

Review

Androgen Receptor Coregulators in Prostate Cancer: Mechanisms and Clinical Implications

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Introduction

The transcriptional activity of androgen receptor (AR) is modulated by coregulators that have a significant influence on a number of functional properties of AR, including the ligand specificity as well as the DNA binding capacity. Because androgens and AR have pivotal roles in the development and growth of normal prostate as well as prostate cancer progression, these coregulators may significantly modulate the events of cancer development and progression. An aberrant regulation or expression of AR coregulators may contribute to androgen and AR-related diseases including prostate cancer. A precise understanding of the roles and mechanisms of individual coregulators may provide valuable information in designing rational therapy against prostate cancer. In this review, we analyzed the up-dated information regarding AR coregulators and their potential roles in prostate cancer progression.

Androgens and AR

Androgens play essential roles in male sexual differentiation, sexual maturation, and the maintenance of spermatogenesis (1, 2). In addition to such vital roles in reproductive organ development, androgens also mediate physiological responses in a wide range of other tissues and organs including the skin, skeletal muscle, cardiac muscle, liver, kidney, central nervous system, and the hematopoietic system (3).

The prostate gland is one of the main target organs for androgens, which mediate their biological responses through the AR, a M_r 110,000 ligand-inducible transcription factor that regulates tissue-specific expression of target genes. The AR belongs to the nuclear receptor (NR) superfamily, which also includes estrogen receptor, glucocorticoid receptor, progesterone receptor, vitamin D receptor, thyroid receptor, and retinoic

acid receptor (4). It is ubiquitously expressed in human organs with the exception of the spleen and bone marrow, which show complete absence of AR expression. The AR gene is composed of eight exons encoding four functional domains similar to other members of the NR superfamily: a conserved DNA-binding domain, a hinge region, a COOH-terminal ligand-binding domain (LBD), and the less conserved NH₂-terminal domain [A/B domain (5)]. Mutational and deletion analyses revealed two transcriptional activation functions including an NH₂-terminal activation function (AF-1) involved in ligand-independent activation of AR and a ligand-dependent AF-2 function in the LBD. The AF-2 seems to be involved in more general transcriptional activity because mutation or deletion of the AF-2 domain dramatically reduces the transcriptional activation of AR (6, 7).

AR and Prostate Cancer

Since cloning of its cDNA structure in 1988 (8), the AR has been extensively studied to elucidate its biological role in the development and progression of prostate cancer. The growth, differentiation, and maintenance of prostate tissue are dependent on AR and androgens, particularly testosterone and dihydrotestosterone (DHT). Overactivation of the AR signaling pathway may link to the progression of prostate cancer, which remains the most frequently diagnosed malignancy in aging men. Androgens and the AR play key roles in this malignancy, and each year about 30,000 men lose their lives to this malignancy in the United States (9). In 1941, Huggins and Hodges (10) first reported that the normal prostate and early stages of prostate cancer were highly dependent on androgens. Since then, androgen ablation, either by surgical castration or by pharmacological/chemical castration, such as treatment with luteinizing hormone-releasing hormone analogs, has been the cornerstone of treatment in patients with locally advanced or metastatic prostate cancer. Androgen deprivation or the blockade of androgens at the level of the AR usually results in a favorable clinical response and a dramatic tumor regression (11, 12). Although complete disappearance of symptoms is achieved initially, in most cases the response does not imply cure. Upon continued treatment, the therapy fails, and most of the patients develop androgen-independent spread of the disease despite androgen ablation. At present there is no effective therapy for such an androgen-independent prostate cancer. One reason for failure of this androgen ablation therapy may be because of the presence of residual adrenal androgens in the circulation. Castration only abolishes testicular androgens, but the circulating adrenal androgens are readily available and can be converted to testosterone or DHT, the more active form of testosterone, in the prostate. To overcome this effect, antiandrogens are given in combination with castration (the so-called maximal androgen blockade therapy) to block the effects of androgens derived from the adrenals. However, support for additional benefit from this combined therapy is controversial, and a recent 5-year

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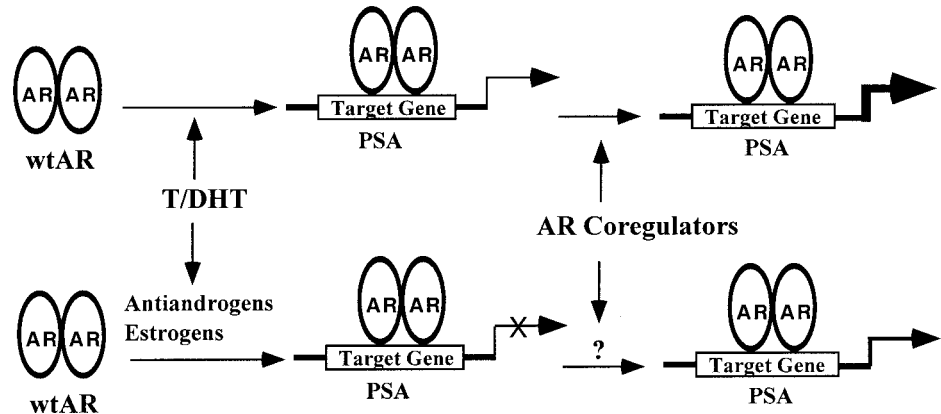
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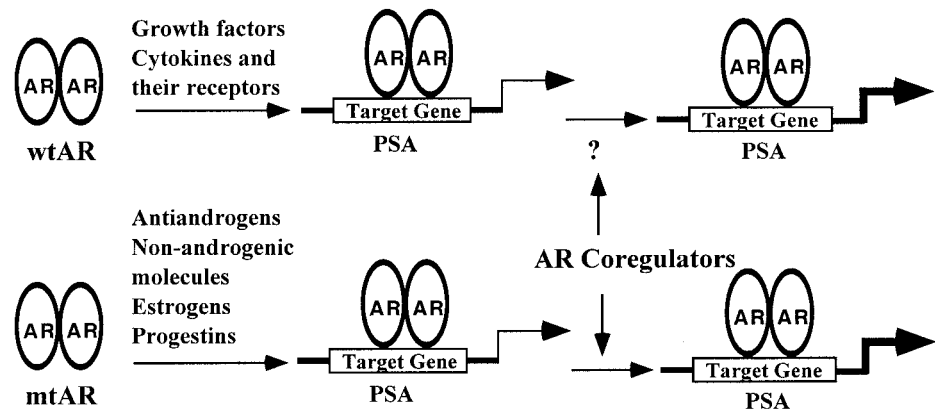
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Fig. 1 Activation of androgen receptor (AR) signaling pathway in prostate cancer. **A**, in an androgen-dependent state, ligand-bound AR activates transcription of target genes such as prostate-specific antigen (PSA). Expression of AR coactivators enhances target gene expression and AR-mediated growth of prostate cancer cells. Androgen ablation and/or antiandrogen therapy inhibits androgen-dependent target gene expression. **B**, in an androgen-independent state, other signaling pathways cross-talk with AR and activate the AR signaling pathway in the absence of androgens. Because of somatic mutations, AR may lose its ligand specificity and become activatable by relatively weak ligands, nonandrogenic steroids including estrogens, and progestin, or even by antiandrogens such as flutamide. Expression of AR co-regulators may play a significant role in these processes. wtAR, wild-type AR; mtAR, mutant AR.

A Androgen-dependent AR Signaling



B Androgen-independent AR Signaling



survival study failed to offer much hope for prostate cancer patients (13). Even after complete blockade of androgen action, the response to therapy is lost, tumors continue to grow, and the disease progresses into an androgen-independent state (14–16). The exact mechanisms of such resistance against ablation therapy remain largely unknown.

One of the hallmarks of androgen-independent prostate cancer is that tumor growth may still depend mostly on a functional AR signaling pathway. Prostate cancers, even when becoming androgen-independent, may still contain many AR-positive cells. Fig. 1 depicts some of the possible mechanisms as to how AR signaling can operate in both androgen-dependent and androgen-independent prostate cancers. One possible mechanism through which prostate cancer cells circumvent ablation therapy is by hypersensitive AR signaling during androgen ablation therapy, part of which can be due to overexpression of 5 α -reductase and AR coactivators in prostate tissues. The 5 α -reductase may help tumor cells rapidly convert available testicular androgens into more active DHT, and the coactivators may render AR more sensitive to usually weak adrenal androgens. Prostate cancer cells are then able to survive and grow at very low levels of androgens. The AR-mediated growth despite low androgen levels could also be achieved via an increased expres-

sion of AR itself, mostly by AR gene amplification, leading to an enhanced ligand occupied AR. Support for this hypothesis comes from the observation that about a third of tumors that become hormone refractory after ablation therapy have an amplified AR gene, compared with none in the primary tumors from the same patients (17–21).

Another possible mechanism by which prostate cancer cells circumvent the androgen requirement is through the development of mutations in the AR gene. The AR LBD, which is responsible for androgen binding and dimerization, may undergo somatic mutations during prostate cancer progression into the hormone-refractory state (14, 15). Mutations of AR allow the cells to circumvent the normal growth regulation by androgens. The AR ligand specificity becomes modulated and broadened so that the receptor now can be activated by nonandrogenic molecules including androgen antagonists, corticosteroids, and 17 β -estradiol (E₂). For example, the AR mutant T877A, isolated from patients with advanced-stage prostate cancer, is activated not only by androgens, the normal ligands, but also by antiandrogens, such as hydroxyflutamide [HF (22, 23)]. Another AR mutant, L701H, has been reported to significantly decrease androgen response but significantly potentiate the responses by glucocorticoids (24). The broadening of AR specificity thus

seems to offer prostate cancer cells a selective growth advantage to evade androgen ablation therapy.

Other growth signaling pathways may cross-talk with the AR signaling pathway and activate AR-mediated growth of prostate cancer cells, bypassing the requirement for androgens. For example, a number of growth factors, including insulin-like growth factor-I, keratinocyte growth factor, epidermal growth factor, and so forth, have been reported to activate AR, inducing AR target genes in the absence of androgens (25). A recent report indicated that interleukin 6 could induce activation of AR via signal transducers and activators of transcription-3. Signal transducers and activators of transcription-3 can physically interact with AR and enhance its transcriptional activity, whereas a dominant-negative signal transducers and activators of transcription-3 mutant blocks interleukin 6-induced AR transactivation (26).

HER2/neu, a transmembrane glycoprotein that belongs to the epidermal growth factor receptor family of tyrosine kinases, is found to be overexpressed in androgen-independent cells isolated from implanted xenografts in castrated mice. Craft *et al.* (27) have shown that HER2/neu can modulate the AR response in the absence of androgens or in the presence of extremely low levels of androgens. This was further supported by the findings that HER2/neu can activate AR signaling through the mitogen-activated protein kinase (MAPK) pathway (28). Sequence analysis revealed that the AR gene possesses putative consensus binding motifs (29) for DNA-dependent protein kinase, protein kinase A, protein kinase C, MAPK, and casein kinase II, suggesting a possibility of AR regulation by these kinases. Such possibilities have been supported by the reports that protein kinase A and protein kinase C can activate AR signaling in the absence of androgens (30, 31). Activation of MAPK signaling was found to be associated with prostate cancer progression (32). When constitutively activated, the Ras/MAPK pathway sensitizes hormone-responsive LNCaP cells to subphysiological levels of androgens (33), indicating a role of MAPK pathway in the acquisition of hormone-refractory growth of cancer cells. Taken together, these studies suggested that aberrant regulation of AR signaling pathways might play a role in developing hormone-independent progression of prostate cancer. The existence of so-called AR coregulators, which can modulate AR functional activity in response to agonists and antagonists, may contribute to all of the possible mechanisms described above, and therefore AR coregulators play significant roles in AR-mediated growth and progression of prostate cancers.

AR Coregulators

Substantial amounts of evidence indicate that NRs function as tripartite receptor systems, involving the receptor, its ligand, and its coregulators (34). Coregulators are biological macromolecules that interact with NR to either enhance receptor activity (coactivators) or reduce transcriptional activity (corepressors), without affecting the basal transcriptional level (35). Although coregulators interact with other DNA-binding proteins such as AP-1 and Smad3, they generally do not bind to DNA. Recently, a number of NR coactivators including SRC/pl60 family members such as SRC-1 (36), GRIP1/TIF2 (37), TIF1 (38), RIP140 (39), PGC-1 (40), SNURF (41), and so forth have been identi-

fied as being able to modulate receptor transactivation. The AR is a ligand-dependent transcriptional factor that has also been found to be modulated by a large number of coregulators (42). Table 1 summarizes those AR coregulators that can influence AR transactivation in prostate cancer cells and play some roles in prostate cancer progression.

The detailed mechanisms of how AR coregulators regulate AR transactivation are unclear and remain to be further investigated. Some AR coregulators, including SRC-1, CBP/p300, and p300/CBP-associated factor, possess histone acetyltransferase activity (43, 44) and may modulate AR activity via their ability to remodel chromatin structure. In addition, posttranslational modifications, such as AR acetylation, may also contribute to modulation of AR activity by these coregulators because it has been shown that AR can be acetylated by CBP *in vitro*. Mutations on AR acetylation sites dramatically reduce AR activity (45). On the other hand, corepressors, such as histone deacetylase-1, contain histone deacetylase activity and reduce AR activity either directly by affecting AR acetylation status or indirectly by regulating the status of histone acetylation (46, 47). Other AR coregulators lacking such enzymatic activity, such as ARA70, ARA54, ARA55, ARA24/Ran, ARA267, ARA160, supervillin (SV), gelsolin, ARIP3, AES, and TIF2, modulate AR activity via as yet undefined mechanisms (48–50). Modulation of AR activity by these coregulators can be achieved through multiple mechanisms, such as control of ligand/AR binding affinity, AR expression, AR stability, AR nuclear translocation, and transcription machinery recruitment. The filamin and SNURF1 coregulators can bind to the hinge region of AR and enhance AR activity via facilitation of AR nuclear translocation (51).

AR Coactivators in Prostate Cancer

Although the exact biological significance of AR coregulators interaction has yet to be defined, by and large all AR coregulators can either enhance (coactivate) or diminish (corepress) AR functional activity, and some of the AR coregulators seem to play significant roles in prostate cancer progression. It is possible that selective expression of AR coregulators may allow tumor cells to grow and survive in androgen-depleted conditions.

ARA70. ARA70 was the first AR coregulator identified by yeast two-hybrid screening of the human brain cDNA library (48). It is highly expressed in the brain, thymus, adrenal gland, prostate, and testis. Although initially described as relatively specific to AR, recent studies have indicated that ARA70 could also interact with peroxisome proliferator-activated receptor (PPAR) α or PPAR γ (52–54). It interacts with AR in a ligand-enhanced manner that can induce AR transcriptional activity up to 5–10-fold in some selective cells. Although ARA70 contains a classical LXXLL motif in its NH₂-terminal domain, it is not essential for interaction with AR because a mutated LXXLL motif has little influence on AR-ARA70 interaction. Instead, ARA70 may use a newly identified signature motif, FXXLF, as described by Hsu *et al.* (55). Although the LXXLL motif does not play any role in AR interaction, it may be necessary for interaction with other NRs such as PPAR α because mutations in the LXXLL motif strongly interrupt the ARA70 interaction with

Table 1 AR^a coregulators involved in prostate cancer

Coregulator	AR region	Significant features	References
ARA24 (Ran)	N-term	Interaction and activity dependent on number of polyglutamine repeats on AR N-term.	93–95
ARA54	LBD	Contains a RING finger and a B-box domain. Enhances wtAR activity by DHT, but mtAR (T877S) is activated by DHT, E ₂ , and HF.	49 and 81
ARA55 (Hic5)	LBD	Contains four LIM domains. wtAR activity is activated by DHT, whereas mtAR (T877A) activity is enhanced by DHT, E ₂ , and HF.	50, 56, 73, and 74
ARA70 (RFG)	DBD-LBD	Enhances the transactivation of both wtAR and mtAR (T877A) in response to DHT, E ₂ , and HF. Activates mtAR (T877S) in response to androstenediol, HF, and bicalutamide.	50, 56–63, and 69
ARA160 (TMF)	N-term	Cooperates with ARA70 to activate AR transactivation.	84, 85, and 62
ARA267	N- and C-term	Contains SET and PHD domains. Enhances mtAR (T877A) in response to androgens.	86
β-Catenin	ND	Enhances activity of wtAR in response to T, androstenedione, and E ₂ . Enhances activity of mtAR (T877A) in response to androgen.	109–112 and 114
Gelsolin	LBD	Enhances AR transactivation in prostate and muscle cells.	96 and 105
Supervillin	N- and C-term	Relatively weak in promoting nonandrogenic steroid-mediated AR transactivation but coordinates with other coregulators, including ARA55 and ARA70.	106 and 107
Smad3/Smad4	ND	May cooperate with HDAC complex to modulate AR acetylation. May mediate cross-talk between TGF-β and AR signaling pathways.	128–131
TR4	ND	Interacts with AR in both the presence or absence of androgen. Represses endogenous AR target gene expression.	146 and 151
ARA67	N-term	Decreases AR nuclear translocation, resulting in the inhibition of AR transactivation.	152 and 153

AR, androgen receptor; N-term, NH₂-terminal; LBD, ligand-binding domain; DBD, DNA-binding domain; ND, not determined; HF, hydroxyflutamide; wtAR, wild-type AR; mtAR, mutant AR; TGF-β, transforming growth factor-β; C-term, COOH-terminal; DHT, dihydrotestosterone; E₂, 17β-estradiol; HDAC, histone deacetylase.

PPARα, suggesting that ARA70 may use distinct domains to interact with different NRs to modulate their transactivating ability. The NH₂-terminal domain (ARA70N) containing amino acids 1–401 shows better coregulator activity than full-length ARA70 and can translocate into the nucleus along with AR. ARA70 can be found both in the cytosol and nuclei of Leydig cells of testes, however, ectopically expressed ARA70 is located predominantly in the cytosol in some cell lines, such as COS-1 cells. Coexpression of ARA70 with AR significantly enhances AR nuclear staining by promotion of AR nuclear translocation and/or stabilization or increase of newly synthesized AR (55).

Apart from the general enhancement of AR transcriptional activity, ARA70 has been extensively characterized as having the capacity to enhance AR transcriptional activity in response not only to testicular or normally weak adrenal androgens (56) but also to the antiandrogen HF and E₂ in prostate cancer cells (57, 58). Using CV-1 cells, Zhou *et al.* (59) also showed that ARA70 could induce E₂-mediated AR transactivation. In contrast, Gao *et al.* (53) reported that E₂ had little induction of AR transactivation in the absence or presence of ARA70 in CV-1 cells. The reasons for these discrepancies using the same CV-1 cells remain unclear. However, using HeLa, PC-3, and TSU-Pr1 cells, other laboratories also demonstrated that ARA70 could induce E₂-mediated AR activity (60–62). In the CWR22 prostate cancer xenograft model, in which the CWR22 progresses

from an androgen-dependent to an androgen-independent state after castration, ARA70 mRNA has been found to be elevated in the recurrent androgen-independent tumors (63). Moreover, adding hypophysiological concentrations of androgens into cells cultured under charcoal-stripped serum conditions or in cancer cells derived from castrated nude mice xenograft results in an increased ARA70 expression. These observations suggest that long-term androgen ablation therapy may result in increased ARA70 expression in tumor cells, which in turn trigger androgen- or antiandrogen-mediated AR activity. Expression of a dominant-negative ARA70 mutant (dARA70) and/or RNA interference-mediated silencing of ARA70 gene reduces the agonist activity of E₂ or HF in LNCaP prostate cancer cells (64). A number of studies have reported the important roles of AR mutations in modulation of AR specificity in prostate cancer cells (65–67). The LNCaP cells used in these studies express a mutant AR (T877A), which is also found in prostate cancer patients, that can change the AR specificity. The inhibition of the agonist activity of antiandrogens in this cell line by dARA70 or ARA70 RNA interference clearly indicates a probable dominant role of ARA70 over AR mutations in the process. Surprisingly, dARA70 showed a very similar effect on DHT, HF, and E₂. These blocking effects suggested that the presence of ARA70 may be able to broaden the specificity of the mutant AR, without reducing the affinity for DHT, so that optimal AR

transactivation can be maintained by any of these molecules in the presence of ARA70. Apart from mutant AR, the expression of ARA70 may provide prostate cancer cells with a selective growth advantage during antiandrogen therapy. Further *in vivo* evidence using the LNCaP xenograft nude mice model may be needed for conclusive verification of such phenomena.

ARA55. ARA55 is another AR coregulator that has been characterized as having the capacity to modulate AR specificity in response to agonists and antagonists (50). It was identified from a human prostate cDNA library by yeast two-hybrid screening and has >90% homology with mouse hic-5, a hydrogen peroxide-inducible gene (68). The four LIM domains of ARA55 are similar in sequence and organization to the LIM cytoskeleton-binding protein paxillin and zyxin, consistent with the localization of ARA55 to the nuclear matrix and focal adhesions (69, 70). Previous reports described LIM proteins functioning as bridging molecules between transcription factors, and a number of coregulators have been described to interact with LIM proteins to modulate their transcriptional activity (71, 72). It is also possible that ARA55 may recruit LIM coregulators or other transcription factors to regulate AR transactivation in response to extracellular signals.

ARA55 has been reported to enhance AR transcriptional activity in response to normally weak adrenal androgens, an antiandrogen HF, and other nonandrogenic steroids including E₂ (56, 57). A frequently occurring AR mutation (T877A) in prostate cancer patients promotes hypersensitivity to ARA55 in the up-regulation of androgen-regulated genes. The commonly used antiandrogens including HF, cyproterone acetate, bicalutamide, and E₂ promote (instead of block) AR transcriptional activity in the presence of ARA55 in prostate cancer cells (56). ARA55 may also be involved in the "superactivation" of AR signaling by HER2/neu in the presence of very low levels of androgens in prostate cancer cells (28). In addition, expression levels of ARA55 were found to be higher in tissue samples from hormone-refractory prostate cancers than in those from androgen-dependent cancers (73). Inactivation of endogenous ARA55 by a dominant-negative ARA55 mutant [COOH-terminal fragment with a point mutation (Ala→Thr) at amino acid 413 (413T)] leads to an inhibition of AR transactivation and a reduction of the agonist activity of antiandrogens in prostate cancer cells (74). The COOH-terminal fragment of ARA55 (413A) also weakly suppressed AR transactivation in prostate cancer cells expressing full-length ARA55. Interestingly, ARA55 (443T), but not ARA55 (413A), was detected in prostate cancer tissues. However, the full-length of ARA55 (both 413A and 413T) can equally enhance AR transactivation (75). These findings clearly indicated a role of ARA55 in the acquired agonist activity of antiandrogens in prostate cells. Similar results obtained by using RNA interference-mediated silencing of the ARA55 gene further strengthen these possible roles of ARA55 in prostate cancer progression.

ARA55, in addition to AR, has also been shown to interact with the cytoplasmic tyrosine kinase CsK, focal adhesion kinase FAK (76), and FAK-related PYK2 kinase (77). The proline-rich tyrosine kinase PYK2, originally identified as a kinase linked to MAPK and c-Jun-NH₂-terminal kinase signaling pathways, has recently been reported as being an ARA55-interacting protein that can repress AR transactivation via inactivation of the

ARA55 coregulator (78). PYK2 phosphorylates ARA55 at tyrosine 43 and impairs its coregulatory function. The disruption of the interaction between AR and ARA55 by PYK2 may also contribute to this inhibition of AR transactivation. PYK2 thus seems to have multiple ways to mediate ARA55-enhanced AR transcriptional activity. PYK2 is expressed in neurons, bone marrow, smooth muscles, and prostate (79, 80). Tissue staining showed that PYK2 was decreased with increased malignancy of prostate cancers (80), indicating a regulatory role of PYK2 on ARA55-enhanced AR-mediated growth of cancer cells. Taken together, ARA55 seems to have an important role in prostate cancer progression.

ARA54. ARA54, a RING finger protein, was initially identified as a coregulator of AR and progesterone receptor (49). In homomeric form, ARA54 interacts with AR and enhances its transcriptional activity in a ligand-enhanced manner. In the presence of 10 nM E₂ or 1 μM HF, ARA54 can further enhance the transcriptional activity of a mutant AR (T877A) in LNCaP cells, but not wild-type AR or another mutant AR (E708K), suggesting that in addition to a particular AR mutation, ARA54 coregulator may contribute to the partial agonist activity of antiandrogens or other nonandrogenic molecules. ARA54 is functional as a dimer, and disruption of ARA54 dimerization by a dominant-negative mutant (dARA54) inhibits ARA54-enhanced AR transactivation, as well as AR-mediated growth of prostate cancer cells (81). The dARA54, although lacking AR interaction, still retains the ability to form a heteromer with ARA54 and thereby prevents ARA54 interaction with AR. Ectopic expression of this dARA54 mutant suppresses both androgen-mediated and antiandrogen-mediated AR activity and prostate-specific antigen (PSA) expression in prostate cancer cells, further strengthening the role of ARA54 in the development of partial agonist activity of antiandrogens in prostate cancer cells.

Recently, ARA54 has been reported to possess ubiquitin ligase activity and ubiquitinate ARA54 itself, which may contribute to its proteosomal degradation (82). ARA54 can interact with E₂-conjugating enzymes through its RING finger domain, and possibly can act as an ubiquitin ligase in the ubiquitination of nuclear proteins. The ubiquitination of cellular proteins plays important roles in multiple biological functions, including cell cycle regulation, apoptosis, transcription, and so forth (83). The major role of ubiquitination is to target substrate proteins for proteosomal degradation. Hence, it is somewhat surprising that ARA54, being an AR coactivator, still possesses ubiquitin ligase activity and may be involved in the degradation of substrate proteins. However, it is quite possible that ARA54 may be involved in the degradation of corepressor(s) involved in the regulation of AR transcriptional activity. Alternatively, targeted degradation of the preinitiation complex by the ubiquitin ligase activity of ARA54 after the initiation of transcription by AR may facilitate the reinitiation of transcription. However, additional studies need to be done to define its role as a ubiquitin ligase as well as AR coregulator with respect to AR transcriptional activity and AR-mediated growth of tumor cells.

ARA160. ARA160, an AR NH₂-terminal-interacting protein (84), can function as a coregulator through interaction with the AR AF-1 domain and shares an identical sequence homology with TATA element modulatory factor (85). Both the

far Western blotting and the immunoprecipitation assays demonstrate that ARA160 interacts directly with AR in a ligand-enhanced manner and enhances its transcriptional activity in prostate cancer cells. ARA160 can also interact (although to a significantly lesser extent) through the AR AF-2 domain to increase AR activity, suggesting that ARA160 may physically and functionally serve as a bridging factor between AR NH₂- and COOH-terminal activation domain. Cotransfection with other AR COOH-terminal coregulators, such as ARA70 and ARA160, results in a cooperative enhancement of AR transactivation. A somatic mutation in the AR gene (E231G) from primary prostate tumor of TRAMP mice showed an increased AR activity in response to androgen but not E₂ in the presence of ARA160, suggesting a role of this coactivator in determining the ligand specificity of mutant AR in prostate cancer (62).

ARA267. ARA267 is a unique AR-interacting protein that contains the SET domain (86), derived from three originally identified proteins named *Su(var)3-9*, *Enhancer-of-zeste*, and *Trithorax* (87, 88). Apart from this evolutionarily conserved motif, ARA267 also contains two LXXLL motifs, three nuclear translocation signal sequences, and four plant homeodomain fingers. In *Drosophila*, these proteins have been suggested to play important roles in homeotic gene expression (89). Human homologs of these genes, such as ALR, huASH, or ALL-1, have also been reported to play important roles in the regulation of transcriptional activity via direct modulation of the chromatin structure, which in turn may control cell growth or disease progression (90, 91).

ARA267 shares 80% homology with NSD1, another SET domain protein (92), which may function as either a coactivator or a corepressor due to two distinct NR-interacting domains. ARA267 can interact with AR LBD and enhances AR transcriptional activity in a ligand-dependent manner. In LNCaP prostate cancer cells, transfection of ARA267 significantly increases expression of PSA, suggesting a role of ARA267 in prostate cancer progression (86). It also can very weakly enhance progesterone receptor activity in response to progesterone. When transfected in the presence of p300/CBP-associated factor, a histone acetyltransferase capable of enhancing AR activity, ARA267 showed an additive increase of AR transcriptional activity (86). These results indicate that ARA267 may function as a general coregulator for NR and modulate the receptor activity via cooperation with other chromatin remodeling factors.

ARA24/Ran. ARA24/Ran, another AR-interacting protein, physically interacts with the polyglutamine region of AR and enhances AR-dependent transcription of target genes (93). ARA24 is an AR NH₂-terminal-associated coactivator that can interact differentially with varying lengths of polyglutamine within AR. It has an identical amino acid sequence as Ran, a Ras-like small nuclear GTPase (94). ARA24/Ran belongs to the superfamily of GTP-binding proteins that function as molecular switches for cycling between the GDP- and GTP-bound states. Recent studies indicated an 81% increase of ARA24/Ran in cancer tissue compared with benign tissue isolated from prostate cancer patients (95). Higher levels of ARA24/Ran in prostate tumor tissues clearly indicated a role for ARA24/Ran in the proliferation of prostate cancer cells. Similar levels of overexpression were also found in high-grade prostate intraepithelial

neoplasia, indicating that aberrant expression of ARA24/Ran may be involved in prostate cancer initiation (95).

Gelsolin. Gelsolin is a multifunctional actin-binding protein well characterized as having implications in cell signaling, cell motility, apoptosis, and carcinogenesis (96). It regulates actin polymerization and depolymerization by sequestering actin monomers, and it can sever and cap actin filaments (97). Gelsolin has also been identified as being a substrate for caspase-3 and possesses a dual role in promoting apoptosis and protecting cells from apoptosis (98, 99). Several reports have indicated a differential expression of gelsolin in various cancers including prostate cancer (100, 101). Gelsolin expression is down-regulated in a number of malignancies including prostate, breast, lung, and bladder cancer (102–104), suggesting it may function as a tumor suppressor. Also, it has been proposed to be a sensitive marker for renal cystadenomas and carcinoma.

Recently, in a yeast two-hybrid screen, gelsolin was identified as an AR-interacting protein that can interact with AR and enhance its transactivation in DU145 prostate cancer cells (105). A novel function of gelsolin as an antiandrogen potentiated AR coactivator has been documented. HF-potentiated AR coactivation and an increased expression of gelsolin after androgen ablation therapy (castration plus antiandrogen HF) suggested a possible role of gelsolin in the development of “flutamide withdrawal syndrome” (105). It is possible that increased expression of gelsolin could enhance AR activity under HF treatment to maintain AR-mediated growth and survival of tumor cells. The interruption of HF-induced interaction between AR and gelsolin could thus offer a potential target for therapeutic design in the treatment of prostate cancer.

SV. SV was initially identified from blood cells as an actin-binding protein and found to be expressed in muscle-enriched tissues such as skeletal muscles and in several cancer cell lines (106). SV has structural homology to gelsolin and villin and has also been identified as an AR-interacting protein (107, 108). The prostate cancer cells DU145 and PC-3 express a low level of endogenous SV, and cotransfection of SV with AR into these cells results in 2–3-fold enhancement of AR transactivation in response to 1 nM testosterone (107). Compared with ARA70 or ARA55, SV is relatively weak in promoting nonandrogenic steroid-mediated AR transactivation but is capable of coordinating with other coregulators, including ARA55 and ARA70, to enhance AR function in prostate cancer cells. Cytologically, SV is localized to the plasma membrane at sites of intracellular contact (108). At low density, SV shows a punctate distribution localizing in the cytoplasm and nucleus, whereas at high density, SV is localized almost exclusively at the plasma membrane (108). SV thus seems to transduce signals from sites of cellular adhesion to the nucleus during cellular proliferation or migration.

β-Catenin. β-Catenin has recently been reported to function as an AR-interacting protein and increase AR transcriptional activity in a ligand-dependent manner (109–112). Yeast two-hybrid experiments provided evidence that AR interacts with β-catenin, which may shuttle β-catenin to the nucleus. Although the function of β-catenin signaling in normal as well as prostate cancer is largely unknown, the documentation of β-catenin in about 5% of primary prostate cancers and the nuclear accumulation of β-catenin in about 20% of metastatic

prostate cancers from patients who have developed resistance to androgen ablation therapy suggest a role for β -catenin in prostate cancer progression (113, 114). Aberrant expression of β -catenin has been documented in multiple tumor types (115–117), and a number of mutant β -catenins have been found in primary prostate cancers (118). The β -catenin mutant S33F increases AR sensitivity to normally weak adrenal androgens androstenedione and dehydroepiandrosterone. In the presence of the mutant β -catenin, both of these ligands activate AR transcriptional activity comparable with that of testosterone or DHT (109). Moreover, S33F β -catenin makes AR responsive to other nonandrogenic molecules including E_2 . The mutation of β -catenin in prostate cancer may thus enable the tumor cells to survive in the presence of low serum levels of testicular androgens.

AR Corepressors in Prostate Cancer

Corepressors were first identified as proteins associated with unliganded or antagonist-bound NRs that mediate transcriptional repression. These proteins function through the formation of a nonproductive interaction with general transcription factors (119) or through recruitment of histone deacetylase complexes (120–122). The two classical and the best-characterized corepressors, NCoR and silencing mediator of retinoid and thyroid hormone receptor (SMRT), preferentially interact with antagonist-bound estrogen receptor, glucocorticoid receptor, or progesterone receptor and repress transcription. Much less is known regarding the role of NCoR and SMRT in the regulation of AR transcriptional activity. However, NCoR was recently reported to interact directly with AR and repress DHT-stimulated AR transcriptional activity (123, 124). The NCoR-related corepressor SMRT can also interact directly with both the liganded and unliganded AR *in vitro* as well as *in vivo* and represses AR activity by disrupting AR NH₂- and COOH-terminal interaction and/or by competition with the p160 coactivators (46). Taken together, these findings indicated a role for NCoR and SMRT in regulating AR transcriptional activity.

Three AR corepressors, cyclin D1, calreticulin, and HBO1, have been identified and characterized as being able to interact with androgen-bound AR. Cyclin D1 binds to AR in the presence of androgen and represses ligand-dependent AR function in prostate cancer cells (125). The suppression of AR activity in pRB-negative cells and the failure of a pRB binding-deficient mutant to rescue AR function suggests a mechanism that could be independent of the cell cycle-regulatory function of cyclin D1. Experimental evidence suggests that cyclin D1 acid-rich COOH termini bind to the AR hinge region and repress ligand-dependent AR activity by directly competing for limiting cellular p300/CBP-associated factor (125). The calreticulin binding to AR prevents its interaction with response elements, resulting in inhibition of AR activity (126). HBO1 is a member of the MYST protein family that has been characterized by a homologous zinc finger and an acetyltransferase domain (127). The MYST family proteins comprise both transcriptional silencers and activators. HBO1 is predicted to function by acetylating other nonhistone proteins involved in AR transcriptional regulations, which may reduce the ability of these proteins to facilitate androgen-induced AR transactivation. Although the physiological roles of repression of AR function remain to be

determined, some of the AR corepressors may be associated with progression of prostate cancers.

Smad3/Smad4. Smad3 and Smad4 have been reported to physically associate with AR and function as AR coregulators. Both immunoprecipitation and glutathione *S*-transferase pull-down assays confirmed an association of AR with Smad3, and binding of Smad3 to AR either enhances (128) or represses AR functional activity (129, 130), depending on the availability of Smad4. Smad4, along with Smad3, also can interact with both AR DNA-binding domain and LBD, thereby modulating ligand-induced AR transactivation. In prostate cancer PC-3 and LNCaP cells, addition of Smad3 enhances AR transactivation, whereas cotransfection of Smad3 and Smad4 represses AR transactivation, followed by a reduction in PSA mRNA levels (131). Smad4 can decrease AR-Smad3 interaction and repress Smad3-enhanced AR transactivation. Reversal of Smad3/Smad4-mediated AR inhibition by histone deacetylase inhibitors raises the possibility that Smad3/Smad4 may cooperate with histone deacetylase complex to modulate AR acetylation (131).

Binding of activated Smad3 and Smad4 with ligand-bound AR may also mediate the cross-talk between transforming growth factor (TGF)- β and AR signaling pathways, which may have important roles in regulating AR-mediated growth of prostate cancers. TGF- β plays a dual role in tumorigenesis. On one hand, TGF- β negatively regulates AR signaling pathways and functions as a potent inhibitor of prostatic epithelial cells and induces cell cycle inhibitors such as p15^{INK4B} and p21^{WAF1/CIP} and inhibits carcinogenesis of the prostate (132–137). Loss of normal TGF- β receptors at the RNA and protein levels, leading to an inactive TGF- β pathway, has been reported in prostate cancers (136–138). Overexpression of TGF- β receptors in prostate cancer cells that were refractory to TGF- β has been reported to inhibit cell proliferation and suppress *in vitro* tumorigenicity (139, 140). On the other hand, TGF- β can promote the malignant process during late stages of tumorigenesis (141, 142). TGF- β is overexpressed in various tumors of epithelial origin, facilitates tumor invasion, and promotes cancer progression to metastasis (143). In several studies, significantly elevated plasma TGF- β levels were reported in patients, which correlated well with increased serum PSA levels (144, 145). The exact molecular events that determine such discrepancies between growth *versus* inhibition by TGF- β and AR signaling in prostate cancers, however, remain unclear. Further study needs to be done to determine the physiological significance of the cross-talk between TGF- β and AR signaling as mediated by Smad3 and Smad4, two intracellular signaling mediators of TGF- β in prostate cancer progression.

The Human Testicular Orphan Receptor 4 (TR4)

TR4 was isolated by screening human prostate and testis cDNA libraries (146). As a homodimer, TR4 can recognize AGGTCA direct repeat sequences on target genes and modulate their expression. It has been reported to modulate many signal transduction pathways, including those involving retinoic acid (147), thyroid hormone (148), vitamin D₃ (149), and ciliary neurotrophic factor (150). Recently, TR4 has been shown to physically interact with AR in both the presence and absence of DHT and to repress the transcriptional activity of AR in prostate cancer cells (151). Immunocytofluorescence studies indicate that AR translocates into the nucleus in the presence of TR4, providing

Table 2 Potential clinical implications of AR^a coregulators in prostate cancer

Functions	Agents	Mechanisms
Inhibitors	Small molecules	Disruption of AR-coregulator interactions
Suppressors	Small molecules	Suppression of expression of coregulators
Gene therapeutics	Dominant-negative mutants	Inactivation of coregulators
	RNAi	Silencing the expression of mRNA
	Ribozyme	Degradation of mRNA
	Antisense	Blocking the translation of mRNA
Prognostic markers	Selective coregulators	Overexpressions

^a AR, androgen receptor; RNAi, RNA interference.

further *in vivo* evidence for an association between TR4 and AR. The physiological significance of these AR-TR4 heteromer formations can be predictable because of their similar expression patterns in many tissues, including testis, hypothalamus, and prostate (146, 151). Ectopic expression of TR4 represses AR target gene activation and expression both *in vitro* and *in vivo*. The repression of endogenous PSA may suggest a role of TR4 in prostate cancer progression. Further study is warranted to determine the exact biological significance of TR4-mediated repression of AR function in both normal prostate growth and prostate cancer progression.

ARA67. ARA67 is a novel AR-associated protein that can function as a corepressor for AR by interacting with the AR NH₂ terminus and thus inhibits AR transcriptional activity (152). Sequence alignment revealed its identity with the amyloid precursor protein tail 1 [PAT1 (153)]. ARA67 traps AR in the cytosol and blocks its nuclear translocation, resulting in inhibition of AR function. It has been well documented that ligand binding translocates AR from the cytosol to the nucleus, where AR activates the expression of target genes to elicit biological responses. A decrease in AR nuclear translocation leads to inhibition of AR activity and androgen-mediated cell growth (153). ARA67 is distributed in both the cytosol and nucleus. It shares a sequence homology with kinesin light chain, which interacts with microtubules (a cytoskeleton structure) and is involved in protein trafficking (153). ARA67/PAT1 also can bind to microtubules, and this binding can be enhanced 5–10-fold in the presence of Mg-ATP (153), raising the possibility that ARA67/PAT1 may be a component of the microtubule network and utilizes this network to trap AR in the cytosol. Although relatively weak, ARA67 also interacts with AR LBD and enhances AR NH₂- and COOH-terminal interaction and produces a mild increase in AR protein level expression, indicating a potential role for ARA67 to enhance AR transactivation under certain conditions. The cellular microenvironment may determine which of these two opposing effects dominate in particular cell types or conditions. Further study is needed to unravel the biological roles of ARA67 in the regulation of AR-mediated growth of prostate cancer cells.

Clinical Implications and Future Perspectives

Androgen-bound AR functions as a transcription factor to regulate genes involved in a diverse array of physiological responses, including reproductive functions and sexual behaviors. The transcriptional activity of AR is modulated by coregulators that are widely expressed in AR-expressing tissues and

organs. A number of AR functional properties, including ligand selectivity and DNA binding capacity, are influenced by these AR coregulators. A large array of AR coregulators have been identified, which demonstrated multiple modes of mechanisms to influence AR transcriptional activity. Aberrant regulation of coregulator activity due to mutations or altered expression levels may contribute to androgen-related diseases. Substantial evidence suggested a role of AR coregulators in tumorigenesis and in the development of hormone-refractory prostate cancers. Additional studies are warranted to fully understand the roles of AR coregulators in normal development in response to physiological stimuli and in disease processes in response to pathological insults. Recent studies using dominant-negative AR coregulators have indicated that some AR coregulators may be involved in the acquired agonist activity of antiandrogens in prostate cancer cells (64, 74, 81). Similar studies are needed to examine the relative roles of all AR coregulators. Animal models, including targeted disruption of coregulators, will be valuable to further dissect the relative importance of AR coregulators in different tissues or in pathological conditions. Clinical studies examining expression levels of different coregulators in histological samples such as prostate cancer specimens and a comparison in normal *versus* disease states will help to understand their physiological roles *in vivo*. A clear understanding of the mechanisms and relative importance of different AR coregulators will help to design and develop more effective therapies against androgen- and AR-related diseases, including prostate cancer.

Because AR coregulators physically interact with AR to modulate its transcriptional activity, disruption of AR-coregulator interaction could be an important strategy to regulate AR-mediated growth of prostate cancer cells. The expression of selective AR coregulators may offer a growth advantage to tumor cells in androgen ablation and/or antiandrogen therapy. AR coregulators can modulate AR functional properties in both the presence and absence of androgens; therefore, therapeutic interventions based on AR-coregulator interactions can be designed to block both androgen-dependent and androgen-independent growth of tumor cells. The disruption of AR-coregulator interactions or suppression of coregulator expressions by small molecules may represent a feasible approach to regulate AR-mediated growth of tumor cells. *In vivo* gene therapies using dominant-negative mutants, RNA interference, ribozymes, and antisense RNAs against selective AR coregulators can be evaluated in prostate cancer model systems as potential treatments. Recent reports have indicated that selective AR coregulators

might be overexpressed in tissues from advanced-stage and/or androgen-independent prostate cancers compared with normal prostate or androgen-dependent tumors. Therefore, these approaches may be beneficial for patients with androgen-independent prostate cancer as well. Individual treatment strategies will be able to be decided after evaluation of expression of each AR coregulator (63, 73). In addition, the potential use of such selective coregulators as prognostic markers in monitoring advanced prostate cancer progression should thus be examined. Table 2 summarizes some of the potential clinical implications for AR coregulators.

In conclusion, AR coregulators may be necessary for optimal AR function in the normal development of target organs as well as progression of AR-related diseases. Substantial evidence now supports the role of AR coregulators in the development and progression of cancers, in particular, in the development of hormone resistance in prostate cancers. An aberrant expression or improper regulation of these coregulators may affect the normal function of the AR and may lead to the development and progression of prostate cancers. Because AR coregulators physically interact with AR and modulate its transcriptional activity, the interruption of AR-coregulator interactions offers a feasible target for developing new therapeutic agents to regulate both androgen-dependent and androgen-independent but AR-mediated progression of prostate cancer.

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