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Androgen Receptor Action in Hormone-Dependent and Recurrent Prostate Cancer

Irina U. Agoulnik and Nancy L. Weigel*

Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas 77030

Abstract The importance of androgens and androgen receptors (AR) in primary prostate cancer is well established. Metastatic disease is usually treated with some form of androgen ablation, which is effective for a limited amount of time. The role of AR in prostate cancers that recur despite androgen ablation therapy is less certain. Most of these tumors express prostate specific antigen (PSA), an androgen-regulated gene; moreover, AR is generally highly expressed in recurrent prostate cancer. We propose that AR continues to play a role in many of these tumors and that it is not only the levels of AR, ligands, and co-regulators, but also the changes in cell signaling that induce AR action in recurrent prostate cancer. These pathways are, therefore, potential therapeutic targets. J. Cell. Biochem. 99: 362–372, 2006. © 2006 Wiley-Liss, Inc.

Key words: androgen receptor; prostate cancer; co-activator; cell signaling

Prostate cancer is the most frequently diagnosed noncutaneous cancer and the second most common cause of death from cancer in American men. Based on estimates by the American Cancer Society in 2005, about 232,090 men will be diagnosed and 30,350 will die from prostate cancer. Because prostate cancer is initially an androgen-dependent disease, reducing the levels of circulating androgens and/or administration of an androgen receptor (AR) antagonist to diminish AR activity is the primary treatment for metastatic prostate cancer. Although initially responsive to androgen deprivation, most tumors become resistant to this therapy and additional means of inhibiting tumor growth are required. As with many cancers, prostate cancer is a very heterogeneous disease. However, recent studies point to the AR and its actions as a key factor in many androgen ablation-resistant tumors despite the reduction in circulating testosterone. We summarize, here, the evidence for changes in expression and activity of factors that potentiates AR action. Although a great deal of attention has been paid to levels of proteins, there is good evidence indicating that the activities of AR and its co-regulators are highly regulated by post-translational modifications including phosphorylation. As a result, the alterations in the AR signaling pathway that permit AR to function under these conditions and alternative means of blocking AR activity are of profound interest to prostate cancer researchers. The challenge is not simply to find a target that contributes to AR action, but to find a means to inhibit its potentiation of AR activity without an unacceptable loss of other critical functions of the protein or signaling pathway.

ANDROGEN ACTION IN PROSTATE AND PROSTATE CANCER

Development and maintenance of normal prostate is androgen-dependent, with stromal cells providing the necessary androgen-regulated growth factors for growth and differentiation of the epithelial cells (reviewed in [Arnold and Isaacs, 2002]). The epithelial cells also express AR, which is a hormone-activated transcription factor that regulates transcription of mRNAs encoding proteins such as prostate specific antigen (PSA). Prostate cancers arise from epithelial cells; at some point, the tumor cells develop the means to produce autocrine growth factors in an androgen-dependent manner and are no longer dependent upon

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*Correspondence to: Nancy L. Weigel, PhD, Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030. E-mail: nweigel@bcm.edu

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stromal cell growth factors. The androgen-regulated gene product, PSA, is normally secreted into the lumen of the prostate glands. As tumors develop, the cells come in greater contact with the blood supply and serum PSA levels rise. With improved screening for serum PSA, many prostate cancers are being detected when they are confined to the prostate and these can be treated successfully by surgical removal of the prostate or, in some cases, by radiation. Tumors that have escaped the confines of the prostate are typically treated with some form of therapy to reduce androgen action. These include treatments to reduce the production of testosterone (luteinizing hormone releasing hormone (LHRH) analogs or orchiectomy), administration of anti-androgens, or a combination of these two approaches. Most tumors are responsive to this treatment initially, but eventually become resistant and recur as tumors that are termed hormone independent or hormone refractory. A variety of changes in growth factor signaling as well as increases in expression of anti-apoptotic proteins have been described. Although the loss of the requirement for normal levels of androgens suggested that the tumors have activated growth factor signaling pathways that bypass AR signaling, there is now a wealth of evidence that shows that many tumors remain AR-dependent and that the activated cell signaling pathways are acting through AR. Indeed, recurrence is typically detected by rising levels of serum PSA, an androgen-regulated protein. Consequently, a detailed analysis of AR action with the aim of developing new means to disrupt AR function has become a high priority in the prostate cancer field.

ANDROGEN RECEPTOR STRUCTURE AND FUNCTION

The AR, a member of the nuclear receptor family of ligand-activated transcription factors, exhibits the typical structure of the members of this family [McEwan, 2004]. These receptors contain DNA-binding domains (DBD) comprised of two zinc finger motifs that determine the DNA sequences recognized by the receptors and a carboxyl terminal hormone (ligand) binding domain. This domain also contains a region, termed activation function 2 (AF-2), which is important for the transcriptional activity of the receptor (Fig. 1). The DNA and hormone-binding domains are linked by a hinge region, which contains a nuclear localization signal. The amino-terminal domains of steroid receptors are the least conserved regions varying in length from a few amino acids to more than 500 amino acids as is the case for AR. The amino-terminus also contains a region important for transcriptional activation, termed AF-1. The relative importance of these two activation functions is receptor- and context-dependent. In AR, AF-1 appears to be the major transactivation domain. Unique to AR are the amino-terminal poly-glutamine, poly-glycine, and poly-proline repeats. The length of the poly-glutamine tract is variable (typically 18–22 amino acids) and has been associated with levels of receptor activity. Extremely long tracts ($\geq$40) cause spinal and bulbar muscular atrophy [Walcott and Merry, 2002]. Within the normal range of about 18–22 repeats, AR with shorter tracts are more active. There is some evidence that the length of the poly-glutamine tract correlates with prostate cancer risk, but the results are conflicting (reviewed in [Linja and Visakorpi, 2004]). An early study indicated that men with shorter tracts were more likely to develop prostate cancer at an earlier age, but others have found no association. African-American men have a higher incidence of prostate cancer and shorter poly-glutamine tracts. However, a recent study found no correlation between length of this tract and prostate cancer risk in African-American men [Gilligan et al., 2004].

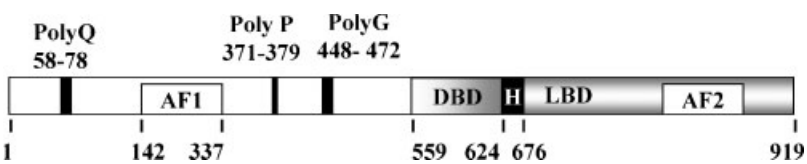

Fig. 1. The structure of AR. The relative locations of the DNA-binding domain (DBD), hinge (H), ligand-binding domain (LBD), activations functions (AF1, AF2), Poly-Q, Poly-P, and Poly-G tracts are shown. The numbering is based on an assumed length of 919, although actual AR lengths will depend upon the length of the Poly-Q tract.

In the absence of hormone, the receptor is cytoplasmic and is associated with heat shock protein (HSP) complexes, which maintain the receptor in a conformation capable of binding hormone and likely also protect the receptor from proteolysis. Binding of hormone (testosterone or dihydrotestosterone) favors dissociation of the complex, receptor dimerization, and induces nuclear translocation (reviewed in [Edwards and Bartlett, 2005a]). In the best characterized mode of receptor action, the receptor binds to specific DNA response elements and recruits a series of co-activator complexes that modify chromatin structure, recruit RNA polymerase II, and induce transcription [Heinlein and Chang, 2002] (Fig. 2). There is evidence that receptors also induce transcription by binding to other transcription factors [Lu et al., 2000] and essentially function as co-activators, themselves, through the recruitment of other co-activators. AR also directly represses expression of some genes.

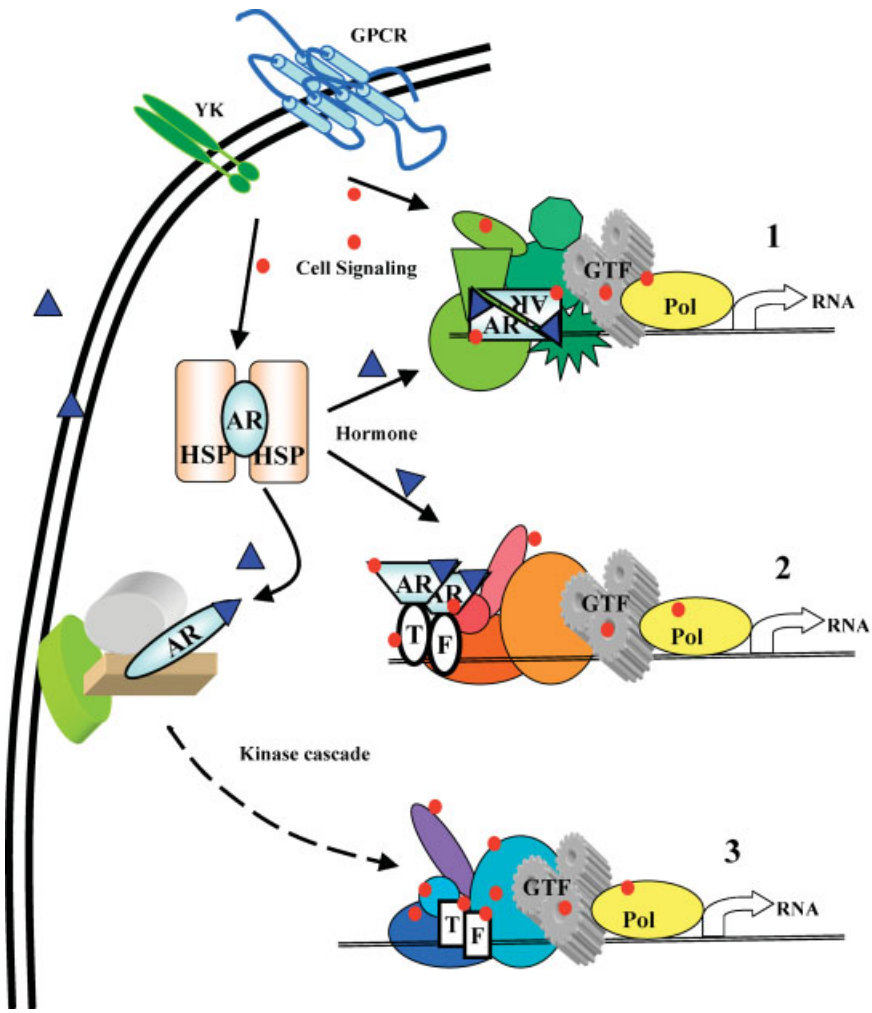

Fig. 2. AR action. In the absence of an activating signaling AR monomers are associated with a complex of HSPs. Androgens are hydrophobic and can freely diffuse through the membrane. Binding of hormone leads to dissociation of the AR HSP complex, dimerization, and translocation to the nucleus. 1. AR binds to specific DNA sequences termed androgen response elements, recruits a series of co-activator complexes and general transcription factors (GTF) to enhance transcription. AR and many of its associated proteins are phosphoproteins. Signals emanating from cell signaling cascades including those initiated by G-protein coupled receptors (GPCR) and receptors with tyrosine kinase activity (YK) can potentiate the activity of AR through phosphorylation (red dots) even at extremely low levels of hormone. 2. AR can also modulate transcription by binding to other transcription factors, T, F, presumably by bringing additional co-activators to the promoter. 3. Upon dissociating from the HSP complex, AR can also interact with proteins such as MNAR and src causing activation of a kinase cascade, which results in the activation of transcription factors in the absence of AR binding to the target gene.

Finally, recent studies reveal that upon binding hormone, AR can stimulate activation of downstream kinases including p42/p44 MAPK and PI3K through interaction of AR with modulator of nongenomic action of estrogen receptor (MNAR) and src [Castoria et al., 2004; Unni et al., 2004]. The individual contributions of these modes of action to androgen-dependent cell growth have not been elucidated, but there is new evidence that the kinase activation pathway contributes to cell growth [Unni et al., 2004].

In addition to the classical hormone-dependent pathway, there is good evidence (discussed in more detail below) that increasing the activity of a subset of cell signaling pathways is sufficient to induce AR activity at very low levels of hormone and perhaps in the absence of measurable hormone. Although the precise mechanisms by which this activity is induced have not been determined, both AR and many, if not all, of its co-activators are phosphoproteins whose activities can be altered by phosphorylation.

EVIDENCE TO SUPPORT A ROLE FOR ANDROGEN RECEPTORS IN ANDROGEN-INDEPENDENT PROSTATE CANCER

Long before the identification of AR, the critical role of androgens in prostate cancer was recognized and Dr. Charles Huggins was awarded the Nobel prize in 1966 for his work on castration [Huggins et al., 1940] and the use of estrogens in the treatment of prostate cancer. It is only relatively recently that investigators have considered that AR continues to play a role in androgen ablation-resistant prostate cancer. Evidence supporting this concept comes from studies of human prostate cancer, xenograft tumor models, and in vitro cell culture studies. Tumors that recur are typically detected by increases in serum PSA levels, an androgen-regulated protein. Although it is formally possible that there are changes in the regulators of its expression, the frequency of the return of PSA expression and the lack of an identified survival function for PSA suggest that AR functioning in the absence of normal circulating levels of androgens is responsible for its expression. In support of this finding is the elevation in AR mRNA in androgen-independent tumors relative to androgen-dependent tumors and a re-expression of some androgen-regulated genes when the tumors become androgen refractory [Balk, 2002; Holzbeierlein et al., 2004]. Studies in human tumor xenografts comparing matched androgen-dependent and androgen-independent derivatives also show an increase in AR [Chen et al., 2004].

Studies in cell lines permit a more direct test of this idea. Tindall's group has shown that use of a ribozyme against AR or microinjection of AR antibodies into AR positive androgen refractory prostate cancer cell lines blocks growth in vitro [Zegarra-Moro et al., 2002]. Agoulnik et al. [2005] subsequently demonstrated that reducing AR expression using siRNA for AR in androgen-independent C4-2 cells grown in androgen-depleted medium blocked both cell growth and basal hormone-independent expression of PSA. The C4-2 cells are a derivative of the LNCaP cells and thus contain the T877A mutation in the AR as do the androgen-dependent LNCaP cells. This mutant responds to other steroids including glucocorticoids [Chang et al., 2001], but the use of serum depleted of steroids argues that the AR is acting in the absence of hormone or in the presence of vanishingly low levels (pmolar or less) unless the cells have developed the capacity to synthesize androgens.

FACTORS THAT CAN FACILITATE ANDROGEN RECEPTOR ACTION IN THE ABSENCE OF NORMAL CIRCULATING LEVELS OF ANDROGENS

The activity of AR is dependent not only on levels of hormone and receptor, but also on the levels and activities of co-activators/co-repressors. In addition, recent studies demonstrate that cell signaling pathways modulate receptor and co-activator activities contributing to overall activity. Misregulation of any of these components has the potential to elevate AR activity.

Hormone Levels After Androgen Ablation

Androgen receptor activity is regulated by its two major ligands, testosterone and 5-α-dihydrotestosterone (DHT) (for a brief review of androgen metabolism, see Debes and Tindall [2002]). The major circulating androgen, testosterone, is produced primarily in the testis with a small amount produced in the adrenal glands. In prostate, testosterone is converted by 5-α-reductase to the more potent androgen, DHT.

Because chemical or surgical androgen ablation dramatically reduces the levels of circulating testosterone and initially causes a reduction in serum PSA, it was believed that tissue levels of androgens were correspondingly reduced. However, a recent study by Mohler et al. [2004] revealed that tissue levels of testosterone in locally recurrent prostate cancer were similar to those in tissue from patients who had not received androgen ablation therapy. Although DHT levels were much lower in the recurrent samples (8.13 nM for untreated and 1.45 nM for recurrent), studies in cell lines indicate that the residual DHT should be sufficient to activate AR significantly. Thus, this residual hormone, in combination with other factors that potentiate AR activity, is likely to be sufficient to cause AR-dependent tumor growth. Whether recurrent prostate cancer metastases contain comparable levels of androgens and their metabolites is unknown. However, the studies of Holzbeierlein et al. [2004] suggest that the recurrent metastases may be capable of producing the necessary steroids. They found elevated levels of mRNA for steroid metabolizing enzymes including squalene monooxygenase, a rate limiting enzyme for steroid biosynthesis and a corresponding increase in expression detected by immunohistochemistry. Thus, one potential means for compensating for the reduced levels of circulating testosterone is elevated endogenous biosynthesis of androgens. Although these results would suggest that a combination of androgen ablation and an anti-androgen should be effective in blocking the activity of the residual steroids, patients typically also become resistant to combination therapies using an anti-androgen such as flutamide. As discussed in subsequent sections, there are a number of changes in other components of the AR signaling pathway that can reduce the effectiveness of antagonists.

Androgen Receptor Expression and Prostate Cancer

Androgen receptor expression in prostate cancer is heterogeneous and despite numerous studies, there has been debate regarding correlations between AR expression and prostate cancer (reviewed in [Balk, 2002] and [Edwards and Bartlett, 2005a]). Li et al. [2004] examined AR expression using tissue microarrays containing samples of normal and tumor tissue obtained from radical prostatectomies of patients who had not yet received hormonal treatment and found that AR expression was generally lower in the tumor tissue than in the normal tissue. However, a comparison of AR expression in tumor samples with markers of clinical aggressiveness and time to biochemical recurrence revealed that elevated AR expression was correlated with a shorter time to biochemical recurrence highlighting the importance of AR in prostate cancer. Moreover, as described above, AR expression is increased in direct comparisons of androgen-dependent and androgen-independent prostate cancers. The *AR* gene is amplified in about 20–30% of androgen-resistant prostate cancers (reviewed in [Edwards and Bartlett, 2005a]), accounting for a fraction of these cases, but many cases exhibited elevated AR levels without amplification [Holzbeierlein et al., 2004]. The cause(s) of elevated AR expression in the other cases has not been determined. However, Gregory et al. [2001b] demonstrated increased AR stability in androgen-independent cell lines compared to androgen-dependent lines suggesting that this contributes to the increase in AR levels. Mellinghoff et al. [2004] reported that inhibition of HER-2 signaling decreased AR stability implicating elevated cell signaling as a means of increasing AR stability and resulting levels of AR.

Androgen Receptor Mutations in Prostate Cancer

Because the *AR* gene is located on the X chromosome, most prostate cancer cells will have a single copy of the gene and mutations in the AR will have more profound effects than a mutation in genes with two copies/cell. There has been a great deal of interest in determining whether mutations in AR contribute to prostate cancer and, in particular, to androgen independence (see [Balk, 2002] and [Feldman and Feldman, 2001] for reviews and http://www.androgendb.mcgill.ca/prost.gif for a listing of the identified mutations). Although the percent of mutations found varies from study to study, there is general agreement that mutations are less frequent in patients who have not received hormone ablation therapy and that they are most frequent in patients who have failed treatment that includes an anti-androgen. Even under these conditions, the majority of the tumors express wild-type AR. Analysis of tumors from the TRAMP mouse model of prostate cancer revealed a number of AR

mutations [Han et al., 2001] and expression of one mutation, E231G, but not wild-type AR in transgenic mice was sufficient to cause prostate cancer [Han et al., 2005]. Thus, AR mutations are at least theoretically capable of inducing prostate cancer. The greatest frequency of mutations is found in patients who have been treated with flutamide [Balk, 2002]. Typically, the mutations found in these tumors cause AR to respond to the flutamide metabolite, 4-OH flutamide, as an agonist. The best known of these mutations, T877A, is also found in the LNCaP cell line and has been found repeatedly in flutamide-treated patients [Balk, 2002]. The mutations are somatic mutations presumably arising due to the selective pressure for AR activity. These mutations are significant contributors to the failure of flutamide treatment and withdrawal of flutamide often results in a short-term response.

Androgen Receptor Co-Activators/Co-Repressors in Prostate Cancer

Co-activators were originally identified as proteins that interact with agonist-bound steroid receptors enhancing their transcriptional activity. Numerous co-activators have been identified, some of which play roles in enhancing the activities of wide ranges of transcription factors while others are more restricted in their targets [Heinlein and Chang, 2002]. Co-activators exhibit a remarkable range of enzymatic activities. The first steroid receptor co-activators to be identified were found to be histone acetyl transferases (HATs) or to be capable of recruiting other co-regulators with HAT activity such as p300/CBP (CREB binding protein) or P/CAF (P300/CBP associated factor), but more recent studies have revealed numerous other activities including methyltransferase activity, kinase activity, and ubiquitin ligase activity [Smith and O'Malley, 2004]. Recent studies utilizing chromatin immunoprecipitation (ChIP) assays have revealed that steroid receptors recruit a series of protein complexes that modify chromatin and facilitate binding and activation of the polymerase complex. Although many candidate co-activators have been identified, the relative contributions of most of these proteins to AR action have not been determined. While some of these proteins may be required for optimal AR function under all conditions, others likely contribute to tissue and/or target gene-specific actions of AR. Some of these proteins can potentiate AR action at low concentrations of hormone, enable AR to use other hormones, weak agonists, and antagonists as agonists, or enhance ligand-independent action of AR in response to altered cell signaling. Many of the proteins identified as AR co-activators, such as cyclins and β-catenin, are proteins with established functions independent of AR activation and thus they influence multiple cellular functions.

The potential importance of co-activators in AR action has led to numerous studies of their expression and role in cell growth and AR function. Many of the co-activators have been identified relatively recently; antibodies suitable for immunohistochemistry are often not commercially available leading to studies of expression at the mRNA level in cell lines and in human and xenograft samples. Interpretation of the tumor and tissue results is often confounded by the use of mRNA measurements without consideration of the proportion of tumor versus normal tissue and epithelial versus stromal cell components. The same limitations are of concern in experiments using Western blotting to assess expression. Some studies have used an insufficient number of samples to produce statistically valid results; others have used benign prostatic hyperplasia (BPH) samples rather than normal tissue for comparisons. Although such a comparison may reveal differences between the two diseases, BPH is also androgen sensitive so AR co-activators may also be elevated in this disease. These different approaches have led to conflicting results and the conclusions and limitations of the studies should be considered carefully when interpreting the data. Often a general comparison of levels in primary tumors versus normal tissue reveals no differences, whereas a comparison of tumor expression levels with time to biochemical recurrence or with markers of aggressive disease reveals significant correlations. For example, mRNA analyses revealed no differences in levels between tumor and BPH tissues for a battery of co-activators [Linja et al., 2004], but studies of protein expression levels revealed correlations with tumor aggressiveness as summarized below.

There is evidence that expression of the p160 co-activators plays a role in prostate cancer. This family is comprised of three members, steroid receptor co-activator-1 (SRC-1), SRC-2 (TIF2/GRIP-1), and SRC-3 (AIB1/RAC3). SRC-1

and SRC-3 have intrinsic HAT activity and all three bind and recruit additional HATs inducing histone acetylation that changes the chromatin conformation making binding sites on the DNA more accessible. There is good evidence that SRC-1 is important in normal prostate function. SRC-1 null mice exhibit impaired testosterone-dependent growth of the prostate [Xu et al., 1998]. In a study of a small number of prostate cancer specimens, Gregory et al. [2001a] found that SRC-1 expression was enhanced in recurrent prostate cancer relative to primary prostate cancer or BPH. Agoulnik et al. [2005] using tissue microarrays from more than 500 patients found that increased expression of SRC-1 was correlated with clinical markers of aggressive disease. SRC-1 expression in normal tissue was heterogenous, but there was a strong correlation between high levels of SRC-1 expression in tumor tissue with high levels in normal tissue suggesting that high levels of SRC-1 expression in normal tissue increases the risk for aggressive prostate cancer. In studies elucidating the role of SRC-1 in AR-dependent cell growth and gene expression, Agoulnik et al. [2005] found that SRC-1 is required for optimal growth of androgen-dependent LNCaP cells, androgen-independent, but AR-dependent C4-2 cells, but is not required for the growth of AR negative PC-3 or DU145 prostate cancer cells. Consistent with its role in modulating the actions of AR, reducing expression of SRC-1 using siRNA not only reduced AR-dependent cell growth, but also reduced expression of androgen-regulated genes including PSA. Remarkably, SRC-1 was also required for AR-dependent repression of maspin expression. As outlined below, SRC-1 activity is regulated by cell signaling and SRC-1 is also important for the ligand independent actions of AR [Ueda et al., 2002]. There is also evidence that SRC-2/TIF2 is overexpressed in prostate cancer [Gregory et al., 2001a]. SRC-3 is also important for the growth of prostate cancer cells, although many of its actions appear AR independent. SRC-3 is overexpressed in prostate cancer [Zhou et al., 2005] and its overexpression is correlated with increased Ki67 expression, reduced apoptosis, and elevated Akt signaling. Overexpression of SRC-3 increases Akt activity [Zhou et al., 2003]. SRC-3 stimulates AR-independent cell growth as a reduction in SRC-3 expression reduces growth of not only AR-positive LNCaP cells, but also AR-negative PC-3 and DU145 cells [Zhou et al., 2005]. The effects of reducing SRC-3 expression on AR target gene expression have not been reported, so its contribution to AR action remains to be determined. Interestingly, reducing expression of SRC-3, reduces expression of Bcl-2 and induces apoptosis [Zhou et al., 2005]. Thus, SRC-3 may be important for the survival of prostate cancer cells in the absence of a functioning AR signaling pathway.

There is also good evidence that the closely related co-regulators p300 and CBP play a role in androgen action in prostate cancer although their broad roles as co-regulators of numerous transcription factors support a role in general growth regulation. Debes et al. [2003] have shown a correlation between prostate cancer proliferation and p300 expression and previous studies from this group showed that p300 is important both for hormone-dependent and IL-6-dependent activation of AR [Debes et al., 2002]. Also of interest is the finding that p300 acetylates AR; preventing this acetylation by site-directed mutagenesis reduces AR transcriptional activity [Fu et al., 2002].

A series of structurally unrelated AR co-activators termed AR associated proteins (ARA70, ARA55, ARA54 etc.) have been identified by the Chang group [Heinlein and Chang, 2002]. Although originally identified as AR interacting proteins, many of these serve as co-activators for other steroid receptors as do the p160 co-activators and other co-regulators. Expression of a dominant negative form of ARA54 in LNCaP cells reduces cell growth and PSA expression implicating ARA54 in AR action [Miyamoto et al., 2002].

Despite the limited information regarding the relative contribution of specific co-activators to AR activity in prostate cancer, one fairly consistent finding is that artificial overexpression of a variety of co-activators induces AR activity in the presence of a broad range of metabolites including ligands for other receptors (estradiol), adrenal androgens, androgen metabolites, and anti-androgens (see Edwards and Bartlett [2005b] for a review). These findings may be a reflection of the ability of a variety of ligands to bind to AR and to induce nuclear localization and DNA binding. The overexpression of co-activators, in many cases, will enhance AF-1 activity. Thus, overexpression of co-activators is a candidate cause for anti-androgen resistance.

Modulation of Androgen Receptor Activity by Alterations in Cell Signaling and Effects on Prostate Cancer Cell Growth

There is ample evidence demonstrating that AR activity is regulated by cell signaling pathways and that enhancing the activity of specific cell signaling pathways can lead to androgen independence. In a number of instances, the studies support a continued role for AR despite the elimination of the requirement for high levels of androgens. AR is a phosphoprotein and a number of confirmed and candidate phosphorylation sites have been identified (reviewed in [Gioeli, 2005]). Many, if not all, of the co-activators are also phosphoproteins. Thus, there are many targets in the AR signaling pathway.

Numerous studies have described elevated expression of a variety of growth factors in prostate cancer cells leading to an elevation in autocrine growth factor signaling; elevated levels of p42/p44 MAPK have been detected in advanced prostate cancer (reviewed in [Gioeli, 2005]). Several of the cell signaling pathways that are activated by the growth factor receptors have been implicated in the regulation of AR activity and cell growth. Early studies using reporter assays showed that in some cells, treatment with growth factors, forskolin (a PKA activator) or IL-6 induced AR activity in the absence of added hormone (reviewed in [Gioeli, 2005]). Androgen-independent sublines of androgen-dependent LAPC-4 cells express higher levels of HER-2/neu (an EGF receptor family member) than do the androgen-dependent cells [Craft et al., 1999]. Artificial overexpression of HER-2/neu induces hormone-independent growth and activates AR in LAPC-4 cells, implicating this pathway as a means of developing androgen independence. Inhibition of HER-2 using PKI-166, inhibited AR activity and AR recruitment to the PSA promoter as well as reduced AR expression levels. Inhibition of Akt, one of the kinases activated by HER-2 did not recapitulate the actions of PKI-166 implicating another downstream kinase in the regulation of AR activity [Mellinghoff et al., 2004]. HER-2 signaling is also implicated in androgen independence in an LNCaP lineage. Late passage androgen-independent LNCaP cells, termed C-81 cells, exhibit elevated HER-2 signaling and androgen-independent PSA secretion. Inhibition of this pathway restores androgen dependence of cell growth and PSA expression [Lee et al., 2003; Lee et al., 2004]. These studies implicated p42/p44 MAPK as a downstream kinase required for hormone-independent PSA expression.

Although, as described above, there is extensive evidence to support the concept that altered cell signaling potentiates AR activity and facilitates prostate cancer cell growth when suboptimal levels of hormones are present, the targets of the cell signaling pathways that induce AR activity under these conditions have not been well defined. In some cases, AR may be the direct target, but in others the co-regulators may be targets. Activated ras working through the p42/p44 MAPK pathways decreases the requirement for androgens in LNCaP cells [Weber and Cioeli, 2004], but known phosphorylation sites in AR are not regulated by these kinases [Gioeli, 2005]. However, this signaling pathway potentiates the activity of AR in both LNCaP lineages and in CWR22 cells. In addition to some evidence for receptor stabilization as a result of activation of this pathway, there is good evidence that MAPK-dependent phosphorylation of the two p160 co-activators SRC-1 [Ueda et al., 2002] and TIF2 [Gregory et al., 2004] contributes to the potentiation of AR activation through these pathways.

An additional means of cell signaling altering the activity of AR is through its effects on co-repressor activity. Co-repressors have classically been considered to be proteins that interact with antagonist-bound steroid receptors recruiting histone deacetyalases. However, recent studies have indicated that co-repressors can reduce the activity of agonist-bound AR [Agoulnik et al., 2003]. Elevated-MEKK1 activity through growth factor signaling induces phosphorylation of one co-repressor (SMRT), dissociation from nuclear receptors, and relocalization of the co-repressor to the cytoplasm [Jonas and Privalsky, 2004]. Thus, activation of this cell signaling pathway would not only reduce the effectiveness of antagonists but also enhance the activity of agonist bound receptor.

Other studies have suggested that Akt can potentiate AR activity, but the findings in this area are contradictory indicating that the relationship between AR activity and Akt is complex. Activated-Akt is associated with advanced prostate cancer [Zhou et al., 2005] and there are reports that Akt activation enhances AR activity [Lin et al., 2003; Lu

et al., 2006] but others describe inhibition of activity [Lin et al., 2003]. There are multiple Akt enzymes and most of the studies have relied on inhibition of the upstream activator PI-3 kinase to evaluate the role of this signaling pathway in AR action. This pathway regulates AR activity at multiple points and the relative importance of these contributions may determine the net effect on AR activity in a particular cellular context. AR is degraded through an mdm2-dependent proteasome degradation pathway and Akt phosphorylation activates mdm2. Thus, Akt activation will reduce the level of AR [Lin et al., 2002] unless there is a compensating signal to block this degradation. In contrast, Akt may stimulate AR activity through its phosphorylation and consequent activation of the HAT activity of p300 [Huang and Chen, 2005], a co-regulator important for both hormone-dependent and IL-6-dependent activation of AR and for acetylation of AR [Fu et al., 2002].

Whether Akt directly phosphorylates AR regulating its activity has been controversial. AR can be phosphorylated by Akt in vitro on sites including Ser^{213}. Activation of PI-3K (which activates Akt) inhibits the transcriptional activity of wild-type AR, but not of AR with a non-phosphorylatable alanine substituted for Ser^{213} [Taneja et al., 2005]. However, Gioeli [2005] found no evidence of phosphorylation of Ser^{213} in LNCaP cells. A recent study utilizing a newly developed antibody to the phosphorylated Ser^{213} epitope provides some clues to this discrepancy [Taneja et al., 2005]. Ser^{213} is phosphorylated in some tissues and cells, but not in others. Phosphorylation of wild-type AR was detected in LAPC-4 prostate cancer cells, but not in LNCaP cells, which express a mutant AR T877A. When the phosphorylation of wild-type AR and T877A AR were compared directly in transfected cells, the T877A mutant was only weakly phosphorylated compared to wild-type AR. Thus, in addition to its broadened ligand specificity, this mutant may be less susceptible to inhibition of its activity by Akt.

SUMMARY

Recent studies have revealed a variety of factors that can contribute to the potentiation of AR activity at low levels of hormone or can cause antagonists to act as agonists. Increased expression of AR and some of its co-activators certainly can contribute and increased expression has been detected in prostate cancer. However, other studies have highlighted the importance of cell signaling in modulating AR activity through effects on its expression, stability, and transcriptional activity. While some of these alterations are due to direct AR phosphorylation, many are a result of altered co-regulator phosphorylation. Consequently, levels of co-regulators (or of other proteins) are insufficient to predict the activation of AR. Specific co-regulator phosphorylations in the absence of any increase in expression will also have profound effects on AR activity. A better understanding of the interplay between the cell signaling pathways and AR and its co-regulators is needed to develop means to target these pathways to inhibit AR action.

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