. 2001. Contribution of the androgen receptor to prostate cancer predisposition and progression. Cancer Metastasis Rev 20:207–223.

Castoria G, Lombardi M, Barone MV, Bilancio A, Di Domenico M, Bottero D, Vitale F, Migliaccio A, Auricchio F. 2003. Androgen-stimulated DNA synthesis and cytoskeletal changes in fibroblasts by a nontranscriptional receptor action. J Cell Biol 161:547–556.

Che M, Grignon D. 2002. Pathology of prostate cancer. Cancer Metastasis Rev 21:381–395.

Claverie JM. 2005. Fewer genes, more noncoding RNA. Science 309:1529–1530.

Clegg N, Eroglu B, Ferguson C, Arnold H, Moorman A, Nelson PS. 2002. Digital expression profiles of the prostate androgen-response program. J Steroid Biochem Mol Biol 80 :13–23.

de Launoit Y, Veilleux R, Dufour M, Simard J, Labrie F. 1991. Characteristics of the biphasic action of androgens and of the potent antiproliferative effects of the new pure antiestrogen EM-139 on cell cycle kinetic parameters in LNCaP human prostatic cancer cells. Cancer Res 51: 5165–5170.

DePrimo SE, Diehn M, Nelson JB, Reiter RE, Matese J, Fero M, Tibshirani R, Brown PO, Brooks JD. 2002. Transcriptional programs activated by exposure of human prostate cancer cells to androgen. Genome Biol 3: RESEARCH0032.

Desai KV, Michalowska AM, Kondaiah P, Ward JM, Shih JH, Green JE. 2004. Gene expression profiling identifies a unique androgen-mediated inflammatory/immune signature and a PTEN (phosphatase and tensin homolog deleted on chromosome 10)-mediated apoptotic response specific to the rat ventral prostate. Mol Endocrinol 18:2895–2907.

Eder IE, Culig Z, Ramoner R, Thurnher M, Putz T, Nessler-Menardi C, Tiefenthaler M, Bartsch G, Klocker H. 2000. Inhibition of LncaP prostate cancer cells by means of androgen receptor antisense oligonucleotides. Cancer Gene Ther 7:997–1007.

Eder IE, Haag P, Basik M, Mousses S, Bektic J, Bartsch G, Klocker H. 2003. Gene expression changes following androgen receptor elimination in LNCaP prostate cancer cells. Mol Carcinog 37:181–191.

Febbo PG, Lowenberg M, Thorner AR, Brown M, Loda M, Golub TR. 2005. Androgen mediated regulation and functional implications of fkbp51 expression in prostate cancer. J Urol 173:1772–1777.

Feldman BJ, Feldman D. 2001. The development of androgen-independent prostate cancer. Nature Rev Cancer 1:34–45.

Gao S, Lee P, Wang H, Gerald W, Adler M, Zhang L, Wang / SNM> YF, Wang Z. 2005. The androgen receptor directly targets the c-FLIP gene to promote the androgen-independent growth of prostate cancer cells. Mol Endocrinol 24:24.

Grossmann ME, Huang H, Tindall DJ. 2001. Androgen receptor signaling in androgen-refractory prostate cancer. J Natl Cancer Inst 93:1687–1697.

Haag P, Bektic J, Bartsch G, Klocker H, Eder IE. 2005. Androgen receptor down regulation by small interference RNA induces cell growth inhibition in androgen sensitive as well as in androgen independent prostate cancer cells. J Steroid Biochem Mol Biol 24:24.

Heemers H, Verrijdt G, Organe S, Claessens F, Heyns W, Verhoeven G, Swinnen JV. 2004. Identification of an androgen response element in intron 8 of the sterol regulatory element-binding protein cleavage-activating protein gene allowing direct regulation by the androgen receptor. J Biol Chem 279:30880–30887.

Heinlein CA, Chang C. 2004. Androgen receptor in prostate cancer. Endocr Rev 25:276–308.

Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ. 2004. Cancer statistics, 2004. CA Cancer J Clin 54:8–29.

Jiang F, Wang Z. 2003. Identification of androgen-responsive genes in the rat ventral prostate by complementary deoxyribonucleic acid subtraction and microarray. Endocrinology 144:1257–1165.

Kaaks R, Lukanova A, Sommersberg B. 2000. Plasma androgens, IGF-1, body size, and prostate cancer risk: A synthetic review. Prostate Cancer Prostatic Dis 3:157–172.

Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogen JA, Katzenellenbogen BS, Roberson PK, Weinstein RS, Jilka RL, Manolagas SC. 2001. Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: Dissociation from transcriptional activity. Cell 104:719–730.

Kurita T, Wang YZ, Donjacour AA, Zhao C, Lydon JP, O'Malley BW, Isaacs JT, Dahiya R, Cunha GR. 2001. Paracrine regulation of apoptosis by steroid hormones in the male and female reproductive system. Cell Death Differ 8:192–200.

Labrie F, Luu-The V, Labrie C, Simard J. 2001. DHEA and its transformation into androgens and estrogens in peripheral target tissues: Intracrinology. Front Neuroendocrinol 22:185–212.

Lamb DJ, Weigel NL, Marcelli M. 2001. Androgen receptors and their biology. Vitam Horm 62:199–230.

Liao X, Tang S, Thrasher JB, Griebling TL, Li B. 2005. Small-interfering RNA-induced androgen receptor silencing leads to apoptotic cell death in prostate cancer. Mol Cancer Ther 4:505–515.

Lilja H. 2003. Biology of prostate-specific antigen. Urology 62:27–33.

Lin B, Ferguson C, White JT, Wang S, Vessella R, True LD, Hood L, Nelson PS. 1999. Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. Cancer Res 59:4180–4184.

Lin B, White JT, Lu W, Xie T, Utleg AG, Yan X, Yi EC, Shannon P, Khrebtukova I, Lange PH, Goodlett DR, Zhou D, Vasicek TJ, Hood L. 2005. Evidence for the presence of disease-perturbed networks in prostate cancer cells by genomic and proteomic analyses: A systems approach to disease. Cancer Res 65:3081–3091.

Litvinov IV, De Marzo AM, Isaacs JT. 2003. Is the Achilles' heel for prostate cancer therapy a gain of function in androgen receptor signaling? J Clin Endocrinol Metab 88:2972–2982.

Meehan KL, Sadar MD. 2004. Quantitative profiling of LNCaP prostate cancer cells using isotope-coded affinity tags and mass spectrometry. Proteomics 4:1116–1134.

Migliaccio A, Castoria G, Di Domenico M, de Falco A, Bilancio A, Lombardi M, Barone MV, Ametrano D,

Zannini MS, Abbondanza C, Auricchio F. 2000. Steroid-induced androgen receptor-oestradiol receptor beta-Src complex triggers prostate cancer cell proliferation. EMBO J 19:5406–5417.

Nantermet PV, Xu J, Yu Y, Hodor P, Holder D, Adamski S, Gentile MA, Kimmel DB, Harada S, Gerhold D, Freedman LP, Ray WJ. 2004. Identification of genetic pathways activated by the androgen receptor during the induction of proliferation in the ventral prostate gland. J Biol Chem 279:1310–1322.

Navarro D, Luzardo OP, Fernandez L, Chesa N, Diaz-Chico BN. 2002. Transition to androgen-independence in prostate cancer. J Steroid Biochem Mol Biol 81:191–201.

Nelson PS, Ng WL, Schummer M, True LD, Liu AY, Bumgarner RE, Ferguson C, Dimak A, Hood L. 1998. An expressed-sequence-tag database of the human prostate: Sequence analysis of 1168 cDNA clones. Genomics 47: 12–25.

Nelson PS, Clegg N, Arnold H, Ferguson C, Bonham M, White J, Hood L, Lin B. 2002. The program of androgen-responsive genes in neoplastic prostate epithelium. Proc Natl Acad Sci USA 99:11890–11895.

Oosterhoff JK, Grootegoed JA, Blok LJ. 2005. Expression profiling of androgen-dependent and -independent LNCaP cells: EGF versus androgen signalling. Endocr Relat Cancer 12:135–148.

Oudes AJ, Roach JC, Walashek LS, Eichner LJ, True LD, Vessella RL, Liu AY. 2005. Application of Affymetrix array and Massively Parallel Signature Sequencing for identification of genes involved in prostate cancer progression. BMC Cancer 5:86.

Pandini G, Mineo R, Frasca F, Roberts CT, Jr, Marcelli M, Vigneri R, Belfiore A. 2005. Androgens up-regulate the insulin-like growth factor-I receptor in prostate cancer cells. Cancer Res 65:1849–1857.

Pfundt R, Smit F, Jansen C, Aalders T, Straatman H, van der Vliet W, Isaacs J, van Kessel AG, Schalken J. 2005. Identification of androgen-responsive genes that are alternatively regulated in androgen-dependent and androgen-independent rat prostate tumors. Genes Chromosomes Cancer 43:273–283.

Rokhlin OW, Taghiyev AF, Guseva NV, Glover RA, Chumakov PM, Kravchenko JE, Cohen MB. 2005. Androgen regulates apoptosis induced by TNFR family ligands via multiple signaling pathways in LNCaP. Oncogene 24:6773–6784.

Ronquist G, Nilsson BO. 2004. The Janus-faced nature of prostasomes: Their pluripotency favours the normal reproductive process and malignant prostate growth. Prostate Cancer Prostatic Dis 7:21–31.

Roth SY, Denu JM, Allis CD. 2001. Histone acetyltransferases. Annu Rev Biochem 70:81–120.

Roy-Burman P, Tindall DJ, Robins DM, Greenberg NM, Hendrix JC, Mohla S, Getzenberg RH, Isaacs JT, Pienta KJ. 2005. Androgens and prostate cancer: Are the descriptors valid? Cancer Biol Ther 4:4–5.

Segawa T, Nau ME, Xu LL, Chilukuri RN, Makarem M, Zhang W, Petrovics G, Sesterhenn IA, McLeod DG, Moul JW, Vahey M, Srivastava S. 2002. Androgen-induced expression of endoplasmic reticulum (ER) stress response genes in prostate cancer cells. Oncogene 21:8749–8758.

Shi XB, Ma AH, Tepper CG, Xia L, Gregg JP, Gandour-Edwards R, Mack PC, Kung HJ, deVere White RW. 2004. Molecular alterations associated with LNCaP cell progression to androgen independence. Prostate 60:257–271.

Steers WD. 2001. 5alpha-reductase activity in the prostate. Urology 58:17–24; discussion 24.

Sung SY, Chung LW. 2002. Prostate tumor-stroma interaction: Molecular mechanisms and opportunities for therapeutic targeting. Differentiation 70:506–521.

Swinnen JV, Heemers H, van de Sande T, de Schrijver E, Brusselmans K, Heyns W, Verhoeven G. 2004. Androgens, lipogenesis and prostate cancer. J Steroid Biochem Mol Biol 92:273–279.

Thome M, Tschopp J. 2001. Regulation of lymphocyte proliferation and death by FLIP. Nat Rev Immunol 1:50–58.

Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA, Chinnaiyan AM. 2005. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310:644–648.

Vaarala MH, Porvari KS, Kellokumpu S, Kyllonen AP, Vihko PT. 2001. Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. J Pathol 193:134–140.

Velasco AM, Gillis KA, Li Y, Brown EL, Sadler TM, Achilleos M, Greenberger LM, Frost P, Bai W, Zhang Y. 2004. Identification and validation of novel androgen-regulated genes in prostate cancer. Endocrinology 145:3913–3924. Epub 2004 May 6.

Waghray A, Feroze F, Schober MS, Yao F, Wood C, Puravs E, Krause M, Hanash S, Chen YQ. 2001. Identification of androgen-regulated genes in the prostate cancer cell line LNCaP by serial analysis of gene expression and proteomic analysis. Proteomics 1:1327–1338.

Xu LL, Su YP, Labiche R, Segawa T, Shanmugam N, McLeod DG, Moul JW, Srivastava S. 2001. Quantitative expression profile of androgen-regulated genes in prostate cancer cells and identification of prostate-specific genes. Int J Cancer 92:322–328.

Ye D, Mendelsohn J, Fan Z. 1999. Androgen and epidermal growth factor down-regulate cyclin-dependent kinase inhibitor p27Kip1 and costimulate proliferation of MDA PCa 2a and MDA PCa 2b prostate cancer cells. Clin Cancer Res 5:2171–2177.

Zegarra-Moro OL, Schmidt LJ, Huang H, Tindall DJ. 2002. Disruption of androgen receptor function inhibits proliferation of androgen-refractory prostate cancer cells. Cancer Res 62:1008–1013.

Zhivotovsky B, Orrenius S. 2005. Caspase-2 function in response to DNA damage. Biochem Biophys Res Commun 331:859–867.