

The Cell Fate Determination Factor Dachshund Inhibits Androgen Receptor Signaling and Prostate Cancer Cellular Growth

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Abstract

Initially isolated as the dominant suppressor of the mutant epidermal growth factor receptor (*ellipse*), the *Dachshund* gene plays a key role in metazoan development regulating the Retinal Determination Gene Network. Herein, the *DACH1* gene was expressed in normal prostate epithelial cells with reduced expression in human prostate cancer. *DACH1* inhibited prostate cancer cellular DNA synthesis, growth in colony forming assays, and blocked contact-independent growth in soft agar assays. *DACH1* inhibited androgen receptor (AR) activity, requiring a conserved DS Domain (*Dachshund* domain conserved with *Ski/Sno*) that bound NCoR/HDAC and was recruited to an androgen-responsive gene promoter. *DACH1* inhibited ligand-dependent activity of AR mutations identified in patients with androgen-insensitive prostate cancer. The DS domain was sufficient for repression of the AR wild-type but failed to repress an AR acetylation site point mutant. These studies show a role for the Retinal Determination Gene Network in regulating cellular growth and signaling in prostate cancer. [Cancer Res 2009;69(8):3347–55]

Introduction

Prostate cancer is the most frequently diagnosed cancer of men in the United States and the second leading cause of male cancer deaths (1, 2). Hyperactivation of the androgen receptor (AR) is a fundamental driver of prostate cancer progression (3), and AR antagonists are the frontline of therapeutic intervention (4). Increased expression and/or activity of the AR is a common feature during prostate cancer onset and progression. Androgen ablation therapy results in a 60% to 80% initial response rate. Understanding the molecular mechanisms governing the growth property of the AR is essential to devise targeted therapies for prostate cancer, including androgen-insensitive prostate cancer.

The AR is a ligand-activated transcription factor that belongs to the superfamily of steroid receptors (5). These receptors have similar protein structures and are composed of an NH₂-terminal domain that contains an activation function (AF-1), a DNA-binding domain, a hinge region, and a ligand binding domain that contains a second activation function domain (AF-2). Upon binding of ligands, such as dihydrotestosterone (DHT), the AR translocates to the nucleus and undergoes a conformational change that results

in formation of a homodimer and the recruitment of multiple transcription factors that activate the expression of androgen-dependent genes (5). Activity of the AR is mediated via a balance between the function of coactivators [CBP/p300, Tip-60, and AR activator proteins (ARA); refs. 6, 7] and corepressor proteins [including NCoR, Smads, and histone deacetylases (HDAC, SIRT1; refs. 8–10)]. In contrast with the wild-type (wt) AR, mutant AR that arise in patients with prostate cancer are promiscuously activated by a variety of hormones (estrogen, progesterone) that would normally have no effect on the wt AR (11). These AR ligand binding mutations cause an androgen antagonist such as flutamide to behave as androgen agonist (12).

Upon ligand binding to the AR, posttranslational modifications are induced including phosphorylation, acetylation, and ubiquitination, which in turn determine transcriptional and growth regulatory functions (13). The AR, like several other nuclear receptors, is directly acetylated at a motif that is widely conserved among various species (14–17). AR acetylation, which is induced by agonists including DHT and bombesin (18), governs prostate cancer cellular growth (15). Thus, gain of function mutants of the AR-acetylation site promote growth of human prostate cancer cells in tissue culture and when implanted in nude mice (15). The enhanced cellular growth is mediated by reduced MAPK kinase kinase 1-induced apoptosis (16) and enhanced cellular proliferation through induction of cell-cycle regulatory genes (16).

Conversely, ligand-dependent AR activity is repressed by HDACs of both the TSA-dependent type (class I and II) and by the class III HDAC. Corepressors of the AR, including HDAC1, HDAC3, and NCoR can bind the AR in the context of local chromatin and disengage in the presence of ligand (19–21). The class III HDACs are homologous to the transcriptional repressor Sir2P. It is known that class I and class II HDACs are sensitive to the inhibitor Trichostatin A (TSA). Class III HDAC activity is nicotinamide-adenine dinucleotide-dependent and cannot be inhibited by TSA. SIRT1 represses ligand-dependent AR activity and SIRT1 deacetylates the AR with similar kinetics and K_m to that described for p53 (10). Inhibition of class III HDACs activity in prostate cancer cells enhances liganded AR activity and AR expression (10), suggesting an important biological role for endogenous class III HDACs in AR signaling (22). SIRT1 inhibits prostate cancer cellular growth in the presence of the AR (10).

Although AR corepressors inhibit AR function, studies to date have not revealed significant alteration in the abundance of AR corepressor proteins during prostate cancer onset and progression. Recent studies have identified a protein resembling the NCoR/Ski family that physically associates with HDAC1 and HDAC3, known as Dachshund1 (23). *DACH1* repression function has been linked to its association with HDAC1, HDAC3, Sin3, and NcoR, which colocalized with *DACH1* at the promoters of target

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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genes repressed by DACH1 (23, 24). The *Dachshund1* (*DACH1*) gene was initially identified in *Drosophila* as a dominant suppressor of *Ellipse*, a constitutively active epidermal growth factor receptor (EGFR; ref. 25). The *Dac* gene is known to play a key role in metazoan development regulating ocular, limb, and gonadal development. In the *Drosophila* eye, *Dac* encodes a key component of the Retinal Determination Gene Network. Loss of function mutations of the gene result in defective eye development, whereas ectopic *Dac* expression is sufficient for the induction of eye development implicating *Dac* in organizing cellular fate (25).

The *DACH1* gene encodes a protein with winged helix-Forkhead subgroup structure of the helix-turn-helix family (26). Although no specific DNA binding characteristics have been identified, *DACH1* has been identified in the context of local chromatin by chromatin immunoprecipitation (ChIP) assays within distinct DNA binding sites for the transcription factors Smad, c-Jun, cAMP-responsive element binding protein, and *Six1* (23, 24, 27). Thus, the *DACH1* gene product uses other DNA-binding transcription factors to regulate gene expression. A conserved domain (Dachshund domain 1) has significant identity with the Ski/Sno (Dachshund DS domain), which is conserved from *Drosophila* to human. *DACH1* gene expression is lost in metastatic human breast cancer (23). The loss of *DACH1* expression correlates with poor prognosis. *DACH1* inhibited cellular migration and invasion of oncogene (Ras, Myc, ErbB2)-transformed human mammary epithelial cells. Interleukin-8 is a critical target of *DACH1*-mediated breast cancer cellular migration *in vitro* and metastasis *in vivo* (28). In ovarian cancer, *DACH1* is overexpressed and may promote tumor progression by antagonizing transforming growth factor β signaling (29, 30).

The role of *DACH1* gene expression in prostate cancer was unknown. In the current studies, *DACH1* abundance was reduced in human prostate cancer. Re-expression of *DACH1* inhibited prostate cancer cellular growth and colony formation in soft agar assays. *DACH1* inhibited DHT-dependent AR activity requiring the conserved DS domain. *DACH1* inhibited the activity of mutant AR derived from patient samples. The *DACH1* gene is a novel inhibitor of prostate cancer cellular growth, inhibiting ligand-dependent AR activity.

Materials and Methods

Plasmid construction, reporter genes, expression vectors, reagents, and kits. The expression plasmids encoding an NH₂-terminal FLAG peptide linked to *DACH1*, *DACH1* DS-domain alone (DS) or *DACH1* DS-domain deleted (Δ DS), the tet-inducible *DACH1* expression vector, and *DACH1*-VP16 fusion protein were previously described (27, 30). The Ski cDNA was subcloned into the 3 \times FLAG-CMV7.1 vector (Sigma). Expression vectors for human TIP-60, SRC-1, ARA70, and SIRT1 were previously described (10, 15, 16). The androgen-responsive synthetic reporter constructs [MMTV-LUC, PSA-LUC, Sc-ARE (ARE)₄LUC, pG₅LUC] and the expression vectors (pCMVHA-p300, pCDNA3AR-GFP, hAR_{wt}, the AR carboxyl terminal ligand binding domain mutants, and the AR point mutants AR_{K630T}, AR_{K630G}) were previously described (10, 11, 15, 17, 31, 32). DHT, R1181, TSA, and Nicotinamide were purchased from Sigma. Free PSA in the culture medium was measured by Free-PSA ELISA kit from BioQuant.

Cell culture, DNA transfection, and luciferase assays. Cell culture, DNA transfection, and luciferase assays were performed as previously described (15–17). The CV1, DU145, LNCaP, and HEK293T cell lines were cultured as previously described (10). Transfection and data analyses were as described in Supplementary Data.

DNA synthesis analysis and colony formation assay. DNA synthesis was analyzed by ³H-dTh incorporation. Cells (1×10^5) were plated into 24-well plate and cultured for 36 h. ³H-DTH (1 μ Ci) was added to each well and culture continued for 2 h. Cells were washed and fixed with incorporation of ³H-DTH measured by liquid scintillation. For contact-dependent growth, 4×10^3 LNCaP cells were plated in triplicate in the presence or absence of 2 μ g/mL doxycycline into 6-cm dish and medium was changed every 3 d. The colonies were visualized after 2-wk of growing by staining with 0.04% crystal violet in methanol for 1 h. For contact-independent growth assays, 4×10^3 LNCaP cells were plated in triplicate in 2 mL of 0.3% agarose in complete growth medium in the presence or absence of 2 μ g/mL doxycycline overlaid on a 0.5% agarose base, also in complete growth medium. Two weeks after incubation, colonies >500 μ m in diameter were counted using an Omnicon 3600 image analysis system.

Western blot analysis, tissue microarrays, and immunohistochemistry. Western blot analysis using antibodies to FLAG, AR (Santa Cruz Biotechnology), and the loading control β -tubulin was conducted as described (10). Immunohistochemistry for *DACH1* was performed as previously described (23, 30). A human prostate tissue array consisting of 32 paired prostate cancer tissues with corresponding normal tissues was from **AccuMax Array (MD, USA, ISU Abxis)**. The immunostaining intensity was scored as negative, 0; minimally positive, 1; positive, 2; strong positive, 3; respectively. The *DACH1*-positive cells were counted and recorded by a pathologist.

RNA extraction from formalin-fixed paraffin-embedded human prostate tissue specimens, real-time quantitative PCR. RNA was extracted from formalin-fixed paraffin-embedded archival human prostate tissue blocks containing 10 normal human prostates and 10 matched prostate tumor tissue specimen, using RecoverAll Total Nucleic Acid Isolation kit for formalin-fixed paraffin-embedded Tissues (Applied Biosystems). Subsequent analysis was as described in Supplementary Data (28).

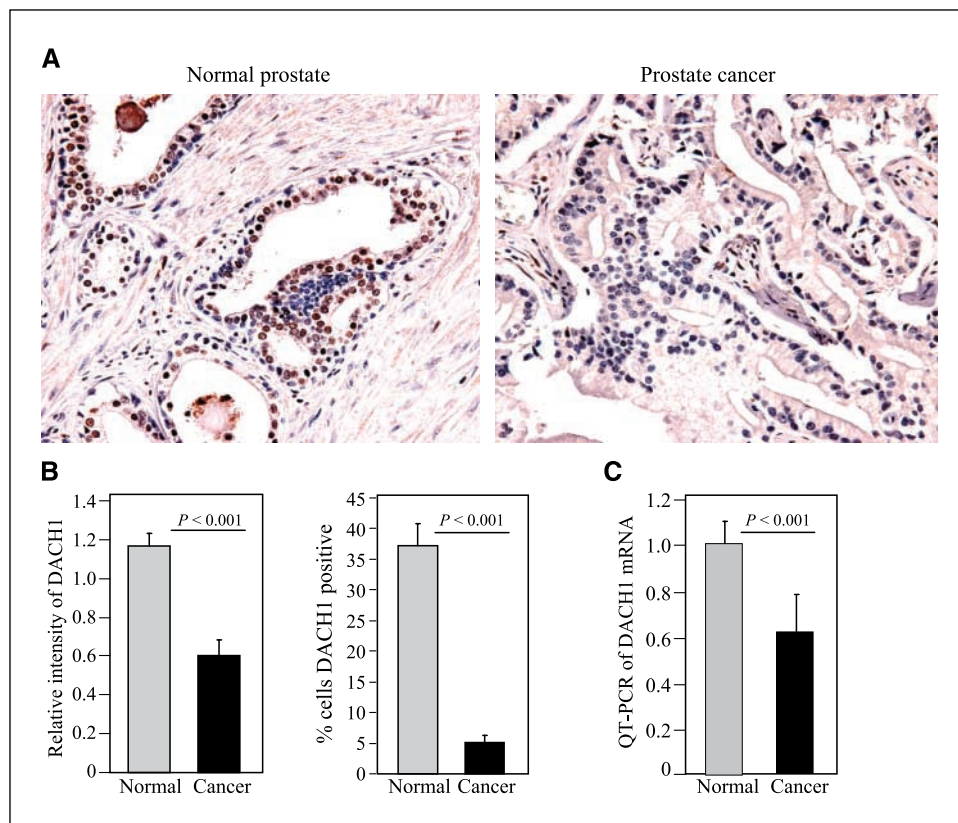
ChIP assays. Endogenous PSA promoter ChIP assays using antibodies directed to the FLAG epitope of *DACH1*, AR, Sirt1, NCoR, HDAC3, and control IgG were performed as previously described (refs. 9, 23, 28, 33; see Supplementary information).

Results

***DACH1* expression is reduced in human prostate cancer.** To determine the relative abundance of *DACH1* in normal and prostate cancer samples, immunohistochemical staining was performed on paired normal and prostate cancer tissues, using a previously well-characterized antibody (23). *DACH1* staining was identified in the nucleus and cytoplasm of normal prostate epithelial cells (Fig. 1A). Approximately 35% of epithelial cells stained positively for *DACH1* in the normal prostate. The relative intensity of the immunohistochemical staining was quantitated for each sample. Relative mean intensity was compared between those individual samples staining positively for *DACH1* in the normal and tumorous tissues. The relative intensity of immunostaining was significantly reduced (\sim 2-fold) in the prostate tumors, compared with the corresponding normal prostate epithelium (Fig. 1B). The percentage of cells staining for *DACH1* was also significantly reduced (\sim 7-fold; Fig. 1B). The relative abundance of *DACH1* mRNA was assessed in matched prostate tissue comparing normal and prostate cancer samples from each patient (Fig. 1C). The relative abundance was normalized for epithelial cells using KLK3 (PSA) mRNA. *DACH1* mRNA was reduced in prostate cancer samples compared with the normal prostate tissue of the individual. Thus, the relative abundance of *DACH1* is reduced in prostate cancer epithelium compared with normal prostate epithelium.

***DACH1* inhibits human prostate cancer cellular growth.** To determine the role of *DACH1* in prostate cancer cellular growth,

Figure 1. Immunohistochemical staining and mRNA expression of DACH1 in human prostate cancer. *A*, representative example of DACH immunostaining of normal prostate and prostate cancer ($\times 400$). *B*, quantification of DACH1 relative immune intensity and percent of cells staining for DACH1. *C*, DACH1 mRNA determined by quantitative PCR. Comparison was made between matched normal and tumorous prostate samples.



the LNCaP cell line was used to create tetracycline inducible *DACH1* gene expression. The addition of doxycycline induced DACH1 expression as characterized by Western blot analysis to the FLAG epitope of the DACH1 protein (Supplementary Fig. S1A). β -Tubulin, used as a loading control for protein abundance, showed similar protein levels. Doxycycline did not affect ^3H thymidine uptake in LNCaP cells. DACH1 expression inhibited LNCaP cell proliferation by >60% (Fig. 2A). Contact-dependent growth of LNCaP cells was characterized by colony formation assay. LNCaP cells formed colonies in tissue culture (Fig. 2B). The

number and size of colonies were reduced by >85% upon expression of DACH1. The induction of DACH1 expression upon doxycycline treatment reduced colony formation in soft agar by >85% (Fig. 2C). Dihydrotestosterone (10^{-8} M) induced PSA secretion ~ 9.5 -fold (Fig. 2D). Expression of DACH1 reduced DHT-dependent induction of prostate-specific antigen (PSA) by $\sim 60\%$ (Fig. 2D). Collectively, these studies show that DACH1 inhibits LNCaP cell contact-dependent growth and DNA synthesis.

DACH1 repression of ligand-dependent AR activity involves the DS domain. As DACH1 expression had inhibited PSA secretion

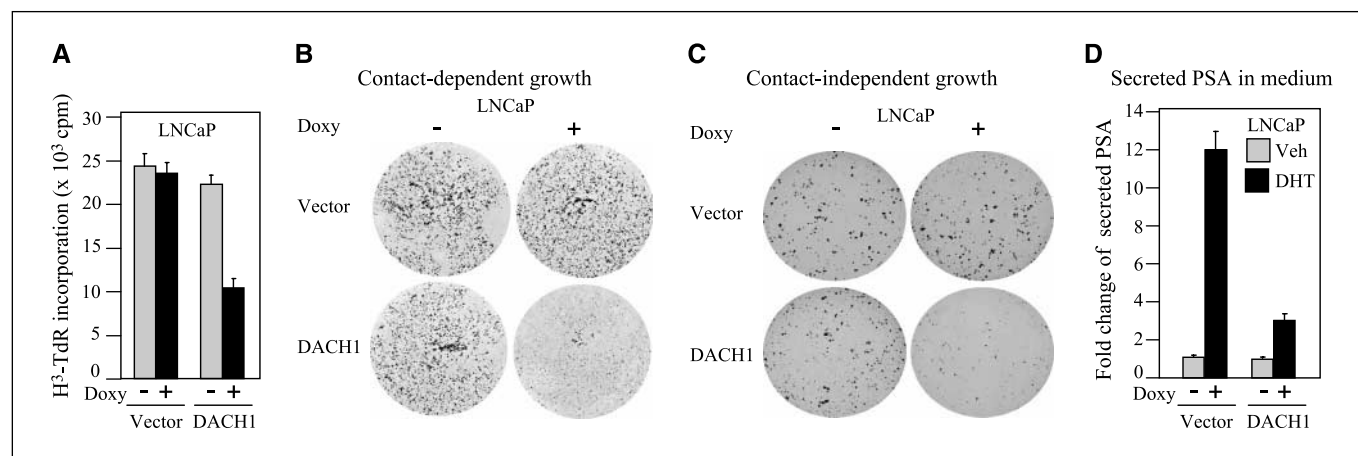
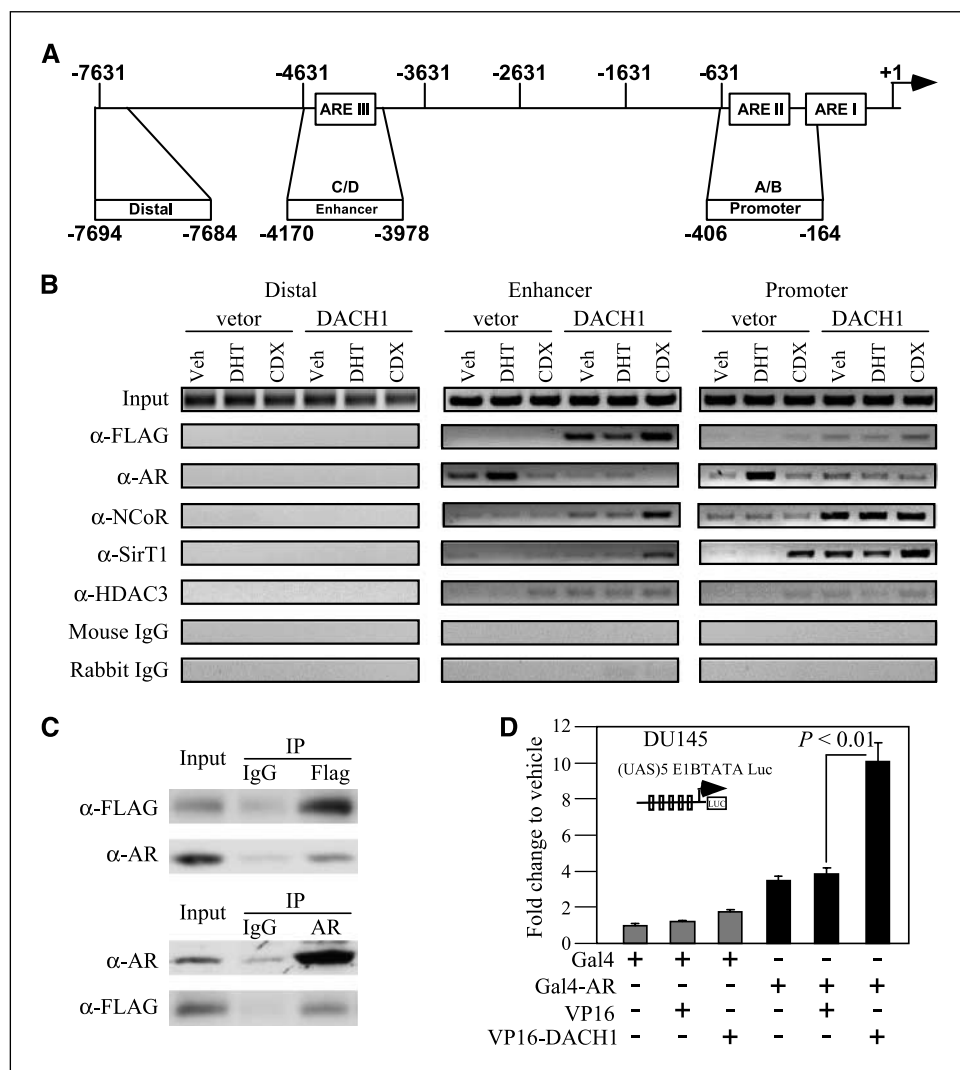


Figure 2. DACH1 inhibits prostate cancer cellular proliferation and growth. *A*, DNA synthesis analysis of LNCaP DACH1-doxycycline-inducible cell line. Columns, mean for five separate experiments; bars, SE. The doxycycline (*Doxy*)-inducible DACH1 LNCaP cell line was assessed for (*B*) colony formation, (*C*) contact-independent growth in soft agar, and (*D*) PSA secretion in the media determined by ELISA. Columns, mean throughout ($n \geq 5$); bars, SE. Veh, vehicle.

Figure 4. DACH1 associates with the AR and is corecruited to an endogenous ARE. *A*, schematic representation of the human PSA promoter indicating the ARE (I-III). *B*, ChIP analysis of LNCaP cells stably transduced with a doxycycline-regulated DACH1 expression vector. Cells were treated for 12 h with either DHT (10 nmol/L), Casodex (CDX; 15 μmol/L), or vehicle control. ChIP assays were conducted with the antibodies indicated. *C*, immunoprecipitation-Western blot assay in LNCaP cell. *D*, association of DACH1 and AR in mammalian two-hybridization assay.



conducted in LNCaP cells. A doxycycline-inducible retrovirus expression vector encoding FLAG-tagged DACH1 was used to transduce LNCaP cells. ChIP analyses were conducted in the presence of vehicle, DHT (10 nmol/L), or the DHT antagonist Casodex (15 μmol/L). Comparison was made between the PSA proximal promoter (ARE I-II), the enhancer (ARE III), and a distal region of the PSA promoter that does not bind the AR as a form of negative control (Fig. 4A). ChIP conducted to DACH1 showed Tet-induced DACH1 expression correlated with DACH1 recruitment to the PSA ARE I-II and ARE III but not the control distal promoter region (Fig. 4B). DHT reduced and Casodex enhanced DACH1 recruitment to ARE I-II/III. In the presence of DHT, the AR abundance was increased at ARE I-II/III, which was reduced by Casodex or DACH1 expression (Fig. 4B). NCoR recruitment was enhanced by DACH1 expression at ARE I-II/III with further recruitment by Casodex at ARE III. NCoR and DACH1 associate with class II TSA-sensitive HDACs. Recent studies showed SirT1 represses the AR through deacetylation and is recruited to the ARE (10, 34). Consistent with previous studies, Casodex enhanced SirT1 abundance at the ARE I-II. DACH1 expression enhanced SirT1 recruitment in the basal state and in the presence of DHT at ARE I-II. DACH1 increased SirT1 recruitment in the presence of Casodex

to ARE III. Immunoprecipitation-Western blot analysis detected the increased association of AR with DACH1 in the presence of DHT and recruitment of the corepressor protein HDAC1 and NCoR (Fig. 4C). Mammalian two-hybrid analysis using Gal4-AR and VP16-DACH1 showed their association (Fig. 4D).

DACH1 DS domain-mediated AR repression involves the AR acetylation site. To determine the AR domains required for DACH1-dependent repression, a series of point mutants of the AR were examined in cotransfection experiment. In some patients with prostate cancer, point mutations arise in the carboxyl terminal ligand binding domain and contribute to promiscuous activation of the AR by different ligands. It has been considered that these mutations may thereby contribute to androgen antagonist failure (11). Using the androgen-responsive reporter PSA-luc in DU145 cells, comparison was made with point mutations of the AR acetylation site, as mutation of this site has been identified in prostate cancer and this site is a key determinant of androgen-dependent prostate tumor cellular growth (15, 16). As DACH1 repression of the wt AR was reduced by deletion of the DS domain and the DS domain was sufficient for repression of the wt AR (Fig. 3A), we tested the effect of DACH1 on the AR acetylation site point mutants using the DACH1 DS domain. Point mutation of the

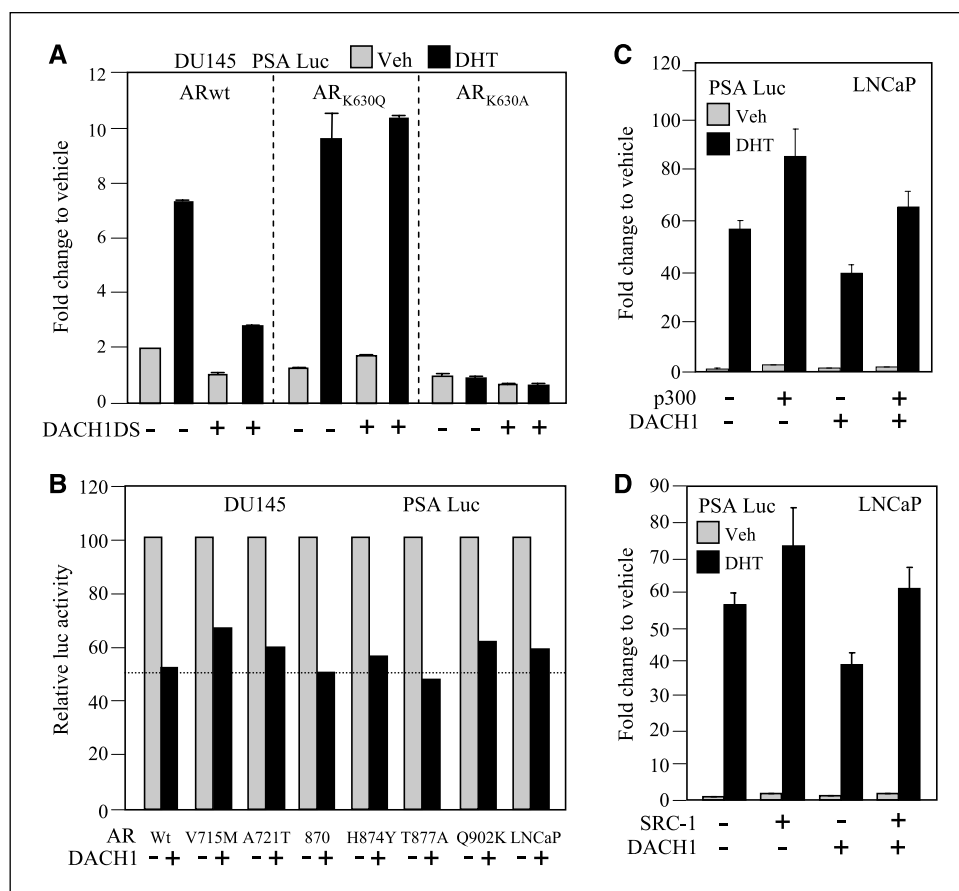


Figure 5. DACH1 inhibition of AR reporter gene activity via the AR acetylation site and overcome by p300. *A*, luciferase reporter gene assays were conducted using the AR-responsive PSA-Luc reporter gene. Cells were cotransfected with the AR wt or AR mutants and DACH1 DS domain as indicated. The AR K630 site is acetylated in response to ligand. *ARK630Q*, a gain-of-function mutant; *ARK630A*, an acetylation-dead mutant. *B*, AR wt or carboxyl terminal ligand binding mutants were assessed for DACH1 repression. p300 (*C*) and SRC-1 (*D*) enhanced AR activation and overcome DACH1 repression. Columns, mean of five separate transfections; bars, SE.

AR acetylation site enhanced ligand-induced transactivation (Fig. 5A) as previously described (15), inducing PSA activity ~10-fold. In contrast with AR wt, expression of the DACH1 DS domain failed to repress the gain-of-function AR acetylation point mutant. These studies suggest involvement of the AR acetylation site in AR repression by the DACH1 DS domain. An acetylation site dead mutant (*ARK630A*) failed to induce AR activity. No significant interaction was observed upon the expression of DACH1 (Fig. 5A).

The role of the AR carboxyl (C)-terminus was next examined. A series of point mutations in the AR COOH terminus were examined for DACH1 repression. The AR wt and ligand-binding domain point mutants each activated PSA activity (Fig. 5B). Ligand-dependent activity was normalized to 100% to enable an assessment of DACH1 repression. DACH1 inhibited ligand-dependent activation of the *PSA* promoter assessed using either the wt AR or each of the ligand binding domain AR point mutants (Fig. 5B). Collectively, these studies suggest the interaction between DACH1 and the AR does not involve the COOH-terminal ligand binding domain and may involve the AR acetylation site.

DACH1 repression of AR signaling involves class III deacetylases. AR activity is determined by coactivators including p300, SRC-1, TIP60, and ARA-70. Reporter gene assays were conducted to examine the effect of these coactivator proteins on DACH1-mediated repression of the AR. Ligand-dependent augmentation of AR activity was induced by p300 and SRC-1, and the expression of p300 and SRC-1 overcame the effect of DACH1 repression on PSA luciferase activity in the presence of DHT in both LNCaP and DU145 cells (Supplementary Fig. S2C, lanes 6

versus 8; Fig. 5C and D). Similarly, expression of pCAF, TIP60, or ARA-70 was capable of overcoming DACH1 repression of AR activity in the presence of ligand in both LNCaP and DU145 cells (Supplementary Fig. S2A–E, lanes 6 versus 8). Collectively, these studies show AR coactivators may either partially or completely rescue DACH1-dependent AR repression.

The AR acetylation site gain-of-function mutant *ARK630Q* evaded repression by the DACH1 DS domain. Endogenous SIRT1, a class III HDAC, constitutively represses AR activity in prostate cancer cells (10, 34, 35). We examined the role of endogenous Sirt1 in DACH1 repression using Sirt1 inhibitors (nicotinamide, Sirtinol, splitomycin). AR activity was assayed using the *PSA* promoter in HEK293T cells. AR activity was induced ~4.5-fold by DHT and repressed 50% by DACH1. The addition of Nicotinamide (5 μ mol/L) reversed the DACH1-dependent repression in the presence of ligand (Fig. 6A). DACH1 repression of DHT-dependent AR activity was not effected by the addition of TSA (Fig. 6B). Coexpression of either HDAC1 or HDAC3 augmented DACH1-dependent repression of the AR responsive *PSA* promoter (Fig. 6C). In addition to the known association of class I/II HDACs with DACH1, the current studies suggest class III HDACs are involved in DACH1-repression of the AR.

Discussion

The current studies show that DACH1 inhibits DHT-dependent AR signaling. DACH1 inhibited DHT-dependent DNA synthesis, cellular proliferation, and contact-dependent growth. DACH1

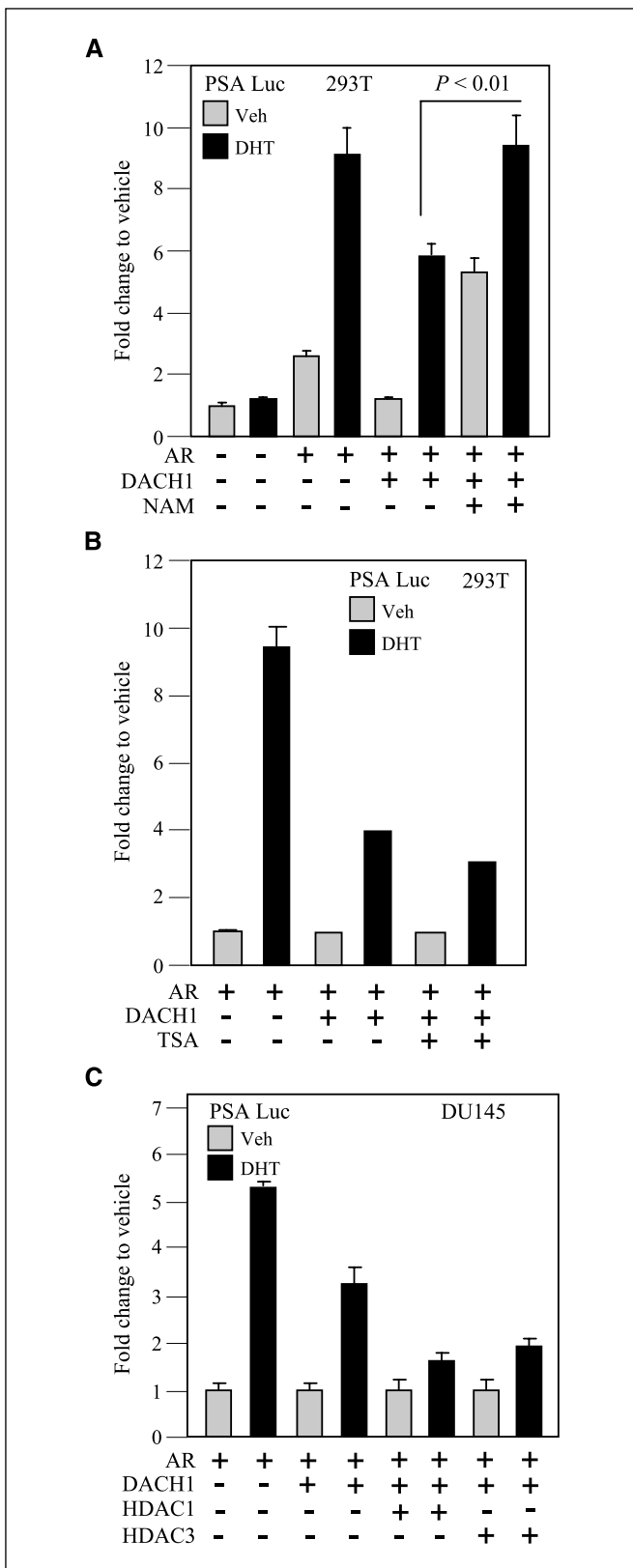


Figure 6. DACH1 inhibition of AR reporter gene activity was mediated through sirt1 dependent. Luciferase reporter gene assays were conducted using the AR-responsive PSA-Luc reporter gene. Cells were cotransfected with the AR and DACH1 as indicated and treated with (A) nictotinamide or (B) TSA, or (C) cotransfected with HDAC. Columns, mean of five separate transfections; bars, SE.

repression of the AR was dependent on the ARE, the presence of the AR, and the presence of the AR ligand DHT. Repression of AR activity required the conserved DS domain of DACH1. This region of DACH1 is conserved with the Sno/Ski oncogenes. However, neither Ski nor Sno repressed ligand-dependent AR activity. The DS domain alone was sufficient for repression of ligand-dependent AR activity. The DS domain is known to physically associate with NCoR/HDAC1 and HDAC3 (23, 24). ARE activity is determined by the relative abundance of coactivators and corepressor proteins that either encode or recruit HDAC activity. The AR corepressors, including NCoR, SMRT, HDAC1/3, and SIRT1 (8, 9, 19, 36, 37), play a key role in regulating ligand-dependent activity (38). The current studies are consistent with a model in which DACH1 recruits known AR corepressors to maintain the AR in a repressed state.

The DS domain of DACH1 was sufficient for repression of AR activity. Point mutation of the AR within the carboxyl terminus did not affect the magnitude of repression by DACH1. Thus DACH1, like SIRT1 (10, 34), inhibits the activity of mutant AR that arise in patients that are resistant to androgen ablation therapy. Point mutation of the AR acetylation site, however, created an AR mutant that was defective in repression by the DS domain. The AR is modified by phosphorylation, acetylation, ubiquitination, and sumoylation (38). The AR acetylation at lysine residues 630, 632, and 633 is known to enhance AR activity and gain-of-function mutations of the AR acetylation site (AR_{K630Q}, AR_{K630T}) convey enhanced growth and reduced apoptosis in human prostate cancer cells in culture and *in vivo* in nude mice (15). Molecular analysis of the gain-of-function AR acetylation site mutants showed enhanced binding of coactivator proteins (p300) and reduced binding of the corepressor complex including NCoR, HDAC1, HDAC3, SMAD6 (15). The finding that the DACH1 DS domain failed to repress the gain-of-function AR acetylation site mutant is consistent with previous studies showing DACH1 binds NCoR, HDAC1/3 (23, 27), and studies showing HDAC1/3 and NCoR (15, 16) have reduced binding affinity to this AR acetylation site mutant.

Herein, the AR was recruited to an ARE in the presence of ligand. In the current studies, DACH1 was recruited to both the proximal promoter (ARE I-II) and the distal enhancer (ARE III) and augmented NCoR recruitment. DACH1 enhanced Casodex-mediated recruitment of NCoR to the distal enhancer ARE III. The mechanisms governing NCoR recruitment are important as transcriptional repression by the AR induced by androgen antagonist is dependent on recruitment of a corepressor complex involving NCoR. Furthermore, NCoR seems to be required for transcriptional repression by androgen antagonists, and Casodex (bicalutamide) may function as an agonist in the absence of this corepressor (35–38). Previous studies have been consistent with a model in which the AR binds to and represses target gene transcription upon binding an androgen antagonist. Antagonist-bound AR undergoes a conformational change, rendering it competent to bind transcriptional corepressors. The current studies show that DACH1 expression is associated with the recruitment of NCoR in the absence of androgen antagonist. DACH1 thus functions to recruit the NCoR corepressor in the absence of antagonist.

The repression of AR activity was dependent on Nicotinamide, adenosine-dinucleotide. The class III HDAC family encoded by the Sirtuin proteins, which regulate diverse biological processes involved in the stress response, metabolism, apoptosis, aging, and nuclear receptor function (22). Sirt1 regulates transcription factors including p53, Foxo proteins, MyoD, nuclear factor-κB, and the

p300 and PGC1 α coactivators (22). Consistent with a prior study (34), SirT1 was recruited to ARE I-II upon Casodex treatment. SirT1 inhibits ligand-dependent AR-transactivation (34) and is required for AR antagonist-induced transcriptional repression (34). Sirt1 deacetylates and represses AR activity (10). In a previous study, SirT1 was recruited in the context of local chromatin to AREs (10, 34). Herein, DACH1 enhanced Sirt1 recruitment to the endogenous ARE of the *PSA* gene. SirT1 is expressed in prostate cancer and represses AR activity and AR-dependent cellular proliferation (10, 34). DACH1 such as Casodex recruits SirT1. DACH1 loss in prostate cancer may uncouple Sirt1 repression. The inhibitions of DACH1 repression by the SIRT1 inhibitors (Sirtinol, nicotinamide) are consistent with the participation of class III HDAC in DACH1 function. Thus, DACH1 repression of the liganded AR involves Type I/II and Type III HDAC activity.

The *Dachshund* gene encodes a protein of the helix-turn-helix family with a winged Forkhead structure (26). Initially described as a dominant repressor of the EGFR, *ellipse* mutation (25). *Drosophila Dachshund (Dac)* encodes one of seven genes required for compound eye specification. The current studies have implications for cross-talk between the Retinal Determination Gene Network signaling pathway and hormonal signaling through the AR. *Drosophila* gonadal development is dependent on *Dac* gene expression, and *Dac* mutants flies have defective external male genitalia and defective ovarian duct formation (39, 40).

Herein, DACH1 expression inhibited DHT-dependent cellular growth, DNA synthesis, and proliferation. Although the molecular mechanisms by which DACH1 regulated DHT-dependent cellular

proliferation remains to be determined, the inhibition of AR transcriptional activity is likely to be the key mechanism. DHT-induced LNCaP cellular proliferation is AR-dependent and DACH1 expression inhibited transcriptional activity of the AR mutant identified in LNCaP cells. DACH1 may inhibit proliferation via additional targets of the AR. DACH1 can inhibit the transcriptional activity of c-Jun and repress expression of cyclin D1 in fibroblasts (23, 27). Given the role of *Dachshund* in both the Retinal Determination Gene Network and AR signaling, it will be of interest to determine the role of the Retinal Determination Gene Network genes in cell fate determination and prostate tumor progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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