FUNCTIONAL ANALYSIS OF ANDROGEN RECEPTOR N-TERMINAL AND LIGAND BINDING DOMAIN INTERACTING COREGULATORS IN PROSTATE CANCER

Shuyuan Yeh, Erik R. Sampson, Dong Kun Lee, Eungseok Kim, Cheng-Lung Hsu, Yuh-Ling Chen, Hong-Chiang Chang, Saleh Altuwaijri, Ko-En Huang, and Chawnshang Chang

Abstract: Several new androgen receptor (AR) coregulators, including ARA70, ARA55, ARA54, ARA160 and ARA24, associated with the N-terminal or the ligand-binding domain (LBD) of AR, have been identified by our group. We first identified the AR–LBD coregulators ARA70, ARA55, and ARA54. Our previous reports suggest that ARA70 can enhance the androgenic activity of 17β-estradiol (E2) and antiandrogens toward AR. It is of interest to compare and determine if the specificity of sex hormones and antiandrogens can be modulated by different coregulators. Our results indicate that, ARA70 is the best coregulator for increasing the androgenic activity of E2. Only ARA70 and ARA55 were able to significantly increase the androgenic activity of hydroxyflutamide, the active metabolite of a widely-used antiandrogen for the treatment of prostate cancer. Furthermore, our results suggest that among the LBD coregulators, ARA70 has a relatively high specificity for AR in the human prostate cancer cell line DU145. Together, our data suggest that the androgenic activity of some sex hormones and antiandrogens can be modulated by selective AR coactivators. In addition to the AR–LBD associated proteins, ARA24 and ARA160 have been identified as AR coregulators, interacting with the AR N-terminal instead of the LBD. Functional analysis revealed that the AR N-terminal coregulator ARA160 could cooperate with the AR LBD-associated coregulator ARA70. Our data indicate that ARA24 could also interact with AR, and that this binding is decreased by an expanding poly-glutamine (Q) length within AR. The length of the poly-Q stretch in the AR N-terminal domain is inversely correlated with the transcriptional activity of AR. Our data suggest that optimal AR transactivation may require interaction of AR with AR coregulators. The identification of factors or peptides that can interrupt androgen-mediated AR–ARA interactions may be useful in the development of better antiandrogens for treating androgen-related diseases, such as prostate cancer.

As a member of the steroid receptor (SR) superfamily, the androgen receptor (AR) functions primarily as a ligand-activated transcription factor that may play critical roles in prostate cancer growth and libido [1, 2, 3]. The AR regulates androgen target genes by binding to androgen response elements with the potential involvement of coregulators [3].

In general, most of the SRs have an N-terminal transactivation domain, a DNA binding domain, a hinge region, and a C-terminal ligand-binding domain.
(LBD). The crystal structures of the LBDs of several receptors reveal that the ligands are almost entirely buried within the conserved core of helices 3, 7 and 10 [4]. A conserved C-terminal helix, known as the activation function-2 (AF-2) domain, is required for ligand-dependent gene activation and becomes folded against the LBD of the agonist-bound nuclear hormone receptor.

Our group and several others have demonstrated that receptors may utilize a group of coregulators to effectively stimulate gene transcription, which would depend on allosteric alterations in the AF-2 helical domain. These coregulators would modulate the SR’s function through the transactivation process and may function as a bridge factor between the receptor and the basal transcriptional machinery to promote changes in chromatin structure [5].

Due to the homology of the receptor interaction domain (RID) and similar functional domains, some of the coregulators have been grouped into the SR coactivator (SRC) / p160 family containing SRC-1/ NCoA-1 [6], GRIP1/TIF2/ NCoA-2 [7, 8], and RAC-3/ ACTR/ P/ CIP/ NCoA-3 [9, 10, 11]. The RIDs of these coregulators are highly conserved and contain three repeats of the consensus sequence LXXLL (where X is any amino acid) [12]. In addition, other receptor coregulators, such as TIF1, RIP140, TAFII30, and PGC-1 also have been identified [13–16]. Some of these coregulators have been shown to bind to multiple receptors, but the others may possess some specificity [16].

The coregulators identified in our lab, including ARA70, ARA55, ARA54, Rb, ARA160, TIF1I1H, and ARA24 have different RIDs, transactivation domains, and signature functional domains, as compared to the SRC / p160 family [17–22]. It is of interest to systematically study the differential effects and characteristics of these unique coregulators and SRC-1. Currently, some other coregulators have also been identified that associate with the AR DNA binding domain or AR N-terminal domain, including CREB-binding protein (CBP), a small nuclear ring finger protein (SNURF), a novel nuclear protein kinase (ANPK), and TFIIF [23–25]. In this review, we will mainly focus on the associated proteins that were identified in our laboratory.

**Identification of the N-terminal and C-terminal AR–LBD Coregulators**

To identify the coregulators by protein-protein interaction, several strategies including the yeast two-hybrid system, the modified yeast one-hybrid system, Far western screening of a phage expression library, and microsequencing of the protein purified by immunoprecipitation, were employed. The hypothesis that a mutation of AR (mtAR) in the LBD may change the antiandrogen specificity and contribute to the progression of prostate cancer from an androgen-dependent to an androgen-independent stage has been widely accepted [26]. Therefore, we were interested in investigating if coregulators were required for the wild type AR (wtAR) or mtARs to exert their distinct functions. The LBD of wtAR or mtAR t877s (codon 877 was changed from threonine to serine) was used as bait to screen out potential positive clones from a human prostate cDNA library by the yeast two-hybrid system. Using this strategy, we have obtained more than 20 candidates including SRC-1. After characterization, three of the positive cDNA clones, ARA70, ARA55 and ARA54, were further studied.

To date, two AR N-terminal associated proteins, ARA160 and ARA24, have also been identified in our laboratory. ARA160 was identified by a Far-western screening assay using the AR N-terminal domain (amino acids 38–643) [21]. The N-terminal domain of nuclear receptors contain a ligand-independent activation function-1 (AF-1), which is under the control of AF-2. The length of the poly-glutamine (Q) stretch in the AR N-terminal domain is inversely correlated with AR transcriptional activity. In addition, an abnormal expansion of the poly-Q stretch [27–29] is linked to neurodegenerative disease, spinal/ bulbar muscular atrophy or Kennedy’s disease. A yeast two-hybrid assay using the AR poly-Q stretch region (amino acids 11–208) as bait identified the AR poly-Q region-associated coregulator ARA24 [22]. The interaction between ARA24 and AR is affected by expanding the poly-Q length within AR. This interaction between the poly-Q length of AR and ARA24 may be linked to the development of Kennedy’s disease.

We will first introduce the functional characterization of the AR–LBD coregulators, followed by the characterization of the N-terminal coactivators.

**Functional Domains of Different AR Coregulators**

The unique sequences of ARA70, ARA55, and ARA54 place these three AR coregulators outside the p160 family of common SR coregulators that includes SRC-1, TIF2/ GRIP1, and RAC3/ ACTR/ AIB1 [6–11]. For example, these three AR coregulators lack such common motifs as the basic helix-loop-helix domain, the Per-AhR-Sim domain and the LXXLL motif, which are shared by p160/ SR coregulators. Furthermore, it has been well documented that LXXLL is the signature
motif used by members of the p160 family of coregulators to interact with SRs [6–11]. Although there is an LXXLL motif in the N-terminal domains of ARA70 and ARA55, this motif is not located in the RIDs of these two coregulators [19]. Moreover, ARA55 has three lin-11, isl-1, mec-3 (LIM) motifs in the interaction domain of the C-terminal region. LIM is a cysteine-rich motif that is found in several proteins (including Trip 6) with diverse functions and subcellular distributions. The biochemical properties and functions of LIM motifs have not been fully defined, but it has been suggested that their main function is in developmental regulation [30, 31]. Schmeichel and Beckerle have reported that LIM motifs might be involved in protein-protein interaction [32]. Therefore, the LIM motifs in the ARA55 C-terminal region may contribute to its interaction with AR.

ARA54 is another interesting AR coregulator with a novel sequence, containing a conserved Really Interesting New Gene (RING) finger motif and a B-box-like structure. Proteins in the RING finger family are ubiquitously expressed in species ranging from humans to viruses, participate in diverse cellular processes, and are possibly involved in some aspects of transcriptional regulation and protein-protein interaction [33]. In addition, it has been reported that mutant promyelocytic leukemia (PML) proteins without the RING finger motif can become potential dominant-negative inhibitors of wild type PML protein [34]. Although the significance of the RING finger domain in ARA54 remains unclear, it is possible that ARA54 might use this domain to interact with other key factors in the activated nuclear receptor-mediated signaling complex and to function as a bridge factor between AR and the general transcription machinery.

There is also no homology among ARA55, ARA54, and ARA70, the AR coregulators first identified [17, 19, 20]. Although these AR coregulators enhance AR transcriptional activity in DU145 cells, their effects on AR are distinct, and this issue will be discussed in following sections. The precise roles of these three coregulators may, therefore, differentially affect physiologic influences on prostate cells. Together, it will be of great interest to further characterize the functions of these three AR coregulators.

**SRC-1 AND SRC-3/ RAC3/ ACTR/ AIB1 ALSO FUNCTION AS AR COREGULATORS**

To investigate whether two other SR coregulators, SRC-1 and SRC-3/ RAC3/ ACTR/ AIB1 [6, 9, 10, 11], can function as AR coregulators, we co-transfected these two cofactors with AR. Our data indicate that these coregulators could enhance AR transcriptional activity in the presence of 1 nM 5α-dihydroxytestosterone (DHT) [35]. Although it has been speculated that SRC-1 and SRC-3/ RAC3/ ACTR are coregulators for many SRs, our results provided the first evidence showing that SRC-1 and SRC-3/ RAC3/ ACTR functionally enhance AR transactivation activity (Fig. 1).

**DHT-MEDIATED AR TRANSACTIVATION IN THE PRESENCE OF DIFFERENT AR COREGULATORS**

Among the cell lines we tested (CHO, DU145, LNCaP, PC-3, HeLa, and MCF7), the human prostate cancer cell line DU145 was used due to its low background level of AR transcriptional activity in the absence of exogenous AR. To compare the relative enhancement...
of DHT-mediated AR transcriptional activity by different AR coregulators, human AR and available AR coregulators (ARA70, ARA55, ARA54, SRC-1, and RAC3) were each inserted into the pSG5 expression vector to establish the same transfection efficiency. When human AR and/or individual AR coregulators were transiently expressed in DU145 cells without exogenous DHT, there was no AR transcriptional activity. AR transcriptional activity could be induced 5- to 7-fold when AR was expressed in the presence of 1 nM DHT. The addition of various AR coregulators, at a 1:3 AR: AR coregulator ratio, (typically 1.5 µg AR: 4.5 µg ARA), could further enhance AR transcriptional activity to 22- to 45-fold in the following order: ARA55 > ARA70 > ARA54 > RAC3 > SRC-1 (Fig. 2). Together, these data suggest that ARA55 and ARA70 are the two most effective AR coregulators in human prostate cancer DU145 cells.

**Coexpression of ARA70, ARA55, or SRC-1 can Additively Enhance AR Transcriptional Activity**

It has been demonstrated that the coexpression of SRC-1 and CBP can stimulate estrogen receptor (ER) and progesterone receptor (PR) transcriptional activity in a synergistic manner [36]. Since both ARA70 and SRC-1 can act as coregulators of AR transcriptional activation [17, 35], we were interested in knowing if the coexpression of ARA54 with ARA70 or SRC-1 would synergistically enhance AR-mediated transcriptional activity. While ARA70, ARA54 and SRC-1 can individually induce AR transcriptional activity in DU145 cells, our data suggest that when any two of these coregulators are expressed simultaneously, AR-mediated transactivation is increased additively, but not synergistically relative to that observed in the presence of any single coregulator [20]. These results indicate that these coregulators do not cooperate in their enhancement of AR-mediated transcriptional activity, and likely utilize distinct coactivational mechanisms.

**Interactions Between AR and ARA70, ARA55, or ARA54 are Androgen-Dependent**

To determine whether the three ARAs identified by our group can interact with AR in an androgen-dependent manner, we first applied a yeast two-hybrid assay. We found that both DHT and testosterone (T) could promote the specific interaction of ARAs with wtAR or mtAR at concentrations greater than 1 nM [19, 20].

Next, we applied a mammalian two-hybrid assay to confirm this DHT-dependent interaction between AR and ARAs in vitro. DU145 cells were co-transfected with a plasmid encoding the LBD of wtAR fused to the GAL4 DNA binding domain (GAL0AR) and a plasmid encoding ARA55, ARA54, or ARA70 fused to the activation domain of VP16. VP16 is a herpes simplex virus protein that can activate transcription in mammalian cells. In the mammalian two-hybrid system, the VP16 activation domain fused to a protein of interest can activate transcription through specific interaction with a DNA-binding Gal4 fusion protein. Interaction was estimated by determining the level of luciferase activity from the reporter plasmid, and the SV40 large T-antigen was used as a negative control. In the absence of androgen, the interaction of GAL0AR and VP16-ARA was negligible. In contrast, a significant level of luciferase activity was induced by the co-transfection of GAL0AR with VP16-ARA70 or VP16-ARA54 in the presence of 1 nM DHT. The induction by VP16-ARA55 was not as high as that of ARA70 or ARA54, but was still over 3-fold. Together, results from the yeast two-hybrid assay and the mammalian two-hybrid assay indicate that the specific interaction between an ARA and AR is an androgen-dependent process. Using the GST pull-down assay however, Alen et al reported that AR could also interact with ARA70 in the absence of androgen [37]. While the cause of this discrepancy is currently
unclear, the use of different assays in different cell environments may be a contributing factor.

**E2-mediated AR Transcriptional Activity in the Presence of Different AR Coactivators**

Previous experiments using the mouse mammary tumor virus-androgen response element–chloramphenicol transferase (MMTV-ARE-CAT) reporter system (CAT assay), have shown that 10 nM E2 can enhance AR transcriptional activity in the presence of ARA70 [35]. We were interested in determining if this new ARA70-mediated E2-AR pathway could also occur in the presence of other AR coregulators. We found that E2 (10⁻⁹ M to 10⁻⁸ M) could not significantly enhance AR transcriptional activity in the absence of AR coregulators. Addition of ARA70 could enhance AR transcriptional activity 2- to 4-fold at 1 nM E2 and about 20-fold at 10 nM E2. Other AR coregulators demonstrated slight induction (2- to 3-fold) at 10nM E2 treatment with the exception of SRC-1, which was able to enhance E2-mediated AR transcriptional activity 7- to 8-fold (Fig. 3).

**Hydroxyflutamide-Mediated AR Transcriptional Activity in the Presence of Different AR Coregulators**

The conversion of antiandrogens, such as hydroxyflutamide (HF), from antagonists to agonists during androgen ablation therapy of prostate cancer has been proposed as one of the reasons why most prostate cancers progress to an androgen-independent stage. The detailed molecular mechanism of this phenomenon, the so-called flutamide withdrawal syndrome, remains unclear. Since ARA70 can enhance the androgenic activity of E2, we were interested in knowing whether AR coregulators could enhance the agonist activity of HF. Our results indicate that ARA70 and ARA55 could enhance HF-mediated wtAR transcriptional activity 3- to 6-fold at 1 to 5 µM of HF [19, 38, 39]. Other coregulators, such as ARA54, SRC-3/ RAC3/ACTR/ AIB1, SRC-1, and Rb, showed only marginal effects on wtAR activity (Fig. 4).
Together, these data indicate that some selective AR coregulators can promote agonist activity by HF at pharmacologic concentrations, and this knowledge may help us to explain why HF can be converted to an agonist during androgen ablation therapy.

The HER2/Neu-MAP kinase signal cascade can regulate the function of AR and promote the interaction between AR and AR-associated proteins

Overexpression of the HER2/Neu proto-oncogene has been linked to the progression of breast cancer. Our previous report demonstrated that the growth of prostate cancer LNCaP cells could also be increased by the stable transfection of HER2/Neu [40]. Using AG879, a HER2/Neu inhibitor and PD98059, a MAP kinase inhibitor, as well as MAP kinase phosphatase-1 [41–43], we found that HER2/Neu could induce PSA, a marker for the progression of prostate cancer, through the MAP kinase pathway at a low androgen level [40]. The phosphorylation site of human AR by MAP kinase was also identified in the N-terminus (amino acids 511 to 515). The amino acid surveys further identified a consensus MAP kinase phosphorylation site that is conserved among human, rat, and mouse ARs. Site-directed mutagenesis studies further confirmed that this consensus site may play an important role in HER2/Neu-MAP kinase-mediated AR transactivation. Compared to wtAR, the mtARs 514a (amino acid 514 was changed from serine to alanine) has lower HER2/Neu-mediated AR transcriptional activity.

Moreover, our data suggested that this HER2/Neu-induced AR transactivation might function through the promotion of interaction between AR and AR coregulators, such as ARA70. To further study the molecular mechanism of how HER2/Neu can induce AR transactivation, we used a mammalian two-hybrid system to study the potential effects of HER2/Neu on the interaction of AR and ARA70N (amino acids 1–401 of ARA70). Transient transfection of AR or ARA70N peptide alone showed negligible activity. On the other hand, the interaction could be induced by cotransfection of AR and ARA70N in the presence of 10 nM DHT. Interestingly, addition of HER2/Neu further promoted the interaction between AR and ARA70N (Fig. 5). These data indicate that the induction of AR transactivation by HER2/Neu may involve the promotion of interaction between AR and ARs. The phosphorylation of the AR N-terminus may also affect the interaction of the AR LBD and associated proteins.

Fig. 5. Promotion of the interaction between AR and ARA70 by HER2/Neu. The human prostate cancer cell line DU145 was transiently co-transfected with 2.5 µg of the reporter plasmid, pG5-LUC, 2.5 µg of the Gal4 DBD fused to ARA70 (amino acids 176–401), and 2.5 µg VP16-AR fusion protein in the presence or absence of HER2/Neu. One nM of DHT was then added 24 h before cells were harvested for the CAT assay.

Can AR, ARA70, ARA55, and ARA54 interact with proteins that exhibit histone acetyltransferase activity?

It has been speculated that specific sets of proteins are recruited by SRs as coregulators, and may function as bridge factors between the receptors and general transcription factors in the preinitiation complex [44–46]. Identification of and understanding the functions of individual components of these complexes are critical in elucidating how nuclear receptors regulate their target genes. More significantly, recent progress in the study of coregulators has further linked the transcriptional activation of steroid receptors to chromatin acetylation. Some of these coregulators, such as CBP/p300 [5], SRC-1 [47], and SRC-3/RAC3/ACTR [9], have been found to either have intrinsic histone acetyltransferase (HAT) activity or the capacity to recruit the p300/CPB-associated factor (P/CAF), which...
Androgen receptor coregulators in prostate cancer cells

has HAT activity [48]. The physiologic significance of these coregulators and their involvement in development, differentiation, and reproductive diseases remain to be studied.

While the HAT P/CAF has been suggested to be part of a transcriptional complex, the physical interaction between P/CAF and AR, or between P/CAF and ARAs, has remained unclear. We have applied the mammalian two-hybrid system and immunoprecipitation to test the chemical and functional association of P300/CBP with ARA70, ARA55, and ARA54. Our results suggest that the essential step of chromatin acetylation during the transcriptional activation of AR could be achieved not only through the CBP-AR interaction, but also through the association of P/CAF with AR or AR coregulators, ARA70, ARA54, and ARA55 [49].

**The Linkage of Androgen Receptor} Poly-Glutamine repeats (Poly-Q) and the Poly-Q Associated Protein, ARA24, to Kennedy’s Neuron Disease**

The AR N-terminal domain contains several polytracts, such as the poly-Q and poly-proline regions [1]. The length of the poly-Q stretch in the AR N-terminal domain is inversely correlated with its transcriptional activity [27]. In addition, abnormal expansion of the poly-Q stretch [43–65] is linked to the neurodegenerative disease, spinal/bulbar muscular atrophy (SBMA). SBMA is often associated with gynecostasia and reduced fertility (Kennedy’s syndrome), suggesting a defect in AR function [28]. The severity of the syndrome is inversely correlated with the length of the poly-Q stretch [29].

One of our previous reports has identified a nuclear membrane trafficking protein, Ran/ARA24, which binds to the AR poly-Q stretch domain [22]. Detailed molecular studies of ARA24 indicate that the binding affinity of ARA24 to the AR N-terminal domain decreases as the poly-Q stretch length increases. The mammalian two-hybrid assay and immunofluorescence microscopy showed that, after androgen treatment, wtAR can bind to Ran/ARA24 and co-translocate to the nucleus, whereas the SBMA AR binds poorly to Ran/ARA24 and is located mainly in the cytoplasm. Moreover, transient transfection assays demonstrate that Ran/ARA24 can also function as a coregulator to enhance AR transcriptional activity. These data suggest that the poor interaction of SBMA AR with Ran/ARA24 not only prevents the translocation of SBMA AR to the nucleus, but also hinders the coactivation of SBMA AR transactivation. Increasing poly-Q length may, therefore, be linked to the development of Kennedy’s disease.

In addition, the AR N-terminal domain has been reported to interact with the general transcription factors TFIIIF and TBP [50]. We have also found that cdk-activating kinase (CAK), the kinase moiety of TFIIH, interacts with the AR N-terminal domain and enhances AR transactivation [51]. Interaction between the AR N-terminal domain and general transcription factors suggests that AR transcriptional activation may par-
potentially be enhanced by the recruitment of the general transcription machinery to the core promoter. AF-1, which is situated within the N-terminus of AR, is very likely involved in this process, but its precise role in AR-mediated transcription needs to be further studied.

**DISCUSSION**

Transcriptional activation or repression by nuclear hormone receptors can be augmented by transcriptional coactivators and corepressors, which can serve as a bridge between the nuclear receptor and the basal transcription machinery. In an effort to understand transcriptional regulation by the AR LBD, we have identified ARA70, ARA55, and ARA54 as ligand-dependent coregulators.

While SRC-1 has been reported as an effective SR coregulator [6], our results from DU 145 cells indicate that SRC-1 is a relatively weak AR coregulator. A previous report indicated that SRC-1 had only a 2-fold coactivator effect in CV-1 cells [11]. Conflicting results were also found with another coactivator, TIF2, with a 20-fold coactivator effect in COS-7 cells vs. only a 4-fold coactivator effect in HeLa cells [37]. These conflicting results suggest that the relative effects of various coactivators may depend heavily on the cell environment. Different cell lines, cell growth conditions, or transfection methods using various vectors and varying ratios of AR to AR coactivators may all contribute to varying coactivator effects. Indeed, we have also found that ARA70 can be a relatively weak AR coactivator in other cell lines, such as PC-3 or HeLa. Therefore, to fully understand the coregulator effects, it will be very important to systematically compare the relative strengths of different coactivators under the same conditions.

Our results indicate that ARA70 exhibits a relatively high specificity for AR when compared with GR and PR in the prostate cancer cell line DU145. On the other hand, ARA55 was able to enhance the activity of most of the classic steroid receptors except ER [19]. As transfection conditions and cell environments may influence coactivator effects, we may expect different specificity patterns in other cell lines. Furthermore, as our data only compares the coactivator specificity among classic steroid receptors, the potential coactivator effect on many new members of the SR superfamily, such as orphan receptors, remains unclear. Indeed, preliminary data indicate that ARA70 can also function as a coactivator to enhance the transactivation of the peroxisome proliferator-activated receptor in DU 145 cells [52]. Therefore, the observed specificity of each coactivator in different cells may preclude cell-specificity for each SR.

Both androgens and the androgen receptor regulate prostate cell proliferation. The functional analysis of the role of AR coregulators in AR transactivation may help us to better understand how AR influences prostate cell growth. Prostate cancer has become the most frequently diagnosed neoplasm in the United States and the second leading cause of cancer-related death in American men. To date, the only effective treatment for metastatic prostate cancer is androgen ablation therapy. A major concern in androgen ablation therapy is that the median duration of response is only 18–36 months. While both HF and bicalutamide are effective antiandrogens for the treatment of prostate cancer, they gradually lose their therapeutic effects after a protracted treatment period. Several studies have tried to explain at a molecular level how antiandrogens lose their antagonist activity and become partial agonists during protracted therapy [40, 53, 54]. In this review, we have discussed results that may provide some clues about this phenomenon. The data discussed here suggest that some selective AR coregulators, such as ARA70 and ARA55, have the unique ability to modulate the partial agonist activity of antiandrogens in the human prostate cancer cell line, DU145. Moreover, we have previously reported that ARA70 promotes E2-mediated activation of AR transactivation activity [35]. These two alternative AR signaling pathways may explain how prostate cancer patients deprived of circulating androgens by chemical and/or surgical methods eventually succumb to the disease. Analysis of the expression patterns of these AR coregulators in different stages of prostate cancer, both before and after antiandrogen treatment, may further strengthen our knowledge of their role in the progression of prostate cancer from an androgen-dependent to an androgen-independent stage.

In summary, the identification of cofactors that can enhance AR transactivation and alter agonist/antagonist activity may allow a better understanding of the scope of AR regulation of prostate cell proliferation and assist in the development of more effective therapeutic agents for controlling prostate cancer.

**REFERENCES**


50. McEwan IJ, Gustafsson J. Interaction of the human androgen receptor transactivation function with the general transcription factor TFIIF. Proc Natl Acad Sci USA 1997;94:8485–90.


