The Androgen Receptor Represses Transforming Growth Factor-β Signaling through Interaction with Smad3*

In the prostate, androgens negatively regulate the expression of transforming growth factor-β (TGF-β) ligands and receptors and Smad activation through unknown mechanisms. We show that androgens (dihydrotestosterone and R1881) down-regulate TGF-β1-induced expression of TGF-β1, c-Fos, and Egr-1 in the human prostate adenocarcinoma cell line, LNCaP. Moreover, 5α-dihydrotestosterone (DHT) inhibits TGF-β1 activation of three TGF-β1-responsive promoter constructs, 3TP-luciferase, AP-1-luciferase, and SBE44-luciferase, in LNCaP cells either with or without enforced expression of TGF-β receptors (TβRI and TβRII). Similarly, DHT inhibits the activation of Smad-binding element (SBE)44-luciferase by either constitutively activated TβRI (T204D) or constitutively activated Smad3 (S3*). Activation of SBE44-luciferase by S3* in the NRP-154 prostate cell line, which is androgen receptor (AR)-negative but highly responsive to TGF-β1, is blocked by cotransfection with either full-length AR or AR missing the DNA binding domain. Immunoprecipitation and GST pull-down assays show that AR directly associates with Smad3 but not Smad2 or Smad4. Electrophoretic mobility shift assays indicate that the AR ligand binding domain directly inhibits the association of Smad3 to the Smad-binding element. In conclusion, our data demonstrate for the first time that ligand-bound AR inhibits TGF-β transcriptional responses through selectively repressing the binding of Smad3 to SBE.

Normal prostatic epithelium depends on androgens for growth, development, secretory function, and survival (1–4). Most remarkably, androgen ablation induces massive apoptosis, differentiation, apoptosis, and carcinogenesis in the prostate (17–20), is under androgenic control. TGF-β signals through a cooperative interaction with two cell surface serine/threonine kinase receptors, TβRI and TβRII (21–25). TGF-β first associates with constitutively active dimeric TβRII, which then recruits and activates TβRI kinase by transphosphorylation at a juxtamembrane glycine-serine repeat (21, 26). With the help of Smad anchor for receptor activation (27), phosphorylated TβRI is able to activate Smads 2 and 3 by phosphorylating their carboxyl-terminal serine-serine-Xaa-serine motifs (28). Active Smads 2 and 3 can form heteromeric complexes with co-Smad4, and either directly or through interactions with transcription factors and co-regulators bind to Smad-binding elements (SBEs) in TGF-β-regulated genes (29–31). Further activation of Smads 2 and 3 is blocked by Smad7, whose expression is induced upon TGF-β stimulation (32).

Androgens negatively regulate TGF-β1 ligand (17, 33) and receptor expression (34, 35), along with Smad expression and activation (36) in the prostate. Recent reports show AR associates with Smad3 and that this association may either enhance...
and SBE.

MATERIALS AND METHODS

Cell Passaging—LNCaP cells (ATCC) were cultured in Dulbecco's modified Eagle's medium/Ham's F-12 (DMEM/F-12) supplemented with 10% fetal bovine serum (FBS) in polylysine-coated 6-well dishes. Following 48 h, wells were washed twice with DMEM/F-12 supplemented with 100 μg/ml bovine serum albumin fraction V, 5.0 μg/ml human transferrin, 20 ng/ml mouse epidermal growth factor, and 10 ng/ml sodium selenite and replaced with 2 ml of the medium above. After 2–3 h at 37°C, the medium was replaced as before, and various test factors were added. Following 48–72 h, conditioned 24 h media was harvested to assay for TGF-β1–3 by sandwich enzyme-linked immunosorbent assay (40–42). Samples were prepared as follows. Conditioned medium was treated with protease inhibitors (2 μg/ml aprotinin, 2 μg/ml leupeptin, and 0.2 mg/ml phenylmethylsulfonyl fluoride) and clarified at 10,000 x g for 10 min. The pellets were washed four times with RIPA buffer and eluted with 50 mM Tris-HCl (pH 7.4) and 10 mM NaCl for 20 min, and then pre-hybridized at 80°C with 50 mM Tris-HCl, 100 mM NaCl, 5 mM EDTA, 0.1% sodium dodecyl sulfate, 50% deionized formamide, and 50% formamide for 30 min, and microcentrifuged at 14,000 x g for 10 min. The resin was washed four times with 500 mM Tris-HCl, 100 mM NaCl, 5 mM EDTA) containing Complete Mini EDTA-free Protease Inhibitor Mix (Roche Molecular Biochemicals), 0.2 mg/ml phenylmethylsulfonyl fluoride, and 2 mM dithiothreitol. Lysates were treated with 5% Nonidet P-40 for 5 min on ice, clarified at 20,000 x g for 15 min at 4°C, and mixed overnight with 1 mg/ml of the ligand binding domain (a.a. 1–403) of Smad3 directionally cloned into pGEX-6P-1 (5 μg each pCMV2-FLAG-S3* and pCMV5-AR using a 5′-Xho I site of pCMV5-AR (Promega) and ethanol-precipitated. 50,000 cpm of labeled 32P-labeled TGF-β1 was added to equal volumes of glutathione-Sepharose-GST-Smad fusions in 50 mM Tris-HCl, 50 mM NaCl, 0.5 mM EDTA containing Complete Mini EDTA-free Protease Inhibitor Mix (Roche Molecular Biochemicals), 0.2 mg/ml phenylmethylsulfonyl fluoride, and 2 mM dithiothreitol. Resin-binding fusion proteins were quantified by Coomassie Blue staining of Tris glycine gels, using bovine serum albumin standards.

Transfection of NRP-154 Cells—Cells (6.0 x 10^5) were plated overnight in 12-well dishes and transfected using a standard calcium phosphate co-precipitate method for 3 h in GM2 (39) made with dextran charcoal-stripped FBS. The calcium phosphate/DNA co-precipitate was washed away, and the cells were glycerol-shocked (15% glycerol in 1 x HEPES-buffered saline) for 90 s. Cells were washed twice with phosphate-buffered saline, allowed to recover overnight in GM3 (47) (made with dextran charcoal-stripped calf serum) ≤ 10 nM DHT (Sigma) (or vehicle, 70% ethanol), and then treated with 10 ng/ml TGF-β1 (R & D Systems) or vehicle (4 μg/ml HCl, 1 mg/ml bovine serum albumin). Luciferase activity was measured 24–48 h later. LNCaP cells were transfected using Promega's Dual Luciferase Assay Kit and a Dynex ML3000 Microtiter Plate Luminoimeter.

Immunoprecipitation—NRP-154 cells (8.0 x 10^5) were plated overnight in 100-mm² dishes with 5 ml of GM2. Cells were transiently transfected with 5 μg each pCMV2-FLAG-S3* and pCMV5-AR using a standard calcium phosphate co-precipitate method. 10 nM DHT (or vehicle) was added after the transfection and again 4 h prior to harvest. Cells were lysed at 4°C with 400 μl of cold radioimmunoprecipitation (RIPA) buffer (containing Complete Mini EDTA-free Protease Inhibitor Mix) containing 3% sodium dodecyl sulfate, 0.2 mg/ml phenylmethylsulfonyl fluoride and 20 ng/ml human transferrin) using a 25-gauge needle. Lysates were pre-cleared with 5 μg of mouse IgG and 20 μl of protein A/G Plus-agarose (Santa Cruz Biotechnology) for 1 h, immunoprecipitated with either 2 μg of mouse anti-FLAG M2 (Sigma) or mouse anti-AR (Lab Vision) overnight in the presence of 10 nM DHT (or vehicle), and treated with 20 μl of protein A/G Plus-agarose for 2 h at 4°C. The resin was washed four times with RIPA buffer and eluted with 50 μl of 1 x SDS-PAGE loading buffer. Immunoprecipitation detection was done as described (47). Antibodies for Western blot detection were from Santa Cruz Biotechnology (human androgen receptor C-19, (1:4000)) and Sigma (FLAG M2 (1:500)).

In Vitro Transcription/Translation and GST Pull Down—Full-length AR cDNA (a.a. 1–919) was cloned into pcDNA3 (Invitrogen) and in vitro transcribed/translated using a T7 TnT® Coupled Reticulocyte Lysate System (Stratagene). 5 μl of the 35S-labeled TnT product was added to equal volumes of glutathione-Sepharose-GST-Smad fusions in buffer A (20 mM Tris (pH 7.8), 180 mM KCl, 0.5 mM EDTA, 5 μM MgCl2, 0.5 mM ZnCl2, 10% glycerol, 0.1% Nonidet P-40, 0.05% milk, 1 μM dithiothreitol, 0.5 μM phenylmethylsulfonyl fluoride) and rotated overnight at 4°C. The glutathione-Sepharose beads were then centrifuged (5 min at 3000 rpm) and washed 3 times with 500 μl of cold buffer A. GST-Smad-AR complexes were eluted with 50 μl of 1 x SDS-PAGE loading dye and heated to 70°C for 10 min prior to resolving the complexes in a 4–12% NuPAGE BisTris gel with 1 x MES buffer (Invitrogen). The proteins were then transferred to nitrocellulose and detected by a DCF488 dye enhanced DIG PhosphorImager.

Electrophoretic Mobility Shift Assay—SBE oligonucleotides (Santa Cruz Biotechnology) were labeled with 32P-ATP using T4 polynucleotide kinase (Promega) and ethanol-precipitated. 50,000 cpm of labeled oligonucleotides were mixed with GST fusion proteins in binding buffer (10 mM Tris (pH 7.8), 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol, 2 mM ZnCl2, 50 ng of polydeoxyinosinic-deoxycytidylic acid),
Androgens Block the TGF-β1-induced Autoinduction of TGF-β1 Ligand and c-Fos and Egr-1 Expression in LNCaP Cells—Androgens negatively regulate expression of TGF-β ligands in prostatic cells in both animals (17) and rat cell culture (48). We determined whether androgens could also down-regulate TGF-β1 autoinduction (49) in the androgen-responsive human prostatic adenocarcinoma cell line, LNCaP, under serum-free conditions, using isoform-specific sandwich enzyme-linked immunoadsorption assays for TGF-β. From a list of common hormones and growth factors added to LNCaP cells, only TGF-β binds substantially elevated protein levels of TGF-β1 (data not shown; referred to as “TGF-β autoinduction”). A physiological concentration of DHT (10 nM), the active metabolite of testosterone, inhibited the induced expression of TGF-β1 protein (Fig. 1A) and mRNA (Fig. 1B). The stable androgen analogue, R1881, also blocked TGF-β2-induced expression of TGF-β1 (Fig. 1, B–D). We used R1881 to study the kinetics of TGF-β1 mRNA loss following an initial (72 h) induction by TGF-β1 (Fig. 1D). Changes in the expression of two transcription factors (c-Fos and Egr-1), shown to be induced by TGF-β and involved in TGF-β autoinduction (50–53), were also determined (Fig. 1D). The induced expression of TGF-β1 ligand, c-Fos, and Egr-1 mRNAs was inhibited by R1881 in a time-dependent manner. Of note, LNCaP cells treated with TGF-β1 will maintain increased mRNA levels of TGF-β1 ligand, c-Fos, and Egr-1 between 2 and 5 days of treatment (data not shown).

The decrease in TGF-β1 expression by R1881 occurred through transcriptional repression and did not require de novo protein synthesis, as demonstrated by loss of TGF-β1 mRNA expression following 24 h of treatment with R1881 in the presence of actinomycin D in cycloheximide (Fig. 1E, right panel). Quantification of the Northern blot in Fig. 1E is also presented (Fig. 1E, left panel). Actinomycin D caused the same loss of TGF-β1 mRNA as did R1881, and co-treatment of R1881 and actinomycin D did not enhance loss by R1881 alone. In the presence of cycloheximide, R1881 caused a smaller loss of TGF-β1 mRNA expression, perhaps due to a decrease in AR expression. Co-treatment of actinomycin D and cycloheximide had the same effect as actinomycin D alone. Together, these data suggest that AR functions through directly repressing transcription by TGF-β.

DHT Inhibits the Transcriptional Activation of Several Response Elements Induced by TGF-β1—We tested the above hypothesis by assaying the effect of DHT on TGF-β1-induced transcriptional activation of various response elements. In LNCaP cells, the TGF-β1-induced 3TP-luciferase (54) activity was blocked by co-treatment with DHT (Fig. 2A). Previous work supports that LNCaP cells express low levels of either TβRI or TβRII (55, 56), potentially accounting for their relatively weak response to TGF-β. Therefore, we co-transfected these cells with either TβRI or TβRII along with 3TP-luciferase. Co-trans
Infection of TβRII resulted in 50-fold enhanced activation of luciferase by TGF-β1 (Fig. 2B). In contrast, TβRI did not enhance TGF-β1-induced 3TP-luciferase activity (data not shown), suggesting that TβRII but not TβRI was limiting in our LNCaP lineage. Overexpression of TβRII did not blunt the ability of DHT to inhibit TGF-β1-induced 3TP-luciferase (Fig. 2C). Additionally, the inhibition of TGF-β1-induced 3TP-luciferase activity was dependent on DHT concentration (Fig. 2D). These data demonstrate that the levels of endogenous AR in LNCaP can fully repress TGF-β1-induced 3TP-luciferase activity, even in the presence of overexpressed TβRII, when ligand-bound.

We tested the above results and the requirement for AR in another prostatic cell line, NRP-154, which is exquisitely sensitive to TGF-β1 but has undetectable levels of AR (39). Transfection of full-length AR (57) enabled DHT to similarly inhibit TGF-β1-induced 3TP-luciferase activity in NRP-154 cells (Fig. 2E). 3TP-luciferase has a complex promoter, consisting of 3TRE elements upstream of a plasminogen activator inhibitor-1 promoter fragment. We co-transfected LNCaP cells with basic promoter constructs, AP-1- or SBE4BV-luciferase (58), to define better the elements responsible for transcriptional inhibition by DHT. TGF-β1 activated both AP-1-luciferase and SBE4BV-luciferase, and 10 nM DHT substantially inhibited these activ-
DHT inhibition of TGF-β1-induced SBE4vβ-luciferase activity occurs through Smad3.

A. LNCaP (left panel) or NRP-154 (right panel) cells were transiently co-transfected with 0.5 μg of SBE4βv-luciferase and 150 ng of pCMV2-S2* or pCMV2-S3* (or pCMV2 control). LNCaP cells were co-transfected with 0.5 μg of SBE4βv-luciferase, 30 ng of pCMV2-FLAG-S3*, and 1.0 μg of pcDNA3.1-DN-Smad4 (B) or pcDNA3.1-Smad7 (C) (or pcDNA3.1 control). LNCaP (D) or NRP-154 (E) cells were transiently co-transfected with 0.5 μg of SBE4βv-luciferase, 30 ng of pCMV2-FLAG-S3* (or pCMV2 control), and 0.5 μg of pCMV5-AR (or pCMV5 control). F. LNCaP cells were transiently co-transfected with 0.2 μg of SBE4βv-luciferase, 0.25 μg of pCMV5-AR, 0.25 μg of pCMV2-Smad3 (or pCMV2), and 0.3 μg of pCMV5-TβRII before 24 h co-treatment with TGF-β1 (or vehicle) and DHT. Cells were treated with 10 nM DHT (or vehicle) for 24 h before harvesting. 12.5 ng of pCMV-Renilla/well was also co-transfected for all luciferase assays. Data shown are averages (± S.D.) of triplicate independent measurements of luciferase/Renilla readings relative to untreated controls.

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To define better the inhibitory role of AR on Smad3, we analyzed the effect of AR on the association of Smad3 to SBE. By expression of wild-type AR or Δ538–614 in the absence of ligand (Fig. 5B). In contrast to full-length AR that had no inhibitory effect without DHT, Δ538–614 repressed the majority of S3* activity without ligand. Expression of DDB or ABC had little to no significant inhibitory effect on S3* activity; and as expected, DHT did not change the effectiveness of these (DBD or ABC) regions on inhibiting S3* activity (Fig. 5B). These data suggest that DHT promotes the inhibitory effect of AR on Smad3 in a DDB-independent manner that requires the LBD or some other region in the AR carboxyl terminus.

The Ligand Binding Domain of the AR Inhibits Smad3 Binding to the Smad-binding Element—Inhibition of Smad3 signaling by DHT could occur through several mechanisms. Upon TGF-β1 treatment, Smad3 translocates to the nucleus where it induces target gene expression; similarly, AR is localized to the nucleus in the presence of DHT. Therefore, we examined the effect of AR on the association of Smad3 to SBE. Electrophoretic mobility shift assays were performed with [32P]-labeled SBE oligonucleotides and purified GST-Smad3 in the presence of different domains of AR (Fig. 6A; amino-terminal GST-AR, GST-AR DBD, or GST-AR LBD). GST-Smad3 was able to bind to the SBE in the presence of the amino-terminal GST-AR and GST-AR DBD; however, GST-AR LBD inhibited this association (Fig. 6B). This result is consistent with the data presented in Fig. 5B, which suggests the carboxyl terminus (i.e. LBD) of AR confers inhibition of S3* activity. We next determined the effect of DHT on the association of GST-Smad3 binding to SBE in the presence of GST-AR LBD (200, 400, and 800 ng). The addition of 10 nM DHT enhanced GST-AR LBD-induced inhibition of GST-Smad3 binding to SBE (Fig. 6C). This inhibition was specific to GST-Smad3 because no loss of the GST-Smad4 SBE complex (Fig. 6D) in the presence of GST-AR LBD (800 ng) + DHT was observed. Also, DHT alone did not abrogate GST-Smad3 binding to the SBE. Together, these results

the substantial S3*-induced SBE4 luciferase activity in LNCaP, additional AR was required to fully repress this response (however, endogenous AR could fully inhibit TGF-β1-induced SBE4 luciferase activity, Fig. 2G). Similarly, in NRP-154 cells, DHT inhibited S3*-induced SBE4 luciferase activity only in AR co-transfected cells (Fig. 3E), demonstrating the dependence of both androgens and AR on this effect. Finally, to determine whether Smad3 was the major target of DHT-induced inhibition of TGF-β1 signaling, we transfected LNCaP cells with wild-type Smad3 in an attempt to reverse the inhibition. As shown in Fig. 3F, expression of wild-type Smad3 fully reversed the inhibition of TGF-β1-induced SBE4 luciferase activity by AR and DHT.

The Androgen Receptor Associates with Smad3 Independent of Ligand—Recent evidence from other laboratories (37, 38) suggests that AR associates with Smad3 in an androgen-independent manner. We confirmed these results in our system, along with determining if S3* was still able to bind AR due to mutation of its carboxyl terminus, by both GST pull-down assays and co-immunoprecipitation, respectively. NRP-154 cells were co-transfected with FLAG-S3* and AR and treated with DHT (or vehicle) before immunoprecipitation 24 h later. Fig. 4A demonstrates that immunoprecipitating against either FLAG or AR can capture S3* complexes independent of DHT addition. Full-length (a.a. 1–919) in vitro transcribed/translated [35S]-labeled AR was preincubated with GST pull-down antibodies. Bands were detected using ECL. SDS-PAGE and immunodetected using anti-FLAG and anti-AR antibodies. Bands were detected using ECL. B, equal volumes of GST-Smad fusions bound to glutathione-Sepharose were mixed with full-length (a.a. 1–919) in vitro transcribed/translated [35S]-labeled AR in buffer A overnight at 4°C. The complexes were washed three times in buffer A before SDS-PAGE and transfer to nitrocellulose. Lanes 1–4 indicate the respective GST-Smad, and GST control indicates GST alone with AR. Bands were detected using a PhosphorImager. A representative blot of three independent experiments is shown.

FIG. 4. AR associates with Smad3 independent of ligand. A, NRP-154 cells were co-transfected with 5 μg each of pCMV5-AR and pCMV2-FLAG-S3*. 10 nM DHT (or vehicle) was added to cells after transfection, and cells were harvested 24 h later. Cell lysates were precleared using non-immunized mouse IgG and immunoprecipitated (IP) with either anti-FLAG or anti-AR antibodies in the presence of 10 nM DHT (or vehicle) overnight at 4°C. Samples were subjected to SDS-PAGE and immunodetected using anti-FLAG and anti-AR antibodies. Bands were detected using ECL. B, equal volumes of GST-Smad fusions bound to glutathione-Sepharose were mixed with full-length (a.a. 1–919) in vitro transcribed/translated [35S]-labeled AR in buffer A overnight at 4°C. The complexes were washed three times in buffer A before SDS-PAGE and transfer to nitrocellulose. Lanes 1–4 indicate the respective GST-Smad, and GST control indicates GST alone with AR. Bands were detected using a PhosphorImager. A representative blot of three independent experiments is shown.

FIG. 5. AR DBD is not required to inhibit Smad3 activity. A, mammalian expression vectors used for different domains of AR. B, NRP-154 cells were co-transfected with 0.5 μg of SBE4 luciferase, 12.5 ng of pCMV-Renilla, 30 ng of pCMV2-FLAG-S3* (or pCMV2 control), and 0.5 μg of different AR domains or full-length AR (or pCMV5 control). 10 nM DHT (or vehicle) was added to cells immediately after transfection and 8 h before harvest. Each point represents the average of at least triplicate determinations ± S.D.
DHT is required for full-length AR to inhibit S3*-induced SBE4int-luciferase (Fig. 5B). Δ538–614 AR, which lacks the DBD, is able to inhibit ~70% of S3* activity independent of ligand and ~90% (similar to wild type) of S3* activity with ligand (Fig. 5B); therefore, the DBD may prevent DHT-independent inhibition of S3*. The mechanism by which Δ538–614 without DHT inhibits S3* activity is unclear; however, data show ARΔDBD (no DBD) is more efficiently translocated to the nucleus compared with an AR nuclear localization mutant (64). This suggests that the nuclear localization signal of AR is bi- or tripartite and may be enhanced in the absence of DBD. Thus, we hypothesize a greater percentage of Δ538–614 may be localized to the nucleus in the absence of DHT, as compared with wild-type AR. The means by which AR DBD alone reduces S3* activity is not known, because the Δ538–614 AR data suggest this region is not necessary for complete inhibition. Furthermore, the EMSA results substantiate that the DBD is not essential for AR-mediated loss of S3* activity (Fig. 6A). AR LBD is able to inhibit GST-Smad3 binding to SBE without DHT; nevertheless, addition of ligand does enhance this effect by ~4-fold, perhaps through an AR LBD conformational change.

The above result parallels data from previous reports that demonstrated LBD alone can bind to Smad3 (37). Interestingly, one report (37) showed the AR DBD or AR LBD could bind to Smad3, although no functional consequence of these interactions was presented. The other study revealed the AR amino-terminal region (a.a. 1–563) associates with the MH2 domain of Smad3 to repress androgen receptor-mediated transcription of murine mammary tumor virus-luciferase (38), but the significance of this interaction (amino-terminal AR-Smad3) to TGF-β signaling was not examined. We are not able to explain the discrepancy that exists among several groups attempting to characterize the association of AR with Smad3. However, we believe our data are solid due to our EMSA assay observing a function of the AR-Smad3 association and not solely the physical interaction. It is important to note that although AR can inhibit Smad3, we cannot yet rule out that this inhibition is exclusive to Smad3 and does not also involve Smads 2 or 4 through indirect means.

The normal prostate requires an intact androgen signaling pathway for growth and function, whereas prostate carcinomas often escape from this dependence through changes likely to involve AR. AR receptor mutants that are unable to activate androgen-responsive genes or change the sensitivity of the receptor to circulating androgens (or other steroids) may suppress androgen dependence in the prostate (65–67). Also, mutations within the AR carboxyl terminus which decrease steroid affinity may produce a ligand-insensitive and yet transcriptionally active AR (68, 69). An abundance of AR polymorphisms without functional significance have been characterized, especially within the amino-terminal region, which are linked to increased incidence of prostate cancer (70–72). The expression of several ARAs have been implicated in altering AR activity in the prostate (12, 13, 73–75), which may dysregulate AR-Smad3 interaction and function. Moreover, it is apparent that prostate tumors, both localized and metastatic, maintain or increase AR expression and sensitivity following androgen ablation therapy (10, 76–79). The data presented here suggest a novel mechanism by which such aberrations in AR can directly antagonize TGF-β effects within the prostate and promote the development and progression of cancer.

Restoration of TGF-β receptor levels by overexpression of wild-type TβRII in LNCaP cells was reported to promote TGF-β responsiveness and suppress tumor growth through reduced cell proliferation and the induction of apoptosis (80).
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Consistent with a tumor-suppressive role of TGF-β in the prostate, we have shown that DN-TβRII promotes malignant transformation of two non-tumorigenic prostate epithelial cell lines (19). Our data showing that TGF-β is a potent inducer of apoptosis in the above cell lines further support TGF-β may suppress prostate tumor growth through the induction of apoptosis (47, 81). With this in mind, the current work proposes that constitutive or enhanced activation of AR, through means described earlier, may cause loss of androgen dependence (e.g., rescue from undergoing apoptosis) partly via loss of TGF-β signaling through inactivation of Smad3, because Smads were shown to be critical to the induction of apoptosis by TGF-β (82). This loss would allow for prostatic epithelial cells to escape growth inhibition and apoptosis by TGF-β, contributing to carcinogenesis of the prostate.

In conclusion, our data demonstrate for the first time that alteration of AR with Smad3 can inhibit the ability of Smad3 to bind SBE and activate transcription. Amplification of AR, or variances within the AR (or its signaling pathway) that promote deregulated and enhanced Smad3 binding, may counteract tumor suppression by TGF-β. Moreover, these findings strengthen our hypothesis that androgens promote viability of prostatic epithelial cells, in part, by preventing TGF-β-induced apoptosis. In view of the numerous binding partners for Smads shown to be critical to the induction of apoptosis by TGF-β signaling through inactivation of Smad3, because Smads were formation of two non-tumorigenic prostate epithelial cell lines may—

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