

Androgen receptor corepressors and prostate cancer

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Abstract

The androgen receptor (AR) mediates the effects of male steroid hormones (androgens) and contributes to a wide variety of physiological and pathophysiological conditions. As such, the regulatory mechanisms governing AR activity are of high significance. Concerted effort has been placed on delineating the mechanisms that control AR activity in prostate cancer, as AR is required for survival and proliferation in this tumor type. Moreover, AR is the central therapeutic target for metastatic prostate cancers, and recurrent tumors evade therapy by restoring AR activity. It is increasingly apparent that AR cofactors which modulate receptor activity can contribute to prostate cancer growth or progression, and this has been particularly well established for AR coactivators. The present review is focused on the role of AR corepressors in governing androgen action, with a specific emphasis on their activities in prostate cancer.

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Introduction

Androgens regulate numerous physiological responses from male sexual development to bone and muscle growth. The biological action of androgens is mediated through the androgen receptor (AR), a ligand-dependent transcription factor that belongs to the nuclear receptor superfamily (Trapman & Brinkmann 1996). There is no sexually dimorphic organ, including the brain, which is not affected by AR during development. A large number of spontaneous mutations have been characterized that affect a broad range of phenotypes of male developmental abnormalities, including androgen insensitivity syndrome and spinal and bulbar muscular atrophy. AR is also necessary for the development of pathologic conditions later in life, most notably prostate cancer.

Prostatic adenocarcinoma is the most commonly diagnosed malignancy and the second leading cause of male cancer death in most western countries, including the United States (Denmeade & Isaacs 2002, Jemal *et al.* 2006). Remarkably, prostate cancer development and progression are dependent upon AR function (Trapman & Brinkmann 1996, Taplin & Balk 2004), and first-line treatment for metastatic (including micrometastatic) disease is designed to specifically inhibit AR activity (Feldman & Feldman 2001, Denmeade & Isaacs 2002). These treatment regimens

are accomplished through either depletion of ligand (testosterone) and/or through the use of antagonists that bind directly to AR (Denmeade & Isaacs 2002, Salesi *et al.* 2005). Both intervention strategies are highly effective at reducing AR function, thereby inducing both cell cycle arrest and apoptosis in AR-dependent prostate cancer cells (Denmeade *et al.* 1996, Agus *et al.* 1999). However, recurrent tumors ultimately arise for which treatment efficacy is limited. Clinical analyses of recurrent tumors and recapitulation of therapeutic resistance in model systems strongly support the hypothesis that aberrant AR reactivation is responsible for tumor recurrence despite hormonal blockade (Feldman & Feldman 2001). Given the importance of AR activity for prostate cancer development, growth, and progression, a concerted effort has been put forth to delineate the factors that regulate AR activity.

Androgen receptor activation

Androgens activate AR by unmasking its ability to bind DNA and thereby activate gene transcription. The AR belongs to the steroid receptor subclass of nuclear receptors, all ligand-activated transcription factors which bind to specific DNA sequences and transactivate a distinct subset of genes (Evans 1988,

Mangelsdorf *et al.* 1995, Gelmann 2002). The AR protein is comprised of four domains: (i) an N-terminal domain (NTD) with the principle transcriptional activation functions (AF-1 and AF-5), (ii) a central DNA-binding domain (DBD), (iii) a hinge region, and (iv) a C-terminal ligand-binding domain flanked at the far C-terminus by another transcriptional activation function domain (AF-2; Jenster *et al.* 1992, Brinkmann *et al.* 1999). A nuclear import sequence flanks the DNA-binding and hinge regions. Like all nuclear steroid receptors, the AR is activated by ligand binding (Wilson 1996, Jenster 1999). Testosterone, secreted by both the testes and the adrenal gland, is the most abundant androgen and ligand for the AR present in the human male circulation. In target tissues, such as the prostate, testosterone is converted to dihydrotestosterone (DHT), through the action of 5 α -reductase. DHT is a high-affinity ligand for the AR, and although present at lower levels than testosterone, DHT is a much more powerful AR agonist (He *et al.* 2006). Prior to activation by ligand (DHT), the AR is present diffusely throughout the cytoplasm and nucleus of prostatic epithelial and adenocarcinoma cells. The inactive receptor is associated with heat-shock proteins that sequester AR in the cytoplasm and prevent the AR from exerting transcriptional activity (Marivoet *et al.* 1992). Upon ligand binding, the receptor dissociates from the heat-shock proteins and translocates to the nucleus (Jenster *et al.* 1993, Zhou *et al.* 1994). Active AR homodimers recognize and bind specific DNA sequences called 'androgen-response elements' (AREs), which are located in the promoter and/or enhancer region of androgen-responsive genes (Trapman & Brinkmann 1996) and can be organized into either spaced palindromic or direct repeats (Verrijdt *et al.* 2003). Subsequent recruitment of cofactors regulates the ability of the AR to stimulate transcription of these target genes (Jenster 1998, Heinlein & Chang 2002).

AR coactivators in prostate cancer

It is evident that ligand-induced cofactor recruitment strongly influences the outcome of receptor activation. AR coactivators serve as critical amplifiers that affect gene expression and transcription complex assembly in a context-specific manner. Since coactivators can demonstrate differential functions dependent on promoter context and/or in the presence of selected AR agonists, it is hypothesized that coactivators assist in controlling the ligand and tissue-specific activities of the

receptor. Several distinct classes of AR coactivators have been identified, and these have been extensively reviewed (Heinlein & Chang 2002, Cullig *et al.* 2004; also see Androgen Receptor Mutations Database, <http://www.androgendb.mcgill.ca/>). In general, coactivators act directly or recruit enzymes to modify chromatin through either covalent histone modification (e.g. acetylation and methylation) or remodeling of chromatin structure (e.g. SWI/SNF complexes). Such modifications foster a state of open chromatin, allowing recruitment of basal transcription machinery and RNA polymerase II (Sommer & Fuqua 2001, Urnov & Wolffe 2001, Heinlein & Chang 2002, Narlikar *et al.* 2002). More specialized AR coactivators serve to modulate association of the AR NTD with the carboxy terminal domain (N/C interaction). The AR N/C interaction is mediated through an FxxLF motif in the receptor NTD, which interacts with and occupies a hydrophobic cleft in the C-terminal AF-2 domain upon ligand binding (Zhou *et al.* 1994, He *et al.* 2000). Successful interaction of these two motifs is important for robust transcriptional activity, and recently it has been shown that the N/C interaction is critical for AR-chromatin association in cells (Li *et al.* 2005). Some coactivators, including steroid receptor coactivator 1 (SRC-1) and translation initiation factor 2 (TIF2), have been shown to facilitate N/C interaction and thus stabilize the AR-ligand association, promote subsequent coactivator recruitment, and enhance overall receptor stability (Ikonen *et al.* 1997, Berrevoets *et al.* 1998). Alternatively, factors that negatively modulate N/C interaction (e.g. MAGE-11) can induce AR activity by facilitating coactivator recruitment to the AF-2 domain (Bai *et al.* 2005). Thus, modulation of N/C interactions represents a major mechanism by which coactivators govern AR activity.

Given the potent role of coactivators in enhancing AR function, it was suspected that AR effectors may be deregulated in a subset of prostate cancers. Emerging evidence supports this concept. For example, aberrant expression of the coactivators SRC-1 and TIF2 is associated with relapse after therapeutic intervention (Fujimoto *et al.* 2001, Gregory *et al.* 2001, Agoulnik *et al.* 2005). Additionally, the androgen receptor-associated proteins 70 (ARA70) and 55 (ARA55) can enhance AR activity in the presence of selected therapeutic AR antagonists (e.g. hydroxyflutamide; Miyamoto *et al.* 1998, Fujimoto *et al.* 1999). ARA70 expression is also induced post-castration in a xenograft prostate cancer model system, suggesting

that this is one mechanism that facilitates the transition to therapeutic resistance (Gregory *et al.* 1998). Most recently, ARA70 expression was shown to be enhanced in the neoplastic prostate as compared with benign tissue, thus further implicating a role for this coactivator in prostate cancer progression (Hu *et al.* 2004). When combined, these observations indicate that cofactor deregulation may have significant impact on prostate cancer growth and progression. Here, we review the mechanisms of AR corepressor function and discuss the role of these cofactors in prostate cancer (Table 1).

AR corepressors: mechanisms of action

The mechanisms of corepressor action are as vast as that of their counterparts, the coactivators. By the classical definition, corepressors are those molecules that bind directly to AR and recruit chromatin-modifying enzymes that preclude transcriptional activation. However, a large number of molecules have been identified that negatively regulate AR activity through alternate mechanisms. For the purposes of this review, corepressors have been subdivided based on their mechanism of repression, including: (i) chromatin modification, (ii) regulation of AR N/C interaction, (iii) abrogation of AR-chromatin association or nuclear translocation, and/or (iv) inhibition of coactivator recruitment (Fig. 1).

Chromatin modifiers: Corepressors that trigger chromatin condensation and/or chromatin modifications typically recruit histone deacetylases (HDACs) to the AR complex. As mentioned, a certain subset of coactivators acetylate lysine residues on histone tails, thus loosening the association of nucleosomes with DNA and facilitating gene transcription (Fu *et al.* 2002a). HDACs counterbalance these effects through deacetylation of the same lysine residues, and this event results in both chromatin condensation and promoter inaccessibility (Sengupta & Seto 2004). However, HDACs lack inherent DNA-binding capability, and also require activation by secondary molecules to induce enzymatic activity (Sengupta & Seto 2004). For example, HDAC3 requires activation by nuclear receptor corepressor (NCoR) or silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) to exert HDAC function (Alland *et al.* 1997, Heinzel *et al.* 1997, Wen *et al.* 2000). It is believed that these regulators exist in large repressor complexes that require additional alternate subunits, such as transducin β -like 1 (TBL1), for function (Yoon *et al.* 2003). Lastly,

evidence suggests that HDACs may impinge on alternate, non-histone substrates to govern AR function. For example, AR itself is known to be acetylated by p300/CBP-associated factor (P/CAF) in key lysine residues within the hinge region, and this modification has been shown to enhance AR activity and subsequent coactivator recruitment (Fu *et al.* 2002b, 2003). It is presumed that a lysine deacetylase (such as HDAC) counterbalances this function. Thus, while it is clear that HDACs act in concert with NCoR and SMRT to elicit chromatin condensation, this class of repressor may directly modify the AR itself or other AR cofactors to modulate receptor activity.

Given the potent ability of HDACs to control chromatin structure, it is not surprising that many AR corepressors recruit HDACs (in addition to NCoR and SMRT) to induce transcriptional repression. Several AR corepressors are known to depend on HDAC function. Cyclin D1, protein inhibitor of activated STAT, PIAS γ , short heterodimer partner (SHP), and AR corepressor -19 kDa (ARR19) have been shown to directly interact with HDACs, and pharmacological inhibitors of HDAC function reduce the efficacy of each AR corepressor (Gobinet *et al.* 2001, Petre *et al.* 2002, Gross *et al.* 2004, Jeong *et al.* 2004). For example, cyclin D1 associates directly with HDAC3, and utilizes this mechanism to repress both AR and thyroid hormone receptor activity (Lin *et al.* 2002, Petre-Draviam *et al.* 2004). In both cases, cyclin D1 corepressor function was partially relieved upon treatment with trichostatin A (TSA), an established HDAC inhibitor (Lin *et al.* 2002, Petre *et al.* 2002). In addition, PIAS γ has been shown to interact with HDAC1, and in this case, AR activity was completely restored in the presence of TSA (Gross *et al.* 2004). Thus, HDAC recruitment is a critical component of this class of corepressor.

Other AR corepressors (such as ErbB3-binding protein (EBP1), DJ1-binding protein (DJBP), and TG-interacting factor (TGIF); (Sharma & Sun 2001, Niki *et al.* 2003, Zhang *et al.* 2005a)) employ Sin3 complexes to exert transcriptional repression, which consist of Sin3a and Sin3b in conjunction with either HDAC1 or HDAC2. The Sin3 complex is pre-assembled prior to chromatin recruitment; therefore, corepressors serve to provide specificity of Sin3 activity by recruiting the complex to appropriate gene targets (Silverstein & Ekwall 2005). More specifically, EBP1 has been shown to be recruited to AR targets in the presence of therapeutic antagonists in concert with HDAC2 (Zhang *et al.* 2005b), thus providing evidence of EBP1 function in prostatic cancer therapy. Interestingly, the recently identified

Table 1

| Corepressor | Interaction | Mechanism | Biological relevance | Citations |
|--------------------------------|-------------------|--|---|--|
| AES | NTD | ND | Prostatic epithelia expression | Yu <i>et al.</i> (2001) |
| ARA67/PAT1 | NTD/DBD/LBD | Cytoplasmic sequestration | PCa cell line expression | Zhang <i>et al.</i> (2004) |
| ARR19/Cmtm2a/ Cklfsf2a | DBD | Recruits HDAC4 | Expression in mouse prostate (weak), not in human prostate | Jeong <i>et al.</i> (2004) and Shi <i>et al.</i> (2005) |
| Calreticulin | DBD | Inhibits DNA binding | ND | Dedhar <i>et al.</i> (1994) |
| Cyclin D1 | AR1-36, 626–666 | Inhibits N/C, Recruits HDACs sequestors coactivators | Altered localization or expression in advanced disease, isoform upregulated in PCa | Knudsen <i>et al.</i> (1999), Drobnjak (2000), Reutens <i>et al.</i> (2001), Petre <i>et al.</i> (2002), Petre-Draviam <i>et al.</i> (2003), Maddison <i>et al.</i> (2004), Burd <i>et al.</i> (2005, 2006) |
| Dax-1/NROB1 | LBD | Inhibits N/C | Expressed in prostatic epithelia, absent in BPH | Yuan <i>et al.</i> (2001), Holter <i>et al.</i> (2002) and Agoulnik <i>et al.</i> (2003) |
| DAXX/DAP6 | DBD | ND | ND | Lin <i>et al.</i> (2004a) |
| DJBP | DBD | Recruits Sin3a/ HDACs | Expressed in testes | Niki <i>et al.</i> (2003) |
| EBP1 | NTD | Recruits Sin3a/HDAC2 | Prostatic epithelia expression | Xia <i>et al.</i> (2001), Zhang <i>et al.</i> (2002, 2004, 2005a,b) |
| FLNA | Hinge | Inhibits N/C, suppresses TIF2 activation | PCa cell line expression | Loy <i>et al.</i> (2003) |
| Fox H1 | NTD | ND | ND | Chen <i>et al.</i> (2005) |
| GSK3β | NTD/LBD | Inhibits N/C, Degrades β-catenin | Overexpressed in PCa cell lines | Wang <i>et al.</i> (2004a,b) |
| HBO1 | DBD–LBD | ND | Prostatic expression | Sharma <i>et al.</i> (2000) |
| Hey1/Hesr1/HRT1/ CHF2/HERP2 | NTD | Possible HDAC recruitment | Cytoplasmic in PCa | Belandia <i>et al.</i> (2005) |
| HOXB13 | ND | ND | Prostatic epithelia expression | Jung <i>et al.</i> (2004) |
| hRAD9 | LBD | Inhibits N/C | Prostatic expression | Wang <i>et al.</i> (2004a,b) |
| LATS2/KPM | LBD | Inhibits N/C | Decreased expression in PCa | Powzaniuk <i>et al.</i> (2004) |
| N-COR | DBD | Recruits HDACs | Critical for antagonist action, deregulated in PCa by inflammation, expressed in the prostate | Cheng <i>et al.</i> (2002), Shang <i>et al.</i> (2002), Berrevoets <i>et al.</i> (2004), Kang <i>et al.</i> (2004), Song <i>et al.</i> (2004), Hodgson <i>et al.</i> (2005), Yoon & Wong (2005) and Zhu <i>et al.</i> (2006) |
| NF–KB | LBD | ND | Increased nuclear localization in PCa | Palvimo <i>et al.</i> (1996), Cinar <i>et al.</i> (2004), Sweeney <i>et al.</i> (2004), Lessard <i>et al.</i> (2005) |
| p53 | ND | Inhibits N/C, DNA binding | Mutated/lost in PCa | Gumerlock <i>et al.</i> (1997), Shenk <i>et al.</i> (2001), Shi <i>et al.</i> (2002), Cronauer <i>et al.</i> (2004), Shi <i>et al.</i> (2004), Tsui <i>et al.</i> (2004) |
| PAK6 | LBD, AR634–668 | Cytoplasmic sequestration | Prostatic expression | Yang <i>et al.</i> (2001), Lee <i>et al.</i> (2002), Schrantz <i>et al.</i> (2004) |
| PIAS3 ^a | AR1-333 | ND | Prostatic expression | Junicho <i>et al.</i> (2000), Gross <i>et al.</i> (2001) |
| PIASy | DBD | Recruits HDACs | Prostatic expression | Gross <i>et al.</i> (2001, 2004) |
| PTEN | DBD/LBD | Cytoplasmic sequestration, AR degradation | Mutated/lost in PCa | McMenamin <i>et al.</i> (1999), Li <i>et al.</i> (2001), Halvorsen <i>et al.</i> (2003), Dreher <i>et al.</i> (2004), Lin <i>et al.</i> (2004b) |
| PYK2/PTK2/FAK2 | Does not interact | Phosphorylates ARA55 and inactivates AR coactivation | PCa cell lines | Wang <i>et al.</i> (2002) |

(continued)

Table 1 continued

| Corepressor | Interaction | Mechanism | Biological relevance | Citations |
|-------------|-------------|---|---|--|
| Rack1 | DBD/LBD | Inhibits DNA binding | ND | Rigas <i>et al.</i> (2003) |
| SHP | NTD/LBD | Competes with TIF2/FHL2, recruits HDAC1 | ND | Gobinet <i>et al.</i> (2001) |
| SMRT | NTD/LBD | Inhibits N/C recruits HDACs | Critical for antagonist action, expressed in the prostate | Dotzlaw <i>et al.</i> (2002), Shang <i>et al.</i> (2002), Liao <i>et al.</i> (2003), Agoulnik <i>et al.</i> (2003), Song <i>et al.</i> (2004) and Yoon & Wong (2005) |
| SRY | 553–635 | ND | Testicular function, lost in prostate cancer | Perincheri <i>et al.</i> (2000) and Yuan <i>et al.</i> (2001) |
| TGIF | 559–624 | Recruits Sin3a/HDACs | ND | Sharma & Sun (2001) |

^aHas been published as a corepressor and coactivator. ND, not determined. Androgen receptor corepressors: NTD, N-terminal domain; DBD, DNA binding domain; LBD, Ligand binding domain.

Hey1 corepressor links the Notch pathway to AR regulation and requires two distinct repressor domains to govern AR function (Belandia *et al.* 2005). While both the C-terminal and bHLH domains of Hey1 utilize HDAC function, the latter can also act in a HDAC-independent manner. As both domains are required to elicit transcriptional repression (Belandia *et al.* 2005), these data indicate that HDACs are not universally sufficient to suppress AR function. In sum, these observations reveal that chromatin-modifying enzymes, such as HDACs, play significant roles in eliciting AR corepressor function, and those corepressor molecules lend specificity to AR regulation.

Modulation of AR N/C interactions: Upon ligand binding, the AR undergoes structural changes, thus providing a second mechanism by which corepressor molecules can dictate receptor function. As discussed previously, ligand binding stimulates an association between the ²³FxxLF²⁷ motif and the hydrophobic surface of the AR LBD that results in enhanced receptor stability and activity (He *et al.* 2000). Moreover, N/C interactions can also modulate coactivator function (He *et al.* 2001, 2002). Recent evidence demonstrates that N/C interaction is essential for AR to effectively bind chromatin (but not naked DNA), thus underscoring the importance of this structural change (Li *et al.* 2005). The biological significance of N/C association is well established, as genetic disruption results in weakened or lost receptor activity and the development of androgen insensitivity syndrome (Langley *et al.* 1998). Thus, N/C interaction represents a major mode of AR regulation that controls chromatin binding, receptor stability, and coactivator function.

Corepressors have been shown to modulate the N/C interaction through multiple mechanisms. First, selected corepressors prevent formation of the N/C interaction through intrinsic motifs that compete for binding. For example, hRad9 was identified as an AR corepressor through a yeast two-hybrid screen designed to identify factors that bind the AR DBD or LBD (Wang *et al.* 2004b). In these studies, an FxxLF motif within hRad9 was shown to compete for binding to the AR LBD, thus inhibiting N/C interaction and blocking AR activity (Wang *et al.* 2004b). A second class of N/C modifiers bind directly to regions of AR that mediate N/C interaction, and are presumed to preclude efficient N/C interaction through steric hindrance. Such a mechanism is proposed for large tumor suppressor 2 (lats2), which associates with a residue in the AR LBD known to be required for N/C interaction (I898; Powzaniuk *et al.* 2004). By contrast, cyclin D1 inhibits N/C interaction by binding directly

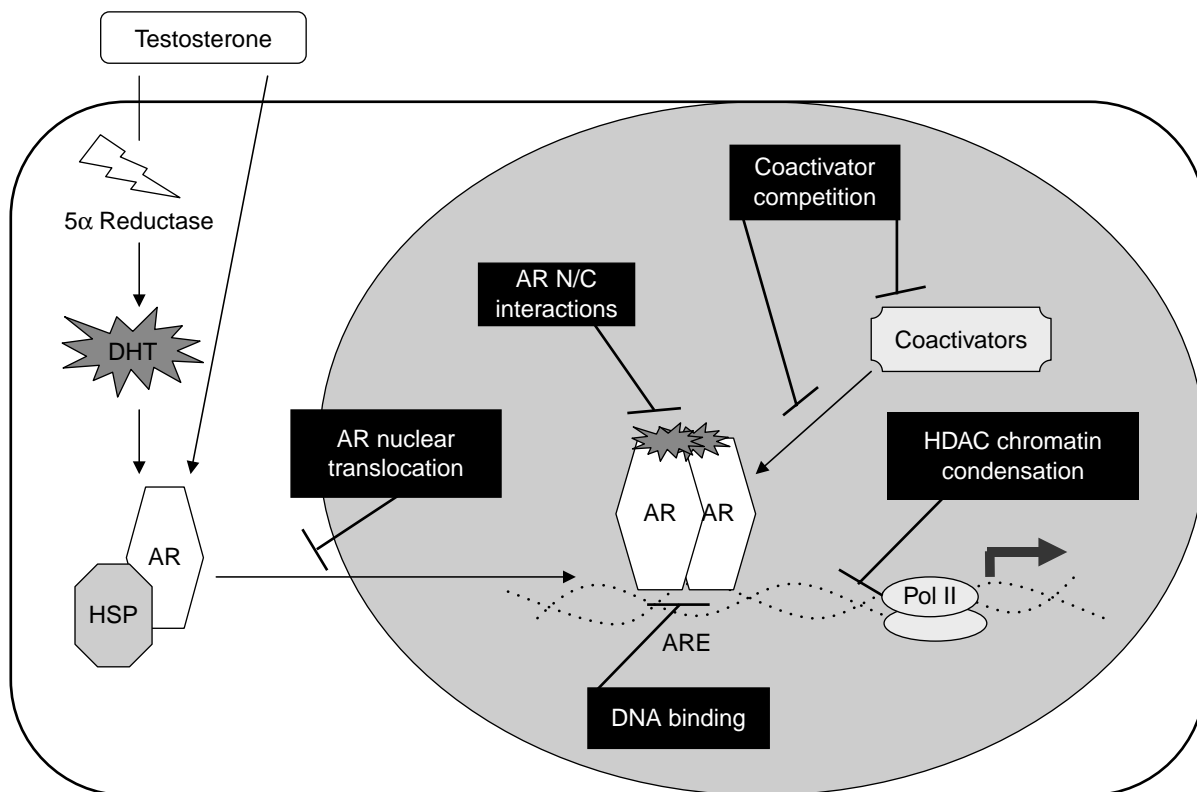


Figure 1 Diverse mechanisms of AR corepressor action. In prostate cancer cells, ligand (androgen) binding induces AR activity through stimulation of conformational changes in the receptor and nuclear translocation, wherein AR binds DNA through androgen-responsive elements. Subsequent coactivator recruitment facilitates gene activation. AR corepressors modulate AR activity through at least four divergent mechanisms, which serve to inhibit: (i) AR nuclear transactivation, (ii) N/C interaction, (iii) DNA binding, and/or (iv) coactivator recruitment. DHT, dihydrotestosterone; AR, androgen receptor; HSP, heat-shock protein; ARE, androgen-responsive element; HDAC, histone deacetylase; PolII, RNA polymerase II.

to the AR FxxLF region. The ability of cyclin D1 to bind the FxxLF motif is critical for its repressor function, and acts in concert with HDAC recruitment to impinge on AR activity (Burd *et al.* 2005). Lastly, several corepressors have been shown to block N/C interaction through as of yet undefined mechanisms. SMRT falls into this class, since the contact sites wherein SMRT interacts with AR (located in both the NTD and the LBD) are not known to be required for N/C interaction (Dotzlaw *et al.* 2002, Agoulnik *et al.* 2003, Liao *et al.* 2003). This is also true for filamin-A (FLNA), which binds the AR hinge region yet inhibits N/C interaction (Loy *et al.* 2003), and for p53, which strongly inhibits N/C interaction through an undefined motif (Shenk *et al.* 2001). Recent evidence suggests that post-translational modifications can also influence the AR N/C interaction, as glycogen synthase kinase 3β (GSK3β; capable of binding both the AR NTD and the LBD) inhibits this interaction through a mechanism dependent on its kinase activity (Wang *et al.* 2004a).

These collective observations indicate that control of the N/C interaction is a major effector point for AR corepressors. Assessing the relative impact of these functions on receptor stability and chromatin association will help to establish the importance of N/C regulation for corepressor activity.

DNA binding/nuclear translocation: As a nuclear receptor, the ability of AR to translocate to the nucleus and bind target gene promoters is essential to its biological function (Jenster *et al.* 1993). A small group of corepressors impinge on these processes to modulate AR activity. An example is the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) tumor suppressor, a phosphatase widely known for its role in preventing unscheduled cell cycle progression and cellular proliferation (Altomare & Testa 2005). PTEN has been shown to sequester AR in the cytoplasm of LNCaP cells (Lin *et al.* 2004b), although the mechanisms by which PTEN exerts this effect are not understood. It has also been shown that PTEN leads to

decreased AR stability and it is hypothesized that this destabilization event is functionally linked to cytoplasmic sequestration of AR (Lin *et al.* 2004b). Inhibition of AR nuclear translocation has also been observed with PAK6 (p21-activated kinase 6) and ARA67 (Schantz *et al.* 2004, Zhang *et al.* 2004). Other corepressors have been shown to block the AR-DNA association using *in vitro* assays, including DAXX and calreticulin (Dedhar *et al.* 1994, Lin *et al.* 2004a); not surprisingly, each of these corepressors interact with the AR DBD. For calreticulin, this interaction is mediated through its KxGFFKR motif, which is homologous to a common motif found in nuclear receptor DBDs (Dedhar *et al.* 1994). As such, it is proposed that calreticulin may act as a general repressor of nuclear receptor action. In addition, Rack1 has been associated with decreased AR promoter occupancy through its activation of the protein kinase C (PKC) pathway (Rigas *et al.* 2003). Together, these corepressors limit AR function by inhibiting AR nuclear translocation and/or DNA binding.

Coactivator competition: A number of AR corepressors function by inhibiting coactivator recruitment to the AR complex, as achieved through association with either AR or the coactivator itself. Both NCoR and SMRT have recently been shown to directly compete for coactivator recruitment by binding to AR. Ablation of either corepressor led to increased recruitment of the coactivators SRC-1 and p300 on endogenous AR targets (Kang *et al.* 2004, Hodgson *et al.* 2005, Yoon & Wong 2005). Several other proteins, including SHP and filamin-A (FLNA) have also been shown to inhibit AR function by competing with coactivators (Gobinet *et al.* 2001, Loy *et al.* 2003). However, most studies rely on transient assays and ectopically expressed AR targets to establish the impact of corepressors on coactivator function. In these model systems, competition is implied by a corepressor's ability to overcome expression of a coactivator, but the mechanisms of action are not revealed by this methodology. For example, it is possible that this function of FLNA may be related to its ability to inhibit N/C interactions, as TIF2 is one of the coactivators thought to stabilize this AR conformation (Alen *et al.* 1999).

An alternate group of corepressors acts directly on coactivators to influence AR activity. In some cases, the besieged coactivator is targeted for degradation. Such is the case with GSK-3 β , which initiates degradation of β -catenin, an established AR coactivator

(Truica *et al.* 2000, Masiello *et al.* 2004). By contrast, the proline-rich tyrosine kinase 2 (PYK2) corepressor phosphorylates the coactivator ARA55, thus preventing its association with AR. PYK2 is unique among the identified corepressors as it does not interact with AR (Wang *et al.* 2002). Cyclin D1 has also been shown to bind to coactivators (SRC-1 and P/CAF; Zwijnen *et al.* 1998, McMahon *et al.* 1999), and a mechanism has been proposed wherein under specific conditions cyclin D1 can suppress coactivator function (Reutens *et al.* 2001). The forkhead box H1 (FOXH1) corepressor has been shown to bind numerous AR coactivators; however, the biological relevance of this activity for FOXH1 repressor function has yet to be determined (Chen *et al.* 2005).

Alternate mechanisms: Lastly, a group of corepressors has been identified for which the mechanisms of action remain elusive. One such corepressor is histone acetyltransferase binding to ORC1 (HBO1), a member of the MYST family that inhibits AR activity through binding the DBD-LBD (Sharma *et al.* 2000). The identification of HBO1 as a repressor was surprising, as this protein contains histone acetyltransferase activity inherent in many coactivators. However, published reports have not challenged the requirement of HBO1 enzymatic activity for its role as a corepressor. A second corepressor whose functions remain elusive is amino-terminus enhancer of split (AES), which represses AR through HDAC-independent means (Yu *et al.* 2001). AES is known to interact with the basal transcription factor, TFIIE, but the relevance of this interaction for AR regulation is unknown. Finally, the PIAS family of proteins may modify AR function through post-translational modifications. The PIAS proteins are implicated in sumoylation of AR and harbor E3 sumoylation activity (Schmidt & Muller 2003). Interestingly, while several PIAS proteins are AR coactivators, selected PIAS family members (e.g. PIAS3) have been shown to exert both coactivator and corepressor activities, dependent upon experimental conditions (Junicho *et al.* 2000, Kotaja *et al.* 2000, Gross *et al.* 2001). The requirement of enzymatic function for corepressor activity has yet to be established, although the SUMO E3 ligase activity has been implicated (Nishida & Yasuda 2002).

In sum, corepressors impinge on virtually every facet of AR regulation, including modulation of AR localization, chromatin accessibility and binding, and cofactor recruitment. Given the importance of AR in prostate cancer, recent studies have begun to challenge the role of AR corepressors in this tumor type.

Corepressors and prostate cancer

Despite the significant advances in identifying AR corepressors and their mechanisms of action, fewer studies have addressed their relevance for human disease. Emerging evidence indicates that alterations in AR corepressor regulation may significantly influence prostate cancer development and progression. As summarized later, present analyses have revealed specific roles for AR corepressors with variant functions in prostate cancer (Fig. 2).

NCoR and SMRT: As described previously, NCoR and SMRT are master regulators of HDAC activity, and repress AR function through their ability to activate these chromatin-modifying enzymes (Cheng et al. 2002, Shang et al. 2002). In the context of prostate cancer, this function of NCoR and SMRT is particularly salient for the treatment of metastatic disease. First-line therapy for disseminated prostate cancer consists of androgen-deprivation therapy used prior to or in combination with AR antagonists, such as bicalutamide (Denmeade & Isaacs 2002, Salesi et al. 2005). It was previously thought that AR antagonists function passively by competing with androgen for binding to the LBD, and thereby precluding formation of an active receptor complex. However, groundbreaking studies demonstrated that antagonists, such as bicalutamide, also initiate

recruitment of corepressors to the AR complex, thus actively repressing transcription from AR target genes (Shang et al. 2002, Song et al. 2004, Yoon & Wong 2005). Analysis of antagonist-induced AR complexes indicated that NCoR and SMRT (in addition to HDACs) are critical components of this process (Shang et al. 2002). Other therapeutic agents, such as the partial antagonists mifepristone and cyproterone acetate specifically induce NCoR recruitment (Hodgson et al. 2005). Elimination of NCoR or SMRT reduced the efficacy of AR antagonists when transcription from endogenous target genes was monitored (Yoon & Wong 2005). Thus, NCoR and SMRT are critical effectors of AR antagonist function.

Most recently, it was shown that inflammation and consequent macrophage infiltration into prostate tumors initiates a signaling cascade that disrupts NCoR and HDAC activity (Zhu et al. 2006). Specifically, in the presence of selective androgen receptor antagonists, macrophages were demonstrated to signal through IL-1 β and MEKK1 to activate TAB2, a ‘molecular beacon’ that is responsive to inflammatory signaling pathways. TAB2 subsequently dismisses the NCoR holocorepressor complex (including TBL1/TBLR1-, Sin3a, and Brg1), from AR, thus resulting in derepression of AR activity. The ability of TAB2 to exert this activity

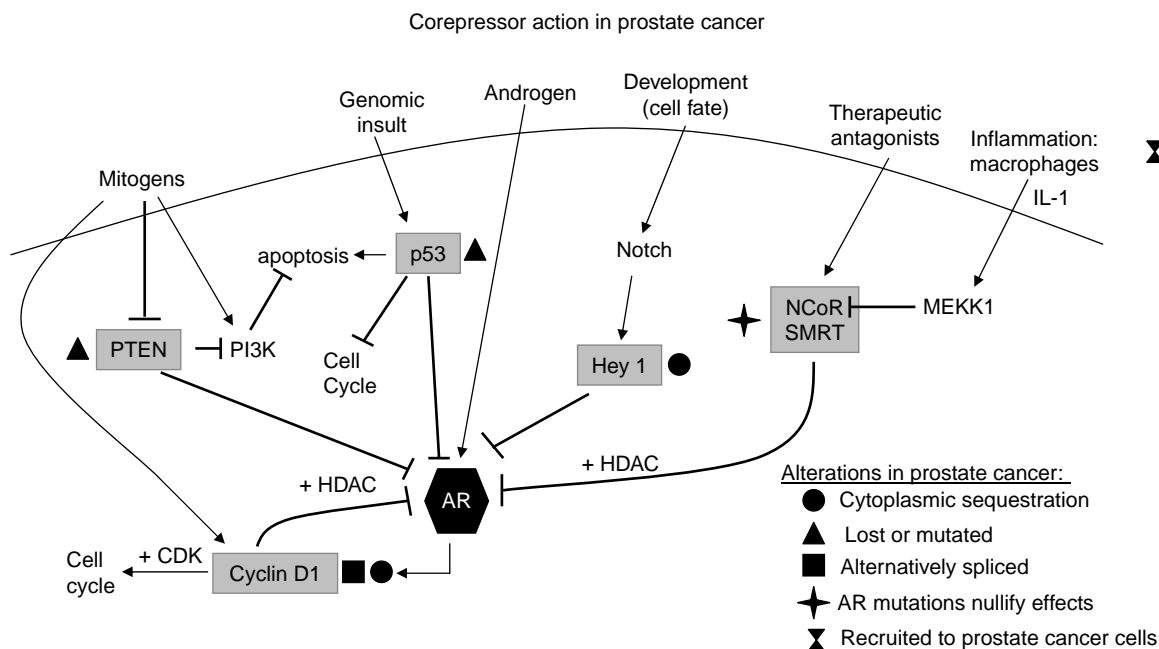


Figure 2 Actions of corepressors in PCa. Numerous AR corepressors (highlighted in gray boxes) are altered in prostate cancer through various diverse pathways. Alterations of corepressor function in prostate cancer include loss of function (cytoplasmic sequestration, loss, mutation, and alternative splicing), a bypass of corepressor sensitivity (through AR mutation), and displacement of corepressors (achieved via signal transduction).

is dependent on an N-terminal, evolutionarily conserved AR motif that is a critical effector of AR function, thus underscoring the importance of this motif (Callewaert *et al.* 2006, Zhu *et al.* 2006). The clinical relevance of these findings is potentially high, as IL-1 β effectively converted therapeutic AR antagonists to agonists (Zhu *et al.* 2006). Together, these observations support the hypothesis that alterations in expression and/or recruitment of NCoR and SMRT may facilitate tumor recurrence after therapeutic intervention. This hypothesis has yet to be confirmed in human tumors, as few published studies have examined corepressor expression during disease progression. One study examining the impact of the vitamin D3 receptor in prostate cancer did demonstrate an increase in SMRT mRNA levels (with no changes in NCoR); however, these studies considered only naïve tumors (Khanim *et al.* 2004). In sum, while there is a clear role for NCoR and SMRT in the molecular response to AR antagonists, the contribution of these corepressors for disease progression and therapeutic efficacy awaits further investigation.

PTEN: The PTEN tumor suppressor is a critical regulator of prostate cancer growth and progression, and has established utility as a prognostic factor in this disease. PTEN is frequently lost in prostate cancer (approximately 20–27% frequency) and its loss is associated with decreased survival rates (McMenamin *et al.* 1999, Halvorsen *et al.* 2003). Moreover, mice heterozygous for PTEN develop prostatic intraepithelial neoplasia (PIN), and combined genetic deletion of factors that control cell cycle progression (such as the CDK inhibitor p27kip1 or the retinoblastoma tumor-suppressor protein, RB) result in carcinoma (Di Cristofano *et al.* 2001, Hill *et al.* 2005). Prostate-specific deletion of PTEN alone induces metastatic prostate cancer (Wang *et al.* 2003), and PTEN heterozygosity promotes tumor progression in the TRAMP prostate cancer model (Kwabi-Addo *et al.* 2001). Interestingly, PTEN disruption also signals to inhibit GSK3 β activity, thereby indicating that PTEN can also regulate the function of other AR corepressors. While it is tempting to speculate that the role of PTEN as a tumor suppressor in this tissue type is attributed to its ability to serve as an AR corepressor, this assumption is confounded by the established role of PTEN in controlling factors that regulate cell survival (e.g. AKT) or cell cycle progression (e.g. p27; Gottschalk *et al.* 2001, Paez & Sellers 2003). It is suspected that PTEN and androgen act in opposition, as the ability of PTEN to induce apoptosis in prostate cancer cells is reversed by androgen. These

observations led to speculation that PTEN loss may result in enhanced AR activity and also promote resistance to therapy-induced cell death (Li *et al.* 2001). Indeed, loss of PTEN is independently predictive of a shortened time to biochemical failure (i.e. rises in PSA after therapeutic intervention) and tumor recurrence (Halvorsen *et al.* 2003). Future studies directed at delineating PTEN action in this tumor type will assist in determining the relevance of its role as an AR corepressor.

Cyclin D1: The role of cyclin D1 in prostate cancer is pleiotropic, as this molecule controls both AR function and cell cycle regulation (Knudsen *et al.* 1998, 1999, Coqueret 2002, Petre *et al.* 2002). It is well documented that in prostate cancer cells, androgen induces cyclin D1 accumulation, and this induction in cyclin D1 is required to stimulate cyclin-dependent kinase 4 (CDK4) activity and concomitant cell cycle progression (Lu *et al.* 1997, Knudsen *et al.* 1998). However, it is known that AR activity is regulated as a function of the cell cycle, wherein AR activity is lowest at the G1-S transition, when cyclin D1 levels peak (Martinez & Danielsen 2002). The ability of cyclin D1 to directly bind and inhibit AR is thought to underlie this regulation, and is proposed to serve as a negative feedback switch to modulate the mitogenic activity of androgen (Petre *et al.* 2002). Supporting this hypothesis, cyclin D1 induction in prostate cancer cells has been shown to inhibit cellular proliferation through its ability to regulate AR (Petre *et al.* 2002, Burd *et al.* 2006). Recent studies indicate that this function of cyclin D1 may be altered in prostate cancer. First, murine models of prostate cancer demonstrate that cyclin D1 expression decreases as a function of disease progression (Maddison *et al.* 2004). It has also been reported that cyclin D1 may be sequestered in the cytoplasm (and potentially precluded from CDK4 or AR regulation) in human tumors (Maddison *et al.* 2004). A mechanism underlying this event could in fact be GSK3 β , which is known to phosphorylate cyclin D1 and thereby mark the protein for rapid nuclear export (Diehl *et al.* 1998). Interestingly, GSK3 β is reported to have increased expression in prostatic cancer cell lines (Wang *et al.* 2004a). Second, it was recently shown that a variant of cyclin D1 that arises from alternate splicing (cyclin D1b) is expressed at high levels in a subset of prostate cancers and pre-cancerous (PIN) lesions (Burd *et al.* 2006). This variant of cyclin D1 was shown to be altered in its ability to regulate AR function, and was unable to attenuate

cellular proliferation in prostate cancer cells (Burd *et al.* 2006). Thus, cyclin D1 corepressor function and expression of cyclin D1 variants may hold significance for the response to androgen stimulation and AR regulation in prostate cancer.

p53: The importance of p53 mutation and loss on cancer development and progression is well established, and in prostate cancer mutations of p53 have been identified in 45% of radical prostatectomy samples (Shi *et al.* 2004). However, as with PTEN, the AR repressor function is confounded by its role as tumor-suppressor protein. A recent study investigating 16 mutant alleles of p53 identified in prostate cancer showed loss of p53 transactivation potential (Shi *et al.* 2002). The ability of these mutants to regulate AR function was not assessed, but the majority of these mutations had been previously identified in other tumor types, suggesting that they result in loss of classical p53 function. Clearly, further experimental consideration is needed to dissect the multiple functions of p53 in prostate cancer.

Hey1: Lastly, the Hey1 corepressor is regulated by the Notch pathway, which controls cell fate (Iso *et al.* 2001). Both Notch and Hey1 are expressed in normal prostatic epithelia, and Notch activation is known to inhibit cellular proliferation in cells of the prostate (Shou *et al.* 2001, Belandia *et al.* 2005). Conversely, Notch and its ligand (jagged) are downregulated by androgen stimulation, whereas negative regulators of the Notch pathway are induced. These observations indicate that Notch signaling negatively regulates androgen action through activation of Hey1. Although Hey1 resides in a region of chromosome 8 known to be amplified in prostate cancer, further investigation revealed that Hey1 localization is altered in cancer. Specifically, Hey1 is sequestered in the cytoplasm of prostate cancer cells, whereas the protein colocalizes with AR in both the cytoplasm and nucleus of non-malignant prostate epithelia (Belandia *et al.* 2005). Therefore, sequestration of Hey1 in prostate cancer cells may represent one mechanism by which tumor cells evade the inhibitory and anti-mitogenic functions of Notch signaling.

Conclusions

The androgen receptor is a critical effector of prostate cancer development and progression. Inhibition of AR activity is the central goal of therapeutic intervention, and recurrent tumors arise with restored AR activity. Given the importance of

AR in this deadly disease, a concerted effort has been implemented to delineate the cofactors that govern AR action, and to identify aberrations in AR control that may facilitate therapeutic bypass. It is clear that AR coactivators can contribute to this process by enhancing AR activity under conditions of androgen deprivation. Identification of AR corepressors significantly lagged behind advances in coactivator function, and prior to the millennium only a handful of AR corepressors had been described. In the last 5 years, there has been an expansion in corepressor discovery. Biochemical analysis of corepressor action revealed that this class of molecule can utilize widely disparate mechanisms to control AR function. These modes of action include those that are general to transcriptional control (e.g. recruitment of enzymes that modify chromatin) or unique to the receptor (e.g. inhibition of N/C interaction). Many AR corepressors have been previously defined as participating in pathways as disparate as signal transduction pathways (e.g. PTEN) or cell cycle control (e.g. cyclin D1), thus indicating that corepressors provide crosstalk mechanisms for pathways that also impinge on proliferation. The concept that AR corepressor function could be compromised in prostate cancer progression (and thus contribute to deregulated AR activity) is emerging, and analyses of effects on influential AR targets in prostate cancer (such as the TMPRSS2-ERG translocation; Tomlins *et al.* 2005) should be considered. Future investigations into the clinical relevance of AR corepressors will provide additional insight into the mechanisms by which tumor cells escape therapy, and could reveal additional execution points for novel therapeutic intervention.

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