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Regulation of Androgen Receptor Levels: Implications for Prostate Cancer Progression and Therapy

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Abstract Androgen deprivation has been the standard therapy for advanced and metastatic prostate cancer for over half a century, as prostate tumors are initially dependent on androgens for growth and survival. Unfortunately, in most patients undergoing androgen ablation, relapse (recurrent tumor growth) eventually occurs. The actions of the principal androgens, testosterone and dihydrotestosterone (DHT), are mediated via androgen receptors (ARs), ligand-activated transcription factors that belong to the nuclear receptor superfamily. Because of the presence of transcriptionally active ARs in tumors from recurrent or androgen-independent disease, there is a heightened interest in new therapeutic paradigms that target the AR and its regulatory pathways. The regulation of AR levels is highly complex with control exerted by several pathways and in a cell-, tissue-, and developmental-stage specific manner. Androgens are important regulators of AR mRNA and protein through transcriptional and post-transcriptional mechanisms. This article reviews the evidence implicating the AR in recurrent prostate cancer and discusses the multiple mechanisms that regulate AR levels in normal and neoplastic cells. The complexity of AR regulation suggests that there will be an ample array of potential new drug targets for modulating levels of this receptor, a key signaling molecule in prostate cancer. J. Cell. Biochem. 95: 657–669, 2005.

Key words: autoregulation; steroid receptor; transcription; androgen response elements; proteasome; degradation; androgen-independence

The androgen receptor (AR), like other steroid hormone receptors, is a ligand-activated transcription factor that belongs to a large family of nuclear receptor proteins, sharing a similar organization of functional domains [reviewed in Tsai and O'Malley, 1994]. AR comprises a divergent amino-terminus that contains a strong transcriptional activation function, a well-conserved DNA binding domain, and a large C-terminal ligand binding domain, which contains a second activation function [Kokontis and Liao, 1999; reviewed in Roy et al., 1999; reviewed in Gelmann, 2002]. Testosterone is the predominant circulating androgen in mammals and is converted to dihydrotestosterone (DHT) by 5α-reductase in certain tissues of the male urogenital tract, skin, and other target cells. DHT binds with highest affinity to AR and together with testosterone promotes AR transcriptional activity thereby ensuring the development and maintenance of male reproductive functions. Androgens also exert rapid effects initiated at the cell membrane but the physiologic significance of these actions is incompletely understood [reviewed in Heinlein and Chang, 2002b]. The unliganded AR resides predominately in the cytoplasm as a heteromeric complex with hsp90 and other chaperone proteins. These chaperone proteins maintain AR in a form that is receptive to ligand binding. Regulation of gene expression by androgen-activated AR occurs through receptor nuclear translocation, dimerization, and binding to androgen response elements (AREs) in the DNA of target genes. AR homodimers recruit a panoply of factors including coactivators and mediator proteins whose enzymatic activities promote chromatin remodeling and transcriptional regulation of target genes leading to cell differentiation, survival, and proliferation [reviewed in Heinlein and Chang, 2002a].

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Androgenic stimulation of the AR is not only essential but is also sufficient for the generation of the prostate gland [Marker et al., 2003] and is strongly implicated in development of prostate adenocarcinoma. Based on the suspected role of AR in prostate cancer initiation and progression, the regulation of AR transcriptional activity in prostate cancer cells and animal models has received substantial research attention and is the subject of several excellent, recent review articles [Culig et al., 2000; Balk, 2002; Heinlein and Chang, 2004; Santos et al., 2004; Taplin and Balk, 2004]. This article will focus on the more poorly understood mechanisms that control AR levels in target tissues and in prostate neoplasia. Recent findings from gene expression profiling analyses in advanced prostate cancers as well as from prostate cell and transgenic mouse models expressing AR strongly support the significance of AR expression and regulation in prostate adenocarcinoma [Stanbrough et al., 2001; Berger et al., 2004; Mellinghoff et al., 2004; Shah et al., 2004].

IMPORTANCE OF AR IN PROSTATE CANCER

Prostate Gland Architecture, Cell Types, and AR Expression

The human prostate gland consists of epithelial-lined acini with an underlying fibromuscular stromal layer. The epithelial cell compartment consists of slow growing well-differentiated secretory (luminal) cells, which are positive for both AR and prostate specific antigen (PSA), and a basal epithelial layer comprised of rapidly proliferating cells expressing low levels of AR and a small number of AR-negative, neuroendocrine cells [Bonkhoff et al., 1994]. Stem cells are presumed to exist within the basal cell population and these progenitors differentiate into basal, secretory, or neuroendocrine lineages. Androgen-directed development of the prostate initiates in the stromal compartment, which contains AR and produces paracrine factors that regulate growth and differentiation of the prostatic epithelium. In contrast, prostate cancer but not normal prostatic epithelial cells xenografted into AR-null nude mice grow in response to androgens suggesting that cancer development may result from an autocrine mechanism [Gao et al., 2001]. However, the cellular origins of prostate adenocarcinoma are unknown.

Androgen Dependence of Prostate Cancer

The dependence of prostate cancer on androgens was established more than half a century ago following Dr. Charles Huggins' Nobel prize-winning discovery that castration results in prostate cancer regression. Androgen deprivation therapy in its various surgical and medical forms continues to be the mainstay treatment for advanced prostate cancer with 80–90% of men undergoing a clinical remission. Complete androgen blockade is often instituted and consists of drugs to decrease testicular testosterone synthesis in conjunction with an AR antagonist. Androgen deprivation therapy is also under investigation in earlier stages of prostate adenocarcinoma in the adjuvant and neoadjuvant settings [reviewed in Hellerstedt and Pienta, 2002]. Despite an initial favorable response to androgen deprivation therapy, nearly all prostate cancer patients progress to androgen-independent or hormone refractory prostate cancer. This form of prostate cancer is incurable.

Because androgen-independent prostate cancer grows in the absence of testicular androgens and prostatic AR levels decrease following androgen ablation in rodents [Prins and Birch, 1993], it was assumed that AR protein would be downregulated in androgen-independent disease. Thus, it was unanticipated that locally progressed and metastatic prostate cancer specimens from patients with androgen-independent disease express AR protein [van der Kwast et al., 1991; Ruizeveld de Winter et al., 1994; Hobisch et al., 1995]. The maintenance of AR in androgen-independent prostate cancer is further supported by a recent study that assessed AR and other markers from metastatic, androgen-independent prostate cancer samples obtained from 30 men as part of a rapid autopsy program [Shah et al., 2004]. Although a high degree of heterogeneity was observed for all markers including AR, the majority of metastatic specimens express AR. Increased expression of AR in advanced prostate cancer can result from AR gene amplification, which occurs in approximately 30% of advanced prostate cancers [Visakorpi et al., 1995]. AR expressed in recurrent cancer appears to be functional based on its nuclear localization and the reacquisition of AR-regulated gene expression such as PSA [Gregory et al., 1998, 2001].

Experimental Models Demonstrate a Central Role for AR in Prostate Cancer Development and Androgen-Independence

To evaluate the role of AR in prostate growth and cancer development, Stanbrough et al. [2001] generated transgenic mice targeting AR (regulated by the prostate-specific probasin promoter) to prostate secretory epithelial cells. Overexpression of AR in prostatic epithelial cells of these mice resulted in enhanced cell proliferation. Although prostate adenocarcinoma did not develop, aged transgenic mice exhibited dysplastic prostate lesions, resembling high-grade human prostatic intraepithelial neoplasia (PIN). PIN is strongly suspected of being the precursor to prostate adenocarcinoma. This study provides direct evidence for the growth-promoting actions of AR and suggests that AR overexpression in prostate initiates oncogenic processes.

Greenberg and colleagues [Han et al., 2005] took a related approach to investigate the potential of AR to initiate prostate cancer. These investigators identified a highly conserved "signature motif" within the amino terminal region of the AR that is implicated in AR interactions with coregulatory proteins. ARs harboring a substitution in this region (E231G) exhibit higher transcriptional activity in the absence of ligand and increased sensitivity to coregulators [Han et al., 2001]. Strikingly, 100% of transgenic mice expressing AR-E231G in prostatic epithelium rapidly exhibited PIN that became invasive and metastatic. These studies demonstrate the critical role played by a variant AR, with a gain-of-function phenotype, in the initiation of aggressive prostate cancer.

The effects of introducing AR into immortalized, normal prostate epithelial cells lacking AR were recently investigated [Berger et al., 2004]. The AR-expressing prostate epithelial cells were tumorigenic when implanted into prostates of nude mice, whereas orthotopically implanted control prostate epithelial cells failed to produce tumors. The immortalized prostate epithelial cells were phenotypically basal cell-like; however, the AR-expressing cells had features of secretory cells when grown orthotopically. In addition, tumors formed from the AR-expressing cells were androgen-dependent for growth. Progression to androgen-independence was not assessed. Together, these studies support a central role for AR in prostate cancer development.

Progression to androgen-independence can be demonstrated experimentally in cell culture and in vivo. The human prostate cancer cell line LNCaP has proven valuable for studies of prostate cancer progression [Kokontis et al., 1994, 1998; Wu et al., 1994; Lu et al., 1999; Igawa et al., 2002]. LNCaP are AR-positive and exhibit growth stimulation at low concentrations of androgen [reviewed in Sobel and Sadar, 2005a]. Androgen induces PSA secretion in these cells. Further, since LNCaP are poorly tumorigenic in castrated nude mice, they are considered androgen-dependent. Extended culture of these cells in androgen-depleted media (or propagation as xenografts in castrated nude mice) results in the emergence of androgen-independent cells. The androgen-independent LNCaP cells express higher levels of AR than the parental LNCaP line, exhibit hypersensitivity to androgens and are tumorigenic in castrated mice [Kokontis et al., 1994, 1998; Umekita et al., 1996]. Disruption of AR by microinjection of hammerhead ribozymes or AR antibodies decreases the growth rate of androgen-independent LNCaP cells in the absence of androgens [Zegarra-Moro et al., 2002]. This study shows that proliferation of androgen-independent cells requires AR signaling.

A variety of prostate xenograft models are currently available that reproduce the transition from androgen-dependence to -independence [reviewed in Sobel and Sadar, 2005b]. A pivotal study by Chen et al. [2004] defined global gene expression changes associated with prostate cancer progression. Seven isogenic pairs of androgen-sensitive and androgen-refractory human prostate cancer xenografts were compared. Androgen-independent sublines were derived from their hormone-sensitive counterparts following serial passaging of xenografts in castrated nude mice. Remarkably, AR was the only gene (of >12,000 probe sets) that exhibited differential expression between all seven hormone-sensitive and refractory human prostate xenograft pairs. AR mRNA and protein were increased in each of the androgen-independent xenografts compared to its parental hormone-sensitive line. This upregulation of AR activity in hormone-refractory cancer is a necessary event in prostate cancer progression as knockdown of AR by RNA interference in refractory sublines dramatically reduced tumor growth in castrated mice. In the reciprocal experiments, introduction of AR into hormone sensitive

sublines resulted in a significantly shorter latency for tumor growth in castrated mice. Thus, AR is sufficient to confer androgen-independence in these model systems. These AR effects were dependent on AR nuclear localization and an intact receptor hormone binding domain. Further, upregulation of AR not only allowed cells to respond to very low levels of androgen but also resulted in limited agonistic action of a clinically used AR antagonist. This study strongly supports the therapeutic goal of reducing AR levels in advanced prostate cancer.

Proposed Mechanisms for Continued AR Function Under Conditions of Androgen Deprivation

As discussed above, androgen-independence results from upregulation of AR in the xenograft models. Clinical data including those showing the striking heterogeneity of metastatic prostate cancer [Shah et al., 2004] suggest that there are multiple mechanisms of androgen-independence. Functional inactivation of AR through mutation was initially proposed to explain maintenance of AR expression but loss of androgen-dependent growth. While somatic mutation of AR occurs with frequencies ranging from 20% to 40% in androgen-independent prostate cancer (particularly in men treated with antiandrogens), the vast majority of these mutations are gain-of-function, not inactivating [reviewed in Taplin and Balk, 2004]. In particular, many AR mutations, including the codon 877 threonine to alanine mutation found in LNCaP cells as well as in clinical samples, occur in the ligand binding domain of the receptor and result in a broadened range of ligand specificity. These mutations thus permit AR to regulate targets genes in response to other hormones or even AR antagonists [reviewed in Feldman and Feldman, 2001]. Additional mechanisms that enhance AR signaling have been proposed including increased levels of AR coactivators or decreased AR corepressors. Crosstalk between AR and other signaling pathways may increase AR sensitivity to low androgens or promote ligand-independent activation of AR. The AR gene contains a CAG repeat in exon 1 whose length is polymorphic (mean length is 21 repeats). Expression of AR containing shorter CAG repeat tracts has been correlated with earlier onset [Hardy et al., 1996] or increased risk of aggressive prostate cancer [Giovannucci et al., 1997]; however, recent reports based on large, prospective studies do not support a significant or strong association between this AR gene polymorphism and prostate cancer risk [Zeegers et al., 2004; Freedman et al., 2005]. Finally, another model suggests that AR may be bypassed in favor of other signaling pathways that drive growth and survival of prostate cancer cells.

The results from experimental models of prostate cancer progression and from analysis of androgen levels in human prostate cancer clinical samples have prompted a reconsideration of terminology [Mohler et al., 2004]. Local levels of androgens in androgen-independent tumors may be sufficient to activate AR, made hypersensitive as a result of mechanisms discussed above. Because of this finding the term androgen-independence will be considered synonymous with recurrent or hormone-refractory prostate cancer.

REGULATION OF AR LEVELS

AR levels vary during development and aging (e.g., see Takane et al. [1991a]; Prins et al. [1996]) and these fluctuations are significant as sensitivity of cells and tissues to androgens is directly related to AR content [Takane et al., 1991a,b; McPhaul et al., 1993]. As discussed above, upregulation of AR can drive progression to androgen-independent prostate cancer [Chen et al., 2004]. Thus, elucidating the varied and cell-specific mechanisms that regulate AR mRNA and protein is essential to understanding hormonal responsiveness and may provide novel therapeutic targets.

AR Promoter

The human AR gene contains a single promoter that lacks typical TATA or CAAAT box motifs [Tilley et al., 1990]. This promoter is utilized in a variety of cell lines and AR-expressing tissues [Tilley et al., 1990]. Cis-acting sequences that contribute to AR promoter activity in humans or rodents include binding sites for Sp1 [Faber et al., 1993; Chen et al., 1997], NFκB [Supakar et al., 1995; Zhang et al., 2004], cAMP response element binding protein [Mizokami et al., 1994], NF1 [Song et al., 1999] as well as sites for unidentified proteins including an age-dependent [Supakar et al., 1993], and a negative regulatory factor(s) that binds in conjunction with NF1 to a composite element [Song et al., 1999]. Two

uncharacterized, single strand DNA binding proteins, mARs [Grossmann and Tindall, 1995] and ssPyrBF [Chen et al., 1997], influence basal mouse AR promoter activity. The human AR gene also contains a suppressor element (ARS); however, this site is located in the 5′-untranslated region. Loss of protein binding to ARS is associated with increased levels of AR mRNA in one model of androgen-independent LNCaP cells [Wang et al., 2004]. Although androgen is a major regulator of AR mRNA levels, no functional AREs have been identified in the AR gene promoter or its 5′-flanking region [Blok et al., 1992; Takane and McPhaul, 1996].

Androgen Regulation of AR Gene Expression

Androgen regulation of AR mRNA levels (autoregulation) occurs in virtually every target tissue and cell line examined. Androgen downregulates AR mRNA in most cells and tissues [Tan et al., 1988; Quarmby et al., 1990; Shan et al., 1990; Krongrad et al., 1991] although there are several examples of androgen-mediated AR mRNA upregulation [Takeda et al., 1991; Nastiuk and Clayton, 1994; Takeuchi et al., 1994; Wiren et al., 1997]. The molecular basis for this differential regulation is unknown but appears to be cell- and tissue-specific. The dynamics of androgen-mediated AR mRNA regulation in target tissues was examined by comparing intact and castrated rats [Quarmby et al., 1990]. Northern blot analysis revealed that levels of AR mRNA in rat kidney, brain, epididymis, and anterior prostate were increased in castrated animals. Testosterone administration to castrated rats caused AR mRNA to decrease to levels lower than those observed in the intact control animals. Since estradiol had no effect on AR mRNA levels, androgen-mediated downregulation of AR mRNA is not due to testosterone conversion to an active estrogen. Consistent with AR-mediation of this autoregulatory process, testicular feminized (*tfm*) mice, which express a truncated, non-functional AR, showed no androgen regulation of AR mRNA.

Studies in LNCaP cells on the mechanism of androgen-mediated AR mRNA downregulation showed that this response is due to decreased transcription of the AR gene [Blok et al., 1992; Wolf et al., 1993]. Despite the transcriptional mode of AR mRNA regulation, neither the 5′-upstream region (including 7 kb of upstream sequences) nor the promoter or 5′-untranslated region of the human AR gene confers androgen regulation [Blok et al., 1992; Mizokami et al., 1994; Takane and McPhaul, 1996]. The lack of autoregulatory sequences in the AR promoter and flanking region in conjunction with the demonstration of steroid receptor autoregulatory sequences represented in the cDNAs of the glucocorticoid and estrogen receptor [Burnstein et al., 1990; Kaneko et al., 1993] led us to ask whether sequences involved in AR mRNA autoregulation are present in the AR coding region. We demonstrated that androgen-mediated up- and downregulation of AR mRNA is reproduced in different cell lines expressing the human AR cDNA and this autoregulation occurs through transcriptional mechanisms [Burnstein et al., 1995; Dai and Burnstein, 1996; Dai et al., 1996]. AREs present within the AR cDNA confer this androgen-mediated upregulation of AR mRNA.

To establish definitively the role of these exonic AREs in autoregulation of AR mRNA, we generated silent mutations of the AREs, which resulted in a functional receptor that is resistant to androgen-mediated upregulation of AR mRNA. AR mRNA autoregulation is due to four AREs, which function synergistically with a myc site (E box) [Grad et al., 1999]. Myc and Max interaction with the E box cooperates with ARs bound to the exonic AREs and is required for androgen regulation of AR mRNA in this model. These regulatory elements are located within exons D and E of the human AR gene. A 6.5-kb genomic AR fragment (or androgen responsive region) encompassing the exonic AREs and myc site is regulated by androgens in cells that exhibit androgen-mediated upregulation of AR mRNA suggesting that these elements participate in the regulation of AR gene expression (Fig. 1) [Grad et al., 1999]. While the DNA region containing these AREs is responsible for

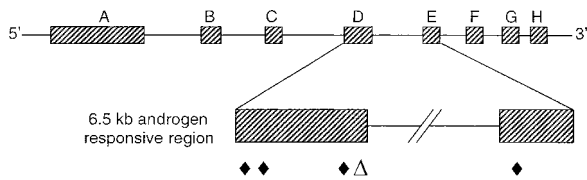

Fig. 1. Schematic of the human androgen receptor gene. The exon/intron structure of the human androgen receptor gene is shown (not to scale) emphasizing the exonic cis elements that are involved in autoregulation of AR mRNA [Dai and Burnstein, 1996; Grad et al., 1999]. Exons are depicted by hatched bars, introns by thin lines, closed diamond symbols represent AREs, and the open triangle is a Myc/Max binding site (E box).

androgen-mediated upregulation of AR mRNA, this region is not involved in AR mRNA downregulation. The finding that mRNA encoded either by the AR cDNA or the native AR gene is subject to differential autoregulation in distinct cell lines and tissues supports the existence of cell-specific factors that dictate this response. Further, we have observed that upregulation of AR mRNA in osteoblastic and some prostate cancer cells is associated with sensitization to hormone, whereas downregulation of AR mRNA is observed in LNCaP cells that undergo desensitization ([Dai et al., 1996] and unpublished data). Thus the identification of the cell-specific factors that participate in androgen-mediated autoregulation is vital to understanding mechanisms of androgen sensitivity.

Transcriptional Regulation of the AR Gene by Signaling Pathways

Androgens regulate gene expression in the liver of the male rat but this hormonal control subsides in aged animals. The decreased androgen responsiveness correlates with downregulation of AR mRNA. Because NF-κB increases with age in the male rat liver and suppresses AR promoter activity, NF-κB may account for decreased levels of AR and diminished hepatic androgen sensitivity in aged rats [Supakar et al., 1995]. The mechanism for transcriptional repression of the AR gene by NF-κB (well-known for transcriptional activation of genes involved in immunity and inflammation) may be due to increased levels and preferential binding of NF-κB subunit p50 homodimers to the AR gene promoter [Supakar et al., 1995; Zhang et al., 2004]. This form of NF-κB can serve as a transcriptional repressor through recruitment of histone deactylase-1 [Zhong et al., 2002] and/or by competing for DNA binding with NF-κB p65/p50 heterodimers, which have high transcriptional activity [Saccani et al., 2003].

NF-κB also regulates the AR promoter in Sertoli cells, which line the seminiferous tubules of the testis. AR levels in Sertoli cells vary during the spermatogenic cycle and dictate androgen regulation of gene transcription required for spermatogenesis [Isomaa et al., 1985; Vornberger et al., 1994]. In contrast to rat liver, NF-κB (p65/p50) increases transcription from the rat AR gene promoter in Sertoli cells of prepubertal [Delfino et al., 2003] and adult rats [Zhang et al., 2004]. Specific binding of the NF-κB heterodimer to an NF-κB consensus sequence was shown using nuclear extracts from adult rat Sertoli cells [Zhang et al., 2004]. NF-κB signaling is also postulated to play a role in prostate cancer as the p65 subunit is overexpressed in human PIN and cancer compared to benign prostate tissue [Sweeney et al., 2004]. It is not known whether NF-κB regulates human AR gene expression.

Post-Transcriptional Mechanisms of Androgen Regulation of AR mRNA

In addition to transcriptional mechanisms of AR mRNA autoregulation, an androgen effect on AR mRNA degradation is implicated in studies of the individual lobes of the rat prostate and in certain cancer cell lines [Prins and Woodham, 1995; Yeap et al., 1999]. Examination of AR mRNA by in situ hybridization and Northern blot analysis of rat prostatic lobes in response to castration revealed increases in AR mRNA in all lobes. While the effect of androgen withdrawal was transient in the dorsal and ventral lobes, the LP2 ducts of the lateral lobe exhibited prolonged elevation in AR mRNA. Nuclear run on assays revealed that increased transcription of the AR gene accounted for the maintenance of AR mRNA in the LP2 ducts. In contrast, castration had no effect on AR gene transcription in the other lobes suggesting that androgen withdrawal influences AR mRNA stability [Prins and Woodham, 1995]. In ventral prostate, testosterone may influence AR mRNA stability through effects on sequestration of AR mRNA on polyribosomes [Mora and Mahesh, 1999].

Yeap et al. [1999] made the intriguing observation that androgen promotes divergent effects on AR mRNA stability in LNCaP cells and in a breast cancer cell line, MDA453. Concurrent with androgen-mediated transcriptional downregulation of the AR gene, AR mRNA half-life is prolonged in LNCaP cells. In contrast, AR mRNA is destabilized in MDA453 cells following androgen treatment. Although the molecular basis for this differential effect on AR mRNA stability is not known, a highly conserved UC-rich sequence was identified in the AR 3′-UTR, which is bound by several widely expressed RNA-binding proteins including HuR (a member of the Elav/Hu family), CP1 and CP2 (heterogeneous nuclear RNP K-homology proteins) [Yeap et al., 2002].

Androgen Binding Stabilizes AR Protein

Discordance between androgen regulation of AR mRNA and protein levels in LNCaP cells [Krongrad et al., 1991], prostatic epithelium as well as certain other target tissues and cell lines prompted investigators to examine androgen effects on AR translation and degradation. While there is limited evidence for androgen regulation of AR protein synthesis [Syms et al., 1985; Mizokami and Chang, 1994], unliganded ARs undergo rapid turnover [Kemppainen et al., 1992]. Ligand binding profoundly increases AR stability thus providing an explanation for the divergent effects of androgen on AR mRNA versus protein levels in some cells and tissues. Ligand-mediated stabilization is relatively unique to AR as other steroid receptors undergo hormone-mediated downregulation. AR protein stabilization can be attributed, at least in part, to the ability of ligand to promote interaction between the AR amino and carboxyl termini [Langley et al., 1995; Zhou et al., 1995; He and Wilson, 2002].

Unliganded ARs appear to be rapidly degraded via the ubiquitin/proteasome system based on studies using the proteasome inhibitor MG132, which causes AR to accumulate in LNCaP and in an AR-expressing human hepatoma cell line, HepG2 [Sheflin et al., 2000]. MG132 had little effect on AR levels in the presence of androgen [Lin et al., 2002a]. In contrast, several steroid and nuclear receptors are subject to ligand-mediated degradation by the proteasome system [Nawaz et al., 1999; Lange et al., 2000; Wallace and Cidlowski, 2001]. For these receptors, including estrogen and progesterone receptors, ligand-mediated transcriptional activity is coupled to proteasomal degradation [reviewed in Nawaz and O'Malley, 2004]. While MG132 did not affect AR levels in the presence of hormone, MG132 reduced AR transcriptional activity, which correlated with decreased AR nuclear translocation and decreased AR-coactivator interactions. Thus, AR transcriptional activity may also be dependent on the proteasome [Kang et al., 2002; Lin et al., 2002a] but ligand-mediated AR degradation may not be linked to this process.

These studies show that androgens can promote up- and downregulation of AR mRNA and protein in a tissue-, cell- and/or developmental stage-specific manner. This autoregulatory process is achieved through a variety of mechanisms even within the same cell type. Since AR levels dictate hormonal sensitivity during development as well as in normal and neoplastic adult tissues, AR autoregulatory mechanisms will affect androgen responsiveness. What factors dictate upregulation versus downregulation of AR mRNA and protein and how do these processes influence AR transcriptional activity? These questions are significant from both a developmental perspective as well as the obvious implications for prostate cancer.

Regulation of AR Degradation by Non-Androgenic Steroids and Signaling Pathways

AR degradation is emerging as a common target for key signaling pathways in prostate epithelial cells although the mechanisms and role of specific AR phosphorylation sites in this process is unclear. As mentioned earlier, unoccupied AR is degraded via the proteasome system [Sheflin et al., 2000]; however, there is evidence for signal-mediated AR degradation as well. Chang and colleagues [Lin et al., 2002b] reported the phosphorylation of AR by Akt in the presence of hormone leading to AR ubiquitylation by the E3 ligase Mdm2 and subsequent proteasome-mediated degradation. This AR degradation correlated with decreased AR transcriptional activity. The opposite effects of PI3-kinase/Akt on AR levels were observed by Manin et al. [2002]. While Akt signaling influences AR degradation, the precise role of Akt in this process requires further study as Gioeli et al. [2002] provide strong evidence that Akt does not directly phosphorylate native or transfected AR in vivo.

The rat prostate gland is sensitive to estrogenic exposure during the neonatal period of prostate development resulting in abnormal patterns of growth, differentiation, and androgen-responsiveness in adult animals [Rajfer and Coffey, 1978; Prins and Birch, 1995]. This imprinting is mediated, in part, through permanent downregulation of AR in epithelial and stromal cells of the ventral lobe of the rat prostate [Prins, 1992; Prins et al., 1993; Prins and Birch, 1995]. This process of AR downregulation in prostates from estrogenized animals is due to increased AR degradation that is proteasome-dependent. Interestingly, prostatic AR degradation in estrogenized

animals is accompanied by decreased Akt activity [Woodham et al., 2003].

AR degradation in prostate cancer cells was recently found to be a downstream event following inhibition of EGFR/HER2 by the small molecule inhibitor, PKI-166 [Mellinghoff et al., 2004]. This exciting finding stemmed from studies addressing the growth stimulatory effects of the EGFR/HER2 receptor tyrosine kinases in prostate cancer xenografts and androgen-independent prostate cancer cells [Mellinghoff et al., 2002]. While targeting HER2 has had a significant impact on breast cancer, this strategy has proven to be more elusive in prostate cancer. Mellinghoff et al. [2004] showed that the dual EGFR and HER2 inhibitor, PKI-166, reduces AR transcriptional activity by promoting AR degradation and decreasing DNA binding by AR. These effects are mediated by inhibition of HER2 and not EGFR and do not involve the downstream kinase, Akt. PKI-166 causes AR degradation in AR-expressing breast cancer cell lines as well.

CONCLUSIONS AND PROSPECTS

The finding that androgen-independent prostate cancer is predominantly AR-dependent has heightened interest in decreasing AR content therapeutically. AR levels can be targeted through several regulatory pathways (Fig. 2). For example, ribozymes, antisense oligomers, and small interfering RNAs directed against AR are being tested in prostate cancer models [Eder et al., 2000; Zegarra-Moro et al., 2002; Wright et al., 2003; Ko et al., 2004]. AR degradation has been achieved using drugs such as geldanamycin that affect hsp90, the AR chaperone [Solit et al., 2002]. Interestingly, COX-2 inhibitors caused decreased levels of AR and several other key proteins in TRAMP mice [Narayanan et al., 2004], a well-characterized transgenic model for prostate cancer [reviewed in Huss et al., 2001]. However, the mechanisms responsible for these effects are unclear. Collectively, these studies illustrate that AR content can be depleted by various means in prostate cancer preclinical models resulting in decreased cell proliferation

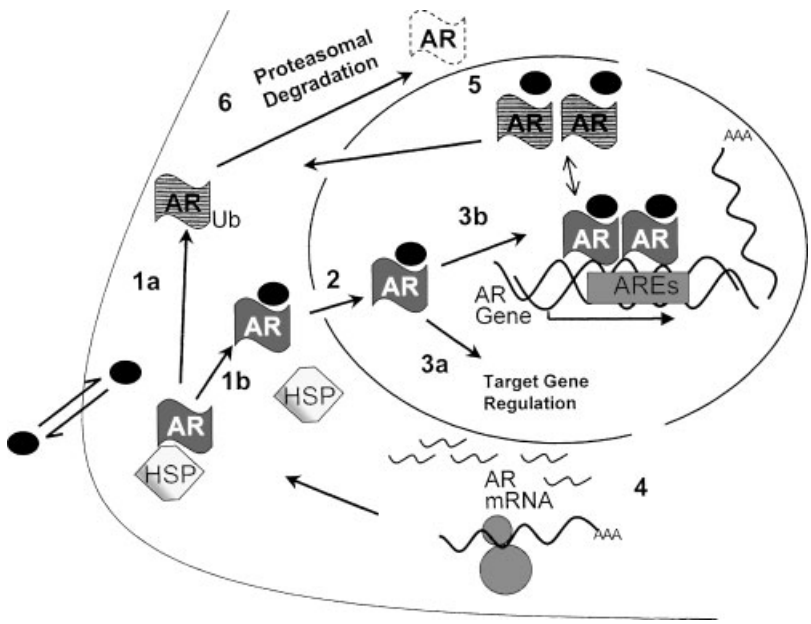

Fig. 2. AR regulation occurs at multiple steps. Prior to androgen binding, ARs exist in complexes (primarily cytoplasmic) containing hsp90 (HSP) and other chaperones (not shown). Unliganded receptors are susceptible to ubiquitylation (Ub) and proteasomal degradation (1a). Hormone that freely transverses the plasma membrane binds AR (1b) and causes a conformational change that releases hsp90 and reveals nuclear localization signals that mediate nuclear import through nuclear pore complexes (2). AR interaction with coactivators (not shown) may occur in subnuclear foci and during chromatin binding. AR homodimers bind AREs associated with target genes (3a) [including exonic AREs within the coding region of the AR gene (3b)]. AR gene transcription is up- and downregulated by AR and by other factors. AR mRNA stability is influenced in a cell-specific manner by androgens via unknown mechanisms (4). ARs are exported from nuclei and this process may be coupled to receptor transcriptional activity (5). While ligand-occupied AR protein is more stable than the aporeceptor; signal-mediated proteasomal degradation of AR has been demonstrated in the presence of hormone although the mechanisms are not well-understood (6). Each of these steps represents a potential target for reducing AR levels or bioavailability.

and tumor growth. A concern with this strategy is the possible detrimental outcomes that may be associated with relieving the pro-differentiation functions of AR. Another potential therapeutic target is AR bioavailability. ARs, like other steroid receptors, shuttle between nuclear and cytoplasmic compartments [reviewed in DeFranco, 1999]. Recent work shows that AR resides transiently within subnuclear foci where interactions with coactivators occur prior to chromatin binding [Black et al., 2004]. Thus, AR nucleocytoplasmic trafficking presents further opportunities for modulating AR action.

There is substantial experimental as well as clinical evidence that AR mRNA and protein levels increase during prostate cancer progression to androgen-independence. However, like other tumor markers, AR expression is heterogeneous in tumor foci of advanced and metastatic cancer [Ruizeveld de Winter et al., 1994; Magi-Galluzzi et al., 1997; Shah et al., 2004]. In the majority of prostate cancer cases, increased AR is not due to AR gene amplification, although this occurs in a significant number of androgen-deprived patients. Since increases in AR mRNA and protein accompany progression to androgen-independence in all prostate xenograft models examined [Chen et al., 2004], androgen deprivation may initiate AR autoregulatory processes. An important consideration is that androgen regulation of AR may be secondary to effects on cell proliferation [Martinez and Danielsen, 2002]. Nevertheless, the pathways leading to AR upregulation in prostate cancer may provide new therapeutic targets. This strategy will require identification of the cell-, tissue- and/or stage-specific factors involved in the regulation of AR mRNA and protein levels in normal cells and elucidation of their dysregulation in neoplasia.

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REFERENCES

Balk SP. 2002. Androgen receptor as a target in androgen-independent prostate cancer. Urology 60:132–138; discussion 138–139.

Berger R, Febbo PG, Majumder PK, Zhao JJ, Mukherjee S, Signoretti S, Campbell KT, Sellers WR, Roberts TM, Loda M, Golub TR, Hahn WC. 2004. Androgen-induced differentiation and tumorigenicity of human prostate epithelial cells. Cancer Res 64:8867–8875.

Black BE, Vitto MJ, Gioeli D, Spencer A, Afshar N, Conaway MR, Weber MJ, Paschal BM. 2004. Transient, ligand-dependent arrest of the androgen receptor in subnuclear foci alters phosphorylation and coactivator interactions. Mol Endocrinol 18:834–850.

Blok LJ, Themmen AP, Peters AH, Trapman J, Baarends WM, Hoogerbrugge JW, Grootegoed JA. 1992. Transcriptional regulation of androgen receptor gene expression in Sertoli cells and other cell types. Mol Cell Endocrinol 88: 153–164.

Bonkhoff H, Stein U, Remberger K. 1994. The proliferative function of basal cells in the normal and hyperplastic human prostate. Prostate 24:114–118.

Burnstein KL, Jewell CM, Cidlowski JA. 1990. Human glucocorticoid receptor cDNA contains sequences sufficient for receptor down-regulation. J Biol Chem 265: 7284–7291.

Burnstein KL, Maiorino CA, Dai JL, Cameron DJ. 1995. Androgen and glucocorticoid regulation of androgen receptor cDNA expression. Mol Cell Endocrinol 115: 177–186.

Chen S, Supakar PC, Vellanoweth RL, Song CS, Chatterjee B, Roy AK. 1997. Functional role of a conformationally flexible homopurine/homopyrimidine domain of the androgen receptor gene promoter interacting with Sp1 and a pyrimidine single strand DNA-binding protein. Mol Endocrinol 11:3–15.

Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL. 2004. Molecular determinants of resistance to antiandrogen therapy. Nat Med 10: 33–39.

Culig Z, Hobisch A, Bartsch G, Klocker H. 2000. Expression and function of androgen receptor in carcinoma of the prostate. Microsc Res Tech 51:447–455.

Dai JL, Burnstein KL. 1996. Two androgen response elements in the androgen receptor coding region are required for cell-specific up-regulation of receptor messenger RNA. Mol Endocrinol 10:1582–1594.

Dai JL, Maiorino CA, Gkonos PJ, Burnstein KL. 1996. Androgenic up-regulation of androgen receptor cDNA expression in androgen-independent prostate cancer cells. Steroids 61:531–539.

DeFranco DB. 1999. Regulation of steroid receptor subcellular trafficking. Cell Biochem Biophys 30:1–24.

Delfino FJ, Boustead JN, Fix C, Walker WH. 2003. NF-kappaB and TNF-alpha stimulate androgen receptor expression in Sertoli cells. Mol Cell Endocrinol 201:1–12.

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.

Faber PW, van Rooij HC, Schipper HJ, Brinkmann AO, Trapman J. 1993. Two different, overlapping pathways of transcription initiation are active on the TATA-less human androgen receptor promoter. The role of Sp1. J Biol Chem 268:9296–9301.

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

Freedman ML, Pearce CL, Penney KL, Hirschhorn JN, Kolonel LN, Henderson BE, Altshuler D. 2005. Systematic evaluation of genetic variation at the androgen receptor locus and risk of prostate cancer in a multiethnic cohort study. Am J Hum Genet 76:82–90.

Gao J, Arnold JT, Isaacs JT. 2001. Conversion from a paracrine to an autocrine mechanism of androgen-stimulated growth during malignant transformation of prostatic epithelial cells. Cancer Res 61:5038–5044.

Gelmann EP. 2002. Molecular biology of the androgen receptor. J Clin Oncol 20:3001–3015.

Gioeli D, Ficarro SB, Kwiek JJ, Aaronson D, Hancock M, Catling AD, White FM, Christian RE, Settlage RE, Shabanowitz J, Hunt DF, Weber MJ. 2002. Androgen receptor phosphorylation. Regulation and identification of the phosphorylation sites. J Biol Chem 277:29304–29314.

Giovannucci E, Stampfer MJ, Krithivas K, Brown M, Dahl D, Brufsky A, Talcott J, Hennekens CH, Kantoff PW. 1997. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. Proc Natl Acad Sci USA 94:3320–3323.

Grad JM, Dai JL, Wu S, Burnstein KL. 1999. Multiple androgen response elements and a Myc consensus site in the androgen receptor (AR) coding region are involved in androgen-mediated up-regulation of AR messenger RNA. Mol Endocrinol 13:1896–1911.

Gregory CW, Hamil KG, Kim D, Hall SH, Pretlow TG, Mohler JL, French FS. 1998. Androgen receptor expression in androgen-independent prostate cancer is associated with increased expression of androgen-regulated genes. Cancer Res 58:5718–5724.

Gregory CW, Johnson RT, Jr., Mohler JL, French FS, Wilson EM. 2001. Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. Cancer Res 61:2892–2898.

Grossmann ME, Tindall DJ. 1995. The androgen receptor is transcriptionally suppressed by proteins that bind single-stranded DNA. J Biol Chem 270:10968–10975.

Han G, Foster BA, Mistry S, Buchanan G, Harris JM, Tilley WD, Greenberg NM. 2001. Hormone status selects for spontaneous somatic androgen receptor variants that demonstrate specific ligand and cofactor dependent activities in autochthonous prostate cancer. J Biol Chem 276:11204–11213.

Han G, Buchanan G, Ittmann M, Harris JM, Yu X, Demayo FJ, Tilley W, Greenberg NM. 2005. Mutation of the androgen receptor causes oncogenic transformation of the prostate. Proc Natl Acad Sci USA 102: 1151–1156.

Hardy DO, Scher HI, Bogenreider T, Sabbatini P, Zhang ZF, Nanus DM, Catterall JF. 1996. Androgen receptor CAG repeat lengths in prostate cancer: Correlation with age of onset. J Clin Endocrinol Metab 81:4400–4405.

He B, Wilson EM. 2002. The NH(2)-terminal and carboxyl-terminal interaction in the human androgen receptor. Mol Genet Metab 75:293–298.

Heinlein CA, Chang C. 2002a. Androgen receptor (AR) coregulators: An overview. Endocr Rev 23:175–200.

Heinlein CA, Chang C. 2002b. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol 16:2181–2187.

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

Hellerstedt BA, Pienta KJ. 2002. The current state of hormonal therapy for prostate cancer. CA Cancer J Clin 52:154–179.

Hobisch A, Culig Z, Radmayr C, Bartsch G, Klocker H, Hittmair A. 1995. Distant metastases from prostatic carcinoma express androgen receptor protein. Cancer Res 55:3068–3072.

Huss WJ, Maddison LA, Greenberg NM. 2001. Autochthonous mouse models for prostate cancer: Past, present and future. Semin Cancer Biol 11:245–260.

Igawa T, Lin FF, Lee MS, Karan D, Batra SK, Lin MF. 2002. Establishment and characterization of androgen-independent human prostate cancer LNCaP cell model. Prostate 50:222–235.

Isomaa V, Parvinen M, Janne OA, Bardin CW. 1985. Nuclear androgen receptors in different stages of the seminiferous epithelial cycle and the interstitial tissue of rat testis. Endocrinology 116:132–137.

Kaneko KJ, Furlow JD, Gorski J. 1993. Involvement of the coding sequence for the estrogen receptor gene in autologous ligand-dependent down-regulation. Mol Endocrinol 7:879–888.

Kang Z, Pirskanen A, Janne OA, Palvimo JJ. 2002. Involvement of proteasome in the dynamic assembly of the androgen receptor transcription complex. J Biol Chem 277:48366–48371.

Kemppainen JA, Lane MV, Sar M, Wilson EM. 1992. Androgen receptor phosphorylation, turnover, nuclear transport, and transcriptional activation. Specificity for steroids and antihormones. J Biol Chem 267:968–974.

Ko YJ, Devi GR, London CA, Kayas A, Reddy MT, Iversen PL, Bubley GJ, Balk SP. 2004. Androgen receptor down-regulation in prostate cancer with phosphorodiamidate morpholino antisense oligomers. J Urol 172:1140–1144.

Kokontis JM, Liao S. 1999. Molecular action of androgen in the normal and neoplastic prostate. Vitam Horm 55:219–307.

Kokontis J, Takakura K, Hay N, Liao S. 1994. Increased androgen receptor activity and altered c-myc expression in prostate cancer cells after long-term androgen deprivation. Cancer Res 54:1566–1573.

Kokontis JM, Hay N, Liao S. 1998. Progression of LNCaP prostate tumor cells during androgen deprivation: Hormone-independent growth, repression of proliferation by androgen, and role for p27Kip1 in androgen-induced cell cycle arrest. Mol Endocrinol 12:941–953.

Krongrad A, Wilson CM, Wilson JD, Allman DR, McPhaul MJ. 1991. Androgen increases androgen receptor protein while decreasing receptor mRNA in LNCaP cells. Mol Cell Endocrinol 76:79–88.

Lange CA, Shen T, Horwitz KB. 2000. Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. Proc Natl Acad Sci USA 97:1032–1037.

Langley E, Zhou ZX, Wilson EM. 1995. Evidence for an anti-parallel orientation of the ligand-activated human androgen receptor dimer. J Biol Chem 270:29983–29990.

Lin HK, Altuwaijri S, Lin WJ, Kan PY, Collins LL, Chang C. 2002a. Proteasome activity is required for androgen receptor transcriptional activity via regulation of androgen receptor nuclear translocation and interaction with coregulators in prostate cancer cells. J Biol Chem 277:36570–36576.

Lin HK, Wang L, Hu YC, Altuwaijri S, Chang C. 2002b. Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt require Mdm2 E3 ligase. EMBO J 21:4037–4048.

Lu S, Tsai SY, Tsai MJ. 1999. Molecular mechanisms of androgen-independent growth of human prostate cancer LNCaP-AI cells. Endocrinology 140:5054–5059.

Magi-Galluzzi C, Xu X, Hlatky L, Hahnfeldt P, Kaplan I, Hsiao P, Chang C, Loda M. 1997. Heterogeneity of androgen receptor content in advanced prostate cancer. Mod Pathol 10:839–845.

Manin M, Baron S, Goossens K, Beaudoin C, Jean C, Veyssiere G, Verhoeven G, Morel L. 2002. Androgen receptor expression is regulated by the phosphoinositide 3-kinase/Akt pathway in normal and tumoral epithelial cells. Biochem J 366:729–736.

Marker PC, Donjacour AA, Dahiya R, Cunha GR. 2003. Hormonal, cellular, and molecular control of prostatic development. Dev Biol 253:165–174.

Martinez ED, Danielsen M. 2002. Loss of androgen receptor transcriptional activity at the G(1)/S transition. J Biol Chem 277:29719–29729.

McPhaul MJ, Deslypere JP, Allman DR, Gerard RD. 1993. The adenovirus-mediated delivery of a reporter gene permits the assessment of androgen receptor function in genital skin fibroblast cultures. Stimulation of Gs and inhibition of G(o). J Biol Chem 268:26063–26066.

Mellinghoff IK, Tran C, Sawyers CL. 2002. Growth inhibitory effects of the dual ErbB1/ErbB2 tyrosine kinase inhibitor PKI-166 on human prostate cancer xenografts. Cancer Res 62:5254–5259.

Mellinghoff IK, Vivanco I, Kwon A, Tran C, Wongvipat J, Sawyers CL. 2004. HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. Cancer Cell 6:517–527.

Mizokami A, Chang C. 1994. Induction of translation by the 5′-untranslated region of human androgen receptor mRNA. J Biol Chem 269:25655–25659.

Mizokami A, Yeh SY, Chang C. 1994. Identification of 3′,5′-cyclic adenosine monophosphate response element and other cis-acting elements in the human androgen receptor gene promoter. Mol Endocrinol 8:77–88.

Mohler JL, Gregory CW, Ford OH3rd, Kim D, Weaver CM, Petrusz P, Wilson EM, French FS. 2004. The androgen axis in recurrent prostate cancer. Clin Cancer Res 10: 440–448.

Mora GR, Mahesh VB. 1999. Autoregulation of the androgen receptor at the translational level: Testosterone induces accumulation of androgen receptor mRNA in the rat ventral prostate polyribosomes. Steroids 64:587–591.

Narayanan BA, Narayanan NK, Pittman B, Reddy BS. 2004. Regression of mouse prostatic intraepithelial neoplasia by nonsteroidal anti-inflammatory drugs in the transgenic adenocarcinoma mouse prostate model. Clin Cancer Res 10:7727–7737.

Nastiuk KL, Clayton DF. 1994. Seasonal and tissue-specific regulation of canary androgen receptor messenger ribonucleic acid. Endocrinology 134:640–649.

Nawaz Z, O'Malley BW. 2004. Urban renewal in the nucleus: is protein turnover by proteasomes absolutely required for nuclear receptor-regulated transcription? Mol Endocrinol 18:493–499.

Nawaz Z, Lonard DM, Dennis AP, Smith CL, O'Malley BW. 1999. Proteasome-dependent degradation of the human estrogen receptor. Proc Natl Acad Sci USA 96:1858–1862.

Prins GS. 1992. Neonatal estrogen exposure induces lobe-specific alterations in adult rat prostate androgen receptor expression. Endocrinology 130:3703–3714.

Prins GS, Birch L. 1993. Immunocytochemical analysis of androgen receptor along the ducts of the separate rat prostate lobes after androgen withdrawal and replacement. Endocrinology 132:169–178.

Prins GS, Birch L. 1995. The developmental pattern of androgen receptor expression in rat prostate lobes is altered after neonatal exposure to estrogen. Endocrinology 136:1303–1314.

Prins GS, Woodham C. 1995. Autologous regulation of androgen receptor messenger ribonucleic acid in the separate lobes of the rat prostate gland. Biol Reprod 53: 609–619.

Prins GS, Woodham C, Lepinske M, Birch L. 1993. Effects of neonatal estrogen exposure on prostatic secretory genes and their correlation with androgen receptor expression in the separate prostate lobes of the adult rat. Endocrinology 132:2387–2398.

Prins GS, Jung MH, Vellanoweth RL, Chatterjee B, Roy AK. 1996. Age-dependent expression of the androgen receptor gene in the prostate and its implication in glandular differentiation and hyperplasia. Dev Genet 18: 99–106.

Quarmby VE, Yarbrough WG, Lubahn DB, French FS, Wilson EM. 1990. Autologous down-regulation of androgen receptor messenger ribonucleic acid. Mol Endocrinol 4:22–28.

Rajfer J, Coffey DS. 1978. Sex steroid imprinting of the immature prostate. Long-term effects. Invest Urol 16: 186–190.

Roy AK, Lavrovsky Y, Song CS, Chen S, Jung MH, Velu NK, Bi BY, Chatterjee B. 1999. Regulation of androgen action. Vitam Horm 55:309–352.

Ruizeveld de Winter JA, Janssen PJ, Sleddens HM, Verleun-Mooijman MC, Trapman J, Brinkmann AO, Santerse AB, Schroder FH, van der Kwast TH. 1994. Androgen receptor status in localized and locally progressive hormone refractory human prostate cancer. Am J Pathol 144:735–746.

Saccani S, Pantano S, Natoli G. 2003. Modulation of NF-kappaB activity by exchange of dimers. Mol Cell 11: 1563–1574.

Santos AF, Huang H, Tindall DJ. 2004. The androgen receptor: A potential target for therapy of prostate cancer. Steroids 69:79–85.

Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghosh D, Zhou M, Macvicar GR, Varambally S, Harwood J, Bismar TA, Kim R, Rubin MA, Pienta KJ. 2004. Androgen-independent prostate cancer is a heterogeneous group of diseases: Lessons from a rapid autopsy program. Cancer Res 64:9209–9216.

Shan LX, Rodriguez MC, Janne OA. 1990. Regulation of androgen receptor protein and mRNA concentrations by androgens in rat ventral prostate and seminal vesicles and in human hepatoma cells. Mol Endocrinol 4:1636–1646.

Sheflin L, Keegan B, Zhang W, Spaulding SW. 2000. Inhibiting proteasomes in human HepG2 and LNCaP cells increases endogenous androgen receptor levels. Biochem Biophys Res Commun 276:144–150.

Sobel RE, Sadar MD. 2005a. Cell lines used in prostate cancer research: A compendium of old and new lines—Part 1. J Urol 173:342–359.

Sobel RE, Sadar MD. 2005b. Cell lines used in prostate cancer research: A compendium of old and new lines—Part 2. J Urol 173:360–372.

Solit DB, Zheng FF, Drobnjak M, Munster PN, Higgins B, Verbel D, Heller G, Tong W, Cordon-Cardo C, Agus DB, Scher HI, Rosen N. 2002. 17-Allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. Clin Cancer Res 8:986–993.

Song CS, Jung MH, Supakar PC, Chatterjee B, Roy AK. 1999. Negative regulation of the androgen receptor gene promoter by NFI and an adjacently located multiprotein-binding site. Mol Endocrinol 13:1487–1496.

Stanbrough M, Leav I, Kwan PW, Bubley GJ, Balk SP. 2001. Prostatic intraepithelial neoplasia in mice expressing an androgen receptor transgene in prostate epithelium. Proc Natl Acad Sci USA 98:10823–10828.

Supakar PC, Song CS, Jung MH, Slomczynska MA, Kim JM, Vellanoweth RL, Chatterjee B, Roy AK. 1993. A novel regulatory element associated with age-dependent expression of the rat androgen receptor gene. J Biol Chem 268:26400–26408.

Supakar PC, Jung MH, Song CS, Chatterjee B, Roy AK. 1995. Nuclear factor kappa B functions as a negative regulator for the rat androgen receptor gene and NF-kappa B activity increases during the age-dependent desensitization of the liver. J Biol Chem 270:837–842.

Sweeney C, Li L, Shanmugam R, Bhat-Nakshatri P, Jayaprakasan V, Baldridge LA, Gardner T, Smith M, Nakshatri H, Cheng L. 2004. Nuclear factor-kappaB is constitutively activated in prostate cancer in vitro and is overexpressed in prostatic intraepithelial neoplasia and adenocarcinoma of the prostate. Clin Cancer Res 10: 5501–5507.

Syms AJ, Norris JS, Panko WB, Smith RG. 1985. Mechanism of androgen-receptor augmentation. Analysis of receptor synthesis and degradation by the density-shift technique. J Biol Chem 260:455–461.

Takane KK, McPhaul MJ. 1996. Functional analysis of the human androgen receptor promoter. Mol Cell Endocrinol 119:83–93.

Takane KK, Husmann DA, McPhaul MJ, Wilson JD. 1991a. Androgen receptor levels in the rat penis are controlled differently in distinctive cell types. Endocrinology 128: 2234–2238.

Takane KK, Wilson JD, McPhaul MJ. 1991b. Decreased levels of the androgen receptor in the mature rat phallus are associated with decreased levels of androgen receptor messenger ribonucleic acid. Endocrinology 129:1093–1100.

Takeda H, Nakamoto T, Kokontis J, Chodak GW, Chang C. 1991. Autoregulation of androgen receptor expression in rodent prostate: Immunohistochemical and in situ hybridization analysis. Biochem Biophys Res Commun 177:488–496.

Takeuchi M, Kakushi H, Tohkin M. 1994. Androgens directly stimulate mineralization and increase androgen receptors in human osteoblast-like osteosarcoma cells. Biochem Biophys Res Commun 204:905–911.

Tan JA, Joseph DR, Quarmby VE, Lubahn DB, Sar M, French FS, Wilson EM. 1988. The rat androgen receptor: Primary structure, autoregulation of its messenger ribonucleic acid, and immunocytochemical localization of the receptor protein. Mol Endocrinol 2:1276–1285.

Taplin ME, Balk SP. 2004. Androgen receptor: A key molecule in the progression of prostate cancer to hormone independence. J Cell Biochem 91:483–490.

Tilley WD, Marcelli M, McPhaul MJ. 1990. Expression of the human androgen receptor gene utilizes a common promoter in diverse human tissues and cell lines. J Biol Chem 265:13776–13781.

Tsai MJ, O'Malley BW. 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451–486.

Umekita Y, Hiipakka RA, Kokontis JM, Liao S. 1996. Human prostate tumor growth in athymic mice: Inhibition by androgens and stimulation by finasteride. Proc Natl Acad Sci USA 93:11802–11807.

van der Kwast TH, Schalken J, Ruizeveld de Winter JA, van Vroonhoven CC, Mulder E, Boersma W, Trapman J. 1991. Androgen receptors in endocrine-therapy-resistant human prostate cancer. Int J Cancer 48:189–193.

Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, Palotie A, Tammela T, Isola J, Kallioniemi OP. 1995. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 9:401–406.

Vornberger W, Prins G, Musto NA, Suarez-Quian CA. 1994. Androgen receptor distribution in rat testis: New implications for androgen regulation of spermatogenesis. Endocrinology 134:2307–2316.

Wallace AD, Cidlowski JA. 2001. Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. J Biol Chem 276: 42714–42721.

Wang LG, Ossowski L, Ferrari AC. 2004. Androgen receptor level controlled by a suppressor complex lost in an androgen-independent prostate cancer cell line. Oncogene 23:5175–5184.

Wiren KM, Zhang X, Chang C, Keenan E, Orwoll ES. 1997. Transcriptional up-regulation of the human androgen receptor by androgen in bone cells. Endocrinology 138: 2291–2300.

Wolf DA, Herzinger T, Hermeking H, Blaschke D, Horz W. 1993. Transcriptional and posttranscriptional regulation of human androgen receptor expression by androgen. Mol Endocrinol 7:924–936.

Woodham C, Birch L, Prins GS. 2003. Neonatal estrogen down-regulates prostatic androgen receptor through a proteosome-mediated protein degradation pathway. Endocrinology 144:4841–4850.

Wright ME, Tsai MJ, Aebersold R. 2003. Androgen receptor represses the neuroendocrine transdifferentiation process in prostate cancer cells. Mol Endocrinol 17:1726–1737.

Wu HC, Hsieh JT, Gleave ME, Brown NM, Pathak S, Chung LW. 1994. Derivation of androgen-independent human LNCaP prostatic cancer cell sublines: Role of bone stromal cells. Int J Cancer 57:406–412.

Yeap BB, Krueger RG, Leedman PJ. 1999. Differential posttranscriptional regulation of androgen receptor gene expression by androgen in prostate and breast cancer cells. Endocrinology 140:3282–3291.

Yeap BB, Voon DC, Vivian JP, McCulloch RK, Thomson AM, Giles KM, Czyzyk-Krzeska MF, Furneaux H, Wilce

MC, Wilce JA, Leedman PJ. 2002. Novel binding of HuR and poly(C)-binding protein to a conserved UC-rich motif within the 3′-untranslated region of the androgen receptor messenger RNA. J Biol Chem 277:27183–27192.

Zeegers MP, Kiemeney LA, Nieder AM, Ostrer H. 2004. How strong is the association between CAG and GGN repeat length polymorphisms in the androgen receptor gene and prostate cancer risk? Cancer Epidemiol Biomarkers Prev 13:1765–1771.

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.

Zhang L, Charron M, Wright WW, Chatterjee B, Song CS, Roy AK, Brown TR. 2004. Nuclear factor-kappaB activates transcription of the androgen receptor gene in Sertoli cells isolated from testes of adult rats. Endocrinology 145:781–789.

Zhong H, May MJ, Jimi E, Ghosh S. 2002. The phosphorylation status of nuclear NF-kappa B determines its association with CBP/p300 or HDAC-1. Mol Cell 9:625–636.

Zhou ZX, Lane MV, Kemppainen JA, French FS, Wilson EM. 1995. Specificity of ligand-dependent androgen receptor stabilization: Receptor domain interactions influence ligand dissociation and receptor stability. Mol Endocrinol 9:208–218.