Pharmacological Uncoupling of Androgen Receptor-mediated Prostate Cancer Cell Proliferation and Prostate-specific Antigen Secretion

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ABSTRACT

The androgen receptor (AR), a member of the nuclear receptor family, is a ligand-inducible transcription factor. In the prostate gland, androgens regulate the transcription of several genes that ultimately result in cell growth and differentiation. With a goal of developing tissue-selective AR modulators that can be used to treat prostate cancer and other androgenopathies, we have taken an approach to identify androgens that function in a manner distinct from the physiological androgens testosterone and dihydrotestosterone. Classical AR agonists function by binding to and inducing a conformational change in the receptor. This facilitates the obligate interaction of the amino and carboxyl terminus of the receptor, recruitment of coactivators, and subsequent regulation of target genes. On the basis of this paradigm, we screened a library of potential AR agonists for compounds that induce an “activating” conformational change in the receptor structure but that do not facilitate a high-affinity intermolecular interaction between the amino and carboxyl terminus. Compounds identified in this manner behaved as partial agonists of AR-mediated transcription in a variety of assays. Additional compounds were identified in this screen that did not allow the activation function-2 coactivator pocket to form and were demonstrated to function as weak agonists of AR-mediated transcription. Surprisingly, when we examined the ability of these compounds to induce cell proliferation, we observed that despite having different degrees of partial agonist activities on classical transcriptional responses (i.e., induction of prostate-specific antigen), these compounds were as efficacious as dihydrotestosterone in stimulating proliferation. The unexpected finding that AR-mediated transcription and proliferation can be uncoupled suggests that AR is not used in the same manner in all androgen-regulated biological processes.

INTRODUCTION

Prostate cancer is the second leading cause of cancer-related mortality among adult males in the United States. Androgen ablation (suppression of gonadal androgens by surgical castration or medical intervention using estrogens or gonadotropin-releasing hormone agonists/antagonists) or total androgen ablation (which combines androgen ablation with the use of antiandrogens such as flutamide) remains the primary therapeutic option for advanced stages of this disease (1–3). Although initially effective, androgen ablation and antiandrogen therapy often fail after 1–2 years when the tumor becomes androgen independent (4). Different mechanisms have been proposed to explain the development of androgen independence in prostate cancer, which include: (a) amplification of AR in ~30% of the patients who failed therapy; and (b) occurrence of somatic mutations in AR that alter its pharmacology (5). We and others (6, 7) have shown that the T877A somatic mutation in the AR LBD that frequently occurs in hormone-refractory metastatic prostate cancer recognizes a number of nonandrogenic compounds such as estrogens, prostegins, glucocorticoids, and even antiandrogens as androgens. This altered ligand specificity has provided an explanation for some treatment failures with antiandrogens such as flutamide. Interestingly, the majority of tumors that fail flutamide therapy respond favorably to drug discontinuation, suggesting that resistance may represent a change in the way the cells recognize this antiandrogen. These observations suggest two opportunities for novel antiandrogens: (a) compounds in which the biocharacter is stable and that cannot acquire the ability to be recognized as agonists; and (b) antiandrogens that can be used to treat flutamide-refractory prostate cancers.

Clearly, there is an unmet medical need for mechanistically distinct modulators for AR that can be used as effective treatments for prostate cancer. The recent discovery of tissue-selective modulators for estrogen receptor and their promising results in treating breast cancer in women have prompted similar research efforts to discover SARMs for AR (8, 9). The challenge, however, is to dissect the different AR-mediated signaling pathways and find targets that are amenable to pharmaceutical intervention and use this information to develop new modulators. AR is a ligand-inducible transcription factor that is responsible for regulating the transcription of several genes, the most notable of which is PSA, a marker for prostate cancer (10, 11). PSA levels have been observed to be elevated in androgen-dependent prostate cancers, and the measurement of PSA has been used for prostate cancer screening and subsequent evaluation of the effectiveness of antihormone treatment (12). However, in the muscle, another androgen target tissue, androgens increase nitrogen retention and thereby seem to increase the size of the muscle cell (13). The exact mechanism for this anabolic activity of androgens is not well understood, and whether AR transcriptional activity is required for this phenomenon is still unclear. Additionally, there is an increasing number of reports that describe the rapid, nongenomic action of androgens, involving kinase-signaling cascades, that may or may not involve AR (14–16). The physiological effect of nongenomic androgen action is yet to be determined. Taken together, the complexity of the androgen signal transduction pathway indicates that androgen action results in a number of distinct intracellular events, and if these events can be separated mechanistically, then we can use that information to screen for or against a certain activity and thereby identify a selective modulator. Here, we have examined two activities of AR: ligand-dependent transcriptional activity and ligand-mediated proliferation activity. We show that these two AR-dependent activities are mechanistically distinct, and, therefore, parameters that determine the transcriptional efficacy and potency of a given ligand cannot be used to predict their effect on prostate cancer cell proliferation.

MATERIALS AND METHODS

Test Ligands. DHT was purchased from Sigma Chemical Co. (St. Louis, MO). Bicalutamide (casodex) was a gift from P. Turnbull (GlaxoSmithKline, Research Triangle Park, NC), and hydroxyflutamide was a gift from K. Gaido.
The antiestrogens tested in these studies have been described previously. The MMTV-luc reporter construct contains the luciferase reporter gene regulated by the MMTV upstream promoter sequence. The PSA upstream promoter sequence was a gift from C. Y-F. Young (Mayo Clinic, Rochester, MN).

**RESULTS**

**Identification and Characterization of Three Mechanistically Distinct Classes of AR Modulators.** On the basis of the current model of androgen-mediated transcription, androgen binds to the COOH-terminal LBD of AR and induces a conformational change that results in receptor dimerization, facilitating a high-affinity intermolecular interaction between the amino and carboxyl terminus (N-C interaction) (22, 23). In general, it has been observed that there is a positive correlation between AR-N-C interactions and the agonist activity of a ligand, because inability of a ligand to facilitate this interaction is associated with weak agonist or antagonist activity (24, 25). In addition, AR has two activation functions, the N-terminal AF-1 is ligand independent, and the COOH-terminal AF-2 is dependent on the repositioning of the LBD helices subsequent to the binding of the agonist. The AF-2 pocket in other steroid receptors such as estrogen receptor and progesterone receptor have been shown clearly to recruit p160 coactivator proteins through a peptide motif represented by the sequence LXXLL (L-leucine, X-any amino acid; Refs. 26 and 27). The mechanism by which p160s are recruited to AR remains controversial (28, 29), although we and others have shown that AR in the presence of agonists can bind short peptides that contain the LXXLL peptide motif, confirming that a functional AF-2 pocket is formed on agonist binding (20).

We have also shown that AR agonist activity can be predicted based on the ability of a compound to facilitate the formation of an AF-2 pocket (7). Thus, N-C interaction and AF-2 formation can be used to screen for AR agonists. Potent agonists such as DHT and R1881 facilitate a strong N-C interaction as well as allow the formation of an AF-2 pocket that permits interaction with nuclear receptor coactivators. These ligands, therefore, represent one class of AR agonists. In contrast, AR partial agonists, such as the clinically used antiestrogen RU486, do not facilitate N-C interaction and also do not allow the formation of an AF-2 pocket. RU486 could, therefore, be considered mechanistically distinct from DHT and belonging to a second class of compounds. We hypothesized that by using a combination of these two assays we may be able to find a third and novel class of ligands that allow the formation of AF-2 without facilitating N-C interaction.

An initial screen to examine AR agonist activity of RU486-based ligands that do not allow the formation of an AF-2 pocket was performed (Fig. 1). The prototype of these ligands is RU486, a compound with potential agonist activity that has a 17β-hydroxy-17α-propynyl n-ring substitution pattern (18).

**Plasmids.** The construction of all of the plasmid vectors used in this study has been described previously (17, 19, 20). The MMTV-luc reporter construct contains the luciferase reporter gene regulated by the androgen-responsive mouse mammary tumor virus promoter. PSA-407E luc reporter containing the PSA upstream promoter sequence was a gift from C. Y-F. Young (Mayo Clinic, Rochester, MN). 2XPRE-Ik-luc contains two copies of the ARE/progesterone response element upstream of a thymidine kinase promoter. pCMVβ-gal plasmid, used for normalization of transcription efficiencies, contains the CMV promoter driving the expression of the β-galactosidase reporter gene. 5X Gal4-luc3 contains the luciferase reporter regulated by five copies of the Gal4 binding site. The N- and COOH-terminal fragments of the AR were expressed from pcDNA AR 1-501 and pcDNA AR 507-919, respectively. VP16-AR contains the full-length wild-type AR, and VP16-ART877A contains the full-length AR containing the point mutation at amino acid 877. VP16 AR 1-660 contains the AR NH2 terminus fused to the VP16 activation domain. AR amino acids 624-919 of the wild-type and mutant AR were fused to the Gal4DBD in pM vector (Clontech, Palo Alto, CA) to generate PM AR 624-919 and PM AR 624-919 T877A, respectively. The Gal4-DBD fusion protein of the D30 peptide, which contains the LXXLL nuclear receptor interaction motif, was also constructed in pM vector. pBSIIKS plasmid (Stratagene, La Jolla, CA). This vector contains the CMV promoter driving the expression of the AR amino acids 624-919 of the wild-type and mutant AR were fused to the Gal4DBD in pM vector (Clontech, Palo Alto, CA) to generate PM AR 624-919 and PM AR 624-919 T877A, respectively. The Gal4-DBD fusion protein of the D30 peptide, which contains the LXXLL nuclear receptor interaction motif, was also constructed in pM vector. pBSIIKS plasmid (Stratagene, La Jolla, CA) was used in transfections to make up for the total amount DNA used in different wells.

**Cell Culture and Transfections.** CV-1 (monkey kidney) cells were maintained in MEM (Invitrogen, Carlsbad, CA) supplemented with 8% FCS (Hyclone Laboratories, Logan, UT). Tissue culture plates were treated with 10% FCS and 1% charcoal dextran-stripped serum. The cells were trypsinized and the cell pellets were frozen in liquid nitrogen. Total RNA was isolated from the pellets using RNeasy kit (Qiagen, Valencia, CA), and quantitative RT-PCR analysis was performed using one-step RT-PCR kit (Qiagen), supplemented with 20 nM FITC and SYBR Green I (Molecular Probes, Eugene, OR). The RT-PCR reactions were performed on iCycler real-time detection system (Bio-Rad Laboratories). The primers for the PSA gene have been described previously (21), and the primers for GAPDH had been described previously (21), and the primers for GAPDH had been described previously (21). (CIT, Research Triangle Park, NC). The antiestrogens tested in these studies have been described previously (17). The 1β-aryl compounds were grouped into six groups based on the functional groups at position 17 (17β-aryl: 6413-001, -002, -003, -016, -018, -031; 17β-nitro: 6413-013, -015, -028, -029E, -029Z, -030, -043, -043ox, -044, -045, -045ox; 17β-methyl thio: 6413-006, -009a, -039, -042; spirothiolane: 6413-050a, -050b, -051a, -051b, -052, -054, -057, -058; spironitro: 6413-055, -056; cyclic ketone: 6413-046a, -046b). The prototype of these ligands is RU486, a compound with potential agonist activity that has a 17β-hydroxy-17α-propynyl n-ring substitution pattern (18).
antiprogestins was established. In Fig. 1A, the formation of an AF-2 pocket was evaluated using a mammalian two-hybrid assay. AR was expressed as a VP16 fusion protein, and its ability to recruit an LXXLL peptide (D30) expressed as a Gal4-DBD fusion protein was assayed on a Gal4-responsive luciferase reporter. In Fig. 1B, the ability of AR ligands to facilitate an N-C interaction was measured. The AR NH2 terminus (amino acids 1–501) was expressed from one expression vector, and the COOH terminus (amino acids 507–919), which codes for the DBD and LBD, was expressed from a second expression vector. The ability of these two AR fragments to interact in the presence of added ligand was evaluated on an androgen-responsive MMTV-luciferase reporter. As expected, DHT exhibited a positive response in both the D30 peptide interaction and the N-C interaction assays. Also, as expected, RU486 was inactive in both the D30 peptide interaction and the N-C interaction assays. AR antagonists such as hydroxyflutamide and casodex also showed a profile similar to RU486. Therefore, in this experiment, a negative response would imply either partial agonist activity, or antagonist activity, or lack of interaction with AR. Interestingly, however, we identified several compounds that exhibited significant activity in the D30 peptide interaction (>20% of DHT response) without facilitating N-C interaction (<20% of DHT response). Notably, 6413-018, 031, 058, 51a, and 45 exhibited D30 interactions of a nature similar to that obtained with DHT.

It is possible that the lack of N-C interaction observed with the compounds in our screen could be caused by the nature of the assay itself that involved the use of truncation mutants containing the DBD and the activation domain of AR on a complex MMTV promoter. Therefore, we used a more rigorous assay to test AR N-C interactions using a system that incorporates a heterologous DBD and activation function (22). Specifically, the AR NH2 terminus amino acids 1–660 were fused to the VP16 activation domain, and amino acids 624–919 (LBD) were fused to the Gal4DBD. The interaction of these two fusion proteins was tested on a 5XGal4-luc3 reporter. As shown in

Fig. 1. Identification of novel AR ligands. A, detection of AF-2 conformation. Mammalian two-hybrid assay was performed in CV-1 cells transfected in T25 flasks with 1600 ng of plasmids expressing VP16-AR and Gal4-DBD-D30 peptide fusion proteins. D30, HPTHSSR1WELLMEATPTM. The interaction between AR and the peptide on addition of ligands (1 μM final concentration) was assayed on 4 μg of transfected 5X Gal4-luc3 reporter plasmid. Eight hundred nanograms of pCMV β gal plasmid served as a normalization control for transfection efficiency. The transfected cells were seeded into 96-well plates for treatment with ligands in quadruplicate. B, detection of AR N-C interaction. CV-1 cells were transfected in 100-mm dishes with a total of 30 μg of DNA containing 2 μg each of pcDNA AR 1–501 and pcDNA3 AR 507–919 plasmids that express the N- and COOH-terminal fragments of AR, respectively. The interaction between the fragments on the addition of test ligands (1 μM final concentration) or DHT (10 nM) was tested on 16 μg of transfected MMTV-luc reporter. One microgram of pCMV β gal was transfected to normalize luciferase activities, and pBSIIKS was used to make up the total amount of DNA used for the transfection. The cells were seeded in 96-well plates 20 h after transfection for treatment with ligands in quadruplicate.
facilitating N-C interaction of ART877A. Compound 6413-001 also exhibited the same characteristics on ART877A as seen with the wild-type AR: lack of formation of the AF-2 pocket and lack of N-C interaction (Fig. 4, A and B). These important findings validated the use of LNCaP cells as a model system to evaluate the new AR ligands. It has been shown previously with DHT that a positive N-C interaction tracks with increased receptor levels, possibly because of the stabilization of the protein. It is, therefore, possible that compounds that did not facilitate N-C interaction may be incapable of stabilizing the receptor levels, which might contribute to decreased agonist activity. We, therefore, examined the relative levels of AR in LNCaP cells by Western blot after treatment with DHT, 6413-018, or 6413-001 (Fig. 4C). The increase in endogenous AR observed in LNCaP cells in the presence of DHT is quite modest (~1.5-fold) in contrast to transfected CV-1 cells, in which we observed DHT-induced AR stabilization of >7-fold (data not shown). Compared with the levels of AR in the absence of treatment (NH) or in the presence of casodex, we observed a similar increase in AR in the presence of DHT, 6413-018, and 6413-001. We, therefore, concluded that the decrease in agonist

Table 1 Chemical structures of RTI 6413-018 and 6413-001

<table>
<thead>
<tr>
<th>RTI No.</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
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<td>-COCH₃</td>
<td>-C₆H₄</td>
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<tr>
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<td>-COCH₃</td>
<td>-C₆H₄</td>
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Fig. 2. DHT, but not 6413-018 or 6413-001 (Table 1), facilitates AR N-C interactions, confirming that these two compounds are mechanistically distinct from DHT.

We anticipated that those compounds that allowed the AF-2 pocket to form without facilitating an N-C interaction would exhibit efficacy and potency lower than that of DHT. To confirm this, we performed a dose-response analysis in LNCaP cells to determine the agonist efficacy of each compound on endogenous AR. As predicted, DHT was the most potent and most efficacious agonist in LNCaP cells on both MMTV-luciferase (Fig. 3A) and PSA-luciferase reporters (Fig. 3B), whereas 6413-018 exhibited partial agonist activity on both promoters. Compound 6413-001, which did not facilitate either an N-C interaction or a D30 peptide interaction (Fig. 1), had no detectable agonist activity on either reporter in LNCaP cells. The pattern of transcriptional activity of all three compounds was also retained on a less complex promoter (2XPRE-tk-luc) containing two copies of the ARE, although the overall response for all ligands was reduced (Fig. 3C).

The endogenous AR expressed in LNCaP cells contains a point mutation at amino acid 877 that affects the pharmacology of AR antagonists, such as hydroxyflutamide. Therefore, we performed experiments to determine whether the identified compounds retained their distinct characteristics on the mutated AR. As observed with the wild-type AR, 6413-018 allowed the AF-2 pocket to form without
activity observed with 6413-018 and 6413-001 is not caused by lower levels of AR and that the T877A mutation did not alter the characteristics of the compounds identified in our screen.

To characterize further the pharmacology of the three classes of AR ligands, we looked at the transcriptional response of an endogenous androgen-responsive gene. LNCaP cells were maintained in phenol red-free medium for 24 h, and the total RNA was isolated to perform a real-time RT-PCR reaction to measure the expression level of the endogenous gene, PSA. We noted that the efficacy of 6413-001 increased with the passage of LNCaP cells, from ~20–30% of the activity of DHT in earlier passages to ~50% in higher passages. The difference in efficacy of activation of an endogenous gene versus a transfected reporter could possibly be caused by the increased sensitivity of the quantitative PCR assay that measures the levels of mRNA over the reporter assay that measures the activity of the translated protein.

We next examined the levels of secreted PSA protein as an additional end point of androgen action in LNCaP cells. As shown in Fig. 5B, the stimulation of secreted PSA was similar to the pattern observed with the reporters, in which DHT behaved as a potent agonist, 6413-018 exhibited partial agonist activity, and 6413-001 exhibited a very weak response. Several conclusions can be drawn from the results of these experiments. First, based on the AF-2 conformation and ability to facilitate N-C interaction, AR ligands can be grouped into three classes. Second, AR transcriptional activation does not require N-C interaction of AR, because both 6413-018 and 6413-001 are capable of activating AR-mediated transcriptional response. N-C interaction, however, increases the efficacy and potency of agonist activity as observed with DHT and not with the other two classes of ligands. Third, the observed partial agonist activity of 6413-001 shows that AR transcriptional activation does not require the formation of an AF-2 pocket. Finally, reporter assays or measurement of translated protein are not the best ways to characterize the phenotype of an activating ligand.

**AR-mediated Transcriptional Response and AR-mediated Proliferative Response Can Be Uncoupled Pharmacologically.** Prostate cancer cells are a good model system to study the actions of
androgens. AR-positive prostate cancer cells such as LNCaP show a marked increase in transcription of several androgen-responsive genes, including PSA, a marker for prostate cancer (30, 31). Additionally, androgens are mitogenic in LNCaP cells (32), and this observation has been used to develop therapeutics to inhibit AR-positive cancer cell growth (33, 34). It has also been observed that potent agonists that stimulate proliferation also increase PSA levels and treatment with antagonists arrests androgen-stimulated cell proliferation and reduces PSA level. Therefore, we anticipated that the two classes of AR ligands that exhibit partial agonist activity on PSA transcription would also be partial agonists for cell proliferation. To test this, we performed proliferation assays in LNCaP cells that were maintained in hormone-free conditions for 3 days, followed by treatment with DHT, 6413-018, or 6413-001 for a period of 6 days. As shown in Fig. 6A, DHT-stimulated cell proliferation reached a maximum at 1 nM and decreased to basal levels at 10 nM. The biphasic pattern for DHT-mediated proliferative response has been reported previously (35) and is thought to be because of the induction by androgens of gene products with antiproliferative activity (36). The efficacy of proliferative response observed in the presence of 6413-018 and 6413-001 was indistinguishable from DHT. This was a rather surprising result, because it indicates that AR-mediated transcription and AR-mediated proliferation can be uncoupled and that parameters used to characterize a partial agonist for AR-mediated transcription do not indicate whether that ligand is a partial or full agonist for proliferation. To verify that the proliferation induced by all three ligands is mediated by AR, we looked at the ability of the AR antagonist casodex to inhibit this response. As shown in Fig. 6B, the addition of 1 μM casodex inhibited the proliferative response induced by the three classes of ligands, confirming that this response is mediated via AR.

**DISCUSSION**

There is renewed interest of late in AR as a drug target fueled in part by extensive off-label usage of both androgens and antiandrogens (9, 37). In addition to prostate cancer, for instance, antiandrogens are likely to see expanded use in the treatment of acne, androgenic alopecia, and benign prostatic hyperplasia (38). AR agonists have even greater potential as treatments for osteoporosis (male and female), anemia, cachexia associated with cancer and AIDS, secondary treatment for metastatic breast cancer (skeletal involvement), and as male contraceptives (39, 40). However, the currently available AR agonists have several problems that make them unsuitable for chronic conditions. The natural androgen testosterone, for instance, has to be delivered by patch or as cream. The orally deliverable synthetic androgens, like fluoxymesterone, have been associated with hepatic toxicities. Evidently, there is a need for orally bioavailable androgens that are liver sparing and display improved anabolic activities.

With the discovery of selective estrogen receptor modulators, there has been a great deal of attention focused on developing SARMs (39, 40). This term describes a set of compounds that display varying degrees of activity in different target tissues rather than functioning as pure agonists or antagonists. A SARM that could function as an AR antagonist in the prostate but could exhibit androgenic activity in other tissues would have a significant advantage over existing therapies. Our primary objective in this study was to look for mechanistically distinct modulators for AR that can be used to treat prostate cancer and other androgenopathies.

It has been established that one of the key steps in AR agonist action is the facilitation of an interaction between the FXXLF motif in the amino terminus of the receptor and the canonical AF-2 pocket in the LBD (29, 41, 42). This interaction stabilizes ligand binding and facilitates the interaction of AR with required cofactors. In this study, we explored whether AR can be activated under circumstances that do not allow the AF-2 pocket to form, or allow AF-2 to form, without facilitating an N-C interaction. We know from previous studies that all known antagonists of PR interacted functionally with AR to some degree (43). In particular, RU486 is known to exhibit AR partial agonist activity, and preliminary studies have indicated that this antiprogestin does not allow the formation of an AF-2 pocket in AR. By inducing subtle changes in the structure of RU486, we have identified several compounds that allow AR AF-2 formation without facilitating the receptor N-C interaction. The ability of these compounds to activate AR-mediated transcription demonstrates that AR N-C interaction is not required for AR-mediated transactivation through canonical ARE-dependent mechanisms. However, the absence of N-C interaction alone decreased the efficacy and potency of activation as shown using 6413-018, and the absence of both AF-2 pocket formation and N-C interaction reduced further the transcriptional response of 6413-001. Surprisingly, we observed that N-C terminal interactions do not seem to be required for ligand-induced AR stabilization.

The observation that 6413-018 stimulates LNCaP proliferation to the same extent as DHT indicates that the lack of N-C interaction affects only AR-mediated transcription, but not the ability of this ligand to stimulate proliferation. Second, 6413-001, which does not allow the AF-2 pocket to form, is equally capable of stimulating cell proliferation. These observations suggest that AR-
mediated proliferation occurs through mechanisms different from transcription, specifically those that are directly mediated by AR-ARE interactions. AR has been shown to inhibit the transcriptional responses of a number of transcription factors such as nuclear factor κB, Ets-1, and cAMP-responsive element binding protein by protein-protein interactions (44–47). It remains to be established whether the compounds identified in this study behave as full or partial agonists in AR-mediated transrepression that results from protein-protein interactions in the absence of AREs. Evidence for the separation of AR-mediated transcription and proliferation also comes from a previous observation with the dose-response curves for DHT-mediated proliferation and PSA activation, in which at saturating concentration the hormone inhibits proliferation while still activating PSA secretion (35). It is possible that the three classes of compounds described here present a common surface on the receptor required to mediate proliferation. In a recent report, Peterziel et al. (16) describe a rapid and reversible mitogen-activated protein kinase activation event induced by androgens that seems to be insensitive to antagonists such as hydroxyflutamide and casodex. In another study, with AR-negative prostate cancer cells, hydroxyflutamide was shown to activate mitogen-activated protein kinase through a membrane-initiated, AR-independent non-genomic event resulting in cell proliferation (48). Our results, in contrast, clearly show the involvement of AR in 6413-018 and 6413-001-mediated LNCaP proliferation because they can be inhibited by casodex, a highly specific AR antagonist. We are now in the process of identifying common and unique surfaces presented on AR in the presence of each of the three classes of compounds using phage-displayed peptide libraries, as well as examining the effect of these compounds on kinase-signaling in LNCaP cells. In addition, gene expression analyses are currently underway to identify subsets of genes that are uniquely activated by DHT, 6413-018, or 6413-001 and genes that are activated by all three ligands. We anticipate that these studies will identify a set of genes that will be useful to further delineate the molecular mechanisms involved in AR-mediated prostate cancer cell proliferation and provide us with more useful drug targets.

ACKNOWLEDGMENTS

We thank Dr. P. Turnbull (GlaxoSmithKline, Research Triangle Park, NC), K. Gaido (CIIT, Research Triangle Park, NC), C. Y-F. Young (Mayo Clinic, Rochester, MN), D. Edwards (University of Colorado Health Sciences, Denver, CO), and Dr. N. Weigel (Baylor College of Medicine, Houston, TX) for providing the reagents used in this study.

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