Prohibitin and the SWI/SNF ATPase subunit BRG1 are required for effective androgen antagonist-mediated transcriptional repression of androgen receptor-regulated genes

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Androgen antagonists or androgen deprivation are the primary therapeutic modalities for the treatment of prostate cancer. Invariably, however, the disease becomes progressive and unresponsive to androgen ablation therapy (hormone refractory). The molecular mechanisms by which androgen antagonists inhibit prostate cancer proliferation are not fully defined. In this study, we identify two molecules which are required for effective prostate cancer cell responsiveness to androgen antagonists. We establish that androgen receptor (AR)-dependent transcriptional repression of AR-responsive genes. The SWI–SNF complex core ATPase BRG1, but not its closely-related counterpart ATPase BRM, is required for this repressive action of prohibitin on AR-responsive promoters. Androgen antagonists induce recruitment of prohibitin and BRG1 to endogenous AR-responsive promoters and induce a physical association between AR and prohibitin and BRG1. The recruitment of prohibitin to endogenous AR-responsive promoters is dependent upon antagonist-bound AR. Prohibitin binding to the prostate-specific antigen (PSA) promoter results in the recruitment of BRG1 and the dissociation of p300 from the PSA promoter. These findings suggest that prohibitin may function through BRG1-mediated local chromatin remodeling activity and the removal of p300-mediated acetylation to produce androgen antagonist-mediated transcriptional repression. Furthermore, in addition to its necessary role in AR-mediated transcriptional repression, we demonstrate that prohibitin is required for full and efficient androgen antagonist-mediated growth suppression of prostate cancer cells.

Introduction
Prostate cancer is the second leading cause of cancer death in men (1,2). Androgen ablation and blockade of androgen actions through the androgen receptor (AR) remain the mainstay of treatment for advanced prostate cancer (3,4). While initial responses to androgen deprivation are the norm, most tumors eventually recur in what is termed an androgen-independent (refractory) state (5–7).

The AR, a hormone-dependent transcription factor, plays a major role in promoting the development and progression of prostate cancer (8–11). The transcriptional activity of the AR is modulated by nuclear coregulatory proteins, designated coactivators and corepressors (12–14). Upon activation by ligands such as dihydrotestosterone (DHT), the AR translocates to the nucleus, whereupon it binds to androgen response elements on target genes and regulates their transcription. Binding of androgens induces recruitment of coactivators such as SRC-1, p300, CBP and pCAF, which contain intrinsic histone acetylase activity (15–18). In contrast, binding of AR antagonists induces the AR to form a complex with corepressors, such as nuclear corepressor (NcoR), SMRT and histone deacetylase (HDAC)-1, -2 and -3 (19–22). A complete understanding of the regulation of AR antagonist-mediated transcriptional repression is of critical importance for the design and development of novel endocrine-based therapies and identification of pharmaceutical targets for treating refractory prostate cancer.

Prohibitin (PHB1) is a highly-conserved protein with homologues in all animal species, yeasts and plants. Prohibitin is recognized as a potential tumor suppressor protein and inhibits cellular proliferation (23,24). Prohibitin interacts with transcriptional repressor proteins, such as NCoR, HDAC1 and retnoblastoma to repress activation of genes regulated by the E2F family of transcription factors (25). Prohibitin protein levels have been reported to be downregulated in the LNCaP prostate cancer cell line following growth stimulation with the androgen DHT (26), and overexpression of prohibitin represses androgen-dependent prostate-specific antigen (PSA) gene transcription and growth in prostate cancer cells (27). In some circumstances, prohibitin acts in concert with the SWI–SNF complex to repress transcriptional activity (28–30). The human SWI–SNF complex is a ubiquitous multimeric complex that regulates gene expression by remodeling nucleosomal structure in an ATP-dependent manner (31–33). The SWI–SNF complex contains one of two core ATPases, BRG1 or BRM. These complexes can interact with sequence-specific transcription factors to either promote or repress target gene activation, depending upon promoter context and the protein subunits comprising the complex (34,35). SWI/SNF activity has been shown to be essential for glucocorticoid receptor (36,37) and estrogen receptor (ER) transcriptional activation (38,39), and BRM, but not BRG1, appears to required for AR-dependent transcriptional activation (40). Our previous studies have demonstrated that prohibitin can co-coordinate with BRG1 and BRM to regulate transcription factor activity and that recruitment of prohibitin, and then both BRG1 and BRM, inhibits E2F activation and causes growth arrest in breast cancer cells treated with estrogen antagonists (28,29).

In the present study, we establish that efficient AR-dependent transcriptional repression by androgen antagonists requires prohibitin and BRG1, but not BRM (in contrast to AR-dependent transcriptional activation). We find that androgen antagonists induce recruitment of prohibitin and BRG1 to AR-responsive promoters and induce a physical interaction among AR and prohibitin and BRG1. The recruitment of BRG1 to, and the dissociation of p300 from, the AR complex at responsive promoters is dependent upon antagonist-bound AR. Prohibitin binding to the prostate-specific antigen (PSA) promoter results in the recruitment of BRG1 and the dissociation of p300 from the PSA promoter. These findings suggest that prohibitin may function through BRG1-mediated local chromatin remodeling activity and the removal of p300-mediated acetylation to produce androgen antagonist-mediated transcriptional repression. Furthermore, we find that prohibitin is required for full and efficient androgen antagonist-mediated growth suppression of prostate cancer cells.

Abbreviations:
AR, androgen receptor; ChIP, chromatin immunoprecipitation; DHT, dihydrotestosterone; ER, estrogen receptor; FBS, fetal bovine serum; HDAC, histone deacetylase; mRNA, messenger RNA; NCoR, nuclear corepressor; PCR, polymerase chain reaction; PSA, prostate-specific antigen; PSA-LUC, prostate-specific antigen promoter-driven luciferase reporter; siRNA, short-inhibitory RNA.

Materials and methods
Cells, plasmids and antibodies
LNCaP and DU145 cells were obtained from the American Type Culture Collection (Manassas, VA) and were maintained in RPMI 1640 medium with 10% fetal bovine serum (FBS) or in 10% charcoal-treated FBS (HyClone, Logan, CO). The prohibitin, BRG1 and BRM expression vectors (pCDNA3.1-prohibitin, BRG1, BRM) and RNAi vectors (pRNAT-U6.1/Neo-Prohibitin, BRG1, BRM) were generously provided by Dr S. Wang (Boston University School of Medicine). The reporter plasmid prostate-specific antigen promoter-driven...
luciferase reporter (PSA-LUC), containing the luciferase gene under the control of a fragment of the human PSA gene promoter, was provided by Dr. A.O. Brinkmann (Erasmus University Medical Center, The Netherlands).

Antibody to prohibitin was obtained from NeoMarkers, Fremont, CA (Cat# MS-261-p1). Antibodies to the androgen receptor (PG-21, Cat# 06-680), BRG1 (H-88) and p300 (N-15) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody to BRM (Cat# 610389) was from BD Biosciences, San Jose, CA. DHT (A-8380) was purchased from Sigma–Aldrich, St. Louis, MO. Bica-lutamide (Casodex) (Cat # S21083) was provided by AstraZeneca, London, UK.

Luciferase reporter assay

LNCaP cells were cultured in six-well plates in RPMI 1640 with 10% charcoal-treated FBS (HyClone Laboratories, Logan, UT) (androgen-deprivation conditions) for 3 days, then cotransfected with PSA-LUC reporter vectors (1 μg), together with prohibitin or BRG1 or BRM expression vectors, using Lipofectamine plus (Invitrogen, Carlsbad, CA). Fresh medium was added after an overnight transfection. Transfected cells were treated with DHT or DHT plus bicalutamide or vehicle control for 24 h before lysis and assay for luciferase activity (Promega Luciferase Assay System, #E1500). The relative luciferase activities were normalized to the activity of a cotransfected β-gal expression vector, as an internal control for transfection efficiency. Each experiment was performed in triplicate and repeated a minimum of three times. These values were used to determine standard deviations, with error bars indicating ±SEM.

Real-time reverse transcription–polymerase chain reaction

Total cellular RNA was isolated using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer’s instructions. A two-step reverse transcription–polymerase chain reaction (PCR) method was employed to synthesize single-stranded cDNA (SuperScript® III First Strand kit, Invitrogen, Carlsbad, CA). Real-time PCR reactions were performed in triplicate on the Applied Biosystems 7500 Fast Real-Time PCR System with SYBR Green I dye (Applied Biosystems, Foster City, CA, 4309155). The primers were used as follows: PSA forward, TGGCAGCGCACAGCACAG; PSA reverse, GTCTCCGTGAGGCAGCACAG; KLK2 forward, CTTGCGAGGTGGCTG; KLK2 reverse, CTGCGGAGACACAGCA; AR forward, GAGGAGACATGCCCAGG; AR reverse, GTGAGTCACTGGGAGACA; TGCCCAG; distal control primer forward, TTCACCGTGTTGGAAGAGACA; TGCCCAG; distal control primer reverse, ATGAGTCACTGGGAGACA; TGCCCAG. PCR conditions were used as follows: PSA or KLK2, 95°C for 10 s, 60°C for 15 s and 72°C for 15 s; AR, 94°C for 10 s, 60°C for 15 s and 72°C for 15 s. For each chIP experiment, triplicate ChIP assays were performed using antibodies directed against prohibitin or the AR. Exposure to the AR antagonist-mediated transcriptional repression by prohibitin, we first determined if prohibitin associates with known AR-binding regions in the promoter of the endogenous PSA gene in a ligand-dependent fashion. Two sets of PCR primer pairs were generated to amplify genomic fragments (~500 bp in size) encompassing the endogenous PSA promoter region or a control region distal to the PSA gene (7 kb upstream of the start site). LNCaP cells were cultured under androgen deprivation for 3 days, followed by treatment with DHT or bicalutamide, an AR antagonist. ChIP assays were performed using antibodies directed against prohibitin or the AR. Exposure to the androgen antagonist bicalutamide significantly increased the recruitment of prohibitin to the endogenous PSA promoter region by >6-fold compared with androgen-deprivation conditions (Figure 2A). In contrast, exposure to DHT produced no statistically significant change in prohibitin occupancy at the PSA promoter. AR recruitment to the PSA promoter was induced by exposure to either DHT or bicalutamide as expected (Figure 2B). Binding of prohibitin or AR to a ‘control’ distal DNA region, located 7 kb upstream of the PSA gene, was not observed (Figure 2A and data not shown). These results indicate that prohibitin and the AR bind to the same general promoter region of the PSA gene and that prohibitin recruitment to the promoter region is stimulated by exposure to androgen antagonists.

Parallel ChIP assays examining PolII recruitment to the promoter as a marker for active transcription demonstrated increases in PolII occupancy after exposure to DHT, but not after exposure to bicalutamide (Figure 2C). PolII occupancy at the endogenous PSA promoter was inversely correlated with occupancy by prohibitin and exposure to bicalutamide uncoupled AR occupancy at the promoter from PolII recruitment.

Prohibitin is required for efficient androgen antagonist-mediated AR transcriptional gene repression

To determine if prohibitin plays a role in androgen antagonist-mediated AR-dependent transcriptional repression, we assessed the effect of altering cellular levels of prohibitin, using ectopic overexpression of prohibitin or prohibitin knockdown by RNAi, on the regulation of an androgen-responsive promoter–reporter vector (human PSA-LUC) in a human prostate cancer cell line (LNCaP). Cotransfection of PSA-LUC with a prohibitin expression vector produced a modest and not statistically significant suppression of DHT-stimulated PSA-promoter-driven luciferase activity, but enhanced bicalutamide-mediated PSA-LUC transcriptional repression by 4-fold (Figure 1A). Conversely, prohibitin knockdown by expression of prohibitin RNAi reversed bicalutamide-mediated PSA-LUC transcriptional suppression (i.e., there was no statistically-significant repression of transcription by bicalutamide when prohibitin levels were knocked down), but resulted in no significant enhancement of DHT-stimulated expression of the PSA-LUC vector (Figure 1B). In parallel studies, using the structurally-distinct androgen antagonist cyproterone acetate, ectopic expression of prohibitin similarly increased cyproterone acetate-acetate-mediated transcriptional repression of PSA-LUC by 4-fold (data not shown). Conversely, prohibitin knock-down by expression of prohibitin RNAi resulted in a 2.5-fold abrogation of cyproterone acetate-acetate-mediated PSA-LUC transcriptional repression (data not shown). Collectively, these data suggest that prohibitin plays a significant role in androgen antagonist-mediated PSA transcriptional suppression, while having only a marginal role in regulating agonist-mediated transcription.

The effect of altering prohibitin protein levels on the transcriptional repression of endogenous AR-responsive genes by AR antagonists was next examined. In prohibitin partial-knockdown cell lines, bicalutamide treatment suppressed AR-dependent PSA and KLK2 gene transcriptional induction by only 55% and 20%, respectively, whereas suppressing PSA and KLK2 transcript levels by 80% and 90% in the control cell lines (Figure 1C). This result suggests that inhibition of prohibitin protein activity can partially release antagonist-mediated transcriptional suppression of endogenous AR-responsive genes. Measurement of the levels of prohibitin protein in those cells transfected with prohibitin RNAi confirmed decreased levels of prohibitin protein (Figure 1C). Collectively, these experiments demonstrate that prohibitin is required for efficient AR antagonist-mediated transcriptional suppression.

**Results**

**Prohibitin is required for efficient androgen antagonist-mediated AR transcriptional gene repression**

To determine if prohibitin plays a role in androgen antagonist-mediated AR-dependent transcriptional repression, we assessed the effect of
We next determined whether the recruitment of prohibitin to the PSA promoter by androgen antagonists required the AR. ChIP assays were performed using anti-prohibitin antibody in DU145 cells, an AR-negative prostate cancer cell line with lower levels of prohibitin expression than LNCaP cells, by cotransfection of AR and prohibitin, or transfection of prohibitin itself, in the presence of DHT or bicalutamide. Exposure to DHT did not induce prohibitin occupancy at the PSA promoter, whether or not the AR was expressed (Figure 2D). Bicalutamide treatment did not induce prohibitin occupancy at the PSA promoter in AR-negative DU145 cells. When the AR was introduced into the cells by transfection, however, exposure to bicalutamide efficiently stimulated recruitment of prohibitin to the PSA promoter. These results suggest that antagonist-bound AR is required for, and likely mediates, prohibitin antagonist-induced prohibitin recruitment to the PSA promoter.

The SWI/SNF core ATPase BRG1 is required for androgen antagonist-mediated AR transcriptional repression. BRM and BRG1, ATPase members of the chromatin remodeling SWI–SNF complex, have been shown to be involved in certain types of steroid-dependent gene transcription, and BRM is required for efficient induction of the PSA promoter by androgens (40). In contrast, no role for BRG1 in the regulation of AR-dependent transcription has been discovered to date. We used short-inhibitory RNA (siRNA) knockdown to determine whether BRG1 or BRM play a necessary role in androgen antagonist-mediated transcriptional repression. Knock down of BRG1 by a specific siRNA significantly reversed bicalutamide-induced transcriptional repression of a PSA promoter-driven reporter (Figure 3A). In contrast, cotransfection of a control (empty) siRNA vector, or of a BRM-specific siRNA, had no effect on the transcriptional repression induced by bicalutamide. We next examined whether BRG1 or BRM is recruited to the PSA promoter in response to exposure to an androgen antagonist. As shown in Figure 3B, amplification of DNA coprecipitated with chromatin complexes by anti-BRG1 antibodies demonstrated an androgen antagonist-dependent recruitment of BRG1 to the endogenous PSA promoter within the same time frame during which transcription is repressed (as determined by prohibitin association and PolII dissociation). In contrast, exposure to androgen antagonists did not induce recruitment of BRM. Rather, BRM recruitment was selectively induced only by exposure to androgen (DHT).
Fig. 2. Ligand-dependent recruitment of prohibitin, AR and RNA polymerase II to the PSA gene promoter region. LNCaP cells were cultured under androgen-deprivation conditions (charcoal-stripped serum) for 3 days and certain cultures were then treated for 4 h with DHT (10 nM) (DHT) or bicalutamide (10 μM) (CDX) or dimethyl sulfoxide as a vehicle control (S). ChIP assays were performed using primers which amplify the PSA promoter region (promoter) or a distal region upstream of known PSA regulatory elements (Distal region). Immunoprecipitations were carried out using antibodies directed against prohibitin, AR, RNA polymerase II (PolII), or an irrelevant protein (Rag1). The bound and input DNAs were analyzed by the ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA) by the ΔΔCt method. The results are presented as the relative level of the protein associated with the PSA promoter, normalized to irrelevant control antibody and input DNA. (A) Prohibitin occupancy at the PSA promoter. Inset photograph shows ethidium-stained, PCR-amplified fragments after separation on an agarose gel and immunoblot analysis of prohibitin protein levels. (B) AR occupancy at the PSA promoter. (C) PolII occupancy at the PSA promoter. (D) Bicalutamide-induced recruitment of prohibitin to the endogenous PSA promoter requires the AR. DU145 cells were cultured in Dulbecco’s modified Eagle’s medium + 10% charcoal-treated FBS. The cells were transfected with prohibitin- and AR expression vectors or prohibitin alone and treated with bicalutamide (CDX) or DHT. ChIP assays were performed using antibodies directed against prohibitin. The immunoblot show the AR and prohibitin expression level in DU145 cells. Asterisks indicate significant differences between two groups (* P < 0.05).
Fig. 3. Role of BRG1 in repression of AR-dependent transcription by androgen antagonists. (A) BRG1 is required for bicalutamide-mediated transcriptional repression. LNCaP cells were cotransfected with PSA-LUC reporter vector and a vector expressing BRG1 siRNA (siBRG1), BRM siRNA (siBRM) or the empty siRNA expression vector (siVector). At 24 h after transfection, the cells were treated with vehicle alone, DHT (10 nM) or DHT plus bicalutamide (CDX) for 24 h, and cell lysates were harvested for luciferase assay. Inset photograph shows the immunoblot analysis of BRG1 and BRM protein levels. (B) LNCaP cells were cultured in charcoal-stripped serum for 3 days and certain cultures were treated for 4 h with DHT (10 nM) (DHT) or bicalutamide (10 μM) (CDX) or dimethyl sulfoxide as a vehicle control (S). ChIP assays were performed using antibodies directed against BRG1 or BRM. The results are presented as the relative level of the protein associated with the PSA promoter, normalized to irrelevant control antibody and input DNA. (C) Prohibitin is required for BRG1 recruitment to the endogenous PSA promoter. LNCaP cells were cotransfected with a vector expressing prohibitin siRNA (siProhibitin) or the empty siRNA expression vector (siVector) and pcDNA3.1-zeosin. The cells were selected with zeosin (50 μg/ml) for 1 week, then cultured in media containing charcoal-stripped serum for 3 days and treated with DHT (1 nM) (DHT) or bicalutamide (CDX) for 4 h. ChIP assays were performed using antibodies directed against BRG1. The results are presented as the relative level of BRG1 protein associated with the PSA promoter, normalized to irrelevant control antibody and input DNA. Asterisks indicate significant differences between two groups (*P < 0.05).
We have demonstrated previously that prohibitin can serve as a transcriptional repressor at certain promoters through direct recruitment of SWI–SNF complexes (29,30), raising the possibility that the occupancy of BRG1 and prohibitin at AR-responsive promoters after exposure to androgen antagonists might be linked. We therefore determined if the recruitment of BRG1 to AR-responsive promoters is prohibitin dependent. As shown in Figure 3C, prohibitin knockdown reduced the recruitment of BRG1 to the PSA promoter by bicalutamide. These data suggest that prohibitin may act through BRG1-mediated chromatin remodeling to regulate bicalutamide-mediated gene transcriptional suppression.

Prohibitin enhances antagonist-mediated dissociation of p300 at an AR-responsive promoter

AR corepressor and coactivator complexes play critical roles in regulating AR activity with precision and efficiency. Binding of androgens induces recruitment of coactivators such as p300 and CBP, which contain intrinsic histone acetylase activity. In contrast, binding of AR antagonists induces the AR to form a complex with corepressors, such as HDAC-1, -2 and -3. To further understand the possible mechanism of prohibitin-mediated transcriptional repression, we determined whether prohibitin has a role in coactivator dissociation or on the balance between coactivators and corepressors in the AR complex. Analysis of p300 occupancy at the endogenous PSA promoter by ChIP assay demonstrated that p300 occupies the PSA promoter in the presence of DHT, but dissociates from the promoter in the presence of bicalutamide (Figure 4A). When prohibitin levels were knocked down by siRNA, this antagonist-mediated dissociation of p300 occupancy was partially reversed (Figure 4B). Thus, prohibitin occupancy at the PSA promoter correlates inversely with p300 occupancy. These results suggest that prohibitin recruitment may result in p300 dissociation from the PSA promoter, and this release of an essential coactivator may be one mechanism contributing to prohibitin-mediated transcriptional repression.

In parallel studies of AR complexes in whole-cell lysates, prohibitin association with the AR complex in vitro was also inversely associated with p300 binding in the presence of bicalutamide (Figure 4). Furthermore, these studies demonstrated an androgen antagonist-inducible association of the AR and BRG1, but not BRM, with prohibitin (Figure 4C). In contrast, in the presence of DHT, AR associated with BRM and p300, but not BRG1 or prohibitin. This finding is consistent with a previous report that the AR associates and colocalizes with BRM in the presence of DHT (41).

Collectively, these results demonstrate ligand (androgen antagonist)-dependent recruitment of prohibitin and BRG1 to AR-responsive promoters and are consistent with a mechanism whereby androgen antagonist-bound AR recruits prohibitin to AR-responsive promoters and prohibitin then facilitates recruitment of BRG1 and dissociation of p300 from the AR complex at the promoter, thus facilitating corepression and suppressing AR-dependent transcription.

Prohibitin is required for efficient androgen antagonist-mediated growth suppression of prostate cancer cell lines

Androgen antagonists, such as bicalutamide and cyproterone acetate, suppress both AR-dependent gene transcription and prostate cancer cell proliferation. While these activities are probably linked, a direct causal relationship has not been established. Therefore, the necessary role of prohibitin in each of these functional outcomes was studied independently. A colony formation assay was carried out to assess whether prohibitin plays a role in bicalutamide-mediated prostate cancer cell growth suppression, using the prohibitin partial knockdown LNCaP cells and control (empty vector-transfected) LNCaP cells. Knockdown of prohibitin significantly ameliorated the sensitivity of LNCaP cells to androgen antagonists, with exposure to bicalutamide resulting in the formation of 3-fold and 6-fold more colonies in the prohibitin partial-knockdown cells than in the control (empty vector) transfected cells (P < 0.05), at two concentrations of bicalutamide (Figure 5). Interestingly, exposure to DHT resulted in a modest but consistent increase in colony formation in the prohibitin partial-knockdown cells compared with the control (empty vector)-transfected cells, suggesting that endogenous levels of prohibitin may exert some tonic antiproliferative effect on the cells even in the

Fig. 4. AR corepressor and coactivator complexes as a function of antagonist or agonist binding. (A) p300 occupies the PSA promoter in the presence of DHT. LNCaP cells were cultured in charcoal-stripped serum for 3 days and certain cultures were treated for 4 h with DHT (10 nM) (DHT) or bicalutamide (10 μM) (CDX) or dimethyl sulfoxide as a vehicle control (S). ChIP assays were performed using antibodies directed against p300. (B) Prohibitin facilitates p300 dissociation from the PSA promoter. LNCaP cells were cotransfected with a vector expressing prohibitin siRNA (siProhibitin) or the empty siRNA expression vector (siVector) and pDNA3.1-zeosin. The cells were selected with zeosin (50 μg/ml) for 1 week, then cultured in media containing charcoal-stripped serum for 3 days and treated with DHT (1 nM) (DHT) or bicalutamide (CDX) for 4 h. ChIP assays were performed using antibodies directed against p300. (C) Ligand-dependent association of antagonist-bound AR with prohibitin and BRG1 and association of DHT-bound AR with BRM and p300. Cell lysates were made from LNCaP cells cultured in DHT and treated or not treated with bicalutamide (CDX) for 4 h. Protein complexes were immunoprecipitated with anti-AR antibody and immunoblotted with anti-prohibitin, anti-BRG1, anti-BRM, anti-p300 or anti-AR antibodies. ‘Input’ represents the relative total amounts of protein in the lysates.
abscence of antagonists, as has been observed by other investigators in different cell types.

Together with the studies presented above, these findings demonstrate that prohibitin is required both for androgen antagonist-mediated AR-dependent gene transcriptional repression and for efficient antagonist-mediated prostate cancer cell growth suppression.

Discussion

A number of studies have elucidated some of the transcription factors and cofactors involved in, or modifying, transcriptional activation by the AR. These have included the SWI/SNF ATPase core subunit BRM, but not BRG1. The factors involved in transcriptional repression have not been as well characterized, despite their clinical relevance to endocrine modalities of prostate cancer treatment. In this study, we demonstrate that prohibitin is required for AR-responsive gene transcriptional repression by androgen antagonists. This requirement for prohibitin was demonstrated using multiple, structurally-distinct androgen antagonists, stable and transient knockdown of prohibitin, and transfected and endogenous AR-responsive genes. In addition, prohibitin is required for efficient AR antagonist-mediated prostate cancer cell growth suppression. Prohibitin is recruited to androgen response elements in endogenous gene promoters by androgen antagonist-bound AR. Finally, we provide evidence to suggest that the mechanism of prohibitin-mediated transcriptional inhibition on AR-responsive genes may be due to chromatin remodeling by BRG1, but not BRM, and the dissociation of acetylase p300 from the promoter.

AR corepressor complexes play a critical role in regulating AR activity with precision and efficiency (12). To date, several corepressors of AR have been characterized, including NCoR, SMRT, HDACs 1–3 and SIRT1 (19–21,42,43). Transcriptional repression mediated by androgen antagonists is through repressor complex formation at the gene promoter. Prohibitin has been shown to be involved in transcriptional suppression through formation of repressor complexes incorporating several distinct transcriptional corepressors. We and others have shown that prohibitin interacts with proteins such as NCoR, HDAC1 and retinoblastoma to repress activation of genes regulated by the E2F family of transcription factors (25,44–46). More recently, we have demonstrated that the recruitment of prohibitin and both BRG1 and BRM inhibits E2F activation and causes growth arrest in breast cancer cells treated with the antiestrogen tamoxifen (29). In this study, we observed significant recruitment of prohibitin to an AR-responsive promoter during exposure to androgen antagonists and found that prohibitin is required for androgen antagonist-mediated transcriptional repression and growth suppression. In the absence of androgen antagonists, however, prohibitin may also play a minor role in the regulation of AR-dependent transcription. Gamble et al. (27) have shown that overexpression of prohibitin can suppress androgen-induced transcription. Constitutive expression of AR and prohibitin in the naturally androgen-unresponsive cells lines, Cos-7, MCF-7 and PC3, significantly suppressed androgen-induced transcription of cotransfected reporter genes. However, in the androgen-responsive cell line LNCaP, knock down of endogenous prohibitin resulted only in a marginal increase in endogenous androgen-dependent gene transcription, consistent with our findings.

We found that the BRG1 associates with prohibitin and AR in an androgen antagonist-dependent fashion in cell lysates, and that BRG1 recruitment to the PSA promoter is dependent on the prohibitin, raising the possibility that the prohibitin suppresses AR-dependent transcription through BRG1-mediated chromatin remodeling in response to androgen antagonists. SWI–SNF complex-mediated remodeling of chromatin is a well-documented mechanism of transcriptional regulation and appears to play a role in transcriptional activation of AR-responsive and ER-responsive genes. Using the AR-deficient and BRM/BRG1-deficient SW13 cell line, Marshall et al. (40) have shown that BRM, but not BRG1, is required for DHT-mediated transcriptional activation of a transfected PSA-LUC reporter. In contrast, we and others have shown that BRG1 (38) and BRM (28,40) are both required for ligand-dependent activation of ER-responsive genes. Interestingly, the SWI–SNF complex can also participate in transcriptional repression. We have recently demonstrated that BRG1 and BRM are each required for both activation and suppression of ER-dependent gene transcription in response to agonists and to antagonists (30). Estrogen and its antagonists both induce the recruitment of BRG1/BRM to ER-responsive promoters, but recruitment of BRG1/BRM induced by antagonists is dependent upon prohibitin, which is not the case for BRG1/BRM recruitment in response to estrogen (an agonist) (30).
Consistent with a previous report which used androgen non-responsive cells rendered responsive by ectopic expression of an AR and BRM (40), we found, using mRNA knockdown in androgen-responsive cells, that depletion of BRM, but not BRG1, strongly reduces DHT-induced transcriptional activation of androgen-responsive reporter genes and of endogenous cellular genes (Y.Dai and D.V.Faller, unpublished data). DHT-bound AR can physically associate with BRM and p300 in the presence of DHT. More interestingly, our data presented herein conversely demonstrate that BRG1, but not BRM, is required for androgen antagonist-mediated transcriptional suppression of AR-responsive genes. BRG1 is recruited to the endogenous PSA gene promoter by androgen antagonists, whereas BRM is recruited to the PSA gene promoter by agonists, such as DHT. These results suggest that BRG1 and BRM function differentially in agonist-dependent transcriptional activation, versus antagonist-mediated transcriptional suppression, of AR-responsive genes, in distinct contrast to their joint requirements for ER-dependent gene regulation.

As discussed above, prohibitin requires association with one or more corepressor proteins, such as HDAC1, to suppress local gene transcription and appears to serve as multivalent recruiter of corepressors rather than possessing any intrinsic corepressor activity. In spite of a demonstrable physical interaction of prohibitin with HDAC1 in a breast cancer cell line, Gamble et al. (27) utilized a general HDAC inhibitor to suggest that HDAC activity is not required for AR-responsive gene repression initiated by overexpression of prohibitin. In contrast, we show here that BRG1, a known binding partner of prohibitin, is recruited to the androgen response element complex in an antagonist-dependent fashion and is required for transcriptional repression. Thus, BRG1 may play the major corepressor role for the transcriptional repression of AR-responsive genes by prohibitin. It is also noteworthy that exposure to androgen antagonists reduces p300 occupancy at AR-responsive promoters and that the absence of prohibitin reduces this effect of antagonists, suggesting that, in addition to facilitating recruitment of BRG1, prohibitin may also act through the removal of p300 acetylase activity to suppress androgen antagonist-mediated transcriptional suppression. We show that suppression of prohibitin or BRG1 protein levels reverses, but does not completely abrogate, the repressive effects of androgen antagonists on AR-dependent transcription. One potential explanation for this is that, although very effective, siRNA techniques do not completely deplete all prohibitin or BRG1 protein from the cell, and the small amount of protein remaining may allow for some repression by antagonists. Alternatively, there may be recruitment of other corepressors induced by exposure to androgen antagonists. Indeed, we have recently reported that the class III HDAC SIRT1 is recruited to the promoter of AR-responsive genes after exposure to androgen antagonists and contributes independently to transcriptional repression (42).

Prohibitin and BRG1 are not DNA-binding proteins, but can be recruited to promoter regions by other transcription factors. We have shown a ligand (androgen antagonist)-dependent physical association of AR and prohibitin and prohibitin and BRG1, and that BRG1 recruitment to endogenous AR-responsive promoters is reduced when prohibitin is knocked down, suggesting that recruitment of prohibitin to an AR-dependent promoter could lead to prohibitin-dependent recruitment of BRG1. Our analysis of AR-responsive promoters in DU145 cells, expressing or not expressing an AR protein, demonstrates that recruitment of prohibitin to the PSA promoter is androgen antagonist-bound AR-dependent.

Collectively, our data demonstrate a critical role for prohibitin in the transcriptional suppression of AR-mediated genes, and in the growth suppression of prostate cancer cell lines, mediated by androgen antagonists. Repression of AR-mediated transcriptional activity is therefore likely to be one mechanism by which prohibitin inhibits androgen-dependent growth of prostate cells. It is interesting to note, however, that we observed significant induction of cellular prohibitin levels following longer exposures (72 h) to bicalutamide (Y.Dai and D.V.Faller, unpublished data). As prohibitin is well-established to interact with the E2F family of transcription factors and to suppress cell proliferation through repression of E2F-mediated transcription of genes required for entry into S phase, we speculate that the upregulation of prohibitin induced by androgen antagonists over time may also suppress cell proliferation by downregulation of E2F activity, thus reinforcing its more immediate repressive effects on AR-dependent gene induction.

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References


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