Activity of Androgen Receptor Antagonist Bicalutamide in Prostate Cancer Cells Is Independent of NCoR and SMRT Corepressors

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Abstract

The mechanisms by which androgen receptor (AR) antagonists inhibit AR activity, and how their antagonist activity may be abrogated in prostate cancer that progresses after androgen deprivation therapy, are not clear. Recent studies show that AR antagonists (including the clinically used drug bicalutamide) can enhance AR recruitment of corepressor proteins [nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid receptors (SMRT)] and that loss of corepressors may enhance agonist activity and be a mechanism of antagonist failure. We first show that the agonist activities of weak androgens and an AR antagonist (cyproterone acetate) are still dependent on the AR NH2/COOH-terminal interaction and are enhanced by steroid receptor coactivator (SRC)-1, whereas the bicalutamide-liganded AR did not undergo a detectable NH2/COOH-terminal interaction and was not coactivated by SRC-1. However, both the isolated AR NH2 terminus and the bicalutamide-liganded AR could interact with the SRC-1 glutamine-rich domain that mediates AR NH2-terminal binding. To determine whether bicalutamide agonist activity was being suppressed by NCoR recruitment, we used small interfering RNA to deplete NCoR in CV1 cells and both NCoR and SMRT in LNCaP prostate cancer cells. Depletion of these corepressors enhanced dihydrotestosterone-stimulated AR activity on a reporter gene and on the endogenous AR–corepressors enhanced dihydrotestosterone-stimulated AR activity and may contribute to AR activation and resistance. [Cancer Res 2007;67(17):8388–95]

Introduction

The androgen receptor (AR) plays a central role in prostate cancer development and progression, and androgen deprivation therapy by suppression of testicular androgen production (surgical castration or administration of luteinizing hormone–releasing hormone superagonists), or by treatment with AR antagonists (flutamide or bicalutamide), is still the standard systemic treatment. The majority of patients have clinical and biochemical improvement but eventually relapse with a more aggressive form of prostate cancer that has been termed hormone-refractory, castration-resistant, or androgen-independent prostate cancer. However, the AR and AR-regulated genes are still expressed at high levels in androgen-independent prostate cancer, indicating that AR transcriptional activity is reactivated in these tumors and that AR remains as a potential therapeutic target (1–4). One mechanism that may contribute to AR reactivation is increased accumulation or synthesis of androgens by prostate cancer cells, and a subset of patients who relapse after castration or luteinizing hormone–releasing hormone agonist treatment will respond to secondary hormonal therapies with AR antagonists such as bicalutamide or to treatments that suppress residual adrenal androgen production such as ketoconazole. However, these responses are usually partial and transient, with AR activity becoming resistant to even high doses of the AR antagonist bicalutamide through unclear mechanisms (5).

Additional mechanisms that may contribute to AR reactivation after androgen deprivation therapy are increased AR expression, including AR gene amplification that occurs in approximately one third of patients, and AR mutations that can enhance responses to nonandrogen steroids and to antagonists (6–9). Increased AR expression can enhance the growth of prostate cancer xenografts in castrated mice and has been reported to enhance the agonist activity of the AR antagonist bicalutamide (10). Mutant ARs that are strongly stimulated by the AR antagonist flutamide have been found in approximately one third of patients who relapse after combination therapy with flutamide, and a distinct mutant AR that is strongly stimulated by bicalutamide has been found in long-term bicalutamide-treated patients, but such mutations are uncommon in patients treated with surgical or medical castration monotherapy (9, 11). Further mechanisms that can enhance AR activity and may contribute to AR activation and resistance to AR antagonists include increased expression of transcriptional coactivator proteins and activation of kinases and signal transduction pathways that can modulate AR function, including the protein kinase A, c-Src, cyclin-dependent kinase 1, Ras-Raf-mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase pathways (12–17). However, the extent to which these or other mechanisms can account for resistance to AR antagonists is unclear.
data show that the agonist-ligated AR can also recruit corepressor proteins such as nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid receptors (SMRT); that recruitment of these corepressors can be enhanced by bicalutamide; and that bicalutamide may function as an agonist in the absence of these corepressors (22–31). The precise mechanisms by which AR antagonists function and how their activity is abrogated in prostate cancer that relapses after androgen deprivation are of central importance for the development of new therapies. Therefore, this study further examines the roles of AR N\(_2\)/COOH-terminal interactions and recruitment of coactivators versus corepressors in mediating AR antagonist activity.

**Materials and Methods**

**Plasmids and reagents.** Expression vectors for AR (pSVARo), VP16-AR, VP16-AR-NTD (amino acids 1–500), AR-DBD/LBD (amino acids 501–919), Ga4-AR-LBD (amino acids 661–919), AR-NTD/DBD, and SRC-1 (pSG5-SRC1) have previously been described (22, 29, 32). The Ga4-4RC1 (amino acids 1,050–1,185) construct was kindly provided by Dr. Frank Claessens (Faculty of Medicine, University of Leuven, Leuven, Belgium; ref. 33).

The mutant dFQNLF (deletion of amino acids 23–27) was generated from pSVARo using the QuickChange site-directed mutagenesis kit (Stratagene). The reporter construct ARE\(_4\)-luciferase, containing four tandem copies of a synthetic ARE, has been described (29). pG5-luciferase, regulated by five tandem Ga4 binding sites, and pRL-CMV, a cytomegalovirus (CMV) promoter regulated Renilla control, were from Promega. DHT, androstenedione, progesterone, and cyproterone acetate were from Sigma and bicalutamide; and that bicalutamide may function as an agonist in the absence of these corepressors (22–31). The precise mechanisms by which AR antagonists function and how their activity is abrogated in prostate cancer that relapses after androgen deprivation are of central importance for the development of new therapies. Therefore, this study further examines the roles of AR N\(_2\)/COOH-terminal interactions and recruitment of coactivators versus corepressors in mediating AR antagonist activity.

**Cell culture and transfection.** CV1 cells were maintained in DMEM supplemented with 5% fetal bovine serum (FBS; Hyclone). Cells in 48-well tissue culture plates in DMEM containing 5% charcoal dextran–stripped FBS (CDS-FBS; Hyclone) were cotransfected using LipofectAMINE 2000 (Invitrogen). Cells were transfected with 50 ng of reporter vector and AR and SRC-1 expression vectors, except AR-NTD/DBD was transfected at 10 ng/well and 1.25 ng of pRL-CMV Renilla vector was used for normalization. After 24 h, medium was replaced with fresh DMEM/5% CDS-FBS containing hormone or drugs at the indicated final concentrations. Following a further 24 h, firefly and Renilla luciferase activities were assayed with the dual-luciferase assay system from Promega as per supplier’s instructions. All samples were in triplicate and firefly luciferase activities were normalized for cotransfected Renilla activity.

**RNA interference.** Vectors expressing NCoR small hairpin RNAs (shRNA) under the control of the U6 promoter were previously described (22). The target sequence for NCoR was 5’-GGGCTTATGGAGGACTATGAG-3’. To assess AR transactivation in a reporter system, CV1 cells in six-well plates were cotransfected with 1 ng of this shRNA plasmid (pBSU6-NCoR) or a control plasmid (pBSU6). 400 ng of AR expression vector, 400 ng of ARE\(_4\)-luciferase reporter plasmid, and 3 ng of pCMV-\(\beta\)Gal plasmid for normalization. After 24 h, medium was replaced with fresh DMEM/10% CDS-FBS containing either 10 nmol/L DHT or 1 \(\mu\)mol/L bicalutamide. After another 24 h, luciferase and \(\beta\)-galactosidase activities were measured. All samples were in triplicate and luciferase activities were normalized for cotransfected \(\beta\)-galactosidase activity.

To assess the role of corepressors in AR regulation of the endogenous PSA gene, a prostate cancer cell line (LNCaP) was used. LNCaP cells in six-well plates in 2 mL of RPMI 1640 containing 10% CDS-FBS (Hyclone) were transfected using LipofectAMINE 2000 (Invitrogen) with 40–80 pmol (20–40 pmol/L NCoR1, NCoR2, SMRT, or negative control STEALTH small interfering RNA (shRNA; Invitrogen). After 24 h, the medium was replaced with fresh RPMI/10% CDS-FBS containing either 10 nmol/L DHT or 1 \(\mu\)mol/L bicalutamide. After 24-h incubation, RNA from the cells was extracted using TRIzol reagent (Tel-Test), and 1 \(\mu\)g was reverse transcribed with random hexamer primers using RT-for-PCR kit (BD Biosciences). Quantitative PCR was done in MX3000P Real-time PCR System (Stratagene). TaqMan Gene Expression Assays for PSA and cyclophilin (endogenous control) were purchased from Applied Biosystems. The data presented are the mean of three biological replicates normalized by cyclophilin mRNA expression.

**Results**

**Partial agonist activities of weak androgens and AR antagonists are dependent on the AR N\(_2\)/COOH-terminal interaction.** The physiologic high-affinity ligands for AR (testosterone and DHT) induce conformational changes in the LBD and a strong interaction with the AR N\(_2\) terminus. In contrast, previous studies have found no detectable interaction between the bicalutamide-ligated AR LBD and the N\(_2\) terminus, which may account for the lack of agonist activity of bicalutamide (21). However, AR transcriptional activity can be stimulated by other steroid hormones or drugs such as cyproterone acetate that do not mediate clearly detectable AR N\(_2\)/COOH-terminal interactions, suggesting that the agonist activities of these drugs may not be dependent on the N\(_2\)/COOH-terminal interaction (19). The interaction between the AR LBD and N\(_2\) terminus is mediated by a phenylalanine motif at amino acids 23 to 27 (FQNLF), which binds tightly to the LXXLL coactivator cleft in the AR LBD (18). Therefore, we first examined whether deletion of this motif in AR (dFQNLF) impaired AR activity in response to DHT versus weak agonists or drugs.

Consistent with previous data, deletion of FQNLF markedly impaired AR transcriptional activity in response to DHT (Fig. 1A). We presume that this largely reflects loss of the NH\(_2\)/COOH-terminal interaction (although additional effects of the deletion cannot be ruled out) and that residual activity is independent of this interaction, is mediated by another site, or is due to bridging of the NH\(_2\) and COOH termini by p160 coactivators (34). It should be noted that the fold induction can be further increased at higher DHT concentrations but that activity is similarly impaired by the deletion of FQNLF (data not shown). Significantly, the FQNLF deletion also markedly impaired AR activity in response to a weak androgen (androstenedione) and to progesterone, both used at micromolar concentrations (Figs. 1B and C). Moreover, the deletion abrogated the partial agonist activity of the AR antagonist drug cyproterone acetate (Fig. 1D). In contrast, bicalutamide did not stimulate the wild-type or mutant ARs (data not shown).

These results suggested that the agonist activities of the above steroid hormones and of cyproterone acetate were dependent on the AR NH\(_2\)/COOH-terminal interaction. Therefore, we next carried out mammalian one- and two-hybrid protein interaction assays to determine whether cyproterone acetate could induce a detectable AR NH\(_2\)/COOH-terminal interaction. CV1 cells were cotransfected with expression vectors encoding an NH\(_2\)-terminal domain (NTD)–deleted AR (AR-DBD/LBD), the AR NTD linked to the VP16 transactivation domain (VP16-AR-NTD), and an ARE regulated onto nitrocellulose membranes. Blots were probed with a 1:500 dilution of an affinity-purified anti-NCoR antisera or anti-SMRT antibody (BD Biosciences) in TBS containing 5% nonfat milk and 0.05% Tween 20, followed by horseradish peroxidase–conjugated antirabbit antibody (Amersham Biosciences) at 1:1,000 dilution. The blots were visualized with the use of ECL Plus Western blotting detection system (Amersham Biosciences).

**Real-time PCR.** Total RNA from LNCaP cells was extracted using STAT-60 reagent (Tel-Test), and 1 \(\mu\)g was reverse transcribed with random hexamer primers using Advantage RT-for-PCR kit (BD Biosciences). Quantitative PCR was done in MX3000P Real-time PCR System (Stratagene). TaqMan Gene Expression Assays for PSA and cyclophilin (endogenous control) were purchased from Applied Biosystems. The data presented are the mean of three biological replicates normalized by cyclophilin mRNA expression.
Consistent with the above results, cyproterone acetate (containing five tandem copies of the Gal4 binding element). CV1 cells were cotransfected with the Gal4-AR-LBD reporter gene (ARE4-luciferase). As previously shown, DHT induced a strong interaction between the AR NTD and DNA binding domain (DBD)/LBD fragment (Fig. 2A). Significantly, cyproterone acetate also induced an interaction, although it was clearly weaker than the DHT-stimulated response and required higher concentrations (1–10 nmol/L for DHT versus 1–10 μmol/L for cyproterone acetate; Fig. 2A). In contrast, there was no detectable interaction in response to bicalutamide. Consistent with previous data, the AR DBD/LBD fragment by itself had no detectable transcriptional activity in response to DHT or antagonists (data not shown).

To confirm the interaction between the AR NTD and the cyproterone acetate–liganded AR LBD, we next carried out two-hybrid experiments using the AR LBD alone fused to the Gal4 DBD (33, 35). Significant interaction was not dependent on the AR LBD (Fig. 3C). Cotransfection of SRC-1 enhanced the transcriptional activity of AR-NTD/DBD. Significantly, cotransfection of SRC-1 with the unliganded AR LBD and the NTD may abrogate the intrinsic transcriptional activity of the AR NTD (39). To test this hypothesis, we expressed the glutamine-rich domain of SRC-1 (amino acids 23–27) deleted (dFQNLF), ARE4-luciferase reporter, and a control CMV reporter gene (ARE4-luciferase). As previously shown, DHT induced a strong interaction between the NTD and the LBD (Fig. 2B). Taken together, these data indicate that the AR NH2/COOH-terminal interaction is critical for AR transcriptional activity mediated by weak androgens and partial agonist drugs. Moreover, the failure of bicalutamide to mediate this interaction is consistent with the lack of partial agonist activity of this drug.

**SRC-1 coactivator can interact with bicalutamide-ligated AR NH2 terminus but does not stimulate transcriptional activity.** Previous studies have shown that AR recruitment of p160 SRC proteins (in particular, SRC-1) is mediated primarily by the AR NTD, with one proposed function for the NH2/COOH-terminal interaction being to structure the NTD and thereby enhance coactivator binding (35–38). Consistent with this hypothesis and previous data, SRC-1 can coactivate both the DHT and cyproterone acetate–liganded AR but not the bicalutamide-ligated AR (Fig. 3B). To further assess whether SRC-1 fails to coactivate the bicalutamide-ligated AR due to the lack of an NH2/COOH-terminal interaction, we examined SRC-1 coactivation of an LBD deletion mutant (AR-NTD/DBD). Significantly, cotransfection of SRC-1 enhanced the transcriptional activity of AR-NTD/DBD on an ARE4-luciferase reporter, showing that SRC-1 could associate with the AR NTD independently of the NH2/COOH-terminal interaction (Fig. 3B).

This result indicated that absence of an NH2/COOH-terminal interaction in the bicalutamide-ligated AR was not the basis for lack of SRC-1-mediated coactivation, and suggested that there may be an inhibitory interaction between the AR NTD and the bicalutamide-ligated LBD that prevents SRC-1 binding. Indeed, previous studies have shown that an inhibitory interaction between the unliganded AR LBD and the NTD may abrogate the intrinsic transcriptional activity of the AR NTD (39). To test this hypothesis, we expressed the glutamine-rich domain of SRC-1 (amino acids 1,050–1,185), which mediates SRC-1 binding to the AR NTD (but does not mediate transactivation), as a fusion protein with the Gal4 DBD (33, 35). Significantly, the Gal4-SRC-1(1,050–1,185) fusion had no transcriptional activity on the pG5-luciferase reporter in transfected CV1 cells, but could be strongly activated by the cotransfected AR NTD, further showing that the AR/SRC-1 interaction is critical for AR transcriptional activity.
The Gal4-SRC-1(1,050–1,185) fusion protein was also strongly activated by the full-length AR fused to VP16 (VP16-AR) in the absence of added ligand or in the presence of DHT (Fig. 3D). Importantly, this activation by VP16-AR was not diminished by bicalutamide (Fig. 3D). Taken together, these results show that the bicalutamide-ligated AR LBD does not interfere with SRC-1 binding by the AR NTD, although full-length SRC-1 does not coactivate the bicalutamide-ligated full-length AR on an ARE reporter gene.

**Bicalutamide antagonist activity is independent of NCoR and SMRT corepressors.** As the above results indicated that SRC-1 recruitment may not be directly blocked by the bicalutamide-ligated AR LBD, we next considered other mechanisms by which bicalutamide may block coactivator recruitment or induction of transcriptional activity. One such possible mechanism is clearly corepressor recruitment, as previous studies indicate that bicalutamide can enhance NCoR recruitment by the AR and that bicalutamide may function as an agonist in the absence of NCoR (23, 27, 28). A previous study also indicated that AR overexpression in prostate cancer cells could by itself alter the response to bicalutamide and make it function as an agonist (10). The molecular basis for this latter effect of AR overexpression was not clear but could possibly reflect a relative decrease in the levels of corepressors. Therefore, we first examined whether NCoR negatively regulates the agonist-liganded AR (Fig. 4B; ref. 22). In contrast, NCoR shRNA did not reveal substantial bicalutamide agonist activity, although it may very weakly enhance both the unliganded and bicalutamide-ligated ARs (Fig. 4B).

To further determine whether NCoR contributes physiologically to the antagonist activity of bicalutamide on the endogenous AR in prostate cancer cells, we examined whether NCoR down-regulation by siRNA would stimulate bicalutamide agonist activity on the endogenous AR-regulated PSA gene in LNCaP prostate cancer cells. It should be noted that LNCaP cells express a mutant AR (T877A) that is stimulated by the AR antagonist hydroxyflutamide but is still repressed by bicalutamide. Transfection with 20 or 40 nmol/L of an NCoR siRNA duplex (NCoR1 siRNA) substantially decreased NCoR protein expression in LNCaP cells relative to control siRNA, with no clear effect on SMRT (Fig. 5A). LNCaP cells cultured in steroid hormone–depleted medium were then transfected with NCoR1 or control siRNA and stimulated with DHT or bicalutamide. Expression of the PSA gene was then assessed by real-time RT-PCR. Significantly, treatment with the NCoR siRNA did not stimulate PSA gene expression in response to bicalutamide (Fig. 5A). In contrast, DHT stimulated PSA gene expression by ~100-fold, although this DHT-stimulated PSA expression was not further enhanced by the NCoR1 siRNA.

We next examined another NCoR siRNA duplex (NCoR2 siRNA), which almost completely suppressed NCoR protein expression (Fig. 5B). Interestingly, SMRT protein expression was increased in response to this siRNA, possibly reflecting compensation by the
cells for the marked decline in NCoR levels. Significantly, maximal PSA gene expression at higher DHT concentrations was enhanced by this NCoR2 siRNA (Fig. 5B), confirming another recent report showing that NCoR negatively regulates the endogenous agonist–liganded AR in prostate cancer cells (24). In contrast, bicalutamide agonist activity was still not stimulated by the NCoR2 siRNA or by SMRT siRNA, or by the combination of both siRNAs (Fig. 5C). Taken together, these results indicate any substantial partial agonist activity of bicalutamide is not being blocked through recruitment of the NCoR or SMRT corepressors.

**Discussion**

AR antagonists such as bicalutamide are effective at blocking AR activity and tumor growth in primary prostate cancer but are not effective at blocking the reactivated AR in prostate cancer that recurs after androgen deprivation therapy. Recent studies have indicated that the AR antagonist activity of bicalutamide may be mediated by NCoR corepressor recruitment and that bicalutamide can function as an AR agonist in response to high-level AR expression or removal of NCoR from the AR complex (10, 23, 25–28). However, the data presented here do not support these conclusions because NCoR and SMRT down-regulation enhanced DHT-stimulated AR activity but did not reveal any clear bicalutamide agonist activity. An alternative general mechanism of action for bicalutamide is that the bicalutamide-liganded AR LBD is unable to effectively recruit coactivators (21). The conformation of the AR LBD may influence coactivator recruitment directly through interactions with coactivators as well as indirectly through the NH2/COOH-terminal interaction. This study shows that the AR NH2/COOH-terminal interaction is critical for the agonist activity of weak androgens and partial agonist drugs, indicating that bicalutamide may fail to recruit critical coactivators due to the absence of this interaction. This study further shows that the NH2 terminus of the bicalutamide-liganded AR can still interact with SRC-1, indicating that ineffective recruitment of other coactivators or chromatin remodeling proteins is responsible for the antagonist activity of bicalutamide. Consistent with this conclusion, a recent study found that the AR NH2/COOH-terminal interaction was required for AR binding to endogenous genes and likely for recruitment of the SWI/SNF complex (40).

The physiologic importance of NCoR and SMRT recruitment by the agonist-ligated AR is supported by results in previous studies that showed increased agonist-stimulated AR transcriptional activity in response to NCoR and SMRT down-regulation (22, 24, 29). A previous study further showed that NCoR and SMRT down-regulation by RNA interference in LNCaP cells could both enhance the DHT-stimulated expression of endogenous AR-regulated genes and decrease the AR antagonist activity of flutamide (24). This result is not inconsistent with the current study because flutamide has substantial agonist activity for the mutant AR (T877A) expressed in LNCaP cells. Therefore, the agonistic activities of both DHT and flutamide in these cells are dependent on the balance between coactivators and corepressors, and on this basis, these ligands may be classified as selective AR modulators. In contrast, we conclude that bicalutamide lacks any substantial partial agonist activity, and its activity is therefore not dependent on corepressors or on coactivator-to-corepressor ratios. Importantly, these ratios may become significant if the bicalutamide-liganded AR in advanced prostate cancer cells can acquire the ability to more effectively recruit coactivators (through increased coactivator expression, AR or coactivator phosphorylation, or other modifications).

Chromatin immunoprecipitation studies have confirmed that NCoR is recruited to endogenous AREs by the agonist-ligated AR and showed that this recruitment can be enhanced by AR antagonists (22–26). Recent studies have further shown that NCoR and SMRT are recruited as part of a complex containing HDAC3, TBL1, TBLR1, and TAB2 (27, 41–44). Significantly, TAB2 phosphorylation by MEKK1 in response to inflammatory signals has been shown to result in the loss of NCoR and HDAC3 from this complex and has been reported to convert...

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**Figure 4.** AR overexpression and NCoR knockdown do not convert bicalutamide into an agonist in CV1 cells. A, cells were cotransfected with the ARE4-luciferase reporter vector, pRL-CMV control, and increasing amounts of full-length AR (25–400 ng). Cells were treated for 24 h with DHT (10 nmol/L) or bicalutamide (10 μmol/L) and firefly versus Renilla luciferase activities were determined from triplicate samples (columns, mean RLU; bars, SE). B, cells were transfected with 400 ng of AR and ARE4-luciferase reporter, 3 ng of control pCMV-βGal, and 1 μg of a control (pBSU6) or NCoR shRNA expression vector (pBSU6-NCoR). Cells were stimulated for 24 h with DHT (10 nmol/L) or bicalutamide (1 μmol/L) and luciferase versus β-galactosidase activities were determined from triplicate samples (columns, mean RLU; bars, SE).
In an experiment, researchers used LNCaP cells to evaluate the effects of bicalutamide and DHT on the androgen receptor (AR). They transfected cells with control siRNA or specific siRNAs targeting NCoR or SMRT and cultured them under different conditions to measure endogenous PSA mRNA levels by real-time RT-PCR. The results showed that down-regulation of NCoR or SMRT by siRNA leads to functionally distinct complexes, with MEKK1 having additional effects that do reveal substantial bicalutamide agonist activity. An alternative explanation may be that the qualitative differences in AR activity are due to the MEKK1/TAB2 mechanism versus NCoR down-regulation by siRNA.

Another study showed that lentivirus-mediated AR overexpression in prostate cancer cells may have distinct effects on cell growth. The interpretation of these latter results is not entirely clear as previous studies indicate that the LXXLL helices in SRC-1 do not contribute significantly to coactivation of the agonist-liganded AR. Nonetheless, these data are consistent with our observation that SRC-1 can interact with the bicalutamide-liganded AR but does not stimulate transcriptional activity. Moreover, they suggest that impaired recruitment of CREB binding protein/p300 and CARM1, possibly secondary to altered SRC-1 recruitment, may be a mechanism contributing to bicalutamide antagonist activity.
transcriptional activity, but its binding is no longer stabilized by the LBD.

terminal interaction. NCoR can still interact weakly with the AR NTD and repress response to DHT. In the presence of bicalutamide (NH2/COOH-terminal interaction and NCoR can bind to both the AR NH2- and COOH-terminal domains. SRC-1 can also interact with the NTD of the complexes. These interactions may be mediated directly by the LBD and/or be proteins in conjunction with additional coactivator and chromatin remodeling complexes. These interactions may be mediated directly by the LBD and/or be dependent on conformational changes in the NTD as a result of the NH2/COOH-terminal interaction. NCoR can still interact weakly with the AR NTD and repress transcriptional activity, but its binding is no longer stabilized by the LBD.

effects on coactivators and corepressors that can account for bicalutamide agonist activity, particularly if this activity is only modest relative to a physiologic agonist. In any case, our NCoR and SMRT siRNA results in LNCap cells indicate that loss of these corepressors is not the mechanism mediating any bicalutamide agonist activity in LNCap cells overexpressing AR.

Previous studies have also shown that long-term growth of LNCap cells in medium with bicalutamide can select for cells that are bicalutamide stimulated, and found that the AR in these cells had an additional mutation that is responsible for the agonist activity of bicalutamide (45, 46). This mutation in codon 741 has been found in patients treated previously with bicalutamide, but is rare overall in patients with relapsed androgen-independent prostate cancer (11). Finally, a recent study investigated bicalutamide resistance in C4-2 cells, which are derived from a LNCaP xenograft that relapsed after castration. Bicalutamide did not have AR agonist activity in these cells but it was unable to inhibit basal AF-2-independent AR transcriptional activity, suggesting that uncoupling of the NH2-terminal AF-1 transactivation function from AF-2 (by unclear mechanisms) may contribute to bicalutamide resistance in androgen-independent prostate cancer (47).

Further studies comparing bicalutamide and DHT agonist activities in prostate cancer cells overexpressing AR and assessing other mechanisms that may mediate bicalutamide agonist activity are clearly warranted. However, it should also be emphasized that data supporting the hypothesis that bicalutamide functions as an agonist in vivo are based primarily on bicalutamide withdrawal responses, which occur in only a small fraction of patients who are treated with this drug as part of their initial androgen deprivation therapy. In contrast, bicalutamide has no effect or has a weak and transient inhibitory effect on the majority of tumors that recur after androgen deprivation by castration or luteinizing hormone–releasing hormone agonist treatment. Therefore, whereas bicalutamide may be an agonist in a small subset of tumors, this agonist activity is not clearly related to the intrinsic bicalutamide resistance observed in the majority of patients who relapse after castration.

In summary, this study further establishes the critical role of the AR NH2/COOH-terminal interaction for AR transcriptional activity, and indicates that whereas NCoR and SMRT are recruited to the bicalutamide-ligated AR, they are not responsible for the antagonist versus agonist activity of this drug. Instead, we suggest that failure to recruit specific coactivators is primarily responsible for bicalutamide antagonist activity and that the molecular basis for any bicalutamide agonist activity that occurs in vivo (in the absence of AR mutation) may be enhanced activity of these coactivators (which may circumvent an AF-2 dependent function). Finally, it should be emphasized that bicalutamide resistance in androgen-independent prostate cancer may not be due to agonist activity but may instead reflect decreased bicalutamide binding to AR and hypersensitivity to low levels of endogenous agonist ligands. This is an important distinction, as the latter hypothesis would predict that bicalutamide-like drugs with higher affinity for AR would be effective whereas the former would indicate the need for drugs that block AR by distinct mechanisms.

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